CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 20-727

Pharmacology Review(s)

A. DeFelice, Ph.D.
HFD-110
June 20, 2005

NDA 20-727

Pharmacology /Toxicology review: mechanism of action labeling.

SUBMISSION: Sponsor requested on 13 June 2005 that Mechanism of Action section of BiDil® package insert include the statement that "In addition, hydralazine is an antioxidant that may act to neutralize excess superoxide production generated by the co-administration of nitrates" and provided two published non-proprietary preclinical studies which they cite in support of their proposed wording.

Executive summary: The rigorous design and execution of the two preclinical studies afforded persuasive evidence that nitrate vasodilator tolerance could be identified *ex vivo* in rabbit aortae; that enhanced endothelial superoxide production at least partially underlies such tolerance; and that suppression of the enzymatic superoxide generation by hydralazine is an important mechanism whereby the latter prevents or attenuates nitroglycerin tolerance. Accordingly Sponsors mechanism of action labeling is empirically supported.

Recommendation: That the statement reflect its pre-clinical basis, and, furthermore, a more precise disclosure of the nature of the "anti-oxidant" activity of hydralazine. It is recommended that the sentence read as follows: **In addition, based on animal studies, hydralazine may act as an indirect anti-oxidant to suppress the NADH- mediated excess superoxide production by nitrates which is experimentally linked to hemodynamic tolerance to the latter.**

CENTER RECEIPT DATE: Published references proposing mechanism of nitrate tolerance and mechanism whereby hydralazine preserves sensitivity to nitrodilators were received on 12/22/2004 (Amend 121 vol 2, ref 3 (Munzel et al, 1995); and as a pdf file included in a June 8, 2005 E-mail submission (Munzel et al, 1996).

Amendment 121, as well as prior amendments, contained published references describing nitrate tolerance *in vitro* and *in vivo* in animals, and its attenuation or prevention by hydralazine. A review of those pharmacodynamic studies, issued by A. DeFelice on 6/25/1997, is appended to this review in its entirety. A synopsis of that review is also provided herein.

REVIEW COMPLETION DATE: 20 June 2005
REVIEWER: Albert DeFelice, Ph.D.
Supervisory Pharmacologist
Division of Cardio-Renal Drug Products (HFD-110)

SPONSOR: Nitromed, Inc.

DRUG PRODUCT: BiDil (hydralazine HCl and isosorbide dinitrate) tablets.
RELATED APPLICATIONS: IND 41,816
PATENT STATUS: Nitromed, Inc. has three patents for this combination product.
DRUG SUBSTANCES: Hydralazine hydrochloride (M.W. 196.64; $C_8H_8N_4 \cdot HCl$); Isosorbide dinitrate (ISDN; M.W. 236.14; C6H8N2O8 .)
Pharmacologic Categories: Arterial (hydralazine) and venous (isosorbide dinitrate) vasodilator agents.
PROPOSED INDICATION: This combination is intended to treat heart failure as an adjunct to standard therapy in black patients in whom ACE-inhibitors are ineffective or contraindicated.

FORMULATION AND ROUTE OF ADMINISTRATION: Each BiDil 20 tablet for oral administration contains 20 mg of ISDN and 37.5 mg of hydralazine hydrochloride. Inactive ingredients include lactose, cellulose, and Opadry orange film coating.
PROPOSED DOSAGE: One BiDil tablet t.i.d. initially, and titrated to a maximum of 2 tablets t.i.d., or to the maximum tolerated dosage.

NONCLINICAL PHARMACOLOGY/TOXICOLOGY STUDIES REVIEWED:

A. Mechanism of hemodynamic tolerance to nitrodilators:

Munzel, T. *et. A.* Evidence for Enhanced Vascular Superoxide Anion Production in Nitrate Tolerance: A Novel Mechanism Underlying Tolerance and Cross-Tolerance. *J Clin Invest,* 1995 95(1): 187-194.

Overview:
This carefully designed, controlled, and rigorously executed study of aortae isolated from nitroglycerin (NTG) -treated rabbits provides persuasive evidence that continuous NTG treatment leads to vascular tolerance demonstrable ex vivo/in vitro ; is associated with appreciably increased vascular $O^{X}_{2}\cdot$ (superoxide) production (twice that of untreated rabbits); that superoxide dismutase restored nitrodilator efficacy; that a flavoprotein – containing oxidase in the endothelium is an important source of the superoxide; that the endothelium was necessary for full development of tolerance to exogenous and endogenous nitrodilators ; and, importantly, that the tolerance was specific to NO- mediated dilations i.e., response to the cAMP dependant vasodilator forskolin remained unaffected in NTG-tolerant tissue.
Experimental design critically included use of normalized reference contractions to assure that changes in relaxation did not reflect differences in baseline tone; a validated, sensitive, calibrated (with xanthine-oxidase/ xanthine) lucigenin chemiluminescence assay for superoxide with which NO does not interfere; the demonstration that both an inhibitor of flavoprotein oxidases and a direct acting superoxide scavenger brought superoxide production back to normal; and that sensitivity of NTG- tolerant aortae to NTG and acetylcholine could be restored with superoxide dismutase all lent credibility to their conclusions. Data strongly indict a flavoprotein oxidase-mediated promotion of superoxide levels in the endothelium at least partially underlies a tolerance to NO-mediated vasodilators. The reviewer concludes that enhanced endothelial superoxide levels underlies hemodynamic tolerance and cross tolerance to nitrodilators in this rabbit model. Since such tolerance is demonstrable/persists *in vitro,* an important role for desensitization at the level of vessel *itself* could be added to that for the extravascular effects (e.g., neurohumoral adaptations; increases in volume and catecholamine/vasopressin content of plasma; activation of RAS) also associated with, and reportedly partially responsible for, tolerance to the vasodilator and cardiac unloading actions of nitrodilators, Accordingly both direct vascular – as well as compensatory systemic factors - may limit chronic use as nitrates as monotherapy.
Methods: Rabbits were treated for three days with NTG patches (0.4 mg/Hr) vs. no treatment, and their aortae were studied pharmacodynamically and biochemically *in vitro.* Vasorelaxation potency of NO-dependent vasodilators (Nitroglycerin; SIN-1, an NO donor; acetylcholine) as well as an NO-independent vasodilator (forskolin, a cAMP –dependant dilator) was determined. Potency was appropriately and informatively expressed as an $ED_{50\%}$ concentration producing 50% of the maximum response to each drug. Critically, all aortae were uniformly pre-constricted with phenylephrine to approx 50% of maximum (high

