controls, and positive controls were plated in triplicate. A dose finding study was first performed using S. typhimurium strain TA100 and E. coli strain WP2uvrA to select doses of NTZ without cytotoxicity. Based on this study, doses of 250, 100,50, 25, 10, and 5μg per plate were used in S. typhimurium studies with S9 and 500, 100, 50, 25, 10, and 5 μg per plate in studies without S9. In the E. coli studies, doses of 600, 300, 100, 50, 25, and 10μg per plate were used in both the presence and absence of S9. A volume of 50 μl per plate was used for NTZ, positive controls, and vehicle control plates and 500 μl S9 for metabolic activation. Bacterial plates were administered the appropriate test solutions with or without S9 for 48h ±8h, then scored for revertants. All positive controls were successful. NTZ caused a positive response with the S. typhimurium strain TA100 in the presence (3.4 fold increase over vehicle control at 100μg/plate) and absence (4 fold increase over vehicle control at 100 μg/plate) of S9. This is a modest effect relative to the positive control (2-aminoanthracene, with S9, 11-fold increase; sodium azide, without S9, 9-fold increase) increases. No other positive responses were seen in this study.

4. Mutagenicity test with desacetyl-nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay; performed by — — study no. — 17567-0-409; batch no. 003; 5 July 1996; GLP

desNTZ was evaluated for the ability to induce reverse mutations at the histidine locus in strains of Salmonella typhimurium and at the tryptophan locus of an Escherichia coli tester strain, both in the presence and absence of metabolic activation by mammalian microsomal enzymes. S. typhimurium tester strains TA98, TA100, TA1535, TA1537, and E. coli tester strain WP2uvrA were used in this study. Positive controls used in S. typhimurium studies were 2-aminoanthracene, 2-nitrofluorene, sodium azide, and ICR-191. Positive controls used in E. coli studies were 2-aminoanthracene and 4-nitroquinoline-N-oxide. The metabolic activation studies used rat liver S9 homogenate prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. Overnight cultures of each tester strain were used for the studies. All doses of NTZ, vehicle controls, and positive controls were plated in triplicate. A dose finding study was first performed using S. typhimurium strain TA100 and E. coli strain WP2uvrA to select doses of NTZ without cytotoxicity. Based on this study, doses of 300, 100,50, 25, 10, and 5 μg per plate were used in S. typhimurium studies with S9 and 100, 50, 25, 10, 5, and 1 μg per plate in studies without S9. In the E. coli studies, doses of 5000, 1000, 500, 250, 100, and 50 μg per plate were used in both the presence and absence of S9. A volume of 50 μl per plate was used for NTZ, positive controls, and vehicle control plates and 500 μl S9 for metabolic activation. Bacterial plates were administered the appropriate test solutions with or without S9 for 48h ±8h, then scored for revertants. All positive controls were

successful. NTZ caused a positive response with the S. typhimurium strain TA100 in the presence (3.1-fold increase over vehicle control at 50 µg/plate) and absence (2-fold increase over vehicle control at 25 µg/plate) of S9. This is a modest effect relative to the positive control (2-aminoanthracene, with S9, 12-fold increase; sodium azide, without S9, 7-fold increase) increases. No other positive responses were seen in this study.

5. Mutagenicity test on nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary; performed by , study no 17348-0-437; batch no. 003; 28 June 1996; GLP

In order to determine the clastogenic potential of NTZ, the in vitro Chinese hamster ovary assay was performed with and without metabolic activation. Chinese hamster ovary cells (CHO-WBL) from a permanent cell line were obtained from the laboratory Positive controls used in this assay were mitomycin C (for assays without metabolic activation), and cyclophosphamide (for assays with metabolic activation). The metabolic activation studies used rat liver S9 homogenate lot #0583) prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. A rangefinding assay was first performed to determine an assay concentration range without cytotoxicity and optimal harvest time of treated cells. Based on these preliminary assays, the concentrations of NTZ used in metabolic activated assays were 121, 80.8, 60.6, 40.4, 20.2, 10.1 and 5.05 µg/ml; 1210, 1010, 808, 606, 404, 202, and 101 µg/ml were used without metabolic activation. An optimal incubation time of 20.1 hours was determined. The cells were cultured for 24 hours prior to treatment. Replicate cultures were used for all doses. Cells were incubated for 17.8 hours, then washed and reincubated with colcemid and for two hours. Cells were harvested and prepared on slides with staining for counting. Cytotoxicity occurred in cultures above 80.8 µg/ml without S9 and above 606 µg/ml. One hundred consecutive metaphases from four dose levels with metaphases with and without S9, and from the positive, negative, and vehicle controls were evaluated for the number of cycles through which cells had progressed while in the presence of colcemid. NTZ was negative for induction of chromosomal aberrations in CHO cells except at 60.6 µg/ml with metabolic activation, which was an equivocal positive (% cells with aberrations, mean =9 cells) while the cyclophosphamide positive control had a mean % cells with aberration=52; solvent mean=1).

6. Mutagenicity test on desacetyl-nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary; performed by ; study no. 17567-0-437; batch no.L07-95110; 27 June 1996 June 1996; GLP

In order to determine the clastogenic potential of desNTZ, the in vitro Chinese hamster ovary assay was performed with and without metabolic activation. Chinese hamster ovary cells (CHO-WBL) from a permanent cell line were obtained from the laboratory —

— Positive controls used in this assay were mitomycin C, for assays without metabolic activation, and cyclophosphamide, for assays with metabolic activation. The metabolic activation studies used rat liver S9 homogenate (— , lot #0583) prepared from Sprague-Dawley rats treated with Araclor 1254. Vehicle (DMSO) controls were used with all tester strains in the presence and absence of S9. A rangefinding assay was first performed to determine an assay concentration range without cytotoxicity and optimal harvest time of treated cells. Based on these preliminary assays, the concentrations of desNTZ used in metabolic activated assays were 999, 799, 599, 400, 200, and 100 μg/ml; 300, 225, 150, 113, 75, 50, and 25 μg/ml were used without metabolic activation. An optimal incubation time of 20.1 hours was determined. The cells were cultured for 24 hours prior to treatment. Replicate cultures were used for all doses. Cells were incubated for 17.8 hours, then washed and reincubated with colcemid and for two hours. Cells were harvested and prepared on slides with staining for counting. Cytotoxicity occurred in cultures above 150 μg/ml without S9. One hundred consecutive metaphases from four dose levels with metaphases with and without S9, and from the positive, negative, and vehicle controls were evaluated for the number of cell cycles through which cells had progressed while in the presence of colcemid. DesNTZ did not produce any positive results in this chromosomal aberration assay.

Summary of the Toxicity, Reproductive, and Genetic Toxicity Studies
Most nonclinical toxicology studies were reviewed previously. Acute toxicity studies were conducted in mice, rats, dogs, and cats. Oral lethal doses by both intraperitoneal and oral routes were determined. In mice, the intraperitoneal LD50 was 105 mg/kg for both males and females. The oral LD50 was 1350 mg/kg in males and 1380 mg/kg for females. The intraperitoneal LD50 in rats was 192 mg/kg in males and 165 mg/kg for females. The oral LD50 was not achieved with the doses used; therefor the LD50 was greater than 10000 mg/kg in both sexes (the lethal oral dose was not achieved in cats). The LD50 was greater than 10 g/kg. The lethal oral dose was also not achieved in dogs. The L\LD50 was greater than 10g/kg. Toxicity was seen as diarrhea and emesis. At necropsy distended gall bladders were seen. Repeat-dose oral toxicity studies were conducted in rats and dogs. Rats were studied for 14 weeks and 6 months. After 14 weeks, rats receiving 450 mg/kg demonstrated intense salivation, increased liver and spleen weights, and decreased thymus weights without histopathological differences. In the 6 month study, a NOAEL of 150 mg/kg/day was observed. The major toxicity seen was an increase in extramedullary hematopoiesis and pigment deposition in the spleens of males and females

receiving 450 mg/kg/day. Hematological assays were affected by treatment in the high dose group. Significant increases in leukocytes, lymphocytes, and mean corpuscular volume and decreases in erythrocyte counts and hematocrit were seen in both males and females. Also seen in high dose females were significant increases in neutrophils and mean corpuscular volume. Clinical chemistry effects were seen in the high dose group. Males had significant decreases in urea nitrogen, sodium, total protein, and calcium and increases in albumin/globulin ratio, total bilirubin, and phosphorus. Females had significantly decreased sodium and glucose and higher total bilirubin. In the 28 day dog study, drug induced effects included weight loss and decreased food consumption, blood in the urine, decreased erythrocyte counts, decreased hematocrit, and decreased organ weights including brain, lung, spleen, heart, lung, kidney, thymus, liver, and testes. Histopathologic findings included lymphoid depletion of the thymus, ulceration of the cecum, and cystic hyperplasia of the gall bladder. A NOAEL was not achieved in this study. In the 90 day oral dog study, similar hematological findings occurred. Both weight loss and decreased food consumption were seen. Loose and/or discolored stool was frequently seen. In males, decreased testicular weights and testicular immaturity were seen. The NOAEL for this study was less then 25 mg/kg. A salicylate–like effect by NTZ may account for the toxicity seen in dogs. In all of the acute and longer term oral studies, yellow material was recovered form the stomach and intestines. NTZ is a yellow powder; these findings are supportive of the pharmacokinetic studies showing poor absorption. Yellow staining of fur in the urogenital region also was seen in most animals.

