CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 20-727

Clinical Pharmacology and Biopharmaceutics Review

CLNICAL PHARMACOLOGY and BIOPHARMACEUTICS REVIEW

Division of Pharmaceutical Evaluation I

NDA 20727 (Amendment No.121) **Submission Date:** December 21, 2004

Type:	Submission of Supplement 121 to NDA 20727
Brand Name:	[ ] (BiDil) 20 Tablets
Dosage Strength:	Hydralazine HCl 37.5 mg/Isosorbide Dinitrate 20 mg
Indication:	Adjunct Treatment of Black Patients with Congestive Heart Failure Who are Intolerant or have a Contraindication to ACE-Inhibitors
Sponsor:	NitroMed, Inc. Lexington, MA
Reviewing Division:	Cardiorenal, HFD-110
Reviewers:	Peter H. Hinderling, MD Lydia Velazquez, Pharm.D.
Team Leader:	Patrick J. Marroum Ph.D.

Reference is made to the non-approvable letter to NDA 20727 of July 1997 BiDil tablets containing hydralazine hydrochloride (H) and isosorbide dinitrate (ISDN) of strength (mg) 37.5 H/10 ISDN, 37.5 H/20 ISDN, 75 H/20 ISND, and 75 H/40 ISND for adjunct treatment of congestive heart failure (CHF). The 1996 submission reported on the results of two pivotal studies, VHeFT I and II, in patients of different races with CHF. In both trials the patients received solid formulations of the two compounds qid. The Cardiovascular and Renal Drugs Advisory Committee did not recommend the application for approval and the Agency concurred with that recommendation.

NDA 20727 submitted in 1996 reported the results of 2 pivotal trials, VHeFT I and VHeFT II, in patients of different races with CHF. In both trials H and ISDN were administered as the individual formulations. The target dose was 75 mg H and 40 mg ISDN qid resulting in daily doses of 300 mg H and 160 mg ISDN. The initial dose was 37.5 mg H and 20 mg ISDN qid. In case of adverse events probably attributable to H or ISND the respective doses could be adjusted. In VHeFT I the dose adjustment for H was achieved by reducing the dose to 37.5 mg qid and the ISDN dose to 20 mg ISDN qid. In VHeFT II the dose regimen adjustments for H were achieved by reducing frequency or size of the administered dose as follows: 37.5 mg bid, 37.5 mg qid, and 37.5 mg qid alternating with 37.5 mg bid. With ISDN only the size of the dose was adjusted as follows: 10 mg qid, 20 mg qid, and 30 mg ISDN qid. H was administered in capsules in VHeFT I and as tablets in VHeFT II. ISDN was administered as tablets in both trials.

VHeFT I compared the combination of H and ISDN to placebo and prazosin and showed fewer deaths in the combination treated population than in the placebo or prazosin treated populations. VHeFT II compared the combination of H and ISDN with enalapril. A placebo control group was not included in the trial. In VHeFT II the combination treatment of H and ISDN was statistically significantly inferior to the enalapril treatment. The information provided in support of the efficacy of the combination in treating patients with CHF was judged to be inadequate and the application was considered non approvable.

The Supplement 121 to NDA 20737 submitted on December 23, 2004, contains the report of the pivotal A-HeFT in African-American patients with CHF. Amendment 121 does not contain an Item 6 or new Clinical Pharmacology or Biopharmaceutics information. As a result, the Office of Clinical Pharmacology and Biopharmaceutics performed its own literature search to update section 6 and the proposed label for both H and ISDN. In the case of ISDN, it was agreed that only areas that were completely omitted in the labeling were to be updated in the interest of time.

The pivotal A-HeFT compared a treatment with BiDil 20 tablets to placebo treatment in African-Americans with CHF. BiDil 20 tablets containing 37.5 mg H and 20 mg ISDN were used in this study and were administered tid. The target single dose of BiDil was 75 mg H and 40 mg ISDN resulting in daily doses of 225 mg H and 120 mg ISDN. The BiDil dose could be adjusted if adverse events occurred that were probably attributable to either H or ISDN. The single doses for H and ISDN could be as low as 18.75 mg and 10 mg ISDN resulting in respective daily doses of 56.25 mg H and 30 mg ISDN. A-HeFT showed fewer deaths in the population treated with BiDil than in the placebo treated population.

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1. EXECUTIVE SUMMARY

1.1 Recommendations

The Office of Clinical Pharmacology and Biopharmaceutics/Division of Pharmaceutical Evaluation 1 (OCPB/DPE1) has reviewed the non-approvable letter of 1997 and the amendments relating to Clinical Pharmacology and Biopharmaceutics issues submitted by the sponsor since that time. Based on the review of the above information OCPB/DPE1 is of the opinion that all Clinical Pharmacology issues previously raised have been adequately addressed. The submitted Clinical Pharmacology and Biopharmaceutic information is acceptable.

It is recommended that the sponsor determines a) the involvement of CYP 450 in the metabolism of H and ISDN and the inhibitory and inductive potential of H and ISDN on CYP 450 in vitro using liver tissues b) whether H and ISDN are substrates and/or inhibitors of P-glycoprotein. The target population receives background therapy including narrow therapeutic range drugs. The identity of a significant fraction of the dose of H that is systemically available has not been determined and the metabolism of H could involve CYP 450. There is evidence for oxidative microsomal metabolism of H. Co-administered digoxin and statins are substrates of P-glycoprotein.

1.2 Phase 4 Commitments

No Phase 4 commitments are proposed.

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

The non-approvable letter for NDA 20727 issued to Medco Research, Inc., in July 1997 contained the following comments and requests for information pertinent to Clinical Pharmacology and Biopharmaceutics:

1. In view of the fact that the 37.5/10 tablet showed a slower dissolution performance compared to the 37.5/20, 75/20 and 75/40 formulations in all 4 media tested, an in vivo bioavailability waiver cannot be granted for the two middle strengths (37.5/20, 75/20). However, the multiple-dose study that you are currently conducting using all strengths in CHF patients could provide the necessary data.

2. When asked to provide the pharmacokinetic parameters for study CB02 in electronic form on diskette the only data submitted was for the normalized parameters to a weight of 65 kg. Upon review, it was discovered that the data on diskette did not match those found in the NDA, and the discrepancy remains unexplained. In future submissions please validate all data sets before they are submitted.

3. Your proposal for inclusion of information regarding food-effect on hydralazine and isosorbide dinitrate based on published literature cannot be accepted. A food-effect study, using to be marketed formulation of BiDil, will be required to support any statement relating to the effect of food on administration of BiDil.

Regarding issue 1: The sponsor used the 37.5/20 strength BiDil tablet in A-HeFT, and the efficacy and safety data reported in Amendment 121 qualifies this formulation and strength. The other pivotal study, VHeFT I, submitted in the original NDA, used the individual hydralazine 37.5 mg capsules and ISDN 10 mg tablets, and the positive results obtained qualifies the two single entity formulations. A demonstration of bioequivalence between the combination and single entity formulations is not required. Therefore issue 1 can be considered resolved.

Regarding issue 2: In the submission of January 8, 2001 the response of NitroMed, Inc., was:" This is a statement by the FDA regarding future submissions. No action is required." This response was acceptable to the Reviewer of DPE1. It should be noted that the inconsistencies concerned data sets in support of the bioequivalence of the single entity formulations and the combination tablets. As discussed in 1) these data are not any longer critical. Thus, this issue can be considered resolved.

Regarding issue 3: In the submission of November 20, 2001, NitroMed agreed to adopt the wording for labeling proposed by the Agency concerning the impact of food:" No information is currently available regarding the effect of food on BiDil tablets." Thus, this issue is resolved.

Unresolved Issues

The results from A-HeFT and VHeFT I suggest BiDil is effective as an adjunct treatment in blacks with CHF. Both, H and ISDN, as individual drugs have been on the market for other indications for years. They are not considered narrow therapeutic range drugs. However, this assessment cannot be necessarily extrapolated to the more vulnerable CHF population receiving both drugs together.

The sponsor has not attempted to determine the optimum dose-ratio and -interval of the proposed fixed dose combination regimen using biomarkers/surrogate endpoints. No dose ranging studies using a factorial design with varied dose ratios and intervals of the H and ISDN components have been conducted in the target population. Thus, the least and maximum effective doses of H and ISDN when administered as a combination have not been determined. The qid regimen used in VeTHFT I and VeHFT II and the tid regimen

employed in A-VeHFT are different. It is unknown whether the different regimens used in A-HeFT and VHeFT I and II optimally minimized the development of tolerance to ISDN since the recommended dose-free interval in the ISDN labels is a minimum of 14 hours.

The Clinical Pharmacology database of BiDil consists of data obtained in a single bioavailability study in healthy subjects who received BiDil tablets of different strengths and different single entity formulations. There is no information on the possible impact of many extrinsic and intrinsic factors including NAT2 polymorphism on exposure and consequently on response to the H component of this fixed dose combination. The potential of H and ISDN to be the cause or the subject of drug interactions has not been investigated, although the target population receiving BiDil will be on several other drugs, some of which have a narrow therapeutic range. The relationship between plasma concentrations and response in the target population has not been determined for the BiDil components either.

It is probable that intrinsic and extrinsic covariates individually and as an aggregate impact the exposure and response to the individual drug entities in CHF patients. The acetylator status determines importantly the absolute bioavailability of H and as a result the mean exposure to H can be from 2.6 to 6.0 times greater in poor metabolizers (PMs) than in extensive metabolizers (EMs). Consequently, the respective concentrations of H and ISDN in the biophase will not be in the same fixed ratio as the respective doses. They will be individually different, but not tailored to the needs of the individual patients. Determination of the major covariates impacting the exposure-response profile of H and ISDN could have contributed to improving the benefit-risk relationship of BiDil. It is likely that the proposed fixed dose regimen is ineffective in some patients and results in adverse events in others, disadvantages that a regimen more tailored to the needs of subpopulations with the target disease would not share.

Given the scarcity of the database for BiDil a literature search was conducted to establish what is known about the Clinical Pharmacology of H and ISDN when administered alone.

For H only PK information from publications that used specific assays for H and metabolites were selected. The information obtained from the reviewed literature indicated that the package insert of H is outdated. The data from the selected publications indicated significant inter-subject variation of the PK of H. The dose requirements of CHF patients treated with H vary by a factor of 8. Acetylator status and food intake can alter the exposure to H importantly. H interacts with beta-blockers and ACE inhibitors, drugs that are likely to be co-administered with BiDil in CHF patients. The review also showed that the impact of many intrinsic and extrinsic factors remains undetermined. Also, it is not known whether the impact of the identified covariates for H is affected by the presence of ISDN.

All publications reviewed utilized some form of a GC method for quantitation of ISDN and its mononitrates. After assessing the label for ISDN products, it was determined that

certain areas of the package insert should be updated. Specifically those areas that are totally not mentioned in the current ISDN product labels. ISDN inter-subject variability is a known phenomenon with nitrate products, including ISDN. Hepatic status, renal impairment, circadian rhythm, and gender do not seem to alter the pharmacokinetics of ISDN or its metabolites to the extent that a dosage adjustment would be warranted. The elimination of ISDN in uraemic patients does not seem to change with haemodialysis. Drugs likely to be administered concomitantly such as some of the beta-blockers do not seem to alter the pharmacokinetics of ISDN or its mononitrate metabolites to warrant a change in the dose. A decrease in diastolic blood pressure (DBP) correlates well with the time course of plasma concentrations of ISDN, and to a lesser extent with 2- and 5-ISMN in healthy volunteers. In many cases an increase in heart rate (HR) was observed as well.

It is recommended that the sponsor determines in vitro a) whether H is a substrate of CYP 450 enzymes and H's inhibitory and induction potential regarding other substrates of CYP 450 and b) whether H is a substrate and/or an inhibitor of P-glycoprotein.

The sponsor should a) determine whether ISDN is a substrate of CYP 450 enzymes in-vitro; b) explore ISDN's inhibitory and induction potential regarding other substrates of CYP 450; and c) investigate whether ISDN is a substrate and/or an inhibitor of P-glycoprotein.

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Clinical Pharmacology & Biopharmaceutics Reviewers

The CPB briefing was held on April 14, 2005, Attendees: Drs. J. Lazor, M. Mehta, F. Frueh, P. Lee, A. Rahman, J. Hunt, P. Marroum, L. Velazquez, P. Hinderling

2. QUESTION BASED REVIEW

2.1 What is the Efficacy and Safety Database for BiDil?

The sponsor performed 3 clinical trials A-HeFT, VHeFT I and VHeFT II. They make up the safety data base. A-HeFT and VHeFT I are placebo controlled trials and represent the efficacy data base.

A-HeFT was a randomized, placebo controlled, parallel group study evaluating efficacy and safety of BiDil 20 tablets containing 37.5 mg H and 20 mg ISDN in 1050 black patients with CHF at 169 centers in the US. All patients were self-identified blacks. They had stable symptomatic NYHA Class III-IV heart failure attributable to ischemia, hypertension or cardiomyopathy while on currently recommended standard therapy including non-aldosterone antagonist diuretics, beta-blockers, ACE inhibitors, digoxin, aldosterone antagonists. Patients were required to have LVEF < 35% or left ventricular internal diastolic dimension >2.9 cm/m2 plus LVEF <45%. Patients were randomized to BiDil (n=518) or placebo (n=532). BiDil was initiated at 1 tablet tid and titrated to a target maximum dose of 2 tablets tid. In the event of adverse events that were probably attributable to H or ISDN, the dose of BiDil could be adjusted. The tablets were to be taken q6 hours during day time with a 12 hour dose- free interval during the night. The primary endpoint was a composite score weighting all-cause mortality, first hospitalization for heart failure during the 18 month follow-up period, and change from baseline in quality of life at six months.

The trial was terminated early, at a mean follow-up of 12 months, due to a statistically significant 43 % reduction in all-cause mortality in the BiDil treated group (p=0.012). Death due to worsening heart failure was reduced by 48 % (p<0.009). The BiDil group also showed an improvement in the event free survival with a 37 % reduction in mortality or first hospitalization for heart failure (p<0.001). Further, the BiDil treated group showed a 39 % reduction in first hospitalization for heart failure and had consistent improvements in quality of life from baseline throughout the trial. The target dose of 6 tablets of the H/ISND combination was achieved in 68.0 % of the patients as compared with 88.9% of the patients in the placebo group. The mean number of the H/ISDN tablets taken was 3.8 (2.5) compared to 4.7 (2.2) in the placebo group. This result indicates that the population average daily doses of H and ISND were 142.5 mg and 76 mg ISDN, respectively.

In the multi-center VHeFT I the combination of H and ISDN was compared to prazosin or placebo in 642 men (186 randomized to H & ISDN) with impaired cardiac function and reduced exercise tolerance while on then standard therapy of digoxin and diuretics. The mean follow-up period was 2.3 years. Mortality in patients treated with H & ISDN was reduced relative to patients on placebo by 38 % at the end of 1 year, 25 % at 2 years (p<0.028) and 23 % at 3 years. Mortality was not different between prazosin and placebo treated groups. In the subset of 128 black VHeFT I patients, the combination of H/ISDN

reduced mortality versus placebo by 47 % (hazard ratio=0.53, p=0.043). Survival in non-blacks was not significantly different between the combination treatment and placebo.

The results of VHeFT I and VHeFT II were submitted as a NDA in 1997. VeFT II in contrast to A-HeFT and VHeFT I was not a placebo controlled study and compared the H/ISDN combination treatment with a treatment with enalapril. As in VHeFT I, the patients in VHeFT II received the combination of H/ISDN as single formulations in the same dose ratios and dose intervals. The efficacy of the combination treatment with H/ISDN was inferior to that of enalapril.

2.2 What are the General Attributes of BiDil?

BiDil is a fixed combination product of H and ISDN. Both, H and ISDN as individual drug products are marketed since years. Both ingredients are available in the U.S. as both single-brand and generic drug products. However, as single agents neither is indicated for the treatment of CHF.

H causes direct relaxation of the arteriolar smooth muscles resulting in vasodilation and lowered blood pressure (Goodman & Gilman 2001). H is approved for the treatment of essential hypertension alone or as an adjunct. The label states that the initial dose to be given for the first 2-4 days of H is 10 mg qid. The dose can be increased to 25 mg qid for the balance of the first week. For the second and subsequent weeks the dose should be increased to 50 mg qid. For maintenance, therapy should be adjusted to the lowest effective dose regimen.

ISDN dilates venous capacitance and arteriolar resistance vessels and decreases preload and afterload (Package Insert, 1999). It is indicated for the prevention of angina pectoris due to coronary artery disease. The strategy of providing plasma concentrations that are above a minimally effective concentration is not appropriate for ISDN. Previous experience seems to indicate that ISDN was no more effective than placebo after 24 hours (or less) of continuous therapy. Dose escalation does not overcome the tolerance seen with continuous therapy either. Only after a drug-free period, do its antianginal properties return. The label states that the usual starting dose for ISDN is 5 to 20 mg bid or tid. The maintenance dose is 10 to 40 mg bid or tid. As with all titrateable drugs, the minimum effective dose regimen should be determined. Due to development of tolerance a daily dose free interval of at least 14 hours should be maintained.

2.2.1 What are the Highlights of the Chemistry and Physico-Chemical Properties of he Drug Substance and the Formulation of the Drug Product as They Relate To The Clinical Pharmacology and Biopharmaceutics Review

BiDil tablets contain hydralazine HCl. Hydralazine is a weak base. The pKa was estimated to be 7.1 or 8.5 (Israeli and Dayton, 1977). Hydralazine HCl is highly water soluble with a solubility of 39.0 mg/mL.

Hydralazine HCl, UPS, is manufactured by [ ] the following site: [ ]. Hydralazine HCl is distributed in the US by [ ]

ISDN is soluble in organic solvents such as acetone, alcohol, and ether; but no very soluble in water (Package Insert, 1999).

The BiDil 20 tablets proposed as commercial formulation are manufactured by Schwarz Pharma Manufacturing, Inc., Seymour, IN. The BiDil 20 Tablets manufactured by the same manufacturer and at the same site were used in A-HeFT and X-A-HeFT (extension of A-HeFT) . BiDil 20 tablets contain hydralazine HCl, USP, and isosorbide dinitrate along with anhydrous lactose, NF, microcrystalline cellulose, NF, sodium starch glycolate, NF, magnesium stearate, NF, and colloidal silicon dioxide, NF. The BiDil 20 tablets are film coated with OPADRY Orange YS-1-6227.

2.2.2. What is the Proposed Mechanism of Action?

The mechanism of action responsible for the beneficial effects of BiDil in the treatment of CHF is not fully understood. H is an antioxidant and arteriolar vasodilator (Goodman & Gilman 2001). ISDN relaxes vascular smooth muscle and consequently dilates peripheral arteries and to a greater extent veins. Dilatation of the veins causes peripheral pooling of blood and decreases venous return to the heart, resulting in left ventricular end-diastolic pressure and pulmonary capillary wedge pressure (preload). Arteriolar relaxation decreases systemic vascular resistance, systolic arterial pressure and mean arterial pressure (after-load). ISDN generates nitric oxide (Freelisch & Kelm 1991). There is evidence suggesting that endothelial dysfunction and impaired availability of nitric oxide resulting in excess production of reactive oxygen species can contribute to the pathophysiology of heart failure (Keith et al. 1998). The antioxidant properties of H may preserve nitric oxide (Muenzel et al 1996, Muenzel et al 1997).

Endothelial dysfunction appears to be more frequent in blacks in whom release of superoxide from endothelial cells and subsequent formation of peroxynitrite results in a decreased availability of nitric oxide (Kalinowski et al 2004). Pre-disposition to impaired endothelial dependent forearm vascular relaxation, attenuation of cyclic nucleotide-mediated smooth muscle relaxation, attenuated responses to NO mediated response, and reduced endothelium dependent and -independent dilation have been reported in blacks (Jones et al 1999, Cardillo et al 1999, Stein et al. 1997, Cardillo et al 1998, Khan et al 2002, Campia et al, 2002)

2.2.3. What are the Proposed Dosages and Route of Administration?

The route of administration of BiDil is oral. Treatment with BiDil should be initiated at a dose of one BiDil 20 Tablet, 3 times a day. BiDil may be titrated to a maximum of two BiDil 20 tablets, three times a day or to the maximum tolerate dose. Although titration of BiDil can be rapid (3-5 days), some patients may experience side effects and may take

longer to reach their highest dose. The dosage may be decreased to as little as one-half BiDil 20 Tablet 3 times a day if intolerable side effects occur. Efforts should be made to titrate up as soon as side effects subside.

2.3 What are the Salient General Clinical Pharmacology Features of BiDil?

2.3.1 What are the Design Features of the Studies in Support of Dosing or Claims?

Three clinical studies VHeFT I, VHeFT II and A-HeFT were conducted in patients with CHF (NYHA Class III-IV). All 3 studies were randomized, parallel group studies. The first 2 studies were conducted in patients of different race. A-HeFT was conducted in Blacks. VHeFT I and A-HeFT were placebo controlled studies, whereas VeHFT II compared the combination of H & ISDN with enalapril. VHeFT I included also a group receiving prazosin.

In the first pivotal trial, the VHeFT I trial, patients of different races received H and ISDN as the individual formulations qid. The target dose was 75 mg H and 40 mg ISDN qid. The initial dose was 37.5 H and 20 mg ISDN qid. In case of adverse events probably attributable to H or ISND the respective doses could be adjusted to 37.5 H & 10 ISDN, 37.5 H & 20 ISDN, or 75 H & 20 ISND. H was administered in capsules and ISDN as tablets. In the second pivotal study, A-HeFT, BiDil tablets containing the fixed combination of 37.5 mg H and 20 mg ISDN were used. BiDil was initiated at 1 tablet tid and titrated to a target maximum dose of 2 tablets tid. The tablets were to be taken q6 hours during day time with a 12 hour dose-free interval during the night. In the event of adverse events that were probably attributable to H or ISDN the dose of BiDil could be adjusted. In VHeFT I the dose adjustment for H was achieved by reducing the dose to 37.5 mg qid and the ISDN dose to 20 mg ISDN qid. In VHeFT II the dose regimen adjustments for H were achieved by reducing frequency or size of the administered dose as follows: 37.5 mg bid, 37.5 mg qid, and 37.5 mg qid alternating with 37.5mg bid. With ISDN only the size of the dose was adjusted to 10 mg qid, 20 mg qid or 30 mg qid.

In both pivotal trials the patients were on concomitant therapy for CHF. In the earlier conducted VHeFT study, concomitant therapy consisted of digoxin and diuretics, whereas in A-HeFT concomittant therapy included non-aldosterone antagonist diuretics, beta-blockers, ACE inhibitors, digoxin, aldosterone antagonists, and angiotensin receptor antagonists.

It can be concluded that no attempt was made by the sponsor to determine the optimal dose regimen in terms of dose-ratio and -interval of the combination product. The target dose regimens were identical for VHeFT I and II, but different in A-HeFT. The prescribed dose adjustments were also different among the three clinical trials.

2.3.2 What is the Basis for Selecting Response Endpoints (Clinical, Surrogate, Biomarker)?

The major endpoints in VHeFT I included 1) mortality during the entire study 2) two-year mortality 3) number and duration of hospitalizations for cardiovascular causes 4) maximum oxygen consumption during peak exercise 5) maximum treadmill exercise time on graded test and 6) duration of exercise on sub-maximal tests. In VHeFT II the same major endpoints 1) 2) 3) and 5) and in addition oxygen consumption at the anaerobic threshold and change in quality of life were specified. In A-HeFT the primary endpoint was as a composite score of weighing all-cause mortality, first hospitalization for heart failure, and change from baseline in quality of life at six months or earlier. Other endpoints measured included all-cause mortality or first hospitalization for CHF, death to worsening CHF and mean days in hospital/patient hospitalized for any cause. Among these all cause mortality is an accepted outcome, number and duration of hospitalizations and quality of life are accepted clinical endpoints and the remainder represent non-validated biomarkers.

2.3.3 What are the Active Moieties of BiDil in Plasma Identified to Measure the Pharmacokinetic Parameters?

Hydralazine

The parent drug, H, is thought to be the main active species and responsible for arteriolar vasodilation and possibly antioxidant action (Goodman & Gilman 2001). One major circulating metabolite, hydralazine pyruvate hydrazone (HPH), has been shown to exert minimal tachycardia in dog experiments (Shepherd et al 1980) and little or no antihypertensive activity in intact rabbits, dogs, and rats (Talseth et al 1979, Shepherd et al 1980, Clementi et al 1981). The activity of the other major metabolite, methyltriazolophthalazine (MTP),has not been determined. The apparent activity of other potentially circulating metabolites possibly including α-ketoglutarate, acetone and acetaldehyde hydrazones, were shown to be due to back transformation to H (Clementi et al 1981). Of the two major metabolites of H excreted in urine, acetyl hydrazino-phthalazinone (AHP) and the free and conjugated 3-hydroxymethyl-triazolophthalazine (3-OH-MTP) (Schmid et al 1981, Dubois et al. 1981), the former is not active (Goodman & Gilman 2001), whereas the activity of the latter has not been determined

Isosorbide Dinitrate

ISDN as well as 2- and 5-ISMN were measured in plasma to assess pharmacokinetic parameters of interest. Both mononitrates have pharmacologic activity. The 5-mononitrate is believed to have similar properties as its parent compound and is believed to have greater activity than 2-ISMN.

2.3.4. Exposure Response

2.3.4.1 What are the Exposure-Response Characteristics of BiDil for Efficacy?

BiDil

Pivotal Trials

Dose ranging trials in the target population using biomarkers/surrogate endpoints and a factorial design to explore the optimal dose regimen, doses and intervals, for the individual compounds when administered as a combination product, were not attempted. A prospective exploration of important covariates impacting the dose-concentration relationship of the two components when given together was also not performed. A retrospective analysis of the impact of different doses and dose ratios of H and ISDN on efficacy and safety of the combination product in the three clinical trials was also not available. Plasma concentrations of the active moieties were not measured in any of the clinical trials.
In conclusion the exposure-response characteristics of the fixed dose combination BiDil have not been satisfactorily explored. An entirely empirical approach was used in defining the dose regimens to be used in the clinical trials.

Hydralazine

The results from the published Clinical Pharmacology studies in CHF patients and hypertensive subjects show that a single oral dose of the combination of 75 mg H and 20 mg ISDN in CHF patients has a shortly lasting greater effect on the central pulmonary system than 75 mg H alone (Leier et al. 1979). The effect on cardiac output by the H & ISDN combination and H alone lasts longer than predicted from the elimination t1/2 of H. Overall hemodynamic effects including an increase in cardiac index and a decrease in systemic vascular resistance were observed after administration of single doses of 75 mg and 100 mg of H in patients with severe chronic CHF who were resistant to treatment with diuretics and digoxin (Packer et al. 1980a). However, when a defined efficacy value was to be attained, the inter-individual dose requirements for H in these CHF patients varied by a factor of 8 (1980b). The duration of the decrease in systemic vascular resistance by H was longer than predicted from the t1/2 of the drug and increases in severe renal impairment. The exposure-response data in hypertensive patients showed that the hypotensive effect of H is log linearly related to Cmax and AUC after single and multiple doses of H (Shepherd et al 1981). The peak hypotensive effect was also related to the acetylator status of the subjects. The hypotensive effect decays much slower than the plasma concentrations of H (Shepherd et al 1981, O'Malley et al 1975). These findings raise the possibility of the presence of unidentified active metabolites or a deep compartment of H.

It can be concluded that a dose-effect relationship for H is only partly established in patients with CHF. Dose requirements to reach efficacy defined as >20% decrease in systemic vascular resistance vary importantly in refractory CHF patients. The existence of dose-and concentration-effect relationships have been demonstrated for H in hypertensive patients. The acetylator status impacts the magnitude of the hypotensive effect of H. The temporal relationship between cardiac performance improving or antihypertensive effects and plasma concentrations of H is complex.

ISDN

Labeling information for ISDN seems to indicate that previous studies of single oral doses of ISDN (total daily doses of 30 to 480 mg) have shown reductions in exercise-related angina for up to 8 hours (one hour after dosing). Multiple dose studies where ISDN was taken every 12 hours or more for a period of several weeks, have demonstrated statistically significant anti-anginal efficacy for up to 2 hours after dosing. Regimens where ISDN was administered once a day and where there was at least a 14-hour drug-free interval (administered at 0800, 1400, and 1800 hours) demonstrated efficacy after the first dose of each day that was similar to single-dose studies. The second and later doses have resulted in smaller and shorter-lasting effects than the first dose. Significant anti-anginal efficacy totaling about 6 hours in a 24 hour period was observed in one trial with 8 patients where they were given a pretitrated dose (average 27.5 mg) of immediate-release ISDN at 0800, 1300, and 1800 hours for 2 weeks.

2.3.4.2 What are the Exposure-Response Characteristics of BiDil for Safety?

BiDil

Hydralazine

Sympathomimetically induced reflex tachycardia induced by vasodilation and decrease in blood pressure, headache, flushing, nausea, dizziness and angina are extensions of the pharmacological effect and known adverse events of H, that can occur shortly after administration. They are dose dependent and disappear usually after 2 weeks of treatment. Co-administration of beta-blockers or diuretics can mitigate the acute side effects of H (Goodman & Gilman 2001). Peripheral neuritis with paresthesia, numbness and tingling has also been observed to occur following treatment with H.

A late occurring adverse event is drug induced systemic lupus erythematodes. Usually, a treatment with H must exceed 6 months for this condition to develop. Its incidence is related to dose, gender, acetylator phenotype, and race (Perry et al 1970, Perry 1973, Strandberg et al 1976). The incidence is four times higher in females than in males and the syndrome is more commonly observed in Caucasians than in African Americans (Goodman & Gilman 2001).

ISDN

ISDN's vasodilatory properties are usually the cause of most of its adverse effects (AE). The most commonly reported AE is headache (it's a marker of the activity of the drug); which can be severe at times and can occur every time ISDN is administered. Lightheadedness due to blood pressure changes and hypotension that may possibly lead to drug discontinuation has been reported. Other reported; but uncommon AEs include syncope, crescendo angina, and rebound hypertension. Methemoglobinemia has been known to occur, though extremely rarely with organic nitrate therapy.

2.3.4.3 Does BiDil Have an Impact on the QT/QTc Interval?

Studies measuring the impact of BiDil, or H and ISDN when administered alone, on the QTc interval in humans have not been conducted.

2.3.4.4 Is the Dose Regimen Selected for BiDil Consistent with the Dose/Concentration-Response Relationship?

BiDil

Studies exploring the dose/concentration exposure-response profile of BiDil have not been conducted. The respective least effective dose of H and ISDN when administered as a combination are unknown. The dose regimens used in the pivotal trials of BiDil were not derived from exposure-response profiles. Also, the dose regimens used in A-HeFT and VeHT I or II were not identical.

Hydralazine

Limited dose-response information in the target population after alone administration of H exists as summarized in Section 2.3.4.1.

ISDN
General dose-response information for ISDN is summarized in section 2.3.4.1 as well. However, no such information in the targeted population was found in the literature.

2.3.5 What are the PK Characteristics of Hydralazine and Isosorbide Dinitrate and Their Major Metabolites after Administration as BiDil?

2.3.5.1 What are the Single and Multiple Dose PK Parameters for Hydralazine and Isosorbide Dinitrate when Administered as BiDil or Co-administered as Individual Formulations?

BiDil

The sponsor did not perform any pharmacokinetic study with the to-be-marketed BiDil 20 tablets containing 37.5 mg H and 20 mg ISDN. The NDA submission reported the results of a single dose bioequivalence/relative bioavailability study with 2 BiDil tablets of different strengths that were compared to single entity formulations of H and ISDN and a solution containing the 2 active substances.

Hydralazine

Because of the known labile nature of H and its major circulating metabolites only publications were accepted for the review that presumably applied appropriate sample handling and used assays selective for H and its major metabolites. There were 4 studies in healthy subjects, 7 in hypertensive patients and 2 in patients with CHF.

The PK parameters for H and its major circulating metabolites, HPH and MTP, referenced to the concentration in plasma obtained in these studies are shown in Tables 1-4. The published PK parameters were alternatively referenced to the concentration of drug in whole blood or plasma. In order to make the results comparable, the parameters referenced to the concentration in whole blood were transformed to parameters referenced to plasma by multiplying with or dividing by the blood/plasma partition coefficient Kb/p=1.65, as appropriate.

The Summary Tables 1-4 list the mean values of the main parameters of H and the major circulating metabolite, HPH, after intravenous and oral administration for healthy subjects, hypertensive patients and patients with CHF. All the PK parameters are referenced to the drug concentrations in plasma. Due to insufficient sensitivity of some of the assays not all parameters could be determined for H after oral administration.

Table 1. Mean PK Parameters of Hydralazine Referenced to the Concentrations in Plasma in Healthy Subjects

Study	Phenot	Route	Dose mg/kg	Formul.^	Vss L/kg	CL/F mL/min/kg	t1/2 min	Cmax/D 10^{-6}/mL	Tmax min	t1/2 min	F %
CB-02	PM	po	0.52	Solution		607[a]		0.82	20	151	
	PM	po	0.52	Capsule		568[a]		1.25	44	143	
	PM	po	0.52	Tablet		1035[a]		0.48	62	123	
	PM	po	0.52	BiDil		596[a]		0.91	58	139	
	PM	po	1.04	BiDil		566[a]		0.60	61	211	
Reece, 1980	EM	iv	1.0	Solution	6.37	148	40				
	PM	iv	0.5	Solution	5.71	130	41				
	All	iv	1.0/0.5	Solution	6.04	139	40				
	EM	po	1.0	Tablet		914[b]		NR	36	26	16.2
	PM	po	0.5	Tablet		367[b]		NR	37	28	35.4
Ludden, 1980	PM	po	0.33/0.66	Solution		312[a,c]		1.84[d]	14	NR	
			0.33	Tablet		304[a,c]		1.19[d]	65	NR	
Semple, 1991	EM,PM	po	0.70	Tablet[e]		355[a]		1.05	30	NR	

^ The formulations used in CB-02 contained H & ISDN, all other studies used formulations that contained H only
[a]Calculated from CL/F= D/AUC [b]Calculated from CL/F =CL· (F/100) [c] AUC after repeat doses of 0.66 mg/kg H [d] Cmax after first dose of 0.33mg/kg H , [e]Only data in fasted state reported NR= Not reported

Table 2. Mean PK Parameters of Hydralazine Referenced to the Concentrations in Plasma in Hypertensive Subjects

Study	Phenot.	Route	Dose mg/kg	Formul.	Vss L/kg	CL/F mL/min/kg	t1/2 min	Cmax/D 10^{-6}/mL	Tmax min	t1/2 h	F %
Ludden, 1980	EM	iv	0.3	Solution	1.68	70.2	58.7[a]				
	PM	iv	0.3	Solution	1.98	75.5	49.5[a]				
	All	iv	0.3	Solution	1.83	72.9	53.7[a]				
Shepherd,1980	EM	po	1.0	Solution		739[b]		0.71	25.0	13.2	9.5
	PM	po	1.0	Solution		244[b]		2.29	17.5	54.3	31.3
	EM	po	1.0[c]	Solution		1064[b]		0.31	21.3	20.3	6.6
	PM	po	1.0[c]	Solution		192[b]		2.14	22.5	40.8	39.3
Ludden, 1983	EM,PM	iv	0.20	Solution	2.31	85.5	NR				
	EM,PM	iv	0.30	Solution	2.03	92.2	NR				
Shepherd, 1981	EM,PM	po	1.0	Tablet		363[d]		1.18	NR	NR	
		po	1.0[c]	Tablet		339[d]		0.98	NR	NR	
Shepherd , 1984	EM	po	0.50	Solution		182[d]		0.21	NR	NR	
	EM	po	1.0			122[d]		0.45	NR	NR	
	EM	po	2.0			54[d]		1.04	NR	NR	
	PM	po	0.25	Solution		78[d]		0.63	NR	NR	
	PM	po	0.50			47[d]		0.86	NR	NR	
	PM	po	1.0			34[d]		1.45	NR	NR	

[a]Harmonic mean [b]Calculated from CL/F=CL/(F/100) [c]Multiple doses [d]Calculated from CL/F=D/AUC NR=Not Reported

Table 2. Mean PK Parameters of Hydralazine Referenced to the Concentrations in Plasma in Hypertensive Subjects Continued

Study	Phenot.	Route	Dose mg/kg	Formul.	Vss L/kg	CL/F mL/min/kg	t1/2 min	Cmax/D 10^{-6}/mL	Tmax min	t1/2 h	F %
Shepherd, 1984	EM,PM	po	1.0	Solution[a]		268		1.37	NR	NR	
Jackson, 1990	PM	po	1.08	Tablet[a]		143		1.36	46	NR	

[a] Only data in the fasted state reported

Table 3. Mean PK Parameters of Hydralazine Referenced to the Concentrations in Plasma in Patients with Congestive Heart Failure

Study	Subjects	Phenot.	Route	Dose mg/kg	Formul.	Vss L/kg	CL/F mL/min/kg	t1/2 min	Cmax/D 10^{-6}/mL	Tmax min	t1/2 h	F %
Crawford, 1985	CHF	EM	iv	0.30[a]	Solution	2.42	51.3	NR				
		PM	iv	0.30[a]		1.90	45.7	NR				
		All	iv	0.30[a]		2.21	48.7	105[b]				
		EM	po	1.1[a]	Tablet		518[c]		NR	NR	NR	9.9
		PM	po	1.1[a]			174[c]		NR	NR	NR	26.2
Hanson, 1983	CHF	EM,PM	po	0.74	Tablet		0.55[d]		4.80	44	136	
	Hypert.	EM,PM	po	0.61	Tablet		0.33[d]		2.56	58	119	

[a] Assumed body weight=70kg [b] Harmonic mean [c] Calculated from CL/F =CL· (F/100) [d] Calculated from CL/F= AUC/D
NR=Not Reported

Table 4. Mean PK Parameters of Hydralazine Pyruvate Hydrazone Referenced to the Concentration in Plasma after Administration of Hydralazine in Healthy Subjects and Hypertensive Patients and Patients with Congestive Heart Failure

Study	Subjects	Phenot.	Route	Dose mg/kg	Formul.	AUC/D 10^2/L·h	t1/2 h	AUCR[a]	AUC/D 10^2·h/L	Cmax/D 10^2/L	Tmax min	t1/2 h	AUCR[a]
Reece,1980	Healthy	EM	iv	1.0	Solution	2.33	3.32	31.6					
		PM	iv	0.5		6.10	3.64	35.5					
		EM	po	1.0	Tablet				NR	NR	NR	ND	6.6
		PM	po	0.5					NR	NR	75	3.85	22.8
Ludden,1980	Hypert.	EM	iv	0.3	Solution	10.5	3.33[b]						
		PM	iv	0.3		12.6	4.95[b]						
		EM,PM	iv	0.3		11.5	3.98[b]						
Shepherd,1980		EM	po	1.0	Solution				1.45	0.31	35	4.05	47.5
		PM	po	1.0					3.99	0.66	60	4.95	38.0
		EM	po	1.0[c]	Solution				1.94	0.42	35	4.23	38.5
		PM	po	1.0[c]					7.39	0.97	95	6.23	56.5
Hanson, 1983	CHF	EM,PM	po	0.74	Tablet				262	1.61	47	3.44	3.2
	Hypert.	EM,PM	po	0.61	Tablet				102	0.75	50	1.88	2.7

[a]Ratio of AUC,HPH to AUC,H [b]Harmonic mean [c]Multiple doses NR=Not reported ND=Not determined

Healthy Subjects

Important inter-study differences in the PK parameters for H were noted (see Table 1). Also, only single dose studies were conducted in healthy subjects. The covered dose range was 0.25 -1.0 mg/kg and 0.33-1.0 mg/kg after intravenous and oral administration, respectively. A first study in healthy subjects determined the PK parameters of H after intravenous and oral administration. Mean Vss and Cl values were 6.04 L/kg and 139 ml/min/kg, respectively, and t1/2 was 40 minutes following intravenous administration (Reece et al. 1980). The CL value exceeds hepatic plasma flow substantially indicating important extra-hepatic elimination of H. After oral administration of the tablet peak concentrations were observed 37 minutes following administration, t1/2 was 27 minutes and tended to be shorter than after intravenous administration. Absolute bioavailability of the tablet was 16.2 % and 35.4 % in EMs and PMs, respectively, indicating the importance of the acetylator status.

Peak concentrations occurring between 20 and 61 minutes after administration and longer t1/2 values ranging between 123 and 211 minutes were observed in Study CB-02 that compared the relative bioavailability/bioequivalence of 2 different strength BiDil tablets, single entity solid and solution formulations in presumed PMs. Cmax and AUC values of the H concentrations with the BiDil tablets of different strength when dose normalized were similar.

A third study determined the relative biovailability of the tablet relative to a solution of H. Peak concentrations of H were observed 65 minutes and 14 minutes, respectively, following administration.

In healthy subjects the pharmacokinetics of H appear to be linear over the limited dose range that can be tested and measured after intravenous administration. There is no important difference between EMs and PMs after intravenous administration. However, after oral administration the pharmacokinetics of H are characterized by an acetylator status dependent first pass effect resulting in an absolute bioavailability of H that is 2.6 times greater in PMs than EMs. A Comparison of the computed CL/F and dose normalized Cmax values of the 3 studies in healthy volunteers indicates large inter-study variation.

After intravenous administration of the parent drug, HPH and MTP are the major circulating metabolites in that order (Table 4). Their concentrations exceed those of the parent drug significantly. The t1/2 of HPH is 3.5 hours and significantly longer than that of the parent drug. MTP decays with a t1/2 of 1.7 hours. After oral administration MTP is the major circulating metabolite, particularly in PMs pointing to the competition between the HPH and MTP metabolic pathways after oral administration of H. The t1/2 values of HPH (PMs) and MTP after oral and intravenous administration are similar.

Hypertensive Subjects

In this population pharmacokinetic information was available after single and multiple doses of H (Tables 2 and 4). The multiple dose information was obtained with bid regimens given for a few days only. The intravenous dose range tested ranged from 0.2 mg/kg to 0.3 mg/kg. The single oral doses administered ranged from 0.25 to 1.0 mg/kg and the multiple dose regimen was 1.0 mg/kg bid. One of the studies tested the possible dose dependency of the pharmacokinetics of H after single doses.

Following intravenous administration of H Vss values of 1.83 L/kg was reported in one study (Ludden et al. 1980) and 2.17 L/kg in the other study (Ludden et al, 1983). The CL values were 72.9 mL/min/kg and 88.9 mL/min/kg, respectively, and similar. A t1/2 value of 53.7 minutes was obtained in the first study which was similar to the value obtained in healthy volunteers. The second study did not report a value for t1/2. In agreement with the data obtained in healthy volunteers, the pharmacokinetics of H in hypertensive EMs and PMs were similar.

After oral administration the respective absolute bioavailability values reported after single and multiple dose administrations of a solution were significantly different between hypertensive PMs and EMs confirming the results obtained in healthy volunteers (Shepherd et al, 1980). After a single dose the respective values were 16.2% and 31.5 % and after multiple doses 6.6 % and 39.3 % in EMs and PMs. There was no evidence of accumulation of H when given as a bid regimen. The t1/2 value of 53.7 minutes reported in just one of the five studies in hypertensive patients was similar to the value in healthy volunteers. Also the respective Tmax values of 17.5- 22.5 minutes for the solution and 46 minutes for the tablet agreed with the values obtained in healthy volunteers. Oral clearance decreased and dose normalized Cmax increased in the dose proportionality study suggesting possibly nonlinear pre-systemic and/or systemic clearance of H (Shepherd et al.1984). However, the CL/F values obtained in this study were smaller than the corresponding values in the other studies with oral administration and in some cases after intravenous administration, suggesting caution in the interpretation of these results. An across study comparison of the CL/F and dose normalized Cmax values indicates significant variation between studies.

After intravenous administration of H the t1/2 of HPH was 3.98 hours in agreement with the value observed in healthy subjects. After oral administration HPH tended to peak in EMs earlier than in PMs and the amounts formed were larger in EMs than in PMs. The t1/2 ranged between 4.05 and 6.23 hours and appeared not to depend on the acetylator status.

CHF Patients

Two studies evaluated the pharmacokinetics of H in CHF patients. In a first study the patients were randomized to receive single doses of 0.3 mg/kg intravenously or an oral dose of 1.1 mg/kg administered as tablet (Crawford et al.1985). On a separate occasion the patients received ascending doses of 75 to 1000 mg H tid. The cardiac ouput had to

rise by > 10% compared to last dose and the pulmonary capillary wedge pressure had to remain < 18 mmg Hg. The second study investigated the pharmacokinetics of H after a single oral dose of 0.74 mg/kg given as tablet in CHF patients and included hypertensive patients as controls (Hanson et al. 1983).

After intravenous administration Vss was 2.21 L/kg, CL 48.7 mL/min/kg and t1/2 105 minutes (Crawford et al. 1985). After oral administration the absolute bioavailability of the tablet formulation was 9.9% and 26.2% in the PMs and EMs, respectively confirming the relevance of the acetylator status for the exposure to H. An increase of the dose from 75 mg up to 1000 mg revealed a clear dose dependency of the dose normalized AUC indicating nonlinear kinetics of H in both EMs and PMs. Up to a 9 fold increase in the dose normalized AUCs were observed when the dose was increased from 75 mg bid to 1000 mg bid. Saturability of the first pass metabolism is the most probable cause.

The second study determined a Tmax of 44 minutes and t1/2 of 136 minutes for H after administration of the tablet (Hanson et al, 1983). The corresponding values in hypertensive patients were 50 minutes and 114 minutes, respectively. The dose normalized Cmax values tended to be larger in the CHF patients than in the hypertensive patients, but none of the parameters for the parent drug was statistically significantly different between the 2 populations. In contrast to the parent drug, the Cmax, AUC and t1/2 values for HPH were significantly greater in CHF patients than in hypertensive patients. However, the CL/F for H was unusually small and the dose normalized AUC for HPH unusually large casting doubts about the selectivity of the assay used in that study.

ISDN

According to labeling for ISDN, bioavailability is quite variable ranging from 10 to 90% (mean 25%). Extensive first-pass hepatic metabolism takes place with concentrations reaching peak levels in about 1 hour. Chronic dosing seems to result in higher bioavailability. Chronopharmacology does seem to affect extent of bioavailability with statistically significant AUC observed between morning and evening ISDN administration (33.9 at 0800 hours versus 25.4 ng/mL/h at 2000 hours). The volume of distribution of ISDN is 2 to 4 L/kg and cleared at a rate of 2 to 4 L/min and a half-life of about 1 hour. Extra-hepatic metabolism seems to occur since the clearance of ISDN exceeds hepatic blood flow.

ISDN has two active metabolites (5-ISMN and 2-ISMN), especially the 5-mononitrate with an overall half-life of about 5 hours. Time for 5-ISMN to reach maximum concentration is about 2 hours. The 2-mononitrate has a half-life of about 2 hours with the same time to reach maximum concentration.

2.3.5.2 How Do the Pharmacokinetics of Hydralazine and Isosorbide Dinitrate Administered as BiDil in Healthy Subjects Compare with Those in Patients with Congestive Heart Failure?

BiDil

No study compared the PK of H and ISND after administration of BiDil in the target population. A single bioavailability/bioequivalence study compared the performance of BiDil tablets and the single entity formulations of H & ISDN.

