CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-494

PHARMACOLOGY REVIEW

PHARMACOLOGIST'S REVIEW OF NDA 21-494

(Amendment Dated October 10, 2003)

Reviewer: David B. Joseph, Ph.D.
Pharmacologist (HFD-180)

Sponsor and Address: Reliant Pharmaceuticals, LLC
Liberty Corner, New Jersey

Drug: AXID® Oral Solution

Other Names: nizatidine; LY139037

Category: histamine H_2 receptor antagonist

Date of Submission: Amendment, October 10, 2003

Date of FDR Receipt: Amendment, October 14, 2003

Date of Review: March 29, 2004

Submission Contents:

1. 28-Day Oral Toxicity and Toxicokinetic Study with Fresh and Degraded Nizatidine Oral Solution in Rats

2. 28-Day Oral Toxicity Study with Fresh and Degraded Nizatidine Oral Solution in Rats

3. Bacterial Reverse Mutation Assay with Fresh and Degraded Nizatidine Oral Solution

4. Chromosomal Aberration Assay with Fresh and Degraded Nizatidine Oral Solution in Human Lymphocytes

Introduction:

The original submission of this application, dated April 10, 2002, was deemed as approvable by the Division of Gastrointestinal and Coagulation Drug Products (letter to Sponsor dated February 10, 2003). Among the deficiencies that need to be resolved is the absence of justification for the acceptance criteria for individual and total impurities in the drug product. Nizatidine is relatively unstable in this formulation, resulting in the accumulation of degradation products during storage. The present submission contains preclinical qualification studies for the degradation products.

Studies Reviewed Within This Submission:

Preclinical Study	Testing Laboratory	Study #	Lot #	Page
GENERAL TOXICOLOGY				
28-Day Oral Gavage Toxicity and Toxicokinetic Study with Nizatidine Oral Solution in Rats	[redacted]	7367-103	XA131 (fresh) XA121A (degraded)	2
28-Day Oral Gavage Toxicity Study with Nizatidine Oral Solution in Rats		7367-106	XA131 (fresh) XA121A (degraded)	5
GENETIC TOXICOLOGY				
***Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with Nizatidine Oral Solution and Degraded Nizatidine Oral Solution**		7367-104	XA131 (fresh) XA121A (degraded)	9
Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes		7367-105	XA131 (fresh) XA121A (degraded)	11

Studies Not Reviewed Within This Submission: None

GENERAL TOXICOLOGY:

28-Day Oral Gavage Toxicity and Toxicokinetic Study with Nizatidine Oral Solution in Rats

Key Study Findings: The toxicity portion of this study was not completed due to a dosing error. Toxicokinetic parameters for fresh nizatidine oral solution and degraded nizatidine oral solution were similar.

Study # 7367-103

Vol. 1, 7367-103

Testing Laboratory: [redacted]

Study Dates: 5/22/03-10/7/03

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ( )No

Drug: Fresh Nizatidine Oral Solution (lot # XA131; ——— of stated nizatidine content)
Degraded Nizatidine Oral Solution (lot # XA121A; ——— of stated nizatidine content)
Nizatidine USP powder (lot # 3ANC700006; ——— pure)

Formulation/Vehicle: Fresh and degraded nizatidine oral solutions (i.e. Axid® Oral Solution) were formulated to contain 15 mg/ml nizatidine. The vehicle is described in the chemistry review of this application. Nizatidine USP powder was administered as a solution containing 15 mg/ml, dissolved in deionized water ———————

Animals: — CD®(SD)IGS BR rats, approximately 7 weeks of age
Males: 222-278 g
Females: 153-188 g

METHODS: This study was designed to compare the toxicity and toxicokinetic profile of degraded nizatidine oral solution to that of fresh nizatidine oral solution at a single dose level, when administered for 28 days. Rats were treated orally with 200 mg/kg/day nizatidine using fresh nizatidine oral solution, degraded nizatidine oral solution, or nizatidine USP powder in solution as an additional comparator. The study groups are listed in the following table.

Group #	Dose (mg/kg/day)	Formulation	# Rats/Sex
	Toxicity Groups		
1	0	Placebo*	10
2	200	Fresh Nizatidine Oral Solution	10
3	200	Degraded Nizatidine Oral Solution	10
4	200	Nizatidine USP Powder in Solution	10
	Toxicokinetic Groups		
5	200	Fresh Nizatidine Oral Solution	12
6	200	Degraded Nizatidine Oral Solution	12
7	200	Nizatidine USP Powder in Solution	12

* Vehicle for Nizatidine Oral Solution.

