

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-818 and 21-498/S-003

PHARMACOLOGY REVIEW(S)



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-498, (also 21-818)
SERIAL NUMBER: S-003
DATE RECEIVED BY CENTER: 12/20/04
PRODUCT: Alinia
INTENDED CLINICAL POPULATION: patients 12 years old and older (NDA 21-498/ S-003), immunocompromised patients (NDA 21-818)
SPONSOR: Romark Laboratories LLC
DOCUMENTS REVIEWED: Vol. 1
REVIEW DIVISION: Division of Special Pathogen and Immunologic Drug Products (HFD-590)
PHARM/TOX REVIEWER: Steven Kunder, Ph.D.
PHARM/TOX SUPERVISOR: Robert Osterberg, Ph.D.
DIVISION DIRECTOR: Renata Albrecht, MD
PROJECT MANAGER: Kristen Miller, PharmD

Date of review submission to Division File System (DFS):

EXECUTIVE SUMMARY

Nitazoxanide (NTZ) was previously submitted by Unimed Pharmaceuticals Inc in 1998 for cryptosporidiosis in AIDS patients (NDA 20-871).

Romark Laboratories, a previous developer of the drug, resubmitted NTZ for cryptosporidial diarrhea in children with a three day course of treatment. The previously submitted preclinical studies were used to support the resubmission (NDA 21-498, approved 11/22/02), with an additional 28-day oral dog toxicity study. The previous preclinical studies demonstrated support for the safety of NTZ. Please refer to reviews of these submissions for the pharmacology and toxicology studies. Overall safety of NTZ is supported by the lack of toxicity in animal studies at doses greater than human exposure for the short clinical treatment times. No additional preclinical studies were included in the current submission. There are no other pharmacology/toxicology issues.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: No additional preclinical pharmacology and toxicology studies were included in this submission. There are no changes with respect to previous pharmacology and toxicology acceptability.

Unresolved toxicology issues (if any): none

Recommendations: none

Suggested labeling: no changes

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

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/s/

Steve Kunder
6/15/05 12:01:35 PM
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-497
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: 28 / 01 / 04
PRODUCT: **Alinia® nitazoxanide**
INTENDED CLINICAL POPULATION: **adults and children**
SPONSOR: **ROMARK PHARMACEUTICALS**
6200 Courtney Campbell Causeway, Suite 200,
Tampa FL 33607

DOCUMENTS REVIEWED: none
REVIEW DIVISION: **Division of Special Pathogen and
Immunologic Drug Products (HFD-590)**
PHARM/TOX REVIEWER: **Steven Kunder**
PHARM/TOX SUPERVISOR: **Stephen Hundley**
DIVISION DIRECTOR: **Renata Albrecht**
PROJECT MANAGER: **Kristen Miller**

Date of review submission to Division File System (DFS): 8 July 2004

PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number: 21-497 (500 mg tablets), 21-498 (oral suspension)

Review number: 001

Sequence number/date/type of submission: 000 / 28 Jan 2004

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Romark Laboratories, LC
6200 Courtney Campbell Causeway
Suite 200
Tampa, Florida 33607

Manufacturer for drug substance

/ / /

Reviewer name: SKunder

Division name: Special Pathogen and Immunologic Drug Products

HFD #: 590

Review completion date: 7 July 2004

Drug:

Trade name: Alinia

Generic name (list alphabetically): nitazoxanide

Code name: PH 5776, NTZ

Chemical name: 2-acetoxy-N-(5-nitrothiazol-2-yl) benzamide

CAS registry number: 55981-09-4

Molecular formula/molecular weight: C₁₂H₈N₃O₅S, / 307.3

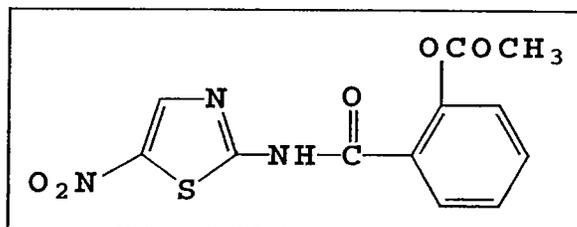
Structure:

Relevant INDs/NDAs/DMFs: IND — , NDA
20871

Drug class: anti diarrheal

Indication: diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

Clinical formulation: 500 mg tablets, oral suspension (100 mg/5 ml)

Route of administration: oral; adults: 500mg tablets bid for 3 days; oral suspension,
children: 1-4 yrs, 5 ml bid for 3 days; 4-11 yrs 10 ml bid for 3 days, ; ≥12 yrs, 25 ml bid
for 3 daysProposed use: treatment of diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia* in adults and children

Executive Summary

Recommendation on Approvability

Nitazoxanide was previously submitted by Unimed Pharmaceuticals Inc in 1998 for cryptosporidiosis in AIDS patients (NDA 20-871).

A previous developer of the drug, Romark Laboratories, resubmitted NTZ for cryptosporidial diarrhea in children with a three day course of treatment (200 mg, bid, approximately 11 mg/kg/day in an 11 year old child). The previously submitted preclinical studies were used to support this submission, with an additional 28-day oral dog toxicity study. The previous preclinical studies demonstrated support for the safety of NTZ. The lack of toxicity in animal studies at doses greater than human exposure supports the safety of this drug for the short treatment time. There are no other pharmacology/toxicology issues.

Recommendation for Nonclinical Studies

none

Recommendations on Labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity studies have not been conducted.

Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. NTZ was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 20 times the clinical adult dose adjusted for body surface area).

PREGNANCY

Pregnancy Category B: Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 26 times the clinical adult dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 2 times the clinical adult dose adjusted for surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to nitazoxanide. There are, however, no adequate and well-controlled studies in pregnant women.

Summary of Nonclinical Findings

Rats receiving 450 mg/kg (HED=75 mg/kg) for 14 weeks demonstrated intense salivation, increased liver and spleen weights, and decreased thymus weights without histopathological differences.

Rats in a 6 month study, demonstrated a NOAEL of 150 mg/kg/day (HED=25 mg/kg). 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 (HED=75 mg/kg). 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. High dose females had 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 first 28 day dog study (doses= 300, 900, 2700/1800 mg/kg, HED= 150, 450, 1350/900 mg/kg), 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.

A second 28-day oral dog study at lower doses (7.5-60 mg/kg, HED=3.7- 30 mg/kg) had only gastrointestinal effects including focal hemorrhages and stomach ulceration.

In the 90 day oral dog study (doses=25, 50, 100 mg/kg; HED=12, 25, 50 mg/kg), hematological findings similar to those of the first 28-day dog study 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 than 25 mg/kg.

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, chromosomal aberration tests with Chinese hamster ovary cell, also with desNTZ and an in vivo mouse micronucleus assay. A positive genotoxic result was seen in the Salmonella-E. coli reverse mutation assay; one strain of *S. typhimurium* (TA 100) showed slightly higher rates of reversion with both NTZ and desNTZ.

In both rats and rabbits, 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. Both species also had maternal effects of decreased food consumption and body weight, possibly due to the bulk amount of drug in the gastrointestinal tract, confounding fetal effects that were seen. A rat study showed no apparent transfer of tizoxanide (active metabolite) to fetuses at 30 mg/kg (HED= 5 mg/kg) while fetuses from 2 of 9 dams receiving 300 mg/kg (HED=50 mg/kg) did show tizoxanide.

Reproductive toxicology and fertility effects are minimal given the low absorption seen with NTZ. Pregnancy Category B designation appears appropriate due to the lack of impaired fertility or harm to the fetus due to nitazoxanide..