KCL-induced) tension before the dose-relaxation response curves were obtained. Aortic steady-state superoxide levels were assessed by a validated specific lucigenin chemiluminescence assay, and the superoxide level in aortae from control vs. NTG-pretreated rabbits was compared. Additionally, the effects of various interventions [denuding the endothelium; specific inhibitors of oxidases, mitochondrial electron transfer, and NO-synthase] on superoxide levels in NTG-tolerant aortae was assessed as well as effect of superoxide dysmutase on response of the tolerant aortae to exogenous and endogenous nitrodilators.

Results: In NTG tolerant aorta, maximum relaxations to the cGMP/NO - dependent vasodilators (NTG, SIN-1, and acetylcholine) were attenuated by up to approx 50% ($p< 0.05$ for all), but response to cAMP-dependant forskolin was not impaired. In tolerant aortae, removal of endothelium markedly restored sensitivity to NTG and SIN-1 (acetylcholine, of course, requires intact endothelium to express its vasorelaxant activity, otherwise it *vasoconstricts*). Vascular steady state superoxide levels were increased two-fold in tolerant vs. control vessels ($p<0.001$). and the excess was prevented with an inhibitor of a flavoprotein-containing oxidase and with a direct scavenger of superoxide. Importantly and critically, since an inhibitor of NO synthase did not affect chemiluminescence signals, one can conclude that NO is not interfering with this assay for superoxide. Perhaps the cardinal observation was that pretreatment of the tolerant aortae with superoxide dismutase restored vasorelaxant efficacy of exogenous (NTG) as well as endogenous (acetylcholine, which vasorelaxes via endothelial NO release) nitrodilators,

B. Indirect antioxidant activity of hydralazine:

Munzel, T. et al. Hydralazine Prevents Nitroglycerin Tolerance by Inhibiting Activation of a Membrane-bound NADH Oxidase. *J. Clin Invest* 1996 98: 1465-1470.

The authors are those who, as described above, reported that nitrate tolerance in rabbits, demonstrable *ex vivo,* was at least partially due excess vascular (primarily endothelial) superoxide production. Importantly, in the study below, the hydralazine (HYD) dosage, 110 mg/m^2 basis reasonably corresponds to that provided clinically by Bidil, namely approx. 80 mg /m^2

Methods. In the subject ex vivo study, the species, protocol, in-life nitroglycerin (NTG) dosage, and methods -including, critically, that for assuring comparability of vasodilation dose-response curves *in vitro* - were similar to that above. Rabbits either received no treatment; NTG patches (1.5 ug/kg/min X 3 days; hydralazine (HYD) alone (10 mg/kg/d in drinking water; or HYD plus NTG. Aortae were studied in vitro for response to NTG and rates of superoxide generation via lucigenin-enhanced chemiluminescence.

Results: NTG exposure in-life markedly inhibited relaxation to NTG in vitro (max. relaxations in treated and untreated: 92% vs 64 %, respectively), and also promoted vascular superoxide formation two-fold ($P<0.05$). Given concomitantly with NTG, HYD completely prevented - as subsequently monitored in vitro - both the development of hemodynamic nitrate tolerance, and the doubling of endogenous superoxide formation Studies of vessel homogenates revealed an NADH-dependent membrane-associated oxidase as the source of superoxide formation. The activity of this oxidase was 67 +/- 12 nmol superoxide/min/ mg protein in aorta homogenates from NTG-treated rabbits vs. corresponding value of 28 +/- 2 in homogenates from non-tolerant control rabbits. Corroboration was provided when aortae isolated from naïve and NTG –tolerant rabbits were acutely exposed to 1 uM HYD (which is, I believe, a clinically-relevant concentration), and superoxide production was suppressed 2 and 6-fold, respectively ($p<0.05$). . Even in aortae isolated from NTG-naïve rabbits, HYD suppressed superoxide production in association with modestly increased sensitivity to NTG,

indicating that basal activity of the oxidase opposes relaxation to nitrodilators even in normal (i.e., non-tolerant) vessels. Another important observation was that HYD had no direct scavenging effect i.e., adding it (1 uM) to homogenates of tolerant or naïve aortae had no effect on NADH oxidase activity.

C. Animal models of hemodynamic tolerance to nitrodilators, and prevention with hydralazine:

The following is a synopsis of several previously reviewed published studies (See also appended Overview of animal studies of hydralazine- nitroglycerin interaction, A. DeFelice, Feb 25, 1997. memo to Division files: NDA 20-727. (BiDil, Medco Research, Inc.)

In vivo:

1. Bauer, J. and H Fung. Concurrent hydralazine administration prevents nitroglycerin-induced hemodynamic tolerance in experimental heart failure. Circulation 84: 35-39, 1991.

In rats with healed severe LV infarcts and elevated LV End Diastol. Pressure (15-20 mmHg vs < 5 mmHg in intact rats), authors demonstrated *in vivo* that a constant nitroglycerin (NTG) infusion of 10ug/min produced a salutary, but transient (<10 hr) reduction in the LV filling pressure. However, efficacy could be fully preserved with two bolus hydralazine (HYD; 0.1 mg) interventions during the NTG infusion. HYD used alone did not reduce LVEDP, but did reduce LV peak systolic pressure (i.e., afterload). Only the interaction of dosages of nitroglycerin and HYD which used alone reduced LVEDP by approx. 50% and 0%, respectively, was reported; and the dose-response for the HYD-NTG interaction was not studied (or reported).