Reproductive toxicity studies were conducted in rats and rabbits. In both species, NTZ did not significantly affect fertility, implantation, pregnancy rates, or cause fetal death. In male rats, histological and functional studies of sperm did not show drug effects. Decreased gravid uterine weights, decreased body weights, and food consumption were seen in females. Fetal abnormalities included protruding tongue, open eyes, and exencephaly in one fetus, macromelia (one fetus), adactylly (one fetus), and protruding tongue and exencephaly (one fetus). Soft tissue malformations included a trend to increased dilation of the brain ventricles and renal pelvic cavitation. Skeletal variations included incomplete skull ossification, fewer than four caudal vertebrae ossified, unossified vertebral centrum, and wavy ribs. These maternal and fetal effects were seen at doses of 600, 1200 and 2400 mg/kg. The maternal effects complicated the interpretation of these data. The range of these doses is equivalent to human doses of 100 to 400 mg/kg, greater than the proposed doses of 33-50 mg/kg. In the rabbit study, no external abnormalities were seen. Soft tissue abnormalities included internal hydrocephaly (one fetus) and

hepatomegaly (one fetus). The NOAEL for the dams was 50 mg/kg and 25 mg/kg for the fetuses, corresponding to 25 and 12 mg/kg, respectively, in humans. In a rat study to determine effects of NTZ on pre- and postnatal development, reduced survival during lactation was seen in pups from mothers receiving 1200 mg/kg. Maternal activity towards the pups was reduced in this group, coinciding with the impaired condition of these dams due to decreased food consumption and body weight. It is unclear whether impaired maternal care and/or in utero and lactation NTZ exposure explain the fetal effects seen in this study. Developmental markers and behavioral assays were not affected. Reproductive performance of the offspring was not affected. The parental NOAEL for this study appeared to be 300 mg/kg/day. It is difficult to determine the F_1 NOAEL due to the maternal effects, otherwise the fetal NOAEL was <300mg/kg/day. The 300 mg/kg day dose is equivalent to a human dose of 50 mg/kg/day.

Genetic toxicology studies demonstrated little potential for mutagenicity or clastogenic activity. The battery of studies performed included Ames tests with and without metabolic activation, and also with desNTZ, and chromosomal aberration tests with Chinese hamster ovary cell, also with desNTZ. Slight activity was seen in the Salmonella-E. coli reverse mutation assay; one strain of S. typhimurium showed slightly higher rates of reversion with both NTZ and desNTZ.

EVALUATION AND CONCLUSION

The evaluation of this application is largely a moot point due to the rejection by the advisory committee. The lack of toxicity in animal studies at doses equivalent to human exposure supports the safety of this drug. The reproductive toxicology studies do not demonstrate NTZ to be a strong teratogen. Given the low absorption seen with NTZ, Pregnancy Category B would be justified. There are no other pharmacology/toxicology issues.

/S/

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:
HFD-590/ADir/RAlbrecht
HFD-590/SPharm/KHastings
Steven C. Kunder/Pharm/

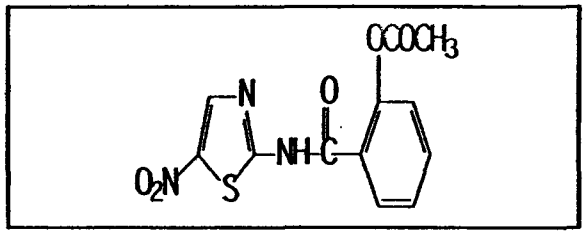
disk:
HFD-590/KHastings
cc:
HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/EFrank
HFD-590/RRoca
HFD-590/GHolbert
HFD-590/SBala
HF
D-345/

PHARMACOLOGIST'S REVIEW

IND # —

DATE SUBMITTED: 11 August 1995
DATE RECEIVED: 16 August 1995
DATE ASSIGNED: 17 August 1995
DATE REVIEW COMPLETED: 8 September 1995
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide
HFD-530

INDICATION: Cryptosporidiosis in AIDS patients



DEFINITIONS
NTZ=nitazoxanide
GI= gastrointestinal
daNTZ = desacetylnitazoxanide

INTRODUCTION
Nitazoxanide is a nitrothiazole antiparasitic which has demonstrated activity against protozoa, helminths, and bacteria. Recently NTZ has shown in vitro and in vivo activity against Cryptosporidium parvum, a parasitic opportunistic infection affecting AIDS patients. The drug is currently marketed outside the U.S. In the proposed clinical protocol, a Phase I/II open label study will treat cryptosporidial diarrhea in patients with AIDS. The objectives of the protocol are: (1) to determine the pharmacokinetic profile of single doses of NTZ in patients with AIDS-related cryptosporidial diarrhea, and determine steady state concentrations of NTZ following repeated dosing, and (2) to assess the safety and efficacy of NTZ for AIDS patients with cryptosporidial diarrhea. This review comprises a safety analysis of the preclinical data in mice, rats, cats, dogs and rabbits, including studies of pharmacokinetics, disposition and toxicity. This protocol proposes to study the safety and pharmacokinetics of NTZ in 4 cohorts of 7 patients each. The drug will be given daily for 14 days; separate cohorts will sequentially receive doses of 500, 1000, 1500, and 2000 mg/day.

PHARMACOKINETICS/TOXICOKINETICS and ADME
A1. A mass balance and tissue distribution study of ^{14}C-nitazoxanide following a single oral dose to male rats (Romark Laboratories; performed by
study no — 6613-100; ^{14}C-NTZ (lot no. 015H9229) and NTZ (lot no. 001); glp; 5/95)
Male CD rats received a single oral dose of NTZ/ ^{14}C-NTZ (30 mg/kg). Three rats per time point were sacrificed at 1, 2, 4, 8, 12, 24, 48, and 72 hours postdose. Urine and feces were collected at 0-4, 4-8, 8-12, 12-24, 48-72 hours postdose from the rats sacrificed at 72 hours. The maximum mean plasma concentration was 20.9 µg/ml at 1 hour. The majority of the dosed radiolabeled NTZ was recovered in the GI tract and its

contents. By 72 hours NTZ was less than 0.235 µg equivalents or undetectable in all tissues except thyroid.
Elimination via feces (52.0%) and urine (38.1%) provided the major routes of excretion of radiolabel (total recovery =90.9%). Of this, most was excreted in the first 24 hours postdose (46.3% feces and 37.1% urine).

A2. Determination of desacetylnitazoxanide in dog and rat plasma samples collected during Romark laboratories studies — .2647114 and 272100 (Romark Laboratories, performed by — Using plasma samples from the 28-day oral toxicity study of NTZ in dogs (see toxicity study C4, below) and the 28-day toxicity study in rats (see toxicity study A1, below) plasma levels of daNTZ were determined and pharmacokinetic parameters derived.

pharmacokinetic parameters from 28-day oral toxicity study of daNTZ in dogs and rats

group	AUC day 1 (0-24 hr)	AUC day 28 (0-24 hr)	Cmax day 1	Cmax day 28
900 mg/kg dog	7.73±4.08	14.65±9.95	1.12±0.55	1.58±1.59
2700/1800 mg/kg dog	37.0 ±18.8	20.0±6.6	3.34±1.03	1.37±0.32
800 mg/kg rat			2.38±0.89	
2400 mg/kg rat			3.62±1.37	
4800 mg/kg rat			5.08±2.54	
400 mg/kg rat				2.34±1.45
1200 mg/kg rat				5.78±2.81
2400 mg/kg rat				6.99±1.28
HUMAN - 7.14 mg/kg (500mg dose)	10.10±5.15		2.73±1.73	

mean group values, mg/L ± s.d.

As the ADME study traces radiolabel and not specific metabolites, correlation with human kinetics is not clear. It cannot be determined from this study whether daNTZ or other

metabolites are involved in elimination.
Species differences in plasma protein binding (which is reported to be about 95% bound in humans) and metabolism may account for the dose difference between dogs and humans yielding similar plasma daNTZ but without complete ADME this cannot be determined

Comment: The basis for the selection of desacetylnitazoxanide as the major metabolite is not made clear by the sponsor. The spectrum of metabolites, their occurrence, activity and toxicity in dogs and rats, as well as humans, would greatly facilitate comparison of pharmacokinetic data between species

Comment: There is a considerable difference between the amount of daNTZ excreted in urine by humans (7.43% in the first 48 hours) and dogs (37.1% in 24 hours).

NONCLINICAL TOXICOLOGY STUDIES
Toxicity studies summary:
A. Subacute toxicity studies
A1. 28-day toxicity study in rats (Romark Laboratories, Tampa, FL; performed by — — lot # NTZAPURE-002; 4/27/95; glp)

B. Acute Studies
B1. Acute intraperitoneal study in mice (— — ; batch #1or — No. 2970; 12/78; not glp)
B2. Acute oral toxicity study in mice (— — , batch #1or — No. 2970;12/78; not glp)
B3. Acute intraperitoneal study in rats (— — ; batch PCA. 101 or — No. 862; 2/15/77; not glp)
B4. Acute oral toxicity study in rats. (— — batch #1or — No. 2970;12/78; not glp)
B5. Acute oral toxicity in cats (— — , batch # PCA. 101 or — No. 862; 5/2/77; not glp)
B6. Acute oral toxicity study in dogs (— — ; batch PCA. 101 or — No. 862; 5/2/77; not glp)
B7. Acute dermal toxicity study in rabbits — — ; batch — No. 2970; 1/79; not glp)
B8. Primary eye irritation/corrosiveness study in rabbits — — , batch — No. 2970; 1/79; not glp)

C. Repeat dose studies

C1. 8-day pilot oral toxicity study of NTZ in rats (— — laboratory project identification: — \ 2647-111; 11/23/92; glp; drug lot# C6682)
C2. 14-week oral toxicity study in the rat (' — — , batch PCA. 101; 5/78; not glp)
C3. Rising dose tolerance study via oral administration of NTZ in dogs (— — project identification; — 2647-112; glp; lot # C6682; 11/23/92)