Dosing was performed twice daily, such that the drug-treated groups were administered 100 mg/kg/day b.i.d. The two daily doses were separated by 8 hr. The study groups were treated via gavage, using a dose volume of 6.7 ml/kg/dose. Due to a dosing error on day 17 (group 3 rats were treated with the incorrect formulation), the toxicity portion of this study was terminated. All toxicity groups (# 1-4) were sacrificed on day 18 without necropsy or collection of blood or tissue samples. The toxicokinetic groups were treated for 28 days. The basis of dose selection was not stated. The following parameters were recorded:

Clinical Signs: twice daily

Bodyweight: once weekly starting on day 1

Food Consumption: weekly

Ophthalmoscopy: pre-study

Toxicokinetics: blood was collected at 0.25, 0.5, 1, 2, 4, 8, 10, and 24 hr after the initial daily dose on days 1 and 28; plasma drug concentrations were measured using HPLC/MS

RESULTS:

Mortality: One male in group 6 (a toxicokinetic group) died on day 6. Blood was collected twice from this animal on day 1. A necropsy was not performed.

Clinical Signs: None.

Bodyweight: Weight gain was not affected in the drug-treated groups. The mean bodyweight in the control males and females was 242 ± 11.9 g and 170 ± 7.6 g, respectively, on day 1, and 337 ± 17.8 g and 213 ± 12.5 g, respectively, on week 3.

Food Consumption: Food intake was increased by 11% and 18% in the group 4 females on weeks 1 and 2, respectively.

Toxicokinetics: Plasma kinetic parameters are shown in the following table.

		First Daily Dose		Second Daily Dose			
Formulation	**Sex**	**t_{max} (hr)**	**C_{max} (ng/ml)**	**t_{max} (hr)**	**C_{max} (ng/ml)**	**AUC_{0-t} (ng•hr/ml)**	**$AUC_{0-8\ hr}$ (ng•hr/ml)**
	Day 1						
Fresh Nizatidine Oral Solution	M	1.0	3030	2.0	3023	14599	11122
	F	0.5	3470	2.0	4867	20864	15110
Degraded Nizatidine Oral Solution	M	2.0	2540	2.0	3247	16588	12917
	F	2.0	3933	2.0	4350	19294	14668
Nizatidine USP Powder in Solution	M	0.5	4207	2.0	4533	21045	16020
	F	0.5	5717	2.0	3993	21870	17745
	Day 28						
Fresh Nizatidine Oral Solution	M	2.0	4043	2.0	3973	17823	13796
	F	1.0	5513	2.0	5480	23571	18091
Degraded Nizatidine Oral Solution	M	1.0	3920	2.0	3517	19407	15494
	F	1.0	6487	2.0	4610	21162	16552
Nizatidine USP Powder in Solution	M	0.5	8120	2.0	3850	19938	16088
	F	0.25	13840	2.0	5280	29647	24325

Values were determined from 3 rats/sex/group/time-point.

The $t_{1/2}$ values were not provided. However, the elimination of drug from plasma occurred rapidly with each formulation. The plasma drug concentrations at 8 hr after the initial daily dose were a small fraction of the peak concentrations. No drug was detected in any group at 24 hr

after the initial daily dose. Each of the plasma kinetic parameters observed with degraded nizatidine oral solution was similar to those observed with fresh nizatidine oral solution. After 28 days of administration, substantial increases in the C_{max} values for the initial daily dose were observed with each formulation. Slight increases in the AUC values were also observed on day 28. These results may be indicative of plasma accumulation. For each formulation, peak drug levels and plasma exposure were slightly higher in females. Nizatidine USP powder in solution produced higher C_{max} and AUC values than either fresh or degraded nizatidine oral solution.

Conclusions: The toxicokinetic parameters for fresh nizatidine oral solution and degraded nizatidine oral solution were similar at the single dose level tested in this study (100 mg/kg b.i.d). The results indicate that plasma accumulation of drug may have occurred during the 28 days of administration. No conclusions about the relative toxicity of fresh and degraded nizatidine oral solutions can be derived from this study, since the toxicity portion of this study was terminated prematurely without necropsy. However, there were no differences in the in-life parameters associated with the fresh and degraded oral solutions.