In vitro:

1. Bauer and Fung, above, also concluded that the HYD-NTG interaction could not be shown in vitro using isolated rat aorta in which response to NTG was monitored after pre-incubation with NTG, NTG+HYD, or buffer per se,. However, this conclusion is unwarranted absent assurance that baseline tension of the isolated artery, and level of phenylephrine-induced precontraction was standardized, and reference responses (typically, contraction to High-K^+) for normalizing data were obtained.

2. Unger, P et al. (Interaction between hydralazine and nitrovasodilators in vascular smooth muscle. J. cardiovasc. Pharmacol. 1993. 21(3): 478-83.) were able to show that in aortas isolated from rats rendered nitrate intolerant ***in vivo*** (50 mg/Kg s. c. daily for 4 days), hydralazine partially, but significantly ($p<0.05$) attenuated tolerance.

In toto, their data reveal that HYD could potentiate the vasorelaxant activity of NTG in n NTG-intolerant tissue, but not the relaxation induced by cGMP or forskolin. thereby indicating that guanylyl cyclase levels, and it's role in relaxation, was *not* promoted by HYD - persuasive evidence that HYD is acting "proximal" to the cyclase.

3. Munzel et al (Hydralazine prevents nitroglycerin tolerance by inhibiting activation of a membrane-bound NADH oxidase. J.Clin Invest. 98: 1465-70. 1996.) , studied relaxation of aortae isolated from rabbits pre-treated for three days with NTG patches (1.5 ug/Kg/min); HYD alone (10 mg/kg/day via drinking water); both agents concurrently; or no in-life pretreatment. Importantly, arteries were preconstricted with phenylephrine to within 30-50% of their maximal (KCl-induced) tone prior to comparing "relaxability". They convincingly showed that marked tolerance to NTG – and also cross-tolerance to acetylcholine and exogenous NO - could be demonstrated in *vitro* in arteries removed from NTG-pretreated cohort. Such tolerance to NTG and to exogenous NO could be completely prevented by concurrent exposure to HYD in-life. What is particularly convincing is the extent of the prevention of the tolerance by HYD, and the fact that the tissue bath did *not* contain added HYD. Normalization of baseline tone to a nominal

50% percent of a reference maximal contraction to KCl was a cardinal aspect of the experimental design, without which the experiment would be uninterpretable, and results *a priori,* invalid.

Accordingly, hydralazine is persuasively able to prevent or attenuate tolerance to nitroglycerin a) in the rat *in vivo* using LVEDP as the metric and b.) in both the rat and rabbit *in vitro* (i.e., aorta isolated from rats and rabbits rendered NTG tolerant in-life, and studied *in vitro* with care taken to normalize baseline tone – indispensable to comparing relative vasorelaxation efficacy and potency.

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/s/

Albert Defelice
6/20/05 04:05:28 PM
PHARMACOLOGIST

NDA 20-727

PHARMACOLOGY/TOXICOLOGY REVIEW

SUBMISSION: dated 21 December 2004.This is amendment no. 121, and asserted to be a complete response to a non-approval letter of July 2, 1997.

CENTER RECEIPT DATE: 23 December 2004

REVIEW COMPLETION DATE: 20 April 2005

REVIEWER: Albert DeFelice, Ph.D.
Supervisory Pharmacologist
Division of Cardio-Renal Drug Products (HFD-110)

SPONSOR: Nitromed, Inc. Original sponsor of NDA 20-727 was Medco Research Inc, Research Triangle Park, NC

DRUG PRODUCT: BiDil (hydralazine HCl and isosorbide dinitrate) tablets.

RELATED APPLICATIONS: IND 41,816

PATENT STATUS: Nitromed, Inc. has three patents for this combination product.

DRUG SUBSTANCES: Hydralazine hydrochloride (M.W. 196.64; $C_8H_8N_4 \cdot HCl$); Isosorbide dinitrate (ISDN; M.W. 236.14; C6H8N2O8.)
Pharmacologic Categories: Arterial and venous vasodilator agents.

PROPOSED INDICATION: This combination is intended to treat heart failure as an adjunct to standard therapy in black patients in whom ACE-inhibitors are ineffective or contraindicated.

FORMULATION AND ROUTE OF ADMINISTRATION: Each BiDil 20 tablet for oral administration contains 20 mg of ISDN and 37.5 mg of hydralazine hydrochloride. Inactive ingredients include lactose, cellulose, and Opadry orange film coating.

PROPOSED DOSAGE: One BiDil tablet t.i.d. initially, and titrated to a maximum of 2 tablets t.i.d., or to the maximum tolerated dosage.

NONCLINICAL PHARMACOLOGY/TOXICOLOGY DATA: Sponsor provided no animal toxicology studies of hydralazine combined with ISDN. .This submission was intended to provide a complete response to the July 2, 1997 Not Approvable Letter which identified three Chemistry and three Pharmacokinetic deficiencies, and the need of an additional clinical trial. The former have been addressed in a series of prior submissions. Rather, the subject submission provides results of the African American Heart failure trial (A-HeFT) in black CHF patients.

EVALUATION: No animal safety studies of the combination product are deemed necessary in view of the very extensive clinical experience with the monotherapies, and the unequivocal results of two placebo-controlled clinical trials of the combination therapy in 1,692 subjects (1,230 black) with mild to severe heart failure treated for up to 18 months. The A-HeFT trial, which evaluated BiDil vs. placebo in 1,050 blacks with symptomatic NYHA classIII-IV heart failure, was terminated early in view of a 43% reduction in all-cause mortality (p=0.012); a 48% reduction in death associated with worsening heart failure; and a 73% reduction in pump failure death.

