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MAR 30 1990

Review and Evaluation of Nonclinical Biologic Data

NDA 19-546

Sponsor: Sandoz Pharmaceuticals

Amendments Dated: 11/28/88 and

11/23/89

Reviewer: Robert H. Harris

Drug Code Name: PN 200-110 Capsules

Generic Name: Isradipine

Trade Name: Dynacirc

Indication: Hypertension

Related INDs:

The sponsor has provided two amendments to NDA 19, 540. The first (11/23, 83) provides a draft summary basis of approval. This has been reviewed and edited for accuracy. A revised nonclinical section of the SBA for isradipine has been attached to this review.

A second amendment (11/23 89) addresses the question on drug-relatedness of isradipine treatment to the development of hepatocellular carcinomas in male CD-1 mice. The sponsor has taken the position that isradipine not be considered a hepatocarcinogen based on five lines of evidence regarding the biological significance of the findings as follows: (1) new morphological classifications (2) historical control rates, (3) target organ toxicity, (4) structure-activity relationships, and (5) biological behavior of the lesions. A prior analysis (Peto) conducted by the Division of Biometrics had shown that a significant positive trend ($p < 0.004$) existed between the incidence of hepatocellular carcinomas and isradipine dose. This relationship was largely attributable to an increased incidence of neoplasms and impaired survival in the high dose treatment group.

Because the accepted nomenclature for classifying mice liver lesions has changed since the original reading of the slides, the sponsor had two independent consultants blindly reread the slides and jointly agree as to diagnoses of any lesions. Table 1 displays the original tumor incidences and the newly determined incidences.

Table 1. Liver Tumor Incidences Among Male Mice

| <u>First Reading</u> | <u>Control 1</u> | <u>Control 2</u> | <u>Low</u> | <u>Mid</u> | <u>High</u> |
|--------------------------|------------------|------------------|------------|------------|-------------|
| Nodular Proliferation | 8 | 6 | 3 | 7 | 11 |
| Hepatocellular Carcinoma | 7 | 10 | 12 | 7 | 17 |
| <u>Second Reading</u> | | | | | |
| Hepatocellular Adenoma | 10, 2 | 11 | 11, 7 | 10 | 16, 10 |
| Hepatocellular Carcinoma | 1 | 1 | 5 | 2 | 7 |

() parenthesis denotes number of animals having only adenomas.

The data indicate much lower numbers of diagnosed carcinomas in each treatment group without any effect on the rank order of treatment groups with respect to number of tumors. Formerly 54 male mice had diagnosed hepatocellular carcinomas and with the new classification system only 20 had tumors of this type.

Statistical analysis of the data showed that the pairwise comparison of hepatocellular adenomas between the control and high dose treated males was statistically significant (Birnch-Cox: not Fisher exact). Among terminally sacrificed animals, a significant linear trend was observed ($p < 0.018$) and the high dose group versus control group difference was significant ($p < 0.042$).

Examining the hepatocellular carcinoma data the statistical significance was achieved only when the natural dying animals were considered. There was a significant overall trend ($p < 0.013$) and significant intergroup

difference between the control and high dose group males ($p < 0.019$,
Birch-Cox: $p < 0.037$, Fisher exact . The combined category of
hepatocellular adenoma or carcinoma showed a significant overall trend
with dose ($p < 0.031$) and a significant difference between the high dose
and control by the Birch-Cox test ($p < 0.024$, but not Fisher exact test.

A Peto analysis was not reported for these new data. Because the impaired
survival among the high dose males was not factored into the analyses and
because numerically increased incidences of both hepatocellular adenomas
and carcinomas were observed it is impossible to determine whether the
second evaluation of the slides provides any meaningful differences from
the results of the first evaluation.

The sponsor also provided historical control data from the color studies,
from those mentioned by L. Sher in his article [Spontaneous Tumors in
Control F 344 and Charles River CD Rats and Charles River CD-1 and B6C3Hf1
Mice], from the Charles River database and from the Sandoz Research
Institute. The most meaningful historical control data is generally that
with the fewest differences from the present study. In three studies
conducted
at Sandoz there were no diagnosed hepatocellular carcinomas among over 150
mice. Thus it cannot be argued from this data (as the sponsor has) that
an inordinately low number of liver tumors in male mice from the
concurrent Control Group I was responsible for the significant differences

between groups. The historical control data from other sources is less reliable because of differences in facilities, in source of animal supply, and in animal caretakers and pathologists.

In a third argument for the lack of biological significance the sponsor states that as a general rule most chemical carcinogens induce damage to the target cells at carcinogenic levels and PN 200-110 does not induce liver damage. They point to subchronic and chronic studies in rats and dogs which show little evidence of drug induced hepatotoxicity. No mouse study data are offered as support their conclusions but there is presented the expert assessments of Drs. Squire and Hardisty who feel that there are no indications of hepatotoxicity in the male mice of the present study. However, the incidences of acidophilic and basophilic foci and of hepatocellular adenomas are all greater or comparable in the high dose treatment group relative to either of the control groups in the study. It should also be pointed out that if there were significant drug-induced toxicity at the highest dose then one could argue that the maximal tolerated dose had been exceeded.

The structure-activity analysis failed to find any structures related to PN 200-110 or four metabolites in the TOPKAT program of Health Designs, Inc. Thus no meaningful information can be gleaned from this source.

CONCLUSION

The sponsor has provided additional data to support its claim that the liver tumors observed in mice treated with isradipine do not predict a tumorigenic potential for patients treated with therapeutic doses of the drug. None of the arguments of themselves possess sufficient weight to persuade one to their conclusions. However, taken collectively some merit must be given to their position. Still the statements about liver tumors should remain in the labeling and the ultimate approvability of the drug should rest on its perceived benefit relative to its residual risk of possessing a tumorigenic potential for man.

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IV. Pharmacology

A. In Vitro Activities Related to Mechanism and Specificity of Action

1. Vascular Smooth Muscle

Isradipine, a calcium entry blocker, is a potent inhibitor of depolarization-induced contraction as demonstrated in isolated rabbit aortic ring and dog coronary artery preparations. The calcium antagonism was selective for depolarization-induced as compared to receptor (noradrenaline and serotonin)-mediated contractions in rabbit aorta. Isradipine also inhibited depolarization-induced calcium uptake in rabbit aorta; it had no effect on noradrenaline-induced calcium uptake or on calcium content of nonstimulated resting smooth muscle.

Isradipine, like other calcium antagonists, was found to inhibit serotonin (5-HT)-induced contractions of canine cerebral (basilar) and peripheral (mesenteric) arteries. It showed a marked selectivity, inhibiting the sensitivity of basilar arteries to 5-HT at concentrations lower than those required to antagonize the mesenteric artery. In vitro blocking activity against blood-induced spasms of dog basilar arteries as well as antagonism of depolarization- and blood-induced spasms on human anterior cerebral arteries have also been observed with isradipine.

2. Cardiac Tissue

Investigations using guinea pig and cat cardiac tissue demonstrate that isradipine lowers heart rate (sinus rate of isolated guinea pig or cat atria) at concentrations that are not negatively-inotropic (paced left guinea pig atria or paced cat papillary muscle).

In the globally-ischemic Langendorff rabbit heart model, isradipine exhibited cardioprotective activity in the absence of negative inotropic effects.

B. In Vivo Effects of Isradipine on the Cardiovascular System

1. Effects on the Heart and Peripheral Circulation

Hemodynamic and regional blood flow studies were

conducted with isradipine in anesthetized cats and conscious rabbits at intravenous doses of 1-30 ug/kg and 10-30 ug/kg, respectively. Isradipine was also examined in anesthetized dogs for hemodynamic (3 ug/kg i.v.) and electrophysiological cardiac (0.25-8 ug/kg i.v.) effects and in anesthetized rabbits for effects on cerebrocortical vasospasms (1 ug/kg i.v.).

In vivo experiments in cats, dogs and rabbits showed isradipine to be a potent dilator of coronary vasculature. Isradipine caused prominent increases of blood flow to the heart and to the left ventricular wall, especially, the subepicardial layer. Significant vasodilation of other vascular beds was noted only for the brain and skeletal musculature. In anesthetized open-chest cats, blood pressure and heart rate were reduced and cardiac output and total peripheral conductance were increased following isradipine administration; myocardial contractility, assessed by peak acceleration of blood in the aorta, was also increased. In anesthetized open-chest dogs, isradipine increased coronary flow, lowered blood pressure, increased cardiac output and tended to reduce heart rate and increased myocardial contractility; myocardial oxygen consumption was lowered.

Isradipine did not induce significant electrophysiological cardiac effects when administered to pentobarbital anesthetized dogs alone or in combination with the beta-blocker pindolol.

In anesthetized rabbits, isradipine at 1 ug/kg i.v. antagonized autologous plasma-induced cerebrocortical vascular spasms. The dose used in this test was ten times lower than the lowest vasodilating dose in experiments with conscious rabbits.

2. Interaction between Isradipine and Verapamil and Two Beta-adrenergic Blockers on Cardiovascular Parameters in Conscious Rabbits

Calcium antagonists and beta-blockers are frequently used in cardiovascular therapy alone and in combination. The interaction between these two agents in combination may occasionally cause problems. The effects of isradipine (0.01, 0.03 and 0.1 mg/kg i.v.) were compared with those of verapamil (0.1, 0.3 and 1 mg/kg i.v.) in conscious rabbits pretreated with either pindolol (0.3 mg/kg i.v.),

propranolol (1 mg/kg i.v.) or placebo.

3. Antihypertensive Autonomic and Diuretic Effects

Antihypertensive Effects in Spontaneously Hypertensive Rats

In spontaneously hypertensive rats, isradipine at 1.01 and 0.1 mg/kg s.c. and 0.01, 0.1 and 10 mg/kg p.o. produced dose-dependent moderate antihypertensive effects without causing tachycardia. The effects lasted approximately 24 hours. Higher doses, which also lowered blood pressure to normotensive levels, caused marked tachycardia.

Autonomic Effects in Anesthetized Rats

In anesthetized rats i.v. infusion of isradipine (0.001-0.7 mg/kg) produced a decrease in blood pressure, inhibited the pressor responses to carotid occlusion and noradrenaline and the cardiovascular responses to isoprenaline. Responses of the nictitating membrane to preganglionic nerve stimulation were also inhibited at the higher dose levels.

Water and Electrolyte Excretion in Rats

Isradipine (0.3-3 mg/kg p.o. administered alone or in combination with the angiotensin converting enzyme inhibitor captopril, increased water and electrolyte excretion. Nifedipine (0.3-3 mg/kg p.o.) also increased water and electrolyte excretion, but in combination with captopril it was ineffective or caused mild urinary volume and electrolyte retention.

4. Other Pharmacological Effects of Isradipine

Antiproliferative Effects in the Rat Carotid Artery Balloon Catheterization Model

The antiproliferative effect of isradipine on arterial lesion development was investigated at 0.25 and 1 mg/kg s.c. in the rat balloon catheterized carotid artery model. The parameters measured from histological cross-sections of balloon catheterized carotid arteries were intimal lesion height and medial thickness. Neointimal proliferation was inhibited by both doses of isradipine.