C4. 28-day oral toxicity study of NTZ in dogs (— — project identification; — .2647-112; glp; lot # NTZAPURE-002; study # 2647-114; 12/20/93)

C5. Dose range-finding study for segment II study in rats with NTZ (— — ; project identification; — 2647-117; glp; lot# 5186; 4/1/93)

C6. Dose range-finding study for segment II study in rabbits with NTZ (— — project # — 2647-118; glp; lot# 5186; 12/15/93)

Special Studies

D1. Mutagenicity test on NTZ in vivo mammalian micronucleus assay (— — study no. 15356-0-455CO, assay)

D2. Study of potential mutagenic effects of NTZ using the Ames test with and without metabolic activation

Toxicity studies review:

A. Subacute toxicity studies

A1. 28-day toxicity study in rats. CD rats were dosed p.o. with vehicle (0.5% methylcellulose) or NTZ at daily doses of 800, 2400, or 4800 mg/kg (days 1-8) and 400, 1200, and 2400 mg/kg (days 9-28), due to the toxicity observed early in the study. Rats in these dosing groups were divided into the main study (10♂, 10♀/group) and satellite groups (6♂, 6♀/group) for plasma collection. Animals were observed twice daily for mortality and morbidity. Physical examination was performed prior to each dosing and at each weighing. Examination for indications of toxic and pharmacologic effects was performed daily 1-2 hours post dose. Body weights were recorded at randomization, prior to dosing on day 1, and then weekly. Food consumption was determined weekly. indirect ophthalmoscopic examination was performed prior to treatment and on day 28 (main study rats only). Blood and urine were collected during week 5 after fasting for hematology, serum chemistry and urinalysis. Satellite rats were used for collection of plasma for drug level determinations, 3/sex/group on day 1/2 and the remainder on day 28/29 following fasting. At necropsy, gross pathology was performed and tissues preserved for histopathology. Deaths during study : 5 high dose rats: one study male (day 8), decreased food consumption in week 1; two satellite males (day 9), one with hypoactivity for two days prior to death; all three males lost weight prior to death, which appears related to NTZ toxicity; and two satellite females (day 29), with bright yellow urine, their deaths related to fasting and blood collection combined with dosing on the day prior to death. The following were observed during the study: all treatment groups: urine discoloration (earlier, more frequent in males), decreased body weight and food consumption (males >females); decreased thymus weight (males); 2400 and 4800mg/kg: increased spleen weights, increased pigment in spleen, increased sinusoidal cell pigment of the liver, increased liver weights (females), decreased heart weights (males); 4800 mg/kg: slight centrilobular hepatocytic hypertrophy.

The NOEL level for oral NTZ in rats was determined to be 800 mg/kg for the first 8 days and 400 mg/kg for the following 20 days. When the 28-day dose is corrected for surface area, the safe human dose is 63.5mg/kg (400/6.3 = 63.5), which exceeds the doses to be used in the clinical study (maximum dose=28 mg/kg) with an adequate safety margin.

B. Acute Studies

B1. Acute intraperitoneal study in mice. CD-1 mice (5 ♂,5 ♀/group) were dosed i.p. with either vehicle (2% gum tragacanth) or NTZ at single doses of 46.4, 68.1, 100, 147, or 215 mg/kg. Mice were observed frequently following dosing and then daily. Body weights were recorded at day 0, 7, and 14. Gross pathology was performed at necropsy. During the study, weakness and decreased activity were observed. At necropsy at either death or day 14, apparent drug material was found in the abdominal cavity of dead mice and pale kidneys observed in one male survivor in the 147 mg/kg group. The acute intraperitoneal LD_{50} was determined to be 105 mg/kg for both sexes.

B2. Acute oral toxicity study in mice. CD-1 mice (5 ♂,5 ♀/group) were dosed p.o. with either vehicle (2% gum tragacanth) or NTZ at single doses of 625, 1250, 2500, 5000, or 10000 mg/kg. Mice were observed frequently following dosing and then daily. Body weights were recorded at day 0, 7, and 14. Gross pathology was performed at necropsy. During the study, weakness and decreased activity were observed. Necropsy at death showed apparent drug material or yellow fluid was found in the intestines and stomach. No clinical signs were reported at day 14 necropsy. The acute oral LD_{50} was determined to be 1350mg/kg for male mice and 1380mg/kg for female mice.

B3. Acute intraperitoneal study in rats. Acute intraperitoneal study in rats. CD rats (5 ♂,5 ♀/group) were intraperitoneally dosed with either vehicle (1%Tween 80) or NTZ at single doses of 100, 147, 215, or 316 mg/kg. Rats were observed frequently following dosing and then daily. Body weights were recorded at day 0, 7, and 14. Gross pathology was performed at necropsy. During the study rapid respiration was observed after dosing as well as bright yellow urine. Restlessness, distress and decreased activity followed. Necropsy findings at death included congested lungs, dark liver, compound in abdominal cavity, and viscera stained with compound. The acute intraperitoneal LD_{50} was determined to be 192 mg/kg for male rats and 165 mg/kg for female rats.

B4. Acute oral toxicity study in rats. CD rats (5 ♂,5 ♀/group) were orally dosed either with vehicle (2% gum tragacanth) or NTZ at single doses of 625, 1250, 2500, 5000, 10000 mg/kg. Rats were observed frequently following dosing and then daily. Body weights were recorded at day 0, 7, and 14. Gross pathology was performed at necropsy. During the study clinical signs included decrease activity and yellow urine stains during the first 2-3 days post dosing. Among rats that died, one rat had yellow material in the intestine. Necropsy findings in survivors were normal. The acute oral LD_{50} was determined to be greater than 10000mg/kg for both male and female rats.

B5. Acute oral toxicity study in cats. Male and female domestic cats were dosed p.o. with NTZ in gelatin capsules at single doses of 1 (1♂,1♀), 5 (2♂,2♀), and 10(2♂, 2♀) g/kg. Cats were observed for several days before dosing, frequently following dosing and then daily. Body temperature, integrity of reflexes, respiration and heart rate were recorded at day 0 (1 to 3 hours following dosing until fluctuations ceased), 7, and 14. Body weights were recorded at day 0 , 7, and 14. Cats were observed frequently following dosing and then daily. Gross pathology was performed at necropsy. Gross pathology was performed at necropsy. All cats survived 14 days after treatment. During the study body temperature in the 10 g/kg group had a decreasing trend. All cats in this group also had diarrhea after

treatment, with decreased activity and depression lasting up to 48 hours. In the 5 g/kg group the central nervous system depression was lessened and did not exceed 24 hours. Diarrhea also occurred, lasting up to 10 days after treatment. Slight diarrhea was observed in both cats in the 1 g/kg group up to 36 hours after dosing. All cats survived until necropsy. At necropsy, yellow tinting of the renal cortex was seen in the 5 and 10 g/kg groups. The lethal oral dose in cats was greater than 10 g/kg.

B6. Acute oral toxicity study in dogs. Male and female beagle dogs were dosed p.o. with NTZ in gelatin capsules at single doses of 1(1♂), 5(1♂), or 10(1♂, 1♀) g/kg. food and water consumption, fecal consistency, emesis, general appearance and behavior were observed for several days prior to treatment and daily after treatment. Body weights were recorded on days 7 and 14. Body temperature, integrity of reflexes, respiration and heart rate were recorded at day 0(1 to 3 hours following dosing until fluctuations ceased), 7, and 14. During the study, emesis occurred following dosing in all groups, as well as yellow coat staining. Diarrhea was observed in the high dose group. Two dogs, one in the middle and one in the high dose groups showed no pupillary reflex from 3 to 24 hours after dosing. All dogs survived until necropsy. At necropsy, distended gall bladders were seen in all dogs. The lethal oral dose of NTZ in dogs was greater than 10 g/kg.

Comment: It is difficult to evaluate the results in a study which only contains 3 males and 1 female in 3 dose groups with no concurrent vehicle control.

B7. Acute dermal toxicity study in rabbits. Eight male rabbits were clipped and four had their exposed skin abraded. A single 3g/kg dose, consisting of NTZ mixed as a paste with deionized water, was applied. After application, the site was covered for 24 hours. The covering was then removed and the site cleaned. All rabbits survived for 14 days until necropsy. At necropsy, liver masses were found in 3 rabbits. No dermal response to NTZ, such as irritation, was observed .
The dermal LD_{50} in rabbits was greater than 3 g/kg.

B8. Primary eye irritation/corrosiveness study in rabbits. Six female rabbits were treated in one eye with 100 mg NTZ and monitored for 7 days for eye irritation. No rabbit had more than minimal and transient eye irritation relative to that seen in the untreated eye.

C. Repeat dose studies

C1. 8-day pilot oral toxicity study of NTZ in rats. CD rats (5♂, 5♀/group) were dosed p.o. with either vehicle (0.5% methyl cellulose) or NTZ at doses of 200, 800, or 3200 mg/kg for 8 days. Animals were observed twice daily for mortality and morbidity. Examination for indications of toxic and pharmacologic effects was performed daily 1-2 hours post dose. Body weights were recorded at randomization and prior to treatment on days 1 and 8. Physical examinations were performed on days 1 and 8. For plasma drug levels, blood was taken following fasting on day 8. One rat/sex/group was bled at 15, 30, 60, 120 and 240 minutes post dose. Drug level results will be presented in the final report. At necropsy gross pathology was performed and tissues sampled for histopathology. No effects on body weight or clinical signs were seen. One female rat in the 800 mg/kg group was found dead due to intubation error, with necropsy findings including perforated esophagus and apparent drug material in the thoracic cavity. The day 8 necropsies were

unremarkable.