The mutagenic and long-term carcinogenic potential of hydralazine, but not ISDN, has been assessed in animals. While clinical experience does not substitute for the absence of such data for ISDN, we can, however, rely on the prior agency finding of its safety and efficacy under 505(b)(2).

LABELLING:
Reviewer is requiring that the proposed Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy Category C labeling sections be modified to conform to that in the corresponding sections for the individual drugs per PDR 51st edition 1997, and updated to include relative exposures (dosage multiples) based on dosages expressed as mg/ square meter of body surface area:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Isosorbide Dinitrate

No animal studies have been performed to evaluate the mutagenic or long-term carcinogenic potential of isosorbide dinitrate. In a modified two-litter reproduction study among rats fed isosorbide dinitrate at 25 or 100 mg/kg/day (up to 9 times the Maximum Recommended Human Dose provided by BiDil on a body surface area basis) , there was no evidence of altered fertility or gestation..

Hydralazine Hydrochloride

An increased incidence of lung tumors (adenomas and adenocarcinomas) was observed in a lifetime study in Swiss albino mice given hydralazine hydrochloride continuously in their drinking water at a dosage of about 250 mg/kg per day (6 times the MRHD provided by BiDil, on a body surface area basis). In a 2-year carcinogenicity study of rats given hydralazine hydrochloride by gavage at dose levels of 15, 30, and 60 mg/kg/day (up to — times the MRHD provided by BiDil, on a body surface area basis) microscopic examination of the liver revealed a small, but statistically significant increase in benign neoplastic nodules in males (high dosage) and females (high and intermediate dosage). Benign interstitial cell tumors of the testes were also significantly increased at the high-dosage. [ ]

Hydralazine hydrochloride was shown to be mutagenic in bacterial systems, and was positive in rat and rabbit hepatocyte DNA repair studies *in vitro*. Additional *in vivo* and *in vitro* studies using lymphoma cells, germinal cells, and fibroblasts from mice; bone marrow cells from Chinese hamsters; and fibroblasts from human cell lines did not demonstrate any mutagenic potential for hydralazine hydrochloride.

[

] **this sentence should be reviewed by medical officer, and deleted unless Sponsor has epidemiological evidence)**

Pregnancy Category C: Isosorbide dinitrate has been shown to cause a dose-related increase in embryotoxicity (increase in mummified pups) in rabbits at oral doses as low as 70 mg/Kg (12 times times the MRHD provided by BiDil on a body surface area basis).

Hydralazine hydrochloride is teratogenic in mice at 66 mg/Kg and possibly in rabbits at 33 mg/Kg (respectively, — and 3 times the MRHD [ ] by BiDil on a body surface area basis). [ ]

There are no [ ] studies using BiDil or its active ingredients alone in pregnant women. The following italicized wording, submitted by Sponsor, should be reviewed by medical officer:

[ ]

[ ]

A meta-analysis of randomized controlled trials comparing hydralazine hydrochloride with other antihypertensive agents for severe hypertension in pregnancy found that hydralazine hydrochloride was associated with significantly more maternal hypotension, placental abruption, caesarean sections and oliguria, with more adverse effects on fetal heart rate and lower Apgar scores. [71]

A combination of propranolol and hydralazine hydrochloride was administered to 13 patients with long-standing hypertension during 15 pregnancies. These pregnancies resulted in 14 live births and one unexplained stillbirth. The only neonatal complications were two cases of milk hypoglycemia. [72] Hydralazine hydrochloride has been detected in maternal and umbilical plasma in patients treated with the drug during pregnancy. [73]

Isosorbide dinitrate has been used for effective acute and sub-chronic control of hypertension in pregnant women. [74,75,76]

RECOMMENDATION:

Approvable from pre-clinical perspective.

This IND contains no new animal pharmacolodynamic, pharmacokinetic, or toxicology data, and none are needed or anticipated. Reviewer's recommended changes to the proposed **Carcinogenesis, Mutagenesis, Impairment of Fertility** and **Pregnancy Category** sections are indicated above.

It is further recommended that the medical officer refer to the publications provided by Sponsor to support their assertions in the Pregnancy Category C labeling of clinical findings associated with use of these vasodilators in gravid patients.

References 26, 27, and 69-76 are publications identified on pages 101-104 of NDA 20-727 submission 23.1 received 12/23/04. Sponsor also provided copies of these publications are in Section 2.4.2 of the latter.

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/s/

Albert Defelice
4/20/05 04:51:49 PM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION **Public Health Service**
Division of Cardio-Renal Drug Products

Memorandum

DATE : February 24,1997 4/25/97

FROM : Albert DeFelice, Team Leader, HFD-110

THROUGH : Dr. Raymond Lipicky, Div. Director, HFD-110

SUBJECT : Overview of animal studies of Hydralazine-nitroglycerin interaction

TO : Division files: NDA 20-727 (Bidil; Medco Research, Inc.)

This is an overview of an *in vivo* and two *in vitro* animal studies examining whether hemodynamic tolerance to nitrates, induced *in vivo* or *in vitro*, could be prevented or reversed by hydralazine (HYD). Bauer, J. and Fung, H. (1991) compared ability of nitroglycerin (NTG) and HYD, alone and combined, to alleviate elevated pre-load in a conscious rat model of CHF. Two *in vitro* studies examined relaxation by NTG of aortae isolated from rats or rabbits which had been either untreated or rendered nitrate - tolerant *in vivo*: Unger, P. et al. (1993) studied such aortae (rat) with and without a HYD pre-incubation, and also examined the pyridoxal- dependence of the NTG-HYD interaction ; Munzel, T. et al. (1996) studied aorta from rabbits treated with NTG and/or HYD for three days.
A hydralazine-nitroglycerin interaction was consistently demonstrated. The dose-response for the potentiation was not studied.