Pharmacologic Activity

Nitazoxanide exerts antiprotozoal activity by interference with pyruvate:ferredoxin oxidoreductase, an enzyme essential to anaerobic energy metabolism. By this enzyme inhibitory action, sporozoites of *Cryptosporidium parvum* and trophozoites of *Giardia lamblia* are inhibited in growth by NTZ.

Nonclinical Safety Issues Relevant to Clinical Use

Gastrointestinal irritation and bleeding are the major concerns.

Administrative

Reviewer signature: _____

Supervisor signature: Concurrence - _____

cc: list:

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/s/

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PHARMACOLOGIST

Steve Hundley
7/9/04 10:23:05 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number: 21-497 (500 mg tablets), 21-498 (oral suspension)

Review number: 001

Sequence number/date/type of submission: 000/29 May 2002

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Romark Laboratories, LC

6200 Courtney Campbell Causeway

Suite 880

Tampa, Florida 33607

Manufacturer for drug substance

/ /

Reviewer name: SKunder

Division name: Special Pathogen and Immunologic Drug Products

HFD #: 590

Review completion date: 6 Nov 2002

Drug:

Trade name: Cryptaz

Generic name (list alphabetically): nitazoxanide

Code name: PH 5776, NTZ

Chemical name: 2-acetoxy-N-(5-nitrothiazol-2-yl) benzamide

CAS registry number: 55981-09-4

Mole file number: not provided

Molecular formula/molecular weight: $C_{12}H_8N_3O_5S$, 307.3

Structure:

Relevant INDs/NDAs/DMFs: IND — NDA
20871

Drug class: anti diarrheal

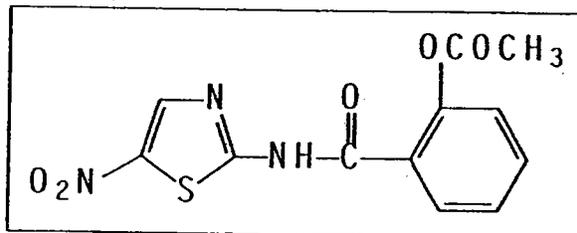
Indication: diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

Clinical formulation: 500 mg tablets, oral suspension (100 mg/5 ml)

Route of administration: oral

Proposed use: children with diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia*

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.



Executive Summary

Recommendation on Approvability

Nitazoxanide was previously submitted by Unimed Pharmaceuticals Inc in 1998 for cryptosporidiosis in AIDS patients (NDA 20-871). The application was not approved due to lack of efficacy. A previous developer of the drug, Romark Laboratories, resubmitted NTZ for cryptosporidial diarrhea in children with a three day course of treatment (200 mg, bid, approximately 11 mg/kg/day in an 11 year old child). The previously submitted preclinical studies were used to support this submission, with an additional 28-day oral dog toxicity study. The previous preclinical studies demonstrated support for the safety of NTZ. The lack of toxicity in animal studies at doses greater than human exposure supports the safety of this drug for the short treatment time. There are no other pharmacology/toxicology issues.

Recommendation for Nonclinical Studies

none

Recommendations on Labeling

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity studies have not been conducted.

Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. NTZ was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 66 times the recommended dose for patients 11 years of age, adjusted for body surface area).

PREGNANCY

Teratogenic Effects

Pregnancy Category B:

Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 48 times the clinical dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 3 times the clinical dose adjusted for body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. There are, however, no adequate and well-controlled studies in pregnant women.

Summary of Nonclinical Findings

Brief Overview of Nonclinical Findings

Rats receiving 450 mg/kg (HED=75 mg/kg) for 14 weeks demonstrated intense salivation, increased liver and spleen weights, and decreased thymus weights without histopathological differences.

Rats in a 6 month study, demonstrated a NOAEL of 150 mg/kg/day (HED=25 mg/kg). 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 (HED=75 mg/kg). 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. High dose females had 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 first 28 day dog study (doses= 300, 900, 2700/1800 mg/kg, HED= 150, 450, 1350/900 mg/kg), 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.

A second 28-day oral dog study at lower doses (7.5-60 mg/kg, HED=3.7- 30 mg/kg) had only gastrointestinal effects including focal hemorrhages and stomach ulceration.

In the 90 day oral dog study (doses=25, 50, 100 mg/kg; HED=12, 25, 50 mg/kg), hematological findings similar to those of the first 28-day dog study 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 than 25 mg/kg.

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, chromosomal aberration tests with Chinese hamster ovary cell, also with desNTZ and an in vivo mouse micronucleus assay. A positive genotoxic result was seen in the Salmonella-E. coli reverse mutation assay; one strain of *S. typhimurium* (TA 100) showed slightly higher rates of reversion with both NTZ and desNTZ.

In both rats and rabbits, 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. Both species also had maternal effects of decreased food consumption and body weight, possibly due to the bulk amount of drug in the gastrointestinal tract, confounding fetal effects that were seen. A rat study showed no apparent transfer of tizoxanide to fetuses at 30 mg/kg (HED= 5 mg/kg) while fetuses from 2 of 9 dams receiving 300 mg/kg (HED=50 mg/kg) did show tizoxanide.

Reproductive and fertility issues are less of a factor with children while the reproductive toxicology studies do not demonstrate NTZ to be a strong teratogen. Given the target age population and low absorption seen with NTZ, Pregnancy Category B appears to be justified. In all cases, HED values should be compared to the proposed clinical dose of about 11 mg/kg for 3 days.

Pharmacologic Activity

Nitazoxanide exerts antiprotozoal activity by interference with pyruvate:ferredoxin oxidoreductase, an enzyme essential to anaerobic energy metabolism. By this enzyme inhibitory action, sporozoites of *Cryptosporidium parvum* and trophozoites of *Giardia lamblia* are inhibited in growth by NTZ.

Nonclinical Safety Issues Relevant to Clinical Use

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: Nitazoxanide is poorly absorbed from the gastrointestinal tract. It remains mostly in the gastrointestinal tract, with mainly fecal elimination by 72 hours. Deacetylation produces the major metabolite, desacetylNTZ. Bioavailability was not determined in animals.

Mechanism of action: antiprotozoal action, may result from interference with pyruvate:ferredoxin oxidoreductase, an enzyme essential to anaerobic energy metabolism

Drug activity related to proposed indication: By the above enzyme inhibitory action, sporozoites of *Cryptosporidium parvum* and trophozoites of *Giardia lamblia* are inhibited in growth

Secondary pharmacodynamics: not applicable

Pharmacology summary: Single dose studies demonstrated in vivo activity including analgesia in the PPQ writhing assay, mortality in rats and mice, antiinflammatory activity, slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity 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.

Pharmacology conclusions: Nitazoxanide appears to exert analgesic, hyperlipidemic, antiinflammatory, antisecretory and antiulcer activity, slight antimicrobial activity against *S. aureus* and *P. vulgaris*, and limited antagonism against angiotensin II, leukotriene D4, substance P and acetylcholine.

II. SAFETY PHARMACOLOGY:

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)

Neurological effects: Reflex inhibition, behavioral depression, muscle relaxation, catalepsy, antipentylentetrazol convulsive activity, anti-electroshock, motor activity, analgesia, tetrabenazine antagonism were evaluated in mice. Analgesia was observed at 500 mg/kg.

Cardiovascular effects: Direct blood pressure, heart rate, and anti-arrhythmia were evaluated in mice. No effects were seen. In vitro study with the guinea pig for atrium contractile force also showed no effect.