C2. 14-week oral toxicity study in the rat. Sprague-Dawley rats (15 ♂, 15 ♀/group) were dosed with either vehicle (0.2% methyl cellulose) or NTZ at doses of 50, 150, or 450 mg/kg for 14 weeks. Body weight, food and water consumption, and body temperatures were determined weekly. Blood was taken at 7, 8, 13, and 14 weeks for hematology and serum chemistry. At necropsy, gross pathology was performed and tissues sampled for histopathology. Two female rats, one in the 150mg/kg and one in the 450 mg/kg dose groups, died during the study. Both were considered related to anesthesia during venipuncture. No drug related adverse affects were seen in the 50 and 150 mg/kg dose groups. Stomach ulceration was observed in the 150 and 450 mg/kg groups but it was not significant compared to ulceration in controls. In the 450 mg/kg dose group, rats displayed intense salivation, increased liver and spleen weights, and decreased thymus weights. Differences in organ weights between treated and control rats did not display any histopathological differences.

C3. Rising dose tolerance study via oral administration of NTZ in dogs. Four beagle dogs were treated with NTZ p.o. in gelatin capsules as follows: phase I-one male, one female 2000 mg/kg on day one and 4000 mg/kg day four, phase II (12 days after phase I)- dogs from phase one (1000 mg/kg) plus an additional male and female (3000 mg/kg), receiving NTZ qd for five consecutive doses. Animals were observed twice daily for mortality and morbidity. Examination for indications of toxic and pharmacologic effects was performed daily 1-2 hours post dose. Physical examinations, including body weights, were performed on days 1 and 4 (phase 1) and days 1 and 3 (phase 2). Blood was sampled on days 1 and 5 (phase II), for drug levels, to be reported in final report. At necropsy, gross pathology was performed and tissues samples for histopathology taken. In phase I, occasional emesis was observed. In phase II, emesis, salivation, lacrimation, and/or mucoid feces, and diarrhea were observed. NTZ did not appear to exhibit toxicity in dogs at 1000 and 3000 mg/kg.

C4. 28-day oral toxicity study of NTZ in dogs. Beagle dogs (3 ♂, 3 ♀/group) were dosed with NTZ p.o. in gelatin capsules in groups consisting of controls, 300, 900, and 2700 (days 1-10, males and 1-9 females, followed by 1800 mg/kg day 15 to end). Animals were observed twice daily for mortality and morbidity. Examination for indications of toxic and pharmacologic effects was performed daily 1-2 hours post dose. Detailed clinical observations were made at weighing (days -1, 8♂/7♀, 13♂/12♀, 15, 22, 29) including body weight, food consumption (week 1 only), ophthalmoscopic examination (prior to treatment and 28♂/27♀). Urine and blood were collected on days 10♂/9♀ and 29 for hematology, serum chemistry and urinalysis. Plasma for drug level determinations was collected prior to dosing and at 1,4,8, and 24 hours post dosing and day 28. See "A2. Determination of desacetylnitazoxanide in dog and rat plasma samples collected during Romark laboratories studies — 3.2647114 and 272100" for results. At sacrifice gross pathology observations and organ weights recorded and tissues for histopathology taken. One male in the high dose group was sacrificed for humane reasons on day 23 following loss of weight, hypoactivity, diarrhea, dehydration, and anorexia. Necropsy showed decreased organ weights, dark ares in the jejunum, colon, ileum; dark mesenteric and external iliac lymph nodes; small testes and epididymides; moderate regenerative anemia, marked thrombosis, mild leukocytosis,

mild hypoglycemia; decreased alkaline phosphatase, alanine amino transferase, sodium chloride, and calcium; elevated triglycerides, lactate dehydrogenase, and potassium; and panhypoproteinemia.
All other dogs survived until sacrifice at the end of the study. In the first week of the study, the condition of the dogs deteriorated such that dosing was suspended in the 2700 mg/kg group at day 11(male) and 10 (female) and resumed on day 15 at a reduced dose of 1800 mg/kg. Effects seen during the study included: 300 mg/kg group: weight loss; decreased erythrocytes, hemoglobin, hematocrit, alkaline phosphatase (males day 10), alanine amino transferase (females day 29), gamma glutamyltransferase (females day 29), total serum protein (males day 10, females day 9), albumin (males day 10, females day 9, both day 29), globulin (females day 9), cholesterol (males day 10); increased reticulocytes in males, triglycerides in females day 9; 900 mg/kg: weight loss, decreased food consumption (males week 1), erythrocytes, hematocrit, hemoglobin, alkaline phosphatase (males day 10), alanine amino transferase (females day 9, all day 29), gamma glutamyltransferase (females day 29), total serum protein (males day 10, females day 9, all day 29), albumin (males day 10, females day 9, both day 29), globulin (males day 10, females day 9), albumin/globulin (males day 29), cholesterol (males day 10); increased triglycerides in females day 9; and 2700/1800 mg/kg: weight loss, decreased food consumption (males week 1), erythrocytes, hematocrit, hemoglobin (females), prothrombin time(males), alkaline phosphatase (males day 10, females day 9), alanine amino transferase (females day 9, all day 29), gamma glutamyltransferase (females day 29), total serum protein (males day 10, females day 9, all day 29), albumin (males day 10, females day 9, both day 29), globulin (males day 10, females day 9), inorganic K, Na, and Cl (females day 9), inorganic Na and Cl (males day 10); increased triglycerides in females day 9.
All treated dogs had increased occult blood in urine on days 10 and 29. Treated males had decreased brain, lung, kidney, heart, spleen, thymus, liver and /or testes weights relative to control values. In treated females, lung and thymus weights were decreased; in the 2700/1800 mg/kg group, females had decreased brain, kidney, and heart weights. Treated females had increased liver and adrenal weights. Histopathologic lesions in the 900 and 2700/1800 mg/kg groups included cystic hyperplasia of the gall bladder, lymphoid depletion of the thymus, ulceration/inflammation of the cecum, and/or degeneration of skeletal muscle.
The NOEL was apparently below the lowest dose in the study, 300 mg/kg.

C5. Dose range-finding study for segment II study in rats with NTZ. Female CD rats (6/group) were mated with untreated males. Females were sacrificed on day 20 of presumed gestation and maternal and fetal tissues obtained. Females were treated with either vehicle (0.5% methylcellulose) or NTZ (200, 800, or 3200 mg/kg) on days 6 to 15 of gestation. Animals were observed twice daily for mortality and morbidity. Each fetus was sexed, weighed and examined externally for abnormalities. All females survived treatment. Bright yellow urine was observed after dosing until day 18 in the 800 and 3200 mg/kg groups. Maternal body weights trended lower in the 3200 mg/kg group relative to control group weights, but the difference was not significant. The number of total resorptions was higher, but not significantly, in the 3200 mg/kg group. Fetal effects included one 200 mg/kg female fetus with exencephaly, cleft palate and rachischisis, and one 3200 mg/kg female fetus with an umbilical hernia. These were considered isolated instances and not drug related. No other fetal effects of NTZ treatment were noted. A

NOEL for NTZ in pregnant rats appears greater than 3200mg/kg.

C6. Dose range-finding study for segment II study in rabbits with NTZ. A preliminary study in two nonpregnant female rabbits dosed for three consecutive days at 3200 mg/kg showed marked decreases in body weight, food consumption and water intake with effects on food consumption continuing after cessation of dosing. These findings led to a choice of 1800 mg/kg as a high dose. Female rabbits (6/group) were treated by oral gavage with either vehicle (0.5% methylcellulose) or NTZ (100, 300, or 900 mg/kg) on days 7 to 19 of gestation and a high dose group (1800 mg/kg) on days 7-13. Animals were observed twice daily for mortality, morbidity and signs of inappetence. Each fetus was sexed, weighed and examined externally for abnormalities. One doe (900 mg/kg) was found dead on day 18 and three does (1800 mg/kg) on day 13.
Maternal toxicity: 100mg/kg group included one premature delivery (day 29), anorexia in two does starting on day 13, distension of GI tract; 300mg/kg group included one premature delivery (day 29), two abortions (day 24 and 28), anorexia starting on day 11, weight loss, few feces, black feces, distension of GI tract; 900 mg/kg group included four abortions, (day 19, day 23, two on day 25), anorexia starting on day 10, weight loss, few feces, black feces, distension of GI tract; 1800mg/kg group included three abortions (two day 21 and day 23) anorexia starting on day 9, weight loss, distension of GI tract.
NTZ dose-related fetotoxicity was evidenced by increased postimplantation loss (total, late, and early resorptions) and reduced fetal viability in 100, 300, and 900 mg/kg groups. One 100 mg/kg and one 900 mg/kg female each had no viable fetuses. Fetal weights were decreased in the 300 mg/kg group. None of the fetuses displayed any external effects of NTZ treatment.

Special Studies
D1. Mutagenicity test on NTZ in vivo mammalian micronucleus assay. ICR mice (5♂,5♀/ group/timepoint) were administered a single dose of vehicle (0.5% methylcellulose) or NTZ (250, 500, 1000 mg/kg; lot# LH#24,763) or positive control (cyclophosphamide. — , lot #70H0948, 80mg/kg in distilled water) by oral gavage. Groups of 5/sex were sacrificed at 24, 48, and 72 hr post dosing. Bone marrow was examined for the presence of micronucleated polychromatic erythrocytes. Micronuclei formation was not induced by NTZ at any of the doses examined. The cyclophosphamide control was positive.
D2. Study of potential mutagenic effects of NTZ using the Ames test with and without metabolic activation. The study presents the conclusion that NTZ lacks mutagenic potential with or without metabolic activation but provides the study in French, not English.