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2. Antiatherogenic Activity in the Cholesterol-Fed Rabbit

Isradipine was administered orally at 0.3 mg/kg/day for 10 weeks to male rabbits fed a diet containing 1% cholesterol. At the end of the dietary period, and after a 24 hour fast, the rabbits were anesthetized and the aortas were excised for analysis. Isradipine decreased cholesterol accumulation in the aorta by 13% and significantly reduced the area of atherogenic lesions in the aorta.

3. Effects on Pulmonary Function and Bronchoconstriction in Guinea Pigs

Isradipine at 10 ug/kg i.v. produced no direct bronchodilator effects and caused no significant changes in either lung resistance or compliance. Bronchospasms induced by histamine and acetylcholine were antagonized 66% and 32%, respectively.

4. Effect on Prolactin Secretion in Rats

Isradipine (2-12 mg/kg s.c.) is a very weak inhibitor of prolactin secretion in the rat.

5. Effect on Serum Lipoproteins in Rats

Isradipine was administered to male rats for six days at 0.25% in the diet which provided a daily dose of 200 ug/kg.

The only significant changes observed were moderate reductions in weight gain (56%) and marked reductions in serum triglycerides (58%). All other measured parameters were not affected.

D. Metabolism and Disposition

Male rats were used for blood level, excretion, and tissue distribution studies. Doses of 0.5 mg/kg of isradipine-¹⁴C were given by gavage or parenterally by injection in the tail vein.

After the intravenous dose, the highest concentration of radioactivity was observed at fifteen minutes while peak levels were reached at 1.5 hours after oral dosing. The terminal half-life was estimated to be 24 (oral) to 23 hours (i.v.). All organs and tissues contained appreciable amounts of radioactivity with the highest levels achieved in the liver.

kidney, fat, and lung. Distribution concentrations of radioactivity in most tissues and organs were similar to blood. The livers in males and females exhibited the highest levels, although after 96 hours, only the female livers contained an appreciable concentration.

The calculated accumulation factor of 1.6 based on the average blood half-life and dosing interval in these studies indicates steady state amounts in the various organs will not be substantially greater than those observed after the single dose.

The elimination pattern after oral or intravenous administration in intact animals as well as in bile duct cannulated animals was similar. Fecal (biliary) excretion was the predominant route of elimination with <20% of the dose appearing in the urine after either route of administration. Excretion was essentially complete within 43 hours of dosing with recovery of 93.0-98.7% of the dose.

Blood level and excretion studies were performed in Beagle dogs. An oral dose of 1.0 mg/kg or an intravenous dose of 0.5 mg/kg isradipine-¹⁴C was administered.

Plasma concentrations were approximately 2-3 times higher than blood indicating that isradipine and/or its metabolites are not distributed equally between blood cells and plasma. After the oral dose, radioactive material rapidly appeared in the blood, reaching a broad peak concentration four hours after dosing, while after the intravenous dose, an initial peak plasma level was observed at 5 minutes followed by a second peak at 2 hours. Based on the urinary quotient (oral/i.v.), the extent of absorption was estimated to be 90%. Following the peak concentration, the radioactivity declined gradually with a terminal half-life of 37-38 (blood) to 43-46 hours (plasma). Based on the moderate half-lives (37-46 hours), the accumulation factor would be approximately 2.5, indicating that at steady state, concentration in the dog would not reach abnormally high levels.

The percent of dose excreted in the urine or feces at each collection period was similar after both routes of administration, although the initial urinary excretion rate was slower after parenteral dosing. The recovery of the radioactive dose was good, ranging from 88.8 to 89.2% of the administered dose. Approximately two-thirds of the radioactivity was excreted in the feces and one-third in the urine.

E. Acute Toxicity 6 days

Acute toxicity tests (LD_{50} 's) performed in mice, rats and rabbits, showed no unusual toxic effects when isradipine was administered p.o. or by i.v. route. The i.v. LD_{50} s fall in the range of 1-2 mg/kg in all species; the p.o. LD_{50} s ranged from 58 mg/kg in the rabbit to greater than 3000 mg/kg in the rat. Single dose i.v. administration to dogs up to 0.3 mg/kg did not produce any mortalities. A broad spectrum of ECG changes were noted: sinus tachycardia, sinus standstills, A-V node alterations and P-waves. These normalized after 24 hours. A minimum lethal dose of 2 mg/kg was observed in dogs administered isradipine as an i.v. infusion over 40 minutes. No abnormal findings were revealed on autopsy of the animals. The tolerated i.v. dose level was set between 0.3 and 1 mg/kg.

F. Multiple Dose/Toxicity Studies (Subchronic/Chronic)

In mice administered doses of up to 86 mg/kg/day p.o. (actual in the diet) for 13 weeks in a dose range-finding study for the carcinogenicity study, no drug-related clinical observations nor any effect on body weight or food consumption were recorded. Histopathology was unremarkable.

In rats which were administered doses of up to 41 mg/kg/day p.o. for 4 weeks, no drug-related clinical observations were recorded. A slight lymphocyte reduction at the high dose did not achieve significance and normalized during a 4 week recovery phase at the end of the treatment period. Histopathology was unremarkable. In rats given p.o. (diet) up to 41 mg/kg/day for 26 weeks, all findings were unremarkable except for a slightly inhibited feed intake in males at the highest dose level. RBC parameters were significantly higher among high dose males but these remained in the normal range. Histological evaluation showed no organ toxicity.

In i.v. studies in rats, doses of up to 0.4 mg/kg/day for 2 weeks produced only general toxic effects with tolerance developing following repeated administration. Trace to mild hepatic periportal lipidosis recorded in all dose levels was not dose-dependent. Drug-induced enlargement and reactive hyperplasia of sublumbar lymph nodes was considered to reflect a mild local reaction to injected material.

In dogs treated orally, a no-toxic-effect level was observed between 2-6 mg/kg/day for 4 weeks; 20 mg/kg/day proved lethal. General dose-dependent toxic effects were observed with a spectrum of ECG changes typical of a potent calcium antagonist.

i.e. sinus tachycardia and QT prolongation occurring following doses above 6 mg/kg/day. A dose-dependent decrease in heart rate was noted 24 hours after dosing, indicating a direct inhibitory action on the sinus node. This was consistent with the normal microscopic appearance of the heart, and ECG changes were reversible in the recovery period. Death of the high dose animals was probably due to the depressant effect of isradipine on the myocardium and sinus node. Among dogs dying on study histopathologic changes included: hemorrhaging of the GI tract and lymph nodes; marked congestion in the CNS, myocardium, kidney, liver, and spleen; hepatic necrosis; and reduced spermatogenesis and atrophy of the prostate. These changes were not observed in the high dose recovery animals. The normochromic anemia observed in high dose animals was not accompanied by any microscopic bone marrow changes. Slight reductions in serum glucose values was probably a rebound phenomena which could be due to the inhibitory action on insulin secretion, as known for some calcium antagonists.

Dogs were administered doses of 1, 3.5, or 10 mg/kg/day in two separate studies lasting for 26 and 52 weeks. All dose levels produced sinus bradycardia as well as transient sinus tachycardia early in the studies. In addition, AV block and QT prolongation was observed. The death of one high dose dog after the third administration in the 26 week study was most probably related to an exaggerated pharmacodynamic effect. An additional death of a high dose animal in the one year study occurred on Drug Day 5 (after fourth administration). This death, as well as the death of a mid dose animal in the 26 week study, was associated with extreme anorexia. Gingival hyperplasia was seen at all dose levels in the 52 week study. This effect has also been reported for other calcium antagonists, notably verapamil and nifedipine. Enlargement of mammary glands at all dose levels in the 52 week study was also observed. Histopathology revealed hyperplasia of adrenal cortical zona glomerulosa, which may have been indicative of stress, but there was no evidence of organ toxicity or treatment related changes.

When administered i.v. for 2 weeks a rat receiving the highest dose, 0.1 mg/kg/day, exhibited tonic convulsion on the first day of treatment. Otherwise the animals tolerated the drug well without evidence of histopathology.

G. Carcinogenic Potential

In the mouse, a 104 week p.o. (diet) study with doses ranging from 2.5-30 mg/kg/day showed no drug-related effect on the total incidence of benign and malignant tumors. Survival among

the male mice of the high dose group was less than control. Although the intergroup difference did not reach statistical significance ($p=0.053$), the trend was significant ($p=0.033$) and the mean survival times were 638 days and 725 days, respectively. A statistically significant increase in hepatocellular carcinomas was observed in all high dose male mice and in the subpopulation of naturally dying animals but not in the corresponding terminally sacrificed animals when analyzed by Fisher's Exact test (one-sided). Because of the higher mortality rate among the high dose male a survival adjusted trend test (Peto Analysis) was performed on the incidence data of hepatocellular carcinoma taken alone and in combination with nodular proliferation. The results were highly significant for both tests. There was also a significant increased incidence of hyperplasia of the stomach mucosa among treated male mice of the mid and high dose groups but no increases in stomach tumors were observed in either of these groups.

The sponsor provided further evaluation on the liver tumor issue in the form of a reclassification of the pathology based on more recent diagnostic criteria and an examination of historical control rates, target organ toxicity, structure-activity relationships, and the biologic behavior of the lesions. These data provide some evidence that isradipine does not pose a similar risk for the development of hepatic tumors in man.

In the rat, a two year p.o. (dist) study was conducted which achieved doses ranging from 2.5-62.5 mg/kg/day. No drug-related clinical observations were recorded and no effect on mortality was seen. Significant reductions in body weight gain were noted during the latter half of the study in both male and female rats of the high dose group relative to the controls. A significant increase in erythrocytes, hemoglobin, and hematocrit were observed in high dose females for the first 37 weeks of the study. Significant increases were also seen in BUN and cholesterol in this group during the first half of the study. An examination of non-neoplastic pathology revealed that chronic progressive nephrosis common to aging rats was frequently more severe among high dose male rats dying prematurely. Male rats at the high dose level (156 times the maximum recommended human dose based on a 50 kg man) had a greater incidence of benign Leydig cell tumors in the testes which was statistically significant by an intergroup comparison with the corresponding controls. There was also a numerical increase in the incidence of Leydig cell hyperplasia at the high dose. A trend analysis of both the numbers of animals with

Leydig cell tumors alone and tumors plus hyperplasia showed a clear dose-response relationship. These findings were considered to be hormonally linked rather than reflect a genotoxic effect of the drug on the basis of numerous mechanistic studies discussed in the section entitled 'Special Studies'.

H. Mutagenic Potential

The following in vitro and in vivo mutagenicity tests all showed negative results: mutagenicity test in *Salmonella* (Ames test); mutagenicity evaluation using V79 Chinese hamster cells (HGPRT-assay), induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures; micronucleus assay in mice. The in vitro chromosome aberration test using V79 Chinese hamster cells showed increased frequencies of chromosomal aberrations at the highest dose levels, i.e. 40 and 63 ug/ml, which reduced the mitotic index to values between 14 and 47.5%. Based on these results it is concluded that isradipine has no relevant genotoxic potential at non-cytotoxic dose levels. The clastogenicity observed in vitro is not considered to be of relevance since it was not confirmed in vivo (micronucleus test) nor in any other assay performed. Since it occurred only at cytotoxic dose levels, it might well be a consequence of cytotoxicity, which can lead to chromosome aberrations.

