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RESEARCH**

APPLICATION NUMBER:

21-361

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY REVIEW

NDA number 21-361

Review number 002

Sequence number/date/type of submission 000/25 Nov 2003

Information to sponsor Yes () No (x)

Sponsor and/or agent Salix Pharmaceuticals, Inc

Manufacturer for drug substance

Reviewer name S Kunder

Division name Special Pathogen and Immunologic Drug Products

HFD # 590

Review completion date 22 April 2004

Drug

Trade name Lumenax, —

Generic name Rifaximin

Code name L 105

Chemical name (2S, 16Z, 18E, 20S, 21S, 22R, 23R, 24R, 25S, 26S, 27S, 28E)-5, 6, 21, 23, 25-pentahydroxy-27-methoxy-2, 4, 11, 16, 20, 22, 24, 26-octanethyl-2, 7-(epoxypentadeca[1,11,13]trienimino) benzofuro[4,5-e]pyrido[1,2-•]benzimidazole-1,15(2H)-dione,25-acetate

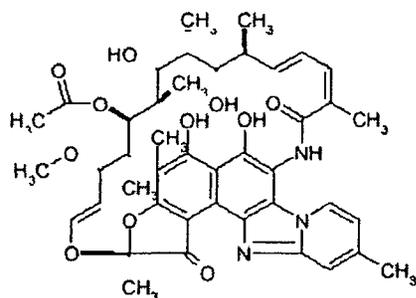
[2S-[2R*,16Z,18E,20R*,22S*,23S*,23S*,24S*,25R*-26S*,27R*,28E)]-25-(Acetyloxy)-5,6,21,23-ttradroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-2,7-[epoxypentadeca[1,11,13]-trienimino]benzofuro[4,5-e]pyrido[1,2-•]benzimidazole-1,15(2H)-dione

CAS registry number 80621-21-4

Mole file number not provided

Molecular formula/molecular weight C₄₃ H₅₁ N₃ O₁₁ 785 89

Structure



Drug class non-aminoglycoside, semi-synthetic derivative of rifamycin SV

Indication traveler's diarrhea

Clinical formulation 200 mg tablet

Route of administration oral

Proposed clinical protocol 200 mg tablet, three times daily, for three days (10 mg/kg/day)

Previous clinical experience Rifaximin has been in use since 1985 in Italy. Since 1985, rifaximin has been approved for use in 12 other countries for indications including acute and chronic intestinal infections due to Gram negative or Gram positive bacteria, diarrheic syndrome, traveler's diarrhea, pre- and post-operative prophylaxis for gastrointestinal surgery, and as a coadjuvant in treatment of hyperammonia. This present submission is a resubmission.

Executive Summary

Recommendation on Approvability

Rifaximin was previously submitted for use in traveler's diarrhea. Preclinical studies have not demonstrated toxicities of clinical concern. The apparent lack of systemic exposure by oral administration in both preclinical and clinical studies appears to limit toxicity, therefore the submission is acceptable regarding pharmacology/toxicology issues. Refer to the previous review of NDA 21-361 for pharmacology/toxicology studies. There are no new pharmacology/toxicology issues, therefore this submission is approvable with respect to pharmacology/toxicology.

SC Kunder PhD, Pharmacology/toxicology reviewer

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/s/

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PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number 21-361

Review number 001

Sequence number/date/type of submission 001/21 Dec 2001

Information to sponsor Yes () No (x)

Sponsor and/or agent Salix Pharmaceuticals Inc

Manufacturer for drug substance

Reviewer name S Kunder

Division name Special Pathogen and Immunologic Drug Products

HFD # 590

Review completion date

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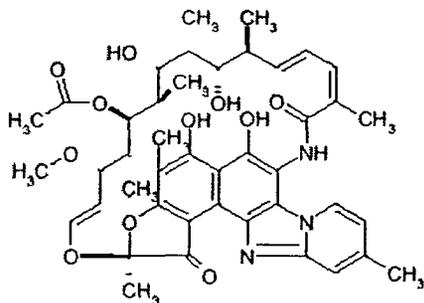
[2S-[2R*,16Z,18E,20R*,22S*,23S*,23S*,24S*,25R*-26S*,27R*,28E)]-25-(Acetyloxy)-5,6,21,23-ttradroxy-27-methoxy-2,4,11,16,20,22,24,26-octamethyl-2,7-[epoxypentadeca[1,11,13]-trienimino]benzofuro[4,5-e]pyrido[1,2- α]benzimidazole-1,15(2H)-dione

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Mole file number not provided

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

Abbreviations

HED= human equivalent dose

i v = intravenous

pp= post partum

p o = per os

i p =intrapertitoneal

I Recommendations

A Recommendation on Approvability

Preclinical studies have not demonstrated toxicities of clinical concern. The apparent lack of systemic exposure by oral administration limits preclinical toxicity and presumably prevents clinical toxicity as well, therefore the submission is acceptable regarding pharmacology/toxicology issues.

B Recommendation for Nonclinical Studies

None

C Recommendations on Labeling

Carcinogenesis, Mutagenesis, and Impairment of Fertility

Carcinogenicity studies were not conducted. Rifaximin was not genotoxic in the bacterial reverse mutation assay, chromosomal aberration assay, rat bone marrow micronucleus assay, and the CHO/HGPRT mutation assay. There was no effect on fertility in male rats following the administration of rifaximin at doses up to mg/kg (approximately times the clinical dose adjusted for body surface area).

Pregnancy

Pregnancy category C Rifaximin was teratogenic in rats at doses of 150 to 300 mg/kg (approximately 2.5 to 5 times the clinical dose adjusted for body surface area) and in rabbits at doses of 62.5 to 1000 mg/kg (approximately 2 to 33 times the clinical dose adjusted for body surface area). These effects include cleft palate, agnathia, jaw shortening, hemorrhage, eye partially open, small eyes, brachygnathia, incomplete ossification, and increased thoracolumbar vertebrae. There are no adequate and well controlled studies in pregnant women. Rifaximin should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus.

Use during lactation

It is not known whether rifaximin is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for adverse reactions in nursing infants from rifaximin, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

II Summary of Nonclinical Findings

A Brief Overview of Nonclinical Findings

Mortality in the intravenous rats and mouse studies demonstrates possible toxicity of rifaximin if available systemically. The mortality finding (below) in a reproductive toxicology study of a male receiving oral rifaximin with extensive internal discoloration and liver pathology is indicative of systemic exposure and exemplifies a toxicity seen in rifampin, liver toxicity. Longer duration studies such as the 26-week rat and 39-week dog show potential immunotoxicity with decreased lymphocyte counts and thymic involution, respectively.

Systemic rifaximin exposure, although small, is in evidence at the end of treatment in these studies (see pharmacokinetics) The oral and intravenous studies together appear to indicate potential rifaximine toxicity from either acute systemic exposure or chronic low level exposure, both of which presumably would be avoided for the proposed indication with three days of treatment

Fetal effects were seen in reproductive toxicology studies, mainly in the rabbit It is difficult to correlate rat and rabbit exposure and fetal effects to clinical exposure due to the poor absorption of rifaximin Perhaps at higher doses rifaximin accumulates in the gastrointestinal tract, impeding nutrition as well as promoting absorption by mass action Antibacterial effects in intestinal flora and inhibition of nutrient absorption, especially at higher doses, may also account for decreased maternal weight and food consumption Fetal effects may correlate with these actions and not with fetotoxic effects of drug

B Pharmacologic Activity

Rifaximin is a semisynthetic derivative of rifampycin which acts through avid binding to DNA-dependent RNA polymerase with inhibition of RNA synthesis

C Nonclinical Safety Issues Relevant to Clinical Use

The apparent lack of systemic exposure is essential to the perception of rifaximin as a relatively non-toxic drug Intravenous studies in rats and mice indicate possible toxicity if substantial systemic exposure is achieved, as well as a rat in a reproductive toxicity study which died, apparently with systemic exposure and liver effects Clinical exposure should be monitored to exclude systemic exposure in patients

Reproductive toxicity occurred at doses 5 times the proposed clinical dose in rats and rabbits, without a NOEL level This is confounded by the issue of absorption, which is observed in a rabbit study, resulting in systemic exposure

III Administrative

A Reviewer signature _____

B Supervisor signature Concurrence - _____

Non-Concurrence - _____
(see memo attached)

cc list

TABLE OF CONTENTS

PHARMACOLOGY	1
SAFETY PHARMACOLOGY	1
PHARMACOKINETICS/TOXICOKINETICS	3
TOXICOLOGY	4
Histopathology Inventory for IND #	13
GENETIC TOXICOLOGY	13
CARCINOGENICITY	14
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	14
SPECIAL TOXICOLOGY STUDIES	
APPENDIX/ATTACHMENTS	37

PHARMACOLOGY

Primary pharmacodynamics Rifaximin is poorly absorbed from the gastrointestinal tract following oral administration. It distributes to the intestinal wall. Metabolism has not been characterized. Radioactivity from radiolabeled rifaximin was predominantly excreted in the feces in dogs and rats.

Mechanism of action As a member of the rifampin class of antibiotics, rifaximin acts through avid binding to DNA-dependent RNA polymerase with inhibition of RNA synthesis. See microbiology review for further details.

Drug activity related to proposed indication antibacterial activity to treat diarrhea

Secondary pharmacodynamics not provided

Pharmacology summary Single dose studies showed no neuromuscular, cardiovascular, respiratory, renal or gastrointestinal effects.

Pharmacology conclusions Rifaximin does not appear to exert pharmacologic effects in single dose studies, probably due to minimal systemic exposure from oral dosing.

SAFETY PHARMACOLOGY

Neurological effects General behavioral effects were assessed in the Irwin dose-range in male ICR mice. Single doses of 0, 100, 300, and 1000 mg/kg, 4/group, were used. Behavior was observed at 30, 90, 150, and 300 minutes after dosing. Mortality and general toxicity were monitored for 7 days. No behavioral effects were seen. Spontaneous activity was evaluated in ICR mice, 10/group, at single doses of 0, 100, 300, and 1000 mg/kg versus a positive control, diazepam (15 mg/kg). Activity was monitored for one hour at 10 minute intervals. Rifaximin caused no effects on activity while diazepam decreased it. Hexobarbital-induced sleeping time was assessed using ICR mice (5/se/dose) at doses of 0, 100, 300, 1000 mg/kg and the positive control chlorpromazine, 15 mg/kg. Following dosing (945 min), an i.p. injection of hexobarbital was administered and onset of sleep time recorded. Chlorpromazine prolonged the sleep time while the 1000 mg/kg dose prolonged the onset of sleep. No effect was seen at the other doses. Proconvulsant activity was evaluated with male ICR mice (10/group) at doses of 0, 100, 300, and 1000 mg/kg and the positive control amphetamine sulphate (30 mg/kg) against metrazol-induced convulsions or bemegride (40 mg/kg) against electroshock-induced convulsions. No proconvulsant activity was seen in either test. Motor coordination was tested using the rotarod test. Female ICR mice (10/group) were given doses of 0, 100, 300, and 1000 mg/kg or mephenesin (400 mg/kg) positive control. No effect was seen due to rifaximin while mephenesin decreased performance. The effect of rifaximin on diazepam-induced protection against metrazol-induced convulsions was examined in male ICR CD-1 mice (20/group) at doses of 0, 100, 300, and 1000 mg/kg. A positive control, flumazenil (25 mg/kg) and metrazol (85 mg/kg) were used. Flumazenil reversed the diazepam-induced protection against metrazol-induced convulsions while rifaximin had no effect.

Cardiovascular effects In anesthetized cats, autonomic effects were studied at a dose of 1000 mg/kg. No effects were seen for basal blood pressure or heart rate, as well as nictitating

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membrane response Responses to bilateral carotid occlusion and i v noradrenaline were not affected In anesthetized female beagle dogs, intraduodenal administration of rifaximin (0, 1000 mg/kg) was examined for cardiovascular and respiratory effects No effects were seen on systolic, diastolic, mean arterial pressure, heart rate, left ventricular dp/dt maximum, electrocardiogram, femoral blood flow, femoral resistance, respiratory tidal volume, respiratory rate and minute volume

Pulmonary effects see cardiovascular effects

Renal effects Single doses of rifaximin (0, 100, 300, 1000 mg/kg) or the diuretic frusemide (20 mg/kg) were employed to determine the effect of rifaximin on urine volume and electrolyte excretion in the Wistar rat (8/group) At the 1000 mg/kg dose, rats produced significantly increased urine output as did frusemide

Gastrointestinal effects Motility effects of rifaximin were determined using the charcoal propulsion test in the ICR CD-1 mouse (10/group) Doses of 0, 100, 300, and 1000 mg/kg were used as well as a positive control, morphine sulphate Morphine inhibited motility while rifaximin had no effect Gastric secretion effects were studied in male Wistar rats (10/group) at doses of 0, 100, 300, 1000 mg/kg or positive control, omeprazole, 10mg/kg Rifaximin had no effect on volume or electrolyte content of the gastric fluid while omeprazole decreased H⁺ and Na⁺ content Gastrointestinal injury by rifaximin oral administration was examined in male Wistar rats (10/group) at doses of 0, 100, 300, and 1000 mg/kg or positive control, acetylsalicylic acid (200 mg/kg) Acetylsalicylic acid produced significant gastric damage while rifaximin had no effect

Abuse liability not evaluated, unlikely

Other not conducted

Safety pharmacology summary No drug-related pharmacologic effects were seen in neurologic, cardiovascular, respiratory, renal or gastrointestinal pharmacology studies

Safety pharmacology conclusions Rifaximin appears to have minimal gross pharmacologic effects, especially at oral doses similar to the proposed clinical dose The lack of effects is in accord with the apparent lack of systemic exposure from oral administration seen in the pharmacokinetic studies (below)

PHARMACOKINETICS/TOXICOKINETICS

PK parameters see tables below

Table 5 Single Dose ¹⁴C-Rifaximin Pharmacokinetic Parameters^a

Route of Administration (Dose)	Rat	Dog
Oral (24 mg/kg)	N = 9	N = 4
C _{max} (µg equivalents/mL)	0.084	0.042
T _{max} (hours postdose)	0.25-0.5	1-3
AUC ₀₋₄ (µg equivalent hours/mL)	0.2	0.1
Systemic availability (% dose)	2	0.5
Intravenous (2.4 mg/kg)	N = 18	N = 4
C _{max} (µg equivalents/mL)	1.039	1.365
T _{max} (hours postdose)	0.08	0.08-0.17
AUC ₀₋₄ (µg equivalent hours/mL)	0.85	2.5

^a Both sexes combined.