NONCLINICAL PHARMACOLOGY STUDIES
Assay of nitazoxanide for general pharmacological activity —
— , 10/9/92; lot # 5186)
NTZ was evaluated in a variety of safety pharmacological screens. These included CNS effects in mice (reflex inhibition, behavioral depression, muscle relaxation, catalepsy, antipentylenetetrazol convulsive activity, anti-electroshock, motor activity, analgesia, tetrabenazine antagonism), acute toxicity in mice and rats, antiinflammatory activity (carrageenan rat paw edema, arachidonic acid ear model, rat gait and antipyretic activity), metabolic assays (cholesterol, glucose tolerance), gastrointestinal (rat antisecretory activity, rat anti-ulcer activity), antimicrobial, cardiovascular (direct blood pressure, heart

rate, anti-arrhythmia, volume diuresis), immunology (immunosuppression, immunoenhancement), antagonism studies (in vitro; ileum: angiotensin II, calcium, leukotriene D4, substance P, acetylcholine, beta (isoproterenol); trachea: bronchodilator (beta II and histamine), guinea pig atrium contractile force; in vivo: platelet activating factor, histamine). In vivo doses were 250 (immunology), 500 or 1000 mg/kg, i.p.; in vitro concentrations ranged from 10µg/ml (antagonism studies) to 20 µg/ml (antimicrobial assays). In vivo activity detected included analgesia (500 mg/kg) in the PPQ writhing assay, mortality in rats (1/3) and mice (1/3) at 1000mg/kg, antiinflammatory activity (500 mg/kg), slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity (500 mg/kg) and slight histamine activity; in vitro activity included slight antimicrobial activity against S. aureus and P. vulgaris, some antagonism against angiotensin II, leukotriene D4, substance P and acetylcholine. The gastrointestinal effects are of interest due to toxicity studies which demonstrate some bowel effects as well.

CLINICAL PROTOCOL: Patients (inclusion: HIV+, CD4 ≤200cells/mm^3, cryptosporidial diarrhea defined by oocysts in stool and chronic diarrhea, stable dose regimens for antidiarrheals and antiretrovirals, 18-65 y.o., male or female (negative pregnancy test 14 days prior to study, abstinence or birth control), life expectancy ≥ 1 month, and ability tolerate food by mouth; exclusion: presence of Salmonella, Shigella, Campylobactor, Yersinia, Giardia lamblia, Entamoeba histolytica, Microsporidia, Isospora, Cyclospora, or Clostridium difficle toxin in stool, Mycobacterium avium infection, intestinal Kaposi's sarcoma, Cytomeglaovirus colitis (unless 28 days therapy with gancyclovir or foscarnet completed, any investigational drug within 14 days, use of any possibly anticryptosporidial drug within 14 days, any treatment/prophylaxis for opportunistic infection without stable dosing regimen, or grade 3-4 toxicity) will be placed in groups of seven starting in the lowest dose group,500 mg, and filling the remaining groups sequentially (1000 (500 mg bid), 1500(500 mg tid), and 2000 (1000 mg bid) mg/kg/day) for 14 days treatment. Following the initial dose, single dose pharmacokinetics will be determined by patient plasma samples at -15 min, 0, 15, 30, 45, 60, 75, and 90 min, 2, 3, 4, 6, 8, 12, and 24 hours. During treatment, patients will maintain a daily diary documenting bowel movement characteristics and antidiarrheal use. Weekly patient evaluation including body weight, Karnofsky status, review of adverse events, concomitant medications, hematology, biochemistry, urinalysis, plasma and stool collection will measure pharmacokinetic and anticryptosporidial activity. Evaluation at day 15 will be used to determine response for a further 14 days of extended therapy to non- and partial responders. Patients exhibiting a complete clinical and parasitological response will discontinue therapy. Clinical and parasitological evaluations of patients will
be performed on day 29.

SUMMARY:

Toxicity: The GI tract was the major organ system affected by NTZ treatment as particularly seen in the dog studies, which were performed at relatively high doses. This may be due to irritant properties of daNTZ, a salicylate-like compound. Toxicity was not seen in the rat studies.

The 8 day rat NOEL > 3200 mg, 28 day rat toxicity NOEL 400 mg/kg (equivalent to 64 mg/kg human dose), 14 week rat NOEL at 150 mg/kg (equivalent to 24 mg/kg human dose), and the 28 day dog NOEL was not determined (<300 mg/kg- lowest dose).

Kinetics: A Cmax for daNTZ of 1-3 mg/L and AUC of 7-20mg/L was found in dogs receiving NTZ (900 and 2700/1800 mg/kg, qd) for 28 days. daNTZ levels from a daily oral dose of 900 mg/kg for 28 days in dogs produced plasma levels comparable to human plasma levels from a single oral dose of 7 mg/kg. Differences in metabolism make this similarity difficult to interpret for toxokinetic purposes considering the differences in dose and apparent half- life. Deactylation is presented as the main route of elimination without sporting studies. Relative activity of the parent compound and this major (?) metabolite are not provided.

Reproductive risks: The preliminary reproductive toxicity studies in rats and rabbits indicated fetotoxicity as postimplantation loss and reduced fetal viability. These two reproductive toxicity studies were dose ranging studies, and not definitive. Maternal toxicity in the rabbit study confounds determination of fetotoxicity at doses ≥100 mg/kg.

Mutagenicity: NTZ was not mutagenic in either the Ames test (with and without metabolic activation, data not provided in English) or the mammalian micronucleus test.

EVALUATION AND CONCLUSIONS:

The protocol as designed is safe to initiate. Animal studies (rats) support clinical dosing of up to 14 weeks duration at the doses currently proposed and up to 28 day usage in nonresponding patients. The clinical protocol will employ a range of 7 to 28 mg/kg for 14 days and potentially up to 28 days duration, which is 2-9 fold less than the NOEL seen in rats treated for 28 days. Previous clinical studies have used doses from 7 to 45 mg/kg safely.

Although there was some toxicity at all doses tested for 28 days in dogs and thus no NOEL, the lowest dose, 300 mg/kg, is well in excess of the highest clinical dose when corrected for surface area (300/ 1.85 =177).

REQUESTS

The following are requested of the sponsor:

The section "Study of potential mutagenic effects of nitazoxanide using the Ames test with and without metabolic activation" should be provided in English instead of French. Antibacterial activity of nitazoxanide should be evaluated against Salmonella typhimurium to ensure a negative Ames result is not due to antibacterial activity.

The 28-day oral toxicity study of nitazoxanide in dogs should be repeated using lower doses (50, 100, 200, mg/kg) to obtain a NOEL. This would also permit comparison of doses comparable to the highest dose used in the clinical protocol.

Reproductive studies in rabbits should be performed, at some time in drug development, at lower doses (25, 50, 100 mg/kg), with at least 8 rabbits per dose group, to avoid the maternal toxicity seen in the segment II study. Maternal toxicity confounds any observed fetotoxicity. Lower doses would permit observation of fetotoxicity in unaffected dams.

To facilitate comparison of dog and human kinetic studies, an ADME study of ^{14}C-nitazoxanide in the dog, with determination of specific metabolites, should be performed. Plasma desacetylnitazoxanide, as well as parent compound, should be determined, with metabolism profiles from liver extracts for dogs and humans. Determination of plasma protein binding in dogs would also be useful for overall understanding of species differences of nitazoxanide toxokinetics.

/S/

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

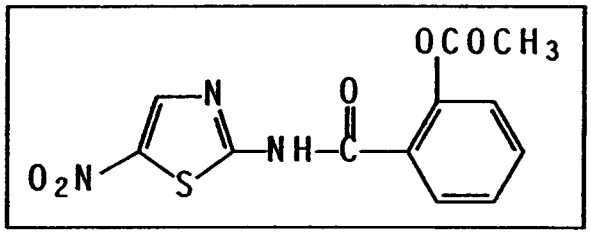
concurrences:
HFD-530/ADir/DFreeman
HFD-530/SPharm/JFarrelly
Steven C. Kunder/Pharm/

disk:
HFD-530/JFarrelly
cc:
HFD-530 (original)
HFD-530 Division file
HFD-340
HFD-530/MTarosky
HFD-530/DLePay
HFD-530/DBoring
HFD-530/SBala
HFD-345/

PHARMACOLOGIST'S REVIEW

IND # —

DATE SUBMITTED: 21 March 1996
DATE RECEIVED: 27 March 1996
DATE ASSIGNED: 2 April 1996
DATE REVIEW COMPLETED: 24 April 1996
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide
RELATED DOCUMENTS: IND [illegible]



INDICATION: Cryptosporidiosis in AIDS patients
DEFINITIONS
NTZ=nitazoxanide

INTRODUCTION:
This submission from Unimed Pharmaceuticals, Inc. is a response to the review of the original submission. The pharmacology section of this submission addresses requests for (1) an English translation of the original Ames test, which was in French, and evidence of antibacterial activity of NTZ against Salmonella typhimurium ; (2) 28-day oral toxicity study in dogs repeated at lower doses (50, 100, 200 mg/kg) to obtain a NOEL; (3) a reproductive study in rabbits at lower doses (25, 50, 100 mg/kg) to avoid maternal toxicity seen in the previous segment II study at higher doses.; and (4) ADME studies with ^{14}C-NTZ in dogs.
In response, the sponsor submitted: (1) the English language version of the Ames test and stated that the Ames test would be repeated with both NTZ and desacetyl-NTZ at a US laboratory; (2) stated that the oral toxicity study in dogs will be repeated at the recommended doses; (3) stated that the reproductive study in rabbits will be repeated at the recommended doses; and (4) stated that an ADME study of ^{14}C-NTZ will be conducted in humans.