I. Special Studies

Tolerance of the intravenous administration of isradipine has been studied both in vitro with human blood and in vivo in rabbits. All results showed that good local tolerance in man can be presumed.

Impurities/By products - Results from acute toxicity studies (LD₅₀'s) carried out with the isolated, identified, impurities/by-products indicated that none of these known impurities/by-products, which occurred at low levels, contributed to the toxicological results obtained with isradipine.

Evaluation of Rat Leydig Cell Neoplasms

Several additional studies evaluated possible hormonal mechanisms which may be responsible for the occurrence of the increased incidence of Leydig cell tumors in the rat carcinogenicity study.

Initial studies focused on a search for changes in circulating hormone levels after acute and 2 weeks' repeated daily

administration in both Sprague-Dawley and Wistar rats were used, but no differences emerged. In a separate study 78-80 week-old males of both strains were treated with the low and high doses of isradipine used in the original carcinogenicity study for 30 weeks. There was a consistent increase in FSH at the high dose level and testicular weight was slightly but insignificantly reduced but no increase in tumors were seen at the end of the study. In other studies LH receptors on Leydig cells were estimated in testicular material after isradipine was given for 4 or 12 weeks. Drug treatment led to significantly reduced Leydig cell LH receptor content in Sprague-Dawley rats, but less so in Wistar rats.

A repeat 2-year study in Sprague-Dawley male rats in which serum hormone levels were measured at repeated intervals integrated some of the above findings. LH and FSH levels were significantly increased after one year and prolactin levels tended to be decreased, from approximately 6 months onwards. Testosterone levels were reduced from 78 weeks onward. Testicular LH receptors were decreased in the treated groups at termination and GnRH receptors were increased.

The results are consistent with a drug-related down regulation of LH receptors, possibly due to the decreased prolactin circulating levels. Consequently, testosterone production is decreased, its feedback to the pituitary diminished, and LH and FSH serum levels increased. As further support, estimations of serum hormone levels have been made in man following single and 7-day repeat dosing in volunteers, and from a population of hypertensive patients receiving isradipine for 1 year in a therapeutic trial. There is no evidence of any drug-related change in circulating hormone levels which correspond in any way to those seen in the rat.

J. Reproductive Toxicity Studies

Teratogenicity Studies

Isradipine was administered to rats (6, 20, or 60 mg/kg/day) and to rabbits (1, 3, or 10 mg/kg/day). In both studies one or two animals died at the two highest dose levels. A decrease in maternal weight gain was also observed in rats at the high dose level and in the 3 and 10 mg/kg/day group of rabbits. This correlated with an increase in embryoletality and an increase in post-implantation losses in the latter two groups and hence resulted in smaller litter sizes. Thus the drug was embryoletal only at doses which were maternotoxic and was not teratogenic to either rats or rabbits.

Pre- and Postnatal Fertility Study

In both studies, rats were administered 6, 20 and 60 mg/kg/day isradipine p.o. No intrinsic effect on fertility was observed in the rat. Increases in mean pregnancy duration at mid and high dose levels were observed, with cases of dystocia in all groups. This was due to the tocolytic activity inherent to calcium antagonists. Mid and high dose groups also exhibited increased pre-, peri- and postnatal loss with peri- and postnatal development of F₁ pups affected. Although mean birth weights were lower with an increase in morphological abnormalities, the decrease in mean body weight and non-acceptance in functional/behavioral tests were only transient, and at the beginning of the F₁ fertility study no deficits were apparent. There was no remarkable effect on the F₂ pups. The low dose level of 6 mg/kg/day caused no adverse effects.

Review and Evaluation of Nonclinical Biologic Data
NDA 19-546

389

Sponsor: Sandoz Pharmaceuticals Original Submission Date: 12/27/85
Amendment Dates: 11/26/86;
3/31/88; 7/6/88; 11/28/88

Reviewer: Robert H. Harris

Drug Code Name: PN 200-110 Capsules

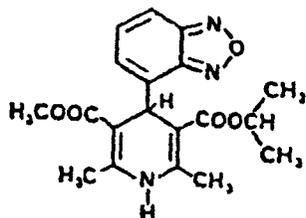
Generic Name: Isradipine

Trade Name: Dynacirc

Indication: Hypertension

Related INDs:

Chemical Structure:



Review

PN-200-110, a dihydropyridine derivative, has been characterized as a calcium influx inhibitor intended for the treatment of cardiovascular diseases, specifically hypertension in this application.

A) Pharmacology

The sponsor has provided a tabulation of the animal pharmacology studies conducted with PN 200-110, which has been appended at the end of this section.

1. Primary in vitro Pharmacology

The sponsor conducted a series of experiments with PN 200-110 to assess the effects on vascular smooth muscle

contraction induced by a variety of agonists. Rabbit aortic rings were exposed to calcium, noradrenaline, potassium or serotonin to induce contractions. PN 200-110 was added serially to determine inhibition of vasoactivity. In some cases nifedipine or verapamil were tested as comparative agents. Both PN 200-110 and verapamil competitively antagonized contractions induced by calcium added to a calcium free media. The potency of PN 200-110 was almost three orders of magnitude greater than that of verapamil ($pA_2 = 10.34$ vs. 7.5). The antagonism of potassium induced contraction was non-competitive with both of the drugs. PN 200-110 displayed a 100 to 1000 fold greater potency than verapamil. Inhibition of noradrenaline induced contractions was minimal with both PN 200-110 and verapamil. PN 200-110 produced virtually no inhibition at 10^{-9} M and only modest noncompetitive inhibition was observed in the presence of serotonin at the same concentration. In contrast, verapamil displayed some degree of competitive antagonism of serotonin induced contractions and the pA_2 value for this inhibition was close to the apparent pA_2 value for this compound for calcium induced inhibition.

Studies were also conducted with canine vascular rings derived from several sources (coronary, basilar, or mesenteric arteries). Graded concentrations of calcium induced contraction of canine coronary artery which was inhibited by increasing concentrations of PN 200-110 (apparent $pA_2 = 11.7$). In contrast to the rabbit the profiles appeared to display mixed competitive inhibition as the curve was shifted to the right but the maximum tension developed was also depressed. PN 200-110 non-competitively inhibited potassium induced contractions in the canine coronary artery at concentrations similar to those obtained with rabbit aortic rings.

In the canine basilar and mesenteric arteries PN 200-110 non-competitively inhibited serotonin induced contractions. The relative potency, however, was 66 times greater in basilar arteries than in mesenteric arteries. Compared to nimodipine, PN 200-110 was more potent in inhibiting serotonin contractions in arteries derived from both vascular beds. Nifedipine was intermediate between the potency of the above two calcium antagonists. With respect to nifedipine and nimodipine the differential potency in inhibiting serotonin contractions in the basilar artery over the mesenteric artery was not as

pronounced as with PN 200-110.

PN 200-110 also proved to be more potent than nifedipine, nisoldipine or nimodipine at inhibiting serum induced contraction of canine basilar artery.

Based on the studies reported in rabbit and canine vascular tissue it can be concluded that PN 200-110 displays a greater potency than referenced calcium antagonists in its inhibition of vasoconstriction of tissue derived from several vascular beds. PN 200-110 also shows a greater preferential inhibition of canine cerebral vasculature than peripheral vasculature relative to the other drugs tested in this in vitro model.

The activity of PN 200-110 was also tested in isolated guinea pig atria. PN 200-110 displayed no effect on the refractory period at concentrations which produced a decrease in rate in spontaneously beating atria and a decrease in contractile force in stimulated left atria from guinea pigs. The effect on heart rate was more prominent at the lower concentrations of PN 200-110. Verapamil was the only one of 4 other Ca^{++} influx inhibitors (verapamil, diltiazam, nifedipine, nisoldipine) to show an increase in refractory period. At particularly high concentrations the inhibition of rate in spontaneously beating atria and contractile force of stimulated left atria was virtually superimposable with verapamil, diltiazem and nifedipine, whereas nisoldipine displayed a distinct preference to inhibit the rate of spontaneously beating atria relative to the contractile force at lower concentrations.

A similar type of experiment was conducted in isolated cat stimulated papillary muscle and spontaneously beating cat right atria. PN 200-110 significantly inhibited the heart rate at lower concentrations than the contractile force of stimulated papillary muscle. However the effect displayed a flat concentration response and consequently the percent change from baseline was much greater at the higher concentrations with respect to contractile force.

The effects of PN 200-110 and nifedipine were examined in the spontaneously beating Langendorff rabbit heart. Both nifedipine and PN 200-110 displayed a significant negative inotropic effect at high concentrations. At lower concentrations, PN 200-110 proved more potent than nifedipine in improving coronary blood flow. Additionally at high concentrations, PN 200-110 displayed a significant

negative chronotropic effect which is not shared by nifedipine. From the limited amount of information it can not be ascertained whether PN 200-110 displays a greater coronary dilatory effect in this model than nifedipine.

Based on studies in the rabbit and guinea pig heart tissue PN 200-110 seemingly has a preferential effect in inhibiting heart rate over contractile force although the results in cat cardiac tissue were equivocal.

2) Primary in vivo Pharmacology

Evaluation of the hemodynamic effects of PN 200-110 was conducted in chloralose-urethane anesthetized cats. The animals were instrumented to obtain measurements of heart rate, blood pressure, right atrial pressure (RAP), peak acceleration (PA), cardiac output and total peripheral conductance (TPC) through the use of electromagnetic probe following radio labelled microsphere injections. PN 200-110 (cumulative doses of 1, 3, 10 and 30 ug/kg) or nifedipine (3, 10 and 30 ug/kg) were administered intravenously at 5 minute intervals. PN 200-110 produced dose related decreases in heart rate and blood pressure and dose related increases in cardiac output, TPC, PA, and RAP. A separate study following a single iv bolus demonstrated that the bulk of the pharmacologic effect reversed by 3 hours. The effect on heart rate and RAP appear to reverse more quickly than those other indicators of hemodynamic functions. Effects with nifedipine were comparable in magnitude and direction to those observed with PN 200-110 in the cat. Evaluation of the regional blood flow distribution following the cumulative administration of PN 200-110 demonstrated that the cardiac and the cerebral circulation were significantly improved whereas there was a modest reduction in the blood flow to the lungs and kidneys. The redistribution following an intravenous bolus of nifedipine was comparable to that observed with PN 200-110.

The hemodynamic effects of PN 200-110 were also investigated in chloralose-anesthetized open-chest dogs. Following a ten minute infusion of PN 200-110 (total dose equals 3 ug/kg) heart rate, blood pressure, total peripheral resistance, coronary resistance, left ventricular work and oxygen consumption were all significantly reduced. The effects on vascular resistance were most prominent. A 10 ug/kg infusion of nifedipine was similarly administered to a series of dogs with

effects on hemodynamic parameters which were relatively short lived and less dramatic compared to the effects of PN 200-110. It could not be determined, however, whether a sufficient dose of nifedipine was administered to demonstrate equipotency with PN 200-110 on at least one of the variables. The direction of changes, however, were comparable between drugs.