Summary of Overview:

A. Rat CHF model:

This *in vivo* study of rats with healed severe myocardial infarcts revealed a salutary , but transient (< 8 hrs.), effect of a constant NTG infusion on elevated left-ventricular filling pressure (i.e.,LVEDP). Efficacy, however, could be fully preserved by two bolus HYD (0.1mg) interventions during the NTG infusion. The HYD regimen , when used alone, did not reduce LVEDP but did decrease LV peak systolic press. (i.e.,afterload). The dose-response for this HYD -NTG was not studied.

I could not interpret author's unrelated *in vitro* study of response of aorta to NTG after pre-incubation with NTG, NTG+HYD, or buffer *per se*. That is, baseline tension of the isolated artery , and level of phenylephrine - induced pre-contraction, was not standardized, and reference responses (typically contraction to high-K+) for normalizing data were not obtained or reported.

B. Isolated Rat Aorta:

Unger *et al* performed three sets of carefully controlled *in vitro* experiments on aortae pre-contracted with phenylephrine: a.) dose-response to NTG, cGMP, and forskolin (an adenylate cyclase activator) for control vs. HYD-preincubated aortae;

b.)dose-response to NTG, with and without HYD pre-incubation, on aortae from control rats vs. rats rendered tolerant to NTG *in vivo* (50 mg NTG s.c../Kg b.i.d./day/4 days), and c.) dose-response to HYD in control aorta vs. tissue rendered tolerant to NTG *in vitro*. Experiments , *in toto*, clearly revealed that HYD could potentiate vasorelaxing activity of NTG in control as well as NTG-tolerant tissue, but not the relaxation induced by cGMP or forskolin. Since pre-incubation with NTG also enhanced sensitivity of tissue to HYD, there is a mutual interaction between NTG and HYD.

C. Isolated Rabbit Aorta:

Munzell *et al.* treated rabbits with HYD (10 mg./Kg/day in drinking water for 3 days), NTG (NTG patch, 1.5 mcg/Kg/min for 3 days, changed daily), both dilators, or tap water alone and then studied aortae with endothelium left intact. Vessels were uniformly preconstricted with phenylephrine, and full cumulative dose-response curves to NTG, syndonimine (SIN-1), and acetylcholine were obtained . *In vivo* treatment with NTG for 3 days markedly attenuated subsequent maximal relaxations in response to NTG, and caused cross tolerance to exogenous nitric oxide (SIN-1) and NO endogenously released by acetylcholine. Concomitant treatment with hydralazine clearly prevented development of tolerance to NTG, and of cross tolerance to both SIN-1 and, to a lesser extent, acetylcholine.

Overview:
Summary of salient results:

A: Rat CHF model.

In vivo: Authors studied conscious catheterized rats with healed massive LV infarcts (ca. 50 % of free LV wall) and LV filling pressures of approx. 15-20 mmHg (vs. < 5mm Hg in intact rats). These values are comparable to what I and others have published. As shown in **Figure 1**., a constant NTG infusion - capable of maximally reducing the LVEDP by approx. 50% within 30 min. without substantial change in heart rate - was completely ineffective within 10 hours. However two intervening boluses of HYD completely prevented tolerance to NTG in association with a persistent concomitant depression of afterload (i.e., LV peak systol. pressure.). Authors showed that the only effect of HYD alone on LV hemodynamics was to moderately depress afterload, but not to the extent or duration of that seen when HYD was superimposed on NTG.
Dose-response, and role of afterload, of the NTG-HYD interaction was not studied.

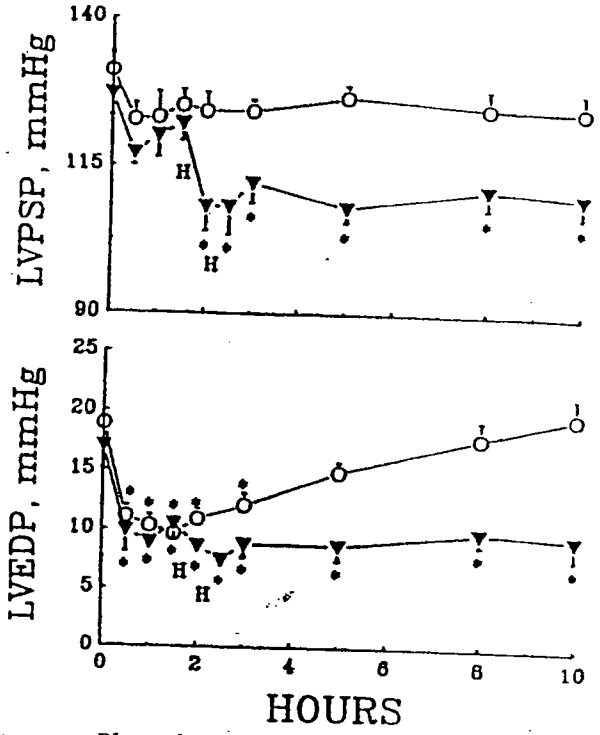

FIGURE 1. *Plots of effects of nitroglycerin infusion alone (○, n=7) or in combination with hydralazine (▼, n=8) on left ventricular hemodynamics in rats with heart failure. Nitroglycerin was infused at 10 μg/min in both groups. Hydralazine was administered at 1.5 and 2.0 hours (0.1-mg bolus) (H) during nitroglycerin infusion. Nitroglycerin alone produced initial reductions in left ventricular end-diastolic pressure (LVEDP) but no effect on left ventricular peak systolic pressure (LVPSP). LVEDP returned to baseline by 8 hours, indicating tolerance development. Hydralazine caused significant reductions in LVPSP, which was maintained during remainder of nitroglycerin infusion. Hemodynamic tolerance to nitroglycerin did not occur during coadministration. Mean±SEM values are shown. *Statistically significant differences from baseline (p<0.05).*

In vitro: Authors suggested that the beneficial effect of HYD on NTG induced tolerance was not a result of localized vascular interaction between the two drugs because the presence of HYD did not prevent development of NTG tolerance *in vitro* (**Figure 2**).