GENETIC TOXICOLOGY:

Study of potential mutagenic effects of nitazoxanide using the Ames test with and without metabolic activation; performed by —
— ; 5 March 1982; no GLP statement

Five strains of Salmonella typhimurium were used in this study to detect mutations caused by either frameshift or substitution mutations. Concentrations of NTZ used were 2.5, 1.0, 0.5, 0.1, and 0.01 mg/ml. Concentrations above 2.5 mg/ml were

bactericidal. NTZ and positive controls (N-methyl-N'-nitro-nitrosoguanidine, 9-amino-acridine, 2-nitrofluorene, methylmethane sulfonate, 2-aminofluorene, and aflatoxin B1) were solubilized with DMSO, except 9-amino-acridine (ethyl alcohol). Assays were performed with and without addition of Araclor 1254 activated rat microsomal preparation, in duplicate. NTZ did not show mutagenic activity with or without metabolic activation at maximum concentration (2.5 mg/ml) similar to human doses (2000 mg/kg/day); positive controls were effective.

CONCLUSIONS:
The studies contained in this submission appear acceptable with respect to pharmacology and do not have any immediate bearing on clinical development of NTZ. No regulatory action is needed.

/S/

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:
HFD-530/ADir/DFreeman
HFD-530/SPharm/JFarrelly
Steven C. Kunder/Pharm/

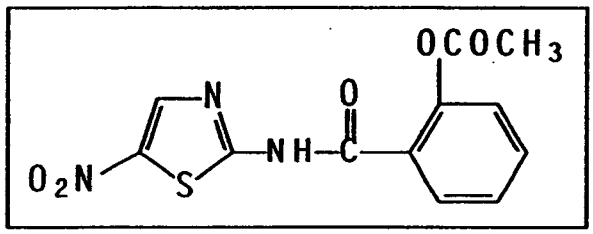
disk:
HFD-530/JFarrelly
cc:
HFD-530 (original)
HFD-530 Division file
HFD-340
HFD-530/MTarosky
HFD-530/DLePay
HFD-530/DBoring
HFD-530/SBala
HFD-345/

PHARMACOLOGIST'S REVIEW

IND # —

DATE SUBMITTED: 6 Mar 1997
DATE RECEIVED: 7 Mar 1997
DATE ASSIGNED:10 Mar 1997
DATE REVIEW COMPLETED: 9 June 1998
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide
HFD-590

INDICATION: Cryptosporidiosis in AIDS patients



DEFINITIONS
NTZ=nitazoxanide

INTRODUCTION
NTZ is indicated for cryptosporidiosis in AIDS patients. This submission included studies of oral toxicity in dogs, developmental toxicity in rabbits, and fertility and developmental effects in rats.

PHARMACOKINETICS/TOXICOKINETICS
90-day toxicity study in dogs with nitazoxanide, performed by — — , report # — 2712-103; batch no. 002; 16 December 1996; GLP

Male and female beagle dogs — , aged 4-6 months, were assigned, 4/sex/dose, to dose groups of 0, 25, 50 and 100 mg/kg/day in gelatin capsules by oral administration for 90 days. Prior to dosing, dogs were given opthalmoscopic examinations, and body weight recorded. Dogs were dosed daily, with observations made prior to, and 1 to 2 hours post dose. Clinical pathology samples (blood and urine) were collected during weeks -2, 6 and 13. Dogs were fasted overnight prior to collection. The tables below list the tissues collected/samples and the assays performed.

Clinical chemistry

red blood cell count	white blood cell count
hematocrit	differential blood cell count
hemoglobin	nucleated red blood cell count
mean corpuscular volume	corrected white blood cell count
mean corpuscular hemoglobin	segmented neutrophil count

mean corpuscular hemoglobin concentration
platelet count
prothrombin time
activated partial thromboplastin time
blood cell morphology
band neutrophil count
lymphocyte count
monocyte count
eosinophil count
basophil count

clinical chemistry

glucose
urea nitrogen
creatinine
total protein
albumin
globulin
albumin/globulin ratio
total bilirubin
cholesterol
triglycerides
aspartate aminotransferase
alanine aminotransferase
alkaline phosphatase
gamma-glutamyl transferase
lactate dehydrogenase
calcium
inorganic phosphorus
sodium
potassium
chloride

urinalysis

volume
specific gravity
pH
protein
glucose
ketones
bilirubin
blood
urobilinogen
microscopic examination of sediment
appearance

At necropsy, macroscopic examination of the external body surface was performed prior to sacrifice. Organ weights were recorded for:

adrenals
brain
epidydimides
heart
kidneys
liver with gallbladder
lungs
ovaries
spleen
testes
thymus
thyroid

The following tissues were collected for histopathology:

adrenals
aorta
axillary lymph node
brain with brainstem
cecum
cervix
colon
duodenum
epididymides
mammary glands (females)
muscle optic nerve
ovaries/testes
pancreas
pituitary
prostate
rectum
salivary glands
sciatic nerve

esophagus
eyes
femur
gallbladder
heart
illeum
illiac lymph node
jejunum
kidneys
liver
lungs with bronchi
lymph nodes (mandibular and mesenteric)
skin
spinal cord
spleen
sternum
stomach
thymus
thyroid
tongue
trachea
urinary bladder
uterus
vagina

Plasma samples for drug level determination were collected from all dogs during the study on day 1 and once during week 13 prior to dosing and at 1, 4, 8 and 24 hours post dosing. Dogs were not fasted prior to collection. During the study, one high dose male was sacrificed due to moribund condition on day 22. All other dogs survived until scheduled sacrifice. During the study, treated dogs in all groups had few, non-formed, liquid, mucoid, discolored (yellow, red or black) feces and discolored (red, orange, yellow/green or black) urine. In the 50 and 100 mg/kg/day groups dogs had incidences of yellow discoloration of fur. Excess salivation was seen in the 100 mg/kg/day group. Body weight was decreased in the 100 mg/kg/day group throughout the study, with significant reductions among males in weeks 4, 5 and 6 and females in weeks 2 through 14. Dogs in the 25 and 50 mg/kg groups also had reduced body weights (relative to control dogs) with significant reductions in the 50 mg/kg/day females in weeks 4, 5, 7, 8, 10 and 14. Food consumption was decreased for middle and high dose dogs with significant decreases in weeks 1 through 7. Opthalmic examinations were not affected by NTZ treatment. Clinical chemistry observations included lower total protein, albumin, calcium, alanine aminotransferase, and mean corpuscular hemoglobin concentration for males and females at all dose levels; lower hemoglobin and hematocrit and higher platelet count for males in the 50 and 100 mg/kg/day groups and females in the 100 mg/kg/day group. Hemorrhage in the mesenteric lymph nodes was seen at all dose levels which supports the above signs of blood loss. Lower mean corpuscular volume and mean corpuscular hemoglobin for males was seen in males at 50 and 100 mg/kg/day, and higher triglycerides in males and females at 100 mg/kg/day. Testicular immaturity and significantly decreased testicular weights were seen in males at all dose levels. At 100 mg/kg/day additional histopathological signs included vacuolation of the neutropil of the brain (minimal in males and minimal to slight in females). The NOAEL for this study was less than 25 mg/kg/day, or 15 mg/kg/day human equivalent dose. The sponsor believes that the known dog sensitivity to salicylates may account for the toxicity seen in this study, as NTZ is the acetylanilide of the aminothiazole ring. Human doses range from approximately 33 to 50mg/kg/day

REPRODUCTIVE TOXICOLOGY STUDIES

Rabbit developmental toxicity study with nitazoxanide; performed by [redacted] study no. [redacted] 2712-102; batch no. 002; 19 Nov 1997; GLP

In this study, mated female rabbits (Hra:(NZW) SPF, [redacted] approximately 5 months old, were assigned, 20/group, to dose groups consisting of vehicle control, 25, 50 and 100 mg/kg/day. Rabbits were dosed by oral gavage on days 7-20 of gestation. Rabbits were observed for mortality and morbidity twice daily. Body weights were measured on gestation day 0, 4, 7, 9, 11, 15, 18, 21 and 29. Food consumption was measured along with body weight from day 4. On day 29, rabbits were euthanized and examined grossly for thoracic, abdominal, and pelvic visceral abnormalities. The uterus of each gravid female was then removed, weighed and examined for number and placement of implantation sites, live and dead fetuses, early and late resorptions, and abnormalities of the uterus or embryonic sacs. Ovaries were examined for the number of corpora lutea. The fetuses were then removed, weighed, examined for external abnormalities, and euthanized. Soft tissue examination of each fetus was performed with a modified Staples technique with sex determination. Cranial examination followed with evisceration and clearing for skeletal examination. Skeletal findings were determined to be variations or malformations. During the study, one dam each from the control, mid, and high dose groups died. The control death was attributed to a gavage accident. Following necropsy, the mid dose female was found to have a mottled liver and distended stomach; the high dose female had no remarkable observations. All other rabbits survived until sacrifice. Decreased feces and bright yellow urine were seen in high dose rabbits. Body weights were generally similar throughout the study. The only significant differences were in body weight changes where mid dose rabbits were higher than that of controls on days 9-11 and lower for high dose rabbits relative to control weight changes on days 15-18. Food consumption was similar for all groups through day 7. Mid and high dose rabbits had significantly lower consumption than controls on days 15-18. Pregnancy rates for control, low, middle, and high dose groups were 90, 85, 95, and 90%, respectively. The mean numbers of corpora lutea and implantation sites were similar across the control and treatment groups. No dead fetuses were seen in any group. Fetal statistics, including means of total, early, late resorptions, number of live fetuses, and covariate-adjusted fetal weights, were unaffected by drug treatment. No external fetal abnormalities were seen. Fetal soft tissue abnormalities included internal hydrocephaly in one high dose fetus and hepatomegaly in one middle dose fetus. The NOAEL for this study was 50 mg/kg/day for the pregnant female rabbits and 25 mg/kg for the fetal rabbits. These are approximately equivalent to human doses of 14 and 7 mg/kg/day, respectively. Human clinical doses range from approximately 33-50 mg/kg/day.