28-Day Oral Gavage Toxicity Study with Nizatidine Oral Solution in Rats

Key Study Findings: no signs of toxicity in any group

Study # 7367-106

Vol. 2, 7367-106

Testing Laboratory: [redacted]

Study Dates: 7/2/03-10/6/03

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ( )No

Drug: Fresh Nizatidine Oral Solution (lot # XA131; [redacted] of stated nizatidine content)
Degraded Nizatidine Oral Solution (lot # XA121A; [redacted] of stated nizatidine content)
Nizatidine USP powder (lot # 3ANC700006; [redacted] pure)

Formulation/Vehicle: Fresh and degraded nizatidine oral solutions (i.e. Axid® Oral Solution) were formulated to contain 15 mg/ml nizatidine. The vehicle is described in the chemistry review of this application. Nizatidine USP powder was administered as a solution containing 15 mg/ml, dissolved in deionized water [redacted]

Animals: [redacted]CD®(SD)IGS BR rats, approximately 7 weeks of age
Males: 195-242 g
Females: 165-197 g

METHODS: This study was designed to compare the toxicity of degraded nizatidine oral solution to that of fresh nizatidine oral solution at a single dose level. Rats were treated orally with 200 mg/kg/day nizatidine for 29 days using fresh nizatidine oral solution, degraded nizatidine oral solution, or nizatidine USP powder in solution as an additional comparator. The study groups are listed in the following table.

Group #	Dose (mg/kg/day)	Formulation	# Rats/Sex
1	0	Placebo*	10
2	200	Fresh Nizatidine Oral Solution	10
3	200	Degraded Nizatidine Oral Solution	10
4	200	Nizatidine USP Powder in Solution	10

* Vehicle for Nizatidine Oral Solution.

Dosing was performed twice daily, such that the drug-treated groups were administered 100 mg/kg/day b.i.d. The two daily doses were separated by 8 hr. The study groups were treated via gavage, using a dose volume of 6.7 ml/kg/dose. The basis of dose selection was not stated. The following parameters were recorded:

Clinical Signs: twice daily

Bodyweight: once weekly starting on day 1

Food Consumption: weekly

Ophthalmoscopy: pre-study and on week 4

Hematology: blood was collected at the scheduled sacrifice

Clinical Chemistry: blood was collected at the scheduled sacrifice

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary (mandibular) glands, seminal vesicles, spleen, testes, thymus, thyroids/parathyroids, uterus; paired organs were weighed together

Gross Pathology: at necropsy (day 30)

Histopathology: The following organs/tissues were examined in groups 1 (vehicle) and 3 (degraded oral solution): adrenals, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur (with bone marrow), gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, lymph node (mesenteric), mammary gland, ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum (with bone marrow), stomach, testes, thymus, thyroids/ parathyroids, tongue, trachea, urinary bladder, uterus, vagina. In groups 2 (fresh oral solution) and 4 (nizatidine powder in solution), examination was limited to gross lesions, heart, liver,

lungs, and kidneys. The selection of organs for examination in groups 2 and 4 includes all target organs of toxicity (i.e. liver and kidneys) that were identified in previous toxicology studies of nizatidine. For the purpose of the present study, the limited selection of organs for examination in groups 2 and 4 is acceptable.

Adequate Battery: (x)Yes ( )No
Peer Review: ( )Yes (x)No

RESULTS:

Mortality: No deaths occurred.

Clinical Signs: Clear oral discharge was observed in 1/20 rats in group 4. Alopecia in the abdominal area occurred in group 2 (1/20 rats).

Bodyweight: Weight gain was not affected in any of the treatment groups. The mean bodyweight in the control males and females was 218 ± 14.3 g and 183 ± 7.0 g, respectively, on day 1, and 396 ± 29.4 g and 250 ± 11.0 g, respectively, at study termination.

Food Consumption: Food intake was increased by 12-22% in group 4 on each week of treatment.

Ophthalmoscopy: No lesions were observed.

Electrocardiography: Not performed.

Hematology: No effects were observed. PT (prothrombin time) and APTT (activated partial thromboplastin time) were not affected.

Clinical Chemistry: No parameters were affected.

Urinalysis: Not performed.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Adrenals: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 16%, 13%, and 10%, respectively, in the group 4 males (absolute weight change was significant).

Lungs: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 20%, 17%, and 17%, respectively, in the group 2 males (not significant). Organ/brain weight ratio was reduced by 11% in the group 3 males (not significant).

Pituitary: Organ/bodyweight ratio and organ/brain weight ratio was decreased by 12% in the group 4 males (not significant).

Prostate: Absolute weight and organ/bodyweight ratio was increased by 11% and 10%, respectively, in the group 2 males (not significant). Organ/brain weight ratio was reduced by 12% in the group 4 males (not significant).

Salivary Glands (mandibular): Absolute weight was reduced by 11% in the group 4 females.

Seminal Vesicles: Weight was increased in all treatment groups. The results are shown in the following table. None of these changes were statistically significant.