Pulmonary effects: In the trachea, bronchodilator antagonism effects (beta II and histamine) were evaluated. Slight antihistamine activity was seen.

Renal effects: Volume diuresis was evaluated in mice. No effects were seen.

Gastrointestinal effects: Antisecretory activity and anti-ulcer activity were evaluated in rats. Gastrointestinal antisecretory and antiulcer activity were seen at 500 mg/kg.

Abuse liability: not determined

Other: Antiinflammatory activity (carrageenan rat paw edema, arachidonic acid ear model, rat gait and antipyretic activity), immunology (immunosuppression, immunoenhancement), antagonism studies (in vitro; ileum: angiotensin II, calcium, leukotriene D4, substance P, acetylcholine, beta (isoproterenol), metabolic assays (cholesterol, glucose tolerance), antimicrobial activity and acute toxicity in mice and rats. Findings included slight effects on increasing HDL and triglycerides, gastrointestinal antisecretory and antiulcer activity (500 mg/kg) and slight histamine activity, and mortality in rats (1/3) and mice (1/3) at 1000mg/kg

Safety pharmacology summary: NTZ was evaluated in a variety of safety pharmacological screens. 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.

Safety pharmacology conclusions:

In vivo activity 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.

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters: see table below

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.

Pharmacokinetic parameters for a single oral dose of C¹⁴ labeled NTZ (100 mg/kg) in dogs
 C_{max}=45.5 µg*eqv/ml, males AUC= 245.4 µg*eqv/hr/ml, males t_{1/2} = 22.5 hours, males
 =32.9 µg*eqv/ml, females =241.2 µg*eqv/hr/ml, females = 27.9 hours, females

Mean (± SD) plasma pharmacokinetic parameter values following administration of a single dose of Nitazoxanide for Oral Suspension with food to pediatric subjects

| Age | Dose* | Tizoxanide (desNTZ) | | | Tizoxanide glucuronide | | |
|-----------------|-------|-----------------------------|-----------------------------|----------------------------------|-----------------------------|--------------------------|----------------------------------|
| | | C _{max} (µg/mL) | T _{max} ** (hr) | AUC _{inf} (µg•hr/mL) | C _{max} (µg/mL) | T _{max} (hr) | AUC _{inf} (µg•hr/mL) |
| 12-47 months | 100mg | 3.11 (2.0) | 3.5 (2-4) | 11.7 (4.46) | 3.64 (1.16) | 4.0 (3-4) | 19.0 (5.03) |
| 4-11 years | 200mg | 3.00 (0.99) | 2.0 (1-4) | 13.5 (3.3) | 2.84 (0.97) | 4.0 (2-4) | 16.9 (5.00) |

NDA 21-497

* Dose: 100 mg/5 mL nitazoxanide, 200 mg/10 mL nitazoxanide

**T_{max} is given as Mean (Range)

The animal studies appear to show greater absorption than pediatric clinical studies.

Absorption: In a single dose C¹⁴-labeled NTZ dog study, tissues had negligible recovery of label despite the appearance of drug in plasma. In a study using pregnant rats, C¹⁴-labeled NTZ was administered orally on days 6-15 of gestation at doses of 30 and 300 mg/kg (HED= 5 and 50 mg/kg). Fetuses were examined for label and found to be below limits of detection in the 30 mg/kg group, while in the 300 mg/kg group, 2 of the 9 litters examined had detectable label (tizoxanide).

Distribution: radiolabeled NTZ administered orally to rats was found mainly in the digestive tract and kidney for the first 4 hours following dosing, then additionally in the large intestine and thyroid at 8 and 12 hours, followed by thyroid, stomach and kidney at 24 to 48 hours and thyroid, kidney and liver at 72 hours.

Metabolism: A metabolic profile of NTZ in animals has not been conducted. A metabolic profile was determined in human liver microsomal preparations. A dog fecal study did not characterize a consistent pattern of metabolites in 3 dogs. This renders correlations between clinical and nonclinical metabolism difficult; the sponsor used the human metabolite identification to perform animal studies employing the major human metabolite (tizoxanide, or desacetylNTZ) without knowledge of the metabolic pattern in animals.

Excretion: In radiolabeled oral studies in rats, 37.1 % of label was recovered in urine by 24 hours, 28.15 by 72 hours. Fecal recovery was 52% by 72 hours. The carcasses had 0.11% remaining by 72 hours. In dogs, urinary recovery of label was 30.4% in males and 21.1% in females after 72 hours while fecal recovery was 38% in males and 53.3% in females after 72 hours.

Other studies: not conducted

PK/TK summary: Pharmacokinetic studies conducted during 28-day oral toxicity studies in rats and dogs showed similar exposure to NTZ in humans receiving lesser doses. Radiolabel studies show little tissue deposition in dogs or fetal exposure in pregnant rats. Distribution in rats occurred in the gastrointestinal tract, then liver, kidneys and thyroid.. Metabolism was not characterized in experimental animals. Excretion of NTZ was demonstrated in dogs and rats as mainly in feces but with substantial urinary excretion as well.

PK/TK conclusions: Absorption appears to be minimal. The pharmacokinetics of NTZ in animals are poorly characterized. The relevance of animal metabolism cannot be correlated accurately with the human metabolic profile as a result.

IV. GENERAL TOXICOLOGY:

See attached NDA, IND reviews for other toxicology studies

Study title: 28-day oral toxicity study in dogs

Key study findings: gastrointestinal irritation (focal hemorrhages and stomach ulceration) at all doses (7.5 -60 mg/kg)

Study no: 0436DR13.001

Volume #, and page #: 11, all

Conducting laboratory and location: (/ /)

Date of study initiation: 29 Sept 1998

GLP compliance: yes

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity: lot no. 003

Formulation/vehicle: solution, 0.25% methyl cellulose

Methods (unique aspects): none

Dosing:

Species/strain: dog, beagle

#/sex/group or time point (main study): vehicle controls, 60 mg/kg, 5; 7.5, 15, 30 mg/kg, 3

Satellite groups used for toxicokinetics or recovery: 2/sex/group, control, 60 mg/kg for 14-day recovery

Age: 5-6 months

Weight: males (7.9-9.3), females (7.3-8.8)

Doses in administered units: 0, 7.5, 15, 30, 60 mg/kg/day (0, 3.75, 15, 30 mg/kg bid)

Route, form, volume, and infusion rate: oral solution, 5 ml/kg bid

Observations and times:

Clinical signs: twice daily

Body weights: day 1, 8, 5, 22, 28, 29 (fasted); recovery day 35, 41, 42 (fasted)

Food consumption: daily

Ophthalmoscopy: not conducted

EKG: not conducted

Hematology: day 29 or day 43

Clinical chemistry: day 29 or day 43

Urinalysis: day 29 or day 43

Gross pathology: day 29 or day 43

Organs weighed: day 29 or day 43, see table below

Histopathology: day 29 or day 43, see table below

Toxicokinetics: not conducted

Other: not conducted

Results:

Mortality: none

Clinical signs: yellow discolored fur/paws, especially in 60 mg/kg group, due to drug in feces

Body weights: no treatment related effects

Food consumption: no treatment related effects

Ophthalmoscopy: not conducted

Electrocardiography: not conducted

Hematology: no treatment related effects

Clinical chemistry: no treatment related effects

Urinalysis: no treatment related effects

Organ weights: no treatment related effects

Gross pathology: petechiae in colon, ileocolic junction, jejunum, duodenum, and stomach throughout treated groups

Histopathology:

| site | 7.5 mg/kg | 15 mg/kg | 30 mg/kg | 60 mg/kg |
|------------------------|------------------|------------------|------------------|----------------|
| focal hemorrhage Colon | 1 female, day 29 | 1 M, day 29 | | |
| Cecum | | 1 female, day 29 | | |
| Duodenum | | | 1 female, day 29 | |
| Stomach ulceration | | | | 1 male, day 29 |

Toxicokinetics: not conducted

Summary of individual study findings: The main drug-related finding was gastrointestinal irritation in the form of focal hemorrhages and stomach ulceration in all treatment groups (7.5-60 mg/kg, HED=3.7- 30 mg/kg).