Hydralazine

A comparison of the mean data of H in healthy subjects and hypertensive patients and patients with congestive heart failure shows consistent results for absolute bioavailability. Absolute bioavailability was phenotype dependent, with PMs displaying average values about 2.6-6.0 times greater than EMs in the 3 groups. There was evidence for nonlinearity of the PK following oral administration in hypertensive patients and in patients with CHF. However, for the other parameters important variation among the groups and within each group existed, particularly after oral administration. The acetylator status, impact of disease, the use of different formulations, different assays and post sampling procedures contribute all to the observed large variation as reflected in the CL/F and dose normalized Cmax values across the oral studies. As result, the inter-study variation cannot be used to delineate the impact of disease on the PK of H. There are too many confounding factors in play.

Only one study compared the PK of H in the target population with a control group of hypertensive patients. Unfortunately, the results of the study are biased.

It can be concluded that based on the data available absolute bioavailability is similar in healthy and hypertensive patients and patients with the target disease. No firm conclusion regarding the impact of CHF on the PK of H and HPH can be drawn.

ISDN

No information was found in the literature.

2.3.5.3 What are the Important Characteristics of Absorption for BiDil, Hydralazine, and ISDN?

BiDil

The absorption characteristics of H from two BiDil tablets containing 37 mg H and 10 mg ISDN or 75 mg H and 40 mg ISDN and solid and liquid single entity formulations containing 37.5 mg H and 10 mg ISDN were investigated in healthy volunteers who were presumably PMs of H.

After administration of the higher and lower strengths BiDil tablets peak concentrations occurred 58 and 61 minutes, respectively, after administration. After ingestion of the single entity capsule and tablet by the same subjects peak concentrations of H were observed 44 and 62 minutes, respectively, after administration. Absorption of H was

fastest after administration of the solution formulation with a Tmax of 20 minutes indicating that dissolution of the solid dosage forms retards or delays absorption. However, peak concentration was greatest for the capsule and smallest for the tablet containing only H. The absorption characteristics of H from the BiDil tablets were intermediate. The AUC values indicated that bioavailability of H was greatest from the high dose BiDil tablet, followed by the capsule. As with Cmax, the AUC from the tablet containing only H was smallest. The bioavailability of H from the lower and higher strengths BiDil tablets relative to the solution was 106 % and 125 %, respectively. The corresponding values of the single entity capsule and tablet were 112 % and 85%, respectively. The BiDil tablets were neither bioequivalent to the capsule nor tablet formulations.

The absolute bioavailability of H from the BiDil 20 tablets has not been determined.

Hydralazine

The estimates for absolute bioavailability for H in healthy subjects and hypertensive and CHF patients with EM phenotype were 16.2 %, 9.5 % and 9.9% after a single dose of a solution or tablet. The corresponding estimates in the PM phenotype were 35.4 %, 31.3 % and 26.2 %, respectively. Repeat administration of the solution formulation changed the absolute bioavailability from 9.5 % to 6.6% in EMs and from 31.3 % to 39.3 % in PMs. At higher dose levels the absolute bioavailability of H may be increased because of a possible saturation of the first-pass effect (Shepherd et al. 1984). The increase in bioavailability appears to occur in both PMs and EMs.

The Tmax for H for solid and fluid dosage ranged widely between 35 and 65 minutes for the tablet and between 14 and 95 minutes for the solution, respectively.

After oral administration of a single dose of 50 mg ^{14}C-H in gelatine capsules to 10 hypertensive subjects (5 EMs and 5 PMs) receiving 50 mg H bid, the recovery of total radioactivity in urine was 66.1(12.6) % and was independent of acetylation capacity (Schmid et al 1981). In 2 healthy subjects receiving 100 mg ^{14}C-H in gelatine capsules orally, the recovery of total radioactivity in urine was 89 % and 83% and in the feces 9% and 12% (Wagner et al 1977). Thus, the absorption of H and/or its metabolites is at least 66 %.

ISDN

According to labeling for ISDN, bioavailability is quite variable ranging from 10 to 90% (mean 25%) with a Tmax of about 1 hour. Chronic dosing seems to result in higher bioavailability.

2.3.5.4 What are the Important Characteristics of Distribution for Hydralazine and Isosorbide Dinitrate?

BiDil

The distribution parameters of H and ISDN in the presence of each other have not been determined

Hydralazine

The plasma protein binding of H measured by equilibrium dialysis at 37 ° C is reportedly 87 % at a plasma concentration of H of 1.0 μg/mL (Lesser & Dayton, 1974) and 90.0 (1.2) % at plasma concentrations of H ranging between 0.45 -2.30 μg/mL (Wagner et al.1977). The corresponding values for the binding of H by albumin in physiological concentrations were 84 % and 73.3 (0.3) %, respectively. Given the instability of H in plasma and aqueous solutions one cannot be sure that the reported values reflect the extent of binding of H to the proteins. Also the concentrations tested exceed those found under clinical conditions significantly. In conclusion the plasma protein binding of H is unknown.

The blood /plasma partition coefficient, Kb/p, measured at 4° C in the blood of hypertensive patients was found to be 1.65 (Ludden et al. 1980).

The steady state volume of distribution of H referenced to the concentration in plasma was 6.04 L/Kg in healthy subjects, 1.83 L/kg and 2.17 L/kg in hypertensive patients and 2.21 (1.31) in CHF patients (Reece et al. 1980, Ludden et al 1980 and 1983, Crawford et al, 1985). These results indicated significant binding to and/or partitioning of H into tissues as predicted from the extensive partitioning into red blood cells.

No distribution parameters have been reported for the metabolites of H.

ISDN

Fung and colleagues reported the free fraction of ISDN to be a mean of 0.72 ± 0.12 (28% protein binding). Schneider et. al. investigated ISDN and metabolite concentrations in human vascular and muscle tissue under steady-state conditions and found vessel wall concentrations to be higher than simultaneous plasma concentrations (molar concentration ratios of 4.9 for pectoral muscle to plasma and 7.21 for saphenous vein wall to plasma). Accumulation of ISDN in vessel walls may contribute to its greater vascular action; but may also facilitate tolerance when administered chronically.

2.3.5.5 Does the Mass Balance Study Suggest Renal or Hepatic as the Major Route of Elimination for Hydralazine and Isosorbide Dinitrate?

Hydralazine

The mass balance study indicates that metabolism is the major route of elimination of H.

ISDN
Most of ISDN is eliminated renally as congugated metabolites.

2.3.5.6 What are the Important Characteristics of the Metabolism for Hydralazine and Isosorbide Dinitrate?

BiDil

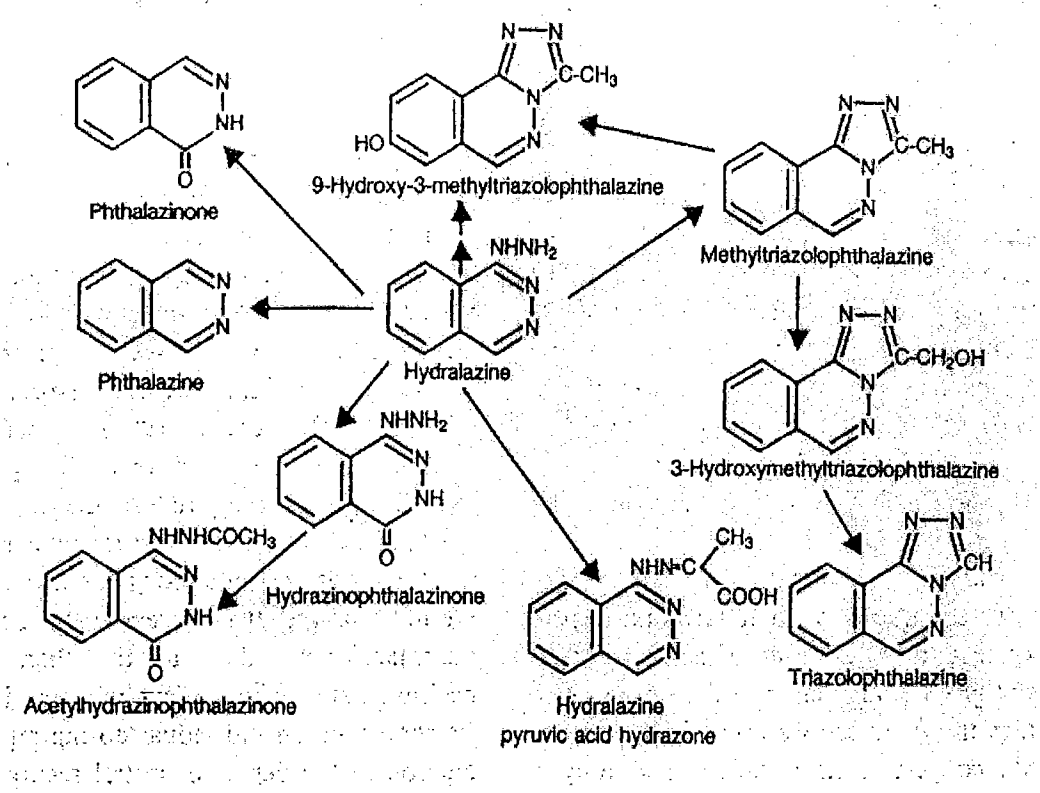
The metabolism of H and ISDN in the presence of each other has not been determined.

Hydralazine

The substrate status and the inhibitory and inductive potential of H have not been studied yet in vitro with human liver tissue. It is recommended that the possible involvement of CYP 450 in the metabolism of H and the inhibitory and inductive potential of H regarding other likely co-administered drugs that are metabolized by CYP 450 are determined.

H is labile and forms hydrazones in vitro and in vivo. The half-life of this transformation in whole blood and plasma is reportedly 11 and 8 minutes, respectively (Reece et al 1978, Reece et al. 1980, Ludden et al. 1982).

The in vivo metabolism of H is complex involving acetylation, aromatic, aliphatic and alicylic oxidation and loss of the hydrazine group. The scheme shown below (Mulrow and Crawford 1989) is one of a number of similar schemes depicting the complexity of the metabolism of H that have been published (Ludden et. al. 1982, Schmid et al. 1981):



H is metabolized along two major pathways: Acetylation and oxidation. Acetylation of H results in the cyclized product methyl-triazolophthalazine, MTP. MTP can be oxidized to 3-hydroxymethyl-triazolophtahalazine, OHMTP, and its product triazolophthalazine, TP, or to 9-hydroxymethyl-triazolophthalazine (Mulrow and Crawford 1989). Alternatively H can be oxidized to hydrazinophthalazinone and then acetylated to yield acetylhydrazinophthalazinone, AHP, or oxidized to phthalazinone or phthalazine. In addition H can form adducts with endogenous acids, aldehydes and ketones. The major metabolite generated by a conjugation with pyruvic acid is HPH. The acetone- and ketoglutaric acid hydrazones are reportedly present in plasma and urine, but have not been quantified (Haegele et al. 1978). The major circulating metabolite of H in plasma is HPH. A second major metabolite quantified in plasma is MTP (Reece et al. 1980).

Total radioactivity recovered in urine was 71.7 (4.8) % and 60.5 (7.8) % of the dose in EMs and PMs, respectively. Of the recovered radioactivity in urine 67.5 (5.9) % and 54.1 (4.3) % were identified as metabolites after enzymic hydrolysis in hypertensive EMs and EMs, respectively (Schmid et al.1981). Thus, 48.4% and 32.7% of the dose represent identified metabolites in EMs and PMs, respectively. However, the disposition of more than 50 % of the dose has not been determined. The major metabolites excreted in urine in free or conjugated form were identified as AHP, OHMTP, and TP. Two studies investigated the urinary excretion of the metabolites in hypertensive EMs and PMs

receiving 100 mg ^{14}C-H by the oral route (Schmid et al. 1981 and Dubois et al. 1981. The respective urinary recoveries of the main metabolites are listed in Table 5.

Table 5. Mean Percent of Dose Recovered in Urine as Acetylhydrazinophthalazine (AHP) and the Sum of Triazolophthalazine (TP), Methyltriazolophthalazine (MTP), and Hydroxymethyltriazolophthalazine (OHMTP) in Hypertensive Extensive (EM) and Poor Metabolizers (PM) of Isoniazide

Compound	EM		PM	
AHP	17.6 [a]	16.0 [b]	12.4[a]	5.9[b]
Σ TP+MTP+OHMTP#	18.1 [a]	15.2[b]	12.2 [a]	8.9[b]

[a] Dubois et al [b] Schmid et al # Measured following enzymic hydrolysis

These results indicate that more AHP and TP+MTP+OHMTP is generated in EMs than in PMs suggesting that both acetylation pathways, the TPM pathway and the hydrazinophthalazinone/AHP pathway, are involved in the first pass of H.

The acetylation capacity determines the relative contribution of the MTP-, hydrazinopthalazinone/AHP- and HPH pathways following oral administration. In EMs compared to PMs the acetylation pathway metabolizes a larger amount of H than the HPH pathway. After intravenous administration the relative contributions of the acetylation and oxidation pathways appear not to be determined by the phenotype.

ISDN

Clearance is primarily by denitration to the 2- (15 to 25%) and the 5-mononitrate (75 to 85%). 5-ISMN is denitrated to isosorbide; glucuronidation to the 5-mononitrate glucuronides; and denitration/hydration to sorbitol. The 2-mononitrate seems to undergo the same metabolic process/pathways.

2.3.5.7 What are the Important Characteristics of Renal Elimination for Hydralazine and Isosorbide Dinitrate?

BiDil

The renal elimination of H and ISDN in the presence of each other has not been determined.

Hydralazine

Only small amounts of H and hydralazine hydrazones were found in urine even with nonspecific methods (Haegele et al 1981, Facchini and Timbrell 1981.

ISDN

ISDN is almost completely metabolized while undergoing first-pass metabolism. As a result, only minimal amounts of ISDN are found in urine.

2.3.5.8. Are the Pharmacokinetics of Hydralazine Linear?

BiDil

The linearity of the PK of H and ISDN in the presence of each other has not been determined

Hydralazine

In hypertensive EMs and PMs receiving ascending doses of 0.50, 1.0 and 2.0 mg/kg and 0.25, 0.50 and 1.0 mg/k, respectively, Cmax and AUC increased more than dose proportionately (Shepherd et al 1984). A similar more than dose proportionate increase in AUC was found in CHF patients receiving ascending doses ranging from 75 mg to 1000 mg tid as shown in the figure below (Crawford et al. 1985):

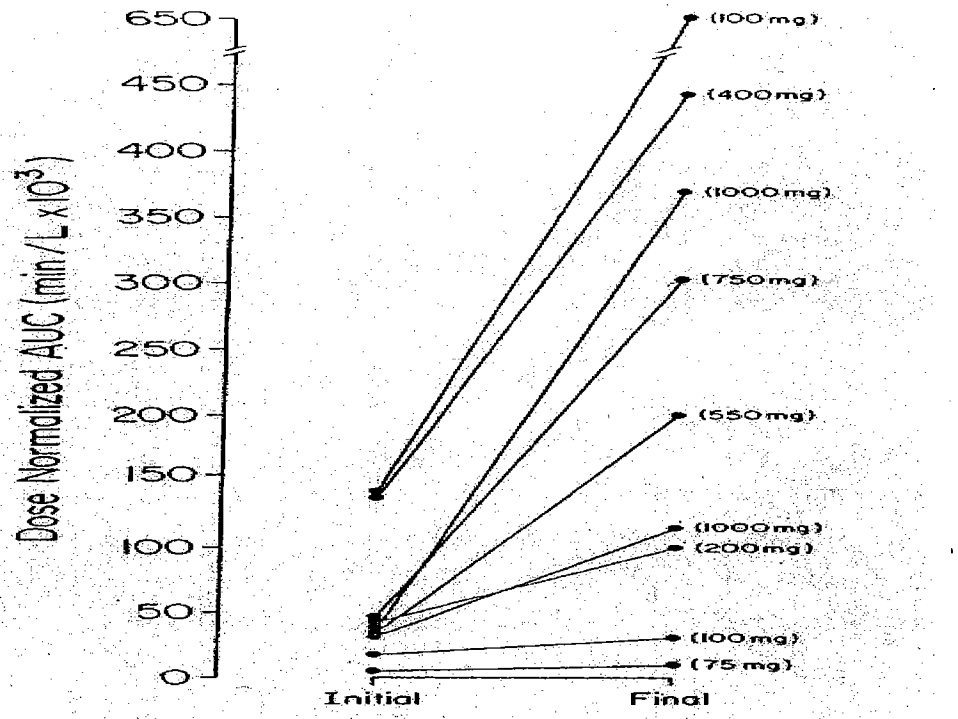


Fig. 2. Dose-normalized AUC after the first and final oral doses of hydralazine in each subject.

In conclusion the PK of H after oral administration in EMs and PMs are nonlinear, most likely because of saturable first pass metabolism.

2.3.5.9 Are the Pharmacokinetics of Hydralazine and Isosorbide Dinitrate Time Dependent?

BiDil

The time dependence of the PK of H and ISDN in the presence of each other has not been determined.

Hydralazine

The AUC and Cmax values in EMs and PMs after a single dose or 5 repeated doses of 1 mg/kg given bid were not different. These results imply time independent PK of H when administered over a short period of time. However, a possible time dependency of the PK of H during chronic administration cannot be ruled out.
The development of pharmacological tolerance after treatment of CHF patients with H alone for 37 weeks has been reported (Packer etal. 1982).

ISDN

Chronopharmacology does seem to affect extent of exposure with statistically significant AUCs observed between morning and evening ISDN administration (33% greater AUC in AM versus PM).

The bioavailability of ISDN seems to increase with chronic dosing (specific figures not provided in package insert).

The development of tolerance has been reported with ISDN administration when a dose-free interval of at least 14 hours is not followed. As a result, an "eccentric dosing" interval is necessary to avoid tolerance.

2.3.5.10 What is the Inter-subject and Intra-subject Variability of PK Parameters in Volunteers and Patients and What are the Major Causes of Variability?

BiDil

The inter-subject and intra-subject variations of H and ISDN when co-administered has not been reported.

Hydralazine

Inter-subject Variation

The inter-subject variation is expressed as coefficient of variation about the mean value of the main PK parameters after intravenous and oral administration of H alone.

After intravenous administration the variation for CL ranged between 10.0-27.1 % in healthy, hypertensive patients and patients with CHF. The inter-subject variation of Vss was comparable in healthy subjects (16.9 %) and hypertensive patients (26.3 %) and tended to be greater in CHF patients (59.0 %). The inter-subject variation of t1/2 was similar in healthy and hypertensive subjects, 25.0 % and 28.8 %, respectively. No estimate for the variation of t1/2 in CHF patients was reported after intravenous administration.

Following oral administration in the fasted state the inter-subject variation for F ranged in the 3 populations between 10.7 % and 49.6 % in PMs and between 28.6 % and 66.7 % in EMs. The inter-subject variation for AUC and Cmax ranged between 46.8% and 100.0% and 32.6-111.3 %, respectively, in the 3 groups (EMs and PMs combined) with no evidence for important differences between the groups.

Intake of a standard breakfast did not appear to increase the inter-subject of variation of AUC in healthy and hypertensive patients. The two estimates for inter-subject variation of 78.1% and 141.9 % obtained for Cmax after intake of a standard breakfast may be indicative for increased variation of the peak concentrations under fed conditions.

In conclusion H after oral administration must be considered a high inter-subject variability drug. The inter-subject variation of H after oral administration is greater than following intravenous administration, indicating that oral first pass and other absorption covariates affect the bioavailability of H. Food intake appears to increase the already high variation of H.

Intra-subject variation

The requisite data for an appropriate determination of intra-subject variation after oral or intravenous administration of H were not available.

ISDN

The bioavailability of ISDN is highly variable ranging from 10 to 90% bioavailability; which may be partially due to extensive first-pass metabolism by the liver.

2.4 Intrinsic Factors

2.4.1 What Intrinsic Factors Influence Exposure and/or Response to BiDil?

BiDil

The importance of intrinsic factors of H and ISDN in the presence of each other has not been determined.

Hydralazine

2.4.2 Age, Gender, Race

The impact of age, gender or race on the PK and PK-PD of H and its metabolites in the presence or absence of ISDN has not been determined. The race of the participants was only identified in a minority of the selected publications. No subgroup analysis of the PK or PD data was attempted in the submitted single bioavailability study or by the selected published studies. Thus, the impact of the congregate of the extrinsic and intrinsic components of race on the PK and the PK-PD of H is unknown.

H is a substrate of the polymorphic NAT2. Thus, a difference in the frequency distribution of PMs among American whites and blacks could impact the exposure to H and consequently efficacy and safety. The frequency of PMs of H in blacks and whites has been examined using determinations of phenotypes or genotypes. Determinations of the phenotypes showed similar frequency distributions of about 50 % PMs in black and white populations in the US (Relling et al. 1989). Determination of the NAT2 genotype showed frequencies of 59.5 % and 51.5 % for PMs in 2 US white population samples, whereas the PM frequency in the only US black population sample evaluated was 37.5% (Lin et al 1993, Gross et al 1999). There is reportedly good concordance between phenotype- and genotype determinations in white populations (Cascorbi et al.1999). The degree of concordance between phenotype and genotype of NAT2 in blacks has not been determined.

It can be concluded that, based on current evidence, the frequency distribution of PMs of NAT2 in American whites and blacks do not explain the postulated greater efficacy of BiDil in blacks. However, it should be noted that other, unidentified, intrinsic and extrinsic racial factors could impact the PK and PK-PD of H.

Published studies have reported evidence for impaired endothelial-dependent forearm vascular relaxation, attenuation of cyclic nucleotide- mediated smooth muscle relaxation, attenuated nitric-oxide mediated responses to mental stress in the forearm circulation and reduced endothelium-dependent and-independent dilation of conductance arteries in American blacks (Jones et al 1999, Cardillo et al 1999, Stein et al 1997, Cardillo et al 1998, Khan et al 2002, Campia et al. 2002). These data may suggest that the responsiveness to H and/or ISDN may be different in American blacks and whites.

ISDN

The effects of gender on the pharmacokinetics of two ISDN formulations and its mononitrate metabolites were explored in a study by Vree and colleagues in 24 volunteers (12 M and 12 F). C_{max}, C_{min}, AUC_{ss}, AUC_{∞}/kg were higher in females when compared to males ($p<0.0001$). A difference in C_{min} between males and females throughout therapy was observed. The differences in AUC_{ss} were even more pronounced when corrected for body weight (AUC_{∞}/kg) regardless of formulation. However, the maximal difference overall even in a delayed half-life of one hour is not of clinical significance.

Table 1. Pharmacokinetic parameters of 60 mg oral isosorbide-5-mononitrate slow release of both formulations I and II in males and females.

Parameter		Males n = 2 × 12	Females n = 2 × 12	p
Age	y	31.0 ± 4.99	28.3 ± 7.00	0.34
Body weight	kg	78.4 ± 11.7	57.0 ± 8.58	0.0024
Body height	cm	178.6 ± 5.84	163.6 ± 9.32	0.0028
BMI	kg/m²	24.34 ± 2.55	21.29 ± 3.13	0.0113
Dose/kg	mg/kg	0.78 ± 0.12	1.07 ± 0.16	< 0.0001
AUC_{ss}	ng × h/ml	6,977 ± 1,343	10,575 ± 1,911	< 0.0001
AUC_{ss}/kg	ng × h/ml/kg	94.1 ± 30.8	192 ± 50.6	< 0.0001
C_{min}	ng/ml	56.3 ± 6.97	92.5 ± 35.6	0.0015
C_{max}	ng/ml	463 ± 74.1	740 ± 110	< 0.0001
t_{max}	h	3.93 ± 0.65	4.16 ± 0.68	0.24
$t_{1/2abs}$	h	3.21 ± 0.94	3.26 ± 0.95	0.85
$t_{1/2a}$	h	0.24 ± 0.29	0.52 ± 0.51	0.024
$t_{1/2\beta}$	h	4.60 ± 0.50	4.69 ± 0.80	0.64
MRT	h	10.1 ± 1.30	10.5 ± 1.07	0.25

Table 2. Pharmacokinetic parameters of 60 mg oral isosorbide-5-mononitrate slow release of both formulations I and II in females. Selection criterion C_{min}.

Parameter		Females n = 2 × 5	Females n = 2 × 7	p
Age	y	28.2 ± 6.14	28.4 ± 8.03	0.96
Body weight	kg	58.6 ± 9.94	57.3 ± 8.30	0.90
Body height	cm	169.2 ± 12.1	164.3 ± 7.80	0.65
BMI	kg/m²	21.28 ± 3.15	21.29 ± 3.47	0.99
C_{min}	ng/ml	59.3 ± 9.16	125 ± 12.2	< 0.0001
C_{max}	ng/ml	729 ± 66.9	748 ± 127	0.69
AUC_{ss}	ng × h/ml	9,354 ± 1,217	11,446 ± 1,862	0.0052
AUC_{ss}/kg	ng × h/ml/kg	171 ± 45.0	205 ± 61.4	0.11
t_{max}	h	3.77 ± 0.52	4.43 ± 0.65	0.0144
$t_{1/2abs}$	h	2.93 ± 0.69	3.50 ± 1.06	0.15
$t_{1/2a}$	h	0.63 ± 0.49	0.45 ± 0.52	0.40
$t_{1/2\beta}$	h	4.19 ± 0.56	5.06 ± 0.76	0.0057
MRT	h	9.40 ± 0.62	11.2 ± 0.55	< 0.0001

C_{min} = males-females low p = 0.18. C_{min} = males-females high p = < 0.0001.

2.4.3 Pediatric Patients

The PK and PK-PD of H and ISDN after treatment with BiDil have not been determined in the pediatric population. Studies investigating BiDil treatment of pediatric patients in the age between 6 and 16 years have been deferred. Studies in pediatric patients in the age between >1 month and <6 years have been waived

Hydralazine

The PK and PK-PD after alone treatment with H have not been determined in the pediatric population.

ISDN

The PK and PK-PD of ISDN have not been determined in the pediatric population.

2.4.4 Body Weight

BiDil

The impact of body weight on the PK of and PK-PD of H and ISDN after administration of BiDil has not been determined

Hydralazine

The impact of bodyweight on the PK of H after administration of H alone has not been determined. The selected published studies reporting PK parameters of H and its metabolites normalized the CL and Vss parameters for body weight assuming a linear relationship without demonstrating the validity of this assumption.

ISDN

The impact of weight on the pharmacokinetics of ISDN has not been determined.

2.4.5 Renal Impairment

BiDil

The PK and PK-PD of H and ISDN after co-administration have not been investigated in patients with renal impairment.

Hydralazine

A study with single and multiple dose administration of H alone was conducted in hypertensive patients with different degrees of renal impairment (Talseth 1976). However, the assay used was not selective for H. However, the results of this study should be considered, because they indicated that renal impairment results in a significant increase of the levels and t1/2 of apparent H (AH). However the dose administered in control patients with normal renal function and patients with a GFR between 5 and 28 mL/min received similar daily doses of H. Only a study using a selective assay can determine which components of AH, i.e. H, HPH or other hydrazones are accumulating in renal impairment.

In a study with CHF patients, the extent and duration of the decrease in systemic vascular resistance was shown to be increased in subjects with severe renal impairment (Packer et al 1980).

ISDN

One study performed two separate pharmacokinetic sub-investigations (Bogaert, 1981) where ISDN (study 1) and then the administration of the mononitrates (study 2) were explored in renal failure. The results of the ISDN study seem to indicate that the half-life of ISDN and its metabolites remained fairly the same (Table 1).

Table 1. Plasma half-lives (in hours) of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of isosorbide dinitrate 10 mg to patients with and without renal failure. Individual values and means (± SEM) are given

Patients with renal failure				Patients without renal failure			
	ISDN	2-ISMN	5-ISMN		ISDN	2-ISMN	5-ISMN
M. M. ♀	0.36	2.60	6.00	H. M. ♀	0.46	2.40	4.46
G. C. ♂	0.32	1.38	3.89	D. C. ♂	0.25	1.85	5.84
L. J. ♀	0.41	3.39	5.15	V. A. ♂	0.35	2.40	5.52
B. D. ♀	0.32	2.10	4.85	E. S. ♀	0.44	3.57	5.48
V. K. ♂	0.44	2.14	4.33				
Mean	0.370 (±0.002)	2.32 (±0.54)	4.84 (±0.65)		0.357 (±0.009)	2.55 (±0.52)	5.32 (±0.36)

The second arm of the study investigated 2- and 5-ISMN administration resulting in similar half-lives between the renal and healthy volunteers (Table 2).

Table 2. Plasma half-lives (in hours) of isosorbide 2-mononitrate (2-ISMN) and of isosorbide 5-mononitrate (5-ISMN) after oral administration of 5 mg doses to patients with renal failure and to healthy volunteers. Individual values and means (± SEM) are given

Patients with renal failure			volunteers			
	2-ISMN	5-ISMN		2-ISMN		5-ISMN
C. H. ♂	2.21	4.85	D. D. ♂	3.21	V. E. ♂	4.00
C. V. ♂	2.43	7.60	D. L. ♂	3.24	D. W. ♂	4.60
V. R. ♀	2.34	5.80	D. G. ♂	2.67	M. B. ♂	4.62
D. I. ♀	2.13	4.39	M. B. ♂	2.20		
B. M. ♀	.1.77	5.49				
Mean	2.17 (±0.06)	5.62 (±1.51)		2.83 (±0.24)		4.41 (±0.12)

Evers and colleagues (1986) explored 5-ISMN chronic dosing pharmacokinetics in renal failure patients resulting in no significant differences between mean values for C_{max} (450±155 ng/mL versus 463±135 ng/mL) on day 2 to day 28 or mean AUC^{ss}_{0-8} values (2158±634 ng·hr·mL^{-1} versus 2270±693 ng·hr·mL^{-1}, respectively). Mean elimination half-lives were 4.29±1.53 hours for day 2 and 4.64±1.66 hours for day 28, indicating no total mean changes between treatment days either.

A third study exploring varying degrees of renal insufficiency after repeated dosing of ISDN was performed by Evers (1989) and colleagues again resulting in no delayed elimination or accumulation seen in this population. The small differences observed do not warrant dosage adjustment.

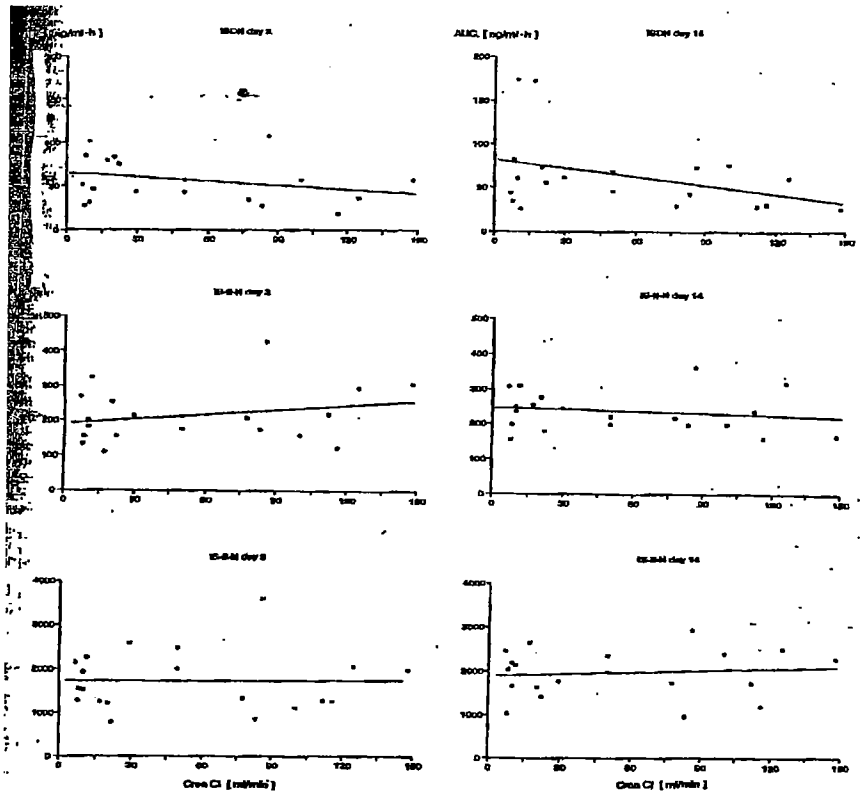


Fig. 1- Relationship between AUC^{ss}_{0-8} and creatinine clearance (on day 2 and day 14) after repeated oral administration of ISDN 20 mg t.i.d.

2.4.6 Hepatic Impairment

BiDil

The PK and PK-PD of H and ISDN after co-administration have not been investigated in patients with hepatic impairment.

Hydralazine

The PK or PK-PD after administration of H alone in patients with hepatic impairment have not been determined.

ISDN

ISDN plasma concentrations in seven cirrhotic patients was compared to 25 volunteers free of hepatic disease (Bogaert 1984) resulting in five out of the 7 cirrhotics having plasma concentrations outside a 95% CI created from the controls. The authors could not find any explanation why two cirrhotic patients had plasma concentrations within the range of the controls since all hepatic patients studied were void of extensive hepatic decompensation. However, some of the patients did have evidence of edema and ascites. No correlation was made between plasma concentrations and parameters of hepatic dysfunction or shunting in the cirrhotics. As a result, additional studies would be required to make a determination on whether dosage adjustment is warranted in this population.

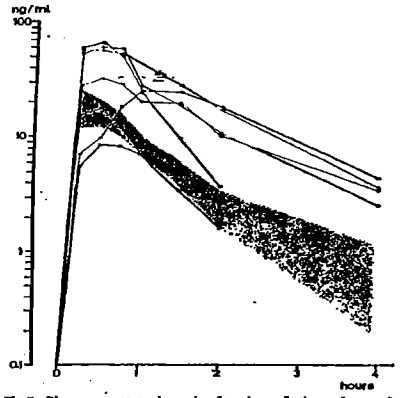


Fig. 7 Plasma concentrations in function of time after oral administration of isosorbide dinitrate, 10 mg, to 7 cirrhotic patients. The shaded area indicates the 95% confidence limit range of the plasma concentrations obtained after administration of the same dose to 25 subjects without hepatic disease. For 3 of the cirrhotic patients, concentrations after 4 hours were below the detection limit (=0.1 ng/ml).

2.4.7 What Pharmacogenetic Information is in the Application and What is its Relevance?

There is no pharmacogenetic information in the submission. However, pharmacogenetic information on the impact of the known polymorphism of NAT2 for the PK and PK-PD of H would be of relevance.

BiDil

The PK of H in the presence of ISDN have been only determined in healthy volunteers presumed to be PMs and only after a single dose. The impact of the acetylator status on the PK and response to BiDil has not been investigated in the target population of black subjects with CHF.

Hydralazine

H is a substrate of NAT. Two N-acetyltranferases have been identified in humans, NAT1 and NAT2. Both NAT1 and 2 are known to be polymorphic (Pompeo et al. 2002). They metabolize different substrates. Whereas NAT1 is ubiquitous, the expression of NAT2 is restricted to liver and intestine (Windmill et al. 2000). EMs are homozygous or heterozygous for the wild-type NAT2 *4 or*12A, * 12B, *12C, *13. PMs are homozygous for NAT2*5A,*5B,*5C, *6A, *6B, *7B or *14A, *14B (Cascorbi et al. 1999, Loktionov et al 2002). PMs do acetylate NAT2 substrates, but at a lower rate than PMs. Compared to EMs a smaller content of NAT2 in the livers of PMs has been found (Grant et al 1990).

The selected published PK studies determined the acetylation phenotype, not genotype. Unlike phenotyping studies with other NAT2 substrates that used the study drug and its acetylated metabolite, H was phenotyped using the proxies isoniazide, sulfamethazine, dapsone or caffeine. It appears that a consensus on a well defined antimode separating EMs and PMs for these NAT2 substrates has not been reached. A possible trimodal distribution has been discussed (Relling 1989). The prevalence of the PM phenotype in American blacks and whites is about 50 % and the one study that phenotyped American blacks and whites the frequency of PMs was lower in the former group (Relling 1989, Lin et al 1993),. Thus, the postulated greater efficacy of BiDil in blacks is not the result of an ethnic difference due to a greater contingent of PMs in blacks than in whites.

The results of the selected published studies show that the bioavailability of H depends on the acetylation phenotype of H in healthy subjects, in hypertensive patients and patients with CHF. When the same dose of H is administered to EMs and PMs drug exposure in PMs will be from 2.6 to 6 times greater than in EMs. The clinical relevance of the acetylator phenotype for the treatment of CHF patients has not been investigated. The acetylator status of the CHF patients participating in A-HeFT and VHeFT I and II was not determined. The clinical relevance of determining the acetylator phenotype of H in hypertensive patients has not been established either. However, there are reports indicating that the dose requirements for H are different for EMs and PMs. A higher incidence of a reversible syndrome similar to systemic lupus erythematodes was observed in hypertensive PMs than in EMs (Perry et al. 1970, 1973, Strandberg et al. 1976 Goodman & Gilman, 2001). Blood pressure control was found to be better in PMs than in

EMs, despite administration of higher dose in the latter (Ramsay et al.1984). Based on these results it has been recommended to restrict the dose of H in hypertensive PMs to 200 mg daily and to determine the phenotype in hypertensive patients who incompletely respond to 200 mg daily.

ISDN

No information was found in the literature on ISDN and pharmacogenetics.

2.4.8 What Pregnancy and Lactation Use Information is in the Application?

BiDil

The impact of pregnancy or lactation on the PK and PK-PD of H and ISDN after co-administration has not been studied.

Hydralazine

Hydralazine has been used in pregnant women with hypertension, particularly in the treatment of severe pre-eclampsia (Liedholm et al. 1982). A single study using a nonselective assay determined maternal and umbilical plasma concentrations of AH at the time of delivery in six mothers and newborns. The mothers were treated with 25 mg to 200 mg H per day. The data showed that the umbilical concentrations of AH exceeded the maternal concentrations indicating that H and/or its major circulating metabolites can cross the placental barrier. The concentration of AH in breast milk could only be measured in one of the women. The result showed substantially smaller concentration of AH in milk than in plasma. On the assumption that all AH is H the computed dose of H in a milk feed is 0.013 mg and negligible.

A study in 10 women with gestational hypertension, co-administration of 25 mg H and 50 mg metoprolol bid resulted in statistically significant increases of Cmax and AUC0-8 of metoproplol of 87.6 % and 37.8%, respectively (Lindeberg et al., 1988). These results indicate that H impacts the exposure of pregnant women to metoprolol under steady-state conditions.

ISDN

According to package inserts on ISDN, it is a pregnancy category C drug. No studies in pregnant women have been performed. However, dose-related increases in embryotoxicity (increase in mummified pups) in rabbits at oral doses of 35 to 150 times the maximum recommended human daily dose has been observed.

Since it is not known if ISDN is excreted in human milk, caution should be exercised when ISDN is given to nursing mothers and has been documented as such in package inserts for the product.

2.4.9 What are other Human Factors that are Important to Understanding of the Drug's Efficacy and Safety?

BiDil and H

No other human factors have been identified that are important for the efficacy and safety of a treatment with BiDil or H.

ISDN

The most commonly reported AE is headache; which is a marker of the activity of the drug. The headaches can be severe at times and can occur every time ISDN is administered. Patients and clinicians should resist the temptation of avoiding these headaches by altering the schedule of dosing or lowering what has been defined as the effective dose. Loss of headache may be associated with simultaneous loss of anti-anginal efficacy. This may also translate in the treatment of CHF.

2.4.10 Under Consideration of All Intrinsic Factors Are Dose Regimen Adjustments Recommended for BiDil in the Following Subpopulations?

The database on the impact of intrinsic factors on the PK and PK-PD of H and ISDN after administration of BiDil is too scarce for recommendations for dose adjustments in subpopulations.

2.5 Extrinsic Factors

2.5.1 What Extrinsic Factors Influence Dose-Exposure and/or Response and What is Impact of Any Difference in Exposure and/or Response to Hydralazine and Isosorbide Dinitrate?

Herbal Products

BiDil

The impact of co-administered herbal products on the PK or PK-PD of co-administered H and ISDN has not been determined

Hydralazine

The impact of co-administered herbal products on the PK or PK-PD of H when administered alone has not been determined

ISDN

The impact of co-administered herbal products on the PK or PK-PD of ISDN has not been determined.

Diet

BiDil

The impact of diet on the PK or PK-PD of co-administered H and ISDN has not been determined

Hydralazine

The impact of diet on the PK or PK-PD of H when administered alone has not been determined

ISDN

The impact of diet on the PK or PK-PD of ISDN has not been determined.

Smoking

BiDil

The impact of smoking on the PK or PK-PD of co-administered H and ISDN has not been determined

Hydralazine

The impact of smoking on the PK or PK-PD of H when administered alone has not been determined

ISDN

The impact of smoking on the PK or PK-PD of ISDN has not been determined.

Alcohol

BiDil
The impact of alcohol on the PK or PK-PD of co-administered H and ISDN has not been determined

Hydralazine

The impact of alcohol on the PK or PK-PD of H when administered alone has not been determined

ISDN

Alcohol has been found to exhibit additive vasodilating effects with ISDN and as such is stated in the precautions section of product labels for ISDN.

2.5.1.1 Is the Impact on Exposure and/or Response to Hydralazine or Isosorbide Dinitrate Significant enough to Warrant and Adjustment of the Dosing Regimen?

In the absence of appropriate data recommendations for dose adjustments cannot be made.

2.5.2 What are the Known Drug Interactions?

2.5.2.1 Is There an in Vitro Basis to Suspect in Vivo Drug-Drug Interactions?

Hydralazine and ISDN

No, in vitro studies with human liver tissues have not been performed to determine the substrate-, inhibitor- or inducer status of H or ISDN.

2.5.2.2.1 Is Hydralazine or ISDN a Substrate of CYP Enzymes?

Hydralazine

This is unknown. Appropriate in vitro experiments were not conducted with H. There is evidence for oxidative microsomal metabolim of H (Streeter and Timbrell, 1985). It is recommended that the sponsor evaluates the possible involvement of CYP 450 in the metabolism of H and the inhibitory and induction potential of H to impact the metabolism of other drugs that are substrates of CYP 450. The identity of a substantial fraction of the systemic available dose of H has not been identified. The target populations is on co-medication with a number of drugs that have a small therapeutic range.

ISDN

No information on this topic could be found in the literature. Appropriate in vitro experiments have not been conducted either. The sponsor should evaluate the possible involvement of CYP 450 in the metabolism of ISDN, its inhibitory/induction potential, and the impact of other drugs that are substrates of CYP 450.

2.5.2.2.2 Is the Metabolism of Hydralazine or Isosorbide Dinitrate Influenced by Genetics?

Hydralazine

Yes, H is a substrate of the polymorphic NAT2.

ISDN

This information is not known.

2.5.2.3 Is Hydralazine or ISDN an Inhibitor and/or an Inducer of CYP Enzymes?

Hydralazine

Theoretically, H could be an inhibitor of other NAT2 substrates including dapsone, isoniazide, procainamide, phenelzine, sulfamethazine, and sulphadimidine. Acetylation of sulphadimidine was found to be decreased in the presence of procainamde (Campbell et al. 1976).

ISDN

No information could be found in the literature on this topic.

2.5.2.4 Is Hydralazine or Isosorbide Dinitrate a Substrate and/or an Inhibitor of P-glycoprotein?

Hydralazine

The interaction between H and P-glycoprotein has not been determined. It is recommended that the sponsor determines whether H is a substrate and/or inhibitor of P-glycoprotein. The target population receives digoxin and statins, which are substrates of P-glycoprotein.

ISDN

The interaction between ISDN and P-glycoprotein has not been determined either. The sponsor should determine if ISDN is a substrate and/or inhibitor of P-glycoprotein as well.

2.5.2.5 Are there Other Important Metabolic/Transporter Pathways?

Hydralazine

In addition to acetylation H is metabolized by ring hydroxylation and by conjugation with endogenous pyruvic acid and possibly with other endogenous aldehydes and ketones. It is not known whether H is a substrate of transporters.

2.5.2.6 Does the Label Specify Co-Administration of Another Drug and if so Has the Interaction Potential between these Drugs Been Evaluated?

Yes, BiDil is a fixed dose combination of H and ISND. The impact of the two drugs on their respective pharmacokinetics has not been evaluated experimentally. The AUC values of H from the higher and lower strength BiDil tablets after normalization of the dose were almost in the same proportion as the amounts of H in the tablets, although the

higher strength BiDil tablet contained a four times larger amount of ISDN than the lower strength BiDil tablet. This result suggested that the presence of ISDN has no major impact on the disposition of H.

2.5.2.7 What Other Co-medications are Likely to be Administered to the Target Population?

Diuretics, beta-blockers, ACE-inhibitors, angiotensin II receptor antagonists, aldosterone antagonists, digoxin, aspirin, and anticoagulants.

2.5.2.8 Are there any in Vivo Drug-Drug Interaction Studies that Indicate the Exposure and/or Exposure-Response Relationships are Different when Drugs are Co-administered?

BiDil

No drug interaction studies were performed with BiDil.

Hydralazine

Drug interaction studies investigating the effect of H administration on the exposure to other drugs have been performed with furosemide, the beta-blockers propranolol, metoprolol (2 studies), acebutolol and nadolol, and the ACE-inhibitors lisinopril and enalapril (Nomura et al 1982, McLean et al 1980, Jack et al 1982, Lindeberg et al 1988, McLean et al 1989). The interaction study with furosemide was conducted in Japanese patients with advanced CHF who received a single dose of 40 mg furosemide in the presence or absence of 0.2 mg/kg H given by the intravenous route. The interaction study with propranolol was a single dose study conducted in healthy volunteers who received propranolol 1 mg/kg in the presence and absence of ascending doses of up to 50 mg H. The impact of co-administering a single dose of 50 mg H with 100 mg metoprolol or 80 mg nadolol or 400 mg acebutolol was investigated in healthy subjects. A second study investigated the impact of 25 mg H on metoprolol 50 mg at steady-state with both drugs given bid. The impact of 25 mg H on the pharmacokinetics of enalapril and lisinopril was studied in a single dose study in healthy volunteers who received 20 mg lisinopril or enalapril in the presence or absence of a single dose of H.

The results of the interaction studies are summarized in Table 6:

Table 6. Mean Changes in Cmax and AUC of Beta-Blockers, ACE Inhibitors and Furosemide when Co-Administered with Hydralazine

Drug	Study	Subjects	Dose, mg		ΔCmax, %	ΔAUC, %
			H	Drug		
Propranolol	Single dose, po	Healthy Vol.	50	1mg/kg	142.8*	77.3*
Metoprolol	Single dose, po	Healthy Vol.	50	100	49.9*	30.5*
Metoprolol	Multiple dose, po	Pregn. Women	25 bid	50 bid	87.6*	37.8*

Nadolol	Single dose, po	Healthy Vol.	50	80	-38.7	-30.7
Acebutolol	Single dose, po	Healthy Vol.	50	400	-0.9	5.6
Lisonopril	Single dose, po	Healthy Vol.	25	20	30.4*	33.2*
Enalapril	Single dose, po	Healthy Vol.	25	20	-1.2	- 3.9
Furosemide	Single dose, iv	CHF Patients	0.2mg/kg	40	#	#

* Statistically significant change # Statistically significant increase in CL of 21.3%

The results indicate that co-administration of H increases the exposure (Cmax, AUC) to propranolol, metoprolol and lisinopril statistically significantly. H increased the CL of furosemide by 21.3%. In contrast, co-administered H had no statistically significant impact on the exposure to enalapril, nadolol and acebutolol.