	SEMINAL VESICLES		
Group	**Absolute Weight (g)**	**Organ/Bodyweight (%)**	**Organ/Brain Weight**
1	1.02 ± 0.19	0.285 ± 0.070	0.502 ± 0.091
2	1.29 ± 0.36 (26)	0.346 ± 0.092 (21)	0.617 ± 0.172 (23)
3	1.17 ± 0.35 (15)	0.321 ± 0.095 (13)	0.560 ± 0.173 (11)
4	1.32 ± 0.32 (29)	0.353 ± 0.095 (24)	0.613 ± 0.140 (22)

Values are the mean ± S.D. of 10 rats.
% Increase relative to the control value (group 1) is shown in ( ).

Thyroids/Parathyroids: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased in the group 2 and group 4 males (not significant). These changes are shown in the table below.

	THYROIDS/PARATHYROIDS (males)		
Group	**Absolute Weight (g)**	**Organ/Bodyweight (%)**	**Organ/Brain Weight**
1	0.018 ± 0.004	0.005 ± 0.001	0.009 ± 0.001
2	0.022 ± 0.004 (22)	0.0059 ± 0.0021 (18)	0.0104 ± 0.0018 (15)
3	0.019 ± 0.005 (6)	0.005 ± 0.001 (0)	0.009 ± 0.002 (0)
4	0.022 ± 0.005 (22)	0.0058 ± 0.0012 (16)	0.0103 ± 0.0026 (14)

Values are the mean ± S.D. of 10 rats.
% Increase relative to the control value (group 1) is shown in ( ).

Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were slightly reduced in the group 4 females (not significant). These results are shown in the table below.

	THYROIDS/PARATHYROIDS (females)		
Group	**Absolute Weight (g)**	**Organ/Bodyweight (%)**	**Organ/Brain Weight**
1	0.017 ± 0.004	0.0074 ± 0.0020	0.0085 ± 0.0022
2	0.016 ± 0.002 (-6)	0.0071 ± 0.0010 (-4)	0.0083 ± 0.0013 (-2)
3	0.015 ± 0.004 (-12)	0.0070 ± 0.0022 (-5)	0.0079 ± 0.0012 (-7)
4	0.014 ± 0.002 (-18)	0.0065 ± 0.0012 (-12)	0.0075 ± 0.0012 (-12)

Values are the mean ± S.D. of 10 rats.
% Change relative to the control value (group 1) is shown in ( ).

Uterus: Organ/bodyweight ratio (expressed as %bodyweight) and organ/brain weight ratio were increased by 12% and 11%, respectively, in the group 4 females (not significant). The control values for %bodyweight and organ/brain ratio were 0.269 ± 0.053% and 0.313 ± 0.067, respectively.

Gross Pathology: No treatment-related effects were observed.

Histopathology: The incidence of lesions that occurred only in the drug-treated groups was extremely limited. These lesions were observed in heart (mineralization of vascular wall in group 2, degeneration in group 4), kidney (dilatation of tubules in group 2), liver (focal necrosis in group 4), pancreas (chronic focal inflammation in group 3), and skin (acanthosis and hyperkeratosis in group 3, ulceration, acanthosis, and inflammation in group 4). In each case, the incidence was 1/20 rats in the affected group(s). None of these effects are considered as treatment-related.

Toxicokinetics: Not performed (see study # 7367-103 in this review for supporting information).

Conclusions: No differences in the toxicity profiles for fresh nizatidine oral solution and degraded nizatidine oral solution were detected at 200 mg/kg/day (100 mg/kg b.i.d.), the only dose tested in this study. No signs of toxicity were observed in any of the drug-treated groups. Since only one dose was tested, this study provides only limited information about the toxicity profiles for the fresh and degraded nizatidine oral solutions.

GENETIC TOXICOLOGY:

***Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with Nizatidine Oral Solution and Degraded Nizatidine Oral Solution**

Key Findings: negative

Study # 7367-104

Vol. 1, 7367-104

Testing Laboratory: [redacted]

Study Dates: 7/8/03-10/9/03

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ( )No

Drug: Fresh Nizatidine Oral Solution (lot # XA131; [redacted] of stated nizatidine content)
Degraded Nizatidine Oral Solution (lot # XA121A [redacted] of stated nizatidine content)

Vehicle: nizatidine oral solution vehicle (see chemistry review of this application for description)