Toxicology summary: 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 LALD50 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 first 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. A second 28-day oral dog study at lower doses had only gastrointestinal effects including focal hemorrhages and stomach ulceration. In the 90 day oral dog study, hematological findings similar to those of the first 28-day dog study occurred. Bleeding from gastrointestinal hemorrhages may be related to the hematological findings such as decreased hematocrit and erythrocyte counts. 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 than 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 from 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.

Toxicology conclusions: Nonclinical studies provided by the sponsor demonstrate nitazoxanide toxicities including hematotoxicity (rats, 6 months, 450 mg/kg and dogs, 28 days, 300-1800 mg/kg and 90 days, 25-100 mg/kg), gastrointestinal toxicity (dogs, 28 and 90 days) and testicular toxicity (dogs, 90 days, 25-100 mg/kg). The proposed clinical dose is about 11 mg/kg for three days.

Histopathology Inventory for NDA # 21-497

| | |
|-------------------------|-------------|
| Study | 28-day oral |
| Species | Dog |
| Adrenals | X* |
| Aorta | X |
| Bone Marrow smear | |
| Bone (femur) | X |
| Brain | X* |
| Cecum | X |
| Cervix | X |
| Colon | X |
| Duodenum | X |
| Epididymis | X |
| Esophagus | X |
| Eye | X |
| Fallopian tube | |
| Gall bladder | X |
| Gross lesions | |
| Harderian gland | |
| Heart | X |
| Ileum | X |
| Injection site | |
| Jejunum | X |
| Kidneys | X* |
| Lachrymal gland | X |
| Larynx | |
| Liver | X* |
| Lungs | X |
| Lymph nodes, cervical | |
| Lymph nodes mandibular | |
| Lymph nodes, mesenteric | X |
| Mammary Gland | X |
| Nasal cavity | |
| Optic nerves | X |
| Ovaries | X* |
| Pancreas | X |

| | |
|------------------|----|
| Parathyroid | X |
| Peripheral nerve | |
| Pharynx | |
| Pituitary | X |
| Prostate | X |
| Rectum | X |
| Salivary gland | X |
| Sciatic nerve | |
| Seminal vesicles | |
| Skeletal muscle | |
| Skin | X |
| Spinal cord | X |
| Spleen | X* |
| Sternum | X |
| Stomach | X |
| Testes | X* |
| Thymus | X |
| Thyroid | X |
| Tongue | X |
| Trachea | X |
| Urinary bladder | X |
| Uterus | X |
| Vagina | X |
| Zymbal gland | |
| Standard List | |
| | |

X, histopathology performed

*, organ weight obtained

V. GENETIC TOXICOLOGY:

See attached NDA, IND reviews for other genetic toxicology studies.

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, chromosomal aberration tests with Chinese hamster ovary cell, also with desNTZ and an in vivo mouse micronucleus assay. A positive genotoxic result was seen in the Salmonella-E. coli reverse mutation assay; one strain of *S. typhimurium* (TA 100) showed slightly higher rates of reversion with both NTZ and desNTZ.

Labeling recommendations: Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. Nitazoxanide was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

VI. CARCINOGENICITY:

Carcinogenicity studies were not conducted.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

See attached NDA, IND reviews

Reproductive and developmental toxicology summary:

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), adactyly (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 11 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 F₁ 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.

Reproductive and developmental toxicology conclusions: Reproductive and fertility issues are less of a factor with children while the reproductive toxicology studies do not demonstrate NTZ to be a strong teratogen. Given the target age population and low absorption seen with NTZ, Pregnancy Category B appears to be justified.

Labeling recommendations:

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 66 times the recommended dose for patients 11 years of age, adjusted for body surface area).

PREGNANCY

Teratogenic Effects

Pregnancy Category B:

Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 48 times the clinical dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 3 times the clinical dose adjusted for body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. There are, however, no adequate and well-controlled studies in pregnant women.

VIII. SPECIAL TOXICOLOGY STUDIES:

Not conducted

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Nitazoxanide appears safe for the proposed three day, 200 mg bid (11 mg/kg in 11 year olds) indication. Pharmacokinetics in animals remain poorly characterized, particularly absorption, distribution and metabolism. Absorption appears to be less than that seen in humans which provides some reassurance. Reproductive effects are confounded by maternal toxicity and incomplete characterization of pharmacokinetics. Possible gastrointestinal irritation is the main concern at doses in dogs most similar to the proposed clinical dose.

General Toxicology Issues: Nitazoxanide appears to cause gastrointestinal irritation, possibly through metabolism to salicylate-like metabolites at doses (dogs HED=12.5 mg/kg) similar to the proposed clinical dose (11 mg/kg).

Recommendations: Off-label use of nitazoxanide in women of reproductive age may necessitate further characterization in animals of fetal exposure correlating with possible fetal effects.

Labeling with basis for findings:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term carcinogenicity studies have not been conducted.

Nitazoxanide was not genotoxic in the Chinese hamster ovary (CHO) cell chromosomal aberration assay or the mouse micronucleus assay. NTZ was genotoxic in one tester strain (TA 100) in the Ames bacterial mutagenicity assay

Nitazoxanide did not adversely affect male or female fertility in the rat at 2400 mg/kg/day (approximately 66 times the recommended dose for patients 11 years of age, adjusted for body surface area).

PREGNANCY

Teratogenic Effects

Pregnancy Category B:

NDA 21-497

Reproduction studies have been performed at doses up to 3200 mg/kg/day in rats (approximately 48 times the clinical dose adjusted for body surface area) and 100 mg/kg/day in rabbits (approximately 3 times the clinical dose adjusted for body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to NTZ. There are, however, no adequate and well-controlled studies in pregnant women.

X. APPENDIX/ATTACHMENTS:

Addendum to review: NDA 20-871 review, IND — reviews

Other relevant materials (Studies not reviewed, appended consults, etc.): none

Any compliance issues: none

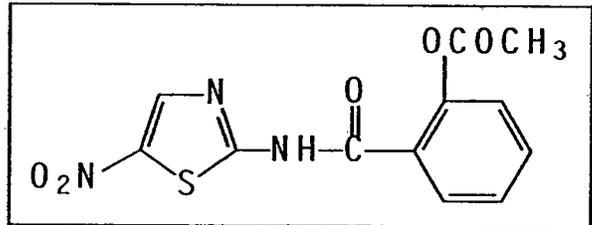
**APPEARS THIS WAY
ON ORIGINAL**

PHARMACOLOGIST'S REVIEW

NDA # 20-871

DATE SUBMITTED: December 30, 1997
DATE RECEIVED: January 5, 1998
DATE ASSIGNED: January 7, 1998
DATE REVIEW COMPLETED: June 11, 1998
HFD-590
SPONSOR: Unimed Pharmaceuticals, Inc.
DRUG: Nitazoxanide