Beta-blockers, ACE inhibitors and furosemide are part of the background therapy in CHF. Except for furosemide, the interaction studies were not conducted in the target population. The doses of H were lower than required for the adjunct treatment of CHF and, except for metoprolol, single doses of the drugs were used. In none of the studies isosorbide dinitrate was present. These additional factors need to be considered when extrapolating the findings of the interaction studies to the target population with CHF. They could have a compounding effect and hence caution is should be exercised when combining BiDil with metoprolol, propranolol and lisinopril.

Theoretically H could decrease the pre-systemic and/or systemic metabolism of other NAT2 substrates including dapsone, isoniazide, procainamide, phenelzine, sulfamethazine, and sulphadimidine. The result of such an inhibition by co-administered H would be an increase in exposure to these drugs (Campbell et al. 1976).

ISDN

Ochs and colleagues (1986) investigated the influence of propranolol and metoprolol on the pharmacokinetics of ISDN and its metabolites (2- and 5-ISMN). Two small studies were conducted in this investigation. The first study evaluated if metoprolol co-administered with 5-ISMN altered the pharmacokinetics of ISMN. The treatment arms were: ISMN (20 mg) given alone and metoprolol given as 100 mg twice daily started 48 hours prior to ISMN administration with the final metoprolol dose given concurrently with ISMN on the day of pharmacokinetic assessment. Results indicate that metoprolol does not seem to influence 5-ISMN pharmacokinetically (C_{max} 429 versus 481 ng/mL with metoprolol; AUC_{total} 2449 versus 2516 ng/mL·h; and Cl_{po} 138 versus 135 mL/min, respectively).

The second portion of the study consisted of ISDN administered as a single 20 mg oral dose in two treatment arms. Treatment arm I was ISDN alone and treatment arm two consisted of propranolol given as 80 mg three times daily started 48 hours prior to ISDN administration with the final propranolol dose given concurrently with ISDN on the day of pharmacokinetic assessment. Differences in total AUC ($p<0.05$, about 20% drop in AUC) and oral clearance (increase, $p<0.1$, 32% increase) for ISDN when administered with propranolol were observed. Peak ISMN levels were reached sooner during propranolol coadministration (35% sooner) with a prolonged apparent half-life (about

27% increase). However, the changes noted were not of sufficient magnitude to conclude clinical significance and the need for dose adjustment.

A study by Bogaert (1983) and colleagues assessing single atenolol (100 mg two hours prior to ISDN) administration on the pharmacokinetics of ISDN (10 mg) resulted in no significant differences in C_{max} and AUC.

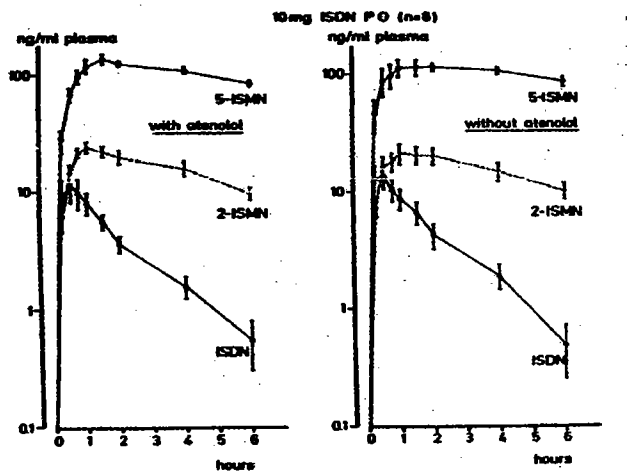


Fig. 1 Mean plasma concentrations (± standard error of the mean) of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of isosorbide dinitrate 10 mg to 8 subjects on 2 occasions, once with and once without previous administration of atenolol, 100 mg.

2.5.2.9 Is there a Known Mechanistic Basis for Pharmacodynamic Drug-Drug - Interactions?

BiDil

H dilates arterioles and ISDN dilates venous mainly as well as arterial vessels. H and ISDN both lower blood pressure (Goodman & Gilman 2001). There is evidence that H increases the availability of NO generated by ISDN in the smooth muscle cells of the arterioles.

2.5.2.10 Are There any Unresolved Questions Related to Metabolism, Active Metabolites, Metabolic Drug Interactions, or Protein Binding?

Hydralazine

H is mainly metabolized. A substantial percentage of systemically available radio-labeled H remains unidentified. The acetylation pathway is of primary relevance, because the

acetylator status is responsible for 2.6 to 6 fold variations in exposure to H. However, H is also metabolized by other routes including oxidation. The respective roles of CYPs and transporters in metabolism and influx and efflux of H has not been determined. The inhibitory potential of H on other drugs and the potential of other drugs to inhibit the metabolism of H have not been determined. Similarly, the induction potential of H towards other drugs and the induction potential of other drugs towards H, when co-administered, have not been determined. The plasma protein binding of H using assay procedures selective for H has not been determined.

ISDN

The role of CYP 450 in the metabolism of ISDN has not been determined. The inhibitory potential of ISDN on other drugs and the potential of other drugs to inhibit the metabolism of ISDN has not been determined either. In addition, induction potential of ISDN towards other drugs and the induction potential of other drugs towards ISDN, when co-administered remain unanswered.

2.5.3 What Issues Related to Dose, Dosing Regimens, or Administration are Unresolved and Represent Significant Omissions?

BiDil

The sponsor has not attempted to determine the optimum dose ratio and interval of the proposed fixed dose combination regimen. The qid regimen used in VeTHFT I and VeHFT II and the tid regimen employed in A-VeHFT are different. It is unknown whether the tid regimen with a less than 12-14 hour dose-free interval during the night does optimally minimize the development of tolerance to ISND. Also, the submission lacks information on the possible impact of many intrinsic and extrinsic factors including NAT2 polymorphism, age, body weight, sex, renal and hepatic function, drug interaction potential, food intake on exposure and consequently on response to one or both components of this fixed dose combination. It is probable that these covariates individually and as an aggregate impact the exposure and response to the individual drug entities in real patients. The acetylator status determines the absolute bioavailability of H and as a result the exposure to H can be from 2.6 to 6 times greater in PMs than in EMs. Consequently, the respective concentrations of the individual components in the biophase are not in a fixed relation as the respective doses of H and ISND are. The ratios in the individuals will be quite different, but not tailored to the individual needs of the patients. One cannot exclude the possibility that the proposed fixed dose regimen is ineffective in some EMs and causes adverse events in some PMs.

2.6 General Biopharmaceutics

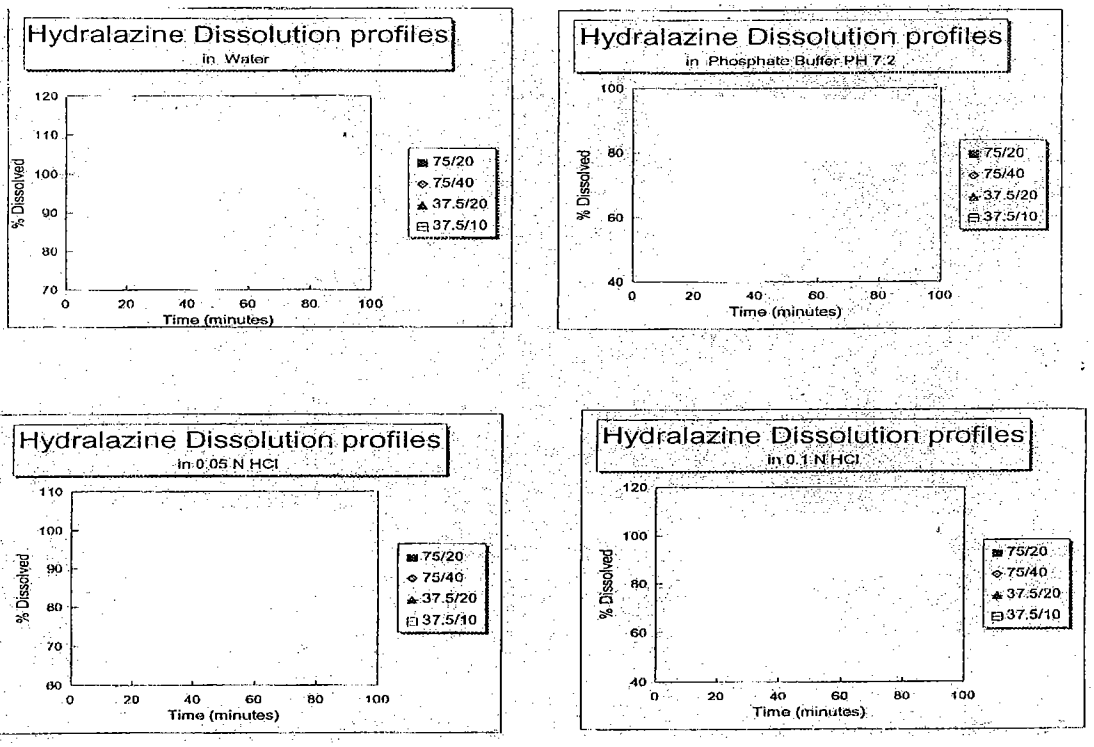
2.6.1 What is the BCS Classification of Hydralazine and Isosorbide Dinitrate?

Hydralazine

Solubility
The water solubility of H is 39 mg/mL. BiDil tablets contain 37.5 mg H HCl. The usual maximum single dose is 2 BiDil tablets. H can be considered a highly water soluble drug. The solubility of H in media with a pH ranging between 1.0 and 7.5 has not been determined. Thus, H cannot be classified according to BCS.

Permeability
The recovery of total radioactivity in urine after oral administration of ^{14}C labeled H is on average 69.1 %. Thus H is not a high permeability drug.

Dissolution
The dissolution of H from a BiDil 37.5/20 tablet in water, 0.1 N HCl, 0.05 N HCl and pH 7.2 phosphate buffer is shown in the adjacent figures:



The Figures indicate that [ ] of H form the BiDil tablets is dissolved in all 4 media after [ ] minutes.

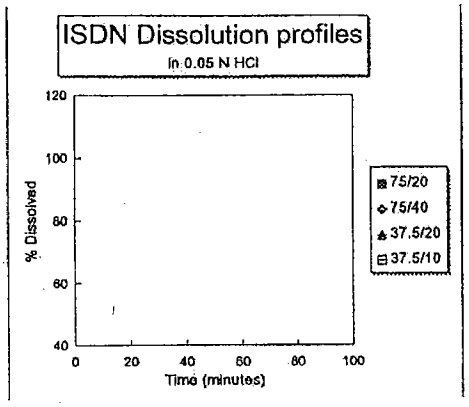
It can be concluded that H is a highly water soluble and low permeability drug. However, the solubility of H has not been determined in aqueous media in the pH range 1.0-7.5. Thus, H cannot be classified according to the BCS system.

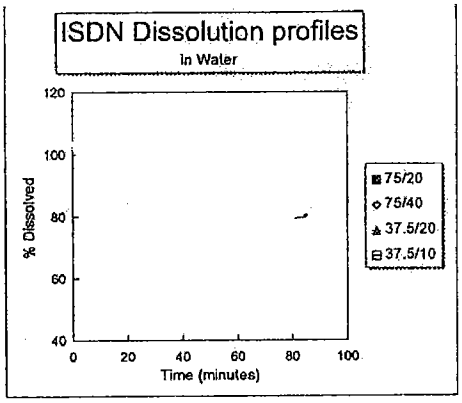
ISDN

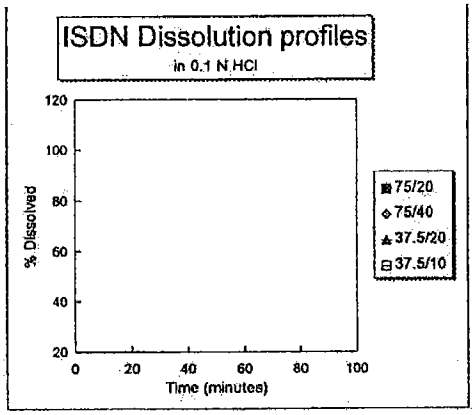
Solubility
According to the package insert on ISDN products, it is not a highly water soluble drug.

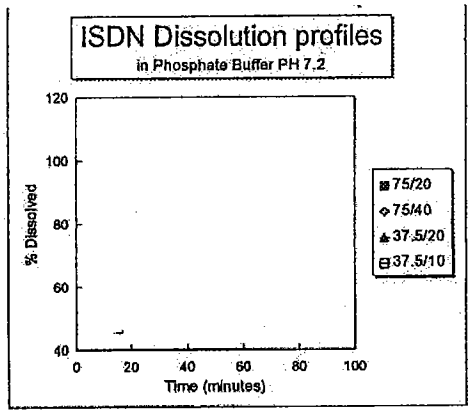
Permeability
Permeability information is not available.

Dissolution
The dissolution of ISDN from a BiDil 37.5/20 tablet in water, 0.05 N HCl, 0.1 N HCl, and pH 7.2 phosphate buffer is illustrated below:









The Figures indicate that [ ] of ISDN is dissolved in all 4 media after [ ] minutes.

It can be concluded that more than [ ] ISDN dissolved in [ ] minutes from the BiDil 37.5/20 tablet and can be considered a rapidly dissolving drug. BCS classification at this time is not possible.

2.6.2 What is Relative Bioavailability of to be Marketed Formulation Relative to the Formulation Used in the Pivotal Trials?

The BiDil 20 tablet containing 37.5 mg H and 20 mg ISDN was used in the pivotal A-HeFT. The single entity capsule of H and a single entity tablet of ISDN were used in VHefT I and the single entity tablets of H and ISDN in V-HeFT II. Due to the demonstration of efficacy in A-HeFT and in VHeFT I the BiDil 20 tablets and the single entity 37.5 mg hydralazine capsule and 20 mg ISDN tablet, respectively, are qualified.

The relative bioavailability/bioequivalence of the formulations used in the 3 efficacy- and safety trials was evaluated in healthy volunteers, presumably PMs. It should be noted that the low dose BiDil tablets tested contain the same amount of H as the BiDil 20 tablets, however only 10 mg of ISDN. Neither of the two different strength BiDil tablets tested was bioequivalent to the single entity formulations. However, because the to be marketed BiDil 20 mg tablet was used in the pivotal A-HeFT this formulation is qualified and the bioinequivalence is no longer an issue.

2.6.2.1.1 What Data Support or Do Not Support a Waiver of in Vivo BE Data?

BiDil

In the NDA submission, the sponsor submitted in vitro dissolution data on the BiDil 20 tablets and the other strength BiDil tablets (75/40, 75/20 and 37.5/10) in support of a waiver. However, because of clearly slower dissolution of the lower strength BiDil tablets the waiver was not granted. However, this is no longer an issue.

2.6.2.2 What Are Safety or Efficacy Issues, if any, for BE Studies that Fail to Meet the 90% CI Using Equivalence Limits of 80-125%?

BiDil

There are no efficacy or safety issues that relate to the bioinequivalence of the single entity formulations and the BiDil formulation

2.6.2.3 If the Formulations Do not Meet the Standard Criteria for Bioequivalence, What Clinical Pharmacology and/or Clinical Safety and Efficacy Data Support the Approval of the-to be Marketed Product?

Bioequivalence is not an issue with BiDil.

2.6.3. What is the Effect of Food on the Bioavailability of the Drug from the Dosage Form?

BiDil

The effect of food on the absorption of H and ISDN after administration of BiDil has not been evaluated.

Hydralazine

Three studies investigated the impact of a standard breakfast on H given in solution or as the single entity tablet formulation of H (Shepherd et al. 1984, Jackson et al.1990, (Semple et al. 1991). All 3 studies used a selective assay for H. One study was conducted in healthy volunteers and 2 studies in hypertensive patients. The study in healthy volunteers enrolled 8 healthy volunteers, 4 females and 4 males and used a tablet formulation of H (Semple et al 1991). H was administered as the intravenous formulation orally to 6 hypertensive patients, 2 EMs and 4 PMs (Shepherd et al 1984). The food administered was a standardized breakfast with known carbohydrate, fat and protein and caloric content. Both studies in the hypertensive patients determined also the possible impact of food intake on blood pressure and heart rate.

The results of the food interaction studies are summarized in Table 7:

Table 7. Mean Percent Change in Cmax and AUC of Hydralazine in Subjects Receiving the Drug in the Fed Sate Relative to the Fasted State.

Subjects	Formul.	Dose	ΔCmax, %	Δ AUC, %
Healthy	Tablet	50 mg	-55.0^*	- 82.8^*
Hypertensive	Solution	1 mg/kg	- 41.5*	- 41.1*
Hypertensive	Tablet	100 mg	- 69.2*	- 47.8*

^ Median * Statistically significant difference

The results of the three studies show a consistent and statistically significant decrease in the bioavailability measures of H by food intake. The reduced blood concentrations of H were accompanied by reduced vasodepressor effects on mean blood pressure and heart rate in the smaller study in hypertensive patients. In the larger study in hypertensive subjects the vasodepressor effects were not different in the fed and fasted state.

These results indicate that food impacts the absorption of H and the effect relates primarily to the substance. The proposed mechanism responsible for the observed food effect is greater intravascular conversion of H to HPH due to increased pyruvic acid plasma concentrations in the postprandial state.

Other studies reported a statistically significant increase in the plasma levels of H in the fed state (Melander et al. 1977, Liedholm et al. 1982) or no impact of food on the bioavailability of H (Walden et al. 1981). However, these three latter studies used non-selective assays for H.

The time interval between drug- and food intake was not defined in the protocols of the pivotal clinical studies. With the proposed tid dose regimen for BiDil it is possible that drug- and food intake will coincide, resulting in a smaller bioavailability of H.

ISDN

The package insert is silent on this topic.

2.6.3.1 What Dosing Recommendation should be Made, if any, Regarding Administration of the Product in Relation to Meals or Meal Types?

The above studies demonstrate that food (standard breakfast) has an impact on the availability of H when H is administered alone. The time of intake of H and food should be in a fixed relation. However, more definitive recommendations should await the results of a study that investigates the impact of food on BiDil.

2.6.4 When would a Fed BE Study be Appropriate and was One Conducted?

The conduct of a fed BE study would not be appropriate. A fed BE study was not conducted.

2.6.5 How do Dissolution Conditions and Specifications Ensure in vivo Performance and Quality of Product?

The following dissolution method and specifications are recommended for the BiDil 20 Tablets: USP Apparatus I (basket) at a speed of 100 rpm, medium: 0.05 N HCl.
Q ≥[ ] in 30 minutes for both, H and ISDN.

The composition of the BiDil 20 Tablet is shown below:

Table 4-10: BiDil® 20 Tablet Core Formulation

Ingredient	% w/w	mg/tab
Hydralazine hydrochloride, USP	18	37.5
Diluted isosorbide dinitrate, USP (ISDN/lactose [25/75])		
Lactose anhydrous, NF		
Microcrystalline cellulose, NF		
Sodium starch glycolate, NF		
Magnesium stearate, NF		
Colloidal silicon dioxide, NF		
Total	100.00	

[ ]

2.6.6 If Different Strength Formulations are not Bioequivalent based on Standard Criteria what Clinical Safety and Efficacy Data support the Approval of the Various Strengths of the to-be Marketed Formulation?

This is not an issue with BiDil

2.6.7 If Unapproved Products or Altered Approved Products were used as Active Controls, how is BE to the Approved Product Demonstrated?

BiDil

No unapproved or altered approved formulations were used in the pivotal trials

2.6.8 What is the Basis for Using either in Vitro or in Vivo Data to Evaluate BE?

Neither *in vitro* nor *in vivo* BE data are relevant for qualifying the formulations used in the pivotal trials.

2.6.9 What Other Significant, Unresolved Issues Related to in Vitro Dissolution or in Vivo BA and BE Need to be Addressed?

There are no significant, unresolved issues.

2.7 Analytical Section

2.7.1 How are Active Moieties Identified and Measured in Plasma in the CP and BP Studies?

Hydralazine

H is presumably the only major active species in plasma or blood. Specific HPLC methods using the matrices plasma and blood were used.

ISDN

ISDN and its mononitrates (2- and 5-ISMN) are the major active species in plasma or blood. Specific GC methods using the matrices in plasma and blood were used.

2.7.2 Which Metabolites were Selected and Why?

Hydralazine

HPH and MTP were measured because they constitute the major circulating metabolites. AHP and OHTPM were measured, because they constitute the major metabolites excreted in urine. Some early data suggested HPH and other hydrazones may be pharmacologically active. The hypotensive effect of H decays much slower than the plasma concentrations of H. It was shown later that HPH has a minor tachycardic effect (Shepherd et al, 1980). Other hydrazones were shown no to directly exert pharmacological activity. Their potential to generate H and the possibility that they remain in the body longer than H renders them relevant.

Given the labile nature of H and some of the generated hydrazones in biological fluids and the specificity problems of the first generation assays, it was important for the more specific second generation assays to quantify the main circulating metabolites and to determine their relationship to H.

ISDN

2- and especially 5-ISMN have been identified as active metabolites of ISDN that contribute to the prolonged effect of ISDN after the parent drug is undetectable in plasma. It is believed that the mononitrate's longer half-lives (1 hour for ISDN, 2 hours for 2-ISMN and 5 hours for 5-ISMN) play a role in the observed prolonged vasodilatory effect.

2.7.3 For all Moieties Measured, is Free, Bound or Total Measured?

Hydralazine

Total concentrations in blood or plasma were measured for H, HPH and MTP.

ISDN

Total concentrations in blood or plasma were measured for ISDN, 5-ISMN and 2-ISMN.

2.7.3.1 What is the Basis for this Decision, if any, and is it Appropriate?

Hydralazine

The plasma protein binding of H and the metabolites using a selective method has not been determined. In the absence of this information an educated decision on whether to measure total or unbound concentrations of the active species cannot be made.

2.7. Analytical Section

2.7.4 What Bioanalytical Methods are used to Assess Concentrations?

Hydralazine

H in plasma and blood is unstable and forms acid labile hydrazones with endogenous acids, aldehydes and acetones. The acid labile hydrazones under acid conditions are hydrolyzed to H (Reece et al 1978). The labile nature of H and its major circulating metabolites posed a challenge to the development of assays and required appropriate sample handling. Assays developed before 1978 were non-specific and, except for one, the selected assays developed after 1978 were specific for H and ensured that degradation of H to HPH and other hydrazones and their subsequent hydrolysis to H was minimized. These second generation assay methods used plasma or whole blood as matrix (Reece et al. 1978 and 1980, Ludden et al. 1979, 1980 and 1983, Semple 1991). Only PK data on H and metabolites derived from plasma or blood concentrations measured by the more specific second generation assays were considered in this review. However, these more specific assays of the early 1980's cannot be considered validated as defined by today's standards (FDA Guidance for Industry, Bioanalytical Method Validation, 2001). Nevertheless, the information provided on the published second generation assays suggested an acceptable performance. The HPLC assay used in the single bioavailability/bioequivalence study performed by the sponsor can be considered validated.

ISDN

Most studies reviewed utilized some form of a GC method. One study measured 5-ISMN concentrations from plasma by a method involving liquid chromatography mass spectrometry with an internal standard. A second study analyzed plasma ISDN concentrations by HPLC. Two studies utilized a gas liquid chromatographic method for analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations. One study modified a GC method developed for nitroglycerin. The same issue exists as with H in that the assays of the early 1980's cannot be considered validated if defined by today's standards (FDA Guidance for Industry, Bioanalytical Method Validation, 2001).

2.7.4.1 What is the Range of the Standard Curve?

Hydralazine, Hydralazine Pyruvate Hydrazones and Methyltriazolophthalazine

The range of the respective calibration curves of the 3 analytes is provided in Tables 8-10

ISDN

The information was not provided.

2.7.4.1.1 How Does it Relate to the Requirements for Clinical Studies?

Hydralazine,Hydralazin,e Pyruvate Hydrazone and Methyltriazolophthalazine

Assays 1, 2, 6 and 7 covered the clinically observed range of H after oral administration adequately (Tables 8-10).

Table 8. Characteristics of the Assays for the Determination of Hydralazine in Plasma or Whole Blood

Assay	Calibration Range ng/mL	Fit	LLOQ ng/mL	ULOQ ng/mL	Accuracy[a] %	Precision[b] %	Selective	QC Samples Used	Sample Stability Tested[b]
1	0.25-200	LR[c]	0.25	200	-1.06	9.06	Yes	Yes	Yes
2	1-1000	LR[c]	NR	NR	NR	2.0-4.0	Yes	No	No
3	5-200	LR[c]	10	10-200	-9.0 - (+)5.4	2.8- 10.0	Yes	No	No
4	2-160	LR[c]	2	2-160	-10.0 - (+)9.0	6.4 – 18.8	Yes	No	No
5	1-160	LR[c]	1	1-160	4.6-18.3	3.7-18.3	Yes	No	No
6	10-1000	LR[c]	1	100	NR	3.0-7.0	No	No	No
7	0.3-640	LR[c]	0.3	640	-13.0- (+)2.5	2.55- 11.97	Yes	No	No

[a] Intra-assay [b] Freeze thaw cycles, benchtop [c] Linear regression

1 COB2 study 2 Reece et al 1980 3 Ludden et al 1979 4 Ludden et al 1980 5 Ludden et al. 1983 6 Hanson et al 1983 7 Semple et al. 1988

Table 9. Characteristics of the Assays for the Determination of Hydralazine Pyruvate Hydrazone in Plasma

Assay	Calibration Range	Fit	LLOQ	ULOQ	Accuracy[a]	Precision[a]	Selective	QC Samples Used	Sample Stability Tested[b]
	ng/mL		ng/mL	ng/mL	%	%			
2	0.2-1000	LR[c]	NR	NR	NR	3.0-7.0	Yes	No	No
4	50-1000	LR[c]	50	1000	-9.0- (+) 5.3	6.4-18.8	Yes	No	No
6	100-2000	LR[c]	100	2000	NR	3.0-5.0	No	No	No

[a] Intra-assay [b] Freeze thaw cycles, benchtop [b] Linear regression

Table 10. Characteristics of the Assay used for the Determination of Methyltriazolophthalazine in Plasma

Assay	Calibration Range	Fit	LLOQ	ULOQ	Accuracy	Precision	Selective	QC Samples Used	Sample Stability Tested[a]
	ng/mL		ng/mL	ng/mL	%	%			
2	0.1-100	LR[b]	NR	NR	NR	2.0-3.0	Yes	No	No

[a] Intra-assay [b] Freeze thaw cycles, benchtop [c] Linear regression

The LOQ of assay 3 was not sufficiently low for H. Assays 2 -5 covered the clinical range of concentrations of H after intravenous administration adequately. The assays for HPH and MTP covered the clinically encountered concentration range adequately.

ISDN

Bioanalytical assay methodology has been consistent and has not been an issue in the determination and quantification of ISDN or its metabolites.

2.7.4.1.2 What Curve Fitting Techniques are Used?

Hydralazine

Linear regressions were fitted to the calibration curves (Tables 8-10)

2.7.4.2 What are the Lower and Upper Limits of Quantification (LLOQ/ULOQ)

Hydralazine, Hydralazine Pyruvate and Methyltriazolophthalazine

See Tables 8-10.

ISDN

A range of 0.5 to 200 ng/mL was observed.

2.7.4.3 What are Accuracy, Precision, and Selectivity at These Limits?

Hydralazine, Hydralazine Pyruvate and Methyltriazolophthalazine

See Tables 8-10.

ISDN

The CV% for ISDN was usually below 10%.

2.7.4.4 What is the Sample Stability under the Conditions Used in the Study (long Term, Freeze-Thaw, Sample Handling, Sample Transport, Auto Sampler?

2.7.4.5 What is The QC Sample Plan?

Hydralazine, Hydralazine Pyruvate and Methyltriazolophthalazine

See Tables 8-10..

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5.2 Clinical Pharmacology and Biopharmaceutics Individual Study Review

Hydralazine

5.2.1 Selected Published Clinical Pharmacology Studies

5.2.1.1 Exposure-Response Studies

On the assumption that the antihypertensive and cardiac performance improving effects of H may have a common mechanistic base, the results of studies in hypertensive patients were included in the review of published data.

Studies in Patients with Congestive Heart Failure

1. Leier CV, Magorien RD, Desch CE, Thompson MJ, Unverferth DV. Hydralazine and isosorbide dinitrate: Comparative central and regional hemodynamic effects when administered alone or in combination. Circulation 1981; 63:102-109

This single oral dose study compared the cardiovascular effects of H and ISDN given alone and in combination as single entity formulations to CHF patients of NYHA Class III and IV. Twelve males and 3 females in the age between 24 and 68 years participated in the study. Eight patients received 75 mg H on study Day 1 and 25 mg ISDN and 75 mg H on study Day 2. The remaining 7 patients received only 75 mg H. Control values of central and peripheral hemodynamic parameters were obtained prior to drug administration in both groups.

H increased statistically significantly mean cardiac output 17-25%, mean renal blood flow 19% and limb blood flow 17%. H decreased also pulmonary capillary wedge pressure. The effects of H became measurable 0.5 hours after administration, peaked at 2 or 3 hours after dosing and lasted for the remainder of the 6 hour observation period (see Figure). H did not change arterial pulmonary pressure and hepatic blood flow statistically significantly. ISDN, except for a transient increase in cardiac output 0.5 h after administration, did not change renal or limb blood flow. ISDN decreased statistically significantly pulmonary capillary wedge pressure and pulmonary arterial pressure. The peak effects of ISDN occurred 0.5 hours after administration and decayed thereafter.

Addition of ISDN to H resulted in a statistically significant enhancement of the responses in pulmonary capillary wedge pressure, pulmonary arterial pressure and total pulmonary vascular resistance during the first 2 hours after administration. The combination of H and ISDN lead to similar changes in cardiac output, renal and limb blood flow as H given alone. The respective effect time curves of the combination and H alone treatments were similar. The increases in renal and limb blood flow observed after administration of H alone and the combination are related to the augmented cardiac output, since heart rate did not change significantly with any of the treatments.

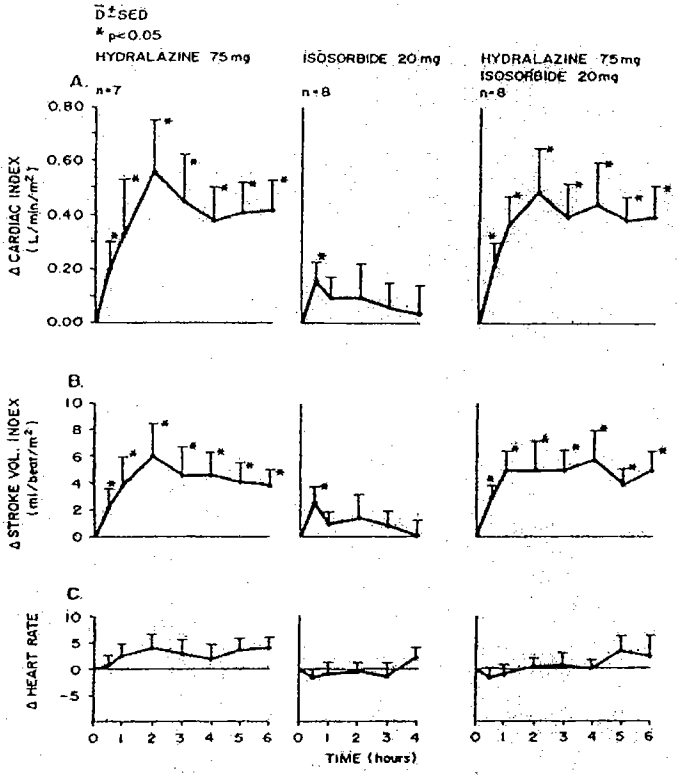

FIGURE 1. *(A) Hydralazine increased the cardiac index over 6 hours after administration. A transient increase of cardiac index was noted 30 minutes after the administration of isosorbide dinitrate. The cardiac index response of combined hydralazine-isosorbide dinitrate did not differ from that of hydralazine alone. (B) The stroke volume responses generally reflected those of the cardiac index (see panel A). (C) Hydralazine and isosorbide dinitrate, alone or in combination, did not significantly change heart rate from control. D ± SED = mean of the difference ± standard error of the difference; VOL = volume.*

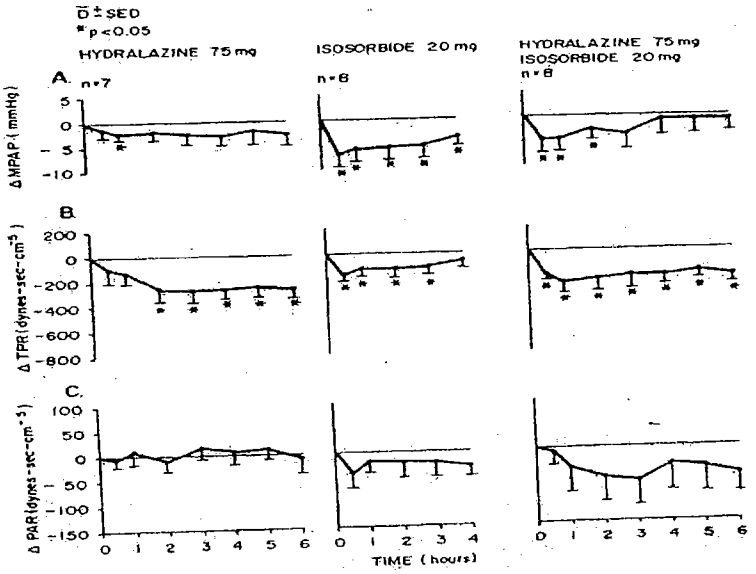

FIGURE 3. *(A) Isosorbide dinitrate alone elicited the greatest decrease in mean pulmonary artery pressure (MPAP). Hydralazine significantly decreased the MPAP at 1 hour only. The addition of isosorbide dinitrate to hydralazine resulted in a greater decrease in the MPAP for the initial 2 hours than hydralazine alone. (B) All three dosing groups significantly decreased total pulmonary resistance (TPR). The addition of isosorbide dinitrate to hydralazine significantly decreased the 30-minute and 1-hour values below the corresponding values for hydralazine alone. (C) Mean pulmonary arteriolar resistance (PAR) was not altered significantly by hydralazine, isosorbide dinitrate, or the combination, suggesting that most of the total pulmonary resistance changes (TPR, panel B) are secondary to a reduction in the PCWP. $\bar{D} \pm SED$ = mean of the difference ± standard error of the difference.*

2. Packer M, Meller J, Medina N, Gorlin R, Herman MV. Hemodynamic evaluation of hydralazine dosage in refractory heart failure. Clin Pharmacol Ther. 1980;27:337-346

This study evaluated the dose-response relationship of H in 18 patients, 15 males and 3 females, of mean age 64 years, with severe chronic CHF resistant to therapy with digitalis and diuretics. Two patients had normal renal function, 10 patients had mild or moderate renal impairment and 5 had severe renal impairment. Three days before the study, patients observed bed rest and received a 2 gm sodium diet, and the co-medication was reduced to digoxin and diuretics. Right heart catheterization with measurements of right atrial, pulmonary arterial, and pulmonary capillary wedge pressure was performed. Arterial blood pressure was measured invasively in some patients and in the remainder by the cuff method. Heart rate was determined from a continuously recording ECG. Arterial blood pressure, heart rate, left ventricular filling pressure, LVFP, mean pulmonary artery pressure, mean right atrial pressure and cardiac output, CO, were determined. The derived parameters included mean arterial blood pressure, MAP, cardiac index, CI, systemic vascular resistance, SVR, stroke volume index, SVI, and stroke work index, SWI. Control values were measured for 3 hours prior to dosing with H. Patients were assigned to receive single doses of 50, 75 and 100 mg H, in randomized order on different days separated by intervals of 24 to 36 hours. Four patients did not receive the 50 mg dose, because of minimal response at higher dose levels of H. Seven patients not responding to 100 mg H subsequently received single doses of 150 mg or 200 mg H. No overall significant hemodynamic effects were observed at the 50 mg dose level of H. After 75 mg, CI increased slightly (+0.36 L/min/m2) with a decrease in SVR (-19%). After 100 mg the increase in CI (+60 mL/min/m2) and decrease in SVR (-31%) were substantial. The changes in MAP at the 100 mg and 75 mg dose level were of the same order - 6.6 mm Hg and -5.0 mm Hg, respectively. Seven patients with no response to 100 mg H all responded to single dose of 150 to 200 mg. The duration of action of H was

statistically significantly longer, 14.3 (1.4) hours, in patients with a creatinine clearance < 35 mL/min than in patients with a creatinine clearance > 50 mL/min (7.9 (0.5)) hours.

3. Packer M, Meller J, Medina N, Gorlin R, Herman VM. Dose requirements of hydralazine in patients with severe congestive heart failure. Am J Cardiol 1980;45;655-680

This study using similar methods and endpoints as Study 2 determined the least effective oral or intravenous dose of H in 45 patients with CHF NYHA Class IV, 34 males and 11 females of mean age 64 (Packer et al 1980 b). A response of H was defined as a decrease in SVR > 20% persisting for >1 hour. Twenty six patients (group A) responded to oral dose of 100 mg H, whereas 19 patients (group B) did not. Of the patients in group B, 14 responded to 150 to 300 mg orally as a single dose, 3 patients responded only to a single dose of 600 or 800 mg orally and 2 patients responded only to intravenous administration of the drug. Despite different dose requirements, the hemodynamic responses elicited by H were similar in the two groups, except that patients in group B had higher control values for mean atrial pressure and LVFP. The results of the study show that the individual dose requirements for H vary substantially.

Studies in Hypertensive Patients

4. Shepherd AMM, McNay JL, Ludden TM, Lin MS Musgrave GE. Plasma concentration and acetylator phenotype determine response to oral hydralazine. Hypertension 1981;3:580-585

The relationship between antihypertensive effect and plasma concentrations of H were investigated in 9 hypertensive patients of mean age 55 years and body weight 83.8 kg. The patients received a single oral dose of 1 mg/kg, a single intravenous dose of 0.3 mg/kg and multiple oral doses of 1 mg/kg (five doses bid). The patients were randomized to receive the single oral and intravenous doses first. For safety reasons the oral multiple dose treatment was administered last. Frequent blood pressure measurements were taken for 2 hours prior to and 8 hours following dosing. Mean arterial blood pressure, MAP, was calculated in the standard fashion. Only the data obtained after oral administration of H are reported.

There were log linear relationships between decrease in MAP and Cmax or AUC of H following single and multiple oral doses as shown in the adjacent figure. The decrease in MAP was also related to acetylation capacity as measured by sufamethazine.

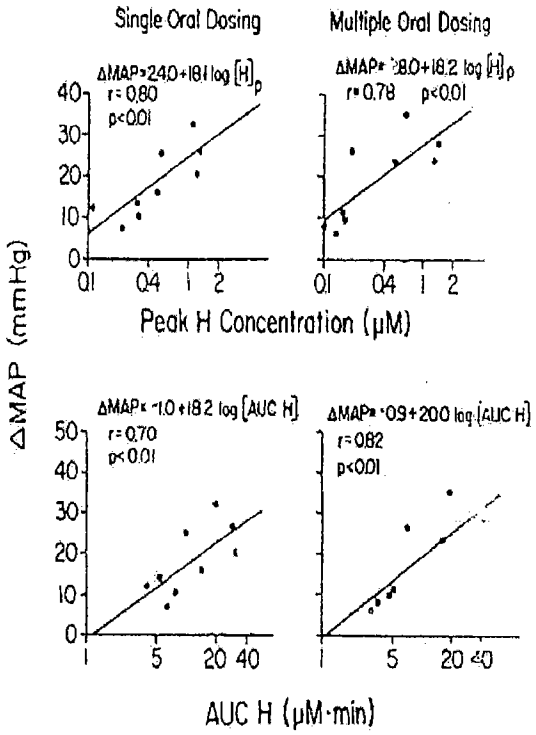

FIGURE 2. *Relationships between peak reduction in mean arterial pressure (MAP) and plasma concentrations of hydralazine following a single dose (left panels) and the fifth dose (right panels) of hydralazine, 1 mg/kg. Hydralazine concentrations are expressed as both peak plasma concentration (upper panels) and area under the plasma concentration-time curves, AUC_H (lower panels).*

5. O'Malley KO, Segal JL,ZH Israeli, Boles M, McNay JL, Dayton PG. Duration of hydralazine action in hypertension. Clin Pharmacol Ther 1975;18: 581-586

The duration of the hypotensive effect of H was investigated in 4 black hypertensive patients, 3 females and 1 male, of mean age 47 years and body weight 85.2 kg (O'Malley et al. 1975). All antihypertensive therapy was stopped at least 3 weeks before dosing with H. The patients were placed on a sodium diet and received placebo tablets. Seven days were allowed for the blood pressure to stabilize. The patients then received multiple daily doses of 300 mg H bid, tid or qid for 14 days. Placebo tablets were administered to the patients for the next 6 days, and the duration of the antihypertensive effect estimated during this interval. The time to half the full antihypertensive effect measured at the end of the active treatment period was estimated to be at least 30 days. This result showed that the antihypertensive effect decays at a much slower pace than the plasma concentrations of H. Estimates of t1/2 of H after oral administration obtained from other studies range between 0.5 and 3.5 hours (Reece et al. 1980, Shepherd et al;. 1980, Hanson et al.1983).

6. Shepherd AMM, Irvine NA, Ludden TM, LinMS, McNay JL. Effect of oral dose size on hydralazine kinetics and vasodepressor response. Clin Pharmacol Ther 1984;36:595-600

The dose response relationship of H was investigated in 9 male hypertensive patients of mean age 56 years and 87.6 kg (Shepherd et al. 1984). Phenotyping with sulfamethazine showed that 4 subjects were EMs and 5 PMs. After all medications except for thiazide diuretics were stopped for at least 7 days, EMs received ascending doses of 0.5, 1.0 and 2.0 mg/kg H. The escalating doses for the PMs were 0.25, 0.5 and 1.0 mg/kg H. The respective doses were administered on 3 different study days which were at least 3 days apart. For safety reasons the order of the doses was not completely randomized. Supine blood pressure and heart rate were measured frequently prior to and after drug administration. Blood pressure was expressed as mean arterial pressure using the standard formula. The control values obtained prior to dosing on each study day showed no statistically significant intra-subject difference.

The results shown in the adjacent figure indicate that a dose response relationship exists for the vasodepressor effect of H. The relationship is steeper than expected for a log linear relationship and it is different for EMs and PMs.

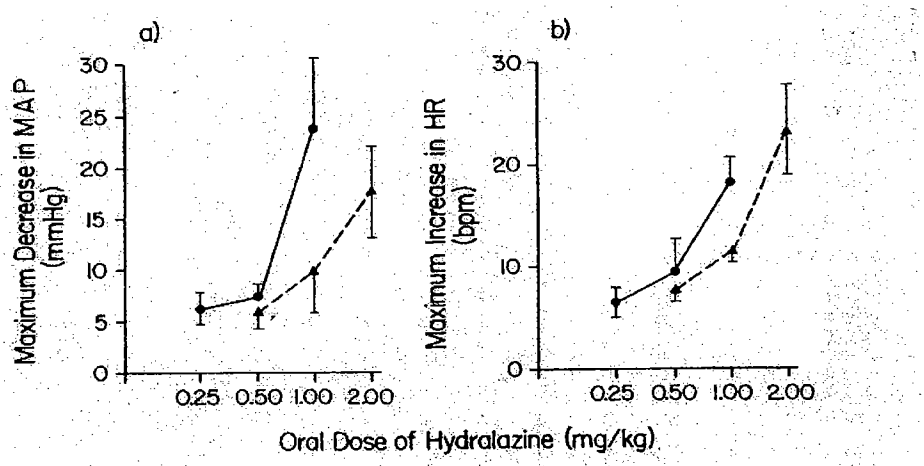

Fig. 3. *a*, Maximum decrease in mean arterial pressure and *b*, maximum increase in heart rate after a range of oral doses of hydralazine in slow (●) and fast (▲) acetylators.

5.2.1.2 Pharmacokinetic Studies

The results from the selected published PK studies indicated that the first generation assays, mostly published prior to 1979, were not specific for H. There were two problems with the first generation assays. The first had to do with the instability of H in plasma and

blood due to rapid conjugation with endogenous pyruvic acid to HPH after sampling. H added in vitro at 37 ° to blood or plasma has a half life of 11 and 8 minutes, respectively. Thus, to avoid degradation of H in biological fluids after sampling, immediate derivatising of H is required (Reece et al. 1978, Reece et al. 1980, Ludden et al. 1982). The second problem was that some of the first generation assays used strong acid conditions when derivatising H resulting in back transformation of HPH to H. Thus, only publications were considered that used second generation assays. Some of the selected publications measured AH in addition to H and the major circulating metabolite, HPH and MTP, to determine whether additional hydrazones or adducts are present in the systemic circulation.

One report (CB-02) determined the PK of H in healthy volunteers after administration of BiDil. Two publications reported the PK parameters after administration of H alone in healthy volunteers. Six publications reported PK parameters of H in hypertensive patients and 2 articles dealt with the PK of H in patients with CHF. Four publications reported the PK parameters of the major circulating metabolite, hydralazine pyruvate hydrazone, HPH and one publication measured additionally TPM.

The PK parameters for H and ISND and their major metabolites are reported as means and standard deviations (SD) or standard error of the means (SEM), unless specified otherwise. SEM values are identified by #. The PK parameters of H and metabolites are referenced to plasma- or whole blood concentrations. The PK parameters of ISDN and metabolites are referenced to the respective concentrations in plasma.

Studies in Healthy Volunteers

7. Final Clinical/Statistical Report Protocol No.:CB-02. The relative bioavailability of low and high dose BiDil, a fixed combination of hydralazine/ISDN, as compared to an oral solution, tablet, and capsule of hydralazine HCl and ISDN (Pivotal Bioequivalence Study)

This single center, open label, randomized, incomplete cross-over study compared the bioequivalence of different strengths of the combination tablet and single entity formulations of H and ISND. The bioavailability of the different solid formulations relative to a solution formulation was also investigated. During Phase A of the study 149 healthy male and female subjects, 18 to 40 years of age, received an oral solution containing 37.5 H and 10 ISDN. During Phase B of the study subjects who completed Phase A and had an oral clearance of H $\leq$4000 L/hr (66667 mL/min) in blood and measurable blood concentrations for at least 4 hour following administration were randomized to receive ISDN BiDil tablets containing 37.5 H/10 ISDN, BiDil tablets containing 75 H/40 ISDN, H tablets containing 37.5 H plus ISND tablets containing 10 ISDN, or H capsules containing 37.5 H plus ISND tablets containing 10 ISDN. The PK of H and ISDN and its metabolites were only evaluated in the 75 subjects completing both phases of the study of the trial. Among the subjects completing the study were 55

males and 20 females, 64 Caucasians, 9 Blacks and 4 Others. Their age ranged between 18 and 40 years and their mean weight was 72.5 kg. All subjects were considered PMs of H, however this claim was not based on phenotyping or genotyping of the subjects. The following results were obtained for H and ISND and its active metabolites:

Hydralazine

Table 1. Mean PK Parameters of Hydralazine in Whole Blood

Formulation	AUC [c] ng·h/mL	t1/2 h	Tmax h	Cmax [c] ng/mL
Solution 37.5 H/10 ISDN [a]	31.61	2.52	0.34	50.8
Capsule 37.5 H/10 ISDN [b]	37.03	2.38	0.74	77.3
Tablet 37.5 H/10 ISDN [b]	24.40	2.05	1.03	29.6
BiDil 37.5 H/10 ISDN [b]	35.11	2.31	0.96	56.3
BiDil 75 H/ 40 ISDN [b]	81.80	3.52	1.02	88.0

[a] Average of 4 groups [b] Mean of each group [c] Normalized to body weight of 65 kg

Among the formulations H was absorbed from the solution fastest. However, peak concentration was greatest for the capsule and smallest for the tablet containing only H. The absorption characteristics of H from the BiDil tablets were intermediate. The AUC values indicated that bioavailability of H was greatest from the high dose BiDil tablet, followed by the capsule. As with Cmax, the AUC from the tablet was smallest. The apparent half lives of H ranged between 2.05 and 3.52 hours after administration of the different formulations.