Table 6 Multiple Dose Oral Pharmacokinetics Parameters in Rats^a

Parameter	50 mg/kg/day (N = 6)		150 mg/kg/day (N = 6)		300 mg/kg/day (N = 6)	
	Day 1	Week 26	Day 1	Week 26	Day 1	Week 26
t _{max} (hour)	~ 1	~ 1	~ 1	~ 1	~ 1	~ 1
C _{max} (ng/mL)	14.21	8.08	20.83	7.57	25.04	6.36
AUC ₀₋₈ (ng h/mL)	50.65	ND ^b	92.35	ND	126.7	ND
t _{1/2} (hour)	2.2	ND	3.1	ND	ND	ND

^a For both sexes combined.

^b ND = Not determined due to sample values below the limit of quantification of ✓

Table 7 Multiple Dose Oral Pharmacokinetics Parameters in Dogs^a

Parameter	100 mg/kg/day (N = 8)		300 mg/kg/day (N = 8)		1000 mg/kg/day (N = 8)	
	Day 1	Week 39	Day 1	Week 39	Day 1	Week 39
T _{max} (hour) ^b	3	2	3.5	3	4	3
C _{max} (ng/mL)	9.29	16.97	20.10	21.82	12.84	21.88
AUC ₀₋₈ (ng h/mL)	41.75	61.35	102.10	75.45	66.05	105.7

^a For both sexes combined.

^b Median values

Table 8 Multiple Dose Oral Pharmacokinetic in Pregnant Rabbits

Parameter	250 mg/kg/day (N = 4)		1000 mg/kg/day (N = 4)	
	Day 6	Day 19	Day 6	Day 19
T _{max} (hour)	1	1	2	1.5
C _{max} (ng/mL)	1.046	1.705	2.200	4.006
AUC ₀₋₈ (ng h/mL)	6.63	8.25	10.95	16.97

^a Median values

A pharmacokinetic profile was not obtained from 9 of the 10 in vivo clinical studies due to low systemic absorption of rifaximin. The single study in which a pharmacokinetic profile was determined provided the following values:

Study No.	Route of Admin./ Dosage Form	Dose	C _{max} (ng/mL)	T _{max} (h)	AUC (ng·h/mL)	T _{1/2} (h)	Urinary Excretion (%)	CL _r (mL/min)
N2164	Oral/ Capsule	400 mg	4.28 ± 2.85	1.88 ± 1.44	19.5 ± 16.5	NC	0.03 ± 0.02	NC

A food effects study provided increased drug concentrations than the fasted study above:

N2270	Oral/ Tablet	400 mg (Fed)	9.6 ± 5.9	1.9 ± 1.5	34.7 ± 9.2	5.84 ± 4.33	0.0509 ± 0.0172	8.78 ± 4.59
		400 mg (Fasting)	3.8 ± 1.3	1.2 ± 0.5	18.3 ± 9.7	5.95 ± 1.88	0.0225 ± 0.0085	7.14 ± 1.23

Absorption In rats, C¹⁴-rifaximin administered orally resulted in absorption of approximately 2% of the delivered dose by comparison of the exposure from intravenous dosing to oral dosing. In dogs, systemic availability was approximately 12%.

Distribution Oral administration of C¹⁴-rifaximin in rats resulted in highest concentrations of radioactivity in the gastrointestinal tract, with approximately 67% in stomach/intestinal contents at 0.5 h, 78% at 1.0 h, 88% at 2.0 h, 47% at 4 h while tissue levels remained at less than 1% in any individual tissue.

Metabolism Due to the low levels of systemic absorption, the sponsor did not characterize the metabolism of rifaximin.

Excretion Intravenous administration of C¹⁴-rifaximin to dogs led to recovery of radioactivity in feces (83%), urine (6%), while oral administration resulted in 88% excretion in feces, 0.5% in urine.

Other studies not conducted.

PK/TK summary

PK/TK conclusions It is central to the claims of the sponsor that rifaximin is a non-toxic antibiotic due to its lack of systemic absorption. Radiotracer studies in rats verify the lack of systemic absorption from an oral dose.

TOXICOLOGY

The following study reviews are attached:

Study title Rifaximin, acute intravenous toxicity to the mouse

Key study findings Two deaths was of unknown etiology, piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity, all treated groups

Study no — 8/971251/AC, N2107

Volume #, and page # v 9, p 85

Conducting laboratory and location

Date of study initiation 11 Feb 1997

GLP compliance yes

QA report yes (x) no ()

Drug, lot #, radiolabel, and % purity AC/0093

Formulation/vehicle PEG 400/saline, 1 1

Methods (unique aspects)

Dosing

Species/strain male and female ICR mice, —

#/sex/group or time point (preliminary, main study) 2, 5

Satellite groups used for toxicokinetics or recovery none

Age 5 to 7 weeks

Weight 21 to 28 g

Doses in administered units 40 mg/kg (preliminary and main study) (HED=3.33 mg/kg)

Route, form, volume, and infusion rate intravenous, tail vein, volume= 10 ml/kg

Observations and times

Clinical signs daily for 7 days, preliminary, 14 days for main study

Body weights days 1, 8, 15

Food consumption not conducted

Ophthalmoscopy not conducted

EKG not conducted

Hematology not conducted

Clinical chemistry not conducted

Urinalysis not conducted

Gross pathology at necropsy

Organs weighed not conducted

Histopathology not conducted

Toxicokinetics not conducted

Other not conducted

Results

Mortality one female in the preliminary study and one female in the main study died within one hour of dosing

Clinical signs piloerection in all mice, hunched posture, unsteady gait, respiratory distress, ptosis, pallid extremities, lethargy, tremors, cold body, prostration were seen in treated mice, with recovery by day 6

Body weights one female (day 4) , one male, one female (day 15) had reduced weight gains

Food consumption not conducted

Ophthalmoscopy not conducted

Electrocardiography not conducted

Hematology not conducted

Clinical chemistry not conducted

Urinalysis not conducted

Organ weights not conducted

Gross pathology no drug-related findings were seen

Histopathology not conducted

Toxicokinetics not conducted

Summary of individual study findings Two deaths was of unknown etiology, piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity, all treated groups

Study title Rifaximin, acute intravenous toxicity to the rat

Key study findings One death, piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity, all treated groups

Study no — 8/971262/AC, N2105

Volume #, and page # v 9, p 114

Conducting laboratory and location

Date of study initiation 7 February 1997

GLP compliance yes

QA report yes (x) no ()

Drug, lot #, radiolabel, and % purity batch no AC/0093

Formulation/vehicle PEG 400/saline (1 1)

Methods (unique aspects)

Dosing

Species/strain male and female Sprague Dawley CD rats (—

#/sex/group or time point (preliminary, main study) 2, 5

Satellite groups used for toxicokinetics or recovery none

Age 4 to 7 weeks

Weight 111 to 145 g

Doses administered 40 mg/kg (preliminary and main study) (HED=6.75 mg/kg)

Route, form, volume, and infusion rate intravenous, tail vein, volume= 10 ml/kg

Observations and times

Clinical signs daily, for 7 days preliminary, 14 days, main study

Body weights days 1, 8, 15

Food consumption not conducted

Ophthalmoscopy not conducted

EKG not conducted

Hematology not conducted

Clinical chemistry not conducted

Urinalysis not conducted

Gross pathology at necropsy, day 8 (preliminary study, day 15 main study)

Organs weighed not conducted

Histopathology not conducted

Toxicokinetics not conducted

Other not conducted

Results

Mortality one male, main study following dosing on day 1

Clinical signs piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity, all treated groups

Body weights one treated female had decreased weight gain, all others similar

Food consumption not conducted

Ophthalmoscopy not conducted

Electrocardiography not conducted

Hematology not conducted

Clinical chemistry not conducted

Urinalysis not conducted

Organ weights not conducted

Gross pathology no drug-related effects were seen

Histopathology not conducted

Toxicokinetics not conducted

Summary of individual study findings One death was of unknown etiology, piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity, all treated groups

Study title Rifaximin acute oral toxicity to the mouse

Key study findings One death, 2000 mg/kg No other drug-related clinical signs were seen

Study no 5/971056/AC, N2106
Volume #, and page # v 9, p119
Conducting laboratory and location

Date of study initiation 29 Dec 1996
GLP compliance yes
QA report yes (x) no ()
Drug, lot #, radiolabel, and % purity batch no AC/0093
Formulation/vehicle 1% methylcellulose

Methods (unique aspects)

Dosing

Species/strain male and female CFLP mice, ICI strain,
Satellite groups used for toxicokinetics or recovery none
Age 4 to 7 weeks
Weight 19 to 22g
Doses in administered units 1000 mg/kg (preliminary), 2000 mg/kg (main study) (HED=
83, 166 mg/kg)
Number/sex/group 2, preliminary, 5 main
Route, form, volume, and infusion rate oral canula

Observations and times

Clinical signs twice daily, 14 days after dosing
Body weights day 1, 8, 15
Food consumption not conducted
Ophthalmoscopy not conducted
EKG not conducted
Hematology not conducted
Clinical chemistry not conducted
Urinalysis not conducted
Gross pathology at necropsy, day 15
Organs weighed not conducted
Histopathology not conducted
Toxicokinetics not conducted
Other not conducted

Results

Mortality main study, 1 male day 11
Clinical signs piloerection, all mice about 5 minutes after dosing, day 1, 2, orange feces,
day 2
Body weights on female had no weight gain by day 15 All others gained weight
Food consumption no drug-related effect was seen
Ophthalmoscopy not conducted
Electrocardiography not conducted
Hematology not conducted

Clinical chemistry not conducted
Urinalysis not conducted
Organ weights not conducted
Gross pathology no drug-related effect was seen
Histopathology not conducted
Toxicokinetics not conducted

Summary of individual study findings One mouse died on day 11 (2000 mg/kg) Piloerection and orange-colored feces indicative of large amounts of rifaximin, were observed on days 1 and 2 No other drug-related signs were seen NOAEL=1000 mg/kg (HED=83 mg/kg)

Study title Rifaximin Preliminary toxicity study by oral capsule administration to beagle dogs for four weeks

Key study findings No drug-related clinical signs were seen

Study no — 025/973149, N2149
Volume #, and page # v 13, p11
Conducting laboratory and location

Date of study initiation 29 May 1997
GLP compliance yes
QA report yes (x) no ()
Drug, lot #, radiolabel, and % purity batch no PP2040
Formulation/vehicle powder in gelatin capsule

Methods (unique aspects)

Dosing

Species/strain beagle dogs,
#/sex/group or time point (main study) 3
Satellite groups used for toxicokinetics or recovery none
Age 34 to 42 weeks
Weight 9.7 to 13.4 kg
Doses in administered units 100, 300, 1000 mg/kg (HED=50, 150, 500 mg/kg)
Route, form, volume, and infusion rate oral, gelatin capsule

Observations and times

Clinical signs twice daily
Body weights weekly during acclimatization, twice weekly during dosing
Food consumption daily
Ophthalmoscopy not conducted
EKG not conducted
Hematology prior to initial dose, week 4 of dosing
Clinical chemistry prior to initial dose, week 4 of dosing
Urinalysis not conducted

Gross pathology at necropsy
Organs weighed see pathology table below
Histopathology tissues collected but not examined
Toxicokinetics not conducted
Other not conducted

Results

Mortality all dogs survived until sacrifice
Clinical signs orange feces, 1000 mg/kg through study, 300 mg/kg female, from week 3 to end of study
Body weights no drug-related effect was seen
Food consumption no drug-related effect was seen
Ophthalmoscopy not conducted
Electrocardiography not conducted
Hematology no drug-related effect was seen
Clinical chemistry no drug-related effect was seen
Urinalysis not conducted
Organ weights no drug-related effect was seen
Gross pathology no drug-related effect was seen
Histopathology not conducted
Toxicokinetics not conducted

Summary of individual study findings Other than orange-colored feces indicative of large amounts of rifaximin, no drug-related signs were seen at any dose NOAEL=1000 mg/kg (HED=500 mg/kg) With two dogs/dose and no histopathology, this study is of superficial value in determining toxicities of rifaximin in dogs

Study title Acute oral toxicity in the rat

Key study findings piloerection and orange feces

Study no — 6/970957/AC (N2104)