Methods: The objective of this study was to compare the mutagenic potential of fresh nizatidine oral solution to that of degraded nizatidine oral solution using the Ames test (Ames et al., Mutation Res, 31, 1975). The histidine-dependent *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537, as well as *E. coli* WP2*uvr*A (tryptophan-dependent) were tested. A preliminary assay was performed on strains TA-100 and WP2*uvr*A. These bacterial strains were treated with fresh or degraded nizatidine oral solution, using dose levels of 6.67, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/plate nizatidine, in the absence or presence of S9 liver fraction (1 plate/dose). In addition, the vehicle was tested at concentrations of 50, 100, 200, 300, and 400 µl/plate. The plate incorporation method was used for this assay. The plates were incubated for 52 ± 4 hr at 37°C. The number of revertant colonies was counted, and the bacterial background lawn growth was evaluated for assessment of toxicity. In the main assay, the bacterial strains were treated with fresh or degraded nizatidine oral solution, using dose levels of 1, 3.33, 10, 33.3, 100, 333, 1000, and 5000 µg/plate nizatidine, in the absence or presence of S9 liver fraction (3 plates/dose). The plate incorporation method was used for this assay, which was performed in the same manner as the preliminary assay. A confirmatory assay was performed using nizatidine dose levels of 100, 333, 1000, 3330, and 5000 µg/plate. The positive control compounds that were used in the main assay are listed in the following table.

Tester Strain	- S9	+ S9
TA-98	2-nitrofluorene, 1 µg/plate	benzo[a]pyrene, 2.5 µg/plate
TA-100	sodium azide, 2 µg/plate	2-aminoanthracene, 2.5 µg/plate
TA-1535	sodium azide, 2 µg/plate	2-aminoanthracene, 2.5 µg/plate
TA-1537	ICR-191, 2 µg/plate	2-aminoanthracene, 2.5 µg/plate
E. coli WP2*uvr*A	4-nitroquinolone-N-oxide, 1 µg/plate	2-aminoanthracene, 25 µg/plate

The vehicle used for the positive control compounds was not stated. Revertant colonies were counted by an automated colony counter or by hand. The S9 liver fraction was purchased from a commercial vendor, and was prepared from male Sprague Dawley rats treated with 500 mg/kg i.p. Aroclor 1254. The vehicle control (400 µl, equal to the volume of test article) was added to the negative control cultures. The criteria for a valid assay were the following: all *Salmonella* strains exhibited sensitivity to crystal violet, confirming the presence of the *rfa* mutation; TA-98 and TA-100 exhibited resistance to ampicillin, confirming the presence of the pKM101 plasmid; the number of revertants in the vehicle control plates were within the range of historical control values; the cell densities of all test strain cultures were at least 0.5 x 10^9/ml; the number of revertants in each bacterial strain was increased by at least 3-fold in the presence of the positive control compounds; a minimum of three non-toxic doses were used. The criteria for a positive response were the following: for strains TA-98, TA-100, and WP2*uvr*A, the test article produced a dose-dependent increase of at least 2-fold in the number of revertants; for strains TA-1535 and TA-1537, the test article produced a dose-dependent increase of at least 3-fold in the number of revertants.

Results: In the preliminary assay, background lawn growth was unaffected by either fresh or degraded nizatidine oral solution. The number of revertant colonies was not affected by either

solution. In the initial and confirmatory mutagenicity assays, neither the fresh nor the degraded nizatidine oral solution produced substantial increases in the number of revertants. Again, no sign of toxicity was observed. The positive control compounds produced the expected increases in the number of revertants. All criteria for a valid assay were fulfilled. It is noteworthy that the actual dose levels of the fresh and degraded solutions in the confirmatory mutagenicity assay were substantially lower than intended, presumably due to a dilution error. Analysis of the solutions used in the confirmatory assay showed that the nizatidine concentrations were only 20-30% of the intended concentration for the following dose levels: 100, 333, 1000, and 3330 µg/plate. However, the drug concentrations in the fresh and degraded solutions used for the highest dose (5000 µg/plate) in the confirmatory assay were approximately equal to the intended concentration.

Conclusions: Fresh nizatidine oral solution and degraded nizatidine oral solution were negative under the assay conditions.

Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Key Findings: negative

Study # 7367-105

Vol. 2, 7367-105

Testing Laboratory: [redacted]

Study Dates: 7/8/03-10/7/03

GLP Compliance: A statement of compliance was included.