Electrophysiologic studies were conducted with PN 200-110 alone and in combination with beta-blocker (pindolol) in dogs. The right ventricle was stimulated to determine the diastolic excitation threshold. PN 200-110 did not change the excitation threshold or any of the measured ECG intervals at doses between 0.25 and 8 ug/kg. Pretreatment with pindolol did not affect any of the electrophysiologic parameters. In contrast, verapamil caused a significant increase in the PQ interval at doses which had minimal effects on heart rate and blood pressure.

A series of experiments examined the effects of PN 200-110 on hemodynamic parameters in conscious rabbits. Intravenous administration of PN 200-110 demonstrated a slight increase in heart rate and fall in blood pressure which was dose-related. In contrast, nitroglycerin and nifedipine produced dose related decreases in blood pressure and increases in heart rate which were linearly related. These findings suggest that PN 200-110 inhibited normal reflex tachycardia in conscious rabbits; a property not shared with the other vasodilators tested.

Anesthetized rabbits were treated with consecutive intravenous doses of 10 and 20 ug/kg and blood flow measurements were made using the microsphere method. PN 200-110 produced dose related increases in heart rate, cardiac output, and total peripheral conductance. There was a dose related decrease in blood pressure. Coronary flow and vascular conductance were improved by greater than 100% following the second dose. The subdistribution of myocardial blood flow showed that the epicardium was preferentially perfused, although perfusion was improved to all left side loci within the cardiac tissue. Evaluating other organs and tissues the increase in blood flow was also pronounced within the brain and intestine and the ear of the rabbit. The only tissue demonstrating a significant reduction in blood flow was the spleen.

When the cerebral cortex of anesthetized rabbits was bathed with autologous plasma, PN 200-110 administered

intravenously inhibited vasospasm.

In spontaneously hypertensive rats, PN 200-110 produced dose related reductions in blood pressure, whether administered subcutaneously (0.1 to 0.1 mg/kg) or orally (0.01 to 10 mg/kg), without causing tachycardia. Higher doses by both routes induced some degree of tachycardia

PN 200-110 and nifedipine were tested for effects on the autonomic nervous system of anesthetized cats. Both drugs produced a fall in blood pressure, inhibited the effects of carotid occlusion and antagonized the effects of noradrenaline and isoprenaline on blood pressure.

However, isoprenaline induced tachycardia was antagonized only by PN 200-110.

In salt loaded rats, PN 200-110 produced a small dose related enhancement of water and sodium excretion but did not affect potassium excretion.

Among the other pharmacologic studies conducted, PN 200-110 showed no significant effect on vascular proliferation of the aorta in balloon catheterized rats. There was, however, a demonstration of a slight delay in the proliferative response in the carotid artery with PN 200-110 treatment.

In anesthetized guinea pigs PN 200-110 produced no direct bronchial dilatation at 10 ug/kg intravenously. PN 200-110 at the same doses, however, inhibited bronchospasms produced by histamine and acetylcholine. Additionally, PN 200-110 did not affect serum lipids with the exception of a marked reduction in serum triglycerides.

PHARMACOLOGY SUMMARY TABLE E.1-1
 STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Admin. | PN 200-110 | Nifedipine | Verapamil | Diltiazem |
|--|-------------------------------|------------|-----------------------------|-----------|
| A. <u>Effects on Vascular Smooth Muscle (in vitro)</u> | | | | |
| 1. Rabbit Aorta Ring Contractions | | | | |
| Induced by: | | | | |
| Calcium* | | | | |
| Antag, pA ₂ value | 10.34 | - | 7.5 | - |
| Potassium** | | | | |
| Inhibitory ED50, M | 1.4 x 10 ⁻⁹ | - | 7 x 10 ⁻⁷ | - |
| Noradrenaline, M | ne @ 10 ⁻⁵ | - | wk antag @ 10 ⁻⁵ | - |
| Serotonin | wk antag @ 10 ⁻⁵ M | - | pA ₂ = 6.9 | - |
| 2. ⁴⁵ Ca ⁺⁺ Uptake by Rabbit Aorta (a) | | | | |
| ⁴⁵ Ca ⁺⁺ content of resting smooth muscle, M | ne @ 10 ⁻⁵ | - | - | - |
| Noradrenaline-induced ⁴⁵ Ca ⁺⁺ uptake, M | ne @ 10 ⁻⁵ | - | - | - |
| KCL-induced ⁴⁵ Ca ⁺⁺ Uptake inhibitory ED50, M | 3.6 x 10 ⁻⁹ | - | - | - |

* Ca⁺⁺ added to Ca⁺⁺-free depolarizing solution.
 ** KCL added in the presence of a normal concentration of Ca⁺⁺ to induce graded depolarization.
 ne = no effect
 wk = weak
 antag = antagonism

(a) data from Hof RP, et al.
 J Cardiovasc Pharmacol 1984; 6:399-406

PHARMACOLOGY SUMMARY TABLE E.1-1 (Continued)
STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Admin. | PN 200-110 | Nifedipine | Verapamil | Diltiazem |
|--|-----------------------|-----------------------------|-----------|-----------|
| 3. Dog Coronary Artery Ring Contractions Induced by: | | | | |
| Calcium* | | | | |
| Antag, pA ₂ value | 11.7 | - | - | - |
| Potassium** | | | | |
| Inhibitory ED50, M | 3.1×10^{-10} | - | - | - |
| 4. Dog Mesenteric Artery strip Contractions Induced by: | | | | |
| Serotonin | | | | |
| Inhibitory ED50, M | 3.7×10^{-9} | $45\% \pm 8 \times 10^{-7}$ | - | - |
| 5. Dog Basilar Artery Strip Contractions Induced by: | | | | |
| Serotonin | | | | |
| Inhibitory ED50, M | 5.6×10^{-11} | 2.2×10^{-9} | - | - |
| Blood Serum | | | | |
| Inhibitory ED50, M | 2.1×10^{-9} | 3.6×10^{-8} | - | - |

• Ca⁺⁺ added to Ca⁺⁺-free depolarizing solution.
 ** KCl added in the presence of a normal concentration of
 Ca⁺⁺ to induce graded depolarization.
 ne = no effect
 †/† = decrease/increase
 antag = antagonism

PHARMACOLOGY SUMMARY TABLE E.1-1 (Continued)

STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Admin. | PN 200-110 | Nifedipine | Verapamil | Diltiazem |
|--|-------------------------------------|---------------------|----------------------------|---------------------|
| 8. Effects on Cardiac Tissue (in vitro) | | | | |
| 1. Spontaneously Beating Right and Stimulated Left Guinea Pig Atria | | | | |
| Refractory Period, M | ne @ 10^{-6} | ne @ 10^{-7} | wk ↑ @ $10^{-6} - 10^{-5}$ | ne @ 10^{-6} |
| Contractile Force (CF) Inhibitory EC ₂₅ , M | $1.5 \times 10^{-8(a)}$ | $\sim 10^{-8}$ | $10^{-7} - 10^{-6}$ | $10^{-8} - 10^{-7}$ |
| Beating Rate (HR) Inhibitory EC ₂₅ , M | $4.5 \times 10^{-10(2)}$ | $10^{-8} - 10^{-7}$ | $\sim 10^{-7}$ | $10^{-8} - 10^{-7}$ |
| Ratio of Negative-Inotropic to Negative-chronotropic EC ₂₅ Values (CF/HR) | 33 | 0.35 | 2.8 | 3.4 |
| 2. Cat Stimulated Papillary Muscle and Spontaneously Beating Right Atria | | | | |
| Papillary muscle Contractile Force | ↑ @ $10^{-7} - 3 \times 10^{-6}$ M | - | - | - |
| Atria Beating Rate | ↑ @ $3 \times 10^{-10} - 10^{-8}$ M | - | - | - |
| 3. Rabbit Langendorff Heart reparation | | | | |
| Contractile Force | ↑ @ 1-10 μg | ↑ @ 1-10 μg | - | - |
| Heart Rate | ↑ @ 1-10 μg | ne @ 10 μg | - | - |
| Coronary Flow | ↑ @ 0.01-1 μg | ↑ @ 1 μg | - | - |

ne = no effect

wk = weak

↑/↓ = decrease/increase

(a) data from Hof RP, et al.

J Cardiovasc Pharmacol 1984; 6:399-406

PHARMACOLOGY SUMMARY TABLE E.1-1 (Continued)
 STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Admin. | PN 200-110 | Nifedipine | Verapamil(b) | Diltiazem(b) |
|---|---|-----------------|-------------------------|---------------------|
| C. Effects on the Heart and Peripheral Circulation | | | | |
| 1. Anesthetized Open-chest Cats (i.v. Injection) | | | | |
| | Approximate Percent Change @ $\mu\text{g/kg}$ | | | |
| Heart Rate | 5-15 ↓ @ 10-30 | ne @ 43 | 15-25 ↓ @ 300-1000 | 10-20 ↓ @ 300-1000 |
| Blood Pressure | 10-40 ↓ @ 1-30 | 15-25 ↓ @ 13-43 | 10-25 ↓ @ 300-1000 | 10-30 ↓ @ 300-1000 |
| Cardiac Output | 50 ↑ @ 10 | 20-40 ↑ @ 13-43 | 10 ↑ @ 1000 | 30 ↑ @ 1000 |
| Total Peripheral Conductance | 20-180 ↑ @ 1-30 | 15-95 ↑ @ 3-43 | 10-50 ↑ @ 100-1000 | 20-80 ↑ @ 100-1000 |
| Peak Acceleration of Blood in Ascending Aorta | 15-40 ↑ @ 3-30 | 20-40 ↑ @ 13-43 | 5 ↑ @ 300 5 ↑ @ 1000 | 20 ↑ @ 1000 |
| Right Atrial Pressure | 15-30 ↑ @ 3-30 | 20-60 ↑ @ 13-43 | 50 ↑ @ 1000 | 40 ↑ @ 1000 |
| Coronary Blood Flow | 50-150 ↑ @ 1-30 | - | - | - |
| Coronary Conductance | 73-300 ↑ @ 1-30 | - | - | - |
| Regional Blood Flow | | | | |
| Heart (total) | 50-150 ↑ @ 1-30 | 100-175 ↑ @ 43 | 25-75 ↑ @ 100-1000 | 75-125 ↑ @ 100-1000 |
| Epi | 75-250 ↑ @ 1-30 | 125-250 ↑ @ 43 | 25-100 ↑ @ 100-1000 | 50-145 ↑ @ 100-1000 |
| Mid | 55-170 ↑ @ 1-30 | 115-200 ↑ @ 43 | 20-115 ↑ @ 100-1000 | 35-125 ↑ @ 100-1000 |
| Endo | 45-100 ↑ @ 1-30 | 75-115 ↑ @ 43 | 10-55 ↑ @ 100-1000 | 15-75 ↑ @ 100-1000 |
| Muscle (leg) | 200 ↑ @ 10 | 240 ↑ @ 43 | ne @ 1000 | ne @ 1000 |
| Brain (total) | 20-90 ↑ @ 1-30 | - | 25 ↑ @ 1000 | 20-50 ↑ @ 300-1000 |
| Liver | 45 ↑ @ 10 | ne @ 43 | ne @ 1000 | 25 ↑ @ 1000 |
| Lungs | 45-55 ↓ @ 10-30 | 45 ↓ @ 43 | ne @ 1000 | ne @ 1000 |
| Kidneys, adrenals, spleen, small intestine | wk or no signif. change | - | - | - |

ne = no effect
 wk = weak
 ↓/↑ = decrease/increase

(b) data from Hof RP. Br J Pharmacol 1983; 78: 375-394.

STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Admin. | PN 200-110 | Nifedipine | Verapamil |
|--|---|------------|-------------------------|
| 2. Anesthetized Dogs | | | |
| <u>i.v. 10 min infusion (open-chest)</u> | | | |
| | 3 µg/kg | 10 µg/kg | |
| Heart Rate (b/min Δ) | 12 ↓ | 10 ↓ | - |
| Blood Pressure (mm Hg Δ) | 40 ↓ | 18 ↓ | - |
| Total Peripheral Resistance (% Δ) | 50 ↓ | 25 ↓ | - |
| Coronary Resistance (% Δ) | 67 ↓ | 40 ↓ | - |
| Left Ventricular Work (% Δ) | 37 ↓ | 10 ↓ | - |
| Oxygen Consumption (% Δ) | 30 ↓ | 20 ↓ | - |
| <u>i.v. injection (closed-chest)</u> | | | |
| Diast. Excit. Threshold | ne @ 8 µg/kg | - | ne @ 1 mg/kg |
| ECC-interval | | | |
| PQ | } ne @ 8 µg/kg | } | mk ↑ @ 0.63 - 1 mg/kg |
| QRS | | | ns @ 1 mg/kg |
| QT _c | | | |
| Heart Rate (b/min Δ) | ne @ 8 µg/kg | - | 61 ↓ @ 1 mg/kg |
| Blood Pressure (mm Hg Δ) | 22-33 ↓ @ 4-8 µg/kg | - | 22-46 ↓ @ 0.5 - 1 mg/kg |
| Left Ventricular dp/dt max (%) | ne @ 8 µg/kg | - | 26 ↓ @ 1 mg/kg |
| Effects following Pindolol (50 µg/kg i.v.) Pretreatment | no interaction @ 8 µg/kg on diast. excit. threshold, ECC, HR, BP or dp/dt max | - | - |

ne = no effect

mk = marked

↓/↑ = decrease/increase

PHARMACOLOGY SUMMARY TABLE E.1-1 (Continued)
 STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Admin. | PN 200-110 | Nifedipine |
|--|---|------------|
| 3. Conscious Rabbits (i.v. injection) | | |
| Reflex Tachycardia | antag | ne |
| | <u>Percent Change @ $\mu\text{g/kg}$</u> | |
| Heart Rate | 10-25 \uparrow @ 10-30 | - |
| Blood Pressure | 10-25 \downarrow @ 10-30 | - |
| Cardiac Output | 15-35 \uparrow @ 10-30 | - |
| Total Peripheral Conductance | 30-75 \uparrow @ 10-30 | - |
| Coronary Flow | 37-75 \uparrow @ 10-30 | - |
| Coronary Conductance | 50-150 \uparrow @ 10-30 | - |
| Regional Blood Flow | changes in regional blood flow were similar to those observed in anesthe- tized open-chest cats | |
| Cortical Vasospasm (anesthetized rabbits) | antag @ 1 $\mu\text{g/kg}$ | - |
| 4. Spontaneously Hypertensive Rats | | |
| <u>p.o. injection</u> | <u>Change @ mg/kg</u> | |
| Blood Pressure (mm Hg Δ) | 12-40 \downarrow @ 0.01-10 93 \uparrow @ 50 | - |
| Heart Rate (b/min Δ) | ne @ 0.01-10 55 \uparrow @ 50 | - |
| <u>s.c. injection</u> | | |
| Blood Pressure (mm Hg Δ) | 16-35 \downarrow @ 0.01-1 97-122 \downarrow @ 1-3 | - |
| Heart Rate (b/min Δ) | ne @ 0.01-0.1 106-127 \uparrow @ 1-3 | - |

ne = no effect
 \downarrow/\uparrow = decrease/increase
 antag = antagonism

PHARMACOLOGY SUMMARY TABLE E.1-1 (Continued)
STUDIES RELATED TO THE THERAPEUTIC ACTIVITY OF PN 200-110

| Parameter/Route of Adm. | PN 200-110 | Nifedipine |
|--|-------------------------------|--------------------|
| 5. Autonomic Responses in Anesthetized Cats (i.v. infusion) | | |
| | <u>Percent Change @ mg/kg</u> | |
| Blood Pressure | 12-42 † @ 0.005-0.7 | 7-33 † @ 0.005-0.7 |
| Heart Rate | 6-39 † @ 0.005-0.7 | ne @ 0.005-0.7 |
| Noradrenaline Pres. Resp. | 34-88 † @ 0.005-0.7 | 37-70 † @ 0.03-0.7 |
| Isoprenaline Depres. Resp. | 25-81 † @ 0.005-0.7 | 35-67 † @ 0.03-0.7 |
| Isoprenaline Tachycardia | 17-66 † @ 0.1-0.7 | ne @ 0.005-0.7 |
| Carotid Occlusion Pres. Resp. | 65-92 † @ 0.005-0.7 | 32-72 † @ 0.03-0.7 |
| Nictitating Membrane Resp. | 11-68 † @ 0.005-0.7 | 17-26 † @ 0.03-0.7 |
| ECG-Interval | | |
| PQ (in ms) | † (8-38) @ 0.03-0.7 | } ne @ 0.005-0.7 |
| QRS | ne @ 0.005-0.7 | |
| 6. Water and Electrolyte Excretion in Conscious Rats | | |
| <u>p.o. injection</u> | | |
| Water | † @ 3 mg/kg | - |
| Na ⁺ | † @ 0.3-3 mg/kg | - |
| K ⁺ | † @ 3 mg/kg | - |
| Cl ⁻ | ne @ 0.1-3 mg/kg | - |
| <u>s.c. injection</u> | | |
| Water | † @ 0.1-1 mg/kg | - |
| Na ⁺ | † @ 0.1-1 mg/kg | - |
| K ⁺ | ne @ 0.01-1 mg/kg | - |
| Cl ⁻ | ne @ 0.01-1 mg/kg | - |

ne = no effect

†/† = decrease/increase

PHARMACOLOGY SUMMARY TABLE E.1-2
OTHER PHARMACOLOGICAL EFFECTS OF PN 200-110

| Parameter | Test Drug Route of Adm. | PN 200-110 |
|--|------------------------------|-------------------------------------|
| A. <u>Antiproliferative Effects on Arterial Lesion Development in Balloon Catheterized Rats</u> | | |
| Aorta | s.c. (daily for 16 days) | ne @ 0.03 mg/kg |
| Carotid (% Δ in lesion height) | | 26 ↓ @ 0.25 mg/kg 46 ↓ @ 1 mg/kg |
| B. <u>Effect on Pulmonary Function in Guinea Pigs</u> | | |
| Lung Resistance and Compliance | i.v. | ne @ 10 μg/kg |
| Bronchoconstriction Induced by: | | <u>% Antag @ 10 μg/kg</u> |
| Histamine | i.v. | 65 |
| Acetylcholine | | 82 |
| Serotonin | | 40 (1 of 2 animals) |
| C. <u>Effect on Prolactin Secretion in Male Rats</u> | | |
| Two-h Pretreatment | s.c. | 50% ↑ @ 2 mg/kg |
| Four-h Pretreatment | | 50% ↑ @ 11 mg/kg |
| D. <u>Effect on Lipoproteins in Rats</u> | | |
| | p.o. (in diet for 6 days) | <u>222 mg/kg/day</u> |
| Cholesterol (total, VLDL + LDL, HDL) | | ne |
| Triglyceride | | 58% ↓ |
| Weight Gain | | 56% ↓ |
| Food Consumption | | ne |
| Liver weight | | ne |

ne = no effect
+/- = decrease/increase
antag = antagonism

B) Toxicology

A conventional toxicology evaluation was conducted with PN 200-110. Tabulations of the studies performed (including design parameters and results) have been provided by sponsor and included, where appropriate, in the sections which follow.

- 1) The acute intravenous and oral toxicity of PN 200-110 was evaluated in mice, rats and rabbits. The LD₅₀s in these species (mg/kg) after 7 days from intravenous treatment or 15 days from oral treatment were as follows:

| Species | i.v. | p.o. |
|---------|---------------|----------------|
| Mouse | 1.2 (0.9-1.5) | 216 (144-313) |
| Rat | 1.8 (1.3-2.6) | > 3000 (0/10+) |
| Rabbit | 1.2 (1.1-1.4) | 58 (4.0-104) |

() 95% confidence limits

+ Number of deaths in the highest dose group

LD₅ and LD₉₅ values (mg/kg) after an observation time of 7 days (i.v.) or 14 days (p.o.):

| Species | LD ₅ | | LD ₉₅ | |
|---------|-----------------|------|------------------|------|
| | i.v. | p.o. | i.v. | p.o. |
| Mouse | 0.7 | 66 | 2.0 | 705 |
| Rat | 0.7 | -- | 4.6 | -- |
| Rabbit | 1.0 | 6.4 | 1.5 | 524 |

Toxic signs included drowsiness, flaccidity, prone position, heavy and accelerated breathing, in all species. Rats also exhibited pale skin and piloerection. All animals which died did so within the first 4 days following treatment and autopsy revealed no remarkable macroscopic pathology.

- 2) The acute toxicity studies in dogs were conducted with a Cremophor-containing formulation and a non Cremophor-containing formulation which was infused over a longer period because of the limited solubility of the compound. In the studies with the Cremophor-formulation side-effects including severe equilibrium disturbances,

sedation, tachypnea, and excessive salivation were observed at 0.3 mg/kg. In addition, persistent sinus standstill was noted within the first 15 minutes and some intravascular hemolysis did occur in this animal. At the lower dose of 0.1 mg/kg, sinus tachycardia with P-pulmonale was observed with the noncremophor formulation. Emesis occurred shortly after infusion with some degree of sinus tachycardia and P-pulmonale observed at 0.3 mg/kg infused over 1 hour period. At the higher dose of 1 mg/kg equilibrium disturbances and sedation were observed in addition to the above effects. At 2 mg/kg death followed 40 minutes after the initiation of the infusion due to total cardiac depression.