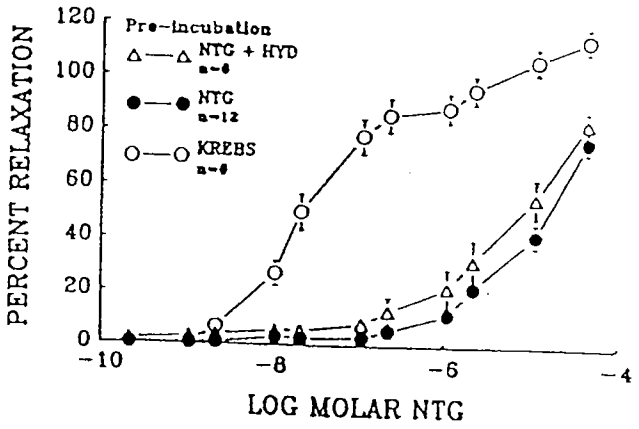

FIGURE 2. *Plot of effect of hydralazine (HYD) on nitroglycerin (NTG) tolerance induced in vitro. Preincubation of rat aortic rings with NTG (0.22 mM) caused a significant shift of concentration/effect curve to the right. This development of vascular tolerance was not affected by presence of HYD (1.0 mM) in preincubation solution. No differences between NTG and the NTG plus HYD groups were detected.*

However it is difficult to interpret the seemingly clear-cut data in figure 2 because the authors do not report whether any attempt was made to apply uniform tension to the isolated aorta , to uniformly pre-contract the vessels prior to developing the NTG dose-response curves , or to normalize relaxation as a percent of a reference contraction e.g., to high-K+. Such precautions are standard practice in studies aimed at accurately assessing changes in responsiveness of isolated vasculature (see below).

B. Isolated Rat Aorta:

For all experiments, authors report that they removed the endothelium in order to place the vessels under optimal conditions for response to vasodilators (Pohl and Busse, 1987). Furthermore, all tissues were consistently placed under 1.5 gms. resting tension, and uniformly precontracted with doses of phenylephrine adjusted to achieve comparable levels of active tone prior to obtaining full dose-responses to NTG:

Effects of HYD incubation: Two adjacent preparations were studied in parallel, one prep. serving as control while the other was incubated for 30 min. with HYD. As shown in **Figure 3**, incubation with HYD , at both 0.01 or 0.1 mM concentrations, potentiated the responses to NTG. However, vasorelaxant potency of SIN-1, cGMP, or forskolin was not affected.

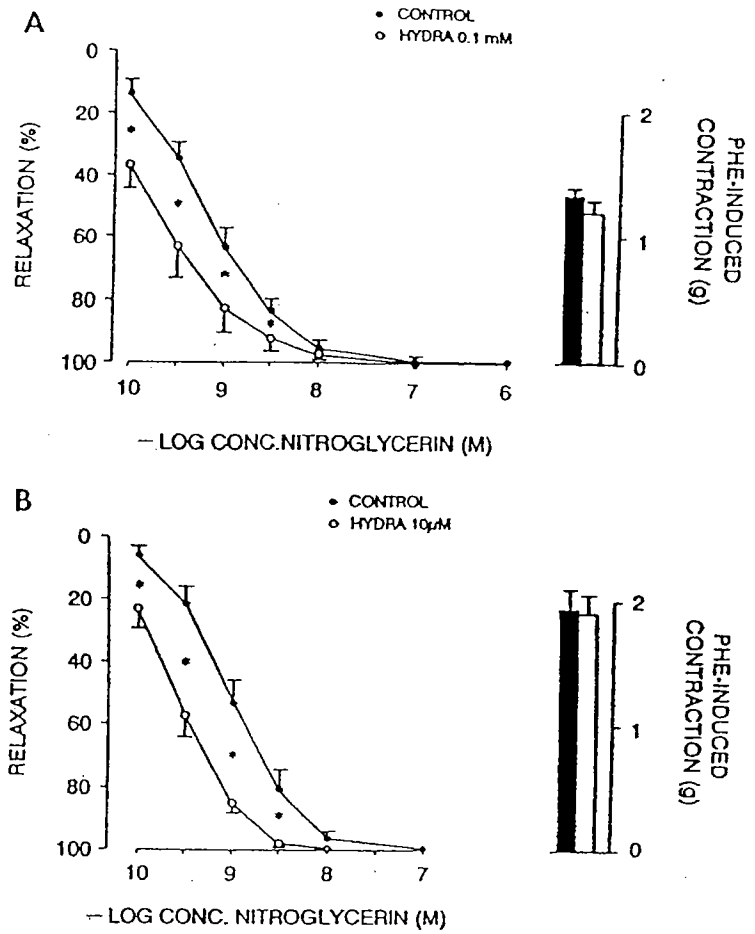

FIG. 3. Concentration–response curve to nitroglycerin (NTG) in aortic rings precontracted with phenylephrine (PHE). In preparations incubated with hydralazine (open circles) at 0.1 m*M* (A, n = 6) and 10 μ*M* (B, n = 6) for 30 min, the responses to NTG were potentiated in comparison to those in control preparations (solid circles). Histograms illustrate the contraction elicited by PHE in control preparations (filled histograms) and in hydralazine-incubated preparations (open histograms) (difference not significant). Results are means; bars = SEM.

For aortae isolated from rats rendered tolerant in vivo to NTG, an incubation with HYD also induced a potentiation of responses to NTG. (**Figure 4**).