QA Report: (x)Yes ( )No

Drug: Fresh Nizatidine Oral Solution (lot # XA131; —— of stated nizatidine content)
Degraded Nizatidine Oral Solution (lot # XA121A; —— of stated nizatidine content)

Vehicle: nizatidine oral solution vehicle (see chemistry review of this application for description)

Methods: The objective of this study was to compare the clastogenic potential of fresh nizatidine oral solution to that of degraded nizatidine oral solution. Blood was obtained from healthy human donors. Lymphocyte cultures were initiated by adding 0.6 ml of blood to 8.9 ml RPMI 1640 complete medium supplemented with 20% heat-inactivated FBS and 2% phytohemagglutinin-M (8.75 ml medium was used for cultures that would be incubated with S9 liver fraction). The cultures were incubated at 37°C for two days. A preliminary toxicity assay was not conducted. In the chromosome aberration assay, lymphocytes were incubated with fresh or

degraded nizatidine oral solution in a volume of 10 ml for 3 hr in the absence or presence of S9 liver fraction, or for 22 hr in the absence of S9 liver fraction (duplicate cultures). For each treatment procedure, the nizatidine dose levels were 257, 368, 525, and 750 µg/ml. The control groups included vehicle-treated cultures and untreated cultures (duplicate cultures). All nizatidine dose levels were evaluated for chromosomal aberrations. Mitomycin C was used as a positive control in the absence of S9 (1 µg/ml in the 3-hour treatment, 0.3 µg/ml in the 22-hour treatment, duplicate cultures). Cyclophosphamide (25 µg/ml) was used as a positive control in the presence of S9 in the 3-hour treatment assay (duplicate cultures). The incubation procedures for the 3-hour and 22-hour treatments are described in the following table.

Without S9			
1. 3 hr incubation with test article or control articles	**2.** Cells were washed and resuspended in fresh medium; incubation was continued for 17 hr	**3.** 0.1 µg/ml Colcemid® was added to medium and incubation continued for 2 hr	**4.** Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations
1. 20 hr incubation with test article or control articles	**2.** 0.1 µg/ml Colcemid® was added to medium and incubation continued for 2 hr	**3.** Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations	
With S9			
1. 3 hr incubation with test article or control articles	**2.** Cells were washed and resuspended in fresh medium; incubation was continued for 17 hr	**3.** 0.1 µg/ml Colcemid® was added to medium and incubation continued for 2 hr	**4.** Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations

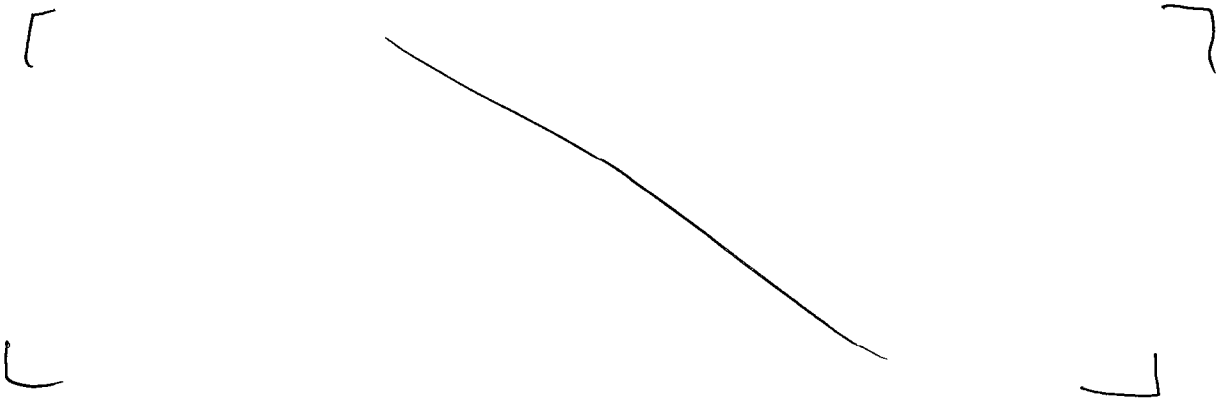
For toxicity assessment, the mitotic index was calculated based on the number of mitotic cells per 1000 cells in the untreated control, vehicle control, and 750 µg/ml nizatidine cultures. The 525 µg/ml concentration was also assessed in the 22-hr treatment. The cells in metaphase (100/culture) were examined for chromosomal aberrations. The incidence of polyploid cells and endoreduplication per 100 metaphase cells was also determined. The S9 liver fraction was prepared from rats treated with 500 mg/kg Aroclor 1254. The assay was considered as valid based on the following criteria: the incidence of cells with structural chromosomal aberrations in the untreated and vehicle control cultures was less than 5%; the proportion of cells with structural chromosomal aberrations in the positive control cultures was increased in a statistically significant manner, relative to the vehicle control; at least three drug concentrations were suitable for evaluation. The criteria for a positive response were a statistically significant increase in the number of cells with aberrations at one or more dose levels (relative to vehicle control cultures), and evidence of dose-dependency. The test article was considered as negative if no significant increase in the number of cells with aberrations occurred. Results that failed to meet the criteria for a positive or negative response were considered as equivocal.