SYNOPTIC TABULATION TABLE E.2-1

ACUTE TOXICITY

| Species | Strain | Initial #/Sex Group | Mode of Administration | Group Avg. Doses (mg/kg/day) | Study Duration (weeks) | Interim Sacrifice (weeks) | Laboratory | Comments |
|---------------|-----------------------------------|---------------------------------|------------------------|------------------------------|---|---------------------------|---|----------------------------|
| mouse | KFM-NMRI (Füllingsdorf, CH) | 5 | oral | 100-1000 | 2a) | N.A. | Sandoz Ltd. Basel Switzer- land | 216 (144-313) () 95% |
| | | | i.v. | 560-3000 | 1 | | | 1.2 (0.9-1.5) Conf. Limits |
| rat | KFM-WIST (*) | 5 | oral | 1000-3000 | 2 | | V A L U E S | 3000 |
| | | | i.v. | 560-3000 | 1 | | | 1.8 (1.3-2.6) |
| rabbit | KFM-HAS (*) | 5 | oral | 56-560 | 2 | | V A L U E S | 5.8 (4.0-104) |
| | | | i.v. | 1-1.8 | 1 | | | 1.2 (1.1-1.4) |
| dog | beagle (Marshall U.S.A.) | 1b (mixed sexes in study) | i.v. Infusion | Cremophor | 2 days observation after iv infusion | N.A. | V A L U E S | Tachycardia. |
| | | | | Form | | | | allergy- like |
| | | | | 0.03 | | | | reaction to |
| | | | | 0.1 | | | | cremophore |
| | | | | 0.03 | | | | |
| Non-Cremophor | " | Tachycardia. | | | | | | |
| Form | | tolerated dose | | | | | | |
| 0.3 | | level is between | | | | | | |
| 1.0 | | 0.5 and 1 mg/kg/ | | | | | | |
| 2.0 | | hour | | | | | | |

a) observation weeks after single application

b) a single animal for each dose level

3) Multidose Toxicity

Thirteen Week Oral (Diet) Dose Range-Finding Study in the Mouse on PN 200-110 (T-1842)

Methods

Study Facility/Dates: Sandoz Research (1982-1983)

Strain/Source: Charles River CD-1 (Charles River Lab., Kingston, NY)

Duration: 13 weeks

Route: Oral (diet)

Dose Levels: 0, 2.5, 10; and 40 (wks 1-8) 60 (wks 9-10), and 80 (wks
11-13) mg/kg [Actual · 2.7, 10.8, and 42.6, 56.9, 85.9
mg/kg]

No. of Animals/Sex/Dose: 10 males/dose

Age at Study Initiation: Not given

Weight Range: Males: 23-30 gm
Females: N/A

Parameters Measured: Mortality, clinical signs of toxicity, body
weight, food consumption, macroscopic and
microscopic examination (latter limited to
liver, kidney, heart and any gross lesion).

Results

There were no treatment related signs, symptoms, or pathology even at
the highest dose in this thirteen week study.

Conclusions

The minimal toxic effect dose was greater than 80 mg/kg/day.

Lifetime Oral (Diet) Carcinogenicity Study
in the Mouse on PN 200-110 (T-1843)

Methods

Study Facility/Dates: Sandoz Research (1983-1985)

Strain/Source: Charles River CD-1 (Wilmington, MA)

Duration: 104 weeks

Route: Oral (diet)

Dose Levels: 0, 2.5, 15, and 80 mg/kg (Actual : 2.6, 15.4, and 81.8 mg/kg)

No. of Animals/Sex/Dose: 70-treated groups/140-controls

Age at Study Initiation: 6 weeks

Weight Range: Males: 26-27 gm
Females: 21-22 gm

Parameters Measured:

Clinical Observations:

- *Mortality
- *Clinical signs of toxicity
- *Physical examinations
- *Body weight
- *Food consumption
- *Tissue mass palpation

Macroscopic Examination:

All relevant organs and tissues

Microscopic Examination:

All relevant organs and tissues

Results:

Body weight gain and food consumption were unaffected by treatment. However, there was an increase in mortality among the high dose male mice in this study and although it did not achieve significance

(p=0.053) there was positive trend with dose (p=0.038). These results indicate that the drug produced some toxicity in the male mouse.

| <u>Parameter</u> | <u>Sex</u> | <u>Control</u> | <u>Low</u> | <u>Mid</u> | <u>High</u> |
|----------------------|------------|----------------|------------|------------|-------------|
| Mortality | Males | 52% (avg) | 54% | 51% | 64% |
| | Females | 52% (avg) | 50% | 56% | 57% |
| Mean Survival (days) | Males | 725 | 722 | 700 | 638 |
| | Females | 721 | 725 | 701 | 695 |

The overall percentage of tumor bearing animals showed a statistically significant increase among the low and mid dose treated males which were terminally sacrificed. However high dose was comparable to control for this sex and the combined percentages for both naturally dying and terminally sacrificed animals was not significantly different from control for this parameter.

| <u>Type of Death</u> | <u>Sex</u> | <u>Pooled Controls</u> | <u>Low</u> | <u>Mid</u> | <u>High</u> |
|-----------------------------|------------|------------------------|------------------|------------------|-------------|
| Naturally Dying | M | 49% | 47% | 47% | 40% |
| | F | 67% | 64% | 57% | 63% |
| Terminal Sacrificed | M | 66% | 88% ^a | 85% ^b | 72% |
| | F | 78% | 79% | 77% | 77% |
| Natural & Terminal Combined | M | 78% | 66% | 66% | 51% |
| | F | 72% | 71% | 66% | 69% |

a p = 0.018, comparison to pooled Controls

b p = 0.030, comparison to pooled Controls

Considering percentages of animals which exhibited a malignant tumor there was a statistically significant increase among the low dose males of the terminally sacrificed group. However there was no significant trend when malignant tumors were analyzed over all dose groups.

The histopathologic evaluation of tissues revealed a number of sites in which a relationship between drug treatment and tumor formation or non-neoplastic changes may exist. These are presented on the attached table.

| Finding | Sex | Treatment Group | | | |
|---|-----|-----------------|------|------|---------|
| | | Control | Low | Mid | High |
| (a) Carcinoma Hepato-cellular | M | 12%+ | 19% | 10% | 25%* |
| | F | 2% | 4% | 0% | 4% |
| (b) Nodular Prolifer-ation of the Liver | M | 10% | 11% | 10% | 16% |
| | F | 5% | 4% | 1% | 1% |
| (c) (a) and (b) combined | M | 22% | 24% | 19% | 31% (*) |
| | F | 7% | 9% | 1% | 4% |
| (d) Hyperplasia in Adrenal Cortex | M | 4% | 6% | 7% | 1% |
| | F | 3% | 0% | 3% | 0% |
| (e) Adenoma & Carci-noma in the Adrenal Cortex | M | 22% | 17% | 31% | 18% |
| | F | 1% | 1% | 3% | 0% |
| (f) (d) and(e) combined | M | 25% | 23% | 34% | 19% |
| | F | 4% | 1% | 6% | 0% |
| (g) Inflammation of the Stomach Mucosa | M | 10% | 17% | 26%* | 20%* |
| | F | 15% | 26%* | 21% | 20% |
| (h) Hyperplasia in the Stomach Mucosa | M | 9% | 13% | 29%* | 19%* |
| | F | 14% | 20% | 19% | 23% (*) |
| (i) Hyperplasia & Neoplasia of the Stomach Mucosa | M | 10% | 13% | 29%* | 20%* |
| | F | 14% | 20% | 21% | 23% (*) |

* Significant increase compared to control; Fisher's exact test $p \leq 0.05$.

(*) Probably significant increase over control since:
 (i) $0.05 < \text{Fisher's } p\text{-value} \leq 0.10$
 and (ii) Mantel-Hanszel $p\text{-value} \leq 0.05$

+ Test for positive linear trend was significant; however, the result is difficult to interpret since the incidence rates do not follow a linear pattern.

A slight increase in the appearance of reticulum cell sarcomas was observed in a number of organs in treated female groups. However,

these malignant sarcomas were localized at multiple centers in just a few animals. The actual number of tumor-bearing animals does not suggest a clear relationship to treatment. The results are presented in Table 1.

Table 1
Multicentric Reticulum Cell Sarcomas

| | Control 1 | | Control 2 | | 2.5 mg/kg | | 15 mg/kg | | 80 mg/kg | |
|---------------|-----------|---|-----------|---|-----------|---|----------|---|----------|---|
| | M | F | M | F | M | F | M | F | M | F |
| Natural Death | 1 | 4 | 0 | 1 | 1 | 1 | 0 | 7 | 0 | 3 |
| Terminal | 1 | 2 | 1 | 2 | 1 | 2 | 3 | 1 | 0 | 4 |
| Total | 2 | 6 | 1 | 3 | 2 | 3 | 3 | 8 | 0 | 7 |

The sponsor indicates that these whole body tumors, considered in combination with other whole body tumors, suggest no carcinogenic potential for PN 200-110.

The liver tumor incidence among treated males does suggest some relationship to treatment. A further breakdown of the distribution of hepatocellular carcinoma and nodular proliferation among treated groups is presented below

Table 2
Liver

| | All Animals | | | | | | | | | |
|-----------------------|-------------|---|-----------|---|-----------|---|----------|---|----------|---|
| | Control 1 | | Control 2 | | 2.5 mg/kg | | 15 mg/kg | | 80 mg/kg | |
| | M | F | M | F | M | F | M | F | M | F |
| Hepatocellular CA | 7 | 2 | 10 | 1 | 13 | 3 | 7 | 0 | 17 | 3 |
| Nodular Proliferation | 8 | 5 | 6 | 2 | 8 | 3 | 7 | 1 | 11 | 1 |
| Naturally Dying | | | | | | | | | | |
| Hepatocellular CA | 3 | 0 | 2 | 0 | 6 | 1 | 4(1)* | 0 | 10(1) | 1 |

* Number in parenthesis - no. of animals with multifocal tumors

The sponsor has provided a complete statistical evaluation of the hepatocellular carcinoma findings in the liver in the attached table

TABLE 7

LIFETIME DIET (DIET) CARCINOGENICITY STUDY IN THE MOUSE
ON 200-110

SANDOGI PROJECT T-1843

TREND AND HOMOGENEITY ANALYSES OF TUMOR INCIDENCE DATA

LIVER CARCINOMA HEPATOCELLULAR

TUMOR RATE IN VARIOUS TIME INTERVALS (NATURAL DEATH)

| DOSE LEVEL | CONTROL 0.0 MG/KG | | LOW DOSE 2.5 MG/KG | | MID DOSE 15.0 MG/KG | | HIGH DOSE 80.0 MG/KG | | P-VALUES OF STATISTICAL TESTS ⁽¹⁾ | |
|--|----------------------|----|-----------------------|------|------------------------|----|-------------------------|------|--|------------------------------|
| | M | F | M | F | M | F | M | F | HOMOGENEITY OF PROPORTION | TEST FOR LINEAR TREND (3) |
| 0 - 12 MONTH | | | | | | | | | | |
| TOTAL TUMOR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| TOTAL # EXAMINED | 8 | 4 | 5 | 5 | 2 | 8 | 8 | 6 | | |
| TUMOR RATE% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% | | |
| P-VALUE (1) | | | | | | | | | | |
| 12 - 18 MONTH | | | | | | | | | | |
| TOTAL TUMOR | 1 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | | |
| TOTAL # EXAMINED | 20 | 22 | 10 | 10 | 9 | 10 | 14 | 10 | | |
| TUMOR RATE% | 5% | 0% | 20% | 0% | 0% | 0% | 21% | 0% | | |
| P-VALUE (1) | | | .25* | | .69* | | .17* | | | |
| 19 - 24 MONTH | | | | | | | | | | |
| TOTAL TUMOR | 4 | 0 | 4 | 1 | 4 | 0 | 7 | 1 | | |
| TOTAL # EXAMINED | 44 | 43 | 20 | 21 | 25 | 19 | 23 | 24 | | |
| TUMOR RATE% | 9% | 0% | 17% | 5% | 16% | 0% | 30% | 4% | | |
| P-VALUE (1) | | | .24* | .31* | .31* | | .002* | .24* | .17* | .02* |
| TUMOR RATE FOR ALL NATURAL DEATH ANIMALS | | | | | | | | | | |
| TOTAL TUMOR | 5 | 0 | 6 | 1 | 4 | 0 | 10 | 1 | | |
| TOTAL # EXAMINED | 72 | 72 | 38 | 31 | 34 | 37 | 43 | 41 | | |
| TUMOR RATE% | 7% | 0% | 16% | 3% | 11% | 0% | 23% | 2% | | |
| P-VALUE (1) | | | .12* | .33* | .34* | | .015* | .31* | .11* | .02* |
| P-VALUE (4) | | | .12* | | .41* | | .01* | | | |