RELATED DOCUMENTS: IND 48620
INDICATION: Cryptosporidiosis in AIDS patients
DEFINITIONS
NTZ=nitazoxanide
GI= gastrointestinal
desNTZ = desacetylnitazoxanide



INTRODUCTION

Nitazoxanide is a nitrothiazole antiparasitic which has demonstrated activity against protozoa, helminths, and bacteria. Recently NTZ was shown to have 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. and has been approved for use in Mexico for infections of the intestinal tract. The drug is manufactured in Columbia. The structure of NTZ demonstrates its relationship to salicylate, an irritant, a factor which may account for the poor tolerance of NTZ by dogs. The metabolism of NTZ is poorly characterized. One metabolite, a deacetylated form (desNTZ) has been recovered in dog studies. Most NTZ appears to be eliminated in feces. The mechanism of action for NTZ is not clear. Since NTZ is not well absorbed, a direct effect on either *C. parvum* or the intestinal wall might be postulated. Direct antimicrobial activity against *C. parvum* is unlikely as demonstrated by the lack of activity by NTZ or desNTZ in in vitro assays. Speculatively, *C. parvum* may enter the surface of cells lining the intestinal wall, so that an irritant action might slough these off. The regulatory history of NTZ included several decisions regarding study requirements. A decision was made at an end of phase II meeting with the sponsor to negate the need for the otherwise required six month dog study in view of the toxicity experienced in the 90 day study. Later, at a pre-NDA meeting, the sponsor agreed to perform carcinogenicity studies as Phase IV a commitment. At an advisory committee meeting, the recommendation was made not to approve NTZ due to the lack of efficacy in humans. Subsequently, the Division of Special Pathogens and Immunologic Drug Products agreed that NTZ was not approvable for lack of demonstrated efficacy.

NONCLINICAL PHARMACOKINETICS**Pharmacokinetics Studies**

1. A mass balance and tissue distribution study of ¹⁴C-nitazoxanide following a single oral dose to male rats
Study no. — 6613-100; study dated 5-4-95
2. Determination of desacetylnitazoxanide in dog and rat plasma samples collected during Romark laboratories studies
Study # 195.505; study dated 5-31-95
3. Plasma pharmacokinetics, elimination and tissue distribution of radioactivity following single oral dose administration of ¹⁴C-nitazoxanide in dogs
Study # 0831XU13.001; study dated 10-8-97
4. Determination of nitazoxanide and tizoxanide in dog plasma samples collected during — study no. — 2712-103
analytical report no. 196.547; study dated 3-7-97

Pharmacokinetics Studies Review

1. A mass balance and tissue distribution study of ¹⁴C-nitazoxanide following a single oral dose to male rats
Study no. — 6613-100
Submitted under IND 48620/000; review dated 9-8-95
2. Determination of desacetylnitazoxanide in dog and rat plasma samples collected during Romark laboratories studies
Study # 195.505
Submitted under IND 48620/000; review dated 9-8-95
3. Plasma pharmacokinetics, elimination and tissue distribution of radioactivity following single oral dose administration of ¹⁴C-nitazoxanide in dogs
Study no. 0831XU13.001
Submitted under IND 48620/029; review dated 6-1-98
4. Determination of nitazoxanide and tizoxanide in dog plasma samples collected during — study no. — 2712-103
Analytical report no. 196.547
Submitted under IND 48620/033; review dated 6-1-98

Summary of Nonclinical Pharmacokinetics Studies

The sponsor attempted to characterize the pharmacokinetic properties of NTZ in radiolabeled studies in dogs and rats. The following table summarizes these studies.

Pharmacokinetic parameters from 28- and 90- day oral toxicity study of desNTZ in dogs and 28-day oral toxicity study in rats

| dose, species | AUC (mg.h/L) | | Cmax (mg/L) | |
|---------------------------------------|--------------------|---------------------|-------------|-----------|
| | Day 1 | Day 90 | Day 1 | Day 90 |
| 25 mg/kg dog | 1.22±0.61 | 1.72±1.26 | 0.59 ±0.23 | 0.58±0.31 |
| 50 mg/kg dog | 4.78±1.06 | 6.25±3.53 | 1.27±0.58 | 1.13±0.52 |
| 100 mg/kg dog | 16.8±14.3 | 13.5±9.26 | 2.10±0.72 | 1.86±1.22 |
| | day 1 (0-24 hr) | Day 28 (0-24 hr) | day 1 | 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 ± s.d.

Metabolism of NTZ has been poorly characterized in animals. Radiolabeled metabolites of NTZ were recovered but, except for the largest fraction described below, uncharacterized. One metabolite desacetylnitazoxanide (desNTZ) has been identified in rats and dogs. Radiolabeled desNTZ has been used to trace

NTZ in the pharmacokinetic studies below. NTZ disappears rapidly in both dogs and rats. Radiolabel concentrations in the plasma peak at about one hour following oral administration. Most radiolabel was found in the gastrointestinal tract. In rats, nearly all radiolabel was eliminated by 72 hours. Most drug was

eliminated by feces and urine in the first 24 hours. In dogs, a single radiolabeled dose was eliminated in feces (0-60%) and urine (0-26%) by 48 hours. Tissue recovery was negligible after 48 hours. As shown in the above table, large oral doses such as 900 and 2700/1800 mg/kg in the dog, do not affect AUC or Cmax when compared to doses such as 25, 50, or 100 mg/kg. Chronic dosing, such as that of the 90 day oral study, did not increase AUC or Cmax either. Plasma protein binding in rats and dogs has not been characterized; human plasma protein binding was reported by the sponsor to be about 95%, thus species differences may produce different pharmacokinetic characteristics between humans and animals. Pharmacokinetic studies employing dosing routes other than oral (i.e. intravenous) would be needed to determine bioavailability of NTZ. Characterization of the remaining metabolites would also permit determination of any changes in the pattern of biotransformation resulting from dose.

NONCLINICAL PHARMACOLOGY

Pharmacology Studies

1. Assay of nitazoxanide for general pharmacological activity
Study # ROM-009; study dated 10-9-92
2. Antiulcerogenic assay in aspirin-treated/pyloric ligated rats
Study # 0251XR13.001; study dated 10-3-96
3. Anti-secretory test in pyloric-ligated Shay rats
Study # 025XR13.001; study dated 10-3-96
4. Study of two compounds on PGE2 secretion
Study # 26034; study dated 11-4-97

Pharmacology Studies Reviews

1. Assay of nitazoxanide for general pharmacological activity
Study # ROM-009;
Submitted under IND 48620/000; review dated 9-8-95
2. Antiulcerogenic assay in aspirin-treated/pyloric ligated rats
Study # 0251XR13.001
Submitted under IND 48620/033; review dated 6-9-98
3. Anti-secretory test in pyloric-ligated Shay rats
Study no. 025XR13.001
Submitted under IND 48620/033; review dated 6-9-98

4. Study of two compounds on PGE2 secretion; performed by _____ study no. 2603; NTZ batch no. 003; desNTZ batch no. L0795110; 4 November 1997; not GLP

NTZ and desNTZ were evaluated for inhibition of PGE2-secretory activity. The assay was performed using differentiated HL60 cells, with indomethacin as a reference compound. The stimulus for inflammation and PGE2 secretion was A 23187 (5 μ M). The test compounds were assayed in duplicate at concentrations of 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , and 10^{-5} M with an incubation of 30 minutes. PGE2 was determined using commercial kits (_____.r), and radioactivity determined with a liquid scintillation counter. The percent inhibition of PGE2 by NTZ ranged from 0 (10^{-9} M) to 96% (10^{-5} M) and by desNTZ from 75% (10^{-9} M) to 89% (10^{-7} M). The IC50 was calculated for NTZ to be 0.10 μ M; the IC50 for indomethacin was 0.0056 μ M. NTZ would not appear to be an effective inhibitor of PGE2 secretion.