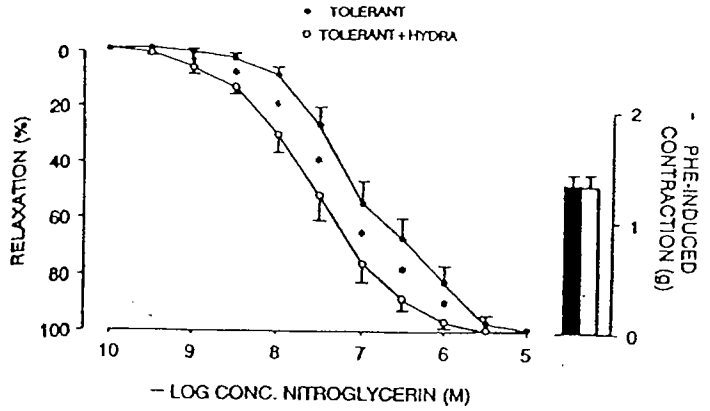

FIG. 4. Concentration–response curves to nitroglycerin (NTG) in aortic rings isolated from rats rendered tolerant in vivo to NTG and precontracted with phenylephrine (PHE) (n = 6). In preparations incubated with hydralazine (0.1 m*M*) for 30 min (open circles), there was a significant potentiation of the responses to NTG compared to hydralazine-untreated preparations (solid circles). Histograms illustrate the contraction elicited by PHE in control preparations (filled histogram) and in hydralazine-incubated preparations (open histogram). Results are means; bars = SEM.

Comparison of figures 3 and 4 shows the extent of the desensitization to NTG after 4 days *in vivo* exposure to NTG (IC50 of 0.3 nM vs. 86 nM, a difference of several orders of magnitude).

Pre-incubation with NTG also potentiated response to HYD by approx. 5-fold (IC50 of 160 microM vs. 29 microM).

Results clearly reveal that a 30 min. exposure to hydralazine potentiates vasorelaxant effect of NTG on aorta isolated from rats rendered tolerant to NTG in vivo as well as on aorta exposed to NTG for the first time in vitro.

Although two levels of HYD were tested, the dose-response for it's NTG- potentiating activity was indeterminate, in my judgment, since the phenylephrine precontraction, i.e. level of active tone imposed prior to obtaining full NTG dose-response curves, were not similar (due to the effect of the HYD incubation).

C. Isolated Rabbit Aorta:

After 3 days *in vivo* exposure to HYD and/or NTG, authors excised the aorta, and obvious care was taken to allow comparison of vasodilating efficacy and potency to be made. Tissues, with endothelium intact, were uniformly placed under 5 gms. of resting tension , and a maximal contractile reference response to high-K+ was obtained . Prior to obtaining full dose-responses to NTG, the vessels were precontracted with a concentration of phenylephrine achieving 30-50% of maximal (KCl-induced) tone. As shown in **Figure 5**, *in vivo* exposure to NTG inhibited potency and maximum relaxations to NTG, and also (to a lesser extent) to SIN-1 and acetylcholine.NTG exposure also increased relative rate of superoxide (.O2 -) production (see below). When given concomitantly with NTG, HYD completely prevented the development of nitrate tolerance, and cross-tolerance. and also prevented the increase in endogenous rates of superoxide production seen with NTG alone.

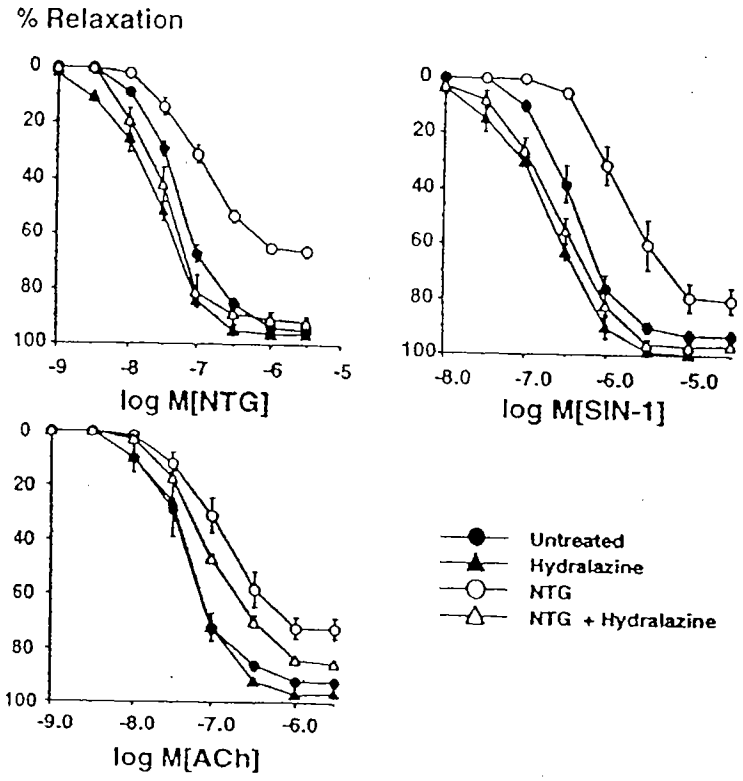

Figure 5. Effects of hydralazine treatment on the relaxations to nitroglycerin, the sydnonimine SIN-1, and the endothelium-dependent vasodilator acetylcholine (*ACh*). The segments were preconstricted with phenylephrine, and relaxations to cumulative concentrations of each drug were examined. Concomitant treatment with hydralazine increased sensitivity to nitroglycerin and SIN-1 in aorta from untreated animals and corrected tolerance and cross-tolerance in nitroglycerin-treated animals. Data are expressed as mean±SEM of five to nine experiments.