- (1) ONE-TAILED COMPARISONS WITH CONTROL USING FISHER'S EXACT TEST. * INDICATES SIGNIFICANCE RESULTING FROM A COMPARISON FAVORING THE DOSED GROUP. SEE FOOTNOTE (2) FOR DEFINITION OF ASTERISKS.
- (2) STATISTICAL SIGNIFICANCE AT THE 0.10, 0.05, 0.01, AND 0.001 LEVEL IS DENOTED BY (*), (*), and (***) RESPECTIVELY. P-VALUES LESS THAN .001 ARE REPORTED AS 0.01.
- (3) COX'S EXACT TEST (ONE-TAILED) FOR LINEAR TREND WAS USED. * INDICATES SIGNIFICANCE RESULTING FROM A NEGATIVE DOSE RESPONSE RELATIONSHIP. + INDICATES SIGNIFICANT LACK-OF-FIT AT THE 0.10 LEVEL.
- (4) ONE-TAILED COMPARISONS WITH CONTROL USING THE BIRCH-COX (EXACT) ANALYSIS OF THE MANTEL-HAENSZEL PROCEDURE TABLES COMBINED WERE THE NATURAL DEATH INTERVALS. * INDICATES ZELLEN'S EXACT TEST FOR INTERACTION WAS SIGNIFICANT AT THE 0.10 LEVEL.

NOTE: MISSING P-VALUES INDICATE THE APPROPRIATE STATISTICAL TEST WAS JUDGED TO BE UNINFORMATIVE DUE TO SMALL SAMPLE SIZE.

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TABLE 7 (Cont'd.)

LIFETIME ORAL (DIET) CARCINOGENICITY STUDY IN THE MOUSE
ON 200-110

SANDOZ PROJECT T-1843

TREND AND HOMOGENEITY ANALYSES OF TUMOR INCIDENCE DATA

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| DOSE LEVEL | CONTROL | | LOW DOSE | | MID DOSE | | HIGH DOSE | | P-VALUES OF STATISTICAL TEST (2) | | | | |
|---|-----------|-----|-----------|------|------------|------|------------|------|----------------------------------|---|---------------------------|---|-------|
| | 0.0 MG/KG | | 2.5 MG/KG | | 15.0 MG/KG | | 83.0 MG/KG | | HOMOGENEITY OF PROPORTION | | TEST FOR LINEAR TREND (3) | | |
| | M | F | M | F | M | F | M | F | M | F | M | F | |
| TUMOR RATE FOR TERMINAL SACRIFICED ANIMALS | | | | | | | | | | | | | |
| TOTAL TUMOR | 12 | 3 | 7 | 2 | 3 | 0 | 7 | 2 | | | | | |
| TOTAL # EXAMINED | 67 | 68 | 32 | 34 | 34 | 31 | 25 | 33 | | | | | |
| TUMOR RATE% | 18% | 4% | 22% | 6% | 9% | 0% | 28% | 7% | | | | | |
| P-VALUE (1) | | | .415 | .342 | .181 | .320 | .217 | .467 | .277 | | | | .148 |
| TUMOR RATE FOR ALL ANIMALS (NATURAL DEATH & SACRIFICED) | | | | | | | | | | | | | |
| TOTAL TUMOR | 17 | 3 | 13 | 3 | 7 | 0 | 17 | 3 | | | | | |
| TOTAL # EXAMINED | 139 | 140 | 73 | 70 | 73 | 68 | 70 | 70 | | | | | |
| TUMOR RATE% | 12% | 2% | 18% | 4% | 10% | 0% | 24% | 4% | | | | | |
| P-VALUE (1) | | | .193 | .318 | .410 | .333 | .023* | .318 | .011* | | | | .021* |
| P-VALUE (4) | | | .133 | .211 | .370 | | .009 | .276 | | | | | |

- (1) ONE-TAILED COMPARISONS WITH CONTROL USING FISHER'S EXACT TEST. * INDICATES SIGNIFICANCE RESULTING FROM A COMPARIS FAVORING THE DOSED GROUP. SEE FOOTNOTE (2) FOR DEFINITION OF ASTERISKS.
- (2) STATISTICAL SIGNIFICANCE AT THE 0.10, 0.05, 0.01 AND 0.001 LEVEL IS DENOTED BY (*), (•), (**), and (***), RESPECTIVELY. P-VALUES LESS THAN .001 ARE REPORTED AS 0.000.
- (3) COX'S EXACT TEST (ONE-TAILED) FOR LINEAR TREND WAS USED. * INDICATES SIGNIFICANCE RESULTING FROM A NEGATIVE DOSE-RESPONSE RELATIONSHIP. + INDICATES SIGNIFICANT LACK-OF-FIT AT THE 0.10 LEVEL.
- (4) ONE-TAILED COMPARISONS WITH CONTROL USING THE BIRCH-COX (EXACT) ANALOG OF THE MANTEL-HAENSZEL PROCEDURE. TABLES COMBINED WERE THE NATURAL DEATH INTERVALS AND THE TERMINAL SACRIFICED ANIMALS. & INDICATES JELEN'S EXACT TEST FOR INTERACTION WAS SIGNIFICANT AT THE 0.10 LEVEL.

NOTE MISSING P-VALUES INDICATE THE APPROPRIATE STATISTICAL TEST WAS JUDGED TO BE UNINFORMATIVE DUE TO SMALL SAMPLE SIZES.

The data shows a significant increase in hepatocellular carcinoma among males of the high dose treatment group whether the comparison with control is limited to those animals dying on study (p=0.018) or encompasses dying as well as sacrificed animals (p=0.023). The COX Exact Test for linear trend also was significant in these subpopulations (p=0.021 for both).

Considering non-neoplastic pathology, there was an increased incidence of hyperplasia of the stomach mucosa among treated male mice. The statistics for mid and high dose groups were p<0.001 and p=0.049, respectively. There was no increase in stomach tumors observed in these groups. The table below presents the findings.

| Group mg/kg/day | Stomach Lesions in Mice | | | | | | | |
|---|-------------------------|-----|------------|-----|-----------|-----|------------|-----|
| | Controls 0 | | Low 2.5 | | Mid 15 | | High 80 | |
| | M | F | M | F | M | F | M | F |
| Naturally Dying Animals | | | | | | | | |
| Examined | 72 | 72 | 38 | 36 | 36 | 37 | 45 | 40 |
| No. with Hyperplasia | 4 | 7 | 4 | 5 | 7 | 4 | 4 | 6 |
| Percentage Rate | 6% | 10% | 11% | 14% | 19% | 11% | 9% | 15% |
| No. with Hyperplasia and/or Neoplasia | 5 | 7 | 4 | 5 | 7 | 4 | 5 | 6 |
| Percentage Rate | 7% | 10% | 11% | 14% | 19% | 11% | 11% | 15% |
| Terminal Sacrificed | | | | | | | | |
| Examined | 67 | 68 | 32 | 34 | 34 | 31 | 25 | 30 |
| No. with Hyperplasia | 9 | 12 | 5 | 9 | 13 | 9 | 9 | 10 |
| Percentage Rate | 13% | 18% | 16% | 26% | 38% | 29% | 36% | 33% |
| No. with Hyperplasia and/or Neoplasia | 9 | 12 | 5 | 9 | 13 | 10 | 9 | 10 |
| Percentage Rate | 13% | 18% | 16% | 26% | 38% | 32% | 36% | 33% |
| Naturally Dying & Terminal Sacrificed Examined | | | | | | | | |
| Examined | 139 | 140 | 70 | 70 | 70 | 68 | 70 | 70 |
| No. with Hyperplasia | 13 | 19 | 9 | 14 | 20 | 13 | 13 | 16 |
| Percentage Rate | 9% | 14% | 13% | 20% | 29% | 19% | 19% | 23% |
| No. with Hyperplasia and/or Neoplasia | 14 | 19 | 9 | 14 | 20 | 14 | 14 | 16 |
| Percentage Rate | 10% | 14% | 13% | 20% | 29% | 21% | 20% | 23% |

The mouse carcinogenicity study was analyzed by CDER's Biometrics Division for differences in survival among treated male mice relative to control and the differences in the incidence of hepatocellular carcinoma and/or nodular proliferations analyzed as a function of dose by the Peto method. This request was made because there appeared to be a dose-related impairment in survival among treated male mice and there was also an increase in hepatocellular carcinoma which failed to reach statistical significance ($p < 0.01$) for common tumor type when analyzed by Fishers Exact Test, but this method does not account for differences in intercurrent mortality.

~~With respect to the male mouse, the statistician found a positive dose-response relationship in intercurrent mortality and a positive dose-response relationship for both hepatocellular carcinoma ($p = 0.004$) and carcinoma pooled with nodular proliferation ($p = 0.011$).~~

In summary, the findings from the two-year oral carcinogenicity study of PN 200-110 in mice suggest a dose-related trend for an increase in hepatocellular carcinoma in male mice with a pairwise significant increase occurring only at the highest dose. The development of hyperplasia of the gastric mucosa was also increased in male mice at the two higher doses of test compound.

A 4-Week Oral Toxicity Study in Rats

Methods

Study Facility/Dates: Sandoz Ltd., Basle (1981-82)

Strain/Source: SPF, NFM-WIST (Fullinsdorf, CH)

Duration: 4 weeks

Route: Oral in diet

Dose Levels: 0, 0.0048% (4 mg/kg), 0.0144% (12 mg/kg), 0.048% (40 mg/kg) [Actual : 4.3, 13, and 42 mg/kg/day]

No. of Animals/Sex/Dose: 15 (10 sacrificed at 4 weeks; 5 permitted recovery for additional 4 weeks)

Age at Study Initiation: 8 weeks

Weight Range: Males: 189-242 g.
Females: 145-190 g.

Parameters Measured:

Clinical Observations included:

- *Mortality
- *Clinical signs of toxicity
- *Physical examinations
- *Body weight
- *Food consumption
- *Behavior/motor effects

Hematology and Clinical Chemistry were Conducted at Week 5:

A complete macroscopic and histologic examination of tissues was conducted at study termination

Results:

There was no mortality among treated animals in this experiment and none of the treated groups exhibited clinical signs or symptoms of drug related toxicity either during the formal treatment period or during the recovery period. Body weights were comparable among groups throughout the course of the treatment. Only changes were a decrease in food intake among the high dose males at study initiation and

females over the course of the whole study. These changes in food consumption normalized during the recovery period among the high dose female group. Hematology results showed no particular changes in the low and mid dose treated groups; however, there was a slight reduction in lymphocyte count among males in the high dose treatment group, although this did not reach statistical significance. This was reversible upon treatment cessation.