Comment: The experimental procedures provided for this study are minimal, limiting its value to evaluation of this pharmacologic activity of NTZ.

Summary of Nonclinical Pharmacology Studies

The majority of the nonclinical pharmacology studies were reviewed previously and the reviews are included in the Appendix. Slight antimicrobial activity was seen against *S. aureus* and *P. vulgaris*. Most notably, NTZ was demonstrated to have analgesic and antiinflammatory activity in rat and mouse in vivo models at a dose of 500 mg/kg, as well as gastrointestinal antisecretory activity and antiulcer activity. Antagonism was demonstrated in vitro against angiotensin II, leukotriene D4, substance P and acetylcholine. The gastrointestinal effects of NTZ are relevant to the potential mechanism of action. These effects were not confirmed in two later studies which examined NTZ antiulcer and antisecretory activity in in vivo rat model. Oral NTZ doses of 100, 300, and 1000 mg/kg were used without any resulting activity.

NONCLINICAL TOXICOLOGY

Toxicity Studies

1. 28-day toxicity study in rats
Study # — 2712-100 ; study dated 4-27-95
2. Acute intraperitoneal study in mice
Study # ROM-010; study dated 12-78
3. Acute oral toxicity study in mice
study dated 12-78
4. Acute intraperitoneal study in rats
Study # ROM-015; study dated 2-15-77

5. Acute oral toxicity study in rats
Study # ROM-011; study dated 12/78
5. Acute oral toxicity in cats
Study # ROM-013; study dated 5/2/77
6. Acute oral toxicity study in dogs
Study # ROM -012; study dated 5/2/77
7. Acute dermal toxicity study in rabbits
Study # ROM-016; study dated 1/79
8. Primary eye irritation/corrosiveness study in rabbits
Study # ROM-017; study dated 1/79
9. 8-day pilot oral toxicity study of NTZ in rats
Study # — 2647-111; study dated 11/23/92
10. 14-week oral toxicity study in the rat
Study # ROM-018; study dated 5/78
11. Rising dose tolerance study via oral administration of NTZ in dogs
Study # HWA 2647-112; study dated 11-23-92
12. 28-day oral toxicity study of NTZ in dogs
Study # 2647-114; study dated 12-20-93
14. 90-day toxicity study in dogs with nitazoxanide
Report # — 2712-103; study dated 12-16-96
15. 6-month oral toxicity study in rats
Study no. 0460RU13.001; study dated 7-9-97

Toxicity Studies Review

1. 28-day toxicity study in rats
Submitted under IND 48620/000; review dated 9-8-95
2. Acute intraperitoneal study in mice
Submitted under IND 48620/000; review dated 9-8-95
3. Acute oral toxicity study in mice
Submitted under IND 48620/000; review dated 9-8-95
4. Acute intraperitoneal study in rats
Submitted under IND 48620/000; review dated 9-8-95
5. Acute oral toxicity study in rats.
Submitted under IND 48620/000; review dated 9-8-95
6. Acute oral toxicity in cats
Submitted under IND 48620/000; review dated 9-8-95

7. Acute oral toxicity study in dogs
Submitted under IND 48620/000; review dated 9-8-95
8. Acute dermal toxicity study in rabbits
Submitted under IND 48620/000; review dated 9-8-95
9. Primary eye irritation/corrosiveness study in rabbits
Submitted under IND 48620/000; review dated 9-8-95
10. 8-day pilot oral toxicity study of NTZ in rats
Submitted under IND 48620/000; review dated 9-8-95
11. 14-week oral toxicity study in the rat
Submitted under IND 48620/000; review dated 9-8-95
12. Rising dose tolerance study via oral administration of NTZ in dogs
Submitted under IND 48620/000; review dated 9-8-95
13. 28-day oral toxicity study of NTZ in dogs
Submitted under IND 48620/000; review dated 9-8-95
14. 90-day toxicity study in dogs with nitazoxanide
Submitted under IND 48620/019; review dated 6-9-98
15. 6-month oral toxicity study in rats
Submitted under IND 48620/029; review dated 6-1-98

REPRODUCTIVE TOXICOLOGY**Reproductive Toxicology Studies**

1. Dose range-finding study for segment II study in rats with NTZ
Study # — 2647-117; study dated 4-1-93
2. Dose range-finding study for segment II study in rabbits with NTZ
Study # — A2647-118; study dated 12-15-93
3. Rabbit developmental toxicity study with nitazoxanide
Study # — 2712-102; study dated 11-19-97
4. Study for the effects of fertility and development in rats with Nitazoxanide
Study # — 2712-104; study dated 12-16-96
5. Study for effects of pre- and postnatal development, including maternal function, in the rat
Study # 6807-100; study dated 10-31-97

Reproductive Toxicology Studies Review

1. Dose range-finding study for segment II study in rats with NTZ
(Study # 2647-117)

Submitted under IND 48620/000; review dated 9-8-95

2. Dose range-finding study for segment II study in rabbits with NTZ
(Study # A2647-118)

Submitted under IND 48620/000; review dated 9-8-95

3. Rabbit developmental toxicity study with nitazoxanide
(Study #. 2712-102)

Submitted under IND 48620/019; review dated 6-9-98

4. Study for the effects of fertility and development in rats with nitazoxanide
(Study # 2712-104)

Submitted under IND 48620/019; review dated 6-9-98

5. Study for effects of pre- and postnatal development, including maternal function, in the rat; performed by _____, study no. 6807-100; batch no. 003; 31 October 1997; GLP

NTZ was evaluated for oral toxicity in rats from implantation through weaning, in pregnant and lactating females, and the development of the offspring. Male and female Sprague-Dawley rats, _____, 9 weeks old, were acclimated for one week and then mated. Breeding was confirmed as judged by observation of vaginal sperm or copulatory plug. Mated females were placed in dose groups (25/group). NTZ doses consisted of 0 (vehicle=0.5% methylcellulose), 300, 600, and 1200 mg/kg/day. Dosing was performed daily by oral gavage from gestation day 6 through lactation day 20. Rats were observed twice daily for morbidity and mortality. Body weights were recorded on days 0, 6, 8, 10, 14, 17, and 20 of gestation and on days 0, 4, 7, 10, 14, 17 and 21 of lactation. Food consumption was measured on gestation days 0-6, 6-8, 8-10, 10-14, 14-17, and 14-20. Females were allowed to deliver and raise their litters to day 21 postpartum. At birth, pups were weighed, sexed, and examined for external abnormalities. On days 0, 4 (precull), 7, 14, and 21 of lactation, litter size and pup weights were recorded. On day 4, litters of greater than 8 pups were culled to eight by randomly removing excess pups to leave four males and four females in each litter. The culled pups were sacrificed and examined for cervical, thoracic, or abdominal visceral abnormalities. Litters were examined daily for abnormal behavior or morbidity. At weaning, 23 males and 23 females were selected from each dose group for mating, with at least one pup/sex from each litter (if possible) without sibling matings. Remaining pups were sacrificed. Mating was arranged at seven weeks with up to 21 days of

cohabitation. Mating was confirmed by observation of vaginal sperm or copulatory plug. Mated females were observed twice daily. Body weights were recorded on days 0, 7, 14, and 20, and on lactation day 0. Natural deliveries followed. On day 0 of lactation, litter size and individual sex and weight were recorded. Pups were examined grossly on lactation days 0 and 1, sacrificed on day 1, and examined for cervical, thoracic, and abdominal visceral abnormalities. The F₀ females were sacrificed following weaning. A gross examination of cervical, thoracic, and abdominal viscera was performed. Uteri, ovaries, and abnormal tissues were collected. The uterus was examined for implantation sites. The F₁ generation was examined for vaginal opening or cleavage of the balanopreputial gland on day 30 and 35 post partum until all pups were positive. Body weight was recorded with this event. Open field activity was recorded on day 22 postpartum and week 5 postweaning. The pupillary reflex was assessed together with the 3 week open field test and with week 3 postweaning water maze and memory tests. F₁ females and males were sacrificed following weaning. A gross examination of cervical, thoracic, and abdominal viscera was performed. Uteri, ovaries, and abnormal tissues were collected from the females. Abnormal viscera and reproductive organs were collected. During the F₀ gestation, one group 4 female was found dead on day 20, with hypoactivity, coldness, and red/black vaginal discharge prior to death.