Volume #, and page # v 9, p143

Conducting laboratory and location

Date of study initiation 20 Dec 1996

GLP compliance yes

QA report yes (x) no ()

Drug, lot #, radiolabel, and % purity AC/0093

Formulation/vehicle 1% methylcellulose

Methods (unique aspects)

Dosing oral gavage

Species/strain rat/Sprague-Dawley CD,

#/sex/group or time point (main study) 5
Satellite groups used for preliminary study-2/sex
Age 4-7 weeks old
Weight 78-90 g
Doses in administered units 2000 mg/kg
Route, form, volume, and infusion rate oral, 10 ml/kg

Observations and times

Clinical signs twice daily, for 7 days (preliminary), 14 days (main)
Body weights days 1, 8, 15
Food consumption not conducted
Ophthalmoscopy not conducted
EKG not conducted
Hematology not conducted
Clinical chemistry not conducted
Urinalysis not conducted
Gross pathology at necropsy
Organs weighed not conducted
Histopathology not conducted
Toxicokinetics not conducted
Other not conducted

Results

Mortality all rats survived until sacrifice
Clinical signs piloerection, all rats immediately after dosing, orange discolored feces
Body weights not affected by treatment
Food consumption not conducted
Ophthalmoscopy not conducted
Electrocardiography not conducted
Hematology not conducted
Clinical chemistry not conducted
Urinalysis not conducted
Organ weights not conducted
Gross pathology no abnormalities
Histopathology not conducted
Toxicokinetics not conducted

Summary of individual study findings

Clinical signs of piloerection and orange colored feces were seen in all rats All rats survived until terminal sacrifice

Toxicology summary

Major rifaximin-related findings in single and multiple dose studies

Species	findings	Study duration	route	Doses of findings Mg/kg	HED Mg/kg
Mouse	Mortality piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity,	1 dose	i v	40	3 33
	Mortality	1 dose	p o	2000	166
rat	Mortality piloerection immediately following dosing, all treated rats, respiratory distress, hunched posture, unsteady gait, tremors, increased activity	1 dose	i v	40	6 66
		1 dose	p o	1000, 2000	
	Orange feces, piloerection	1 dose	p o	2000	333
	Mortality 3 rats (1 control, 1 middle dose, 1 high dose (no rug-related findings) Decreased lymphocyte counts, reversed post recovery Decreased thymus weight No other drug related histopath findings	26 weeks, 4 week recovery	p o	50, 150, 300	8 2, 25, 50
Dog	Orange feces	4 week	p o	100, 300, 1000	50, 150, 500
	Orange feces, decreased thymic weight, involution, reversed post recovery	39 week, 4 week recovery	p o	100, 300, 1000	50, 150, 500

Toxicology conclusions Mortality in the intravenous rats and mouse studies demonstrates possible toxicity of rifaximin if available systemically. The mortality finding (below) in a reproductive toxicology study of a male receiving oral rifaximin with extensive internal discoloration and liver pathology is indicative of systemic exposure and exemplifies a toxicity

seen in rifampin, liver toxicity Longer duration studies such as the 26-week rat and 39-week dog show potential immunotoxicity with decreased lymphocyte counts and thymic involution, respectively Systemic rifaximin exposure, although small, is in evidence at the end of treatment in these studies (see pharmacokinetics) The oral and intravenous studies together appear to indicate potential rifaximine toxicity from either acute systemic exposure or chronic low level exposure, both of which presumably would be avoided for the proposed indication with three days of treatment

Histopathology Inventory for NDA

See individual studies, attached

GENETIC TOXICOLOGY

See attached reviews for the following studies Study of mutagenic activity of the compound L105 with Salmonella Typhimurium, An evaluation of the mutagenic potential of rifaximin in the Ames salmonella/microsome assay, Study of the mutagenic activity of the compound L105 with sacromyces cerevisiae, Study of the mutagenic activity of the compound L105 with Schizosaccharomyces pombe, Rifaximin chromosome aberrations in human lymphocytes cultured "in vitro", Rifaximin micronucleus test in rat bone marrow

Study title An evaluation of the mutagenic potential of Rifaximin in the CHO/HGPRT mutation assay

Key findings

Study no S79-91-06-702, SA3733, N2020

Study type (if not reflected in title) CHO/HGPRT mutation assay

Volume #, and page # v 20, p19

Conducting laboratory and location —

Date of study initiation 1 May 1991

GLP compliance

QA reports yes () no ()

Drug, lot #, radiolabel, and % purity lot # G0704

Formulation/vehicle dimethylsulfoxide

Methods

Strains/species/cell line Chinese hamster ovary cells (subline K₁-BH₄)

Dose selection criteria

Basis of dose selection range finding study

Range finding studies 7 91- 1000 µg/ml, with and without S9 activation, limited by cytotoxicity above 62.5 µg/ml

Test agent stability not provided

Metabolic activation system rat S9 liver fraction activated with Aroclor 1254 treatment

Controls

Vehicle dimethylsulfoxide

Negative controls not used

Positive controls ICR -191 acridine, 3-methylcholanthrene

Comments

Exposure conditions

Incubation and sampling times 7 days incubation, followed by sampling

Doses used in definitive study 10, 25, 60, and 80 µg/ml without activation, 100, 150, 200, and 250 µg/ml with activation

Study design as in rangefinder

Analysis

No of replicates 3

Counting method visual mutant colony counting

Criteria for positive results mutant colony frequency for two successive test article concentrations at least 15 per 1×10^6 clonable cells and a statistically significant increase when compared to the solvent controls

Summary of individual study findings

Study validity positive controls were effective

Study outcome negative, no increase of mutants in treated cell number or when compared with solvent control

Genetic toxicology summary Rifaximin had negative genetic toxicology findings in bacterial reverse mutation assay and yeast mutation assays, chromosomal aberration assay, rat bone marrow micronucleus assay, and the CHO/HGPRT mutation assay

Genetic toxicology conclusions Rifaximin was not genotoxic in the above assays

Labeling recommendations Rifaximin was not genotoxic in the bacterial reverse mutation, chromosomal aberration and rat bone marrow micronucleus assays

CARCINOGENICITY

Studies not conducted

Labeling Recommendations

Carcinogenicity studies were not conducted by the sponsor

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

1 Study title L105 (Rifaximine) study on fertility and reproduction in the rat

Key study findings in a pre-GLP study of questionable quality, no drug-related effects were seen

Study no N2015

Volume #, and page # v 16 p 1

Conducting laboratory and location Alfa Wassermann
Bologna, Italy

Date of study initiation 1983
GLP compliance pre GLP
QA reports yes () no (X)
Drug, lot #, radiolabel, and % purity CR/III
Formulation/vehicle 1% gum arabic

Methods

Species/strain Sprague-Dawley NOS rats, males and females
Doses employed 0, 25, 50, 100 mg/kg (HED= 4, 8, 12 mg/kg)
Route of administration oral gavage
Study design males dosed for 70 days prior to mating, 20 days during mating, sacrificed at end of mating, females 14 days prior to mating, 20 days during mating, during gestation (up to 23 days), and suckling (20 days) Ten females were sacrificed on days 12-14 of gestation The remaining ten females were allowed to deliver naturally and suckle their offspring
Number/sex/group 10 males, 20 females/dose
Parameters and endpoints evaluated All daily mortality and clinical signs Males at necropsy, examination of testicles, epididymis, prostate, seminal vesicles control and high dose), Day 12-14 sacrifice dams uterine examination including number of embryos, resorption, non-viable fetuses, and uterine abnormalities Natural delivery dams bodyweight post delivery day 0, 11, 20, F₁ at birth gross examination, viability (daily until day 35), bodyweight, days 0, 11, 20, 28 35, morphologic development (ear detachment, hair growth, incisor eruption, eyes opening, testicle decent, vaginal opening), functional development (palpebral, corneal, auricular reflexes, days 18 and 24), neuromuscular development (pivoting, walking, righting reflexes, grasping, (day 24), swimming (days 24, 28)), reproductive function (mating of F₁ offspring, natural delivery followed by F₂ bodyweight, day 1, 4 post delivery, gross examination)

Results

Mortality two 50 mg/kg females due to gavage error, all other rats survived until sacrifice
Clinical signs no drug-related clinical signs were seen
Body weight no drug-related effect was seen
Food consumption no drug-related effect was seen
Toxicokinetics not conducted

For fertility studies

In-life observations no drug-related effects were seen
Terminal and necroscopic evaluations males no effects were seen on male reproductive organs in the 100 mg/kg group, females (day 12-14 sacrifice) no drug-related effects were seen in uterine examination, corpora lutea, implantations or resorptions

For peri-postnatal development studies

In-life observations
Dams no drug-related effects were seen
Offspring F₁ No drug-related effects were seen in gross examination, viability, bodyweight, morphologic development, functional development, neuromuscular

development, reproductive function F₂ no drug-related effects on bodyweight or gross examination)

Terminal and necroscopic evaluations

Dams no gross effects were seen

Offspring no gross effects were seen

Summary of individual study findings This report was a sub-optimal translation from Italian of a pre-GLP study It lacked figures, tables and was generally poorly organized The apparent lack of rifaximin-related effects on mothers or offspring in this study provides a maternal NOAEL of 100 mg/kg (HED= 16 mg/kg as well as a fetal NOAEL of 100 mg/kg (HED= 16 mg/kg)

2 Study title Rifaximin preliminary study of effects on fertility and peri- and post-natal development in CD rats by oral gavage administration

Key study findings Food consumption and bodyweight were decreased in 150 and 300 mg/kg treated females One death , a male receiving 300mg/kg

Study no - 034/990015, N2174

Volume #, and page # v 16, p 63

Conducting laboratory and location

Date of study initiation 18 Aug 1998

GLP compliance yes

QA reports yes (x) no ()

Drug, lot #, radiolabel, and % purity batch no PP2040

Formulation/vehicle 1% methylcellulose

Methods

Species/strain male and female Sprague-Dawley CD rats

Doses employed 0, 150, 300 mg/kg (HED=0, 25, 50 mg/kg)

Route of administration oral gavage

Study design males were dosed from 15 days prior to mating to terminal sacrifice as females delivered Females were dosed from 15 days prior to mating to day 9 of lactation

Number/sex/group 8

Parameters and endpoints evaluated mortality (daily), clinical signs (daily), bodyweight, males (twice weekly), females (twice weekly until mated successfully, then daily, food consumption, males, females twice weekly until mating, females days 0-6, 7-13, 14-19 after mating, days 1-3, 4-6, 7-9 during lactation, delivery observations of live/dead births, post natal observations offspring number, bodyweights, sex ratio, mortality, gross examination, terminal observations on day 10 of lactation necropsy, males sacrificed after seven weeks of treatment with necropsy including collection and weighing of pituitary, testes, epididymides, prostate, and seminal vesicles

Results

Mortality one male (300 mg/kg) was sacrificed on day 30 for humane reasons displaying pallor and bodyweight loss. Necropsy showed yellow discoloration of most internal organs (except heart, spleen, kidneys), enlarged spleen, pale liver with reduced left and median lobes, swollen posterior and anterior right lobes, dark thymic, mesenteric, pancreatic and renal lymph nodes, and the pancreas had edema.

Clinical signs No drug-related clinical signs were seen. One occurrence of post-dose salivation was seen in a 300 mg/kg male in the first week of dosing.

Body weight males and females in the 150 and 300 mg/kg groups had decreased weight gain (300 mg/kg males = 84% of controls, females = 39%, 150 mg/kg males = 81% of controls, females = 52%). Female weights (150, 300 mg/kg) narrowed this difference but remained lower than control weights until lactation.

Food consumption 300 mg/kg females were decreased relative to controls in pre-mating, late in gestation and early lactation.

Toxicokinetics not conducted.

For peri-postnatal development studies

In-life observations

Dams No drug-related effects were seen.

Offspring pup mortality was slightly decreased in treated groups, after day 4-10 survival was similar to that in controls, bodyweight was unaffected by maternal treatment.

Terminal and necroscopic evaluations

Males No drug-related effects were seen at necropsy.

Dams No drug-related effects were seen at necropsy.

Offspring No drug-related effects were seen at necropsy.

Summary of individual study findings No overt drug-related effects on reproduction were seen in this study in treatment of males, females or the resulting offspring. Food consumption and bodyweight were decreased in 150 and 300 mg/kg treated females. One death, a male receiving 300 mg/kg, may be drug-related as evidenced by the internal discoloration observed at necropsy. This finding is in contradiction to the apparent lack of absorption seen in other studies and raises the question of rifaximin toxicity if absorbed from the intestine at high doses.

3 Study title L 105 (rifaximine) fetal, perinatal and postnatal toxicity test

Key study findings No apparent adverse drug-related effects were seen in the fetal toxicity study in rats. No apparent adverse drug-related effects were seen in the fetal toxicity study in rabbits. A neonatal study in rats also had no apparent drug-related effects.