I am not familiar with the lucigenin-enhanced chemiluminescence method used to monitor endogenous superoxide production. However, a reference citing it's specificity for superoxide is given and , furthermore, authors showed that nitric oxide did not interfere with the method. The ability of HYD to inhibit vascular .O2- production would represent a novel mechanism of action for that drug, and it's interaction with NTG. This significant biochemical interaction supports my conclusion that NTG-potentiating activity of HYD is not an artifact of possible differences in baseline tone between the various groups of isolated aorta existing or imposed prior to obtaining the NTG dose-response curves. As noted above, authors went to some length to correct for any such differences, and to express size of relaxation as a percentage of individual reference responses (a standard normalizing practice).

Conclusions:

These studies clearly demonstrate, to me, that tolerance to nitroglycerin could be induced *in vivo* in both rats and rabbits, and that hydralazine could convincingly prevent it's development when co-administered with NTG in both species. Moreover, hydralazine could also reverse the phenomenon when nitrate-tolerant rat tissue was

excised and incubated with hydralazine. Furthermore, arterial smooth muscle previously unexposed to NTG could also be rendered significantly and appreciably more sensitive to NTG by a 30 min. incubation with HYD.

A possible mechanism for the HYD-NTG interaction is that hydralazine prevents the increase in rate of superoxide generation seen with NTG alone.

While NTG is usually used clinically at dosages which are relatively selective for venous smooth muscle, the animal studies focused on arterial smooth muscle . However , decreases in systemic vascular (arteriolar) resistance , and tachycardia , are common after nitrate administration to humans (Parmley and Chatterjee, 1993). Moreover, the in vivo rat study monitored pre-load, and it is reasonable, *a priori*, to project clinical relevance of these animal findings.

NDA 20-727

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICIOLOGY CONSIDERED FOR THE 45-DAY FILING SUMMARY

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08/26/96

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6/26/97

SPONSOR: Medco Research, Inc.
P.O. Box 13886
Research Triangle Park

DATE OF SUBMISSION: July 3, 1996
DATE OF RECEIPT: July 3, 1996

DRUG:

Proprietary Name: BiDil®.
Established Names: hydralazine HCl and isosorbide dinitrate.
Proposed Indication for Use: Treatment of Congestive Heart Failure in patients intolerant to ACE Inhibitors.
Dosage Form: Tablets; strengths of 75/40, 75/20, 37.5/20, 37.5/10 (weight of mg hydralazine/mg isosorbide dinitrate per tablet, respectively).
Rationale for development of this drug therapy: This treatment is envisioned as an adjunct to standard therapy in patients who are intolerant to, or have a contraindication to, angiotensin-conversion enzyme (ACE) inhibitiors.

REVIEW OF PRECLINICAL BIOLOGY:

Sponsor has assembled and evaluated the nonclinical biology and chemistry of the proposed fixed-dose formulations. As would be expected, the emphasis of this presentation is on the toxicology and pharmacology. It must be emphasized that no original reserarch was conducted in support of the reported information. The literature was apparently searched and the resources of the Freedom of Information act (FOI) were called into play to provided data used to support the application.

The resulting summary is a reasonable one which covered the following important highlights:

The nonclinical animal pharmacology of hydralazine alone and in combination with isosorbide dinitrate (ISDN).
The nonclinical and animal pharmacology of ISDN alone and in combination with hydralazine.
The mechanism of action and the hemodynamic effect of hydralazine in rats and dogs.
Proposed mechanisms and pharmacological properties of organic nitrates.

Preclinical toxicology studies were not conducted by the sponsor's laboratories, but were based on data deduced from the available scientific literature and that available under the terms of the FOI.

Hydralazine was studied in acute toxicity protocols (single dose) using mice, rats, hamsters, and dogs. Multiple-dose studies were done using dogs. Carcinogenicity studies were done in mice and rats. Reproduction-fertility studies were done in rats and teratology studies were done in mice, rats, and rabbits. *In vitro and in vivo* mutagenicity studies were done in mammals and non-mammals.

ISDN toxicity studies reviewed were from single dose acute, rat toxicity studies, a modified rat fertility study, and a rabbit study of teratology. Various animals were used for the study of the LD_{50} values of both drugs. Under the carcinogenicity sub-topic, a relationship between heart rate and tumorogenicity is suggested (e.g., 300+heart rate) on page 33. This should be explained or eliminated as appropriate. Sponsor reported increased incidence of pulmonary adenomas and adenocarcinomas in a study wherein mice were treated with daily doses of "around" 250 mg/kg of hydralazine over an unspecified period of time. This is obviously of limited value. In a similar manner, sponsor reported increased benign hepatic neoplastic nodules in rats of both sexes dosed with 60 mg/kg/day of hydralazine, and also in females dosed with 30 mg/kg/day. An increased incidence of benign testicular interstitial tumors was also seen in males dosed with 60 mg/kg/day.

Teratogenicity related to oral treatment with hydralazine was studied in rats dosed with 10 to 50 mg/kg; decreased food consumptdion in both sexes receiving 25 and 50 mg/k/day was observed. Oral hydralazine doses of 10 to 50 mg/kg resulted in decreased food consumption by both sexes of the two highest doses and decreased pup weight during litter lactation by these groups. Hydralazine was teratogenic in mice dosed daily with 20-to150mg/k; the most susceptible period being gestation day 11 (GD11). Hydralazine was not teratogenic in rats receiving 180 mg/kg/day (during the period of major organogenesis) in the presence of maternal toxicity.

No drug-related effects were seen in a modified reproduduction-fertility study in rats given daily doses of 25 or 100 mg/kg of ISDN. ISDN administered at daily oral doses of 30 and 150 times the maximum recommended human daily dose (during the period of major organogenesis) resulted in dose-related increase in embryotoxicity, as manifested by mummified fetuses.

Sponsor appears to have quite clearly elucidated the mutagenic and clastogenic properties of hydralazine and ISDN.