Red/black vaginal discharge was also seen in one group 3 female. Yellow staining of the fur of the urogenital region was seen throughout in the treated groups. Dark feces was seen in 19 rats in group 3 and 23 in group 4. Four dams were sacrificed due to the poor condition of their litters, one each in group 1 and 3, and two in group 4. The group 1 pups had no milk visible in their stomachs and died on day 5. The group 3 pups had no remarkable observations prior to death on day 4. One litter of group 4 pups was weak, thin, and without milk visible in their stomachs prior to death on day 9. A second group 4 litter also was weak, thin, and without milk visible in their stomachs prior to death on day 4. The dam also was not attending the litter. Group 4 body weight during gestation was significantly lower than controls on days 6-8, 17-20, 0-20, and 6-20, and higher than controls on days 8-10. During lactation, body weights of the treated dams were significantly higher than controls on day 17. Otherwise, group body weights were similar. Food consumption was similar during gestation for all groups except for days 6-8, when treated groups were significantly lower than controls. Pregnancy rates were similar for all groups. Single litters had stillborn pups in each treated group; the livebirth index was group 1= 100 (291/291), group 2=99(226/229), group 3= 99(269/271) and group 4=99.99(263/264). Four litter deaths occurred during lactation, one each in groups 1 and 3 and two in group 4. The weaning index was: group 1, 94; group 2, 99; group 3, 97; and group 4, 86. More pups died, disappeared/eaten, or were sacrificed in extremis in group 4 than the other groups. Covariate adjusted weights for the group 4 pups were significantly lower than controls throughout lactation. Pathology findings in the F₀

dams included one group 2 dam with mottled kidneys, one group 2 dam with uterine hydrometra (neither pregnant); group 3, two pregnant females with dark areas on the lungs, one dam with distended intestines (sacrificed following total litter death); group 4, one dam found dead with dark kidneys, pale and enlarged adrenals, autolyzed fetuses in the uterus, one dam with an enlarged spleen, one nonpregnant and two pregnant females with distended intestines. Gross pathological findings of the pups included empty stomach (one group 1, one group 2, and three group 4). Bright red or dark lungs were seen throughout, with greatest incidence in group 4. Developmental indicators were unaffected by treatment. One group 4 F₁ female was found dead on day 28 postweaning, with hypoactivity, ataxia, wheezing, ruffled fur, urine stains, and few feces prior to death. Bright yellow stained fur was seen sporadically during the first 3 weeks postweaning. Body weights for group 4 pups of both sexes were significantly lower than controls on postweaning days 0, 7, 14, 21, 28, 35, and 42 (males) and 0, 7, 14, and 21 (females). Open field testing, learning, memory, and reversal learning were unaffected by drug treatment. During gestation, no overt clinical signs were seen in the F₁ females. One group 4 litter died. No other overt clinical signs were seen. F₁ female body weight was unaffected during gestation or lactation. Reproductive performance was unaffected. No gross observations were seen at birth in the litters. One F₁ male (group 2) had a liver mass. Dilated renal pelves were seen in males in all groups (group 1=1, group 2= 2, group 3= 2, group 4=1). Hydrometra was seen in three group 1 females, one group 2 female, and three group 3 females. An ovarian cyst was seen in one group 2 female. The group 4 female found dead had a distended urinary bladder with calculi and mottled kidneys with dilated pelves. Food consumption and body weights were particularly sensitive to drug treatment in this study. Toxicities were seen throughout F₀, F₁, and F₂ generations, particularly in the high dose groups. It is unclear whether NTZ is transmitted via lactation or in utero exposure, or whether drug effects were transmitted from generation to generation. The parental NOAEL for this study appeared to be 300 mg/kg/day. It is difficult to determine the F₁ 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. The proposed clinical doses for NTZ are 33-50 mg/kg/day.

GENETIC TOXICOLOGY

Genetic Toxicology Studies

1. Mutagenicity test on NTZ in vivo mammalian micronucleus assay; Study # 15356-0 -455CO; study dated 1-3-94

2. Study of potential mutagenic effects of nitazoxanide using the Ames test with and without metabolic activation
Study # ROM-003; study dated 3-5-92
3. Mutagenicity test with nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay
Study # — 17348-0-409; study dated 5 July 1996
4. Mutagenicity test with desacetyl-nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay
Study # — 17567-0-409; study dated 5 July 1996
5. Mutagenicity test on nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary
Study # — 17348-0-437; study dated 28 June 1996
6. Mutagenicity test on desacetyl-nitazoxanide: measuring chromosomal aberrations in Chinese hamster ovary
Study # — 17567-0-437; study dated 27 June 1996

Genetic Toxicology Studies Review

1. Mutagenicity test on NTZ in vivo mammalian micronucleus assay
Study no. 15356-0-455CO
Submitted under IND 48620/000; review dated 9-8-95
2. Study of potential mutagenic effects of nitazoxanide using the Ames test with and without metabolic activation
Submitted under IND 48620/007; review dated 4-24-96
3. Mutagenicity test with nitazoxanide in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay; performed by _____
_____ study no. — 17348-0-409; batch no. 003; GLP; 5
July 1996

NTZ 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 Aroclor 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 250, 100, 50, 25, 10, and 5 µg 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 10 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 (3.4 fold increase over vehicle control at 100 µg/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

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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 of _____

_____ 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 $\mu\text{g/ml}$; 300, 225, 150, 113, 75, 50, and 25 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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; therefore 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 LLD50 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 than 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 from 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), adactyly (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 F₁ 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.

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:

HFD-590/ADir/RAIbrecht

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 # 48,620

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

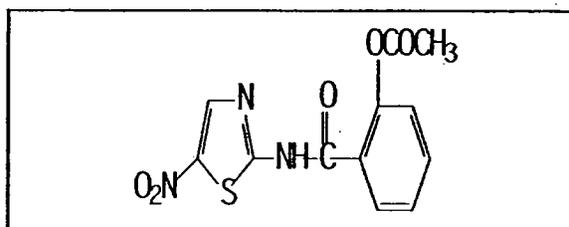
INDICATION: Cryptosporidiosis in AIDS patients

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 ¹⁴C-nitazoxanide following a single oral dose to male rats (Romark Laboratories; performed by _____ study no. — 6613-100; ¹⁴C-NTZ (lot no. 015H9229) and NTZ (lot no. 001); glp; 5/95) Male CD rats received a single oral dose of NTZ/ ¹⁴C-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 n° .2647114 and 272100 (Romark Laboratories, performed by ; 5/95) 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 #1 or _____ No. 2970; 12/78; not glp)

B2. Acute oral toxicity study in mice (_____ , batch #1 or _____ 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. (_____ oatch #1 or _____ 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 (_____ oatch 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 (_____ performed by _____ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 effects 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 _____ 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 areas 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, antipentylentetrazol 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 10 µg/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 \leq 200 cells/mm³, 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 \geq 1 month, and ability tolerate food by mouth; exclusion: presence of *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Giardia lamblia*, *Entamoeba histolytica*, *Microsporidia*, *Isospora*, *Cyclospora*, or *Clostridium difficile* toxin in stool, *Mycobacterium avium* infection, intestinal Kaposi's sarcoma, Cytomegalovirus 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, Kamofsky 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. Deacylation 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 ¹⁴C-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.