Study for the effects of fertility and development in rats with nitazoxanide, performed by [illegible] study no. [illegible] 2712-104; batch no. 002; 16 December 1996 (draft date); GLP

Male and female Sprague-Dawley rats ([illegible], 25/sex/group) were assigned to dose groups receiving 0, 300, 600, 1200, or 2400 mg/kg/day (groups 1-5) by oral gavage. Methylcellulose (0.5%) was used as the vehicle. Parental male rats were dosed 28 days prior to mating, and throughout mating for a total of at least 10 weeks, and females were dosed for 14 days prior to mating, through mating and through gestation day 17. Rats were monitored twice daily for morbidity and mortality. Body weights for males were obtained twice weekly during treatment and at sacrifice. Females were weighed twice weekly prior to, and during mating, and on days 0, 3, 7, 10 14, 18, and 20 of gestation. Those females which were not successfully mated continued with weekly weighings until sacrifice. Food consumption for both sexes was determined weekly prior to mating. Female food consumption was determined following mating on the corresponding body weight days. Mating was performed by the pairing of one male with one female. Females were examined daily for vaginal sperm or copulatory plug. Day 0 of gestation followed from observation of vaginal sperm or copulatory plug. Vaginal smears were made until successful mating was confirmed. Females at day 20 of gestation were sacrificed prior to cesarean section. Cervical, thoracic, and abdominal viscera were examined grossly. The uterus and ovaries were examined for implantations and corpora lutea. The uterus from each gravid female was removed, weighed, and examined for number and placement of implantation sites, live and dead fetuses, early and late resorptions, and abnormalities of the uterus or embryonic sacs. Nongravid females were sacrificed 10 days after the mating period and their viscera examined grossly. Their uteruses were examined for implantation sites. Males were sacrificed after at least 10 weeks of dosing and their viscera examined grossly. The right testis was removed and stored for possible histological examination; the left testis was fixed in Bouin's solution. The right epididymis was removed, weighed, and frozen for sperm motility and morphology evaluation. The fetuses were sexed, weighed, examined externally and sacrificed. Approximately half of each litter were randomly selected and processed for visceral examination by the Wilson technique. The remaining fetuses were eviscerated and processed for skeletal examination. Deaths during the study included two group 5 males on day 10 and two group 3 males on days 6 and 14. One of the group 5 males was observed prior to its death as being thin and pale, exhibiting hunched posture, hypoactivity, and bright yellow staining of the fur of the urogenital region. The other group 5 male also had bright yellow staining of the fur of the urogenital region prior to death. Histopathology revealed pale lungs and distended stomachs and intestines for both of these males. Of the two group 3 males, one exhibited bright yellow staining of the fur of the urogenital region and mottled lungs; the other had no remarkable signs. This staining was also seen among all drug treated rats and was presumably related to excretion of drug, which is a yellow powder.

Mating was performed within four days with nearly all females. Significant body weight changes were seen during days 0-70 in group 3-5 males, which were lower than controls. Female body weights in groups 4 and 5 were significantly reduced relative to control weights during days 0-3. During gestation, the mean total body weight changes were significantly reduced in group 3 over days 18-20, group 4 during days 3-7, and group 5 over days 0-3, 3-7, 14-18, and 18-20. Group 4 and 5 female body weights were significantly lower than those of the control group at day 20 of gestation. Food consumption was significantly decreased for males of groups 3-5 during days 0-7 and groups 4 and 5 during day 7-14 and 0-28 when compared to controls. Females in group 4 had significantly decreased food consumption during days 0-7 while group 5 was decreased over days 0-7 and 0-14 compared to control values. During gestation, food consumption of females in groups 3-5 was significantly increased over days 18-20 compared with that of controls. The last dose was on day 18; seemingly indicative of a drug-related effect on either appetite or gastrointestinal effects not conducive to eating. Fertility was not affected by drug treatment. Histopathology findings for the surviving rats were restricted to increased incidence of distended intestines in group 2-5 males. Mean epididymis weights of group 3-5 were reduced. Sperm analysis did not show any drug-related abnormalities. In group 3 and 5 female rats, gravid uterus weights were significantly reduced when compared with those of controls. Mean preimplantation loss, mean number of total, early, and late resorptions, and postimplantation loss were similar in all dose groups. Corpora lutea, implantation sites, and live fetuses trended lower in groups 3-5. Covariate weights (adjusted for litter size) for groups 4 and 5 were significantly reduced when compared with the control value. External fetal abnormalities included protruding tongue, open eyes, and exencephaly in fetus #14 and macromelia in fetus #11 in one group 5 litter; adactylly in fetus 35 from a group 4 litter; and protruding tongue and exencephaly in fetus #16 of a group 2 litter. Fetal soft tissue malformations showed an increased trend to dilation of the lateral ventricles of the brain in fetuses and litters from groups 4 and 5; renal pelvic cavitation (fetuses in groups 2, 4, and 5), and total increased soft tissue variation in group 5. Skeletal variations included significant increases relative to controls of incomplete ossification of the skull in groups 4 and 5; fewer than 4 caudal vertebrae ossified, groups 3, 4, and 5; unossified vertebral centrum, group 5; wavy/bent ribs, group 4 and 5; and total skeletal variations in groups 4 and 5.
The NOAEL for adults and fetal effects in this study was 300 mg/kg/day, an equivalent human dose of 50 mg/kg/day. Human clinical doses range from approximately 33-50 mg/kg/day.

SUMMARY:
Dogs treated for 90 days with NTZ showed toxicities including hematological effects possibly related to hemorrhage, testicular immaturity, and weight loss. Rabbits did not show significant reproductive toxicity. Rats did show soft tissue and skeletal effects as well as maternal and paternal toxicity, but no impairment of

mating, or fertility. All species showed yellow staining of fur in the urogenital area probably related to excretion of drug.

CONCLUSIONS: This submission is acceptable with respect to pharmacology.

/S/

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:
HFD-590/ADir/RAlbrecht
HFD-590/SPharm/KHastings
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disk:
HFD-590/KHastings
cc:
HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/EFrank
HFD-590/RRoca
HFD-590/GHolbert
HFD-590/SBala
HF
D-345/

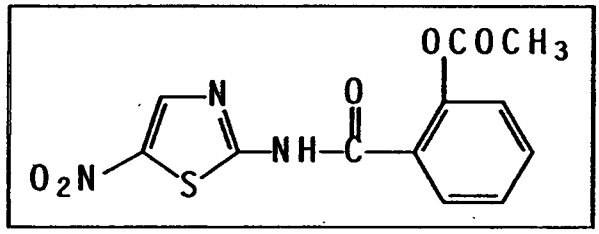
PHARMACOLOGIST'S REVIEW

IND # —

DATE SUBMITTED: 3 October 1997
DATE RECEIVED: 6 October 1997
DATE ASSIGNED: 7 October 1997
DATE REVIEW COMPLETED: 1 June 1997
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide
HFD-590

INDICATION: Cryptosporidiosis in AIDS patients

DEFINITIONS
NTZ = nitazoxanide



INTRODUCTION
The following studies are draft reports. Included are a 6-month oral rat toxicity study and a single oral dose in dogs using 14C radiolabel to estimate pharmacokinetics, routes of elimination, and tissue distribution of 14C-NTZ in dogs.

TOXICITY STUDY
6-month oral toxicity study in rats; performed by —
study no. 0460RU13.001; lot 003; 9 July 1997; GLP

Rats, male and female, (Sprague Dawley, — approximately 5 weeks old, were assigned 20/sex/group to dose groups receiving vehicle (0.5% methyl cellulose), 50 mg/kg/day, 150 mg/kg/day, and 450 mg/kg/day by oral gavage. Prior to study initiation, an opthalamoscopic examination was performed. During the study, daily monitoring of morbidity/mortality and clinical observations were performed. Weekly recordings of body weights and food consumption were made. At necropsy, the following assays were performed:

hematology	
red blood cell count	Mean corpuscular volume
Hematocrit	Platelet count
Hemoglobin	White blood cell count
Mean corpuscular hemaglobin	Differential blood cell count
Mean corpuscular hemaglobin concentration	

clinical chemistry

glucose
Urea nitrogen
Total serum protein
Albumin
Globulin
Albumin/globulin ratio
Total bilirubin
Cholesterol
aspartate aminotransferase
alanine aminotransferase
alkaline phosphatase
calcium
phosphorus
sodium
potassium
chloride

urinalysis

volume
Specific gravity
PH
Protein
Glucose
Ketones
bilirubin
blood
Nitrite
Microscopic examination
Appearance

At necropsy, macroscopic examination of the external body surface was performed prior to sacrifice. Organ weights were recorded for adrenals, brain, ovaries, heart, testes, kidneys, liver

The following tissues were collected:

Adrenals
Aorta
Brain with brainstem
Cecum
Cervix
Colon
Duodenum
Epididymides
Esophagus
Eyes + optic nerve/Harderian gland
Femur
Gallbladder
Heart
Illeum
Illiac lymph node
Jejunum
Kidneys
Liver
Lungs with bronchi
Lymph nodes (mesenteric)
Mammary glands (females)
muscle -thigh
Peripheral nerve
ovaries/testes
Pancreas
Pituitary
Prostate
Rectum
salivary glands
sciatic nerve
Skin
spinal cord
Spleen
Sternum
Stomach
Thymus
Thyroid
Tongue
Trachea
urinary bladder
Uterus
Vagina