Study no N2008

Volume #, and page # v 16, p 158

Conducting laboratory and location Alfa Wassermann
Bologna, Italy

Date of study initiation Aug 1982
GLP compliance pre-GLP
QA reports yes () no (x)
Drug, lot #, radiolabel, and % purity batch 569-LS/2439
Formulation/vehicle gum arabic 5% solution

Methods fetal toxicity in rats

Species/strain male and female Sprague Dawley (NOS) rats
Doses employed 50, 100 mg/kg (HED= 8.3, 16.6 mg/kg)
Route of administration oral gavage
Study design rats were mated, then females dosed on gestation days 5-18, dams sacrificed on day 21, fetuses removed, examined
Number/sex/group 11
Parameters and endpoints evaluated clinical signs, daily, bodyweight, days 1, 4, 7, 10, 13, 16, 19, 21 of pregnancy, hematology, day 21, uterine examination/implantations, day 21, fetuses viability, bodyweight, delivery, external examination, visceral examination, skeletal examination

Results

Mortality all survived until sacrifice
Clinical signs no drug-related signs were seen
Body weight reduced weight gain in treated dams was seen on days 18-21
Food consumption not conducted
Toxicokinetics not conducted

For peri-postnatal development studies

In-life observations

Dams no drug-related signs were seen
Offspring no drug-related signs were seen for viability, bodyweight, delivery

Terminal and necroscopic evaluations

Dams drug-treated dams had slightly higher rate of post-implantation loss
Triglycerides were slightly elevated in treated dams No other drug-related signs were seen
Offspring no drug-related signs were seen for external examination, visceral examination, skeletal examination

Methods fetal toxicity in rabbits

Species/strain female New Zealand rabbits
Doses employed 50, 100 mg/kg (HED=16.3, 33 mg/kg)
Route of administration oral gavage
Study design mated rabbits were administered drug on days 6-18 of gestation Rabbits were sacrificed on day 28, fetuses were delivered and examined
Number/sex/group 6

Parameters and endpoints evaluated clinical signs, daily, bodyweight, weekly, hematology, day 28, uterine examination/implantations, day 28, fetuses viability, bodyweight, delivery, external examination, visceral examination, skeletal examination

Results

Mortality all dams survived until sacrifice
Clinical signs decreased lipids at 100 mg/kg, no other drug-related signs were seen
Body weight no drug-related signs were seen
Food consumption not conducted
Toxicokinetics not conducted

For peri-postnatal development studies

In-life observations

Dams see above clinical signs
Offspring not conducted

Terminal and necroscopic evaluations

Dams no drug-related findings were seen
Offspring no drug-related signs were seen for external examination, visceral examination, skeletal examination

Methods rat neonatal toxicity

Species/strain female rats, Sprague-Dawley (NOS)
Doses employed 50, 100 mg/kg (HED=8.3, 16.6 mg/kg)
Route of administration oral gavage
Study design pregnant rats were administered rifaximin on day 17 of gestation through post-natal day 22, sacrifice on day 22
Number/sex/group 7 controls, 9-50 mg/kg, 8-100 mg/kg
Parameters and endpoints evaluated clinical signs, daily, bodyweight, days 1, 3, 6, 9, 12, 15, 18, 21 of pregnancy, days 1, 4, 7, 10, 13, 16, 19, 22 post-delivery, fetuses viability, bodyweight, delivery, external examination, physical, neuromuscular, sensory and reproductive development

Results

Mortality all dams appear to survive until sacrifice
Clinical signs no drug-related signs were reported
Body weight no drug-related effect was seen
Food consumption not conducted
Toxicokinetics not conducted

For peri-postnatal development studies

In-life observations

Dams no drug related effects were reported
Offspring no drug-related effect was seen regarding viability, bodyweight, delivery, external examination, physical, neuromuscular, sensory and reproductive development

Summary of individual study findings This report was a sub-optimal translation from Italian of a pre-GLP study The lack of detail devalues the study itself No apparent adverse drug-related effects were seen in the fetal toxicity study in rats A maternal and fetal NOAEL may occur at 100 mg/kg (HED= 16 mg/kg) No apparent adverse drug-related effects were seen in the fetal toxicity study in rabbits A maternal and fetal NOAEL may occur at 100 mg/kg (HED= 33 mg/kg) A neonatal study in rats also had no apparent drug-related effects NOAEL = 100 mg/kg (HED= 16 mg/kg)

4 Study title Rifaximin combined study of effects on fertility and embryo-fetal toxicity in CD rats by oral gavage administration

Key study findings Bodyweight gains were decreased in the 300 mg/kg group during gestation Fertility, implantation, fetal survival and fetal development were not significantly affected by drug treatment An increase of incomplete ossification of the cranial centers was seen in fetuses at 150 and 300 mg/kg An increase of hemorrhages were seen in fetuses at 150 and 300 mg/kg

Study no — 038/993391 (N2266)
Volume #, and page # v17 p58
Conducting laboratory and location

Date of study initiation 1 Mar 1999
GLP compliance yes
QA reports yes (x) no ()
Drug, lot #, radiolabel, and % purity batch no PP2040
Formulation/vehicle 1% aqueous methylcellulose

Methods

Species/strain Sprague-Dawley CD rats ' —
Doses employed 0, 50, 150, 300 mg/kg (0, 8 33, 25, 50 mg/kg HED)
Route of administration oral
Study design males dosed from day -29 (prior to mating) until sacrifice (day 20 after mating), females dosed from day -15 (prior to mating) through day 17 of gestation, sacrifice on day 20 and fetal examination
Number/sex/group 22
Parameters and endpoints evaluated clinical signs (twice daily), bodyweight, males (twice weekly), females (twice weekly until mating, daily from day 0-20 after mating), food consumption (weekly until mating, then females on days 0-2, 3-6, 7-9, 10-13, 14-17 and 18-19 after mating), vaginal smears for estrous/mating determination Terminal observations included examination of testes, epididymides, prostate gland and seminal vesicles if a male was infertile, female observations included count of corpora lutea, implantations, resorptions and fetuses Fetal examination included weight, sex, external examination, placental weight and examination, and division of fetuses for either visceral or skeletal examination

Results

Mortality All rats survived until terminal sacrifice
Clinical signs brown staining among treated females
Body weight not affected , males, females receiving 50 and 150 mg/kg, decreased prior to mating, days 0-7 and 0-10 respectively, 300 mg/kg females decreased relative to controls before and after mating
Food consumption not affected
Toxicokinetics not conducted

For fertility studies

In-life observations males, no effects
Terminal and necroscopic evaluations males, 300 mg/kg increased weight, prostate gland

For peri-postnatal development studies

In-life observations
Dams brown fur staining
Offspring

Terminal and necroscopic evaluations
Dams No overt treatment related findings were seen in the females at necropsy
Offspring

Litter data - group mean values on Day 20 of gestation

Group	1	2	3	4
Compound	Control	--	Rifaximin	--
Dosage (mg/kg/dav)	0	50	150	300

Group		Corpora lutea	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre	Post
1	Mean	16.7	15.6	1.3	0.0	1.4	6.8	7.5	14.3	47.1	7.4	9.1
	SD	1.7	1.9				2.2	1.9	2.6			
	n	22	22	22	22	22	22	22	22	22	22	22
2	Mean	16.7	15.7	0.7	0.0	0.7	7.2	7.8	15.0	48.1	6.8	4.2
	SD	1.7	1.7				2.2	2.2	1.7			
	n	22	22	22	22	22	22	22	22	22	22	22
3	Mean	17.8	16.7	1.0	0.1	1.1	8.4	7.1	15.5	54.2	6.5	6.6
	SD	1.9	2.5				2.4	2.4	2.8			
	n	22	22	22	22	22	22	22	22	22	22	22
4	Mean	16.7	15.1	0.9	0.0	0.9	6.4	8.0	14.3	44.1	9.1	6.4
	SD	2.2	2.2				2.2	2.2	2.8			
	n	22	22	22	22	22	22	22	22	22	22	22

SD Standard deviation
 n Number of animals/litters
 No statistical significance (p>0.05)

No drug-related effect was seen regarding # corpora lutea, implantations, resorptions, live young, or implantation loss

Placental litter and fetal weight - group mean values (g) on Day 20 of gestation

Group	1	2	3	4
Compound	Control		-----Rifaximin-----	
Dosage (mg/kg/day)	0	50	150	300

Group		Placental weight	Litter weight	Fetal weights		
				Males	Females	Overall
1	Mean	0.54	51.93	3.77	3.53	3.64
	SD	0.07	9.43	0.22	0.24	0.21
	n	22	22	22	22	22
2	Mean	0.49b	55.13	3.77	3.58	3.67
	SD	0.04	7.42	0.26	0.24	0.24
	n	22	22	22	22	22
3	Mean	0.49b	55.05	3.65	3.44	3.55
	SD	0.04	9.17	0.20	0.20	0.19
	n	22	22	22	22	22
4	Mean	0.48b	50.31	3.64	3.45	3.53
	SD	0.04	10.21	0.24	0.22	0.22
	n	22	22	22	22	22

SD Standard deviation

n Number of litters.

Significant when compared with Group 1 b p<0.01

Placental weight was decreased in all drug-treated groups

Fetal examinations - major abnormalities - group incidences

Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Dose (Rifaximin, mg/kg/day)	0	50	150	300	0	50	150	300
Number examined	314	330	42	314	22	22	22	22
Number affected	1	3	5	2	1	3	1	2
Cranioschisis ablepharia, protruding tongue			1				1	
Cranioschisis ablepharia cleft anterior palate/lip, protruding tongue			1				1	
Craniorachischisis ablepharia short premaxillae and maxillae, cleft anterior palate/lip, protruding tongue		-	1				1	
Craniorachischisis ablepharia, short premaxillae and maxillae protruding tongue lordosis, kyphosis, forelimb flexure			1				1	
Anophthalmia		-		1				1
Small eye retinal irregularities				1				1
Folded retina			1				1	
Duplicated inferior vena cava			1				1	
Linked irregularly ossified ribs marked, bent scapulae and clavicles			1				1	
Thoracolumbar scoliosis, additional vertebral arch/hemiacentrum	1				1			
Termination vertebral column sacral region short threadlike tail		-	1				1	

Fetal examinations - minor visceral abnormalities - group incidences

Group	Dose (Rifaximin, mg/kg/day)	Fetuses				Litters			
		1	2	3	4	1	2	3	4
		157	163	168	155	22	22	22	22
		33	37	48	52	18	16	19	18
Eye(s)	variation contralateral size	4	5	2	1	3	3	2	1
Thyroid	rudimentary/small	2		1		2		1	
Thymus	undescended	4	2	3	3	4	2	3	2
Innocent	absent/rudimentary	1	1	1	1	1	1	1	1
Inferior vena cava	premature branching				1				1
Diaphragm	thin with protruding liver	3	4	6	9	3	3	4	5
Liver	additional lobe	1	2			1	2		
Kidney(s)	rudimentary/absent papilla	3	3		1	2	2		1
Ureter(s)	dilated	2	2	1	1	2	2	1	1
Umbilical artery	left sided	2				2			
Testis(es)	d. placed	4	2	10	3	4	2	8	2
Tail	linked tip			1				1	
Haemorrhages									
Brain/spinal cord		2	2	6	5	2	2	6	5
Eye/surrounding tissue		1			1	1			1
Subcutaneous		8	6	18	19	5	6	10	10
Other	dorsal fat pad		1	1			1	1	
	intra-abdominal	4	5	2	4	4	4	2	4
	within liver	3	6	6	13	3	4	4	7

@ Fetuses with major abnormalities excluded.
 # Individual fetuses/litters may occur in more than one category.

Increased hemorrhages were seen in fetuses in the 150 and 300 mg/kg treatment groups

**APPEARS THIS WAY
ON ORIGINAL**

Fetal examinations - minor skeletal abnormalities/variants - group incidences

Group	Dose (Rifaximin mg/kg/day)	Fetuses#				Litters#			
		1	2	3	4	1	2	3	4
Number examined ^(a)		156	164	169	157	22	22	22	22
Cranial	bone plaque				1				1
	fissure			1	1			1	1
Basisphenoid	misshapen		1				1		
Vertebral element	thoracic	-			3				3
Ribs	medial kinked/thickened	1		1		1		1	
Sternebrae	offset	1	-	2	1	1		2	1
	bipartite/misshapen	1			1	1			1
Costal cartilage	offset/misaligned	1		1	1	1		1	1
	fused				1				1
Appendicular	truncated scapula	2		1	2	2		1	2
Total affected by one or more of the above		4	1	5	7	3	1	5	7
Rib and vertebral configuration									
Cervical rib		2	2		2	2	2		2
Number with 13, 14 or 14.5 ribs		22	33	15	16	10	16	9	8
Complete 14 th rib(s)		2	1			2	1		
	0 thoracolumbar vertebrae	1			1	1			1
	Offset pelvic girdl	1				1			
Incomplete ossification									
Cranial centers				1	11	2	2	2	6
Vertebrae	cervical	1	1		1	1	1		1
	thoracic	1	9		4	6	6	3	4
	sacrocaudal	1	2	9	4	1	2	6	3
Sternebrae	5th and/or 6th	92	110	115	120	20	21	21	21
	other	2	11	11	8	2	7	9	7
	total	92	110	116	120	20	21	21	21
1 st Rib					1				1
Pelvic bones		2	2	2	4	2	2	2	4
Metacarpals/metatarsals		1	1			1	1		
Precocious ossification									
Cervical vertebral centra (>3 ossified)		3	12	3	5	3	5	2	1
Additional observations at necropsy									
Renal dilatation				3	1			2	1
Hydronephrotic		3	1	4	1	2	1	3	1
Oedema					1				1

^(a) Fetuses with major abnormalities excluded # Individual fetuses/litters may occur in more than one category

An increase of incomplete ossification of the cranial centers was seen in the fetuses of the 150 and 300 mg/kg treatment groups

Summary of individual study findings A NOAEL was observed in the adult rats at 150 mg/kg. Bodyweight gains were decreased in the 300 mg/kg group during gestation. Fertility, implantation, fetal survival and fetal development were not significantly affected by drug treatment. An increase of incomplete ossification of the cranial centers was seen in the fetuses of the 150 and 300 mg/kg treatment groups.