Table 2. Bioavailability Measures for the Combination and Single Entity Formulations Relative to the Solution Formulation

Formulations	AUC Ratio
Capsule	1.12
Tablet	0.85
BiDil Low Dose	1.06
BiDil High Dose	1.25

The bioavailability of H from the low and high dose BiDil tablets and the single entity capsule exceeded that from the solution. Only the single entity tablet's bioavailability was smaller than that of the solution.

Table 3.Point Estimates and Confidence Intervals of the Bioequivalence Determinations for the Solid Combination and Single Entity Formulations for Hydralazine

Formulation	Cmax Ratio	Cmax 90% CI	AUC Ratio	AUC 90% CI	AUCR Ratio	AUCR 90%
BiDil Low Dose/Tablet	1.47	0.89-2.40	1.56	1.11-2.19	1.25	0.99-1.58
BiDil Low Dose/Capsule	0.65	0.40-1.07	0.94	0.67-1.32	0.90	0.72-1.44
BiDil High Dose/Tablet	1.30	0.79-2.12	1.79	1.28-2.52	1.46	1.15-1.85
BiDil High Dose/Capsule	0.58	0.35-0.95	1.08	0.77-1.51	1.06	0.84-1.34

The lower and higher dose combination BiDil tablets were not bioequivalent to the tablets and capsules containing only H used in VHeFT I and VHeFT II, respectively. The bioavailability of H from the BiDil tablets of both strengths was smaller than from the capsules used in VHeFT II and greater than from the tablets used in VeHFT II.

8. Reece PA, Cozamanis I, Zacest R. Kinetics of hydralazine and its main metabolites in slow and fast acetylators. Clin Pharmacol Ther 1980; 1980;28:769-777

A single center study investigated the PK of H and the major circulating metabolites in 10 healthy volunteers, 9 males and 1 female, of mean age 29 years and mean body weight 72.9 kg. The acetylation capability of the individuals was tested with sulfamethazine. Five subjects were EM and five subjects PM. All subjects received 0.3 mg/kg over 5 minutes. The oral dose for the EMs and PMs was 1.0 mg/kg and 0.5 mg/kg H, respectively. A tablet formulation was used. The oral and intravenous administrations were given to the volunteers one week apart.

Table 4. Mean (SD) PK Parameters of Hydralazine Referenced to the Concentration in Plasma after Intravenous Administration of 0.3 mg/kg Hydralazine

Phenotype	Vss, L/kg	CL, L/h/kg	t1/2, h
EM	6.37 (1.29)	8.86 (1.81)	0.664
PM	5.71 (0.65)	7.77 (2.10)	0.677 (0.152)
All	6.03 (1.02)	8.32 (1.94)	0.671 (0.168)

The values for Vss, CL and t1/2 were similar in both phenotypes after intravenous administration of H. The CL value which exceeds the liver plasma flow indicated that there is significant extra-hepatic metabolism of H. The values for Vss imply extensive partitioning into and/or binding of H to tissues. Due to the large value of CL the

disposition half-life of H is short, even though the drug is widely distributed in the tissues.

Table 5. Mean (SD) PK Parameters of Hydralazine Referenced to the Concentration in Plasma after Oral Administration of 1.0 mg/kg and 0.5 mg/kg Hydralazine, Respectively, to Extensive and Poor Metabolizers

Phenotype	t1/2, h	Tmax, h	F, %
EM	0.437 (0.069)	0.60 (0.22)	16.2 (6.3)
PM	0.474 (0.112)	0.62 (0.26)	35.4 (3.8)
All	0.456 (0.090)	NR	NR

NR =Not reported

Absorption of H is fast and the disposition half life after oral administration H tends to be shorter than following intravenous administration in both EMs and PMs. The reason for this discrepancy is unknown. The absolute bioavailability, F, of H is clearly smaller in EMs than in PMs. Absolute bioavailability of H is small in both groups. Of all parameters only F depended on the phenotype. All other parameters of H were phenotype independent. This indicates that the acetylation pathway is importantly responsible for the first pass effect of H.

Table 6. Mean (SD) Area under the Whole Blood Concentration Time Curve of Hydralazine (H) and the Main Circulating Metabolites, Hydralazine Pyruvate Hydrazone (HPH), Methyltriazolophthalazine (MTP), and Apparent Hydralazine (AH) after Intravenous Administration of Hydralazine

	AUC μM·h			
Phenotype	H	HPH	MTP	AH
EM	0.273 (0.054)	8.63 (1.70)	1.014 (0.132)	9.26 (1.59)
PM	0.318 (0.078)	11.29 (3.06)	0.501 (0.219)	11.55 (2.82)

Table 7. Mean (SD) Disposition Half-lives of the Major Circulating Metabolites, Hydralazine Pyruvate Hydrazone (HPH), Methyltriazolophthalazine (MTP) and Apparent Hydralazine (AH) after Intravenous Administration of Hydralazine

	t1/2,h		
Phenotype	HPH	MTP	AH
EM	3.32 (0.25)	1.41 (0.20)*	3.08 (0.27)
PM	3.64 (0.51)	1.98 (0.31)	3.86 (0.67)

* Statistically significant difference

The major circulating metabolite after intravenous administration of H is HPH. Its AUC is more than 30 times greater than that of H in EMs and PMs. MTP was the second major circulating metabolite. HPH and MTP made up the overwhelming part of AH in both phenotypes. The apparent disposition half lives of the metabolites HPH and MTP were longer than that of H.

Table 8. Mean (SD) Dose Normalized Area under the Whole Blood Concentration Time Curve of the Major Circulating Metabolites Hydralazine Pyruvate Hydrazone (HPH) and Methyltriazolophthalazine (MTP) and Apparent Hydralazine (AH) after Oral Administration of Hydralazine

		AUC/D *		
Phenotype	H	HPH	MTP	AH
EM	0.119 (0.042)	0.79 (1.11)	3.98 (1.16)	4.20 (1.50)
PM	0.295 (0.054)	6.74 (1.52)	1.50 (0.728)	8.24 (1.77)

* Statistically significant difference

Table 9. Mean (SD) Tmax and t1/2 Parameters of the Main Circulating Metabolites Hydralazine Pyruvate Hydrazone (HPH), Methyltriazolophthalazine (MTP) and Apparent Hydralazine (AH) after Oral Administration of Hydralazine

	Tmax, h			t1/2, h		
Phenotype	HPH	MTP	AH	HPH	MTP	AH
EM	NR	0.65(0.22)	0.60(0.22)	ND	1.49(0.11)	1.87(0.31)
PM	1.25(0.18)	0.70(0.21)	0.70(0.21)	3.85(0.80)	1.62(0.22)	3.62(0.85)

NR=Not reported ND=Not Determined: only 2 EMs had traces of HPH

After oral administration of H, EMs exhibited a smaller formation of HPH than PMs. The ratio of AUC,HPH to AUC,H was 6.6 and 22.8 in EMs and PMs, respectively. This points to a competition between N-acetylation and hydrazone formation during the first pass. There was also a trend for greater MTP formation in EMs than in PMs. MTP is formed by acetylation. In EMs MTP was the major circulating metabolite, whereas HPH was the dominant metabolite in PMs. AH reflects the behavior of HPH and other circulating metabolites.

9. Ludden TM, Rotenberg KS, Ludden LK, Sheperd AMM, Woodworth JR. relative bioavailability of immediate and sustained release tablets. J Pharm Sci. 1988;77: 1026-1032

A study in 12 healthy PMs of mean age 26 years and body weight 74.9 kg determined the bioavailability of the commercial H tablet relative to the intravenous solution administered orally. The relative bioavailability of several pilot modified release

formulations was also tested in this study, but the results are not reported here. The acetylator status of the subjects was verified with sulfamethazine. According to an incomplete block design each subject received 4 of 6 different formulations and all 20 subjects received the oral solution. A first dose of 25 mg H was administered with the subject in the fasted state in the morning followed 6 hour later by a second 25 mg dose with the subjects in a postprandial state. H and AH were measured in whole blood and the metabolites TP, MTP and OH-MTP were measured in urine. The PK parameters of H after administration of the tablet and solution and the urinary recoveries of the metabolites are shown in Tables 10 and 11, respectively:

Table 10. Mean (SD) Parameters of Hydralazine Referenced to the Concentration in Whole Blood after Repeat Administration of the Solution and the Commercial Tablet in Healthy Subjects

	Tablet	Solution
Cmax 1, ng/mL	49.0 (28.6)*	75.9 (47.4)
Cmax 2, ng/mL	8.9 (7.3)	14.8 (8.4)
Tmax 1, h	1.08 (0.61)*	0.26 (0.61)
Tmax 2, h	2.45 (1.04)*	0.43 (0.27)
AUC0-14, ng·h/mL	49.2 (18.3)	47.8 (15.1)

* Statistically significant difference

Table 11. Mean (SD) of Metabolites Excreted in Urine in Percent of the Dose after Repeat Administration of the Solution and Commercial Tablet in Healthy Subjects

	Tablet	Solution
AHP	7.68 (2.34)	8.13 (2.67)
TP	2.36 (0.97)	2.39 (0.60)
MTP	3.67 (1.72)	3.93 (1.17)
OH-TPM	2.74 (1.30)	2.74 (1.44)
Total	16.4 (5.14)	17.2 (4.64)

The results indicated that H is faster absorbed from the solution than from the tablet whose absorption was delayed by a lag time of about 30 minutes. In the postprandial state absorption of H from the solution and tablet is slower. The extent of absorption of unchanged H from the tablet and solution following repeat administration is similar. The bioavailability of H in the postprandial state appears to be smaller than in the fasted state. The blood concentrations of AH showed the opposite. The bioavailability of AH was greater in the postprandial state than in the fasted state. The amounts excreted in urine as metabolites were comparable for tablet and solution.

9. Semple HA, Koo W, Tam YK, Ngo LY, Coutts RT. Interactions between hydralazine and oral nutrients. Ther Drug Monit 1991;13:304-308

This study in 8 healthy subjects, 4 males and 4 females, of mean age 27 years and body weight 71.6 kg, investigated the effect of food on the PK parameters after 50 mg H administered as the marketed tablet. Phenotyping using the molar ratio of the caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil to 1-methylxanthine in urine showed that 5 of the subjects were PMs and 3 EMs. The subjects were assigned randomly to two 4x 4 Latin squares of subjects x treatment. In addition to the fasting condition and treatment with a standard breakfast consisting of 500 kcal containing 470 mL liquid volume, 17.5 g protein, 17.4 g fat and 68.2 g carbohydrates, the subjects received an enteral infusion and bolus of an entirely liquid breakfast of the same composition. Only the results of the study pertaining to the standard fed and fasted treatments are reported as shown in Table 12:

Table 12. Median (Range) of the Bioavailability Measures of H Referenced to the Drug Concentrations in Whole Blood After Administration of 50 mg H as Commercial Tablet in Healthy Subjects in the Fed and Fasted State

	Fed	Fasted
Cmax, ng/mL	15 (3.5-33.9)*	87 (4.5-224)
AUC0-6, ng· min/mL	1189 (202-1737)*	2641 (385-4747
Tmax, min	23 (15-90)	30 (15-30)

* Statistically significant difference

The results indicate that Cmax and AUC0-6 after administration of the commercial tablet are reduced by 82.8 % and 55.0%, respectively, under fed conditions.

Studies in Hypertensive Patients

10. Ludden TM, Shepherd, McNay JL Lin M-S. Hydralazine kinetics in hypertensive patients after intravenous administration. Clin Pharmacol Ther 1980;28:736-742 and 11. Shepherd AMM,Ludden TM, McNay JL, Lin MS. Hydralazine kinetics after single and repeated doses. Clin Pharmacol Ther1980;28: 804-811

This single dose study investigated the PK of H and HPH in 8 male hypertensive patients, 4 Caucasians, 2 Blacks and 2 Hispanics of mean age 56 years and mean weight of 71.9 kg. One patient had moderate renal impairment. The patients were randomized to receive 0.3 mg/kg H intravenously as a bolus or 1.0 mg/kg po. The intravenous and oral administrations were separated by 4 days. One day after completion of the single dose

phase of the study, the patients received an oral maintenance therapy of 1 mg/kg H every 12 hours. After the patients had received at least 5 oral doses of H, a second oral study was carried out. The oral formulation was a solution. All patients received diuretics. The patients were phenotyped with sulfamethazine. There were 4 EMs and 4 PMs. The results are summarized in Tables 13-19:

Table 13. Mean (SEM) PK Parameters of Hydralazine Referenced to the Concentration in Plasma after Intravenous Bolus Administration of 0.3 mg/kg Hydralazine

Phenotype	Vss, L/kg	CL (mL/min/kg	t1/2 min
EM	1.68 (0.19)	70.2 (1.2)	58.7 [a]
PM	1.98 (0.22)	75.5 (7.4)	49.5 [a]
All	1.83 (0.17)	72.9 (4.9)	53.7 [a]

[a] Harmonic mean

Table14. Mean (SEM) PK Parameters of Hydralazine Referenced to the Concentration in Plasma after Oral Administration of 1.0 mg/kg Hydralazine

Admin	Phenotype	Cmax, μM	Tmax, min	t1/2, min	AUC,μM·min[a]	F %
SD	EM	0.32 (0.08)*	25.0 (4.6)	13.2 (0.3)	6.7 (1.2)*	9.5 (2.6)*
	PM	1.03 (0.19)	17.5 (2.5)	54.3(17.2)	23.0 (3.9)	31.3 (4.0)
MD	EM	0.14 (0.02)*	21.3 (2.4)	20.3 (5.6)	4.8 (0.9)*	6.6 (1.7)*
	PM	0.96 (0.22)	22.5 (2.5)	40.8 (6.0)	28.7 (6.5	39.3 (8.0)

a Extrapolation used the terminal slope following intravenous administration

The Vss value indicated significant partitioning into and/or binding of H to tissues. The CL value exceeds hepatic plasma flow significantly indicating extra-hepatic elimination of H. The values of Vss, CL and t1/2 were similar in EMs and PMs.

After oral administration peak concentrations and bioavailability of H are significantly larger in PMs than in EMs. The results indicate that acetylation capability affects only the first pass metabolism of H.

Table15. Mean (SEM) PK Parameters of Hydralazine Pyruvate Hydrazone (HPH) Referenced to the Concentration in Plasma after Intravenous Administration of 0.3 mg/kg Hydralazine

Phenotype	AUCμM·min	Cmax, μM	Tmax, min	t1/2, min
EM	688 (122)	1.65 (0.032)	65.0 (30.2)	200 [a]
PM	825 (257)	1.50 (0.358)	40.0 (12.2)	297 [a]
All	756 134	1.58 (0.223)	52.5 (15.8)	239 [a]

[a] Harmonic Mean

Peak concentrations of HPH were reached 52.5 minutes after intravenous administration of the drug and the harmonic mean half life was 239 minutes and clearly longer than that of the parent drug. There were no differences between EMs and PMs.

Table16. Mean (SEM) PK Parameters of Apparent Hydralazine Referenced to the Concentration in Plasma after Intravenous Administration of 0.3 mg/kg Hydralazine

Phenotype	AUC μM·min	Cmax, μM	Tmax, min	t1/2, min
EM	1030 (198)	3.12 (0.42)	2.00 (0.01)	262 [a]
PM	1276 (281)	4.46 (0.94)	8.50 (4.27)	341 [a]
All	1153 (166)	3.79 (0.54	5.25 (2.33)	296 [a]

[a] Harmonic Mean

Peak concentrations of AH were reached immediately after intravenous administration of a bolus of H. This was in contrast to the significantly later occurring peak concentrations of HPH. The AUC and Cmax values of AH exceeded those of HPH, The apparent terminal half life of AH tended to be greater than that of HPH. These results indicated that additional circulating metabolites are present.

Table 17. Mean (SEM) PK Parameters of Hydralazine Referenced to the Concentration in Plasma after Oral Administration of 1.0 mg/kg Hydralazine

Administr.	Phenotype	AUC,μM·min	Cmax,μM	Tmax, min	t1/2, min	F, %
SD	EM	6.7 (1.2)*	0.32 (0.08)*	25.0 (4.6)	13.2 (0.3)	9.5 (2.6)*
	PM	23.0 (3.9)	1.03 (0.19)	17.5 (2.5)	54.3(17.2)	31.3(4.0)
MD	EM	4.8 (0.9)*	0.14 (0.02)*	21.3 (2.4)	20.3 (5.6)	6.6 (1.7)*
	PM	28.7 (6.5)	0.96 (0.96)	22.5 (2.5)	40.8 (6.0)	39.3 (8.0)

* Statistically significant difference between EMs and PMs

After single and multiple oral doses of H, AUC was significantly greater in PMs than in EMs. Both Cmax and F indicated that more H reaches the systemic circulation in PMs than in EMs. The PK of H after single and multiple dose administration did not change significantly.

Table 18. Mean (SEM) PK Parameters of Hydralazine Pyruvate Hydrazone Referenced to the Concentration in Plasma after Oral Administration of 1.0 mg/kg Hydralazine

Administr.	Phenotype	AUC,μM·min	Cmax, μM	Tmax, min	t1/2 min
SD	EM	318 (27)*	1.13 (0.25)	35.0 (2.9)	243 (51)
	PM	873 (158)	2.34 (0.51)	60.0 (12.2)	297 (101)
MD	EM	425 (115)	1.55 (0.30)	35.0 (2.9)	254 (33)
	PM	1622 (813)	3.55 (0.91)	95.0 (32.8)	374 (141)

* Statistically significant difference between EMs and PMs

Table 19. Mean (SEM) PK Parameters for Apparent Hydralazine Referenced to the Concentration in Plasma after Oral Administration of 1.0 mg/kg Hydralazine

Administr.	Phenotype	AUC, μM·min	Cmax, μM	Tmax, min	t1/2 min
SD	EM	605 (58)	3.95 (0.10)*	32.5 (4.8)*	170 (14)
	PM	1916 (574)	5.61 (0.42)	17.5 (2.5)	294 (69)
MD	EM	754 (140)	5.63 (1.24)	32.5 (4.8)	263 (29)
	PM	3522 (1935)	7.61(1.72)	27.5 (4.8)	538 (218)

* Statistically significant difference between EMs and PMs

Possibly due to wide variation within the groups only AUC was statistically significantly greater in EMs than in PMs for HPH after oral administration of a single dose of H. The AUC values of HPH were between 44 to 89 times greater than those of H indicating that HPH is the major circulating compound. The AUC and Cmax values of AH exceeded those of HPH suggesting the presence of additional circulating metabolites. After single dose administration Cmax of AH was significantly greater and occurred earlier in PMs than in EMs. The apparent terminal half lives of AH and HPH were similar and clearly longer than that of H after oral administration of H.

12. Ludden TM, Shepherd AMM, McNay JL, Lin MS. Effect of intravenous dose on hydralazine kinetics after intravenous administration. Clin Pharmacol Ther 1982;34:148-152

In this study the PK of H and AH were investigated in six male hypertensive patients of mean age 52 years and body weight 91.7 kg (Ludden et al. 1983). The patients received intravenous bolus doses ranging between 0.05-0.60 mg/kg. Initially, doses ranging between 0.100 and 0.375 mg/kg were injected and the dose size was then increased or decreased depending on the hypotensive response. The PK parameters were determined

for all six patients at 2 dose levels of 0.200 mg/kg and 0.300 mg/kg. The acetylator phenotype was determined with sulfamethazine. There were three EMs and three were PMs.

Table 20. Mean (SD) PK Parameters of Hydralazine Referenced to the Concentration in Whole Blood after Intravenous Administration of 0.20 mg/kg or 0.30 mg/kg H by Bolus

Dose, mg/kg	CL, mL/min/kg	Vss, L/kg
0.20	51.8 (12.3)	1.40 (0.56)
0.30	55.9 (14.6)	1.23 (0.58)

There was no difference in the CL and Vss values between EMs and PMs and similar mean values were obtained at the 2 dose levels. These results indicated that the acetylator status did not impact systemic disposition of H. The CL values among the individuals at the different dose levels varied significantly between 26.5 and 84.8 mL/min/kg. The Vss values among the individuals at the different dose levels varied even more and ranged between 0.72 and 4.87 L/kg. However, there was no evidence for a dose dependency.

13. Shepherd AMM, McNay JL, Ludden TM, Lin MS, Musgrave GE. Plasma concentrations and acetylator phenotype determine response to oral hydrazine. Hypertension 1981;3:580-585

In this study nine hypertensive patients of mean age 55 years and body weight 83.8 kg received on 3 separate occasions a single oral dose of 1 mg/kg, a single intravenous injection of 0.3 mg/kg and 5 oral doses of 1 mg/kg every 12 hours (Shepherd et al. 1981). The patients were randomized to receive the single oral and intravenous administrations. A three day interval was maintained between the single dose administrations of H. Following the single oral or intravenous administration the multiple oral dose regimen was started. The oral doses of H were given as tablets. Frequent blood samples were taken after intake of the fifth and last dose of the oral regimen and after the single oral and intravenous doses. The subjects were phenotyped with sulfamethazine and the acetylation capability expressed as percent acetylated sulfamethazine to total sufamethazine.

Table 21. Mean (SEM) Parameters of Hydralazine (H) and Apparent Hydralazine (AH) Referenced to the Concentration in Plasma after Administration of Single or Multiple Doses of 1 mg/kg Hydralazine

Regimen	Cmax H, μM	AUC H μM· min[a]	Cmax AH, μM	AUC AH, μM·min
Single Dose	0.62 (0.23)	14 (3.8)	4.8 (0.37)	1260 (361)
Multiple Dose	0.51 (0.17)	15 (5.0)	6.6 (1.05)	3785 (2032)

[a] Extrapolated by using the terminal slope following intravenous administration

The Cmax and AUC values of H after a single dose and after the 5th dose of a multiple dose regimen were similar. H does not accumulate. However, the corresponding values for AH indicated that the metabolites representing AH accumulate after multiple dose regimen of H. The average ratio of AUC,H/AUC,AH was 0.014 (0.0032) after single dose administration and became even smaller after the multiple oral doses indicating that the metabolites represent the major circulating moieties and not the parent drug.

14. Shepherd AMM, Irvine NA, Ludden TM, Lin Ms, McNay JL. Effect of oral dose size on hydralazine kinetics and vasodepressor response. Clin Pharmacol Ther 1984; 36:595-600

This study investigated the PK of H after single ascending oral doses in 9 male hypertensive patients of mean age 56 years and mean body weight 87.6 kg. Depending on the acetylator phenotype they received ascending oral doses of 0.5, 1.0 and 2.0 mg/kg H (EMs) or 0.25, 0.5 and 1.0 mg/kg (PMs). The intravenous solution formulation was used. The order of the doses was varied, but was not completely randomized because of safety concerns. The subjects'acetylator phenotype was determined using sulfamethazine. There were 4 EMs and 5 PMs.

Table 22. Mean (SEM) Area under the Whole Blood Concentration Time Curve of Hydralazine after Single Ascending Oral Doses of Hydralazine

	AUC, μg·min/mL[a]			
Dose, mg/kg	0.25	0.50	1.0	2.0
EM		3.7 (0.5)	11.0 (2.8)	49.7 (12.0)
PM	4.3 (0.4)	14.2 (2.8)	39.0 (4.0)	

[a] Units of AUC in the publication erroneously indicated as ng·min/mL

Table 23. Mean (SEM) Peak Concentrations in Whole Blood of Hydralazine after Single Ascending Oral Doses of Hydralazine

	Cmax, ng /mL			
Dose, mg/kg	0.25	0.50	1.0	2.0
EM		15.2 (2.2)	64.7 (21.3)	302.0 (105.9)

PM	23.0 (3.5)	62.2 (10.1)	209.3 (33.0)	

The dose normalized AUCs at the common 0.5 mg/kg and 1.0 mg/kg dose levels in PMs were 3.8 and 3.5 times greater than in EMs showing that the acetylator status is an important determinant of the absolute bioavailability of H after oral administration. The values for Cmax and AUC increased more than dose proportionate in both EMs and PMs, indicating saturability of the first pass and/or nonlinearity of the systemic clearance. The respective ratios of Cmax and AUC at the highest to the lowest dose level are greater in EMs than in PMs suggesting a more pronounced degree of nonlinearity of the PK of H in EMs than PMs after oral administration of H.

15. Shepherd AMM, Irvine NA, Ludden TM. Effect of food on blood hydralazine levels and response in hypertension. Clin Pharmacol Ther 1984; 36: 14-18

In this study the effect of food on the PK of H was studied in 6 hypertensive subjects, 4 males and 2 females, of mean age 56 years and body weight 70 kg. Two of the subjects were EMs and 4 PMs as determined by sulfamethazine. At the time of the study they were only on diuretics. The patients were randomized to receive 1 mg/kg H as solution in the fasted or fed state on 2 occasions at least 3 days apart. The standard breakfast contained 473 kcal, 21 g protein, 18 g fat, 58 g carbohydrates. 100 mL coffee was also part of the breakfast. Dosing occurred 45 minutes after start of the breakfast. In the fasted state the patients were dosed with H after an overnight fast.

The PK parameters obtained in the fed and fasted state are summarized in Table 12.

Table 24. Mean (SEM) PK Parameters of H referenced to the Concentration in Whole Blood after Oral Administration of Hydralazine as Solution to Hypertensive Patients in the Fed and Fasted State

	Fed	Fasted
Cmax, μM	0.69 (0.22)*	1.18 (0.22)
AUC, μM· min	18.40 (5.06)*	31.25 (6.13)

* Statistically significant difference

The results indicate that food decreases Cmax and AUC of the drug substance H by 41.5% and 41.1%, respectively. The decrease in bioavailability is large enough to recommend that the time interval between drug and food intake should be fixed.

16. Jackson SHD, Shepherd AMM, Ludden TM, Jamieson MJ, Woodworth J, Rogers D, Ludden LK, Muir KT. Effect of food on the oral availability of apresoline

and controlled release hydralazine in hypertensive patients. J Cardiovasc Pharmacol.1990; 16: 624-628

This study investigated the impact of food on the bioavailability of H when administered as the market tablet formulation to 16 hypertensive patients, 11 males and 5 females, of mean age 52 years and body weight 92.6 kg. In the fed state the FDA standard breakfast (642 kcal, 25 g protein, 43 g fat, 40 g carbohydrate) was to be ingested in 15 minutes and dosing with H followed immediately thereafter. In the fasted state the subjects were fasted overnight. All patients were PMs based on the sulfamethazine test. The patients were on beta-blockers and diuretics. The study was a double blind, randomized four-way crossover. The patients were maintained on 100 mg H for 1-2 weeks prior to the administration of the first test dose and throughout the study. In addition to the marketed immediate release formulation, 3 pilot modified release formulations were also tested in the study, but their results are not reported.

The results of the impact of food on the bioavailability measures of H after administration of the marketed tablet are shown in Table 13:

Table 25. Mean (SD) PK Parameters of H Referenced to the Concentration in Whole Blood after Administration of the Marketed Tablet to Hypertensive Subjects in the Fasted and Fed State

	Fed	Fasted
Cmax, ng/mL	69.0 (97.2)*	224.1 (126.8)
AUC, ng/ml.· h	88.5 (52.1)*	169.4 (73.9)
Tmax, h	1.20 (0.88)*	0.77 (0.60)

* Statistically significant difference

The results indicate that food decreases Cmax and AUC of H by 69.2 % and 47.8%, respectively, after administration of the tablet. These results confirmed the results obtained in a previous study when H was administered as solution. One can conclude that the impact of food on drug substance and formulation is similar.

17. Talseth T. Studies on Hydralazine II. Elimination rate and steady-state concentration in patients with impaired renal function. Eur J Clin Pharmacol 1976;10:311-317

The impact of renal impairment on the pharmacokinetics of H were investigated after a single dose of H in 13 hypertensive patients with impaired renal function (GFR: 6-78 mL/min) and after multiple doses in 49 hypertensive patients. Thirty two of these patients had impaired (GFR: 5-79 mL/min) and 17 normal (GFR>100 mL/min) renal function. In the single dose sub-study the patients received 50 mg H and in the multiple dose sub-study they received 25-200 mg H bid, tid or qid. H was assayed with a non-selective assay that really measured AH.

In the single dose study there was an inverse relationship between t1/2 an GFR. In the multiple dose study there was a nonlinear and inverse correlation between steady-state through concentration and GFR. Despite the accumulation of AH, the doses of the patients with the lowest GFR (5-28 mL/min) and the patients with normal GFR were not statistically significantly different (1.87 (0.24) mg/kg/24 hours versus 1.35 (0.15) mg/kg/24 h) .

Studies in Congestive Heart Failure Patients

18. Crawford MH, Ludden TM, Kennedy GT. Determinants of systemic availability of oral hydralazine in heart failure. Clin Pharmacol Ther 1985; 38: 539-543

The study investigated the PK of H after a single intravenous dose or escalating single doses given orally in 10 male patients of mean age 60 years with chronic congestive heart failure (NYHA Class III). They were on digoxin and furosemide. The patients were randomized to receive an intravenous bolus of 0.3 mg/kg or an oral doses of 1.1 mg/kg. On a separate occasion the subjects received ascending doses of 75, 100, 150, 225, 300, 400, 550, 750 or 1000 mg tid. The cardiac ouput had to rise by > 10% compared to the last dose and the pulmonary capillary wedge pressure had to remain < 18 mmg Hg. The acetylator status of the patients was determined with sulfamethazine. There were 6 EMs and 4 PMs.

Table 26. Mean (SD) PK Parameters of Hydralazine Referenced to the Concentration in Whole Blood

CL	Vss	t1/2	F, %			AUC/Dpo	
mL/min/kg	L/kg	min	EM	PM	All	Initial	Final
29.5 (8.0)	1.340 (0.791)	105[a]	9.9 (6.0)	26.2 (13.0)	16.4 (12.1)	53.5 (50.5)	247.2 (213)

[a] Harmonic mean

The CL exceeded liver blood flow in the congestive heart failure patients. The value of Vss indicated partitioning into and/or binding of H to tissues. The absolute bioavailability of H was small and depended on the acetylator status. The dose normalized AUC values after the last dose of the tid regimens were up to a factor of 9 greater than after the sinl dose of 1.1 mg/kg. If AUC which was measured as AUC0-∞ is not significantly greater than AUC0-8h the result appears to indicate non-linearity in the pre-systemic and/or systemic PK of H. However, it should be noted that with the design used the effects of increasing the dose and repeat dose administration may be confounded. An impact of the background co-medication on the parameters of H can also not be excluded.

19. Hanson A, Johansson BW, Wernersson, Wahlander LA. Pharmacokinetics of oral hydralazine in chronic heart failure. Eur J Clin Pharmacol 1983;25: 467-473

The study examined the PK of H, HPH and AH in 7 patients with congestive heart failure (NYHA Class III or IV), 3 males and 4 females, of mean age 61 years and body weight 68 kg (Hanson et al.1983). The congestive heart failure patients were on digoxin and diuretics. Eight hypertensive patients, 7 males and 1 female, of mean age 57 years and mean body weight 82 kg served as control. All patients received a single 50 mg oral dose of H. A tablet formulation of H was used. The acetylator status of the patients was determined by dapsone and showed that among the congestive heart failure patients 4 were EMs and 3 were PMs, whereas among the hypertensive patients 5 were EMs and 3 PMs.

Table 27. Mean (SD) PK Parameters of Hydralazine Referenced to the Concentration in Plasma after Oral Administration of Hydralazine

Patients	AUC0-4 nM/mL·h	Cmax nM/mL	Tmax h	t1/2 h
CHF	208 (143)	1.5 (0.9)	0.73 (0.26)	2.27 (1.25)
Hypertensives	94 (44)	0.8 (0.3)	0.96 (0.44)	1.99 (1.41)

The values for AUC0-4 and Cmax of H tended to be greater in the CHF patients than in the hypertensive patients, however statistical significance was not reached. The Tmax and t1/2 values were similar in both phenotypes.

Table 28. Mean (SD) PK Parameters of Hydralazine Pyruvate Hydrazone (HPH) Referenced to the Concentration in Plasma after Oral Administration of Hydralazine

Patients	AUC0-4 nM/mL·h	Cmax nM/mL	Tmax h	t1/2 h
CHF	666 (301)*	4.1 (2.0)*	0.79 (0.40)	3.44 (1.23)*
Hypertension	258 (84)	1.9 (0.6)	0.83 (0.49)	1.88 (0.32)

* Statistically significant difference between groups

AUC0-4 and Cmax of HPH were significantly greater than those of H in both groups indicating that HPH is the major circulating moiety. The AUC0-4, Cmax and t1/2 values of HPH in the CHF patients were statistically significantly greater than the corresponding values in the hypertensive patients.

Table 29. Mean (SD) PK Parameters of Apparent Hydralazine (AH) Referenced to the Concentration in Plasma after Oral Administration of Hydralazine

Patients	AUC0-4 nM/mL·h	Cmax nM/mL	Tmax min	t1/2 min
CHF	871 (381)*	5.7 (2.7)*	0.69 (0.23)	3.87 (1.56)*
Hypertension	407 (133)	3.0 (0.8)	0.86(0.48)	2.00 (0.48)

* Statistically significant difference between groups

The AUC0-4 and Cmax values of AH were greater than those of HPH indicating that additional circulating metabolites existed. As with HPH the AUC0-4, Cmax and t1/2 values were statistically significantly greater in the CHF patients than in the patients with hypertension. In addition to disease factors differences in co-medications may have influenced the results.

5.2.1.3 Drug Interaction Studies

Studies on the Impact of Hydralazine on Other Drugs

20. Nomura A, Yasuda H, Katoh K, Akimoto T, Miyazaki K, Arita T. Hydralazine and furosemide kinetics. Clin Pharmacol Ther 1982;32:303-306

Eight Japanese patients 48 years of age with advanced CHF on a regimen with furosemide and digoxin received 40 mg furosemide alone or together with 0.2 mg/kg H (Both drugs were given intravenously, first H and after 30 minutes furosemide. The order of treatments was defined by the order the subjects entered into the study. Statistically significant increases in CL and CLR of 21.3% and 34.1 % occurred in the presence of H. It can be concluded that co-administered H affects the exposure to intravenously administered furosemide. The clinical relevance of this finding is unknown.

21. McLean AJ, Skews H, Bobik A, Dudley FJ. Interaction between oral propranolol and hydralazine. Clin Pharmacol Ther 1980; 27:726-732

Seven healthy subjects of mean age 23 years and 70.1 kg body weight received on different occasions 1 mg/kg propranolol po and 0.2 mg/kg propranolol intravenously alone followed by oral treatments with propranolol 1 mg/kg and 25, 50 or 100 mg H administered in ascending order (McLean et al. 1982). H was administered 15 minutes after propranolol. Statistically significant increases in AUC (62.4%) and Cmax (77.3%) of oral propranolol in the presence of 25 mg H were observed. The corresponding values for propranolol after co-administration of 50 mg H were 97.3 % and 142.8 %, respectively. Because of adverse events the highest dose of 100 mg H could only be

administered to 3 subjects and the results indicated that no further increase of the AUC and Cmax values occurred at the highest dose level.

It can be concluded that co-administration of 25 or 50 mg H increases the exposure to propranolol dose dependently. Considering that higher doses of H are administered with BiDil this interaction may be clinically relevant. An additional pharmacodynamically mediated hypotensive effect of ISDN is likely with BiDil.

22. Jack DB, Kendall MJ, Dean S, Laugher SJ, Zaman R. The effect of hydralazine on the pharmacokinetics of three different beta adrenoceptor antagonists: Metoprolol, nadolol and acebutotol. Biopharm Drug Dispos. 1982:3:47-54

Healthy volunteers of mean age 21 years participated in this single dose trial which was conducted as 3 separate studies. The subjects were randomized to receive the beta-blocking agent alone or in the presence of 50 mg H. Marketed formulations were administered to the volunteers. In the first study 15 volunteers took 100 mg metoprolol, in the second study 7 subjects took 80 mg nadolol and in the third study 7 subjects ingested 400 mg acebutolol alone or together with H.

Table 30. Mean (SD) of Cmax and AUC Values of Metoprolol, Nadolol, Acebutolol and Diacetolol in the Presence and Absence of Hydralazine

Cmax, ng/mL	Drug	AUC, ng/mL·h
211 (109)*	Metoprolol	1263 (925)*
315 (109)	H & Metoprolol	1648 (881)
101 (68)	Nadolol	1026 (692)
70 (46)	H & Nadolol	629 (334)
1589 (403)	Acebutolol	5691 (967)
1574 (343)	H & Acetbutolol	6011 (1281)
1223 (520)	Diacetolol	10639 (2292)
1014 (283)	H & Diacetolol	9198 (1771)

* Statistically significant difference

It can be concluded that co-administration of a single dose of 50 mg H had an impact on metoprolol resulting in statistically significant increases in mean AUC and Cmax of 30.5% and 49.9%, respectively. However, 50 mg H had no effect on nadolol or acebutotlol and its major metabolite diacetolol.

23. Lindeberg S, Holm B, Lundborg P, Regardh CG, Sandstroem B. The effect of hydralazine on steady-state plasma concentrations of metoprolol in pregnant hypertensive women. Eur J Clin Pharmacol 1988;35:131-135

The impact of H on the PK of metoprolol and its active metabolite α-hydroxymetoprolol at steady-state were studied in 10 women with gestational hypertension (diastolic blood pressure ≥ 95 mm Hg. The women were in the 33rd to 37th week of gestation. Their mean age was 27 years and their mean body weight was 80.3. The women received 50 mg metoprolol as an immediate release tablet bid for 3 days. In the morning of the fourth Day after a fast of 8 hours they were administered 50 mg metoprolol and frequent blood samples were taken up to 8 hours after administration. The 50 mg metoprolol bid regimen was then continued until delivery. On Day 5, 25 mg H given bid was added to the regimen. On Day 4 of the combination treatment frequent blood samples were collected up to 8 hours after administration of the drugs. The plasma concentrations of metoprolol and α-hydroxymetoprolol were measured by GC-MS. The results for metoprolol and its active metabolite are shown are shown in Tables 31 and 32, respectively:

Table 31. Median Exposure Parameters of Metoprolol in the Presence and Absence of Hydralazine in Pregnant Women with Gestational Hypertension

Cmax, nMol		Tmax, h		AUC0-8, nMol·h	
M	M & H	M	M&H	M	M&H
72.5	136.0*	1.5	1.0	223.9	308.6*

* Statistically significant difference

Table 32. Median Exposure Parameters of α-Hydroxymetoprolol in the Presence and Absence of Hydralazine in Pregnant Women with Gestational Hypertension

Cmax, nMol		Tmax, h		AUC0-8, nMol·h	
M	M & H	M	M&H	M	M&H
232.5	236.5	1.5	1.0	941.0	965.0

The results indicate that co-administration of 25 mg bid and 50 mg metoprolol bid result in respective increases of 87.6 % and 37.8% in Cmax and AUC0-8 of metoprolol. In contrast, co-administration of H and metoprolol had no impact on the exposure parameters of the active metabolite α-hydroxymetoprolol.
It can be concluded that H increases the exposure to metoprolol, but not to its active metabolite in hypertensive pregnant women.

24. McLean AJ, Drummer OH, Smith HJ, Froomes P, McNeil JJ. Comparative pharmacokinetics of enalapril and lisinopril alone and with hydralazine. J Human Hypertesnion 1989;3:147-151

Sixteen healthy male volunteers in the age between 19-24 years participated in this randomized crossover study using a Latin Square design. The subjects received 20 mg enalapril or lisinopril together with 25 mg H on separate occasions. Marketed formulations were administered to the volunteers.

In the presence of H Cmax (30.4%), AUC (33.2%) and Tmax of lisinopril were statistically significantly increased. Co-administration of H did not impact the bioavailability measures of enalapril. It can be concluded that a single dose of 25 mg co-administered H increases the exposure to lisinopril, but not enalapril.

Isosorbide Dinitrate

1. **INFLUENCE OF BETA-BLOCKER COADMINISTRATION ON THE KINETICS OF ISOSORBIDE MONONITRATE AND DINITRATE.** OCHS H.R., et.al., Klin. Wochenschr 1986; 64: 1213-1216.

This article describes two separate studies evaluating beta-blockers such as propranolol, metipranolol, and metoprolol on the pharmacokinetics of single oral doses of 5-ISMN and ISDN. Since this study used metipranolol oral tablets; which are not approved in the U.S., those results will not be evaluated. However, some tables and figures scanned into the report may have metipranolol data on it; which can not be avoided.

ISMN Study

This study was a three-way, crossover, randomized study conducted with twelve healthy volunteers. 5-ISMN was administered as a single 20 mg oral dose in the fasted state in all three arms of the study. The treatment arms were: 1) 5-ISMN given alone; 2) metipranolol with 5-ISMN; 3) metoprolol given as 100 mg twice daily started 48 hours prior to 5-ISMN administration with the final metoprolol dose given concurrently with 5-ISMN on the day of pharmacokinetic assessment. The wash-out period was at least one week in duration between treatment arms.

Blood samples were collected at baseline before 5-ISMN administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after 5-ISMN administration. Intact 5-ISMN serum concentrations were measured by gas chromatography with electron capture detection. The pharmacokinetic calculations made were C_{max}, $AUC_{0-\infty}$, apparent terminal elimination $t_{½}$, and CL_{total} (calculated as administered dose divided by AUC).

Eight subjects participated in all three arms. As a result, only their data was reported. Two subjects did not participate in each of the other two arms. Assessment of differences in pharmacokinetic variables between the two treatment arms was performed visually by the reviewer through descriptive pharmacokinetics below.

Table 1. Influence of metoprolol on the kinetics of isosorbide-5-mononitrate (mean ± SE)

	Control	With metoprolol
Peak serum concentration (ng/ml)	429 (±39)	481 (±54)
Time of peak (h after dose)	0.63 (±0.08)	0.55 (±0.07)
Terminal elimination half-life (h)	4.56 (±0.19)	4.53 (±0.20)
Total AUC (ng/ml × h)	2,449 (±77)	2,516 (±105)
Oral clearance (ml/min)	138 (±5)	135 (±6)

* Based on eight subjects that completed all three trials; degrees of freedom = 2,14

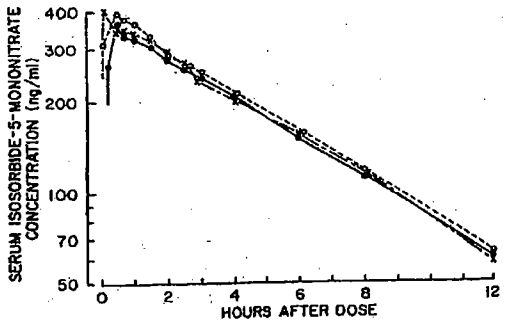

Fig. 1. Mean serum isosorbide-5-mononitrate concentrations at corresponding times during the three phases. •—•, control treatment condition; ×—×, with metipranolol; o---o, with metoprolol

Table 1 shows mean pharmacokinetic variables for 5-ISMN and metoprolol. Figure 1 shows mean serum 5-ISMN concentrations during the two treatment arms of interest that were assessed. Upon visual inspection of all pharmacokinetic variables calculated, no differences among the two treatment arms was observed.

In conclusion, there does not seem to be any pharmacokinetic drug interaction between 5-ISMN and metoprolol.

ISDN Study

The second study was a two-way, crossover, randomized to treatment arm study conducted with ten healthy volunteers. ISDN was administered as a single 20 mg oral dose in the fasted state in both arms. Treatment arm one was ISDN alone with no other drug and arm two consisted of propranolol given as 80 mg three times daily started 48 hours prior to ISDN administration with the final propranolol dose given concurrently with ISDN on the day of pharmacokinetic assessment. The wash-out period was at least one week in duration between treatment arms.

Blood samples were collected at baseline before ISDN administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after ISDN administration. ISDN and 5-ISMN serum concentrations were measured by gas chromatography with electron capture detection. The assay sensitivity limit for ISDN is 2 ng/mL, and between-day replicability is 11.2%. For 5-ISMN, the sensitivity value is 5 ng/mL and the replicability 9.4%. The same pharmacokinetic calculations were made as described above. Differences between the control and the propranolol arm was evaluated using a Student's paired t-test.

Table 2 demonstrates that there were differences in total AUC ($p<0.05$, about 20% drop in AUC) and oral clearance (increase, $p<0.1$, 32% increase) for ISDN when administered with propranolol. Peak 5-ISMN levels were reached sooner during propranolol

coadministration (35% sooner) with a prolonged apparent half-life (about 27% increase). Assessment of differences in pharmacokinetic variables between the two treatment arms is illustrated below in Table 2 and Figure 2:

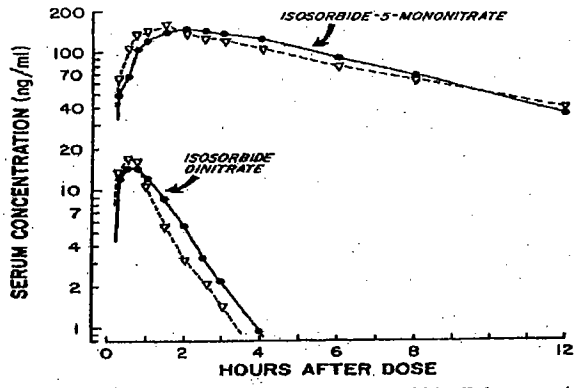

Fig. 2. Mean serum concentrations of isosorbide dinitrate and its metabolite isosorbide-5-mononitrate at corresponding times during the two treatment conditions. ●——●, control treatment condition; ▽---▽, with propranolol

Table 2. Influence of propranolol on the kinetics of isosorbide dinitrate and its metabolite, isosorbide-5-mononitrate (mean ± SE)

	Control	With pro-pranolol	Value of Student's *t*
Isosorbide Dinitrate			
Peak serum level (ng/ml)	19.0 (±1.6)	20.6 (±2.4)	0.55 (NS)
Time of peak (h after dose)	0.75 (±0.16)	0.5 (±0.05)	1.5 (NS)
Total AUC (ng/ml × h)	28.2 (±0.8)	22.6 (±2.3)	2.60 ($P<0.05$)
Oral clearance (liters/min)	12.33 (±0.78)	16.33 (±2.08)	2.11 ($0.05<P<0.1$)
Isosorbide-5-Mononitrate			
Peak serum level (ng/ml)	162 (±11)	158 (±12)	0.30 (NS)
Time of peak (h after dose)	1.83 (±0.17)	1.17 (±0.11)	2.56 ($P<0.05$)
Terminal half-life of disappearance (h)	4.46 (±0.22)	5.65 (±0.47)	2.83 ($P<0.025$)
Total AUC (ng/ml × h)	1,333 (±71)	1,379 (±124)	0.30 (NS)

The author concludes that no drug interaction exist between ISDN or 5-ISMN and propranolol.