PN 200-110: A 26-Week Oral Toxicity Study in Rats

Methods

Study Facility/Dates: Sandoz Ltd., Basle (1981-82)

Strain/Source: SPF, KFM-W157 (Fullinsdorf, CH)

Duration: 26-week

Route: oral in diet

Dose Levels: 0, 2.5, 11, and 42 mg/kg [Actual : 2.5, 10, and 41 mg/kg/day]

No. of Animals/Sex/Dose: 15 (10 sacrificed at 26 weeks; 5 held for 4 week recovery)

Age at Study Initiation: 8 weeks

Weight Range: Males: 183-259 g.
Females: 132-179 g.

Parameters Measured:

Clinical Observations: Conducted during course of study

Hematology: Clinical chemistry, and urinalysis conducted at week 7, 14, and 26.

*Erythrocyte sedimentation

*Activated partial thromboplastin test

A complete macroscopic and microscopic evaluation of tissues was conducted at the end of 26 weeks.

Results

All animals survived the study; feed intake was slightly inhibited among high dose males.

RBC values (erythrocyte, hemoglobin, and hematocrit) trended higher among females in the mid dose group and was significantly higher in the high dose group, although these latter values remained in the normal range.

Blood glucose levels were slightly lower among females at the mid and high dose. These reverted to normal during the recovery period.

There were no consistent dose-related abnormalities in any urinalysis parameter.

Macroscopic examination revealed no significant toxicologic findings. The relative organ weight increases (heart and ovary) among high dose females were explainable on body weight variation or individual animal differences. There was no trend toward increased testes weights among males of any treatment group.

Histopathologic examination of tissues revealed nothing remarkable in treated animals relative to controls.

Two-Year Oral (Diet) Toxicity/Carcinogenicity Study
in the Rat on FN 200-110

Methods

Study Facility/Dates: Sandoz Res. Inst., E. Hanover, NJ (1983-85)

Strain/Source: Charles River-CD (Sprague Dawley Kingston, NY)

Duration: 2 years

Route: oral in diet

Dose Levels: 0, (2), 2.5, 12.5, 62.5 mg/kg/day [Actual : 2.5, 12.7,
and 63.6 mg/kg/day]

No. of Animals/Sex/Dose: 75 (150 controls designated as C-1 and C-2)

Age at Study Initiation: not specified

Weight Range: Males: 128-132 g.
Females: 112-114 g.

Parameters Measured:

Clinical Observations:

- *Mortality
- *Clinical signs of toxicity
- *Physical examinations
- *Body weight
- *Food consumption
- *Water consumption
- *Tissue mass palpation

Ophthalmoscopy:

- *Indirect during weeks 3, 7, 11, 17, 25, 36, 51, 77 and 103

Hematology: (Weeks 4, 8, 12, 18, 26, 38, 52, 78 and 104)

- | | |
|---|---------------------------------|
| *Red blood cell count | *Reticulocyte count |
| *Hemoglobin | *Thrombocyte (platelet) count |
| *Hematocrit | *Prothrombin time |
| *Mean corpuscular volume (MCV) | *White blood cell count |
| *Mean corpuscular hemoglobin concentration (MCHC) | *Differential white blood count |
| *Mean corpuscular hemoglobin (MCH) | |

Clinical Biochemistry: (same wks. as hematology)

| | |
|--|------------------|
| *Blood urea nitrogen (BUN | *Total protein |
| *Serum glutamic oxaloacetic transaminase (SGOT) | *Sodium |
| *Serum glutamic pyruvic transaminase (SGPT) | *Potassium |
| *Glucose | *Chloride |
| *Cholesterol | *Chloride |
| | *Calcium |
| | *Total bilirubin |

Urinalysis: (same wks. as hematology)

| | |
|----------------------------------|-------------------|
| *pH | *Proteins |
| *Glucose | *Occult blood |
| *Ketones bodies | *Specific gravity |
| *Sediment (microscopic analysis) | |

Macroscopic and Microscopic Examination

All relevant organs and tissues.

Results

Survival appeared to be unimpaired by drug treatment. Food consumption and body weight gain were affected, particularly during the latter half of the study. Significant weight reductions of 10% or greater were noted for both male and female rats of the high dose group relative to controls (F, 24%; M, 14%). Female body weights were significantly reduced (relative to current control) by week 8 and male body weights by week 40. A 10% decrement in body weight was achieved by week 38 for the females and week 80 for males of the high dose group relative to controls.

Mortality and Mean Survival Data

| <u>Parameter</u> | <u>Sex</u> | <u>Control</u> | <u>Low</u> | <u>Mid</u> | <u>High</u> |
|----------------------|------------|----------------|------------|------------|-------------|
| Mortality | Males | 54% | 55% | 54% | 64% |
| | Females | 66% | 57% | 54% | 51% |
| Mean Survival (days) | Males | 708 | 716 | 713 | 682 |
| | Females | 677 | 705 | 695 | 729 |

In-life clinical observations and ophthalmoscopic examinations revealed no drug-related toxic events. Palpable masses were comparable between groups.

A significant increase in erythrocytes, hemoglobin and hematocrit was observed among high dose females through week 37 but not thereafter.

Statistically significant increases in BUN (25-53%) and cholesterol (26-47%) were recorded for the high dose females during the first half of the study.

Urinalysis revealed a slight decrease in the specific gravity of the urine of high dose females, again during the first year of study.

Analysis of organ weight data of terminally sacrificed animals revealed several differences from concurrent control. However, only two findings among males of the high dose group were probably significant. These were increases in the absolute and relative (brain weight) weights of the liver and thyroid.

An examination of non-neoplastic histology data demonstrated that chronic progressive nephrosis common to aging rats was frequently more severe among male rats of the high dose group dying naturally on study. There may have been a slight propensity to develop necrotizing arteritis among high dose males at terminal sacrifice (8 vs. 5 and 2 for control groups I and II). Combining males independent of their demise, the incidence of Leydig cell hyperplasia was somewhat increased in the high dose treatment group.

The only tumor type significantly increased with treatment in this study was benign Leydig cell tumors. The tumor and related pathology for all groups is tabulated below:

| Finding | Control 1 (n=75) | Control 2 (n=75) | Low (n=74) | Mid (n=75) | High (n=75) |
|-----------------------|---------------------|---------------------|---------------|---------------|----------------|
| Tumor bearing Animals | 9 | 6 | 5 | 11 | 19 |
| No. with Hyperplasia | 2 | 1 | 1 | 1 | 6 |
| Total* (either or) | 10 | 7 | 6 | 12 | 23 |

*Animals with both tumors and hyperplasia are counted once in total.

Cox's Exact Test (one-tailed) was applied separately to the data recorded for animals dying naturally or terminally sacrificed. The respective p-values are listed below:

| | |
|--------------------------------------|--------|
| Natural Death/Leydig Cell Tumors | p=.001 |
| Sacrifice/Leydig Cell Tumors | p<.001 |
| Natural Death/Tumors and Hyperplasia | p=.001 |
| Sacrifice/Tumors and Hyperplasia | p<.001 |

The sponsor makes the statement that these tumors may have been endocrine linked and cites the decrease in pituitary adenomas in the high dose group (more probably related to body weight reductions) and the decreases in the numbers of parafollicular adenomas and islet cell adenomas (not statistically significant) as evidence of endocrine changes.

Studies to evaluate the relationship between the endocrine changes and the tumor response in the rat were conducted and are presented together on the following pages

A statistical analysis of the rat study by CDER's Biometrics Division (Peto analysis) confirmed that there is a positive dose response relationship in the incidence of Leydig cell tumors and Leydig tumors pooled with testicular hyperplasia ($p < 0.01$).

PN 200-110 and PY 108-068: A 30-Week Oral Toxicity Study in
Old Sprague Dawley Rats

Methods

Study Facility/Dates: Sandoz AG, Basel, Switzerland (1986-87)

Strain/Source: Beagle (SPF, Km: SPRD)/Kleintierfarm Madoren AG

Duration: 30 weeks

Route: oral (feed)

Dose Levels: PN 200-110, 6.6 and 64.0 mg/kg; PY 108-068, 6.5 and
65.0 mg/kg (PY 108-068 is an analog of PN 200-110)

No. of Animals/Sex/Dose: 20 males

Age at Study Initiation: 78-80 weeks

Weight Range: Males: 517-773 g

Parameters Measured:

Clinical Observations: (daily)

- *Mortality
- *Clinical signs of toxicity
- *Body weight
- *Food consumption
- *Tissue mass palpation

Clinical Chemistry: (weeks -2, 5, 9, 13, 17, 21, 25, and 29)

- *Luteinizing hormone
- *Follicle stimulating hormone
- *Prolactin
- *Testosterone
- *Progesterone

Postmortem Examinations:

Complete macroscopic; only microscopic of liver, heart,
testes, spleen, thymus, kidneys, and lungs.

Results

There were no treatment-related effects on mortality or clinical signs. Feed intake and body weight gain were inhibited at the high dose of both drugs particularly during the first weeks of the study.

Among the hormones measured, only plasma FSH was consistently affected. Increased levels were observed at all time points in the high dose PN 200-110 group. Significant increases were recorded at 5 points in PY 108-068 high dose group and 3 points at the low dose of PN 200-110. Levels of the other hormones were variably affected.

~~The absolute testicular weights were slightly but significantly~~ reduced in the PN 200-110 high dose treatment group. One animal treated with the high dose of PY 108-068 showed Leydig cell hyperplasia but there were no other testicular or major organ changes noted among treated rats.

Two-Year Oral (Diet) Target Organ Reproducibility Carcinogenicity
Study in the Rat on PN 200-110

Methods

Study Facility/Dates: Sandoz Res. Inst.; East Hanover, NJ (1985-1987)

Strain/Source: Charles River CD/Charles River Labs (Kingston, NY)

Duration: 2 years

Route: oral (diet)

Dose Levels: 0, 6.25, and 62.5 mg/kg/day PN 200-110

No. of Animals/Sex/Dose: 70 males

Age at Study Initiation: 6 weeks

Weight Range: Males: ca. 155 g

Parameters Measured:

Clinical Observations:

- *Mortality (daily)
- *Clinical signs of toxicity (daily)
- *Body weight (weekly)
- *Food consumption (weekly)
- *Tissue mass palpation (weekly after week 36)

Clinical Chemistry: (weeks 3, 8, 12, 19, 26, 37, 52, 66, 78, 86,
94, and 104)

- | | |
|----------------------|-------------------------------|
| *Luteinizing hormone | *Follicle stimulating hormone |
| *Prolactin | *Testosterone |
| *Growth hormone | |

Biochemical Analyses:

- *Pituitary LH and FSH content
- *Testicular testosterone
- *Testicular LH and GnRH receptor content

Postmortem Examinations:

Macroscopic, organ weight, microscopic evaluation of only
the testes, pituitary, and epididymis.