An increase of hemorrhages were seen in fetuses in the 150 and 300 mg/kg treatment groups The NOAEL for fetuses was 50 mg/kg

5 Study title Rifaximine study of effects on pre- and post-natal development in CD rats by oral gavage administration

Key study findings Reduced maternal bodyweight gain at 150, 300 mg/kg on days 6-9 of gestation

Study no — 036/99736, N2265

Volume #, and page # v17 p219

Conducting laboratory and location

Date of study initiation 26 Oct 1998

GLP compliance yes

QA reports yes (x) no ()

Drug, lot #, radiolabel, and % purity PP2040

Formulation/vehicle 1% methylcellulose

Methods

Species/strain Sprague-Dawley CD rats (—)

Doses employed 0, 50, 150, 300 mg/kg (HED= 8 33, 25, 50 mg/kg)

Route of administration oral gavage

Study design females (F₀) were dosed from day 6 post mating to day 20 of lactation

Following delivery, from 20 litters/dose group, 1 male and 1 female pup were selected

from each litter for F₁ studies On day 4 of age, F₁ litters of >8 were culled to 8

randomly Culled pups were examined if displaying external abnormalities F₁ pups

were mated at 9-10 weeks of age Natural delivery followed F₂ litters were examined at

1 day of age and sacrificed on day 7 F₁ females were sacrificed and examined

macroscopically with implantations counted

Number/sex/group 22 females/dose

Parameters and endpoints evaluated During the study clinical signs were observed twice

daily, bodyweight (F₀) was recorded on days 0, 3, 6, 10, 14, 17 and 20 after mating and

daily until parturition, then during on lactation on days 1, 4, 7, 11, 14, 18 and 21, food

consumption was recorded on days 0-2, 3-5, 6-9, 10-13, 14-16, and 17-19 after mating,

days 1-3, 4-6, 7-10, 14-17, and 18-20 of lactation, post-natal observations included

recording numbers of live/dead offspring, sex, weight and clinical observations at

delivery, mortality (daily), bodyweight (days 1, 4, 7, 11, 14, 18, 21 and 28), physical

development (pina unfolding, hair growth, tooth eruption, eye opening), auditory and

visual development, day 25 (startle response, pupillary response, visual placing

response), locomotor activity (day 26/27), maze swimming, neuromuscular function

(traversing flat and round rods, rotarod, righting reflex, wire hanging, grid-gripping),

clinical signs (daily), bodyweight (F₁ males, weekly, females, weekly until mating,),

sexual maturation, mating interval, F₂ litters examined for live/dead pups at birth, daily clinical signs, mortality and litter size, bodyweight on days 1, 4, 7 At day sacrifice, pups were examined macroscopically

Results

Mortality All F₀ rats survived until sacrifice

Clinical signs alopecia seen in all groups

Body weight Females receiving 300 mg/kg had decreased weight gain relative to controls during the first week of treatment, remaining at lower weight than controls throughout gestation

Food consumption Significantly reduced in 150 and 300 mg/kg females during first four days of treatment (days 6-9)

Toxicokinetics not conducted

For peri-postnatal development studies

In-life observations

Dams all F₁ females were pregnant and delivered live offspring

Offspring One litter (150 mg/kg) had eaten pups, survivors were killed for humane reasons, one litter (300 mg/kg) had pup deaths on days 1, 2, 4, 5 with little milk found in stomachs, the dam having pale and inactive mammary tissue This litter reduced the mean litter weight for the 300 mg/kg group, otherwise bodyweights were unaffected, pup physical development, auditory and visual response, locomotor activity, water maze performance, neuromuscular tests were not affected by maternal treatment, litter size, sex ratio, pup survival were unaffected by F₀ treatment

Terminal and necropsic evaluations

Dams F₀ and F₁ dams had corpora lutea counts unaffected by treatment

One F₁ dam (300 mg/kg F₀) was killed in last week of study following weight loss, abdominal distension and yellow skin Necropsy showed enlarged liver and spleen

Offspring F₁ and F₂ No F₀ treatment-related signs were seen

Summary of individual study findings

Maternal bodyweight gain was decreased in the 150 and 300 mg/kg dams relative to controls No other overt treatment related signs were seen in the F₀ dams The NOEL was 50 mg/kg (HED= 8.33 mg/kg) In the F₁ and F₂ offspring, peri- and post-natal development, behavior and reproductive performance appear unaffected by F₀ maternal treatment

6 Study title Rifaximin study of tolerance in the rabbit by oral gavage administration

Key study findings orange feces, no drug-related effects on implantation

Study no 96/ ~ 001/1040, N2103

Volume #, and page # v 18, p217

Conducting laboratory and location

Date of study initiation 17 July 1996

GLP compliance yes

QA reports yes (x) no ()

Drug, lot #, radiolabel, and % purity lot# AC/0093

Formulation/vehicle 1% methyl cellulose

Methods

Species/strain female New Zealand White rabbits, — ,

Doses employed see study design below

Route of administration oral gavage

Study design group I 2 females (non-pregnant) received escalating doses as follows days 1-2, 25, mg/kg, days 3-4, 50 mg/kg, days 5-6, 100 mg/kg, days 7-8, 200 mg/kg, days 9-10, 400 mg/kg, days 11-12, 800 mg/kg, day 13, 1600 mg/kg Following this preliminary study, group II, 2 pregnant females received 1000 mg/kg on days 6-12 of gestation with sacrifice on day 13

Number/sex/group 2

Parameters and endpoints evaluated Bodyweight and clinical signs were recorded daily
Gross pathology was performed at necropsy

Results

Mortality all rabbits survived until sacrifice

Clinical signs In group I, orange/brown staining under the cage was seen for both females from day 4 (50 mg/kg) until sacrifice, while group II rabbits exhibited this staining on days 12-13 Body swaying was seen in one female from day 4 until sacrifice

Body weight group II rabbits had body weight loss from days 6-9 (initiation of treatment)

Food consumption not recorded

Toxicokinetics non conducted

Dams had no gross pathologies at necropsy Group II females had successful implantation

Summary of individual study findings

Fecal elimination of rifaximine is apparent following prolonged or high doses in rabbits by orange colored feces No other overt drug-related effects were seen

7 Study title Rifaximin preliminary embryo-fetal toxicity study in the rabbit by oral gavage administration

Key study findings Fetal defects in the 100 (jaw shortening, posterior cleft palate) mg/kg group and 300 (agnatha) mg/kg groups yields a fetal NOAEL of <100 mg/kg

Study no 96/ — J02/1263, N2154
Volume #, and page # v 18, p258
Conducting laboratory and location

Date of study initiation 2 Oct 1996
GLP compliance yes
QA reports yes (x) no ()
Drug, lot #, radiolabel, and % purity lot # AC/0093
Formulation/vehicle 1% methylcellulose

Methods

Species/strain female New Zealand White rabbits, —
Doses employed 0, 100, 300, 1000 mg/kg (HED= 0, 33, 100, 333 mg/kg)
Route of administration oral gavage
Study design females were inseminated, dosed starting on day 6 of gestation through day 19 and sacrificed on day 29 At necropsy, dams were examined for gross pathology and pregnancy parameters, fetuses were removed and examined (see below)
Number/sex/group 4
Parameters and endpoints evaluated clinical signs (daily), bodyweight (daily), food consumption, days 1-5, 6-12, 13-19, 20-23, and 24-28, number of corpora lutea, number of implantation sites, number of resorption sites, number and distribution of live and dead fetuses, fetal weights, placental weights, external fetal examination

Results

Mortality all rabbits survived until sacrifice
Clinical signs orange/brown staining in cage pan of treated rabbits
Body weight bodyweight gain was decreased during treatment (days 6-19) for the 300 and 1000 mg/kg groups, followed by increased weight gain after day 19
Food consumption decreased in all treatment groups during treatment, remaining decreased in 100 and 300 mg/kg groups, the 1000 mg/kg group was increased after day 19, greater than controls on days 24-28
Toxicokinetics not conducted

For embryofetal development studies

In-life observations orange/brown staining in cage pan of treated rabbits
Terminal and necroscopic evaluations
Dams no drug-related gross signs were seen at necropsy, corpora lutea, implantation, and litter size fetal and placental weights were unaffected by treatment
Offspring fetal and placental weights were unaffected by treatment External examination showed one 100 mg/kg fetus with jaw shortening, posterior cleft palate, one 300 mg/kg fetus had agnatha

Uterine examination group mean values for females killed on Day 29 of gestation

Group	1	2	3	4								
Compound	Control	Rifaximin										
Dosage (mg/kg/day)	0	100	300	1000								
Group	Number of pregnant animals	Corpora lutea count	Implantations	Viable young			Resorptions			Implantation loss (%)		
				M	F	Total	Early	Late	Total	Pre-	Post	
1	4	Mean SD	13.3 2.1	11.5 3.3	5.3 1.0	4.5 1.9	9.8 2.2	0.3 0.5	1.5 1.2	1.8 1.3	13.2	15.2
2	4	Mean SD	13.0 3.5	11.5 4.0	5.5 1.3	4.8 2.8	10.3 2.6	0.5 0.7	0.8 0.9	1.1 1.1	11.5	10.9
3	4	Mean SD	13.3 4.0	12.5 4.4	5.5 1.7	5.0 2.2	10.5 2.6	1.5 1.2	0.5 0.7	2.0 1.4	5.7	16.0
4	3	Mean SD	12.7 1.5	11.7 3.1	2.7 2.1	6.7 2.3	9.3 4.0	0.3 0.6	2.0 1.4	2.3 1.5	10.3	20.0

SD Standard deviation

Summary of individual study findings The NOAEL for maternal exposure was 1000 mg/kg, the incidence of a structural defect in both the 100 (jaw shortening, posterior cleft palate) and 300 (agnathia) mg/kg groups yields a fetal NOAEL of <100 mg/kg

8 Study title Rifaximin study of effects on embryo-fetal toxicity in the rabbit by oral gavage administration

Key study findings Increased incidence of 20 thoracolumbar vertebrae (all treated groups), subdural hemorrhage (250, 1000 mg/kg), partially open eye (1000 mg/kg), one fetus with small eyes, retinal irregularities, cleft palate, brachygnathia, small displaced kidneys, sternebral irregularities, large atrium, interventricular septal defect (250 mg/kg)

Study no — 009/973155, N2155

Volume #, and page # v 19, p 32

Conducting laboratory and location

Date of study initiation 11 Mar 1997

GLP compliance yes

QA reports yes (x) no ()

Drug, lot #, radiolabel, and % purity PP2041

Formulation/vehicle 1% methylcellulose

Methods

Species/strain female New Zealand White rabbits, —

Doses employed 0, 62.5, 250, 1000 mg/kg (HED=0, 20, 42, 333 mg/kg)

Route of administration oral gavage

Study design Females were mated with New Zealand White male rabbits Dosing began on day 6 following insemination to day 19 of gestation Dams were sacrificed on day 29 and examined for pregnancy parameters, Fetuses were examined grossly, and for skeletal and visceral abnormalities Toxicokinetic satellite dams had blood collected on days 6 and 19 prior to dosing and 1, 2, 4, 6, 8 hr following dosing

Number/sex/group 22, main study, 4 toxicokinetic satellite group

Parameters and endpoints evaluated maternal clinical signs, bodyweight, daily, food consumption, days 1-5, 6-12, 13-19, 20-23, 24-28, at necropsy number of corpora lutea, number of implantation sites, number of resorption sites, number and distribution of live and dead fetuses, fetal weights, placental weights, external, visceral and skeletal fetal examination

Results

Mortality one control, day 25, two control dams aborted day 21, 25, two 250 mg/kg dams aborted, day 23, 29 Aborted dams were sacrificed on same day of aborting, with macroscopic examination, and corpora lutea and implantation sites counted

Clinical signs No other drug-related clinical signs were seen

Body weight All treated groups had reduced bodyweight gain following initiation of treatment, resulting in mean group weight less than control through the treatment period

Food consumption Treated groups had significantly reduced food consumption after the initiation of treatment, gradually improving toward the end of treatment but less than controls

Toxicokinetics

Dosage (mg/kg/day)	C _{max} (ng/ml)		AUC ₀₋₂₄ (ng.h/ml)	
	Day 6	Day 19	Day 6	Day 19
250	— (0.813)	— (0.573)	6.63 (2.19)	8.25 (3.24)
1000	— (1.372)	— (5.760)	10.95 (7.98)	19.38 (7.36)

()= standard deviation

Insufficient quantifiable plasma levels prevented estimation of pharmacokinetic parameters from the 62.5 mg/kg dose group

For embryofetal development studies

In-life observations

Terminal and necroscopic evaluations

Dams

Uterine examination - group mean values for females killed on Day 29 of gestation

Group	1	2	3	4
Compound	Control	Rifaximin		
Dosage (mg/kg/day)	0	62.5	250	1000

Group		Corpora Lutea	Implantations	Viable young			Resorptions			Implantation loss(%)	
				Male	Female	Total	Early	Late	Total	Pre-	Post-
1	Mean	12.9	9.7	3.8	4.1	7.9	0.88	0.94	1.82	25.3	18.8
	SD	2.3	2.9	1.7	1.6	2.5	0.94	0.97	1.35		
	n	17	17	17	17	17	17	17	17		
2	Mean	11.4	10.2	4.9	3.7	8.6	1.14	0.52	1.67	12.2	16.3
	SD	3.2	3.6	1.8	1.4	2.9	1.07	0.72	1.29		
	n	21	21	21	21	21	21	21	21		
3	Mean	12.7	10.3	4.3	4.8	9.1	0.84	0.42	1.26	19.3	12.2
	SD	3.9	3.2	1.4	2.3	2.7	0.92	0.65	1.12		
	n	19	19	19	19	19	19	19	19		
4	Mean	12.2	9.5	3.2	4.2	7.5	1.23	0.77	2.00	23.2	21.2
	SD	3.1	4.0	1.8	2.4	3.6	1.10	0.88	1.41		
	n	22	22	22	22	22	22	22	22		

SD Standard deviation.

n Number of pregnant animals on Day 29 of gestation.