REVIEWER'S COMMENTS:

1. In the 5-ISMN study, two subjects did not participate in each of the other two arms. As a result, only data for 8 subjects was reported. The sponsor should have reported the data for 10 subjects in each of the treatment arms and compared that data to the 5-ISMN arm alone.
2. The sponsor only sampled out to 12 hours and 5-ISMN has a mean terminal elimination half-life of about 4.5 hours in the both studies. In the ISDN study (2^{nd} study), 5-ISMN t_{max} was about one hour delaying the times for 5-ISMN determination and further supporting the need for longer blood sampling times. Blood sampling should have been carried out to at least three half-lives for both drugs (about 16 hours).
3. No clearance calculations were reported in the article for the 5-ISMN study even though it was intended as part of the final analysis and assessment.
4. In the ISDN study, there were differences in total AUC ($p<0.05$, about 20% drop in AUC) and oral clearance (increase, $p<0.1$, 32% increase) for ISDN when administered with propranolol. Likewise, peak 5-ISMN levels were reached sooner during propranolol coadministration (35% sooner) with a prolonged apparent half-life (about 27% increase). However, dosage adjustment is not warranted for the pharmacokinetic changes observed since they are not of clinical significance.

2. **SINGLE ADMINISTRATION OF ATENOLOL DOES NOT INFLUENCE THE KINETICS OF ORALLY GIVEN ISOSORBIDE DINITRATE.** BOGAERT M.G., et.al., Int. J. Clin. Pharm. Res. 1983; III 6: 491-493.

Eight healthy volunteers (6 were between 69 – 84 years, two age 24 years, 5 males and 3 females) were given 10 mg of ISDN in the fasted state on two different randomized occasions with a one week wash-out period between treatments. Atenolol 100 mg was given orally two hours before ISDN administration on one of the treatment arms. Exact blood sample collection times were not given in the article. Upon reviewing the article and figures, it seems at least 7 time points were taken, up to a 6-hour time point. Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations was obtained by a gas liquid chromatographic method with a capillary column and an electron capture detector. The pharmacokinetic calculations made were C_{max}, and AUC.

Table 1 shows individual and mean C_{max} and AUC values for all three moieties with and without atenolol in all eight volunteers. Upon visual inspection of the pharmacokinetic variables calculated below, a great deal of variability is observed within and between treatment arms.

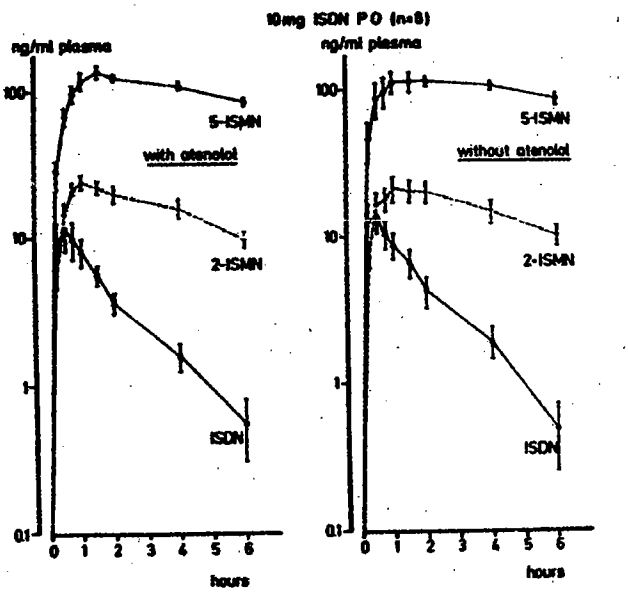

Fig. 1 Mean plasma concentrations (± standard error of the mean) of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of isosorbide dinitrate 10 mg to 8 subjects on 2 occasions, once with and once without previous administration of atenolol, 100 mg.

Table I *Area under the curve (from 0 to 6 hours) of isosorbide dinitrate and isosorbide mononitrates after oral administration of isosorbide dinitrate, 10 mg, to 8 subjects on 2 occasions, once with and once without previous administration of atenolol, 100 mg. Individual values and mean (± s.e.m.) are given. Between brackets, the corresponding peak plasma concentrations are given.*

		With atenolol			Without atenolol		
		ISDN	2-ISMN	5-ISMN	ISDN	2-ISMN	5-ISMN
EK	♀	16.2	141.9	620.5	8.4	112.5	567.6
		(8.6)	(34.5)	(147.7)	(4.5)	(26.6)	(119.5)
HC	♂	11.8	114.6	637.7	21.3	149.3	626.9
		(5.8)	(25.6)	(155.0)	(9.0)	(34.0)	(139.1)
HD	♀	45.1	121.9	653.4	47.9	138.4	756.8
		(34.3)	(36.0)	(187.9)	(23.8)	(40.4)	(197.7)
VT	♀	40.9	103.5	720.2	41.2	114.8	624 9
		(22.5)	(24.6)	(148.1)	(32.6)	(29.2)	(170.1)
JV	♂	20.8	69.1	515.2	20.4	75.8	533.4
		(11.2)	(18.4)	(126.4)	(21.2)	(18.3)	(130.0)
FL	♂	20.3	97.4	540.1	22.0	48.3	315.9
		(11.0)	(30.3)	(117.8)	(7.7)	(12.9)	(77.0)
AJ	♂	11.9	60.3	698.0	22.3	71.2	734.3
		(6.7)	(23.0)	(171.6)	(21.0)	(21.7)	(204.6)
JV	♂	6.7	61.5	527.7	20.1	57.1	651.4
		(5.3)	(15.9)	(138.0)	(20.6)	(15.9)	(126.3)
Mean		21.7	96.3	614.1	25.5	95.9	601.4
		(13.2)	(26.0)	(149.1)	(17.6)	(24.9)	(145.5)
s.e.m.		4.9	10.7	27.7	4.5	13.4	48.7
		(3.6)	(2.5)	(8.1)	(3.4)	(3.3)	(15.1)

The author states that there were no significant differences in C_{max} or AUC. However, the author recommends that future studies be performed with betalytics being administered in a chronic fashion in order to assess ISDN changes pharmacokinetically.

REVIEWER'S COMMENTS:

1. Sampling was not carried-out long enough to characterize the pharmacokinetics of ISDN or its metabolites. The investigators should have gone out to at least 16 hours to capture a better concentration versus time curve.

3. **FATE OF ISOSORBIDE DINITRATE AND MONONITRATES IN PATIENTS WITH RENAL FAILURE.** Bogaert M.G., et.al., Eur. J. Clin. Pharmacol. 1981; 21: 73-76.

Two separate studies were conducted in this literature report in which one study involved the administration of a single 10 mg dose of ISDN and the second study involved the single administration of 2-ISMN and 5-ISMN at a dose of 5 mg each after an overnight fast.

ISDN Study

The first small study involved 5 patients with renal failure (CL_{CR} 6 - 14 mL/min; 2 males, 3 females) between the ages of 25 – 75 years and the four normal volunteers enrolled had a CL_{CR} 60 - 112 mL/min (2 males and 2 females). The renal failure group was taking other medications that were stopped the day of the study just for that day. Exact blood sample collection times were not given in the article. However, upon reviewing figures, it seems at least 9 time points were taken, up to an 8-hour time point. Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations was obtained by a gas liquid chromatographic method using a Ni-electron capture detector. According to the author, inter-day reproducibility had a coefficient of variation of < 10%. The pharmacokinetic calculation made for comparisons between groups was $t_{1/2}$ and concentration versus time figures.

As seen in Table 1, much more variability in the half-lives of the renal failure group is seen compared to the volunteers without renal failure. However, no difference is observed when the concentration versus time data for ISDN, 2-ISMN, and 5-ISMN is examined in Figure 1 below.

Table 1. Plasma half-lives (in hours) of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of isosorbide dinitrate 10 mg to patients with and without renal failure. Individual values and means (± SEM) are given

Patients with renal failure				Patients without renal failure			
	ISDN	2-ISMN	5-ISMN		ISDN	2-ISMN	5-ISMN
M. M. ♀	0.36	2.60	6.00	H. M. ♀	0.46	2.40	4.46
O. C. ♂	0.32	1.38	3.89	D. C. ♂	0.25	1.85	5.84
L. J. ♀	0.41	3.39	5.15	V. A. ♂	0.35	2.40	5.52
B. D. ♀	0.32	2.10	4.85	E. S. ♀	0.44	3.57	5.48
V. K. ♂	0.44	2.14	4.33				
Mean	0.370 (±0.002)	2.32 (±0.54)	4.84 (±0.65)		0.357 (±0.009)	2.55 (±0.52)	5.32 (±0.36)

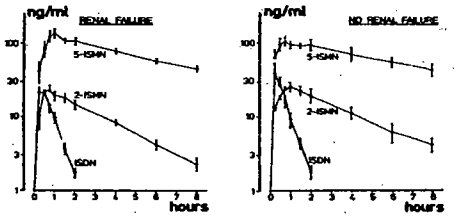

Fig. 1. Mean plasma concentrations (± SEM) of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of isosorbide dinitrate 10 mg to 5 patients with and 4 patients without renal failure

According to the author, no differences between renal and volunteer subjects is observed with the administration of a single 10 mg dose of ISDN.

2- and 5-ISMN Study

Five patients with renal failure (CL_{CR} 7 - 15 mL/min; 2 males, 3 females) between the ages of 49 – 71 years were enrolled in this study. The ISMN dose of 5 mg was administered simultaneously to all the healthy volunteers. The 2-ISMN 5 mg dose was given to 4 healthy males and the 5-ISMN 5 mg dose was given to 3 healthy male volunteers. Reasons for the discrepancy was not explained in the article. The same approach to concomitant medications being taken by the renal failure was taken in this portion of the study. Exact blood sample collection times were not given in the article and was difficult to assess by reviewing the figures provided. Blood sampling was collected up to an 8-hour time point for the renal patients and 10 hours for the volunteers. Analysis of 2-ISMN and 5-ISMN concentrations was obtained by the same methods stated earlier. The same pharmacokinetic calculations were made for comparisons between groups.

According to the author, the plasma concentration versus time curves for the 2-ISMN group seem similar; but the 5-ISMN group seem to be higher when compared to the volunteers. The half-lives of 5-ISMN in the renal patient group are higher as well (5.62 versus 4.41 hours).

Table 2. Plasma half-lives (in hours) of isosorbide 2-mononitrate (2-ISMN) and of isosorbide 5-mononitrate (5-ISMN) after oral administration of 5 mg doses to patients with renal failure and to healthy volunteers. Individual values and means (± SEM) are given

Patients with renal failure			volunteers			
	2-ISMN	5-ISMN		2-ISMN		5-ISMN
C.H. ♂	2.21	4.85	D.D. ♂	3.21	V.E. ♂	4.00
C.V. ♂	2.43	7.60	D.L. ♂	3.24	D.W. ♂	4.60
V.R. ♀	2.34	5.80	D.G. ♂	2.67	M.B. ♂	4.62
D.I. ♀	2.13	4.39	M.B. ♂	2.20		
B.M. ♀	1.77	5.49				
Mean	2.17 (±0.06)	5.62 (±1.51)		2.83 (±0.24)		4.41 (±0.12)

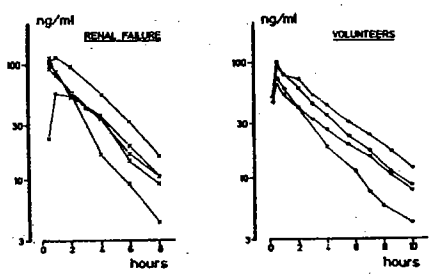

Fig. 2. Plasma concentrations of isosorbide 2-mononitrate (2-ISMN) after oral administration of 5 mg to 5 patients with renal failure and to 4 healthy volunteers

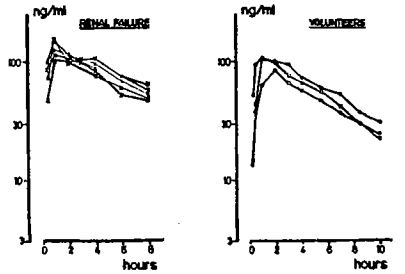

Fig. 3. Plasma concentrations of isosorbide 5-mononitrate (5-ISMN) after oral administration of 5 mg to 5 patients with renal failure and to 3 healthy volunteers

In conclusion, even though the 5-ISMN renal group given the 5 mg dose seem to have greater half-lives, the half-lives of the renal group for 5-ISMN in the ISDN study did not seem to display any differences.

REVIEWER'S COMMENTS:

1. Sampling was not carried-out long enough to characterize the pharmacokinetics of ISDN or its metabolites. The investigators should have gone out to at least 16 hours to capture a better concentration versus time curve.
2. The only method of assessing pharmacokinetic changes was assessment of half-life.
3. The renal failure groups were taking medications up to the day before the study was performed. No information was given as to what medications were being taken and their possible implications when assessment of the study results was made.
4. It remains unclear how multiple dosing in this patient population would affect the pharmacokinetics of ISDN and its metabolites.
5. In the ISDN study, normal renal function volunteers and those with mild renal impairment were combined for the results illustrated in figure1 making an assessment not possible and possibly canceling out any differences that do exist.
6. Differences in C_{max}, AUC, apparent clearance (CL/F), renal clearance (CL_R), and apparent volume of distribution (V_z/F) were not calculated nor was a statistical method implemented to quantitate the differences that may be present between the renal and volunteer subjects.

4. **PHARMACOKINETICS OF ISOSORBIDE-5-NITRATE IN RENAL FAILURE.** Evers J., et.al., Eur. J. Clin. Pharmacol. 1986; 30: 349-350.

Twenty patients with stable chronic renal failure and coronary artery disease (Table 1) were given 20 mg of ISDN three times daily for 28 days. Patients on dialysis or with liver disease were not included in the study. The first doses on days 2 and 28 were administered after an overnight fast due to pharmacokinetic assessment. Blood samples were collected immediately before drug administration and at 0.5, 1, 2, 4, 6, and 8 hours after administration. Analysis of plasma ISDN concentrations was obtained by HPLC with a thermal energy analysis detector. The pharmacokinetic calculations made were C_{max}, t_{max}, AUC_{0-8} at steady state, and $t_{1/2}$ (after curve fitting by NONLIN assuming a one-compartment open body model with a lag-time in absorption). A Student's t-test was utilized for statistical analysis.

Table 1. Details of the patients (mean ± SD; range)

Patients	Creatinine clearance [ml·min⁻¹]	Sex	Age [years]	Height [cm]	Weight [kg]
Group I n=10	66.4 ± 23.3 (30 – 100)	2f/8m	62 ± 11.9 (46–85)	165 ± 6.1 (154–173)	69 ± 6.7 (62–72)
Group II n=10	15.1 ± 8.5 (5 – 30)	6f/4m	59 ± 10.7 (45–76)	166 ± 6.2 (154–178)	69 ± 13.9 (51–98)
Total	5 – 100	8f/12m	61 ± 11.1	166 ± 7.0	69 ± 10.6

Details of pharmacokinetic parameters between the two treatment groups and the two assessment time points (day 2 to day 28) are illustrated in Table 2 below.

Table 2. Pharmacokinetic parameters of IS-5-N after oral administration of 20 mg tablets (i.d.) mean ± SD (range)

Parameter	Day 2		Day 28	
	Group I	Group II	Group I	Group II
C_{max} [ng·ml⁻¹]	437 ± 126 (270 ± 720)	462 ± 185 (240 – 770)	471 ± 188 (290 – 610)	455 ± 169 (270 – 680)
AUC_{0} [ng·h·ml⁻¹]	2131 ± 514 (1630 –3150)	2186 ± 764 (1250 –3490)	2261 ± 492 (1539 –3050)	2279 ± 907 (1020 –3693)
$t_{1/2}$ [h]	3.86 ± 0.76 (2.5 – 5.4)	4.70 ± 1.59 (2.0 – 7.2)	4.41 ± 0.84 (3.1 – 5.8)	4.90 ± 2.31 (2.1 ± 9.6)

There were no significant differences between mean values for C_{max} (450±155 ng/mL versus 463±135 ng/mL) on day 2 to day 28 or mean AUC^{SS}_{0-8} values (2158±634 ng·hr·mL^{-1} versus 2270±693 ng·hr·mL^{-1}, respectively). Mean elimination half-lives were 4.29±1.53 hours for day 2 and 4.64±1.66 hours for day 28, indicating no total mean changes between treatment days as well. Table 2 illustrates mean values between renal groups on days 2 and 28.

No delayed elimination or accumulation appeared in patients with renal failure after repeated dosing of ISDN. No dose adjustments seem necessary in this patient population.

REVIEWER'S COMMENTS:

1. Sampling was not carried-out long enough to characterize the pharmacokinetics of ISDN. The investigators should have gone out to at least 16 hours to capture a better concentration versus time curve.
2. Based on the information provided in the study, the reviewer concurs with the author's findings.

5. **PHARMACOKINETICS OF ISOSORBIDE DINITRATE, ISORSOBIDE-2-NITRATE AND ISOSORBIDE-5-NITRATE IN RENAL INSUFFICIENCY AFTER REPEAT ORAL DOSAGE.** Evers J., et.al., Klin. Wochenschr. 1989; 67: 342-348.

Twenty patients with stable coronary heart disease and varying levels of renal function were enrolled in this study. The two groups were based according to renal function with Group I (6 males and 4 females, ages 41 – 77 years) having a CL_{cr} of 5 to 30 mL/min and Group II (6 males and 4 females, ages 45 – 84 years) having a CL_{cr} of 50 to 150 mL/min. Only four patients in Group II were not taking concomitant medications. Volunteers received 20 mg of ISDN three times daily for 14 days. The first doses on days 2 and 14 were administered after an overnight fast due to pharmacokinetic assessment. Blood samples were collected immediately before drug administration and at 0.5, 1, 2, 3, 5, and 8 hours after administration. Analysis of plasma ISDN, 2- and 5-ISMN concentrations was obtained by electron capture capillary gas chromatographic method with isomannide dinitrate and isomannide mononitrate as the internal standard. The recovery for ISDN over the range of 0.5 – 50 ng/mL was 89% ± 4%, 82% ± 4% for 2-ISMN, and 85% ± 8% for 5-ISMN, respectively. The pharmacokinetic calculations derived were C_{max}, t_{max}, AUC_{0-8} at steady state, and $t_{1/2}$ (after curve fitting by NONLIN assuming a one-compartment open body model with a lag-time in absorption). A Student's unpaired t-test was utilized for statistical analysis between day 2 and 14. The relationship between AUC^{ss}_{0-8} and $t_{1/2}$ with renal function were assessed by linear regression analysis.

Details of pharmacokinetic parameters between the two treatment groups and the two assessment time points (day 2 to day 14) are illustrated in Table 1, 2, and 3 below.

Table 1.
Pharmacokinetic parameters of unchanged ISDN after oral dose of 20 mg ISDN t.i.d. as tablets. Group I (above): CrCl 5–30 ml/min; Group II (below): CrCl > 50 ml/min

	Day 2				Day 14			
	c_{max} (ng/ml)	t_{max} (h)	AUC^{ss}_{0-8} (ng/ml h)	$t_{1/2}$ (h)	c_{max} (ng/ml)	t_{max} (h)	AUC^{ss}_{0-8} (ng/ml h)	$t_{1/2}$ (h)
	14.8	0.5	27.5	1.0	11.6	1.0	33.3	1.7
	20.1	2.0	79.9	2.5	32.1	0.5	61.4	0.8
	13.1	2.0	63.8	2.7	36.8	1.0	56.1	0.8
	20.4	1.0	45.9	1.0	15.5	1.0	34.2	0.1
	16.4	1.0	45.2	1.3	38.5	1.0	65.1	0.7
	36.9	0.5	53.9	0.7	26.0	0.5	46.8	0.9
	26.1	1.0	75.3	1.5	40.8	1.0	150.0	2.7
	11.3	1.0	30.7	1.6	48.1	0.5	171.7	2.5
	38.1	0.5	75.4	1.1	59.9	1.0	82.8	0.5
	34.9	0.5	78.6	1.2	29.3	1.0	73.0	1.1
	23.2	1.0	57.6	1.5	33.9	0.9	77.4	1.2
Q.	10.2	0.6	19.8	0.7	14.4	0.2	46.9	0.9
	20.6	0.5	48.3	1.3	33.4	0.5	72.0	1.0
	10.5	0.5	37.0	–	13.6	1.0	34.3	1.0
	17.8	1.0	52.4	1.5	25.3	1.0	64.7	1.3
	18.3	0.5	40.6	0.9	19.3	0.5	42.8	1.1
	8.7	1.0	28.0	1.0	11.0	1.0	35.1	1.4
5	14.5	0.5	36.8	1.0	20.3	0.5	43.0	0.8
6	15.0	0.5	44.9	1.1	26.9	0.5	60.2	1.0
7	12.5	1.0	36.5	1.2	12.9	0.5	36.2	1.5
9	42.1	0.5	106.9	1.7	17.5	1.0	73.1	2.0
0	9.8	3.0	41.2	2.3	33.9	1.0	68.6	1.0
	17.0	0.9	47.3	1.3	21.4	0.8	53.0	1.2
Q.	9.7	0.8	22.0	0.4	8.2	0.3	16.2	0.4

Table 2. Pharmacokinetic parameters of IS-2-N after oral dose of 20 mg ISDN t.i.d. as tablets. Group I (above): CrCl 5–30 ml/min; Group II (below): CrCl > 50 ml/min

Patient	Day 2				Day 14			
	c_{max} (ng/ml)	t_{max} (h)	AUC^{SS}_{0-8} (ng/ml h)	$t_{1/2}$ (h)	c_{max} (ng/ml)	t_{max} (h)	AUC^{SS}_{0-8} (ng/ml h)	$t_{1/2}$ (h)
1	28.3	1.0	124	2.6	31.0	1.0	140	2.9
2	36.5	3.0	167	1.8	49.2	1.0	204	3.0
3	26.3	3.0	141	1.8	46.9	2.0	173	1.9
4	55.6	1.0	288	3.3	70.3	1.0	296	3.0
5	47.6	1.0	203	2.2	46.2	1.0	239	3.9
6	55.2	1.0	237	3.3	72.2	1.0	292	2.8
7	18.0	0.5	97	5.2	38.5	2.0	213	4.2
8	34.8	1.0	177	3.3	38.8	0.5	202	4.9
10	38.8	1.0	181	2.9	38.3	2.0	181	2.8
18	52.1	1.0	230	2.0	66.5	1.0	269	2.6
$\bar{x}$	39.3	1.4	185	2.8	49.8	1.3	221	3.2
SD	13.0	0.9	57	1.0	14.7	0.5	52	0.9
9	33.3	1.0	147	2.8	34.8	3.0	187	2.0
11	31.2	1.0	159	3.8	42.1	1.0	217	4.4
12	27.5	3.0	167	1.7	38.8	2.0	205	2.3
13	36.2	1.0	149	3.0	44.3	1.0	187	3.2
14	26.3	2.0	119	2.0	27.4	1.0	140	2.7
15	40.3	1.0	162	1.9	54.0	1.0	195	2.2
16	44.1	1.0	242	3.2	67.2	1.0	291	3.0
17	39.0	1.0	177	3.0	51.6	1.0	208	2.6
19	50.3	3.0	302	4.1	49.2	5.0	287	3.2
20	31.9	3.0	168	2.0	46.6	2.0	219	2.7
$\bar{x}$	36.0	1.7	179	2.8	45.6	1.8	214	2.8
SD	7.5	0.9	53	0.8	11.0	1.3	46	0.7

Table 3. Pharmacokinetic parameters of IS-5-N after oral dose of 20 mg ISDN t.i.d. as tablets. Group I (above): CrCl 5–30 ml/min; Group II (below): CrCl > 50 ml/min

Patient	Day 2				Day 14			
	c_{max} (ng/ml)	t_{max} (h)	AUC^{SS}_{0-8} (ng/ml h)	$t_{1/2}$ (h)	c_{max} (ng/ml)	t_{max} (h)	AUC^{SS}_{0-8} (ng/ml h)	$t_{1/2}$ (h)
1	184.0	1.0	1136	7.5	164.0	2.0	983	6.1
2	270.0	3.0	1463	2.4	277.0	1.0	1433	4.3
3	132.0	3.0	706	3.5	263.0	2.0	1454	3.6
4	302.0	2.0	1930	6.5	333.0	1.0	1954	6.3
5	299.0	2.0	1784	5.3	283.0	2.0	1751	7.8
6	315.0	0.5	1891	6.5	324.0	1.0	2106	7.7
7	195.0	3.0	1308	7.6	348.0	3.0	2357	7.7
8	320.0	3.0	1886	4.3	320.0	0.5	1989	9.3
10	301.0	3.0	1917	6.0	309.0	2.0	1882	6.0
18	212.0	2.0	1126	4.7	280.0	2.0	1572	4.5
$\bar{x}$	253.0	2.3	1515	5.4	290.1	1.7	1748	6.3
SD	66.6	0.9	432	1.7	52.2	0.7	397	1.8
9	191.0	2.0	1095	4.7	291.0	3.0	1807	6.1
11	202.0	2.0	1229	4.9	246.0	1.0	1552	6.7
12	192.0	3.0	1325	14.0	287.0	3.0	1928	7.8
13	289.0	1.0	1793	8.4	344.0	1.0	2069	7.2
14	177.0	1.0	1102	6.0	216.0	3.0	1347	4.0
15	152.0	1.0	843	3.9	199.0	1.0	981	4.2
16	267.0	2.0	1718	6.0	389.0	1.0	2317	5.2
17	209.0	1.0	1118	5.2	265.0	2.0	1567	6.0
19	346.0	5.0	2309	6.5	379.0	5.0	2454	4.1
20	126.0	5.0	751	3.0	223.0	2.0	1342	5.9
$\bar{x}$	215.1	2.3	1328	6.3	283.9	2.2	1736	5.7
SD	66.7	1.6	478	3.1	67.6	1.3	465	1.3

There are small differences between all pharmacokinetic parameters in both groups between days 2 and 14. However, there were no statistically significant differences between mean values for C_{max} on day 2 to day 14 or mean AUC^{SS}_{0-8} values and half-life values as well. No relationship was established between AUC^{SS}_{0-8}, and $t_{1/2}$ with renal function after repeated ISDN administration.

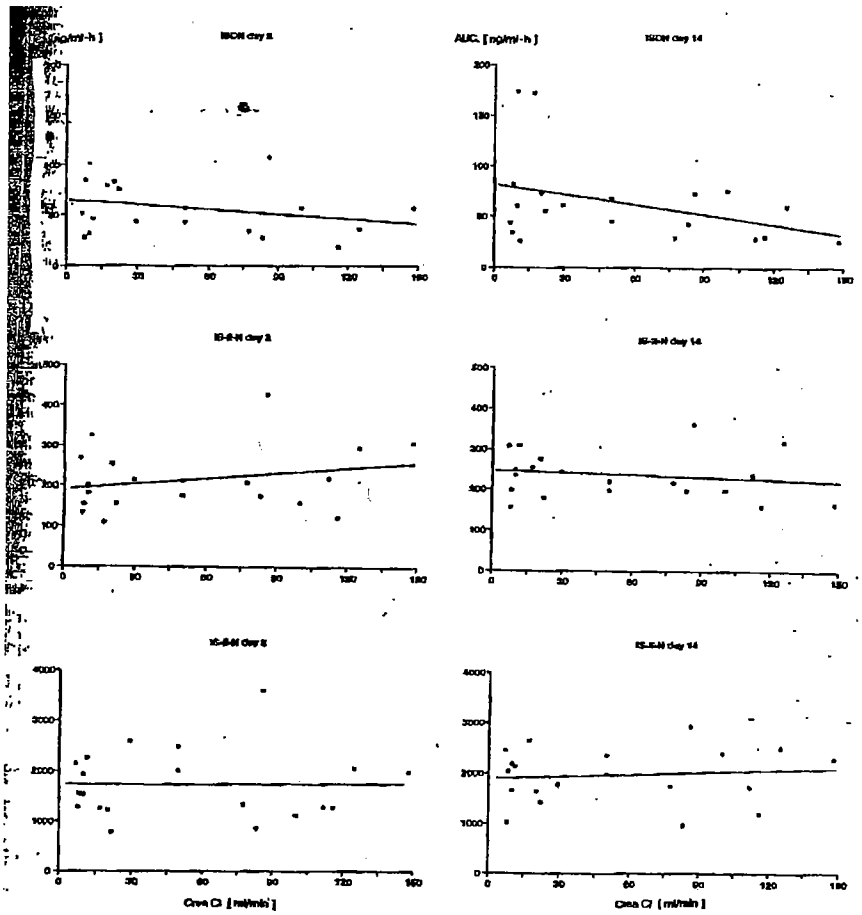

Fig. 1- Relationship between $AUC^{ss}_{0-\tau}$ and creatinine clearance (on day 2 and day 14) after repeated oral administration of ISDN 20 mg t.i.d.

No delayed elimination or accumulation appeared in patients with renal failure after repeated dosing of ISDN. The small differences observed do not warrant dosage adjustments in this patient population.

REVIEWER'S COMMENTS:

1. Sampling was not carried-out long enough to characterize the pharmacokinetics of ISDN. The investigators should have gone out to at least 16 hours to capture a better concentration versus time curve.
2. Based on the information provided in the study, the reviewer concurs with the author's findings.

6. **ISOSORBIDE DINITRATE IN PLASMA AND DIALYSIS DURING HAEMODIALYSIS.** Bauer. H., et.al., Eur. J. Clin. Pharmacol. 1986; 30: 187-190.

Ten patients (5 males and 5 females, age 59 to 75 years) with coronary heart disease and 0 – 5 angina pectoris attacks per week were enrolled in this study. All patients were end stage renal disease who had been on regular hemodialysis for 1 – 6 years. Every patient received 60 mg of ISDN (sustained-release form) daily for a minimum of two weeks. The last dose was taken about 2 hours prior to dialysis. Blood samples from the venous and arterial blood line were collected before and at hourly interval during dialysis. Simultaneous dialysate samples were collected as well. Analysis of plasma and dialysate ISDN concentrations were analyzed by a gas chromatographic method with an electron capture detection system and internal standardization. The reproducibility for ISDN was 8.7% in plasma and 14.8% in dialysate. The pharmacokinetic calculations used for assessment were concentration, AUC_{0-5} at steady state, and hemodialyzer clearance of ISDN in plasma and dialysate.

Plasma and Dialysate ISDN concentrations

Concentrations in the arterial blood line represent the ISDN concentrations in the circulation of the patient with ISDN concentrations in plasma from arterial and venous lines and dialysate in Table 1 below. Figure 1 shows that ISDN levels plateaued during dialysis; but varied considerably between patients (5.1 to 22.8 ng/mL, average 14.2 ng/mL). No influence of hemodialysis on ISDN levels in the arterial blood line was observed.

Table 1. Concentrations (X ± SEM) of ISDN during haemodialysis after ISDN 60 mg po

Time (h)	Number of experiments	Arterial plasma (ng/ml)	Venous plasma (ng/ml)	Dialysate (ng/ml)
0	10	8.14±1.62		
1	10	9.13±1.88	4.48±0.87	2.09±0.44
2	10	11.49±2.14	5.82±0.98	2.35±0.46
3	10	10.74±1.90	5.77±0.86	2.36±0.48
4	10	11.09±1.36	5.59±0.64	2.24±0.36
5	10	10.12±1.80	4.77±0.67	2.25±0.54

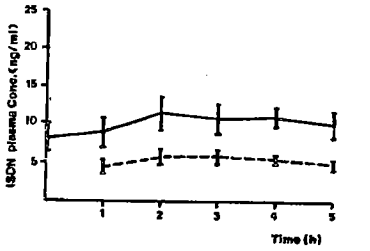

Fig. 1. Concentration of ISDN in arterial (——) and venous (- - -) plasma during haemodialysis after oral administration of 60 mg ISDN Retard. Mean ± SEM

Haemodialyzer Clearance of ISDN

This was obtained by the following equation:

CL_{av} (CL derived from arterio-venous conc) = ca-cv/ca X Qb

and

CL_D (CL derived from dialysate conc) = cd/ca X Qd

Ca and cv are ISDN concentrations in the arterial and venous blood line, respectively. Cd is ISDN concentration in the dialysate, and Qb and Qd are blood and dialysate blood

flow, respectively. Clearances are illustrated in Table 2 below. The mean CL_{av} was 92.3 ± 27.0 mL/min and for CL_D it was calculated as 106.6 ± 31.5 mL/min derived from the equations above.

Table 2. Haemodialyzer clearance of ISDN derived from arterial and venous concentrations and from arterial and dialysate concentrations

Time (h)	CL_{av} (ml/min)	CL_D (ml/min)
1	91.8 ± 29.4	121.6 ± 45.8
2	89.6 ± 27.2	103.0 ± 17.1
3	89.8 ± 23.4	107.0 ± 32.7
4	94.0 ± 29.4	97.5 ± 23.8
5	96.1 ± 30.2	103.6 ± 31.8

ISDN Elimination by Haemodialysis

Was calculated by the AUC using the following equation:

mav (total ISDN eliminated from arterial and venous conc) = $(AUC_a - AUC_v) X Qb$

and

md (total ISDN eliminated from dialysate conc) = $AUC_d X Qd$

The total amounts of ISDN removed (derived from above equations) were 0.356 ± 0.296 mg and 0.274 ± 0.160 mg, respectively. The amount of ISDN removed by 5 hours of hemodialysis was about 0.5% of the administered dose. Since the bioavailability of ISDN is lower with a sustained release formulation, the relative amount of available drug removed from the circulation was much higher.

Table 3. Areas under curve (ng/ml x h)

Patient nos.	Arterial plasma	Venous plasma	Dialysate
1	82.0	34.6	10.3
2	66.2	27.0	15.5
3	53.0	20.8	9.6
4	88.4	35.7	19.5
5	26.9	13.0	3.9
6	17.5	12.2	3.2
7	42.5	16.3	7.7
8	48.7	19.0	8.7
9	73.1	31.2	10.1
10	17.4	9.1	2.7
Mean	51.59	21.89	9.13
±SD	25.80	9.69	5.34

In conclusion, the elimination of ISDN in uraemic patients is unchanged with haemodialysis even when the lower bioavailability of the sustained-release formulation is taken into consideration.

REVIEWER'S COMMENTS:

1. The reviewer concurs with the author's findings.

7. **FATE OF ORALLY GIVEN ISOSORBIDE DINITRATE IN CIRRHOTIC PATIENTS.** BOGAERT M., et.al., Int. J. Clin. Pharmacol. Ther. Toxicol 1984; 22: 491-492.

Seven patients (age 32 to 67 years) with a clinical diagnosis of cirrhosis and signs of portosystemic shunting were enrolled in this study. A control group was also enrolled consisting of 25 volunteers and patients age 21 to 78 years and not suffering from hepatic disease. No changes to the cirrhotic population's medication took place within a week of study initiation and all medications were withheld on the day of the blood sampling until the last sample was collected. Every subject received 10 mg of ISDN (2 X 5 mg of Cedocard® tablets, Byk, Belgium) after an overnight fast. Exact times when blood samples were drawn were not provided in the article. However, it seems from Figure 1 that samples were taken out to the 4-hour time point. Analysis of plasma ISDN concentrations were analyzed by a gas chromatographic method. The plasma concentrations and AUC_{0-2} from cirrhotic patients and the controls were compared.

The plasma concentrations of ISDN in the 7 cirrhotic patients are illustrated in Figure 1 below. Five out of the 7 patients' plasma concentrations fell outside the 95% confidence interval (CI) calculated from the controls. The AUC_{0-2} for the cirrhotics was 43.2 ± 9.3 versus 18.1 ± 9.2 $ng \cdot mL^{-1} \cdot h$ ($p<0.02$, Mann-Whitney U-test).

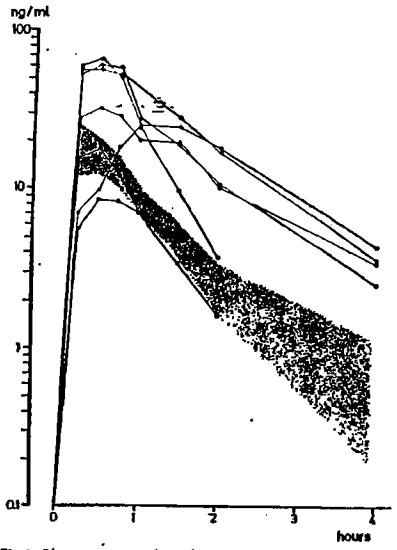

Fig. 1 Plasma concentrations in function of time after oral administration of isosorbide dinitrate, 10 mg, to 7 cirrhotic patients. The shaded area indicates the 95% confidence limit range of the plasma concentrations obtained after administration of the same dose to 25 subjects without hepatic disease. For 3 of the cirrhotic patients, concentrations after 4 hours were below the detection limit (=0.1 ng/ml).

No correlation was made between plasma concentrations and parameters of hepatic dysfunction or shunting in the cirrhotics.

Reasons hypothesized as to why increased plasma concentrations in 5 cirrhotic patients were observed: 1) increased bioavailability due to decreased hepatic first-pass as a consequence of shunting, or 2) decrease in systemic clearance also due to shunting and to hepatic cell dysfunction. The authors could not find any explanation why two cirrhotic patients had plasma concentrations within the range of the controls since all hepatic patients studied were void of extensive hepatic decompensation; but some of the patients did have evidence of edema and ascites. The authors conclude that predicting dosage adjustments in this population is not possible at this time since no correlation was made between plasma concentrations and effect.

REVIEWER'S COMMENTS:

1. The reviewer concurs with the author's findings.

8. **SEX RELATED DIFFERENCES IN THE PHARMACOKINETICS OF ISOSORBIDE-5-MONONITRATE (60 MG) AFTER REPEATED ORAL ADMINISTRATION OF TWO DIFFERENT PROLONGED RELEASE FORMULATIONS.** VREE T.B., et.al., Int. J. Clin. Pharmcol. Ther. 2004; 42 (8): 463-472.

This was a randomized, single-center, open, 2-sequence, 2-way, cross-over, multiple-dose bioequivalence study in 24 Caucasian volunteers (12 M and 12 F, ages 18 – 45 years) designed to identify differences in the pharmacokinetics of 5-ISMN between males and females. All subjects received a single daily oral dose of 5-ISMN 60 mg prolonged-release tablet formulation (formulations I, Euderma® and II, Imdur®) for 6 days in each treatment group with a wash-out period of 1 week between treatments. All subjects were nonsmokers or did not smoke more than 5 cigarettes per day (Table 1). No alcohol, caffeine, xanthine or grapefruit-containing food or drinks were allowed within 48 hours before each drug administration and during the confinement post-dose periods.

Table 1. Pharmacokinetic parameters of 60 mg oral isosorbide-5-mononitrate slow release of both formulations I and II in males and females.

Parameter		Males $n = 2 \times 12$	Females $n = 2 \times 12$	p
Age	y	31.0 ± 4.99	28.3 ± 7.00	0.34
Body weight	kg	78.4 ± 11.7	57.0 ± 8.56	0.0024
Body height	cm	178.6 ± 5.84	163.8 ± 9.32	0.0028
BMI	kg/m²	24.34 ± 2.55	21.29 ± 3.13	0.0113
Dose/kg	mg/kg	0.78 ± 0.12	1.07 ± 0.16	< 0.0001
AUC_{ss}	ng × h/ml	6,977 ± 1,343	10,576 ± 1,911	< 0.0001
AUC_{ss}/kg	ng × h/ml/kg	94.1 ± 30.8	192 ± 50.6	< 0.0001
C_{min}	ng/ml	56.3 ± 6.97	92.5 ± 35.6	0.0015
C_{max}	ng/ml	483 ± 74.1	740 ± 110	< 0.0001
t_{max}	h	3.93 ± 0.66	4.16 ± 0.56	0.24
$t_{1/2abs}$	h	3.21 ± 0.94	3.26 ± 0.95	0.85
$t_{1/2\alpha}$	h	0.24 ± 0.29	0.52 ± 0.51	0.024
$t_{1/2\beta}$	h	4.60 ± 0.50	4.69 ± 0.80	0.64
MRT	h	10.1 ± 1.30	10.8 ± 1.07	0.25

All subjects were drug free from any other medications while on the study. On the final day of study drug administration, all subjects fasted for a minimum of 10 hours until 4 hours post 5-ISMN administration for pharmacokinetic purposes. Blood samples were collected at baseline, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 24, 30, and 36 hours post-dose. The pharmacokinetic analysis was determined by non-compartmental and 2-compartmental analysis. Parameters calculated included C_{max}, t_{max}, AUC_{ss}, AUC_{∞}/kg (AUC corrected for body weight), elimination $t_{1/2\beta}$, $t_{1/2abs}$ (absorption half-life), $t_{1/2\alpha}$ (half-life of the rapid elimination phase), and MRT (mean residence time). Analysis of variance of the pharmacokinetic parameters was carried out by ANOVA, 2-tailed, Gaussian distribution and significance was defined at $p<0.05$. Analysis of plasma 5-ISMN concentrations were analyzed by a validated method involving liquid chromatography mass spectrometry with an internal standard. The lower limit of quantitation was 5 ng/mL (5.2% CV) with an intra-assay precision of 2.6% and -7.4% accuracy at 566 ± 14.7 ng/mL. The interassay precision was 1.9% and accuracy of -6.7% at 560 ± 10.4 ng/mL. The plasma extraction recovery was 78.2% for 5-ISMN and 89.6% (6.1% CV) for the internal standard at 200 ng/mL.

The mean plasma concentration time curves for both formulations were identical in both males and females (Figures 1 and 2) and they appear to run parallel to each other. Statistical comparisons between the two formulations were nonsignificant.

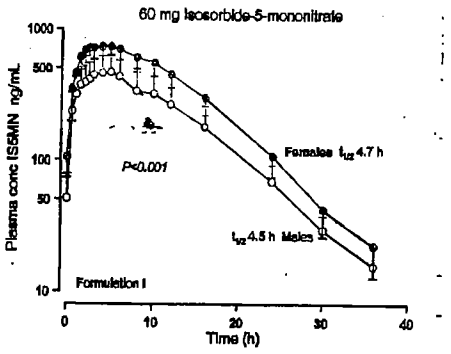

Figure 1. Mean (± SD) plasma concentration-time curves of isosorbide-5-mononitrate in males (open dots) and females (solid dots) after an oral dose of 60 mg prolonged release isosorbide-5-mononitrate (formulation I, Eudemine).

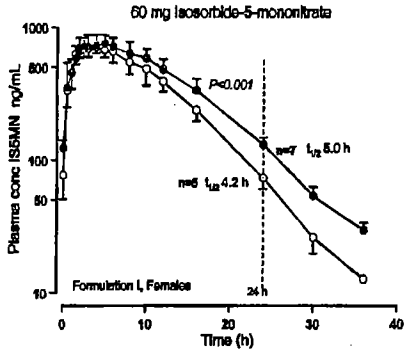

Figure 2. Plasma concentration-time curve (mean ± SD) of isosorbide-5-mononitrate after an oral dose of 60 mg prolonged release isosorbide-5-mononitrate (formulation I, Eudemine) to females (n = 12). At t = 0 = 24 h, a group of 7 females (solid dots) had a higher plasma concentration than a group of 5 females (open dots, p < 0.0001, Table 3). The plasma concentrations differ from t = 16 h (p < 0.0001).

As Table 1 indicates, body weight, length, body mass index, and dose/kg shows differences between males and females that are of statistical significance. C_{max}, C_{min}, AUC_{ss}, AUC_{∞}/kg were higher in females when compared to males ($p<0.0001$). A difference in C_{min} between males and females (56.3 ± 6.97 ng/mL in males versus 92.5 ± 35.6 ng/mL for females, $p<0.0015$) throughout therapy was observed (Table 1 and 2). When further investigated, differences in C_{min} were observed within the females (Table 2) that was twice as high in seven females than the other five (125 ± 12.2 ng/mL versus 59.3 ± 9.16 ng/mL, $p<0.0001$). Regardless of formulation, the same 7 females had a longer $t_{1/2\beta}$ and MRT than the other 5 females as illustrated below in Table 2. The male group are similar to the female group with 5 subjects. The two groups of females had the same weight, height, and body mass index. Elimination curves for the two groups are illustrated in Figure 2.

Table 2. Pharmacokinetic parameters of 60 mg oral isosorbide-5-mononitrate slow release of both formulations I and II in females. Selection criterion C_{min}.

Parameter		Females n = 2 + 5	Females n = 2 + 7	p
Age	y	28.2 ± 6.14	28.4 ± 8.03	0.96
Body weight	kg	56.6 ± 9.94	57.3 ± 8.30	0.90
Body height	cm	163.2 ± 12.1	164.3 ± 7.80	0.85
BMI	kg/m²	21.26 ± 3.15	21.29 ± 3.47	0.99
C_{min}	ng/ml	59.3 ± 9.16	125 ± 12.2	< 0.0001
C_{max}	ng/ml	729 ± 86.9	748 ± 127	0.69
AUC_{ss}	ng × h/ml	9,354 ± 1,217	11,446 ± 1,862	0.0052
AUC_{ss}/kg	ng × h/ml/kg	171 ± 45.0	206 ± 61.4	0.11
t_{max}	h	3.77 ± 0.52	4.43 ± 0.65	0.0144
$t_{1/2abs}$	h	2.93 ± 0.69	3.50 ± 1.06	0.16
$t_{1/2\alpha}$	h	0.63 ± 0.49	0.48 ± 0.52	0.40
$t_{1/2\beta}$	h	4.19 ± 0.56	6.06 ± 0.76	0.0057
MRT	h	9.40 ± 0.62	11.2 ± 0.65	< 0.0001

C_{min} = males-females low p = 0.16. C_{min} = males-females high p = < 0.0001.

Females also demonstrated a higher AUC_{ss} than males for both formulations (Figure 3, $p<0.0001$). When the AUC_{ss} were corrected for body weight, the differences in AUC_{∞}/kg between males and females was even greater (Figure 4) and differences were identical in both formulations.

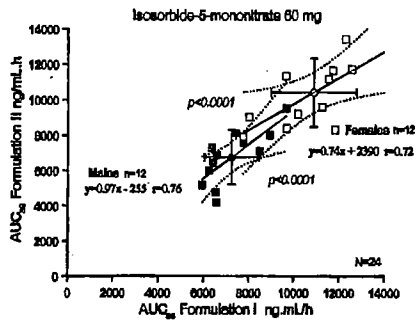

Figure 3. AUC_{ss} formulation I vs AUC_{ss} formulation II of isosorbide-5-mononitrate in 12 males (solid markers) and 12 females (open markers) (actual data and means ± SD). Both regression lines contain the origin in the 95% confidence interval. The differences in AUC_{ss} between males and females in both formulations I and II are both $p < 0.0001$. Dotted lines are the 95% confidence intervals.

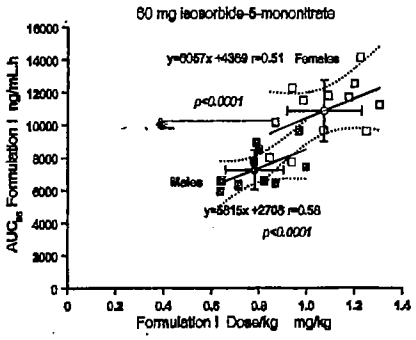

Figure 4. Dose/kg plotted versus the resulting AUC_{∞} of 60 mg isosorbide-5-mononitrate prolonged release of formulation I in males (n = 12) and females (n = 12). Females show a higher resulting AUC_{∞} than males ($p < 0.0001$) because of the higher dose/kg ($p < 0.0001$). Dotted lines are the 95% confidence intervals.

Although all differences discussed are statistically significant, the maximal difference even in elimination half-life of 1 hour overall will be clinically nonsignificant.