Results: No significant increase in chromosomal aberrations occurred in the presence of either fresh or degraded nizatidine oral solution. In the cultures treated with fresh nizatidine oral

solution, the highest drug concentration (750 µg/ml) produced no toxicity under any treatment conditions, as indicated by the mitotic index. However, the degraded solution produced a 28% reduction in mitotic index at the same concentration in the presence of S9. Generally, the maximum drug concentration used in this assay should produce a reduction of at least 50% in the mitotic index. However, the highest nizatidine concentration in this study should be considered as the maximum feasible dose, given that the undiluted oral solution contains 15 mg/ml (0.5 ml of drug solution was added to 9.5 ml of cultured lymphocytes). Therefore, the dose selection for this assay is acceptable. The positive control compounds produced the expected increases in chromosomal aberrations. The study was considered as valid based on the stated criteria.

Conclusions: Fresh nizatidine oral solution and degraded nizatidine oral solution were negative under the assay conditions. However, the degraded solution produced greater toxicity than the fresh solution at the highest concentration in the presence of S9 liver fraction, based on the reduction in mitotic index.

LABELING:



SUMMARY AND EVALUATION:

The original submission of this application, dated April 10, 2002, was deemed as approvable by the Division of Gastrointestinal and Coagulation Drug Products (letter to Sponsor dated February 10, 2003). Among the deficiencies that need to be resolved is the absence of justification for the acceptance criteria for individual and total impurities in the drug product. Nizatidine is relatively unstable in this formulation. During storage, the concentration of nizatidine may decrease by approximately —. relative to the original concentration. The rate of degradation is dependent on temperature. The Sponsor stated that the appearance of degradation products is also due to decomposition of the excipients methylparaben and propylparaben. Based on the Sponsor's proposed acceptance criteria and the maximum recommended dose, the daily intake of total impurities from a "degraded" product could be as much as 15 mg of nizatidine degradation products — of methylparaben degradation products, and — of propylparaben degradation products. The structures of these degradation products have not been characterized or identified.

The present submission contains preclinical qualification studies for the degradation products that accumulate in this formulation during storage. These studies include the following: a comparative 28-day oral toxicity study of fresh and degraded solutions in rats, a bacterial reverse mutation assay using fresh and degraded solutions, and a chromosomal aberration assay in human lymphocytes using fresh and degraded solutions. The submitted studies are consistent with the qualification studies that are recommended in the ICH guidance Q3B entitled "Impurities in New Drug Products" (November 1997).

A 28-day oral toxicity study in rats was conducted using fresh nizatidine oral solution and degraded nizatidine oral solution. Nizatidine USP powder in solution was also tested as a comparator. A single dose level of 200 mg/kg/day (100 mg/kg b.i.d.) was tested for each formulation. No signs of toxicity were observed in any of the drug-treated groups. Since only one dose was tested, this study provides only limited information about the toxicity profiles for the fresh and degraded nizatidine oral solutions. To obtain a more useful comparison of the toxicity profiles, at least three dose levels for each formulation would be needed, such that toxicity would occur in at least one dose group. However, the dose selection in this study was limited by the use of the drug product, which contains a single fixed concentration of nizatidine (15 mg/ml). Administration of the drug product, rather than a preclinical formulation with multiple drug concentrations, was necessary for the purpose of this study. Dose selection was likely based on the maximum dose volume that can be safely administered to rats, which is generally considered to be 10 ml/kg/dose. Therefore, the maximum feasible dose for the drug product in rats could be estimated as 300 mg/kg/day, assuming that the product is administered twice daily. In the present study, the rats were dosed twice daily using 6.7 ml/kg/dose, resulting in a total daily dose of 200 mg/kg/day. Therefore, the dose selection is considered as adequate, since the dose used was almost as high as the maximum feasible dose. It is noteworthy that a 3-month dietary study of nizatidine in rats yielded a NOAEL (no observed adverse effect level) of 740 mg/kg/day in males and 805 mg/kg/day in females (Pharmacologist's Review of NDA 19-508 dated November 17, 1987). Based on these results, the nizatidine dose level used in the present study would not be expected to produce toxicity.