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 # 48,620/007

DATE SUBMITTED: 21 March 1996

DATE RECEIVED: 27 March 1996

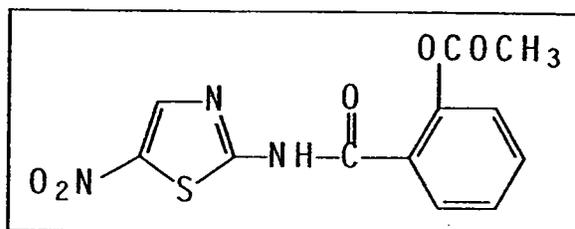
DATE ASSIGNED: 2 April 1996

DATE REVIEW COMPLETED: 24 April 1996

SPONSOR: Unimed Pharmaceuticals, Inc.

DRUG: Nitazoxanide

RELATED DOCUMENTS: IND 48,620/000



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 ¹⁴C-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 ¹⁴C-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 —

— ; 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.

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 # 48,620/019

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.

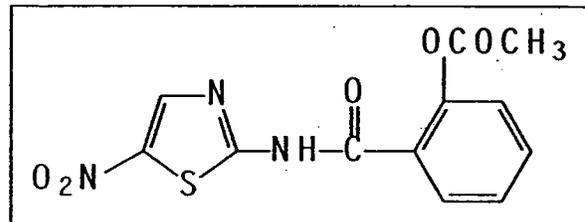
DRUG: Nitazoxanide

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 ophthalmoscopic 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
hematocrit
hemoglobin
mean corpuscular volume
mean corpuscular hemoglobin

white blood cell count
differential blood cell count
nucleated red blood cell count
corrected white blood cell count
segmented neutrophil count

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

clinical chemistry

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

urinalysis

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

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

| | |
|------------------------|---------|
| adrenals | lungs |
| brain | ovaries |
| epididymides | spleen |
| heart | testes |
| kidneys | thymus |
| liver with gallbladder | thyroid |

The following tissues were collected for histopathology:

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

| | |
|---|-----------------|
| esophagus | skin |
| eyes | spinal cord |
| femur | spleen |
| gallbladder | sternum |
| heart | stomach |
| ileum | thymus |
| iliac lymph node | thyroid |
| jejunum | tongue |
| kidneys | trachea |
| liver | urinary bladder |
| lungs with bronchi | uterus |
| lymph nodes (mandibular and mesenteric) | 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. Ophthalmic 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 neutrophil 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 _____
_____ ; study no. _____ 2712-102; batch no. 002; 19 Nov 1997;
GLP

In this study, mated female rabbits (Hra:(NZW) SPF, _____, 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 _____ study no. — 2712-104; batch no. 002; 16 December 1996 (draft date); GLP

Male and female Sprague-Dawley rats _____ (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.

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:

HFD-590/ADir/RAIbrecht
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 # 48,620/029

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.

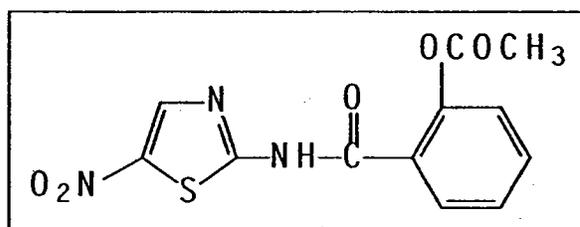
DRUG: Nitazoxanide

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 ophthalmoscopic 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

Hematocrit

Hemoglobin

Mean corpuscular hemaglobin

Mean corpuscular hemaglobin concentration

Mean corpuscular volume

Platelet count

White blood cell count

Differential blood cell count

clinical chemistry

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

urinalysis

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

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 | muscle -thigh |
| Brain with brainstem | Peripheral nerve |
| Cecum | ovaries/testes |
| Cervix | Pancreas |
| Colon | Pituitary |
| Duodenum | Prostate |
| Epididymides | Rectum |
| Esophagus | salivary glands |
| Eyes + optic nerve/Harderian gland | sciatic nerve |
| Femur | Skin |
| Gallbladder | spinal cord |
| Heart | Spleen |
| Ileum | Sternum |
| Iliac lymph node | Stomach |
| Jejunum | Thymus |
| Kidneys | Thyroid |
| Liver | Tongue |
| Lungs with bronchi | Trachea |
| Lymph nodes (mesenteric) | urinary bladder |
| Mammary glands (females) | 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. Ophthalmoscopic 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 _____, study no. 0831XU13.001; lot no. 003; ¹⁴C-NTZ lot no. CFO 9286; GLP; date not provided

Beagle dogs (3 males, 3 females; _____) 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 91 μCi/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 14 to 34 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.

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:

HFD-590/ADir/RAIbrecht
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
HFD-345/

PHARMACOLOGIST'S REVIEW

IND # 48,620/033

DATE SUBMITTED: 20 November 1997

DATE RECEIVED: 24 November 1997

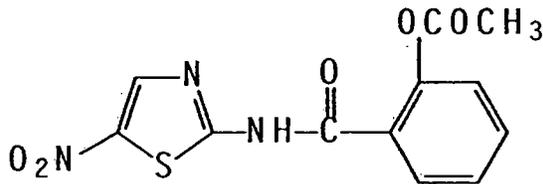
DATE ASSIGNED: 25 November 1997

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 _____ μ g/ml and the limit of quantification in plasma samples was _____ μ g/ml. The following NTZ/desNTZ pharmacokinetic parameters were derived from the plasma samples.

| dose (mg/kg/day) | Cmax (mg/L) | | AUCt (mg.h/L) | |
|---------------------|-------------|-----------|---------------|-----------|
| | 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 _____ study no. 0251XR13.001; batch no. 3; 3 Oct 1996; no GLP statement

Sprague Dawley rats (_____ 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 _____ study no. 025XR13.001; batch no. 3; 3 Oct 1996; no GLP statement

Sprague Dawley rats (_____ 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.

Steven C. Kunder, Ph.D.
Reviewing Pharmacologist

concurrences:

HFD-590/ADir/RAIbrecht
HFD-590/SPharm/KHastings
Steven C. Kunder/Pharm/

disk:

HFD-590/KHastings

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HFD-590 (original)
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HFD-590/EFrank
HFD-590/RRoca
HFD-590/GHolbert
HFD-590/SBala
HF
D-345/

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this page is the manifestation of the electronic signature.**

/s/

Steve Kunder
11/26/02 02:01:19 PM
PHARMACOLOGIST

Kenneth Hastings
11/26/02 02:08:08 PM
PHARMACOLOGIST

Steve Kunder
11/26/02 02:18:49 PM
PHARMACOLOGIST