REVIEWER'S COMMENTS:

1. The reviewer concurs with the author's findings.
2. Dosage adjustment in females does not seem warranted.

9. **PLASMA DISPOSITION AND HEMODYNAMIC EFFECTS OF A SINGLE ORAL DOSE OF ISOSORBIDE DINITRATE IN HUMAN MALES AND FEMALES.** Nakatsu J.F., et.al., Biopharm. Drug Disposit. 1992; 13: 357-367.

Fourteen healthy volunteers (18-28 years, 7 M, and 7 F) were enrolled in the study. All subjects underwent an overnight fast prior to ingesting 325 mg of acetaminophen at 0820 hours. At 0830 hours, a single 10 mg dose of ISDN was administered as a tablet provided by Novopharm Ltd. In Scarborough, Ontario. A standard liquid breakfast was given at 0700 hours prior to study drug administration and a light lunch was served at 1130 hours. Diet contents were not provided. Venous occlusion was performed automatically (5s on, 5s off) by an automatic cuff inflation system that was placed on one forearm with a corresponding wrist arterial cuff inflated to 200 mmHg. Around the bulk of that forearm, a strain gauge was placed and connected to a plethysmograph recorder. An automatic sphygmomanometer was placed on the contralateral arm to record arterial blood pressures (SBP, DBP, and Mean BP). Forearm blood flow (FBF) was recorded on a chart for later analysis with forearm vascular conductance (FVC) calculated as FBF/MBP (Mean BP). Then venous capacitance (Cv) was measured on the same forearm after a short pause by inflating the venous cuff to about 50 mmHg for 5 min and recording the changes in forearm circumference with the plethysmograph. Each subject rested quietly for 30 minutes prior to taking any measurements. After a baseline measurement, preparation for blood sampling was performed by inserting a catheter into the antecubital vein in the contralateral arm that was used for plethysmography. All hemodynamic measurements were collected before each blood sample collection time point.

Blood sampling was performed at baseline, then at 15, 30, 45, 60, 90, 120, 240, 360, and 480 min after the administration of ISDN. The pharmacokinetic parameters calculated included C_{max}, AUC, and elimination $t_{1/2}$. Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations were analyzed by gas-liquid chromatography utilizing large-bore capillary columns. The lower limit of quantitation was 1.9, 1.8, and 1.8 ng/mL, respectively. The within-day CV were 2.4, 7.7, and 9.2% for ISDN, 2-ISMN, and 5-ISMN. The respective recoveries were 86, 75, and 66% at a concentration of 80 nM.

The data were analyzed by Student's t-test for unpaired data whenever two groups were compared. Analysis of the effects of ISDN on FBF, Cv, and FVC was performed by repeated measures ANOVA with Newman-Keuls post hoc test and significance was defined at $p \leq 0.05$. Two different approaches were utilized for the analysis of correlation between plasma drug concentration and hemodynamic effects: 1) the plasma concentration of ISDN alone was used in conjunction with the individual, hemodynamic variables to obtain the line of best fit by linear regression analysis using the least squares method; and 2) taking into account the pharmacological effect of 2-ISMN and 5-ISMN by calculating ISDN equivalent concentrations (formula used was {ISDN} + 0.018{2-ISMN} + 0.083{5-ISMN}). The plasma concentrations of 2-ISMN and 5-ISMN were multiplied by potency constants obtained from previous experiments conducted on rabbit isolated aorta by the author.

The AUC for ISDN was significantly less that for 2-ISMN or 5-ISMN (Figure 1) with values of 2.95 ± 3.26, 57.1 ± 35.5, and 391 ± 182 $ng \cdot h \cdot mL^{-1}$, respectively. This resulted in an AUC rank order of 5-ISMN>2-ISMN>ISDN ($p<0.05$). The mean apparent C_{max} of ISDN was 2.0 ± 1.97 ng/mL compared to 17.8 ± 10.0 ng/mL for 2-ISMN and 69.5 ± 33.45 ng/mL for 5-ISMN ($p<0.05$).

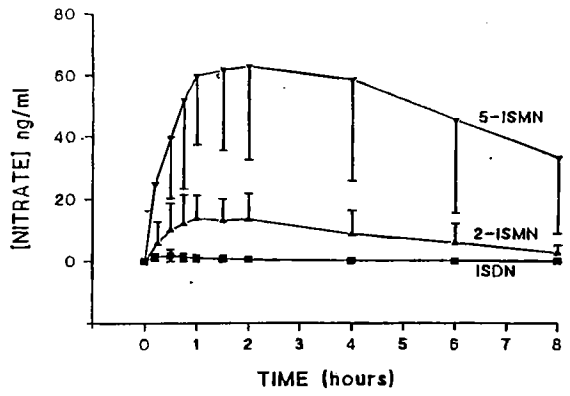

Figure 1. Plasma disposition of ISDN after oral administration of a single 10 mg tablet to seven male and seven female normal volunteers. Samples were taken at the times indicated on the abscissa and analyzed as described in Methods and Materials. Points represent the group mean ± SD; ■ ISDN, ▲ 2-ISMN, ▼ 5-ISMN

There were no gender differences in plasma concentration time curves or AUC values for any of the three moieties (Figure 2). When the data is normalized for body weight between males and females, the plasma concentration time curves become even more similar than they appear in Figure 2. The author stated that the elimination half-lives calculated in males was the same as females: 41 ± 34 for males versus 39 ± 22 min for females for ISDN, 2.3 ± 0.6 compared to 2.7 ± 1.5 hours for 2-ISMN and 7.8 ± 3.5 versus 5.1 ± 1.4 hours for 5-ISMN.

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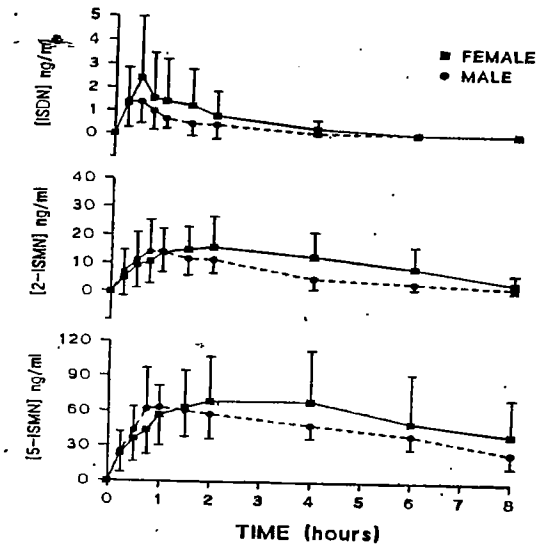


Figure 2. Comparison of the plasma disposition of orally administered ISDN in seven male and seven female normal volunteers. Points represent the group mean ± SD; ● male, ■ female. Upper panel, ISDN; middle panel, 2-ISMN; lower panel, 5-ISMN

DBP was decreased significantly at 30, 45, and 60 min after ISDN administration (Figure 3). However, there were no statistically significant differences in changes in FBF, Cv, or FVC from baseline (Table 1a).

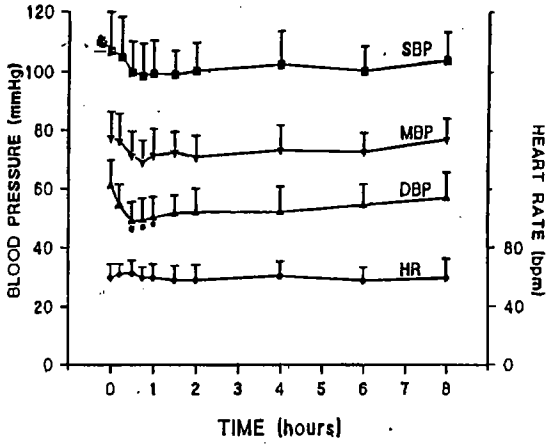


Figure 3. Effects of a single oral 10 mg dose of ISDN on hemodynamic variables in the 14 volunteer subjects. From top to bottom: systolic blood pressure, SBP; mean blood pressure, MBP; diastolic blood pressure, DBP; heart rate, HR. The data are presented as group means ± SD. *DBP was decreased significantly at 30, 45, and 60 min after ingestion of ISDN ($p<0.05$)

Table 1(a). Correlation between plasma ISDN concentration and hemodynamic variables. The correlation coefficients of the 14 individual subjects are reported for each of the measured hemodynamic variables

	FBF	FVC	C_v	DBP	SBP	MBP	HR
Males							
1	0·21	0·20	0·17	0·42	0·29	0·02	0·48
2	0·48	0·47	0·60	0·00	0·03	0·13	0·26
3	0·18	0·20	0·16	0·64*	0·32	0·55	0·78†
4	0·15	0·12	0·18	0·32	0·40	0·04	0·27
5	0·23	0·05	0·05	0·66*	0·65*	0·65*	0·14
6	0·56	0·48	0·57	0·43	0·33	0·23	0·21
7	0·57	0·56	0·49	0·34	0·31	0·30	0·25
Females							
1	0·45	0·69*	0·58	0·74*	0·35	0·52	0·11
2	0·21	0·47	0·49	0·33	0·22	0·45	0·60
3	0·45	0·46	0·30	0·69*	0·60	0·61	0·28
4	0·41	0·40	0·00	0·49	0·56	0·00	0·22
5	0·36	0·25	0·03	0·44	0·12	0·52	0·23
6	0·40	0·33	0·00	0·52	0·45	0·34	0·79†
7	0·16	0·09	0·08	0·34	0·24	0·26	0·56

*$p<0.05$, †<0.01.

Circadian rhythm effects on the measured variables was also investigated. As a result, six randomly selected blinded subjects (3 M and 3F) were given placebo tablets. The blood pressure measurements taken before receiving placebo were the same as those after the administration of placebo. When the placebo values for FBF, Cv, and FVC were subtracted from those same individuals after receiving ISDN, no differences were detected in their measurements either.

Time of ISDN C_{max} occurrence did coincide with the time of maximum DBP effect; which preceded the time of C_{max} of 2- and 5-ISMN. No correlation between plasma concentrations of ISDN or ISDN equivalents and hemodynamic variables observed could be made consistently (Tables 1a and 1b); not even when logarithmic concentration conversions were made.

Table 1(b). Correlation between ISDN equivalents and hemodynamic variables

	FBF	FVC	C_v	DBP	SBP	MBP	HR
Males							
1	0·52	0·56	0·03	0·24	0·84†	0·57	0·55
2	0·65*	0·71*	0·26	0·40	0·64*	0·44	0·50
3	0·10	0·33	0·41	0·35	0·55	0·56	0·84†
4	0·64*	0·64*	0·19	0·61	0·48	0·14	0·36
5	0·07	0·24	0·14	0·44	0·35	0·58	0·50
6	0·66*	0·51	0·59	0·54	0·47	0·29	0·47
7	0·67*	0·53	0·63*	0·58	0·05	0·02	0·02
Females							
1	0·14	0·52	0·47	0·65*	0·91†	0·83†	0·12
2	0·30	0·53	0·56	0·26	0·29	0·41	0·60
3	0·41	0·32	0·56	0·27	0·40	0·18	0·03
4	0·22	0·17	0·24	0·60	0·44	0·45	0·24
5	0·08	0·06	0·16	0·66*	0·53	0·64*	0·59
6	0·49	0·41	0·24	0·62	0·48	0·25	0·24
7	0·09	0·19	0·44	0·46	0·36	0·20	0·51

In conclusion, no differences were found between males and females in the pharmacokinetics of ISDN, 2-, and 5-ISMN according to the author. Decreases in DBP correlated well with the time course of plasma concentrations of ISDN, and to a lesser extent 2- and 5-ISMN. However, no correlation could be made with FBF, Cv, and FVC.

REVIEWER'S COMMENTS:

1. No pharmacodynamic assessment of differences between males and females were made.
2. Dosage adjustment in females does not seem warranted.
3. The reviewer concurs with all the author's findings of no gender or circadian differences based on the analysis that was performed.
4. However, the absence of a PK/PD correlation remains unclear and further studies would be necessary in the targeted population to make such a claim.
5. The 2- and 5-ISMN potency constants were calculated using animal data. As a result, the second approach used to correlate plasma drug concentration and hemodynamic effect by calculating ISDN equivalent concentrations is flawed and can not be used to make any conclusions about the pharmacological contributions of 2- and 5-ISMN in this type of analysis.

10. EFFECTS AND PHARMACOKINETICS OF ISOSORBIDE DINITRATE IN NORMAL MAN. Spröl-Rdum, G., et.al., Eur. J. Clin. Pharmacol. 1980; 18: 237-244.

Eighteen healthy volunteers (mean 30.9 ± 2.1 years, 10 M, and 8 F) were enrolled in this placebo-controlled study. All subjects underwent an overnight fast of 12 hours before a single 5 mg sublingual (SL) dose of ISDN was administered. Plethysmographic measurements (DPG) were taken 29 different times from 1 hour prior to drug administration to 2 hours after; which were recorded along with ECGs and heart rates (HR). Other hemodynamic measurements taken were blood pressure by a sphygmomanometer, and Schellong's tests (orthostatic tilting test). Blood pressure measurements were made at baseline and at 0.25, 0.5, 0.75, 1, 2, 4, 6, and 8 hours after dosing. HR was determined from the ECG charts as previously mentioned and at 4 and 8 hours post-dose. Schellong's tests were performed 1 hour prior to dosing and 2, 4, and 8 hours after drug administration. A positive test was defined as when subjects were not able to remain standing for the 5-min period, or a > 2-fold HR difference between supine and standing was observed, or a SBP fall > 5 mmHg compared to the place induced change was induced. Six blinded subjects were given placebo SL tablets and the same hemodynamic measurements were taken.

Blood sampling for ISDN, 2-, and 5-ISMN concentrations was performed at baseline, then at 5, 10, 20, 30, 45 min and 1, 1.5, 2, 4, and 8 hours after the administration of ISDN. The pharmacokinetic parameters calculated included C_{max}, AUC, and elimination $t_{1/2}$.

The concentration versus time curves of all three moieties were fitted to equation 1 below:

$$c_t = A\, t'^{P_1} e^{-P_2 t' P_3}$$

c_t = serum concentration at time t
t = time after administration
t' = time minus lag-time
A = linear factor
P_1, P_2, P_3 = parameters which define the shape of the curve (1)

The time course of the PDG amplitude was fitted according to equation 2 below:

$$y = B_2[e^{-q_2 t'} - e^{-q_1 t'} - (q_1 - q_2)\, t' e^{-q_3 t'}] + B_1 t' e^{-q_3 t' + B_0}$$

y = height of the amplitude of the a-wave of the first derivative of the DPG
t = time after administration
t' = t minus lag-time
B_0, B_1, B_2 = linear factors

q_1, q_2, q_3 = parameters which define the shape of the curve (2)

Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations were analyzed by gas-liquid chromatography with an internal standard. The lower limit of quantitation was about 2 for ISDN and 2-ISMN, and 5 ng/mL for 5-ISMN. The CV for serum drug concentrations in the range of 10 to 20 ng/mL were 20 to 30%.

Pharmacokinetic Data Derived from Fitted Curves:

ISDN was rapidly absorbed and metabolized to its active metabolites (with a $t_{1/2}$ of about 29 min) and reached a C_{max} of 17.9 ± 3.2 ng/mL. 2- and 5-ISMN had $t_{1/2}$ of 1.75 and 7.6 hours and C_{max} of 9.0 ± 0.8 ng/mL for 2-ISMN and 52.1 ± 9.8 ng/mL for 5-ISMN (Table 1, Figure 1). The AUC ratios were 1: 3.6 : 41.7 for ISMN, 2-, and 5-ISMN, respectively (Table 1). Data from 3 individuals with intermittent bradycardia are illustrated separately in the same table.

Table 1. Pharmacokinetic parameters of ISDN, 2-ISMN and 5-ISMN derived from fitted curves after ISDN 5 mg s.l.; T_E represents the average value from 10 volunteers (x ± SEM) who showed intermittent tachycardia; the data from the subjects with intermittent bradycardia are given as individual values (B_1, B_2, B_3). c_{max} = maximum concentration; t_{max} = time of maximum concentration; $t_{½}$ = terminal half-life; F-tot = area under the concentration-time-curve extrapolated to infinity. In the remaining 5 subjects the data could not be fitted to the curves

Parameter		c_{max} [ng/ml]	t_{max} [min]	$t_{½}$ [hour]	F – tot [ng · ml^{-1} · h]
ISDN	T_E	17.9 ± 3.1	10.0 ± 1.8	0.48 ± 0.13	9.0 ± 1.5
	B_1	3.04	16.2	0.25	1.8
	B_2	31.59	3.7	0.75	19.3
	B_3	13.09	12.7	0.48	7.3
2-ISMN	T_E	9.0 ± 0.8	52.1 ± 9.8	1.76 ± 0.15	32.8 ± 3.6
	B_1	4.79	111.3	1.56	19.8
	B_2	9.69	70.7	1.83	42.9
	B_3	7.96	50.4	1.84	31.8
5-ISMN	T_E	38.4 ± 3.4	87.6 ± 14.6	7.56 ± 1.63	375.3 ± 35.0
	B_1	28.03	144.5	4.16	242.12
	B_2	39.42	89.7	8.12	453.69
	B_3	32.42	104.0	12.72	565.25

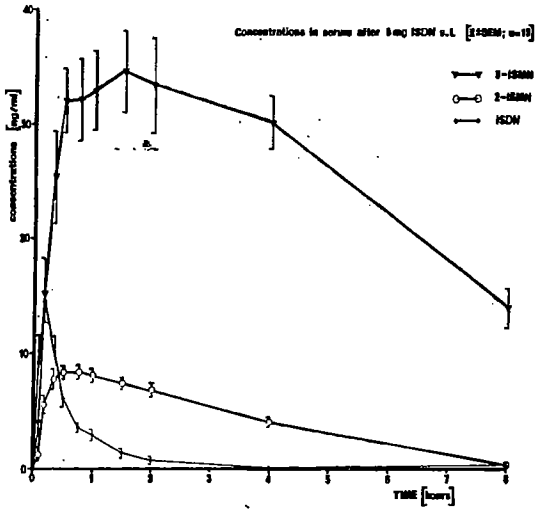


Fig. 1. Average serum concentrations (x ± SEM) of isosorbide dinitrate (ISDN), 2-exo-isosorbide mononitrate (2-ISMN) and 5-endo-isosorbide mononitrate (5-ISMN) after administration of isosorbide dinitrate 5 mg (Corvitin® rapid) s. l. to the 15 human subjects who reacted normally to it

Pharmacodynamic Data Including Data from Fitted Curve

No changes were observed in the amplitude of PDG either after placebo or during the pre-drug period. The amplitude increase from 6.0 ± 1.2 mm to 19.1 ± 2.9 mm after about 4 min post ISDN administration and reaching its maximum value within 14.1 ± 1.3 min, then declining rapidly afterwards. By the two-hour time-point, 9 out of the 18 subjects had returned to baseline (Table 2, Figure 2).

Table 2. Pharmacodynamic parameters of the first derivative of digital plethysmography (DPG) after ISDN 5 mg s. l.; a_0 = average value from six measurements of the a-wave during the predrug period, a_{max} = maximal amplitude in the postdrug period, t_{max} = time of maximal amplitude

Parameter		a_0 [mm]	a_{max} [mm]	t_{max} [min]
DPG	T_F	6.0 ± 1.24	19.1 ± 2.91	14.1 ± 1.25
	B_1	3.62	20.35	47.3
	B_2	20.83	39.96	12.1
	B_3	5.63	20.14	21.0

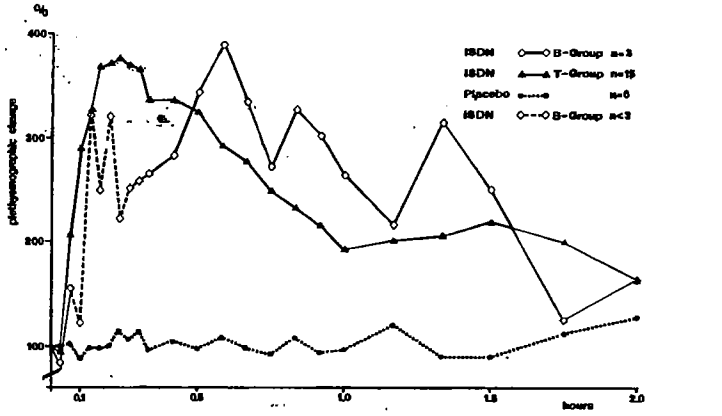


Fig. 2. Mean finger plethysmographic amplitude changes, expressed as per cent of the predrug level, during the 2 h after administration of ISDN or placebo. The B-group represents subjects suffering from intermittent bradycardia, and T-group individuals with no unexpected cardiovascular reactions. As can be seen from the graph (◇—◇), measurements could only be made in part during severe hypotension and bradycardia (from 6 to 18 min after administration)

SBP dropped within 8 min from 116.0 ± 2.9 mmHg to 108.0 ± 2.0 mmHg and HR rose from 63.0 ± 2.1 to 75.6 ± 2.7 beats/min with no change observed after placebo. Schellog's test was positive in 13 of 18 volunteers 2 hours after the administration of ISDN with 4 subjects still testing positive 4 and 8 hours post-dose.

Relationship Between Serum Concentration Time-Course and PDG Measurements

Concentration and effect was fitted to a polynomial curve in 10 subjects. The curve of the DPG measurements did not correlate with ISDN plasma concentrations or its metabolites. Different weighing factors for possible intensities of effect of the 3 moieties were also used in a superimposed curve; but no correlation was discovered either. In fact, the times for the high concentrations of 2- and 5-ISMN occurred after DPG had returned to baseline (Figures 1 and 2).

In conclusion, no correlation could be made between ISDN, 2-, and 5-ISMN and changes in PDG amplitude. ISDN did lower blood pressure and increase HR in most subjects; but 3 subjects where severe hypotension occurred with severe, reversible bradycardia.

REVIEWER'S COMMENTS:

1. Data from five subjects was not used because it could not be fitted to a concentration time curve equation. All data should have been plotted for visual examination.
2. The absence of a PK/PD correlation remains unclear and further studies would be necessary in the targeted population to make such a claim.

11. PHARMACOKINETICS AND HAEMODYNAMIC EFFECTS OF ISDN FOLLOWING DIFFERENT DOSAGE FORMS AND ROUTES OF ADMINISTRATION. Vogt D., et.al., Eur. J. Clin. Pharmacol. 1994; 46: 319-324.

ISDN pharmacokinetics and pharmacodynamics were explored following the administration of single doses of ISDN sublingual spray (2.5 mg, SLS), a sublingual tablet (5 mg, SL), and per oral tablet (10 mg, PO) after an overnight fast in 16 healthy male volunteers (mean age 25 ± 3 years). Intra-individual differences were explored in this randomized, placebo-controlled, double-blind, four-way, cross-over study with a minimum of 5 a day wash-out period between treatments. Haemodynamic quantification was performed using the a/b ratio of the finger pulse wave (a=height of the systolic peak and b=the high of the dicrotic wave), SBP, and heart rate (HR) under orthostatic conditions. Pharmacokinetic blood sampling and finger pulse recordings were performed at baseline and at 1, 3, 5, 7.5, 10, 15, 20, 30, 60, 90, 120, 150, 180, 240 and 360 min post drug administration. Blood pressure and HR was determined using an orthostatic challenge after rising from the supine position, at 5 and 10 min after drug administration and then during the times of blood sampling. Determination of the a/b ratio was calculated by taking the mean of 10 consecutive waves. Pharmacokinetic parameters calculated are illustrated in Table 1 below.

Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations were analyzed by gas-liquid chromatography with an electron capture detector. The lower limit of detection was about 0.5 for ISDN, 1 for 2-ISMN, and 5 ng/mL for 5-ISMN.

Testing for period and sequence effects was performed through a two-way analysis of variance. Differences in PK and PD parameter comparisons was through application of the Wilcoxon-Pratt-test with $p<0.05$ being statistically significant.

The C_{max} of SLS ISDN was higher than the other two formulations (39 ng/mL versus 22.8 and 16.9 ng/mL for SL and PO, Table 1) and t_{max} was shortest with the SLS formulation. The ISDN AUCs of all formulations were similar. However, the AUCs for 2- and 5-ISMN increased from the SLS to SL and the highest being PO (Table1). Metabolite plasma concentrations increased in proportion to the increase in dose indicating that the fraction of the dose absorbed was similar for the three formulations; but first-pass metabolism increased from SLS<SL<PO.

Table 1. Pharmacokinetic parameters of ISDN and metabolites following administration of ISDN as sublingual spray, sublingual tablet, and peroral tablet (mean with (SD), $n = 16$)

Parameter	Sublingual spray 2.5 mg	Sublingual tablet 5.0 mg	Peroral tablet 10.0 mg
$t_{lag,\ ISDN}$ (min)	1.31 (0.89)	5.48 (1.75)*	10.0 (4.1)*,**
$t_{max,\ ISDN}$ (min)	3.9 (1.0)	13.8 (12.8)*,**	25.6 (12.8)*,**
$C_{max,\ ISDN}$ (ng·ml^{-1})	39.0 (11.6)	22.8 (13.3)*	16.9 (7.9)*,**
AUC_{ISDN} (µg·ml^{-1}·min)	1.03 (0.273)	0.879 (0.290)	0.997 (0.233)
$AUC_{IS-2-MN}$ (µg·ml^{-1}·min)	1.09 (0.166)*	2.68 (0.485)*	5.67 (0.702)*,**
$AUC_{IS-5-MN}$ (µg·ml^{-1}·min)	14.3 (3.47)*	27.3 (5.29)*	59.6 (15.9)*,**
$AUC_{total\ metab.}$ (µmol·l^{-1}·min)	85.1 (18.9)*	160.3 (30.1)	345.6 (83.3)
Clearance/f_{ISDN} (l·min^{-1})	2.7 (1.2)	6.3 (2.2)	10.6 (2.6)
f_{ISDN} (%)	~100[b]	46 (22)	27 (15)

* $P < 0.01$ vs. subl. spray; ** $P < 0.01$ vs. subl. tablet
[a] $n = 15$; since in one subject metabolite concentrations were below the detection limit
[b] Based on comparison with literature data where clearance values of 1.6–4.1 l·min^{-1} were obtained following intravenous infusion of ISDN (Morrison et al. 1983a, 1983b; Platzer et al. 1982; Taylor et al. 1982; Abshagen et al. 1985; Straehl and Galeazzi 1985)

The maximal effect of the a/b ratio of the finger pulse was highest (e_{max} of 130%) and the time to e_{max} shortest (te_{max} of 16.6 min) for the spray when compared to the SL or PO formulations (Table 2). The total effect per dose (AUEC) and duration of effect were similar between the 3 formulations. The PO route had the largest decrease in SBP, increase in HR, and longest duration despite the similar ISDN concentrations (Figure 1) indicating the possible contribution of ISDN's metabolites.

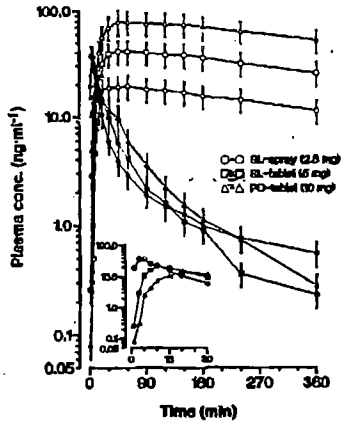


Fig. 1. Plasma concentration-time course of ISDN *(closed symbols)* and its metabolite IS-5-MN *(open symbols)* following administration of a single dose of ISDN as 2.5 mg sublingual spray *(circles)*, 5 mg sublingual tablet *(squares)* and 10 mg peroral tablet *(triangles)*. Mean values with SEM of 16 subjects. *Inset:* Mean plasma concentrations of ISDN during the first 30 minutes after administration of the three dosage forms

Table 2. Haemodynamic measurements (a/b-ratio of finger pulse wave, systolic blood pressure and heart rate in orthostasis) and derived parameters following administration of ISDN as sublingual spray, sublingual tablet and peroral tablet (mean with (SD))

Parameter	Sublingual spray 2.5 mg	Sublingual tablet 5.0 mg	Peroral tablet 10.0 mg
a/b-ratio			
e_{max} (%)	130 (89.6)	84.8 (50.0)	90.2 (60.6)*
t_{max} (min)	16.6 (21.6)	27.5 (24.0)	31.3 (21.2)*
Onset (min)	3	5	7.5
Duration (min)	120	120	120
$AUEC_{0\rightarrow 120}$ (% · min)	3.220 (2.400)	4.390 (4.120)	4.350 (4.300)
Heart rate following orthostatic challenge			
e_{max} (%)	41.0 (22.7)	40.3 (20.2)	48.4 (25.4)*
t_{max} (min)	17.5 (17.9)	19.4 (20.4)	34.1 (32.3)*,**
Onset (min)	5	5	5
Duration (min)	90	120	150
$AUEC_{0\rightarrow 120}$ (% · min)	1.540 (2.480)	1.950 (2.540)	3.270 (2.710)*,**
Systolic blood pressure following orthostatic challenge			
e_{max} (%)	−21.2 (8.4)	−22.2 (8.3)	−25.9 (11.0)
t_{max} (min)	16.3 (8.3)	26.3 (17.7)*	30.0 (19.2)*
Onset (min)	5	10	5
Duration (min)	30	30	60
$AUEC_{0\rightarrow 120}$ (% · min)	357 (1.070)	455 (824)	946 (761)*,**

* $P < 0.05$ vs. subl. spray; ** $P < 0.05$ vs. subl. tablet

Orally administered ISDN produced greater SBP and HR AUECs; but not for the a/b ratio of the finger pulse. This may be due to sensitivity differences of venous and arterial blood vessels to nitrates.

REVIEWER'S COMMENTS:

1. The reviewer concurs.

12. CHRONOPHARMACOLOGY OF ORAL NITRATES IN HEALTHY SUBJECTS. Scheidel B., et.al., Chronobiology International 1991; 8 (5): 409-419.

The pharmacokinetics and pharmacodynamics (blood pressure, HR) were explored following the administration of a single dose of ISDN (20 mg) after an eight-hour fast in 6 healthy male volunteers (age 24 – 45 years) in order to explore whether time of day of drug administration influences the PK or PD of ISDN and 5-ISMN. Two additional studies were conducted with other formulations of 5-ISMN (IR and SR); which will not be reviewed since it has no relevance to the NDA. ISDN was administered at 0800 and at 2000 hours. Thirteen blood samples were taken in the supine position up to the 12 hour time point. Pharmacokinetic parameters calculated are depicted in Table 1 below. Haemodynamic effects were measured in the supine position after standing for 3 minutes. The effects were calculated as the difference in the individual circadian control values determined under identical conditions. The maximum effect (E_{max}) and the time to E_{max} were evaluated.

Analysis of plasma ISDN, and 5-ISMN concentrations were analyzed by a capillary gas chromatographic method. Statistical evaluations were performed using the Newman-Keuls test.

No differences between the morning and evening dose could be observed (Figure 1). However, the AUC was statistically different ($p<0.05$) between morning and evening administration for ISDN (33.9 at 0800 hours versus 25.4 ng/mL/h at 2000 hours). There were no statistically significant differences in C_{max}, t_{max} , and $t_{1/2}$ of ISDN nor in the pharmacokinetics of 5-ISMN.

TABLE 1. *Pharmacokinetic parameters of ISDN and the main metabolite IS-5-MN (mean ± SD, n = 6, median in parentheses) after administration of the immediate-release formulation (20 mg) in the morning or evening*

	AUC (ng/ml/h)	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2}$ (min)
ISDN				
08:00 h	33.9 ± 17.1[a] (28.7)	30.7 ± 18.6 (28.8)	47 ± 34 (30)	38 ± 16 (37)
20:00 h	25.4 ± 11.0 (26.2)	25.3 ± 16.0 (27.2)	45 ± 26 (38)	64 ± 29 (59)
IS-5-MN				
08:00 h	1,263 ± 253 (1,343)	206 ± 42 (205)	2.1 ± 2.0 (1.5)	6.7 ± 4.1 (5.4)
20:00 h	1,183 ± 109 (1,130)	201 ± 22 (207)	1.6 ± 1.3 (1.1)	6.4 ± 2.6 (6.9)

Data from refs. 16 and 17.
[a] Statistically significant between 08:00 and 20:00 h ($p < 0.05$).

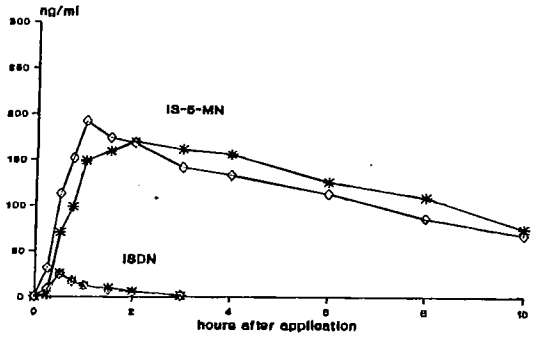


FIG. 1. Mean (n = 6) plasma concentration–time curves of ISDN and the metabolite IS-5-MN after administration of the immediate-release formulation (20 mg) in the morning or evening. –•–, 08:00 h; -◇-, 20:00 h. Data from refs. 16 and 17.

Effects (decrease in SBP, $p<0.05$) were more pronounced in the evening as compared to morning administration (Table 2). T_{max} did not change with time of day. There did not seem to be any haemodynamic differences in the morning dose compared to the evening dose when time to peak effect (T_{max}) and time to peak concentration (t_{max}) was compared (Figure 2).

TABLE 2. *Hemodynamic effects of ISDN (mean ± SD, n = 6, median in parentheses) after administration of the immediate-release formulation (20 mg) in the morning or evening*

	BP_{Sys}		BP_{Dia}		HR	
	E_{max} (mm Hg)	T_{max} (h)	E_{max} (mm Hg)	T_{max} (h)	E_{max} (beats/min)	T_{max} (h)
08:00 h	−22 ± 8[a] (20)	0.6 ± 0.2 (0.7)	−16 ± 10 (13)	0.6 ± 0.1 (0.7)	23 ± 9 (20)	0.8 ± 0.2 (0.9)
20:00 h	−31 ± 11 (36)	0.8 ± 0.3 (0.9)	−23 ± 9 (19)	1.2 ± 0.7 (0.9)	25 ± 10 (30)	0.7 ± 0.2 (0.6)

Shown are peak effects (E_{max}) and time to peak effects (T_{max}) in blood pressure (BP) decrease and heart rate (HR) increase in comparison to circadian control values.
Obtained after 3 min of standing upright.
[a] Statistically significant between 08:00 and 20:00 h ($p < 0.05$).

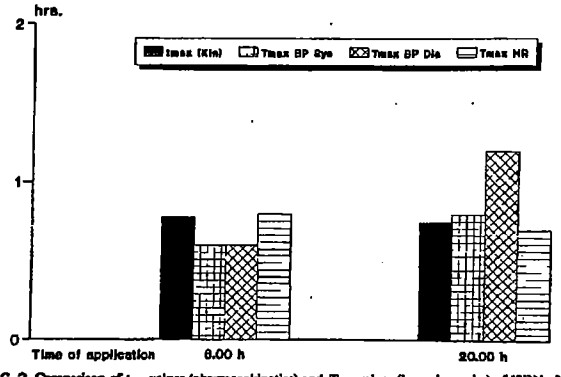


FIG. 2. Comparison of t_{max} values (pharmacokinetics) and T_{max} values (hemodynamics) of ISDN after administration of the immediate-release formulation (20 mg) in the morning or evening (median, n = 6).

Drug-induced haemodynamic effects occurred earlier than maximum drug concentrations when ISDN was given in the evening.

In conclusion, the time of day of drug administration does influence the PK and PD of ISDN when given as a single dose. However, the changes observed are not of such magnitude that advising specified time of day drug administration in the labeling is warranted.

REVIEWER'S COMMENTS:

1. The reviewer concurs that time of day specific drug administration is not warranted.

13. INFLUENCE OF EXERCISE ON PLASMA CONCENTRATIONS OF ISOSORBIDE DINITRATE. Bogaert M.G., et.al., Eur. J. Clin. Pharmacol. 1988; 35: 213-215.

ISDN is often administered in conjunction with exercise for diagnostic and therapeutic purposes. As a result, the influence of bicycle exercise on the pharmacokinetics of ISDN were investigated since ISDN is a high extraction drug and may be influenced by the decrease in hepatic blood flow that is typically observed during exercise. Seven healthy volunteers (6 M and 1F, aged 22- 25 years) were given a single 10 mg dose of ISDN after an overnight fast on two occasions (once on a rest day and once on an exercise day) with a one week wash-out period between treatments. All subjects were to exercise for one hour following a 45 minute rest post ISDN administration. None of the subjects were smokers nor taking any other medications. Blood samples were collected taken at baseline and then every 15 minutes for 2.5 hours with HR and blood pressure determinations at the same times. Pharmacokinetic parameters calculated were AUC, C_{max}, and t_{max}.

Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations were analyzed by a GC-EC method. Day-to-day reproducibility was 10% CV and accuracy 98 to 103%. Statistical evaluations on differences were performed by the Wilcoxon signed ranks test with significance of $p<0.05$.

No differences in the pharmacokinetics of ISDN between the rest and exercise day was observed (Figure 1 and Table 1). The metabolite concentrations did seem lower on the exercise day; but that difference was present prior to exercise initiation. No explanation for the differences in concentrations was evident to the authors.

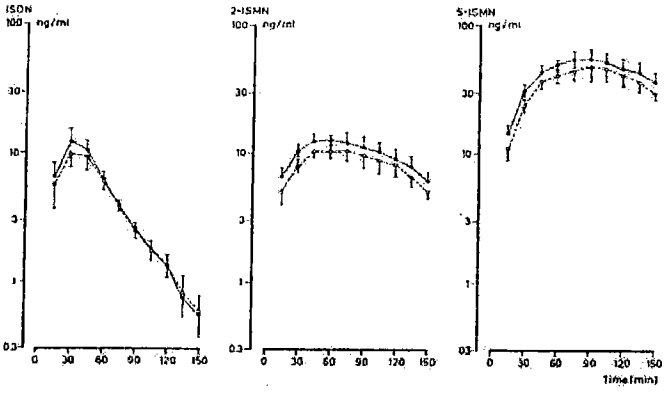


Fig. 1. Plasma concentrations of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of isosorbide dinitrate 10 mg at time 0 to 7 fasting volunteers on 2 different days. *Full line* - rest day; *interrupted line* - day with bicycle exercise from the 45 th to the 105 th min after intake. Means (± SEM) are given

Table 1. Pharmacokinetic parameters of isosorbide dinitrate (ISDN), isosorbide 2-mononitrate (2-ISMN) and isosorbide 5-mononitrate (5-ISMN) after oral administration of ISDN 10 mg

	Rest	Exercise
ISDN		
AUC ($ng \cdot ml^{-1} \cdot min$)	697.8 ± 100.1	618.9 ± 122.1
C_{max} ($ng \cdot ml^{-1}$)	13.0 ± 2.6	10.9 ± 1.9
t_{max} (min)	36.4 ± 3.0	36.4 ± 3.0
2-ISMN		
AUC ($ng \cdot ml^{-1} \cdot min$)	1441.2 ± 181.7	1213.8 ± 174.0[a]
C_{max} ($ng \cdot ml^{-1}$)	14.5 ± 2.1	13.0 ± 2.1
t_{max} (min)	51.4 ± 7.2	55.7 ± 7.8
5-ISMN		
AUC ($ng \cdot ml^{-1} \cdot min$)	6237.8 ± 858.3	5299.9 ± 815.3[a]
C_{max} ($ng \cdot ml^{-1}$)	61.3 ± 11.2	54.8 ± 11.3
t_{max} (min)	79.3 ± 6.3	75.0 ± 10.3

Mean values (± SEM) for 7 volunteers; [a] $p < 0.05$ significance of difference between results on exercise and rest days

In conclusion, exercise does not seem to have a pharmacokinetic effect on ISDN or its metabolites.

REVIEWER'S COMMENTS:

1. The reviewer concurs.

14. CONCENTRATIONS OF ISOSORBIDE DINITRATE, ISOSORBIDE-2-MONONITRATE, AND ISOSORBIDE-5-MONONITRATE IN HUMAN VASCULAR AND MUSCLE TISSUE UNDER STEADY-STATE CONDITIONS. Schneider, G., et.al., Eur. J. Clin. Pharmacol. 1990; 38: 145-147.

Eight male patients (aged 51 – 71 years, mean age 61 years) undergoing coronary bypass surgery were enrolled in a consecutive manner into this clinical trial. ISDN, 2-ISMN, and 5-ISMN concentrations were measured from plasma (PL), the saphenous vein wall (SV), and the pectoral muscle (PM). All subjects were pretreated with daily ISDN 240 mg doses (4 doses of 40 mg at 0600, 1000, 1400, and 1800 hours and then one 80 mg dose at 2200 hours). However, they had all been on chronic ISDN therapy where doses of 120 mg daily or higher was being taken. All patients were negative for signs of heart failure, liver disease, renal insufficiency, or other disorders that may interfere with the study's outcome. Concomitant therapy was discontinued after the evening dose the day before surgery. No one was taking digitalis, aspirin, or diuretics. Surgery was performed between 0800 and 1000 hours. As a result, 10 to 12 hours elapsed since the last ISDN dose.

Pectoral muscle or saphenous vein tissue (150 – 350 mg) was taken from the surgical site and frozen immediately in liquid nitrogen and stored. When analysis took place for ISDN tissue concentrations, the tissue was thawed and blotted dry on filter paper before weighing so that 100 mg of tissue could be measured and then chopped and homogenized by hand for 5 minutes. Determination of ISDN and metabolite concentrations was measured in a similar fashion to plasma described below. Slopes and intercepts of the tissue were almost identical to those obtained for human plasma.

Analysis of plasma ISDN, 2-ISMN, and 5-ISMN concentrations were analyzed by a GC method. Recovery was 66% and the CV for within-day and between-day variability were usually <10%.

ISDN mean concentrations in tissue were higher than in plasma (Figure 1, Table 1) with molar concentration ratios of 4.9 (PM/PL) and 7.21 (SV/PL). However, the mononitrate concentrations in plasma and tissues were virtually the same (ratios of 0.88, PM/PL and 0.99 SV/PL for 2-ISMN; 0.85 PM/PL and 1.06 SV/PL for 5-ISMN).

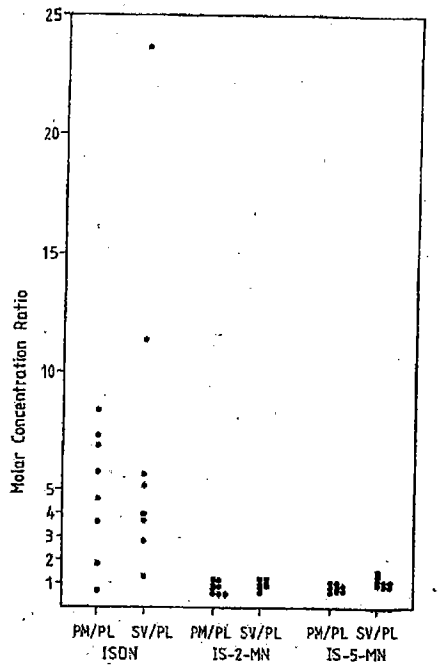


Fig. 1. Molar concentration ratios (muscle/plasma: PM/PL; vein wall/plasma: SV/PL) for ISDN, isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) in 8 patients pretreated with 240 mg ISDN daily for 2 days

Table 1. Concentrations of ISDN, IS-2-MN and IS-5-MN in plasma, saphenous vessel wall and pectoral muscle (upper part) and (tissue/plasma) molar concentration ratios (lower part) in patients undergoing coronary bypass surgery. $n = 8$; mean and (SD)

	Concentration		
	Plasma nmol/l ($n=8$)	Pect. muscle nmol/kg ($n=8$)	Saphen. vein nmol/kg ($n=8$)
ISDN	26.3 (10.9)	123 (83.3)	176 (153)
IS-2-MN	178 (61.8)	153 (62.9)	157 (87.2)
IS-5-MN	2703 (647)	2251 (412)	2797 (559)
	Molar Concentration Ratio		
	Pectoral muscle/ Plasma		Saphenous vein/ Plasma
ISDN	4.90 (2.70)		7.21 (7.28)
IS-2-MN	0.88 (0.23)		0.99 (0.20)
IS-5-MN	0.85 (0.12)		1.06 (0.22)

According to the authors, accumulation of ISDN in vessel walls may contribute to its greater vascular action; but may also facilitate the development of tolerance when administered chronically.

REVIEWER'S COMMENTS:

1. The reviewer concurs.

15. KINETICS OF ISOSORBIDE DINITRATE AND RELATIONSHIPS OF PHARMACOLOGICAL EFFECTS. Fung, H.-L., et.al., Br. J. Clin. Pharmacol. 1981; 11: 579-590.

Review of this article will only involve the portion of the study that investigated protein binding of ISDN since the remainder of the report involves topics that are already included in the package insert for ISDN. Plasma (fresh and previously frozen) from normal healthy volunteers and from four patients with chronic stable angina pectoris due to atherosclerotic heart disease was spiked with ISDN (concentration range 1 to 100 ng/mL) and the free fraction of ISDN was calculated.

Analysis of plasma ISDN concentrations were analyzed by a modified assay for nitroglycerin utilizing a GC method with an electron capture detector. Percent recovered at each concentration was about 92% with a CV% of 9.4%.

In 17 samples tested, the free fraction of ISDN was found to be a mean of 0.72 ± 0.12.

REVIEWER'S COMMENTS:

1. The reviewer concurs.

5.3 Cover Sheet and OCPB Filing/Review Form (2-3pages)

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	20727 Amendment 121	Brand Name	[ ] (BiDil)
OCPB Division (I, II, III)	I	Generic Name	Hydralazine/isosorbide dinitrate
Medical Division	Cardio-Renal Drug Products	Drug Class	Cardiac Perfomance improving drug
OCPB Reviewer	Peter Hinderling and Lydia Velazquez	Indication(s)	Treatment of CHF
OCPB Team Leader	Patrick Marroum	Dosage Form	Tablets
		Dosing Regimen	37.5 mg hydralazine/20 mg isosorbide dinitrate,
Date of Submission	July 30, 2004	Route of Administration	Oral
Estimated Due Date of OCPB Review	October 15, 2004	Sponsor	NitroMed
PDUFA Due Date	February 2, 2005	Priority Classification	P
Division Due Date	December 1, 2005		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies				
HPK Summary				
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:				
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				

fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -	[redacted]			
solution as reference:	x			
alternate formulation as reference:	x			
Bioequivalence studies -				
traditional design; single / multi dose:	X			
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:	X			
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies	[redacted]			

Filability and QBR comments

	"X" if yes	Comments
Application filable?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm?		Comments have been sent to firm (or attachment included). FDA letter date if applicable.
QBR questions (key issues to be considered	**Scarce Clinical Pharmacology data base, impact of most extrinsic and intrinsic factors on PK and PK-PD of hydralazine and isosorbide dinitrate after administration of [ ] unknown.**	

Other comments or information not included above	
Primary reviewer Signature and Date	**Peter H. Hinderling , 03-26-05**
Secondary reviewer Signature and Date	**Lydia V. Velazquez, 03-31-05**

CC: NDA 20727, HFD-860 (Electronic Entry), HFD-110(CSO), HFD-860(Marroum,Mehta, Rahman), CDR (B. Murphy)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Peter Hinderling
4/15/05 10:44:18 AM
BIOPHARMACEUTICS

Lydia Velazquez
4/15/05 10:49:01 AM
BIOPHARMACEUTICS

Patrick Marroum
4/15/05 10:53:51 AM
BIOPHARMACEUTICS

MAR 20 1997

Clinical Pharmacology/Biopharmaceutics Review

NDA: 20-727

ISDN/Hydralazine
Bidil
37/5/10, 37.5/20, 75/20 and 75/40 mg tablets
Medco Research.