No apparent drug effect was present on uterine/implantation/resorption parameters

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Offspring

Fetal evaluation malformations - group incidence by type

Group	1	2	3	4				
Compound	Control	Rifaximin						
Dosage (mg/kg/day)	0	62.5	250	1000				
Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Number examined	134	180	172	164	17	21	19	22
Number affected	6	3	3	3	6	3	3	3
<u>Description</u>								
Cebocephaly persistent posterior cardinal vein	-	1	-	-	-	1	-	-
Hydrocephaly, absent olfactory lobes and upper incisors fused nares nasal tract irregularities, anury		1	-	-	-	1	-	-
Marked dilated 4th ventricle and spinal canal		1				1	-	-
Fused frontals to parietals		1		-	-	1	-	-

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Fetal evaluation malformations - group incidence by type

Group	1	2	3	4				
Compound	Control	Rifaximin						
Dosage (mg/kg/day)	0	62.5	250	1000				
Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Number examined	134	180	172	164	17	21	19	22
Number affected	6	3	3	3	6	3	3	3
<u>Description</u>								
Absent left kidney and ureter, large right kidney	1	-	-	-	1	-	-	-
Persistent posterior cardinal vein	-	1	-	2	-	1	-	2
Lumbar scoliosis due to hemivertebra	-	-	1	-	-	-	1	-
Spina bifida occulta	1	-	-	-	1	-	-	-
Anury	1	-	-	-	1	-	-	-
Sternebral irregularities	-	1	-	-	-	1	-	-

Fetal evaluation malformations - group incidence by type

Group	1	2	3	4				
Compound	Control	Rifaximin						
Dosage (mg/kg/day)	0	62.5	250	1000				
Group	Fetuses				Litters			
	1	2	3	4	1	2	3	4
Number examined	134	180	172	164	17	21	19	22
Number affected	6	3	3	3	6	3	3	3
<u>Description</u>								
Partially open eye	-	-	-	1	-	-	-	1
Small eyes, retinal irregularities, cleft palate, brachygnathia, small displaced kidneys, sternbral irregularities, oedema, large atrium dilated pulmonary trunk, interventricular septal defect.	-	-	1	-	-	-	1	-
Cleft palate	-	1	-	-	-	-	1	-

Fetal evaluation head anomalies - group incidence by type

Group	1	2	3	4
Compound	Control	Rifaximin		
Dosage (mg/kg/day)	0	62.5	250	1000

Group	Heads				Litters			
	1	2	3	4	1	2	3	4
Number examined	45	60	59	54	17	21	19	22
Number affected #	0	0	2	2	0	0	2	2

Descriptions

Subdural haemorrhage		-	1	1	-		1	1
Folded retina	-	-	1		-	-	1	-
Dilated 4th ventricle	-	-	-	1	-	-	-	1

Fetus/litters may occur in more than one category
 α Necropsy findings not included

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Fetal evaluation - skeletal variants; group values

Group	1	2	3	4
Number litters examined	17	21	19	22
Number fetuses examined	134	180	172	164
Ribs				
Number of fetuses with 12 ribs	63	31	28	17
Number of fetuses with 12/13 or 13/13 ribs	71	149	144	147
Mean % fetuses per litter with 12/13, 13/13	53.8	83.6	80.9	89.5
20 Thoracolumbar vertebrae				
Number of fetuses affected	25	80	81	71
Mean % fetuses per litter	17.8	41.6	46.6	42.7
Sternebrae				
No of fetuses with 1 to 5th sternebrae	13	11	2	7
No of fetuses with 1 to other sternebrae	3	9	4	1
Total number of affected fetuses	15	20	6	7
Mean % fetuses per litter with 1 to sternebrae	10.8	11.2	3.4	7.0

10 Incomplete ossification

Summary of individual study findings

Increased incidence of 20 thoracolumbar vertebrae (all treated groups), subdural hemorrhage (250, 1000 mg/kg), partially open eye (1000 mg/kg), one fetus with small eyes, retinal irregularities, cleft palate, brachygnathia, small displaced kidneys, sternebrae irregularities, large atrium, interventricular septal defect (250 mg/kg) Maternal NOAEL was 62.5 mg/kg (HED=21 mg/kg), fetal defects at all doses preclude a NOEL (<62.5 mg/kg) Systemic exposure of rifaximine is minimal (AUC= not obtainable, 62.5 mg/kg=6.63-8.25 ng hr/ml, 1000 mg/kg=10.95-19.38 ng hr/ml) It is unclear whether the fetal effects observed in this study are due to systemic drug exposure, maternal effects, or within normal background levels of defects

Reproductive and developmental toxicology summary

This summary is based mainly on studies conducted under GLP by _____, not the pre-GLP studies by Alfa Wassermann with questionable translations

Major rifaximin-related findings in reproductive toxicity studies

Species	Maternal findings	Fetal effects	Other findings	Doses of findings Mg/kg	HED Mg/kg
rat	↓ food consumption, body weight		Mortality, male	150, 300 300	25, 50 50
	↓ bodyweight gain	↑ incomplete ossification ↓ hemorrhage		300 150, 300 150, 300	50 25 50 25 50
	↓ weight gain			150, 300	
rabbit		Jaw shortening, cleft palate Agnathia		100 300	33 100
	Systemic exposure of rifaximine is minimal (AUC= not obtainable, 62.5 mg/kg, 250 mg/kg=6.63-8.25 ng hr/ml, 1000 mg/kg=10.95-19.38 ng hr/ml)	120 thoracolumbar vertebrae subdural hemorrhage partially open eye one fetus with small eyes, retinal irregularities, cleft palate, brachygnathia, small displaced kidneys, sternebral irregularities, large atrium, interventricular septal		62.5, 250, 1000 250, 1000 250	21, 83, 333 83, 333, 83

Reproductive and developmental toxicology conclusions

It is difficult to correlate rat and rabbit exposure and fetal effects to clinical exposure due to the poor absorption of rifaximin. A pharmacokinetic study within a rabbit reproductive toxicity study indicate slightly increased exposure between days 6 and 19 of treatment. Perhaps at higher doses rifaximin accumulates in the gastrointestinal tract, impeding nutrition as well as promoting absorption by mass action. Antibacterial effects in intestinal flora and inhibition of nutrient absorption, especially at higher doses, may also account for decreased maternal weight and food consumption. Fetal effects may correlate with this action and not with fetotoxic effects of drug.

Labeling recommendations

Pregnancy category C Rifaximin was teratogenic in rats at doses of 150 to 300 mg/kg (approximately 2.5 to 5 times the clinical dose adjusted for body surface area) and in rabbits at doses of 62.5 to 1000 mg/kg (approximately 2 to 33 times the clinical dose adjusted for body surface area) These effects include cleft palate, agnathia, jaw shortening, hemorrhage, eye partially open, small eyes, brachygnathia, incomplete ossification, and increased thoracolumbar vertebrae There are no adequate and well controlled studies in pregnant women Rifaximin should be used during pregnancy only if the potential benefit outweighs the potential risk to the fetus

SPECIAL TOXICOLOGY STUDIES

Not conducted ADDENDUM TO REVIEW
(if necessary)

APPENDIX/ATTACHMENTS

IND 52980 reviews # 000, 004, 006, 028, 032

1 Rifaximine report on the toxicological properties of the product L/105, by Alfa Wassermann, Milan, Italy, Salix control N2001, batch no *442-LS/2245 and *534-LS/2380, 1 June 1981, pre glp

Male and female rats (NOS, Sprague -Dawley, 200g), 8/sex/dose, were administered L/105 by oral gavage. Control rats received vehicle (5% water solution of gum arabic). Treatment groups received a single dose of 500, 1000 or 2000 mg/kg. Neurological tests were administered during the 4 hours following dosing. Rats were observed for mortality for the next 14 days. On day 14, rats were sacrificed and necropsied. All rats survived until necropsy. Clinical signs were seen in the 2000mg/kg dose group which exhibited excitement following dosing and hepatic steatosis at necropsy. The NOEL for this study was 1000mg/kg.

2 Subacute toxicity of the product L/105 administered orally to beagle dogs, 7 days out of 7, for 13 weeks at doses of 25-50-100 mg/kg/day, performed by Alfa Wassermann, Milan, Italy, Salix control #N2006, batch no *1-LS/2291, 12 May 1981, pre glp

Male and female beagle dogs (supplier, age not listed), 3/sex/group, were administered L/105 by oral capsule. Control dogs received 2 capsules each, containing 200 mg lactose. Treatment groups received capsules containing L/105 at doses of either 25, 50 or 100 mg/kg daily for 13 weeks. Parameters measured during the study included mortality and symptomology, ophthalmoscopic examination, body weight, food consumption, urinalysis, fecal observation, hematology and blood chemistry. All dogs survived through the study. During the study, no clinical signs were seen. At necropsy, a battery of tissues were collected for histopathologic examination. Ophthalmic examinations were normal. Body weight, food consumption, urinalysis, feces, blood chemistry and hematology were unaffected by drug treatment. At necropsy, no gross or histopathologic drug-related abnormalities were observed. The NOEL in this study was >100 mg/kg, equivalent to approximately 55 mg/kg human dose.

3 Acute toxicity in the dog, performed for Alfa Wassermann, Milan, Italy by _____ Salix control #N2005, batch no *LS/2291, 11 Sept 1980, pre glp

One male and one female Beagle dog (_____, _____), were administered L/105 in water at doses of 2 g/kg/day (male) and 3 g/kg/day (female) daily for 7 days, then observed for 7 more days. Slight prostration was observed on day 2 in the 2 g/kg/day dog. No other clinical sign was seen. On day 14, the dogs were sacrificed and examined by gross necropsy. No lesions were observed.

Comment No route of administration is described for this study. The lack of detail provided in this study limit its value.

4 L 105 (Rifaximine) report on chronic toxicity in dogs, performed by Alfa Wassermann, Milan, Italy, Salix control #N2007, batch no *569-LS/2439, 16 Feb 1983, pre glp

Male and female beagle dogs (12-18 months old, supplier not listed, 3/sex/dose) were administered L/105 orally in gelatin capsules daily for 26 weeks. Dose groups consisted of O (lactose control), 25, 50 and 100 mg/kg. Parameters measured during the study included mortality and symptomology, ophthalmoscopic examination, body weight, food consumption, urinalysis, fecal observation, hematology and blood chemistry. All dogs survived through the

study No clinical signs were seen during the study Ophthalmic examinations were normal Body weight, food consumption, urinalysis, feces and hematology were unaffected by drug treatment Cholesterol levels were increased in 100 mg/kg females At necropsy, a battery of tissues were collected for histopathologic examination Some erosion of the intestinal mucosal membrane was observed in the drug treated dogs No other drug-related histopathologic signs were seen The NOEL in this study was 50 mg/kg (approximately 27.7 mg/kg equivalent human dose)

5 Report on the toxicological properties of the product "L/105" (Rifaximine)-subacute toxicity in the rodent (3 months), performed by

— for Alfa Wassermann, Milan, Italy, Salix control #N2002, batch no *4 LS/2245 and *534-LS/2380, 1 June 1981, pre glp

Sprague-Dawley rats (—, 60 days old) in groups of 12 males and females each, were administered L/105 (0, 25, 50, 100 mg/kg) or Neomycin sulfate (100 mg/kg) by oral gavage daily for three months Drugs were dissolved in a 5% aqueous solution of gum arabic Parameters measured during the study were mortality and symptomology, body weight, food consumption, urinalysis, fecal observation and hematology During the study, 4 rats died By macroscopic examination these deaths were attributed to non-drug related causes 1 control female, week 12, pneumonia ab ingestis, 1 male 25 mg/kg, week 9, pneumonia, 1 male, 100 mg/kg, week 11, pasteurellosis, 1 female, 100 mg/kg, week 1, undetermined At necropsy, the following clinical signs were seen 100 mg/kg, hepatic steatosis, hepatomegaly Histologic examination showed the following 100 mg/kg, mucosal ulceration, hepatic connectival proliferations, slight nephrosis No other drug related clinical signs were seen The NOEL in this study was 50 mg/kg (approximately 8 mg/kg equivalent human dose)

6 L/105 (rifaximine) report on chronic toxicity in rats, performed by Alfa Wassermann, Milan, Italy, Salix control #N2004, batch no *569-LS/2439, 22 Mar 1983, pre glp