Although dose selection in the 28-day oral toxicity study in rats was limited due to the use of the drug product, the study does provide useful information for safety assessment of degraded nizatidine oral solution. The NOAEL in this study was 200 mg/kg/day (100 mg/kg b.i.d.), a dose that includes nizatidine and all its degradation products. The maximum recommended human dose is 300 mg/day (once daily or 150 mg b.i.d.), which is equal to 6 mg/kg/day in a 50-kg individual. Thus, the NOAEL for degraded nizatidine oral solution in the 28-day rat study exceeded the maximum human dose by 33-fold. In rats treated with degraded nizatidine oral solution, the dose of nizatidine degradation products was approximately 24.4 mg/kg/day, based on information provided from product analysis (i.e. the reduction of nizatidine concentration during storage). In humans, the maximum daily dose of nizatidine degradation products is estimated to be 15 mg, or 0.3 mg/kg/day in a 50-kg individual. Therefore, the dose of nizatidine degradation products at the NOAEL in the 28-day rat study exceeded the maximum potential human dose by approximately 81-fold. These results suggest that the nizatidine degradation products which accumulate in Axid® Oral Solution do not present a significant risk of toxicity in humans at the recommended dose levels. The same conclusion also applies to the degradation products of methylparaben and propylparaben. The estimated dose of methylparaben

degradation products in the 28-day rat study, 3.3 mg/kg day, exceeded the estimated maximum human dose (0.1 mg/kg/day in a 50-kg individual) by a factor of 33. The estimated dose of propylparaben degradation products in the 28-day rat study, 0.08 mg/kg/day, exceeded the estimated maximum human dose (0.01 mg/kg/day in a 50-kg individual) by a factor of 8.

The mutagenic potential of fresh nizatidine oral solution and degraded nizatidine oral solution was compared using the bacterial reverse mutation assay (i.e. Ames test). Both the fresh and degraded solutions tested negative. The fresh and degraded solutions were also tested in the chromosomal aberration assay in human lymphocytes, for comparison of clastogenic potential. Both the fresh and degraded solutions were negative in this assay. The negative results that occurred in these assays are consistent with previous genetic toxicology studies of nizatidine. Each of these studies provided an adequate comparison of the mutagenic or clastogenic potential of the fresh and degraded solutions.

The proposed stability specifications for Axid® Oral Solution include the following acceptance criteria:

Test	Acceptance Criteria
Nizatidine Assay	—— of labeled amount
Related Substances (impurities)	Unidentified Individual: —— Total of Unidentified: ——
Methylparaben Assay	—— of labeled amount
Propylparaben Assay	—— of labeled amount

* Relative to amount of drug substance

The same lot of degraded nizatidine oral solution (lot # XA121A) was used in each of the submitted qualification studies. This lot contained 87% of the labeled nizatidine concentration (88.5% of the original nizatidine concentration as measured by HPLC), a level that is below the lower limit of the acceptance criterion. The original level of total impurities (excluding the degradation products of methylparaben and propylparaben) in the degraded nizatidine oral solution was 0.32%. At the time of the qualification studies, the level of total impurities in the degraded solution had increased to 4.6%, which is close to the acceptance criterion of 5.0%. In addition, the degraded solution contained two individual impurities at levels which exceeded the acceptance criterion of 2.0%. The concentration of methylparaben and propylparaben was 75.2% and 81.9% of the labeled amount, respectively, at the time of the qualification studies. Both of these values are within the acceptance criteria, but are close to the lower limits. Clearly, the degraded drug product that was used in the qualification studies was sufficiently degraded to justify its use, given the proposed acceptance criteria in the stability specifications. The fresh nizatidine oral solution (lot # XA131) that was used as a comparator in each study contained concentrations of nizatidine, methylparaben, and propylparaben that were close to the labeled concentrations.

RECOMMENDATIONS:

The total degradation products that are present in the Sponsor's drug product should be considered as qualified material, with respect to the proposed acceptance criteria. From a preclinical viewpoint, the application is recommended for approval with a provision that the labeling will be changed as described in the Labeling section.

David B. Joseph, Ph.D.
Pharmacologist, HFD-180

Date

Comment:

Jasti B. Choudary, B.V.Sc., Ph.D.
Supervisory Pharmacologist, HFD-180

Date

cc:
Orig NDA 21-494
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Joseph
HFD-180/Dr. Frankewich
HFD-180/Dr. Zhou

R/D Init.: J. Choudary 3/19/04

DJ/dbj: 3/29/04
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David Joseph
3/29/04 03:20:00 PM
PHARMACOLOGIST

Jasti Choudary
4/1/04 02:08:45 PM
PHARMACOLOGIST