Submission Date: July 3, 1996.
August 9, 1996
January 23, 1997
January 27, 1997
February 3, 1997
February 12, 1997

Reviewer: Patrick J Marroum.

Type of submission: New Drug Application.

SYNOPSIS:

The sponsor has conducted one pilot bioavailability study and a pivotal bioequivalency study to establish the link between the to be marketed formulation and the two clinical formulations used in VHEFT-I and VHEFT-II. The results of this pivotal study seem to indicate that the bioavailability of the Bidil formulation is lower than the capsule formulation used in VHEFT I but higher than the tablet formulation used in VHEFT II. The sponsor is currently conducting a study to characterize the steady state pharmacokinetics of Bidil at each proposed dose level. The sponsor however, did not characterize the effect of food on the bioavailability of either hydralazine or isosorbide dinitrate from this Bidil formulation.
The sponsor has adequately validated the assay used in the pivotal bioequivalence study.

RECOMMENDATION:

The studies and dissolution data submitted in this NDA for Bidil do not completely fulfill the Division of Pharmaceutical Evaluation I requirements.

BACKGROUND:

Bidil contains hydralazine hydrochloride, USP, a peripheral vasodilator with antihypertensive properties, and diluted isosorbide dinitrate, USP, an organic nitrate with vasodilator effects on both the arteries and veins. The sponsor intends to market Bidil as a fixed combinations of tablets in the following strengths:
37.5/10, 37.5/20, 75/20 and 75/40 mg hydralazine/isosorbide dinitrate.
Hydralazine is already available on the US market in dosage strengths of 10, 25, 50 and 100 mg.
Isosorbide dinitrate is available as 5, 10, 20, 30 and 40 mg tablets.
Medco is seeking the approval for Bidil for the treatment of chronic congestive heart failure as an adjunct to standard therapy (digitalis glycosides and diuretics) in patients who are intolerant or

have a contraindication to ACE inhibitors.
It is to be noted that the finished product is manufactured by Global Pharmaceuticals Inc. in Canada.

Note:In a meeting between Dr. Ludden Director Division of Biopharmaceutics and Dr. Lipicky, Director Division of Cardio-Renal Drug Products, which was held on October 28 1994, it was agreed that the firm would not be required to meet the strict 90 % confidence interval criteria that is usually required for bioequivalency due to I) the fact that both hydralazine and ISDN exhibit a large degree of variability, ii) a cross-over study design was not feasible as shown in Study CB01 due to large drop-out rate caused by side effects (mainly headaches). A parallel study design was therefore mutually agreed upon. Dr. Lipicky conveyed that as long as the sponsor is able to show that the bioavailability of the formulation they intend to market is similar to the bioavailability of the formulations tested in the clinical trials, this would be considered sufficient for meeting the bioequivalency requirement for the NDA.
Moreover, to cover the dose range that the sponsor intends to market, it was decided that the sponsor needed to conduct the bioavailability study on the lowest and highest strength. An in vivo bioavailability waiver could be granted if the two middle strength the 37.5/20 mg and 75/20 mg tablets exhibited similar dissolution profiles in several media to the lower and upper strengths that were tested in the pivotal relative bioavailability study.

TABLE OF CONTENTS:

SUMMARY OF BIOAVAILABILITY/PHARMACOKINETICS:

A-Relative Bioavailability:

Study CB-02 showed that the relative bioavailability of hydralazine from the low dose Bidil tablet relative to a solution was estimated to be 100 %. However, the high dose Bidil 75 mg/40 mg provided higher hydralazine bioavailability; 116 % relative to a 37.5 mg solution. This increased bioavailability is most probably due to saturation of the first pass metabolism for hydralazine especially in the slow metabolizing subjects that were used in this study.
The same trend of results were obtained for ISDN, its relative bioavailability from the low Bidil tablet relative to 10 mg ISDN in solution form was 95 % while the highest dose Bidil gave a relative bioavailability of 119 % most probably due to the non linearities introduced by the pre-systemic metabolism that ISDN is known to undergo.

B-Bioequivalence:

Study CB-02 showed that both the low and high dose Bidil formulation did not meet the criteria for bioequivalence to the two formulations used in VHEFTI and VHEFTII as far as hydralazine and ISDN are concerned. The Bidil formulation seems to have a lower bioavailability than the capsule formulation but gave higher plasma levels than the tablet formulation used in VHEFTII. A summary of the 90 % confidence intervals is given in Tables 1 to 4.

TABLE 1

Hydralazine	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil /tablet	1.47	0.89-2.4	1.25	0.99-1.58	1.56	1.11-2.19
Low dose Bidil /capsule	0.65	0.4-1.07	0.9	0.72-1.44	0.94	0.67-1.32
High dose Bidil /tablet	1.3	0.79-2.12	1.46	1.15-1.85	1.79	1.28-2.52
High dose Bidil /capsule	0.58	0.35-0.95	1.06	0.84-1.34	1.08	0.77-1.51

AUCR= AUC normalized to the AUC of the solution arm in Phase A of the study.
Note: The 90 % confidence intervals were constructed on the AUC and CMAX normalized by 65 kg of weight.

TABLE 2

ISDN	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil /tablet VHEFTII	1.21	0.86-1.71	1.12	0.98-1.27	0.97	0.82-1.15
Low dose Bidil /tablet VHEFTI	1.06	0.75-1.5	1.04.	0.92-1.19	0.98	0.83-1.17
High dose Bidil /tablet VHEFTII	0.82	0.59-1.15	1.41	1.24-1.6	1.12	0.95-1.32
High dose Bidil /tablet VHEFTI	0.72	0.51-1.01	1.32	1.16-1.5	1.13	0.96-1.34

TABLE 3

IS-2MN	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil /tablet VHEFTII	1.01	0.88-1.16	0.92	0.88-0.96	0.9	0.84-0.96
Low dose Bidil /tablet VHEFTI	0.95	0.83-1.09	0.93	0.89-0.97	0.95	0.88-1.01
High dose Bidil /tablet VHEFTII	0.85	0.74-0.98	1.1	1.05-1.15	1.07	1.0 -1.14
High dose Bidil /tablet VHEFTI	0.8	0.70-0.92	1.11	1.06-1.17	1.12	1.05-1.2

TABLE 4

IS-5MN	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil /tablet VHEFTII	0.98	0.88-1.1	0.93	0.87-1	0.88	0.81-1.1
Low dose Bidil /tablet VHEFTI	0.94	0.84-1.06	0.92	0.86-0.99	0.95	0.88-1.03
High dose Bidil /tablet VHEFTII	0.81	0.73-0.91	0.96	0.89-1.02	0.91	0.84-0.98
High dose Bidil /tablet VHEFTI	0.78	0.69-0.87	0.95	0.89-1.02	0.98	0.91-1.06

C-Food Effect:

The sponsor did not study the effect of food on this fixed combination of hydralazine and ISDN. It is to be noted that during several teleconference calls, it was conveyed to the sponsor that a food effect study on the Bidil formulation should be conducted. Instead of performing a food effect study (the sponsor claimed that the food effects have already been characterized for hydralazine and ISDN) the sponsor opted to provide literature articles on the effects of food on hydralazine and ISDN, in support of information related to this issue.

Upon review of the literature articles submitted by the firm, no definitive conclusion can be drawn regarding the effect of food on hydralazine. Of four food-effect references for hydralazine, 3 seemed to show a decrease in AUC and Cmax while 1 showed a 2-3 fold increase in both AUC and Cmax. The current PDR labeling states "Administration of hydralazine with food results in higher plasma levels." The sponsor is requesting to incorporate "Peak serum concentrations and AUC are reduced when hydralazine is administered comcomitantly with food." In view of the approved Apresoline (approved hydralazine) labeling and literature provided, no definite conclusion can be made regarding the effect of food on hydralazine from Bidil. One review article on ISDN indicated no change in AUC and a decrease in Cmax when administered with food. ISDN labeling is silent regarding this information. Based on the above information, a definite conclusion regarding effect of food on Bidil cannot be drawn.

PHARMACOKINETICS:

Single Dose:

The single dose pharmacokinetics of hydralazine after oral administration of Bidil were

characterized by a high degree of intersubject variability (% CV values as high as 93 %) due to the extensive polymorphic phenotype-dependent acetylation that it undergoes. A summary of the mean pharmacokinetic parameters is presented in Table 4 in Appendix I.
ISDN was rapidly absorbed after single dose administration of the Bidil formulation with a TMAX of 0.6 hour. The plasma oral clearance after administration of 37.5/10 mg tablet as estimated to be 528 l/hr/65 kg while the half-life was estimated to be 1.3 hrs. CMAX was in the order of 30 ng/ml/65 kg.

Multiple dose:

The sponsor is currently conducting a multiple dose study in patients with congestive heart failure whereby each subject will be dosed to steady state in a dose escalation design until all the 4 different strengths are tested up to the highest tolerated dose. Full steady state pharmacokinetic profile will be obtained for each dose strength tested.

FORMULATIONS:

A summary of the compositional formula for the 4 strengths of the Bidil tablets can be found in Appendix II. The 75/40 mg tablet is compositionally proportional to the 37.5/20 mg tablet and the 75/20 mg tablet is exactly double in composition to the 37.5/10 mg tablet.

DISSOLUTION:

The sponsor is proposing a dissolution method using USP Apparatus I at a speed of 100 rpm in 900 ml of 0.05 N HCl. A specification of not less than [ ] at 30 minutes for hydralazine and not less than [ ] for ISDN in [ ] minutes were requested by the sponsor.
The dissolution method proposed by the sponsor is acceptable with the following dissolution specifications: not less than [ ] in 30 minutes for both hydralazine and ISDN.

ASSAY:

Unchanged Hydralazine was assayed using HPLC with UV detection at 365 nm. The internal standard was 4-methyl hydralazine. Hydralazine was derivatized by adding p-nitrobenzaldehyde to form the corresponding hydrazone which is subsequently measured.
Plasma samples were analyzed for ISDN, IS-5MN and IS-2MN by a modified gas chromatography electron capture method. Overall, the analytical validation for hydralazine, ISDN and its two active metabolites IS-5MN and IS-2MN was satisfactory.

COMMENTS TO BE SENT TO THE FIRM:

1-The number of subjects per treatment group (18 per arm) might not provide the study with enough power to detect differences in formulations among the four different treatments due to the very high variability in pharmacokinetic parameters.

2-The results of the study seem to indicate a trend that the Bidil formulation has lower bioavailability than the capsule formulation used in VHEFTI but is more bioavailable than the tablet formulation used in VHEFTII.

3-The clinical significance of this difference in bioavailability between the two formulations used in both clinical trials is unknown from the efficacy stand point. Note that the clinical formulation in VHEFTI had a higher relative bioavailability than the tablet formulation used in VHEFTII.

4-The sponsor constructed the 90 % confidence interval on both AUC and CMAX normalized to 65 kg of weight. However, sponsor did not provide the needed confidence intervals on the unnormalized CMAX and AUC. Normalizing the data to weight would tend to reduce variability in the results and would increase the probability of meeting the criteria for the confidence intervals.

5- The firm's proposal for inclusion of information regarding food-effect on hydralazine and ISDN based on published literature cannot be accepted. The labeling should state "No information is currently available regarding the effect of food on Bidil."

6-The dissolution performance of hydralazine and ISDN for both the lots that were used in the pivotal bioequivalence study at the 15 minute time point was erratic and characterized by a relatively high %RSD as high as 45 % in some instances. However, the process optimization batches showed a much more rapid and complete dissolution with %RSD lower than 5 %.

7- In view of the fact that 37.5/10 mg tablet showed a slower dissolution performance compared to 37.5/20, 75/20 and 75/40 in all 4 media tested, an in-vivo bioavailability waiver cannot be granted for the 2 middle strengths (37.5/20, 75/20). However, the sponsor is currently conducting a multiple dose study on all proposed strengths in CHF patients. Approval of the middle strenghts will be based on the outcome of this study.

8-Based on the dissolution data submitted by the sponsor, the following dissolution method and specifications are recommended for Bidil:
USP Apparatus I (basket) at a speed of 100 rpm
Medium: 0.05 N HCL

Hydralazine and ISDN, not less than [ ]in 30 minutes.

9- When the sponsor, was asked to provide the pharmacokinetic parameters for study CB02 in electronic form on diskette, the sponsor only submitted the data for the normalized parameters to a weight of 65 kg. When the accuracy of the data was checked by taking the raw data presented in the NDA and normalizing to a weight of 65 kg, the data found in the NDA and the data in the diskettes did not match. The two data sets were of by a certain factor that the sponsor could not explain. Therefore, for future submissions, the sponsor is asked to validate all data sets before submition to the Agency.

DEFICIENCIES:

1-The sponsor failed to show that to be market Bidil formulation is similar in bioavailability to either the formulations used in VHEFTI or VHEFTII. The results of the pivotal bioequivalence study CB02 seem to indicate that these differences are formulation related. The point estimates for the mean pharmacokinetic parameters in some instances differ by almost 50 % (e.g the ratio of the low dose Bidil to the hydralazine tablet AUC is 1.56 and for CMAX is 1.47).

Patrick J Marroum Ph.D

3/19/1997

RD/FT initialed by Ameeta Parekh Ph.D. 3/20/97

Clinical Pharmacology/Biopharmaceutics Briefing on 2/26/1997 (Malinowski, Mehta, Parekh, Marroum, M.L. Chen, Ganley, S. M. Huang, Lazor, Balch)

cc: NDA 20727, HFD 110, HFD 860 (Marroum), Barbara Murphy (CDER document room, Pkln) , HFD 340 (Viswanathan).

APPENDIX I

The 36 hour relative bioavailability of Bidil, a fixed combination of hydralazine/ISDN, compared to equivalent doses of reference products.

STUDY: CB-01. Volume:1.10 Pages:1-277

Investigators:

Clinical: [redacted]

[redacted]

Objectives:

1- To compare the relative bioavailability of a fixed combination formulation containing 75/40 mg hydralazine/ISDN vs two reference products of hydralazine (2x37.5 mg tablets or 2x37.5 mg capsules) coadministered with a 40 mg total dose of ISDN (2x20 mg tablets).

2-To conduct a pilot study to demonstrate the feasibility of continuing to a larger study.

3-To verify sample size for conducting a larger study.

Formulation:

-Bidil 75/45 mg tablets batch # F-032-044 manufactured by [redacted]
-20 mg ISDN tablet batch # 9933060 manufactured by Wyeth Ayerst.
-37.5 mg hydralazine tablets batch # E-15119 manufactured by Ciba-Geigy.
-37.5 mg hydralazine capsules batch # E-15358 manufactured by Ciba-Geigy.

Study Design:

This was a single center, open-label, randomized, three treatment, three period crossover study. 12 male or female healthy volunteers between the ages of 18-40 years who met the inclusion exclusion criteria were randomly assigned to one of the three treatment sequences to allow for at least 8 evaluable subjects completing the study.
In a crossover design, each subject received the three treatments shown below:
-Bidil tablets 75/40 mg.
-2x37.5 mg hydralazine tablets +2x20 mg ISDN tablets (V-HeFT II material).
-2x37.5 mg hydralazine capsules+2x20 mg ISDN tablets (V-HeFT I material).
The subjects were dosed with their assigned formulation beginning at 8:00 AM.
12 ml of blood was collected at 0, 10, 30, and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24

and 36 hours post dose administration.

Assay:

Unchanged Hydralazine was assayed using HPLC. Plasma samples were analyzed for ISDN, IS-5MN and IS-2MN by a modified gas chromatography electron capture method.
No assay description or validation data was included in the study report.

Data Analysis:

A poly exponential or noncompartmental pharmacokinetic method, whichever is appropriate will be applied to determine the pharmacokinetic profile of the parent drugs and their metabolites.

Results:

Following completion of the first treatment period 9 out of 12 subjects refused to return for crossover into the next treatment period due to adverse events. Therefore, the study was prematurely terminated.
Table 1 summarizes the main pharmacokinetic parameters for hydralazine while Table 2 gives the same parameters for ISDN and its 2 metabolites.. Figures 1 to 4 show the mean plasma concentrations of hydralazine, ISDN and its two metabolites for all treatments.

Conclusion:

Since this study was interrupted prematurely, no definite conclusions could be drawn by this reviewer.

Text Table 1
Summary of Pharmacokinetic Parameters of Unchanged Hydralazine by Treatment Group

Parameter	Treatment A Mean (SD) N=4	Treatment B Mean (SD) N=4	Treatment C Mean (SD) N=4
AUC (ng•hr/ml)	65.93 (41.59)	63.78 (32.97)	49.03 (38.42)
C_{max} (ng/ml)	59.28 (48.58)	64.28 (54.89)	95.50 (84.88)
T_{max} (hr)	1.63 (1.09)	1.00 (0.41)	1.21 (1.21)
$t_{\frac{1}{2}}$ (hr)	4.11 (1.53)	3.47 (1.80)	3.18 (1.92)
K_{el} (/hr)	0.19 (0.07)	0.27 (0.19)	0.30 (0.20)
Cl/F (l/hr)	2796.25 (3763.66)	1673.00 (1354.87)	4578.00 (6253.32)

Note: These data were derived from Table 2.0 (section 10). Treatment A=BiDil™ tablet; treatment B=hydralazine tablets/ISDN tablets; treatment C=hydralazine capsules/ISDN tablets.

Text Table 2
Summary of Pharmacokinetic Parameters of ISDN, IS–2–MN, and IS–5–MN by Treatment Group

Parameter	Moiety	Treatment A Mean (SD) N=4	Treatment B Mean (SD) N=4	Treatment C Mean (SD) N=4
AUC (ng•hr/ml)	ISDN	104.93 (34.79)	121.18 (44.34)	90.50 (18.34)
	IS–2–MN	395.27 (126.58)	441.47 (171.13)	366.40 (94.08)
	IS–5–MN	3544.57 (1284.29)	3532.25 (1477.21)	3628.82 (880.20)
C_{max} (ng/ml)	ISDN	56.45 (29.55)	67.83 (5.82)	77.05 (42.50)
	IS–2–MN	77.00 (30.92)	102.35 (25.56)	97.10 (30.81)
	IS–5–MN	398.32 (180.07)	395.42 (110.40)	450.62 (127.45)
T_{max} (hr)	ISDN	0.94 (0.38)	0.65 (0.29)	0.52 (0.17)
	IS–2–MN	1.81 (0.94)	1.31 (0.55)	1.13 (0.60)
	IS–5–MN	2.88 (1.03)	2.38 (0.75)	1.50 (0.41)
K_{el} (/hr)	ISDN	0.53 (0.10)	0.75 (0.35)	0.65 (0.26)
	IS–2–MN	0.30 (0.05)	0.29 (0.03)	0.31 (0.01)
	IS–5–MN	0.15 (0.01)	0.13 (0.02)	0.13 (0.02)

Note: These data were derived from Table 2.0 (section 10). Treatment A=BiDil™ tablet; treatment B=hydralazine tablets/ISDN tablets; treatment C=hydralazine capsules/ISDN tablets.

Figure 1
Mean Hy...lazine Concentrations

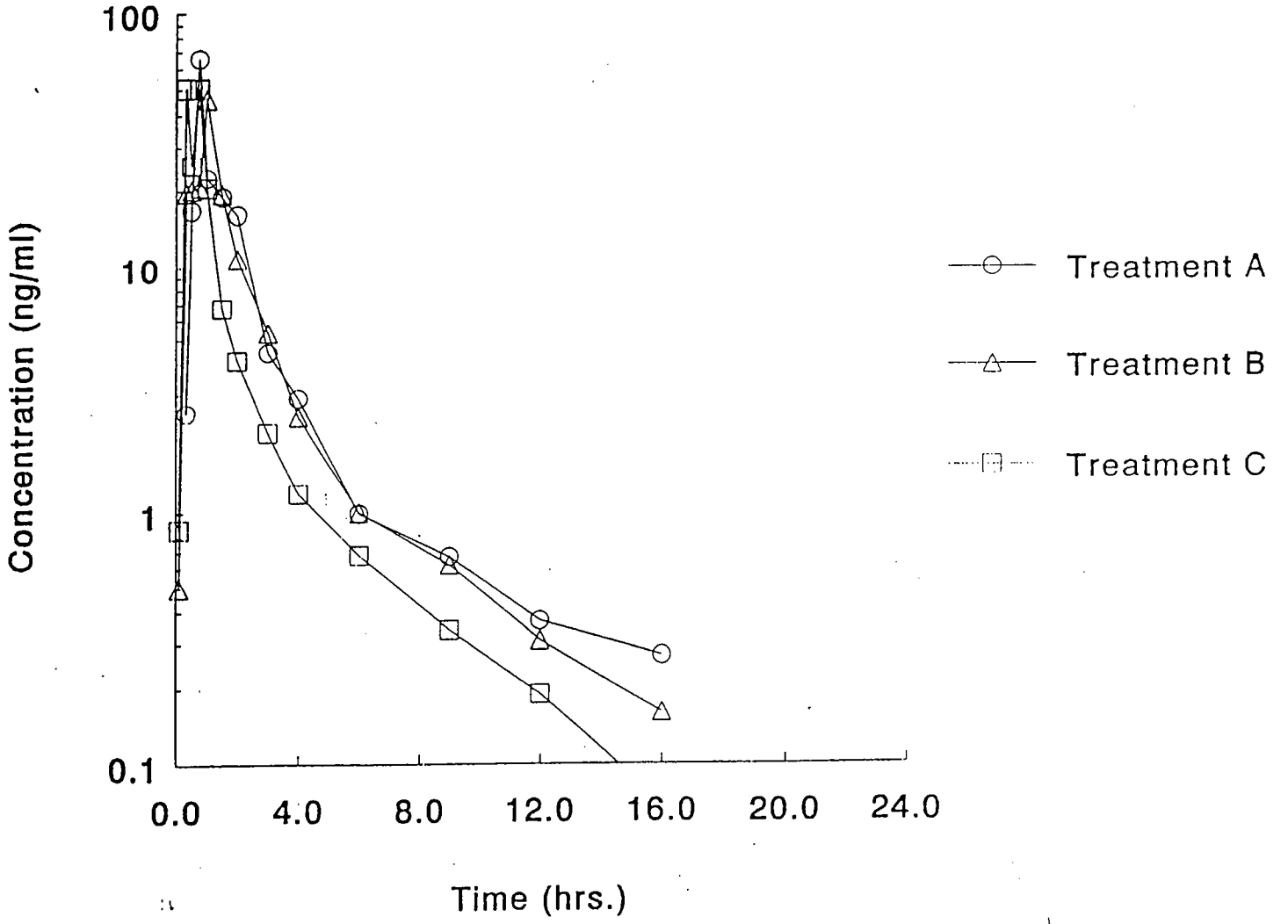
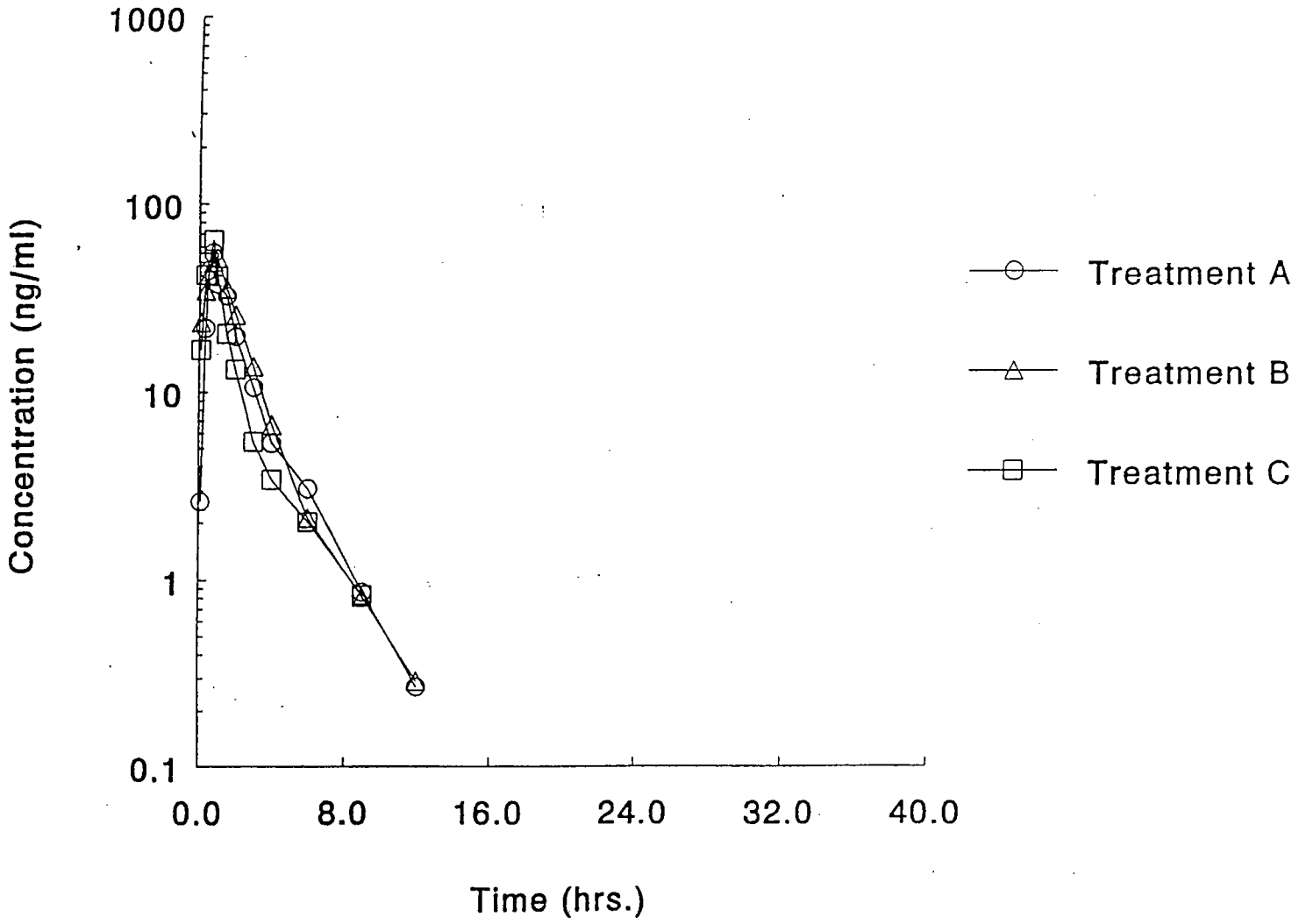


Figure 3
Mean orbide Dinitrate



Mean Isc de 5-Mononitrate

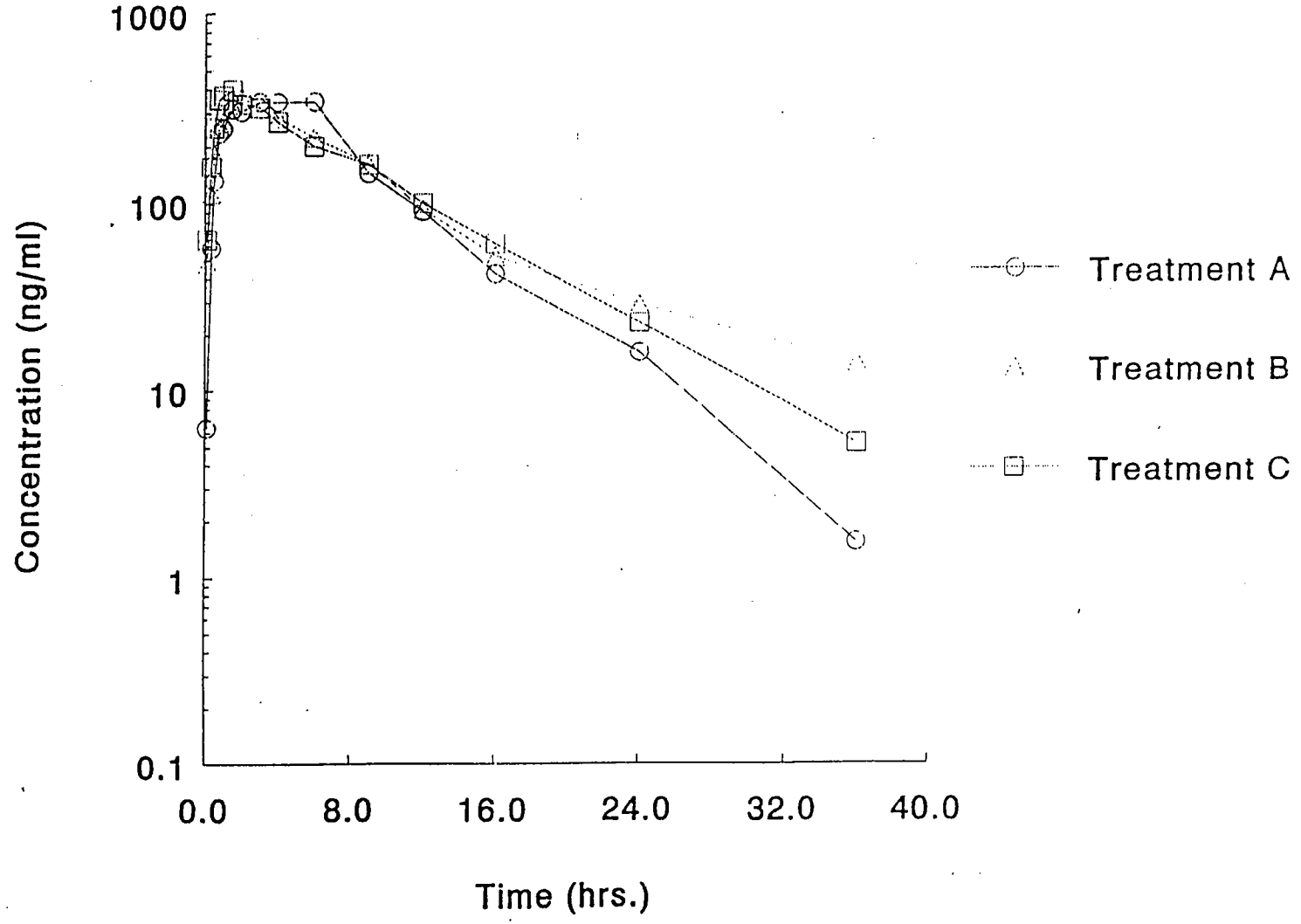
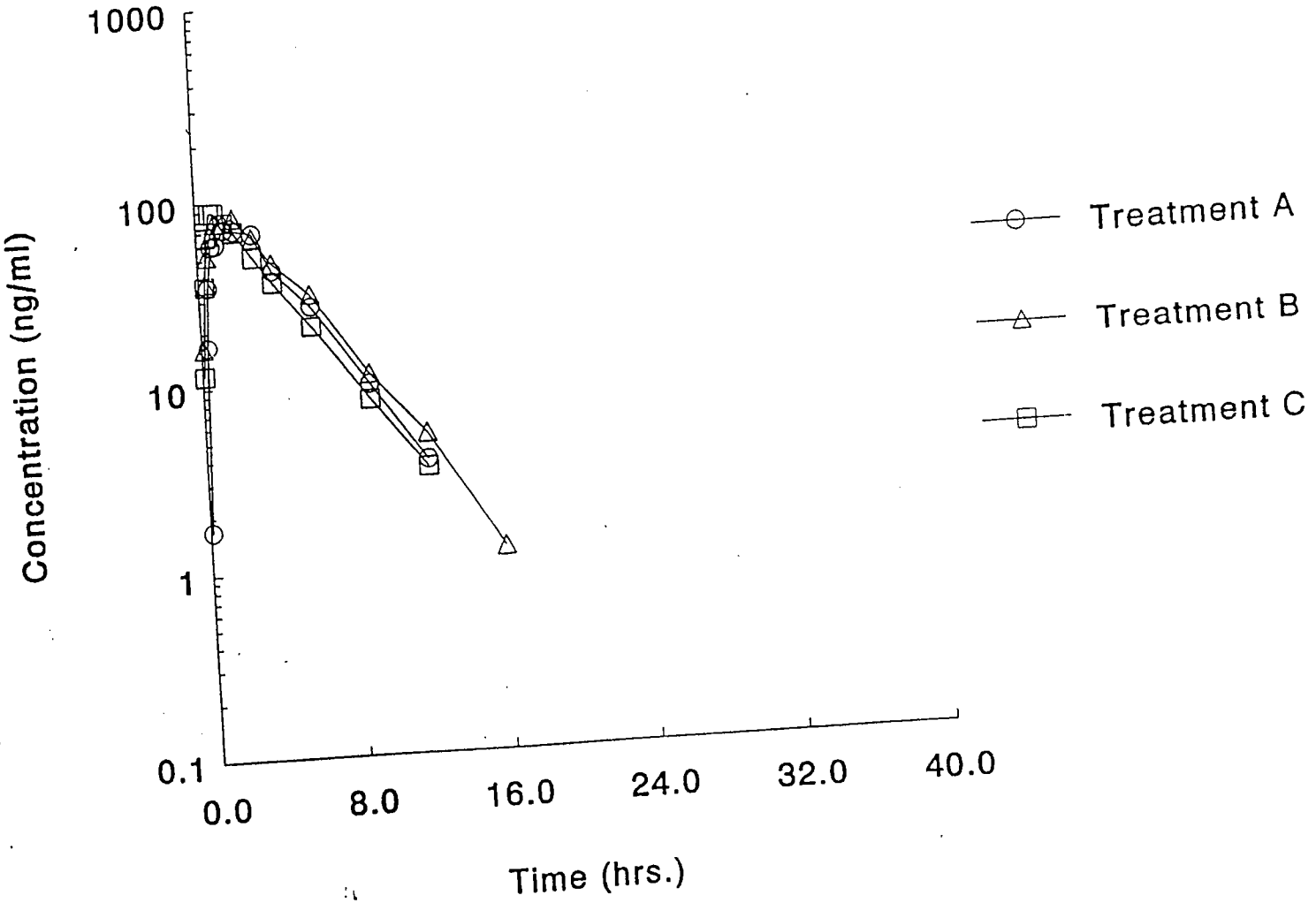


Figure 4
Mean Isosorbide 2-Mononitrate



The relative bioavailability of low and high dose Bidil, a fixed combination of hydralazine/ISDN, as compared to an oral solution, tablet and capsule of hydralazine and ISDN (pivotal bioequivalence study).

STUDY: CB-02. Volume:1.11-1-15 Pages: 1-11-3-1-15-163.

Investigators:

Clinical: [ ]

]

Objective:

To evaluate the bioequivalence of an oral reference solution of hydralazine HCl 37.5 mg and ISDN 10 mg to 4 oral formulations: 1-low dose 37.5/10 mg Bidil tablets, 2-high dose 75/40 mg Bidil tablets; 3-a standard formulation of hydralazine 37.5 mg tablets manufactured by Ciba taken with 10 mg ISDN tablets (Wyeth); 4-hydralazine 37.5 mg capsules manufactured by Ciba taken with a 10 mg ISDN tablet (Wyeth).

Formulation:

-Bidil 75/45 mg tablets batch # CW 75 manufactured by [ ] Lot size [ ] tablets.
-Bidil 37.5/10 mg tablets batch # CW 74 manufactured by Global Pharmaceuticals. Lot size [ ] tablets.
-Isordil 10 mg tablet batch # 9933011 manufactured by Wyeth Ayerst.
-37.5 mg hydralazine tablets batch # E-15119 manufactured by Ciba-Geigy.
-37.5 mg hydralazine capsules batch # E-15358 manufactured by Ciba-Geigy.
-Hydralazine injection 20 mg/ml batch# 950635 manufactured by SoloPack.

Study Design:

This was a single center, open-label, randomized, incomplete crossover study in healthy male volunteers. The study was conducted in two phases:

Phase A: the volunteers received a single oral dose of a reference solution of hydralazine HCl 37.5 mg made from commercially available injectable hydralazine HCl (SoloPack) and ISDN 10 mg (Isordil) tablets dissolved in water. Subjects were continually enrolled into Phase A of the study until at least 72 subjects were randomized to receive of the following test formulations:
A-Bidil 37.5/10 mg tablets.
B-Hydralazine 37.5 mg tablets + ISDN 10 mg tablets.
C-Hydralazine 37.5 mg capsules + ISDN 10 mg tablets.
D-Bidil 75/40 mg tablets.
Subjects were confined for approximately 48 hours, beginning 12 hours before the dose of study drug and remaining in the Clinical Research Center for 36 hours after the dose. As early as 1 week but no later than 4 weeks after completing Phase A , eligible subjects returned to the Clinical Research Center to enter the Phase B, which had an identical confinement to Phase A. The actual number of subjects enrolled was 75 subjects , out of which 74 completed the study. A minimum of 18 subjects per group should have completed the study. Only subjects with an oral clearance of less 4,000 l/hr or have hydralazine concentrations at 4 hours or beyond will be allowed to continue to the Phase B portion of the study.
A minimum of 7 days and a maximum of 4 weeks were to separate the administration of study drug for each treatment sequence.
Following administration of the drug 10 mls of blood samples were collected for ISDN and metabolite measurements at: 10, 20, 30, and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24 and 36 hours. For hydralazine measurements 7 ml blood samples were collected at: 10, 15, 20, 30 and 45 minutes and at 1, 1.25, 1.5, 1.75, 2, 3, 4; 6, 9 and 12 hours post dose administration.

Assay:

Hydralazine:
Unchanged Hydralazine was assayed using HPLC with UV detection at 365 nm. The internal standard was 4-methyl hydralazine. Hydralazine was derivatized by adding p-nitrobenzaldehyde to form the corresponding hydrazone which is subsequently measured. Table 1 and 2 summarizes the assay validation data for hydralazine.

ISDN and its metabolites:(2ISMN and 5 ISMN).
Plasma samples were analyzed for ISDN, IS-5MN and IS-2MN by a modified gas chromatography electron capture method. O-Nitrobenzyl alcohol was used as an internal standard for both ISDN and 2ISMN.
Table 3 summarizes the validation data for the above assay .

Data Analysis:

Non compartmental analysis was used to determine the pharmacokinetic parameters of interest.

SHAM (slope, height, area, moment) analysis was used to compute the pharmacokinetic values. AUC1 was calculated by linear trapezoidal rule from time 0 to the time after which all subsequent concentrations were consecutively decreasing, then by the log-linear trapezoidal rule from that time to the time of the last detectable concentration. AUC and CMAX were normalized by dose (treatment D) and weight (data were normalized to 65 kg), thus the resultant units were l/hr/65 kg for Cl/F and ng/hr/ml/65 kg for AUC.

The AUC from the solution phase of the study (Phase A) was used to clearance normalize the solid dosage form AUC values. The AUC ratio (AUCR) was computed taking the ratio of the solid phase AUC over the AUC of the Phase A.

For each of the four treatments, summary statistics (the median, arithmetic mean, CV% and the 90 and 95 % confidence intervals) were computed for the pertinent parameters. Statistical inference was also performed on the relative bioavailability of the solid dosage forms compared to solution. The Ln(AUCR) values were obtained. For each treatment group, the "point estimate" (measure of central tendency) was the antilog of the mean. Three a posteriori probabilities were computed: the probability that the true mean was below Ln(0.8), $p(\mu < Ln(0.8))$, the probability that the true mean was greater than Ln(1.25), $p(\mu > Ln(1.25))$, and the complement to these, $p(Ln(0.8) < \mu < Ln(1.25))$. Confidence intervals (90 and 95 %) computed for these results are asymmetric about the geometric mean. Significance of differences in relative bioavailability were evaluated by testing the mean Ln(ratios) vs zero using a t-test. To improve robustness of assumptions of normality and homoscedasticity, log transformations were used in developing inference regarding equivalence and significance of differences in the independent contrast groups. If the ANOVA was significant, post hoc testing of the four pre-planned contrasts were performed. The method of Bonnferonni was used to "decay the alpha".

Point estimates and confidence intervals (90 and 95 %) for the ratios (A/B,A/C, D/B, and D/C) were also constructed.

Results:

Table 4 summarizes the main pharmacokinetic parameters for hydralazine, ISDN and its two metabolites IS-2MN and IS-5MN. Figure 1 shows the mean hydralazine concentrations for Phase A of the study while Figure 2 shows the mean hydralazine concentrations for all 4 treatments of phase B of the study. Figures 3 to 5 show the corresponding plasma concentrations time profiles for ISDN and its two active metabolites for all 4 treatments in Phase B of the study.

Relative Bioavailability/Bioequivalence:

Tables 5 to 8 summarize the bioequivalence parameters with the respective 90 and 95 % confidence intervals for the AUC ratios (normalized by the solution AUC to correct for clearance differences), CMAX and AUC values. Table 9 summarizes the relative bioavailability of the four different treatment in Phase B to the solution administered in Phase A.

It can be seen from the results that both the AUC and the AUC ratios as well as the CMAX for hydralazine are outside the confidence intervals for bioequivalence.

Where is Table 9?

On the other hand, the results for ISDN showed bioequivalence between the low dose Bidil and the clinical capsule and tablet formulations as far as AUC is concerned. However, the high dose Bidil was more bioavailable than the reference tablet or capsule clinical formulations. This difference in bioavailability might not be due to a difference in formulation performance but might be due to the fact that at higher doses, there might be a saturation of the first pass effect leading to a more than proportional increase in AUCs. The results of this study also show that none of the formulations used in Phase B of this study meet the bioequivalency criteria as far as CMAX is concerned.

As for the two metabolites of ISDN, IS-5DN and IS-2MN, the low dose Bidil was deemed bioequivalent to both the capsule and tablet formulations used in the clinical trials. The same could not be said for the high dose Bidil since the 90 % confidence intervals for CMAX for both metabolites were skewed to the left and were outside the allowable limits indicating lower values compared to both these formulations.

Comments:

1-The pharmacokinetic parameters calculated from this study show a high degree of variability reflected in very wide confidence intervals ranging sometimes from 0.39 to 5.48.

2-The number of subjects per treatment group (18 per arm) might not provide the study with enough power to detect differences in formulations among the four different treatments due to the very high variability in pharmacokinetic parameters.

3-The results of the study seem to point out to a trend that the Bidil formulation has lower bioavailability than the capsule formulation used in VHEFT I but is more bioavailable than the tablet formulation used in VHEFT II.

4-The clinical significance of this difference in bioavailability between the two formulations used in both clinical trials is unknown from the efficacy stand point specially when the results of the two clinical trials point out in different directions and there is a marked difference in bioavailability between the capsule formulation used in VHEFT I and the tablet formulation used in VHFET II.

TABLE 1

Parameters	Samples	Results
Linearity	Hydralazine[1]	0.250-200 ng/ml
Sensitivity	Limit of Quantitation[1]	0.25 ng/ml
	Limit of Detection[2]	0.158 ng/ml
Accuracy	Calibrators: Overall[1]	-0.03% RE
	Quality Controls: Overall[1]	-4.96% RE
Precision	Calibrators: Overall[1]	4.27% RSD
	Quality Controls: Within-Day[2]	5.15% RSD
	Quality Controls: Overall[1]	8.13% RSD
	Within-Sample[2]	0.83% RSD
	Inter-Lot (Inter-Subject)[2]	4.37% RSD
Relative Recovery	Hydralazine[2]	98.9 %
Stability	Bench-Top[2]	[ ]
	Autosampler[2]	[ ]
	Freeze-Thaw[2]	[ ]
	Long-Term[3] at	[ ]

[1] Referenced from the study phase results of this study
[2] Referenced from the assay validation report
[3] Referenced from PRB-189-085

TABLE 2

Nominal Concentrations of the Hydralazine Calibration Samples (ng/ml)	% RSD of the Observed Concentrations
200	3.84
160	3.35
100	3.52
50.0	3.50
20.0	3.18
5.00	4.81
2.00	5.74
0.500	7.16
0.250	3.34
Overall	4.27

Nominal Concentrations of the Hydralazine Quality Control Samples (ng/ml)	% RSD of the Observed Concentrations
150	6.52
15.0	6.62
1.00	11.26
Overall	8.13

TABLE 3

Parameters	Samples	Analytical Phase	ISDN	2-ISMN	5-ISMN
Linearity	Calibration Range	ASP1	0.50-50.0 ng/ml	0.50-50.0 ng/ml	1.50-200 ng/ml
		ASP2	1.00-50.0 ng/ml	1.00-150 ng/ml	4.00-600 ng/ml
Sensitivity	Limit of Quantitation (ng/ml in EDTA human plasma)	ASP1	0.50 ng/ml	0.50 ng/ml	1.50 ng/ml
		ASP2	1.00 ng/ml	1.00 ng/ml	4.00 ng/ml
	Limit of Detection (ng/ml in human plasma,)	Validation	0.035 ng/ml; 45 fg	0.021 ng/ml; 27 fg	0.076 ng/ml; 97 fg
Accuracy	Calibrators: Overall	ASP1	0.02% RE	0.02% RE	0.03% RE
		ASP2	0.08% RE	0.10% RE	0.01% RE
	Quality Controls: Overall	ASP1	-2.80% RE	1.95% RE	2.56% RE
		ASP2	-5.26% RE	2.69% RE	-7.00% RE
Precision	Calibrators: Overall	ASP1	4.82% RSD	4.54% RSD	5.51% RSD
		ASP2	3.83% RSD	3.47% RSD	4.93% RSD
	Quality Controls: Within-Day	Validation	6.91% RSD	4.62% RSD	9.24% RSD
	Quality Controls: Overall	ASP1	8.12% RSD	7.14% RSD	11.89% RSD
		ASP2	8.90% RSD	6.26% RSD	8.72% RSD
	Within-Sample	Validation	1.14% RSD	1.21% RSD	1.28% RSD
	Inter-Subject	Validation	8.67% RSD	4.31% RSD	4.17% RSD
Recovery	Absolute	Validation	99.6%	95.1%	74.3%
	Relative	Validation	96.0%	99.8%	96.4%
Stability	Bench-Top	Validation	[		]
	Prepared Sample	Validation	[		]
	Freeze-Thaw	Validation	[		]

ASP1 = Part 1 of Analytical Study Phase; ASP2 = Part 2 of Analytical Study Phase

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Summary of Pharmacokinetic Parameters (Means) of Unchanged Hydralazine and ISDN and Its Metabolites by Treatment Group

	Treatment A		Treatment B		Treatment C		Treatment D	
Moiety/ Parameter	Phase A	Phase B	Phase A	Phase B	Phase A	Phase B	Phase A	Phase B[3]
Hydralazine								
AUC[1] (ng•hr/mL)	33.01	35.11	28.63	24.40	32.99	37.03	31.81	40.90
AUC Ratio (B/A)		1.10		0.86		1.19		1.27
C_{max} (ng/mL)[1]	48.9	56.3	48.5	29.6	60.0	77.3	45.8	44.0
T_{max} (hr)	0.40	0.96	0.30	1.03	0.28	0.74	0.38	1.02
$t_{½}$ (hr)	2.22	2.31	3.17	2.05	2.53	2.38	2.17	3.52
K_{el} (hr^{-1})	0.39	0.39	0.41	0.54	0.37	0.39	0.42	0.27
Cl/F[1] (L/hr)	1301.03	1406.3	1735.8	2445.8	1390.4	1342.5	1308.1	1339.0
ISDN								
AUC[1] (ng•hr/mL)	27.1	26.0	30.2	26.1	29.2	26.7	25.8	30.9
AUC Ratio (B/A)		0.97		0.87		0.93		1.23
C_{max} (ng/mL)[1]	33.6	30.0	44.0	22.8	42.7	26.5	33.9	19.0
T_{max} (hr)	0.32	0.64	0.28	0.75	0.24	0.65	0.33	1.05
$T_{1/2}$ (hr)	1.15	1.26	1.57	1.46	1.62	1.75	1.08	2.15
Cl/F (L/hr)[1]	487.9	528.7	405.2	499.2	471.6	522.3	556.1	463.1
IS–2–MN								
AUC[1] (ng•hr/mL)	107.0	97.6	109.9	108.6	106.1	103.8	106.7	116.3
AUC Ratio (B/A)		0.92		1.00		0.98		1.10
C_{max} [1](ng/mL)	31.6	29.5	32.4	28.7	34.8	30.7	32.7	24.4
T_{max} (hr)	0.56	0.89	0.62	0.76	0.44	0.80	0.68	1.61
$T_{1/2}$ (hr)	2.38	2.26	2.47	2.50	2.32	2.38	2.31	2.51
IS–5–MN								
AUC[1] (ng•hr/mL)	891.8	818.0	944.0	932.3	869.4	861.1	894.3	843.4
AUC Ratio (B/A)		0.93		1.00		1.00		0.96
C_{max} (ng/mL)[1]	125.2	110.2	127.5	112.7	128.9	118.0	126.4	91.0
T_{max} (hr)	0.73	1.63	0.93	1.34	0.57	1.24	0.82	2.20
$T_{1/2}$ (hr)	5.40	5.40	5.73	5.82	5.27	5.45	5.28	5.45

Note: A=BiDil® tablet, low dose; B=Hydralazine *tablet* plus ISDN; C=Hydralazine *capsule* plus ISDN; D=BiDil® tablet, high dose.