Male and female Sprague-Dawley rats (approximately 70 days old), in groups of 20 males and females each, were administered L/105 by oral gavage daily for 26 weeks Doses of 0, 25, 50 and 100 mg/kg were used Drug was dissolved in 1% gum arabic suspension Parameters measured during the study were mortality and symptomology, body weight, food consumption, urinalysis, fecal observation and hematology Four rats died during the study, all attributed to pneumonia ad ingestis One control (day 37), one 25 mg/kg (day 98), one 50 mg/kg (day 122) and one 100 mg/kg rat (day 43) died Otherwise, no clinical signs were observed during the study Body weight, food consumption, urinalysis, hematology were all unaffected by drug treatment In male rats in the 50 mg/kg dose group and female rats in the 50 and 100 mg/kg dose groups cholesterol was elevated At necropsy, a battery of tissues were collected for histopathologic examination Observations included hepatomegaly and infiltrate of connective cells into liver parenchyma and regression of renal parenchyma in the 50 and 100 mg/kg groups Catarrhal enteritis was found in all treated groups The NOEL level in this study was 25 mg/kg

7 Subacute toxicity in the rat, performed by Alfa Wassermann, Milan, Italy, Salix control #N2001, batch no *442-LS/2245 and *534-LS/2380, 21 Jan 1994, not glp

Male and female Sprague-Dawley rats (200±10 g) were grouped into 8 males and 8 females per dose group Rats were placed into groups receiving 0, 500, 1000 or 2000 mg/kg L/105 in 5% gum arabic by oral gavage Rats received a single dose and were observed for 14 days On day 14, rats were sacrificed under ether anesthesia and their organs studied by

macroscopic examination No rats died during the study Hepatic steatosis was observed in 2 of 8 males in the 2000 mg/kg group The LD₅₀ in this study was >2000 mg/kg for both sexes Individual animal data was not provided for this study

8 Acute toxicity in the rat, performed by Alfa Wassermann, Milan, Italy, Salix control #N2097, batch not listed, date not listed, not glp

L/105 was applied on a shaved area of Sprague -Dawley rats at doses of 500, 1000 and 2000mg/kg

Comment The sponsor does not provide information on the age, sex, number of rats used, as well as formulation or how a dosage per body weight was derived for this application These shortcomings limit the value of this study

The L/105 ointment was applied to less than 10% of the body surface area and covered by a semi-occlusive bandage for 4 hours Rats were observed for 14 days and sacrificed No clinical signs were seen following dosing or at necropsy

9 Local tolerance studies-evaluation of reversible or irreversible cutaneous damage, performed by Alfa Wassermann, Milan, Italy, Salix control #N2099, batch not listed, date not listed, not glp

L/105 was applied on the both healthy and abraded dorsal skin area of guinea pigs in ointment at concentrations of 0 (excipients), 2.5, 5 and 10% A second administration was applied one week later No skin reaction was detected

Comment The sponsor does not provide information on the age, sex, number of guinea pigs used, as well as formulation These shortcomings limit the value of this study

10 Subcutaneous toxicity in the rat, performed by Alfa Wassermann, Milan, Italy, Salix control #N2098, batch not listed, date not listed, not glp

L/105 5% ointment was applied on a shaved area of Sprague-Dawley rats at doses of 0, 25, 50 and 100 mg/kg for 28 days (5 days/week) No clinical signs were seen following treatment

Comment The sponsor does not provide information on the age, sex, number of rats used, as well as formulation These shortcomings limit the value of this study

11 Special toxicity studies, performed by Alfa Wassermann, Milan, Italy, Salix control #N2100, batch not listed, date not listed, not glp

L/105 was applied to skin of the New Zealand rabbit in single dose to several animals in 5% ointment with each rabbit serving as its own control in a model described by Draize for evaluation of cutaneous injuries Bandages covered the area tested and were removed prior to examination at 0, 5, 10, 24, 48, 72 hr and 14 days

Comment The sponsor does not provide information on the age, sex, number of guinea pigs used, as well as formulation Results are not included These shortcomings limit the value of this study

NONCLINICAL PHARMACOLOGY STUDY

Report on the toxicological properties of the product L/105 (rifaximne) evaluation of the effects exerted on the cardiovascular and respiratory systems performed by Alfa Wassermann, Milan, Italy, Salix control # 2003, batch no *442-LS/2245 and *534-LS/2380, date not listed, pre glp

Activity on the cardiovascular system in the rat

Not reviewed

Activity on respiratory system in the guinea pig

Not reviewed

SPECIAL STUDIES

Genetic Toxicology

1 Study of the mutagenic activity of the compound L/105 with Salmonella typhimurium, performed by Alfa Wassermann, Milan, Italy, Salix control # 2016, batch no *L 499, 21 Jan 1994, glp

Five strains of Salmonella typhimurium were used in this study to detect mutations caused by either frameshift or substitution mutations due to L/105. Concentrations of L/105 (in DMSO) used were 500, 50, 5, 0.5 µg/0.1 ml.

Concentrations less than 50 µg/0.1 ml were not bactericidal. L/105 and positive controls (controls: hydrazine, doxorubicin, 9-aminoacridine, 2-aminofluorene) were performed with and without addition of Aroclor 1254 activated rat microsomal preparation, in triplicate. L/105 did not show mutagenic activity with or without metabolic activation at maximum concentration, positive controls were effective.

2 An evaluation of the mutagenic potential of rifamixin in the Ames Salmonella Microsome assay, performed by — Salix control # 2019, batch no *G0704, 12 July 1991, glp

Five strains of Salmonella typhimurium were used in this study to detect mutations caused by either frameshift or substitution mutations due to rifamixin transformed by Aroclor 1254-induced rat liver S9 fractions. Positive controls included (without S9) sodium azide, 2-nitrofluorene, ICR-191 acridine, with S9 2-aminoanthracene was used. Concentrations of rifamixin in DMSO used were 0.2 to 100000 µg/ml (0.01 to 5000 µg/plate). Concentrations of > 10 µg/ml were cytotoxic.

In the limited range of concentrations without toxicity, no significant increase in numbers of revertant colonies of drug treated plates, with or without metabolic activation relative to vehicle controls. Positive controls were effective.

3 Study of the mutagenic activity of the compound L/105 with Saccharomyces cerevisiae, performed by Alfa Wassermann, Milan, Italy, Salix control # N2017, batch # *L 499, 21 August 1994

This test of L/105 for induction of genic conversions of loci Ade 5 and Trp 5 on different chromosomes of Saccharomyces cerevisiae. Increase in the frequency of genic conversions reflects mutagenic activity. Four hour incubations were performed, with and without metabolic

activation by Araclor 1254-induced Sprague-Dawley rat S9 liver fractions Drug was dissolved in DMSO Concentrations of L/105 of 250, 500 and 1000 mg/ml were used Methylmethanesulphonate was used as a positive control in the non-activated study, cyclophosphamide was used in the activated study L/105 did not increase genic conversions in this test system, positive controls were effective

4 Study of the mutagenic activity of the compound L/105 with Schizosaccharomyces pombe, performed by Alfa Wasserman, Milan, Italy, Salix control # N2018, batch # * L 499, 21 January 1994

This test of L/105 for induction of mutations in the biosynthesis of adenine of the mutated haploid yeast Schizosaccharomyces pombe, scattered on five different chromosomes Reversion of mutations in this pathway results in production of white instead of red colonies Four hour incubations were performed, with and without metabolic activation by Araclor 1254-induced Sprague-Dawley rat S9 liver fractions Drug was dissolved in DMSO Concentrations of L/105 of 250, 500 and 1000 mg/ml were used Methylmethanesulphonate was used as a positive control in the non-activated study, dimethylnitrosamine was used in the activated study L/105 did not increase mutations in this test system, positive controls were effective

5 Rifaximin chromosome aberration in human lymphocytes cultured in vitro, performed by Alfa Wasserman, Milan, Italy, Salix control # N2021, batch # * 920531, 21 January 1994

Rifaximin was evaluated in human lymphocytes, attempting to induce chromosomal aberrations in the presence and absence of metabolic activation Fresh heparinized blood provided lymphocytes for culture Rifaximin concentrations of 0.1, 1, 10 and 100 µg/ml (dissolved in DMSO) and positive controls mitomycin C (dissolved in water), cyclophosphamide (dissolved in DMSO) and colchicine (dissolved in water) were used In the subsequent metabolic activation study, Sprague-Dawley rat S-9 reaction mixture, induced by Araclor 1254, was added to the lymphocyte culture Due to cytotoxicity, a second study using Rifaximin concentrations of 0.01, 0.1, and 10 µg/ml were used Cells from culture were placed on microscope slides for optical examination of changes in mitotic indices relative to controls None of the concentrations of Rifaximin showed increased incidence of mutation with or without metabolic activation, positive controls were effective

6 Rifaximin micronucleus test in rat bone marrow, performed by Alfa Wasserman, Milan, Italy, Salix control # N2022, batch # 920406, 21 January 1994

Clastogenic potential of Rifaximin was evaluated in the bone marrow micronucleus assay Drug was administered orally to male and female Sprague Dawley rats (— —), 6 weeks old, 5/dose/sex/timepoint Doses of 1000 and 2000 mg/kg were used Drug treated rats were sacrificed at 18, 42 and 66 hours after treatment Positive controls received mitomycin-C, with 5/dose/sex sacrificed at 42 hours Following sacrifice, a femur was removed from each rat, marrow aspirated and prepared for examination for the presence of micronucleated polychromatic erythrocytes Rifaximin did not induce increases in frequency of multinucleated cells, the positive control was effective

**7 An evaluation of the mutagenic potential of rifamixin in the CHO/HGPRT mutation assay, performed by Salix control #
N2022, batch # G 0704, 27 August 1991**

In the CHO/HGPRT mutation assay, Rifamixin was evaluated for mutagenic potential, with and without metabolic activation. Drug concentrations of 10-80 µg/ml without metabolic activation and 100-250 µg/ml with metabolic activation. The positive controls ICR-191 acridine, without metabolic activation, and 3-methylcholanthrene, with metabolic activation, were used. CHO cells were cultured with drug for 20-24 hours (without metabolic activation). Metabolic activation studies employed CHO cells with rat liver S-9 reaction mixture, induced by Aroclor 1254, were incubated with drug for 4 hours, then washed and incubated with drug an additional 16 to 20 hours. Subcultures were prepared from all cultures to determine cytotoxicity. None of the concentrations of Rifamixin showed increased incidence of mutation with or without metabolic activation, positive controls were effective.

Steven C Kunder, Ph D
Reviewing Pharmacologist

concurrences

HFD-530/DeputyDir/GChikami
HFD-530/PharmTL/JFarrelly
Steven C Kunder/Pharm/

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HFD-530/JFarrelly

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HFD-530 (original)
HFD-530 Division file
HFD-340
HFD-530/RRoca
HFD-530/NSchmuff
HFD-530/SOhanian
HFD-530/

PHARMACOLOGIST'S REVIEW

IND # 52980/004

DATE SUBMITTED 23 June 1998

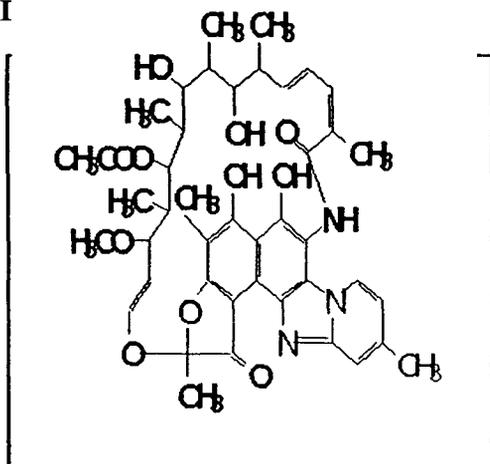
DATE RECEIVED 26 June 1998

DATE ASSIGNED 2 July 1998

DATE REVIEW COMPLETED 2001

SPONSOR Salix Pharmaceuticals, Inc

I



HFD-590

FORMULATION  200 mg tablets[†]

RELATED DOCUMENTS DMF# 

INDICATION 

BACKGROUND INFORMATION Rifaximin is a semisynthetic derivative of rifamycin with minimal systemic absorption from oral administration. It appears similar to other rifamycin derivatives such as rifampin.

TOXICOLOGY STUDIES

- 1 Rifaximine Preliminary toxicity study by oral gavage administration to CD rats for 4 weeks
- 2 Rifaximine Preliminary toxicity study by oral gavage administration to beagle dogs for 4 weeks
- 3 Rifaximine preliminary embryo-foetal toxicity study in the rabbit by oral gavage administration

TOXICOLOGY STUDY RECIEWS

Rifaximine Preliminary toxicity study by oral gavage administration to CD rats for 4 weeks, study no N2148, conducted by England, batch no pp2040, 8 Oct 1997, GLP

CD rats (males and females, 21-28 days old, 90-103 g, were assigned to treatment groups (5/sex/dose) receiving either 0 (10% aqueous methylcellulose vehicle control), 100, 300 or 1000 mg/kg (HEV=0, 16.6, 50, and 166 mg/kg) in a single daily dose by oral gavage for 4 weeks. Parameters recorded during the study included clinical signs (twice daily), bodyweight, food consumption and food conversion efficiency (weekly). Blood was collected during week 4 of treatment for a standard battery of hematology and clinical biochemistry assays. At necropsy, a macroscopic examination was conducted followed by removal of visceral organs for weighing and preparation of tissues for future histopathology examination. All rats survived until necropsy. Orange fecal pellets were produced by males and females in the 1000 mg/kg group. Bodyweight gains were decreased in the 1000 mg/kg group with respect to controls (by 23% in males, 45% in females). In the 300 mg/kg group, female bodyweight gains were decreased by 33% compared with controls. Food consumption was decreased in the 1000 mg/kg group in males (12%) and in males receiving 300 mg/kg (8%). Females had decreased food consumption at the 300 mg/kg (7%) and 100 mg/kg (10%) doses. Hematology effects included slight elevations of packed cell volumes, hemoglobin and erythrocytes in rats receiving 1000 mg/kg and female rats receiving 300 mg/kg. Lymphocyte counts were decreased in both males and females at 300 and 1000 mg/kg, significantly in high dose females. Platelet counts were decreased significantly in both sexes at all doses except low dose females. Clinical chemistry effects included increased alanine amino transferase activity in high dose females, elevated plasma glucose in all treatment groups, decreased triglycerides in high dose males, and increased triglycerides in middle and high dose females. Organ weight changes included decreased spleen weights in low dose females, middle dose males and females, and high dose male and females, decreased thymus weights were seen in all groups and were dose-related, and uterine weights were decreased in all treated females. A NOAEL was not observed in this study.