Histopathology was evaluated on all control and high dose tissues and spleens and gross lesions from the low and mid dose groups. During treatment, one control male and one female 50 mg/kg were found dead. The control male was found to have a spontaneous renal tumor (nephroblastoma). The 50 mg/kg female death was caused by a gavage accident. Clinical signs seen during the study included yellow discharge/staining (consistent with the drug solution), and watery/soft stools in all treated groups. Incidence of soft stool was higher in males and dose related in both sexes. Body weights were significantly lower in high dose females with respect to control females on days 154, 168 and 175. High dose males had reduced weight gain in comparison with control male weight gains (on days 77 and 126, as well as high dose females on day 179) and reduced total weight gain. Food consumption was significantly reduced in high dose males on days 21 and 56 and high dose females on days 21 and 56. Opthalmoscopic exams were all normal. Hematological assays were affected by treatment in the high dose group. Significant increases in leukocytes, lymphocytes, and mean corpuscular volume and decreases in erythrocyte and hematocrit counts were seen in both males and females. Also seen in high dose females were significant increases in neutrophils and mean corpuscular volume. Clinical chemistry effects were seen in the high dose group. Males had significant decreases in urea nitrogen, sodium, total protein, and calcium and increases in albumin/globulin ratio, total bilirubin and phosphorus. Females had significantly decreased sodium and glucose and higher total bilirubin. Urinalysis findings included amber and bright yellow urine with amorphous crystals in all treated groups (consistent with the drug in solution) and stained fur. Urine specific gravity was increased in the middle and high dose groups. At necropsy no drug related gross effects were seen. Organ weights were unaffected by treatment. As a percentage of body weight, significant increases were seen in liver and testes weights in high dose males and in kidney and liver weights in high dose females. Histopathology findings included an increase in extramedullary hematopoiesis and pigment deposition in the spleens of high dose males and females. Other findings seen in the high dose group included distension of the large intestine and increased incidence of submucosal polymorphonuclear leukocytes and submucosal edema in the stomach. The NOAEL for this study was 150 mg/kg/day; the clinical dose in humans ranged from 33 to 50 mg/kg/day.

PHARMACOKINETIC STUDY

Plasma pharmacokinetics, elimination and tissue distribution of radioactivity following single oral dose administration of 14C-nitazoxanide in dogs; performed by [redacted] study no. 0831XU13.001; lot no. 003; 14C-NTZ lot no. CFO 9286; GLP; date not provided

Beagle dogs (3 males, 3 females; [redacted]) were administered single oral doses of 14C-NTZ/NTZ. The drug preparation consisted of 7.972 g NTZ and 0.036 g 14C-NTZ (specific activity 91μCi/mg) and was placed in gelatin capsules on the day of administration. A dose of 100 mg/kg (40 μCi/kg) was used. Dogs were

fasted overnight prior to dosing. Blood was sampled at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 32, and 48 h post dosing. Urine was collected from cages over time periods of 0-8, 8-24, and 24-48 h post dosing. Feces were cage-collected. Whole blood and derived plasma were assayed for total 14C-radioactivity. Urine, feces, and cage wash were assayed for 14C radioactivity. Dogs were euthanized 48h post dose and tissues removed for 14C radioactivity determination. Plasma concentrations peaked at approximately 1 to 2h following dosing. Plasma half-life ranged from — h. At 24h, feces 14C recovery ranged from 0-36%; at 48 h, 2-60%. Urine 14C recovery ranged from 0-26% at 8h, 0-17% at 24h and 0-2% at 48h. Tissue recovery was negligible.

SUMMARY: Rats dosed with NTZ for 6-months demonstrated a NOAEL of 150 mg/kg/day. The major toxicity seen was an increase in extramedullary hematopoiesis and pigment deposition in the spleens of males and females receiving 450 mg/kg/day. Dogs receiving a single dose of 14C-NTZ had negligible tissue levels and label was eliminated primarily through feces.

CONCLUSIONS: This submission is acceptable with respect to pharmacology. Upon receipt of the final copies, a comparison will be made and any changes/discrepancies noted.

/S/

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:
HFD-590/ADir/RAlbrecht
HFD-590/SPharm/KHastings
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disk:
HFD-590/KHastings
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HFD-590 (original)
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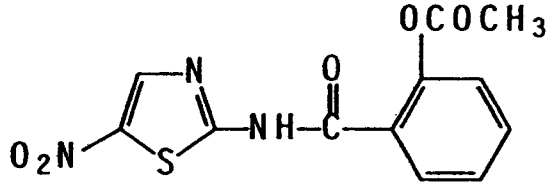
HFD-340
HFD-590/EFrank
HFD-590/RRoca
HFD-590/GHolbert
HFD-590/SBala
HFD-345/

PHARMACOLOGIST'S REVIEW

IND # —

DATE SUBMITTED: 20 November 1997
DATE RECEIVED: 24 November 1997
DATE ASSIGNED: 25 November 1997
DATE REVIEW COMPLETED: 9 June 1998
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide
HFD-590



INDICATION: Cryptosporidiosis in AIDS patients
DEFINITIONS
NTZ = nitazoxanide
desNTZ = desacetyl NTZ
INTRODUCTION
NTZ is indicated for cryptosporidiosis in AIDS patients. This submission examines the beginning and end timepoints of dog plasma from the dog 90 day oral dosing study, the antiulcerogenic activity of NTZ, and the antisecretory activity of NTZ.

PHARMACOKINETIC STUDY
Determination of nitazoxanide and tizoxanide in dog plasma samples collected during — study no. — 2712-103; performed by — analytical report no. 196.547; batch 002; 7 March 1997; GLP

Plasma samples from the 90 day oral dog study were assayed for the metabolite desacetyl nitazoxanide (tizoxanide). Frozen plasma samples (315; samples from a high dose dog on day 90 were missing) were compared with solutions of NTZ (— batch 003), desNTZ (— , batch 280394), and internal standard (nifuroxazide, — batch F1995FA). Samples were extracted with acetonitrile and NaCl. The organic layer was used for assay. Assay consisted of desNTZ determination by high performance liquid chromatography with UV absorbance detection. The mobile phase used was — acetonitrile and — 0.02 M pH 2.5 phosphate buffer; UV absorbance for detection was used at — nm. The limit of detection was estimated to be 0.015µg/ml and the limit of quantification in plasma samples was — µg/ml. The following NTZ/desNTZ pharmacokinetic parameters were derived from the plasma samples.

dose	Cmax (mg/L)		AUCt (mg.h/L)	
(mg/kg/day)	Day 1	Day 90	Day 1	Day 90
25	0.59 ±0.23	0.58±0.31	1.22±0.61	1.72±1.26
50	1.27±0.58	1.13±0.52	4.78±1.06	6.25±3.53
100	2.10±0.72	1.86±1.22	16.8±14.3	13.5±9.26

Values are means ± standard deviation; n=8 except 90 mg/kg, day 90, n=7

The pharmacokinetics of desNTZ appear linear with respect to dose for both Cmax and AUC, both at day 1 and day 90.

PHARMACOLOGY STUDIES

Antiulcerogenic assay in aspirin-treated/pyloric ligated rats; performed by [redacted]; study no. 0251XR13.001; batch no. 3; 3 Oct 1996; no GLP statement

Sprague Dawley rats ([redacted], males, 7 weeks old) were placed in groups of 10. After a 24 hour fast, rats received a single dose of either vehicle (0.25% methylcellulose), cimetidine (25 mg/kg), or NTZ-30, 100, 300, and 1000 mg/kg 30 minutes prior to pyloric ligation. Immediately following surgery, aspirin was administered orally. Four hours later, the rats were sacrificed. The rats' stomachs were bisected at the greater curvature and the mucosa viewed for gastric lesions. The positive control cimetidine produced a 74% reduction in gastric ulceration from control; only the 30 mg/kg dose of NTZ produced any reduction (26%). NTZ does not appear to have antiulcerogenic activity.

Anti-secretory test in pyloric-ligated Shay rats; performed by [redacted]; study no. 025XR13.001; batch no. 3; 3 Oct 1996; no GLP statement

Sprague Dawley rats ([redacted], males, 7 weeks old) were placed in groups of 10. After a 24 hour fast, rats received a single dose of either vehicle (0.25% methylcellulose), scopolamine (1.5 mg/kg), or NTZ-30, 100, 300, and 1000 mg/kg 30 minutes prior to pyloric ligation. Four hours later, the rats were sacrificed. The rats' stomachs were removed and the contents assayed for volume, titratable acidity, pH, and total acidity. The positive control scopolamine decreased gastric volume, increased pH, decreased titrant volume, titratable acidity, and total acidity significantly from control values. NTZ at all doses did not cause any significant changes in the above parameters. NTZ does not appear to have antiulcerogenic activity.

SUMMARY
The major metabolite of NTZ, desNTZ, or tizoxanide, was measured by HPLC assay from plasma of the earlier 90 day oral dog study. Over the 90 day period, desNTZ exhibited linear pharmacokinetics with respect to Cmax and AUC. In two gastric ulcer models in rats, NTZ did not exhibit antiulcerogenic activity.

CONCLUSIONS: This submission is acceptable with respect to pharmacology.

/S/

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:
HFD-590/ADir/RAlbrecht
HFD-590/SPharm/KHastings
Steven C. Kunder/Pharm/

disk:
HFD-590/KHastings
cc:
HFD-590 (original)
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/s/

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