[1] AUC, C_{max} and Cl/F are normalized to 65 kg.

Figure 1

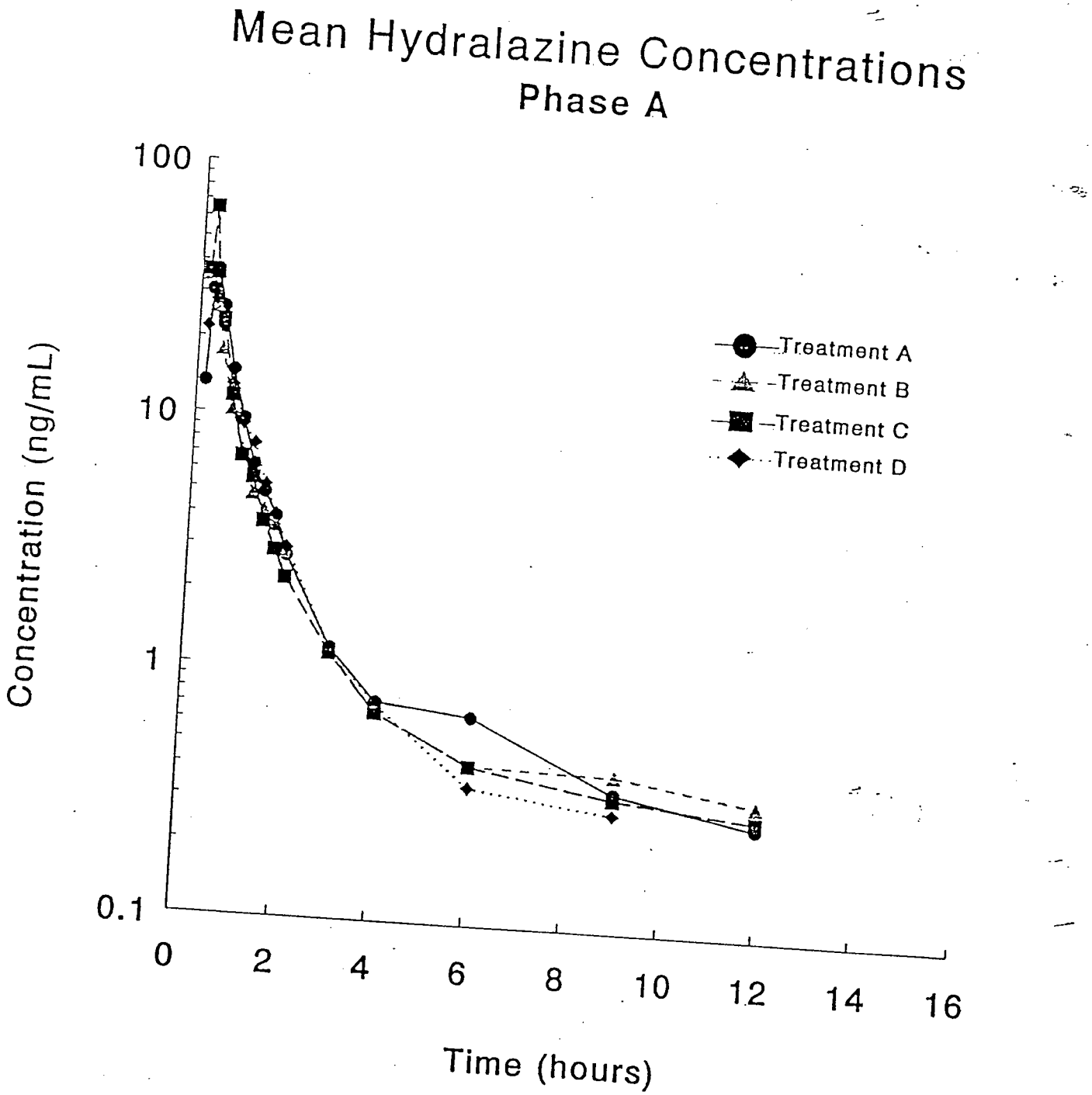

Mean Hydralazine Concentrations
Phase A
100
10
1
0.1
Concentration (ng/mL)
0
2
4
6
8
10
12
14
16
Time (hours)
Treatment A
Treatment B
Treatment C
Treatment D

Figure 2

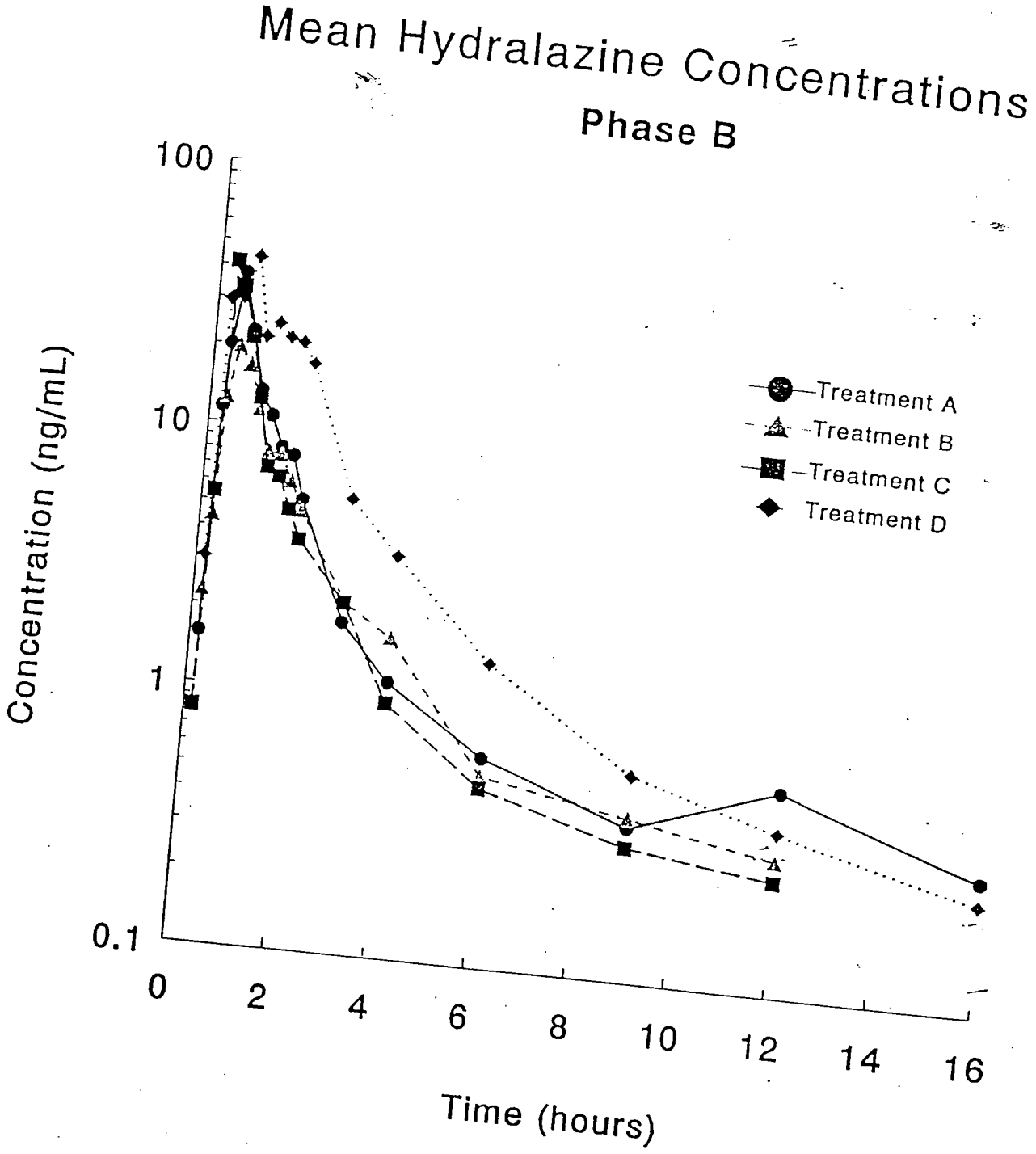

Mean Hydralazine Concentrations
Phase B
100
10
1
0.1
Concentration (ng/mL)
0
2
4
6
8
10
12
14
16
Time (hours)
Treatment A
Treatment B
Treatment C
Treatment D

FIGURE 3

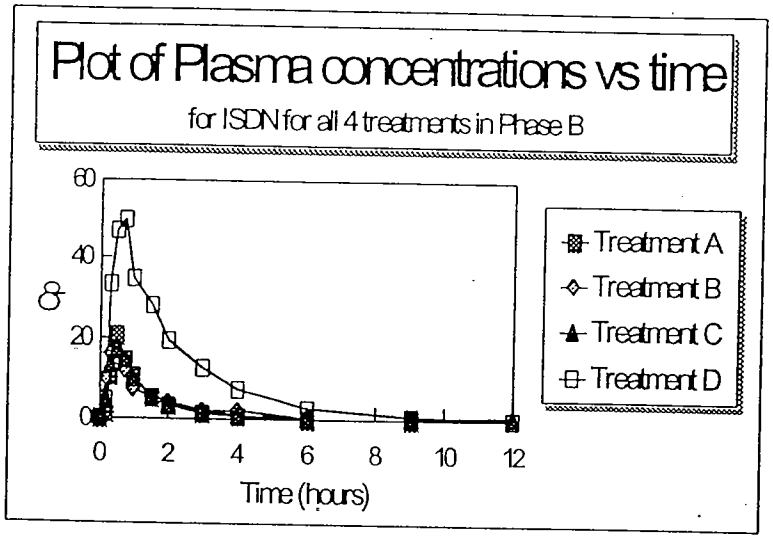


FIGURE 4

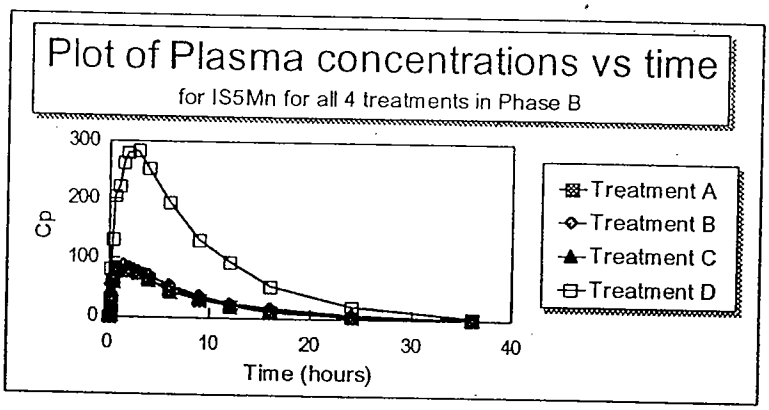


FIGURE 5

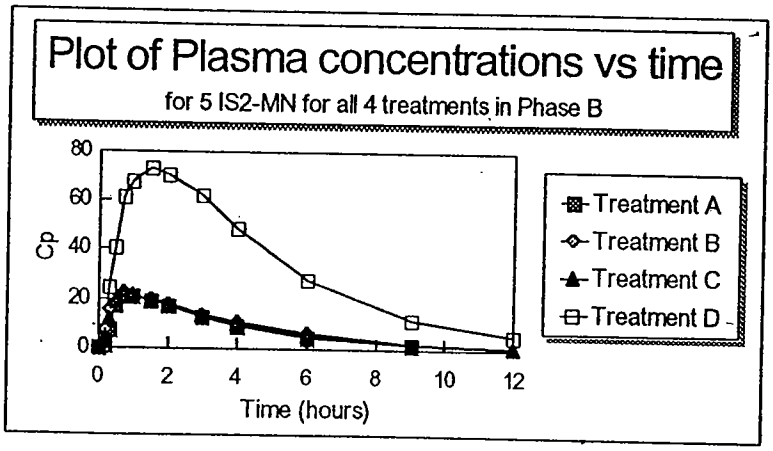


TABLE 5

Hydralazine	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil/tablet	1.47	0.89-2.4	1.25	0.99-1.58	1.56	1.11-2.19
Low dose Bidil/capsule	0.65	0.4-1.07	0.9	0.72-1.44	0.94	0.67-1.32
High dose Bidil/tablet	1.3	0.79-2.12	1.46	1.15-1.85	1.79	1.28-2.52
High dose Bidil/capsule	0.58	0.35-0.95	1.06	0.84-1.34	1.08	0.77-1.51

AUCR= AUC normalized to the AUC of the solution arm in Phase A of the study.

TABLE 6

ISDN	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil/tablet VHEFTII	1.21	0.86-1.71	1.12	0.98-1.27	0.97	0.82-1.15
Low dose Bidil/tablet VHEFTI	1.06	0.75-1.5	1.04.	0.92-1.19	0.98	0.83-1.17
High dose Bidil/tablet VHEFTII	0.82	0.59-1.15	1.41	1.24-1.6	1.12	0.95-1.32
High dose Bidil/tablet VHEFTI	0.72	0.51-1.01	1.32	1.16-1.5	1.13	0.96-1.34

TABLE 7

IS-2MN	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil/tablet VHEFTII	1.01	0.88-1.16	0.92	0.88-0.96	0.9	0.84-0.96
Low dose Bidil/tablet VHEFTI	0.95	0.83-1.09	0.93	0.89-0.97	0.95	0.88-1.01
High dose Bidil/tablet VHEFTII	0.85	0.74-0.98	1.1	1.05-1.15	1.07	1.0 -1.14
High dose Bidil/tablet VHEFTI	0.8	0.70--0.92	1.11	1.06-1.17	1.12	1.05-1.2

TABLE 8

IS-5MN	CMAX Ratio	CMAX 90 % CI	AUCR Ratio	AUCR 90 % CI	AUC Ratio	AUC 90 % CI
Low dose Bidil/tablet VHEFTII	0.98	0.88-1.1	0.93	0.87-1	0.88	0.81-1.1
Low dose Bidil/tablet VHEFTI	0.94	0.84-1.06	0.92	0.86-0.99	0.95	0.88-1.03
High dose Bidil/tablet VHEFTII	0.81	0.73-0.91	0.96	0.89-1.02	0.91	0.84-0.98
High dose Bidil/tablet VHEFTI	0.78	0.69-0.87	0.95	0.89-1.02	0.98	0.91-1.06

Dissolution Method:

The sponsor is proposing to use the following dissolution test condition:
Apparatus: USP Rotating basket (Apparatus I)
Rotation: 100 RPM.
Medium: 900 ml of 0.05 N HCl
Specifications: Hydralazine Q of [ ] at 30 minutes.
ISDN Q of [ ]% at [ ] minutes.

The sponsor submitted dissolution profiles for the two batches used in the pivotal bioequivalence study (CW74 and CW75) and for the process optimization batches (DG29 and DG30). Tablets were tested at two laboratories [ ] using the same method.
The dissolution results are presented in Tables 1 to 8 and the corresponding dissolution profiles are presented in Figures D1-D8.
Figures D9 to D16 show the dissolution profiles for hydralazine and ISDN in 0.05 N HCL, 0.1 N HCL, water and phosphate buffer PH 7.2 for all the four strenghts tablets of the Bidil formulation. The results show that there is almost complete dissolution by 30 minutes of both hydralazine and ISDN for all the four media tested across all strengths.

Comments:

1-The dissolution performance of hydralazine and ISDN for both the two lots that were used in the pivotal bioequivalence study at the 15 minute time point was very erratic and characterized by a relatively high %RSD as high as [ ] in some instances. However, the process optimization batches showed a much more rapid and complete dissolution with %RSD lower than [ ]

2-The 37.5/10 mg tablet of Bidil consistently showed slower dissolution at 15 minutes compared to the higher strenths tablets in all 4 media. However, in most cases, complete dissolution was achieved by 30 minutes.

3-It is to be noted that the USP method for hydralazine is:
900 ml 0.1 N HCl using Apparatus I with a speed of 100 rpm. The specifications are not less than [ ] % in [ ] minutes.
As for ISDN, the USP dissolution method is:
1000 ml of water using USP paddle at a speed of 75 rpm with a specification of not less than [ ] % in 45 minutes.

4-Based on the dissolution data submitted by the sponsor, the following dissolution method and specifications are recommended for Bidil:
USP Apparatus I (basket) at a speed of 100 rpm
Medium: 0.05 N HCL
Hydralazine and ISDN, not less than [ ] in 30 minutes.

BiDil Tablets
Table 2 Dissolution Profiles of Hydralazine Hydrochloride

CW75
BiDil Tablets, 75/40 mg

Sample	Time 15	30	90
[			]
*Mean	97.3	98.2	98.5
%RSD	1.8	2.3	2.3
[			]
**Mean	97.5	102.5	100.3
%RSD	6.4	3.2	4.1

*First set of 6 tablets tested by [ ]
**Second set of 6 tablets tested by [ ]

Conditions:

Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 230nm

BiDil Tablets

Table 3. Dissolution Profiles of Isosorbide Dinitrate

CW74
BiDil Tablets, 37.5/10 mg

Sample	Time 15	30	45	90
1	[			]
2				
3				
4				
5				
6				
*Mean	59.2	98.2	99.8	101.5
%RSD	41.6	1.9	2.2	2.0
1	[			]
2				
3				
4				
5				
6				
**Mean	50.3	94.5	95.5	94.8
%RSD	44.8	2.1	2.5	2.4

*First set of 6 tablets tested by [ ]
**Second set of 6 tablets tested by [ ]

Conditions:

Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 225nm

BiDil Tablets
Table 3. Dissolution Profiles of Isosorbide Dinitrate

CW74
BiDil Tablets, 37.5/10 mg

Sample	Time 15	30	45	90
[				]
*Mean	59.2	98.2	99.8	101.5
%RSD	41.6	1.9	2.2	2.0
[				]
**Mean	50.3	94.5	95.5	94.8
%RSD	44.8	2.1	2.5	2.4

*First set of 6 tablets tested by [ ]
**Second set of 6 tablets tested by [ ]

Conditions:

Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 225nm

BiDil Tablets
Table 4 Dissolution Profiles of Isosorbide Dinitrate

CW75
BiDil Tablets, 75/40 mg

Sample	Time 15	30	45	90
1	[			]
2				
3				
4				
5				
6				
*Mean	80.9	98.7	100.3	101.7
%RSD	13.6	1.1	1.5	1.6
1	[			]
2				
3				
4				
5				
6				
**Mean	67.3	89.5	94.8	97.3
%RSD	22.8	9.5	4.6	1.2

*First set of 6 tablets tested by [ ]
**Second set of 6 tablets tested by [ ]

Conditions:

Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 225nm

BiDil Tablets

Table 5. Dissolution Profiles of Hydralazine Hydrochloride

DG29

BiDil Tablets, 37.5/10 mg

Sample	Time 15	30	90
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
*Mean	97	99	100
%RSD	6.9	2.0	2.3

	15	30	45
1			
2			
3			
4			
5			
6			

only six tablets tested

**Mean	98.0	97.8	97.8
%RSD	1.9	1.8	2.0

*First set of 12 tablets tested by [ ]

**Second set of 6 tablets tested by [ ]

Conditions:
Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 230nm

BiDil Tablets

Table 6. Dissolution Profiles of Isosorbide Dinitrate

DG29
BiDil Tablets, 37.5/10 mg

Sample	Time 15	30	45	90
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
*Mean	94	100	100	101
%RSD	12.2	1.8	2.0	2.0

	15	30	45	90
1				
2				
3				
4				
5				
6				

only six tablets tested

**Mean	96.0	100.2	99.8	-
%RSD	4.7	2.0	1.7	-

*First set of 12 tablets tested by [illegible]
**Second set of 6 tablets tested by [illegible]

Conditions:
Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [illegible]
Media: 0.05 *N* HCl
Mobile Phase: [illegible]
Rotation Speed: 100RPM
Flow: [illegible]
Detection: UV at 225nm

BiDil Tablets

Table 7. Dissolution Profiles of Hydralazine Hydrochloride

DG30

BiDil Tablets, 75/40 mg

Sample	Time 15	30	90
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
*Mean	100	100	101
%RSD	1.8	1.7	1.6

Sample	15	30	45
1			
2			
3			
4			
5			
6			

only six tablets tested

**Mean	99.3	98.8	98.7
%RSD	0.8	1.2	1.0

*First set of 12 tablets tested by [ ]
**Second set of 6 tablets tested by [ ]

Conditions:
Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 230nm

BiDil Tablets

Table 8. Dissolution Profiles of Isosorbide Dinitrate

DG30
BiDil Tablets, 75/40 mg

Sample	Time 15	30	45	90
1	[			]
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
*Mean	95	98	98	100
%RSD	1.7	2.5	2.6	2.5

	15	30	45	90
1	[			]
2				
3				
4				
5				
6				

only six tablets tested

**Mean	94.2	97.8	98.3	
%RSD	1.2	1.0	1.2	

*First set of 12 tablets tested by [ ]
**Second set of 6 tablets tested by [ ]

Conditions:
Apparatus: USP Rotating Baskets (Apparatus 1)
Column: [ ]
Media: 0.05 *N* HCl
Mobile Phase: [ ]
Rotation Speed: 100RPM
Flow: [ ]
Detection: UV at 225nm

FIGURE

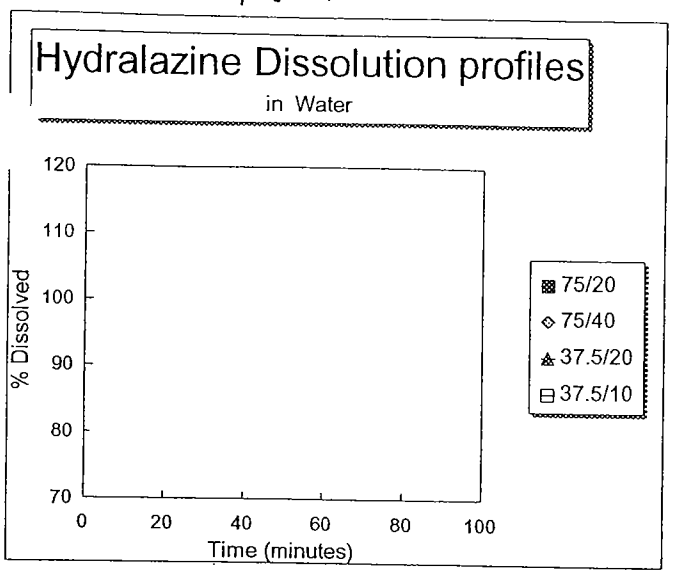


FIGURE D-2

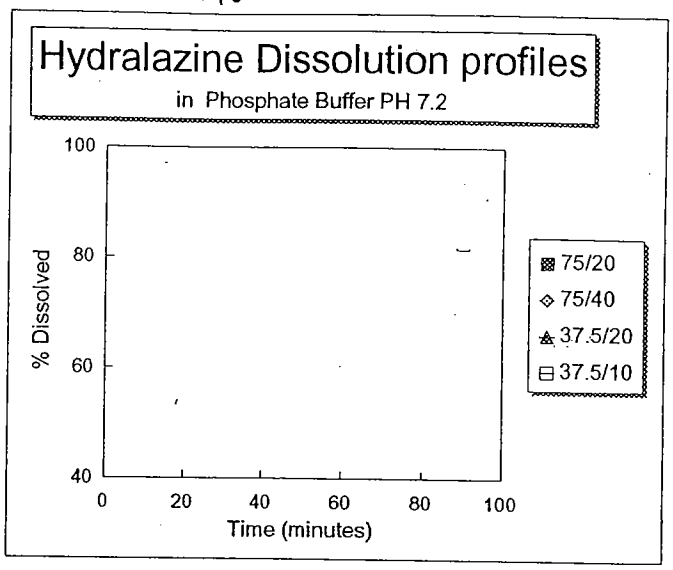


FIGURE D-3

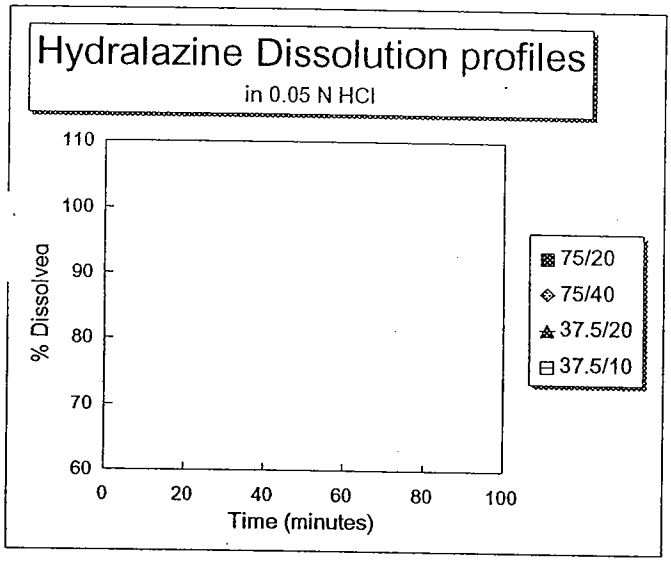


FIGURE D-4.

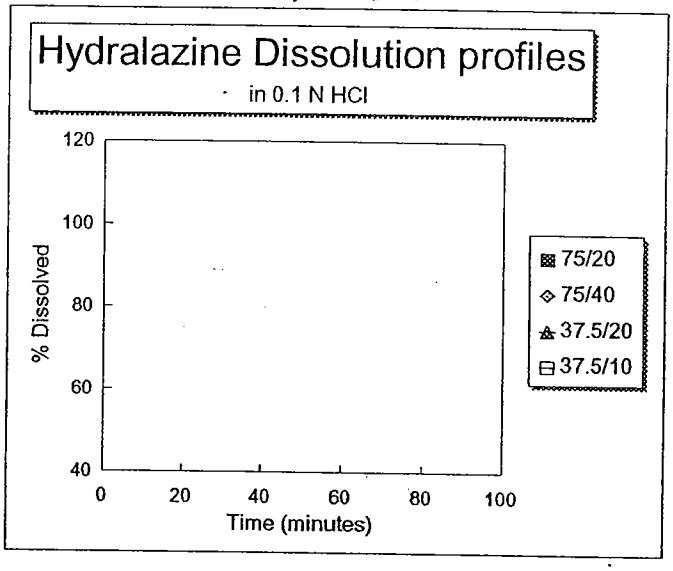


FIGURE D-5

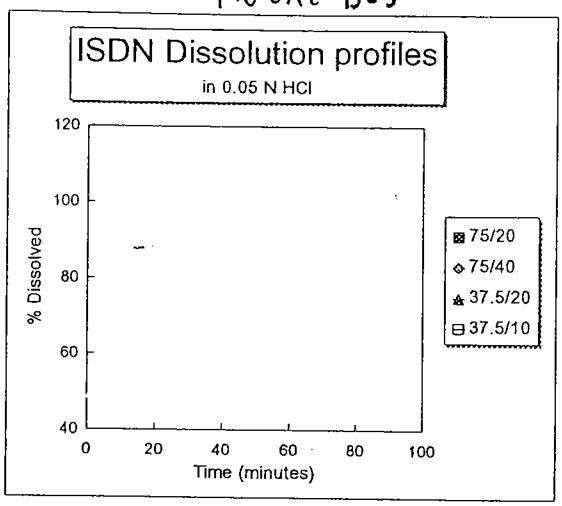


FIGURE D-6

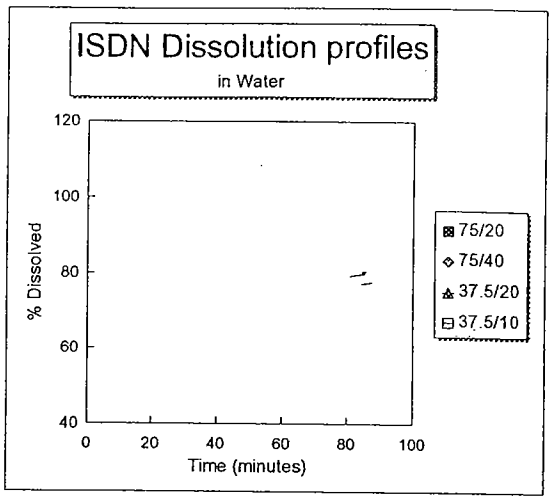


FIGURE D-7

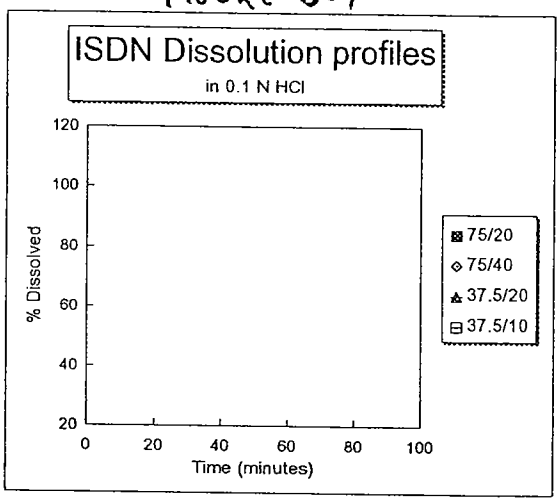
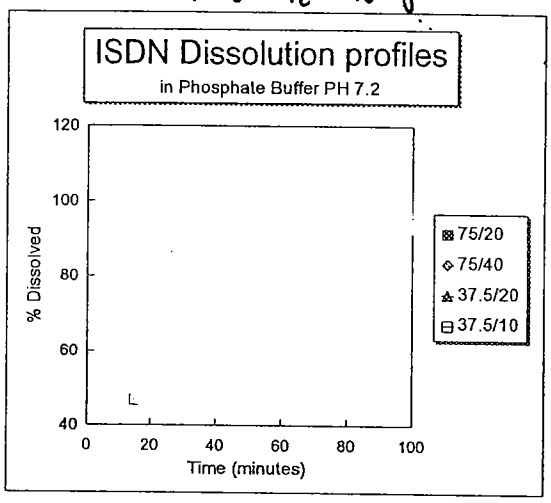


FIGURE D-8



APPENDIX II

3.2.3 Composition

BiDil® (hydralazine HCl/isosorbide dinitrate) Tablets, 37.5/10 mg

	per tablet (mg)	function
Tablet Core:		
Hydralazine HCl, USP	37.5	active
Diluted Isosorbide Dinitrate, USP	40.0	active
Anhydrous Lactose, NF		
Microcrystalline Cellulose, NF		
Sodium Starch Glycolate, NF		
Magnesium Stearate, NF		
Colloidal Silicon Dioxide, NF		
Total		
Tablet Coating:		
Purified Water, USP		

3.2.3 Composition

BiDil® (hydralazine HCl/isosorbide dinitrate) Tablets, 37.5/20 mg

	per tablet (mg)	function
Tablet Core:		
Hydralazine HCl, USP	37.5	active
Diluted Isosorbide Dinitrate, USP	80.0	active
Anhydrous Lactose, NF		
Microcrystalline Cellulose, NF		
Sodium Starch Glycolate, NF		
Magnesium Stearate, NF		
Colloidal Silicon Dioxide, NF		
Total		
Tablet Coating:		
Opadry Orange, YS-1-6227		
Purified Water, USP		

3.2.3 Composition

BiDil® (hydralazine HCl/isosorbide dinitrate) Tablets, 75/20 mg

	per tablet (mg)	function
Tablet Core:		
Hydralazine HCl, USP	75.0	active
Diluted Isosorbide Dinitrate, USP	80.0	active
Anhydrous Lactose, NF		
Microcrystalline Cellulose, NF		
Sodium Starch Glycolate, NF		
Magnesium Stearate, NF		
Colloidal Silicon Dioxide, NF		
Total		
Tablet Film-Coating:		
Purified Water, USP		

3.2.3 Composition

BiDil® (hydralazine HCl/isosorbide dinitrate) Tablets, 75/40 mg

	per tablet (mg)	function
Tablet Core:		
Hydralazine HCl, USP	75.0	active
Diluted Isosorbide Dinitrate, USP	160.0	active
Anhydrous Lactose, NF	[redacted]	[redacted]
Microcrystalline Cellulose, NF		
Sodium Starch Glycolate, NF		
Magnesium Stearate, NF		
Colloidal Silicon Dioxide, NF		
Total	[redacted]	
Tablet Film-Coating:		
Opadry Orange, YS-1-6227	[redacted]	
Purified Water, USP		

11 Page(s) Withheld

____ § 552(b)(4) Trade Secret / Confidential

____ § 552(b)(5) Deliberative Process

✓ § 552(b)(4) Draft Labeling

B.B

JAN 16 1997

Clinical Pharmacology/Biopharmaceutics Review.

IND: 41816 (N,PN014) Submission Date: December 23,1996.
ISDN/Hydralazine.
Bidil^{R}
Medco Research.

Reviewer: Patrick J Marroum.

Type of submission: revised protocol for a multiple dose pharmacokinetic study.

BACKGROUND:

Bidil^{R} is a fixed combination formulation consisting of 2 active ingredients, hydralazine HCl and isosorbide dinitrate (ISDN) which is being developed for the treatment of congestive heart failure. Hydralazine is an antihypertensive agent that lowers blood pressure by exerting a peripheral vasodilating effect on the arterial vascular bed. ISDN is a vasodilator which has effects primarily on the venous system. The effects of the coadministration of both these drugs were studied in 2 large multi center clinical trials.
On July 3rd 1996, a New Drug Application was submitted for Bidil for the treatment of congestive heart failure in patients intolerant to ACE inhibitors. As discussed in several telephone calls and as recommended in the OCPB review dated December 4th 1997, the sponsor is amending the protocol for the agreed upon multiple dose pharmacokinetic study in congestive heart failure patients to comply with the Agency's recommendations.

RECOMMENDATION:

The sponsor amended the protocol in a way that a full pharmacokinetic profile is obtained at each dose level. The new sampling scheme is the one recommended by the Office of Clinical Pharmacology and Biopharmaceutics.
Since the sponsor adopted all the Agency's recommendations, the revised protocol is acceptable to the Division of Pharmaceutical Evaluation I.

Patrick J Marroum Ph.D. 1/16/1997

RD initialed by A Parekh Ph.D. Ameeta Parekh 1/16/97

850

cc:IND 41816, HFD 110, HFD 860 (Marroum), HFD ~~870~~ (Chron, Drug, Reviewer c/o ~~Clarence Bott:room 13b31 PKLN~~). Mira Millison
Room 3070 WOC II

DEC 4 1996

Clinical Pharmacology/Biopharmaceutics Review.

IND: 41816 (N,PN013) Submission Date: November 6,1996.
ISDN/Hydralazine.
Bidil[R]
Medco Research.

Reviewer: Patrick J Marroum.

Type of submission: protocol for a multiple dose pharmacokinetic study.

BACKGROUND:

Bidil[R] is a fixed combination formulation consisting of 2 active ingredients, hydralazine HCl and isosorbide dinitrate (ISDN) which is being developed for the treatment of congestive heart failure. Hydralazine is an antihypertensive agent that lowers blood pressure by exerting a peripheral vasodilating effect on the arterial vascular bed. ISDN is a vasodilator which has effects primarily on the venous system. The effects of the coadministration of both these drugs were studied in 2 large multi center clinical trials.
On July 3rd 1996, a New Drug Application was submitted for Bidil for the treatment of congestive heart failure in patients intolerant to ACE inhibitors. As discussed in several telephone calls and as committed to within the NDA, the sponsor is submitting a draft protocol for the agreed upon multiple dose pharmacokinetic study in congestive heart failure patients.
A summary of the proposed protocol is submitted as Appendix I.

COMMENTS:

1-As per several discussions with the Agency, it was agreed that full pharmacokinetic profile be obtained at each dose level and not only peak and trough levels for the lower doses as the sponsor intends. The firm is recommended to obtain full pharmacokinetic profiles at each dose levels.

2-Another problem that might be encountered in the proposed protocol is that full profiles will only be obtained at the highest tolerated dose level. Thus if there are a few patients (as few as two or three) dropping out at each dose level, one would end up with very few subjects that have a full pharmacokinetic profile characterized. However, if a full pharmacokinetic profile is obtained at each dose starting with the lowest dose, then at least one would have sufficient subjects to provide descriptive information at least at the lower doses.

3-The proposed number of samples to be collected by the sponsor is quite extensive. It is suggested to the firm that the number of samples taken from each patient at each dose level be cut down. The following sampling scheme is suggested: at baseline (trough concentration, prior to final administration of highest tolerated regimen), 20, 40 minutes and at 1, 1.5, 2, 4 and 6 hours after dose administration.

RECOMMENDATION:

In order to evaluate the steady-state pharmacokinetics of hydralazine and ISDN over the dose range, it is recommended that full pharmacokinetic profiles be obtained at each dose level and not only peak and trough levels for the lower doses as the sponsor intends.
The proposed number of samples to be collected by the sponsor is quite extensive. It is suggested to the firm that the number of samples taken from each patient at each dose level be cut down. The following sampling scheme is suggested: at baseline (trough concentration, prior to final administration of highest tolerated regimen), 20, 40 minutes and at 1, 1.5, 2, 4 and 6 hours after dose administration.

12-3-1996

Patrick J Marroum Ph.D.

RD initialed by A Parekh Ph.D. Ameeta Parekh 12/4/96

cc:IND 41816, HFD 110, HFD 860 (Marroum), HFD 870 (Chron, Drug, Reviewer c/o Clarence Bott:room 13b31 PKLN).

Appendix I

Multiple-Dose Pharmacokinetic Evaluation of Bidil tablets in patients with congestive heart failure.

Investigator:

Objectives:

To evaluate the steady state pharmacokinetic characteristics of Bidil tablets after multiple dosing of each tablet strength in patients with NYHA class I-III congestive heart failure.

Formulations:

-Bidil (hydralazine/ISDN) 37.5 mg/10 mg tablets.
-Bidil (hydralazine/ISDN) 37.5 mg/20 mg tablets.
-Bidil (hydralazine/ISDN) 75 mg/20 mg tablets.
-Bidil (hydralazine/ISDN) 75 mg/40 mg tablets.

Study Design:

This is an open label, sequential dosing in twelve male or female patients with congestive heart failure between the ages of 18 to 79 years inclusive.
In phase I, patients will begin therapy with Bidil 37.5/10 mg tablets q.i.d. After at least two days of q.i.d. dosing, patients will present to the pharmacokinetics laboratory for the determination of peak and trough hydralazine, ISDN, IS-2MN and IS-5MN concentrations.
In phase II, patients will begin therapy with Bidil 37.5/20 mg tablets q.i.d. After at least two days of q.i.d. dosing, patients will present to the pharmacokinetics laboratory for the determination of peak and trough hydralazine, ISDN, IS-2MN and IS-5MN concentrations.
In phase III, patients will begin therapy with Bidil 75/20 mg tablets q.i.d. After at least two days of q.i.d. dosing, patients will present to the pharmacokinetics laboratory for the determination of peak and trough hydralazine, ISDN, IS-2MN and IS-5MN concentrations.
In phase IV, patients will begin therapy with Bidil 75/40 mg tablets q.i.d. After at least two days of q.i.d. dosing, patients will present to the pharmacokinetics laboratory for the determination of complete pharmacokinetic parameters as described below.
If at any time, it is determined by the principal investigator or the patient's primary care physician or cardiologist that the patient cannot tolerate sequentially higher dose of either hydralazine or ISDN, the patient will be reinitiated at the next lower dose, and the complete pharmacokinetic study will occur after at least two days treatment (at the highest dose tolerated).

10 ml. blood samples for ISDN and metabolites measurement will be collected at baseline (trough concentration, prior to final administration of highest tolerated regimen), 15, 30, 45 minutes and at 1, 1.5, 2, 3, 4 and 6 hours after dose administration.
Seven ml blood samples for unchanged hydralazine measurements will be collected at baseline (trough concentration, prior to final administration of highest tolerated regimen), 15, 30, 45 minutes and at 1, 1.25, 1.5, 2, 3, 4 and 6 hours after dose administration.

Analytical Assay:

The concentrations of unchanged hydralazine will be analyzed by a sensitive and specific HPLC assay. Concentrations of ISDN, isosorbide-2-mononitrate and isosorbide-5-mononitrate will be analyzed by a sensitive and specific GC assay.

Data Analysis:

Pharmacokinetic parameters will be calculated using standard noncompartmental methods.
AUC will be determined by the log linear trapezoidal rule.

AUG 2 1 1996

Clinical Pharmacology/Biopharmaceutics Review.

NDA: 20727(BB)
ISDN/Hydralazine.
Bidil[R]
Medco Research.

Submission Date: August 9, 1996.

Reviewer: Patrick J Marroum.

Type of submission: protocol for a multiple dose pharmacokinetic study.

BACKGROUND:

Bidil[R] is a fixed combination formulation consisting of 2 active ingredients, hydralazine HCl and isosorbide dinitrate (ISDN) which is being developed for the treatment of congestive heart failure. Hydralazine is an antihypertensive agent that lowers blood pressure by exerting a peripheral vasodilating effect on the arterial vascular bed. ISDN is a vasodilator which has effects primarily on the venous system. The effects of the coadministration of both these drugs were studied in 2 large multi center clinical trials.
On July 3rd 1996, a New Drug Application was submitted for Bidil for the treatment of congestive heart failure in patients intolerant to ACE inhibitors. As discussed in several telephone calls and as committed to within the NDA, the sponsor is submitting a draft protocol for the agreed upon multiple dose pharmacokinetic study.
A summary of the proposed protocol is submitted as Appendix I.

COMMENTS:

1-The sponsor intends to use the lowest strength tablets for this multiple dose study. Since these two drugs exhibit non linear characteristics in their absorption, it is recommended that the sponsor uses the highest strength tablets (the 75/40 mg). The study with the highest strength would give an idea on the highest plasma concentrations that are achieved with Bidil following chronic administration. Moreover, the results that might be obtained from the lowest strength tablets might not be extrapolatable to the higher strength due to the nonlinearities that might be caused by saturation of the first pass effects

2-Giving the highest dose of Bidil to normal volunteers might result in excessive hypotension and headache which would lead a lot of subjects to drop out. One way to avoid this problem is to start with the lowest strength and slowly escalate the dose of Bidil until the highest strength is reached. This would allow the subjects to build tolerance to the side effects and thus would minimize the probability of dropping out. If the sponsor opts to, sparse blood samples could be collected to obtain an idea on the plasma levels achieved.

RECOMMENDATION:

The above two comments should be addressed by the sponsor before initiation of this study.

Patrick J Marroum Ph.D. 8/21/1996

RD initialed by A Parekh Ph.D. Ameeta Parekh 8/21/96

cc:NDA 20-727, HFD 110, HFD 860 (Marroum), HFD 870 (Chron, Drug, Reviewer c/o Clarence Bott:room 13B31 PKLN).

Appendix I

Multiple-Dose Pharmacokinetic Evaluation of Bidil tablets (Hydralazine Hcl 37.5 mg/Isosorbide dinitrate 10 mg).

Investigator: To be determined.

Objectives:

To evaluate the multiple-dosing steady-state pharmacokinetic characteristics of Bidil (hydralazine Hcl 37.5 mg/Isosorbide Dinitrate 10 mg) tablets.

Formulations:

-Bidil (hydralazine/ISDN) 37.5 mg/10 mg tablets.

Study Design:

This is a single center, open label,sequential dosing pharmacokinetic evaluation of Bidil tablets in healthy male and female volunteers. A total of 12 subjects with hydralazine oral clearances of less than 4000 l/hr and measurable hydralazine concentrations for at least 4 hours will complete the study.
The study will be conducted in two phases:
-In phase A volunteers will receive a single dose Bidil 37.5/10 mg tablet.
-In phase B, subjects who completed phase A will receive 5 doses of Bidil 37.5/10 mg tablets one tablet every 6 hours for 24 hours. All subjects will receive a single dose of the study drug in the clinical research unit in the fasted state beginning at 8:00 AM on day 1. All subjects will receive a dose of the study drug beginning at 8:00 AM on day 2 and at 14:00 and 20:00 hours day 2 and 02:00 and 8:00 AM on day 3. A 24 hour drug free period will separate the administration of study drug for the treatment sequences.
In phase A of the study, 10 ml blood samples for ISDN and metabolite measurement will be collected at 0, 15, 30 and 45 minutes and at 1, 1.5, 2, 3, 4, 6, 9, 12, 16, and 24 hours post dose administration. 7 ml blood samples for unchanged hydralazine measurement will be collected at 15, 30, 45 minutes and at 1, 1.25, 1.5, 1.75, 2, 3, 4, 6 and 9 hours post dose administration.
In phase B of the study, 10 ml blood samples for ISDN and metabolite measurements will be collected (trough concentration, prior to day 3 8:00 hours drug administration), 15, 30 and 45 minutes and at 1, 1.25, 1.5, 1.75, 2, 3, 4, and 6 hours post dose administration.
7 ml blood samples for unchanged hydralazine measurement will be collected (trough concentration prior to day 3 8:00 hour drug administration), 15, 30 and 45 minutes and at 1, 1.25, 1.5, 1.75, 2, 3, 4 and 6 hours post dose administration.
If the subject's hydralazine oral clearance has not been previosly determined, the subject will undergo a pharmacokinetic analysis of unchanged hydralazine. The subject will be tested for hydralazine oral clearance using a test solution of 37.5 mg hydralazine for injection.

Concentrations of unchanged hydralazine will be analyzed to determine the AUC and the oral clearance will be calculated.

Analytical Assay:

The concentrations of unchanged hydralazine will be analyzed by a sensitive and specific HPLC assay. Concentrations of ISDN, isosorbide-2-mononitrate and isosorbide-5-mononitrate will be analyzed by a sensitive and specific GC assay.

Data Analysis:

Pharmacokinetic parameters will be calculated using standard noncompartmental methods. Appropriate statistical tests will be applied to the within subject and between subject comparison of AUC(single dose) vs AUC (multiple dose).