Rifaximine Preliminary toxicity study by oral gavage administration to beagle dogs for 4 weeks, study no N2149, conducted by batch no pp2040, GLP, 30 October 1997

Not reviewed

Rifaximine preliminary embryo-foetal toxicity study in the rabbit by oral gavage administration, study no N2154, conducted by batch no AC/0093, GLP, 15 May 1997

Not reviewed

Steven C Kunder, Ph D
Reviewing Pharmacologist

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concurrences.

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Steven C Kunder/Pharm/

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HFD-590/KHastings

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HFD-590 (original)

HFD-590 Division file

HFD-340

HFD-590/JPowers

HFD-590/NSchmuff

HFD-590/SBala

HFD-590/DWillard

PHARMACOLOGIST'S REVIEW

IND # 52980/006

DATE SUBMITTED 16 JULY 1998

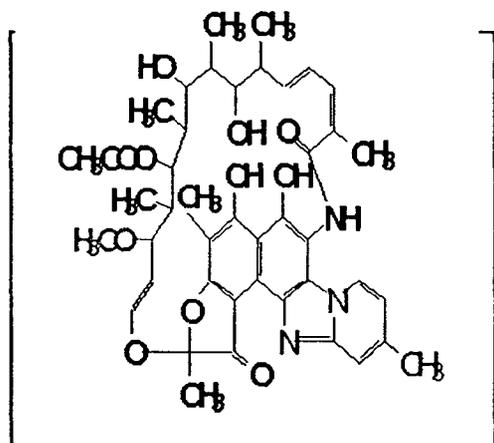
DATE RECEIVED 22 JULY 1998

DATE ASSIGNED 28 JULY 1998

DATE REVIEW COMPLETED 7 Dec 2001

SPONSOR Salix Pharmaceutical Inc

DRUG — (rifaximin)



HFD-590

FORMULATION solution in 1% methylcellulose

RELATED DOCUMENTS

INDICATION — diarrhea

ABBREVIATIONS

Hed= human equivalent dose

BACKGROUND INFORMATION Rifaximin is a semisynthetic derivative of rifamycin with minimal systemic absorption from oral administration. It appears to be similar to other rifamycin derivatives such as rifampin.

TOXICOLOGY STUDY

Rifaximin study of effects on embryo-fetal toxicity in the rabbit by oral gavage administration, Study no N2155, performed by —

— lot no PP2041, GLP, 22 February 1998

Rifaximin was administered by oral gavage daily to pregnant female New Zealand White rabbits (18-26 weeks old) on day 6 to 19 of gestation. Rabbits were assigned 22/dose group for treatment and 4/dose group for toxicokinetic study. Dams were mated with males, with the day of mating considered day 1 of gestation. Doses of control (1% methylcellulose vehicle), 62.5, 250 and 1000 mg/kg/day were used (human equivalent doses= 21, 83 and 333 mg/kg, respectively). During the study, animals were observed and weighed daily and food consumption was recorded on days 1-5, 6-12, 13-19, 20-23, and 24-28. Blood samples were collected on days 6 and 19 from toxicokinetic groups at 0, 1, 2, 4, 6 and 8 hours post-dosing. At day 29 caesarian delivery of the litters was performed, with examination of the reproductive tract including counts of corpora lutea, implantation sites, resorption sites, and live and dead fetuses. Fetuses and placentae were weighed and examined externally. The fetuses were examined for skeletal and visceral drug-related effects.

During the study, 1 control dam was found dead (day 25). Two control dams (days 21, 25) and two receiving 250 mg/kg aborted (days 23, 29). Orange material in the feces, likely drug, was seen in all treated groups, typical of rifamycin-like drugs. Treated groups had bodyweight loss on day 6 through day 8, after day 8 bodyweight for all treated groups paralleled but was slightly less than the weight of the control group. Food consumption from days 6-12 was significantly decreased in all treated groups and decreased on days 13-19 and 20-23. Four rabbits were not pregnant: 1 control, 1@ 62.5 mg/kg, and 1@250 mg/kg. Uterine examination provided the following:

Day 29 uterine examination

Dose (mg/kg)	# pregnant	Corpora lutea	Implantations	Viable young			Resorptions			Implantation Loss (%)	
				Male	female	total	Early	late	total	Pre-	post-
Control	17	12.9	9.7	3.8	4.1	7.9	0.88	0.94	1.82	25.3	18.8
62.5	21	22.4	10.2	4.9	3.7	8.6	1.14	0.52	1.67	12.2	16.3
250	19	10.3	10.3	4.3	4.8	9.1	0.84	0.42	1.26	19.3	12.2
1000	22	9.5	9.5	3.2	4.2	7.5	1.23	0.77	2.00	23.2	21.2

All values are means unless otherwise noted.

Toxicokinetic values were determined as follows:

Dose (mg/kg)	Cmax (ng/ml)		AUC (ng h/ml)	
	Day 6	day 19	Day 6	day 19
62.5	nd	nd	nd	nd
250			6.63	8.25
1000			10.95	19.38

nd= not determined due to insufficient concentration

Fetal examinations showed no gross external findings. Skeletal examination showed an increase in the treated groups of additional 13th rib(s), 20 thoracic/lumbar vertebrae and sternbral centers (incomplete ossification) and offset pelvic girdles.

CONCLUSIONS

Loss of maternal bodyweight at gestation days 6-8 at all doses (10-17%) while food consumption was also decreased in drug treated versus control rabbits on days 6-12. Drug absorption at the 62.5 mg/kg dose was minimal, absorption at 250 and 1000 mg/kg increased over the treatment period. The NOAEL for this study was less than 62.5 mg/kg, based on these and the fetal skeletal ossification effects seen in fetuses at all doses.

Steven C Kunder, Ph D
Reviewing Pharmacologist

concurrences

HFD-590/DeputyDir/RAAlbrecht
HFD-590/PharmTL/KHastings
Steven C Kunder/Pharm/

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HFD-590/KHastings

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HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/DWillard
HFD-590/MSeggel
HFD-590/SBala
HFD-590/

Histopathology Inventory for IND #

Study	1234			
Species	rat			
Adrenals	X*			
Aorta	X			
Bone Marrow smear				
Bone (femur)	X			
Brain	X			
Cecum	X			

Cervix				
Colon	X			
Duodenum	X			
Epididymis				
Esophagus				
Eye	X			
Fallopian tube				
Gall bladder				
Gross lesions	X			
Harderian gland				
Heart	X*			
Hypophysis				
Ileum	X			
Injection site	X			
Jejunum	X			
Kidneys	X*			
Lachrymal gland				
Larynx				
Liver	X*			
Lungs	X*			
Lymph nodes, cervical	X			
Lymph nodes mandibular				
Lymph nodes, mesenteric				
Mammary Gland	X			
Nasal cavity				
Optic nerves				
Ovaries				
Pancreas	X*			
Parathyroid	X			
Peripheral nerve				
Pharynx				
Pituitary	X			
Prostate				
Rectum	X			
Salivary gland				
Sciatic nerve	X			
Seminal vesicles				
Skeletal muscle	X			
Skin	X			
Spinal cord	X			
Spleen	X			
Sternum				

Stomach	X			
Testes	X*			
Thymus	X*			
Thyroid	X*			
Tongue				
Trachea				
Urinary bladder	X			
Uterus				
Vagina				
Zymbal gland				

* organ weight obtained

gelatin capsules of 0, 100, 300 or 1000 mg/kg for 39 weeks. These doses are approximately equivalent to 50, 150, or 500 mg/kg doses in humans, adjusted for body surface area. Additional dogs (2/sex/group) received rifaximin at either 0 or 1000 mg/kg for 39 weeks and an additional 4 week recovery period. Observations during the study included clinical signs at dosing and throughout the day. Body weight was measured each week for the initial 14 weeks and every 4 weeks thereafter, and weekly during recovery. Food consumption was recorded daily through week 14, for one week out of the remaining four, and daily during recovery. Ophthalmoscopic examinations were conducted prior to treatment, and during weeks 12, 25 and 38 of treatment. Electrocardiographic examinations were conducted prior to treatment, and during weeks 1, 12, 25 and 38. Hematology and blood chemistry studies were conducted from blood samples collected during weeks 12, 26 and 39. Urinalysis was conducted on samples collected prior to treatment and during weeks 12, 25 and 38 of treatment. Blood samples for toxicokinetic studies were collected on day 1 and week 39 at 0, 1, 2, 4, 6 and 8 hours after dosing. All dogs survived through the study and recovery period. No clinical signs were seen during the study or recovery period. Orange feces and stained fur were seen in most treated dogs, typical of rifamycin-related drugs. Bodyweight, food consumption, ophthalmoscopy, and electrocardiography appeared not affected by drug treatment. Thymus weights of treated dogs were smaller than those of control dogs. Histologic examination showed thymic involution and atrophy. These findings were reversed following the 4 week recovery period. Pharmacokinetic findings included

Dose (mg/kg/day)	Cmax(ng/ml)				AUC(ng h/ml)			
	Day 1		week 39		Day 1		week 39	
	Males	females	males	females	Males	females	males	females
100		—			5.8	76.7	80.0	42.7
300		—			93.8	110.4	102.5	48.8
1000		—			70.8	61.3	102.7	108.7

Exposure increased with dose from the 100 to the 300 mg/kg dose but was not increased proportionately at the 1000 mg/kg dose, probably indicating a saturation of the absorptive capacity of the orally administered drug.

A NOAEL was not achieved in this study due to the thymic effects.

Steven C. Kunder, Ph.D.
 Reviewing Pharmacologist

concurrences

HFD-590/DeputyDir/RAIbrecht
HFD-590/PharmTL/KHastings
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HFD-590/KHastings

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HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/JPowers
HFD-590/NSchmuff
HFD-590/SBala
HFD-590/

Histopathology Inventory for IND #52980/028

Study	
Species	
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	X
Harderian gland	
Heart	X*
Hyphophysis	

Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	
Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
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

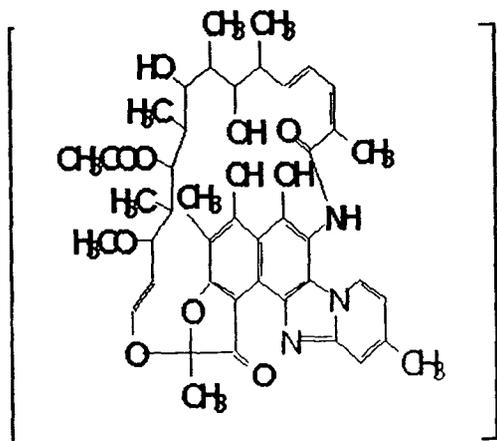
* organ weight obtained

PHARMACOLOGIST'S REVIEW

IND # 52980/032

DATE SUBMITTED 14 March 2000
DATE RECEIVED 15 March 2000
DATE ASSIGNED 16 March 2000
DATE REVIEW COMPLETED 18 January 2001

SPONSOR Salix Pharmaceuticals, Inc
DRUG (rifaximin)



HFD-590

FORMULATION — 200 mg tablets

RELATED DOCUMENTS DMF# —

INDICATION — diarrhea

BACKGROUND INFORMATION Rifaximin is a semisynthetic derivative of rifamycin with minimal systemic absorption from oral administration. It appears to be similar to other rifamycin derivatives such as rifampin.

TOXICOLOGY STUDY

Rifaximin toxicity study by oral gavage administration to CD rats for 26 weeks followed by a 4 week recovery period, study no. — 032/984432 (N 2165), performed by —
GLP, 1 December 1999 Batch no PP2041

Male and female CD rats (— 26 to 30 days old, 130 to 189 g body weight) were assigned to dose groups (20/sex/dose) receiving rifaximin by daily oral gavage doses of 0, 100, 150 or 300 mg/kg for 26 weeks. These doses are approximately equivalent to 16, 24, or 48 mg/kg doses in humans, adjusted for body surface area. Additional rats (5/sex/group) received rifaximin at either 0 or 300 mg/kg for 26 weeks and an additional 4 week recovery period. Observations during the study included clinical signs at dosing and throughout the day. Body weight was measured each week for the initial 14 weeks and every 2 weeks thereafter, and at necropsy. Food consumption was recorded weekly through week 14, and for one week out of each remaining four. Ophthalmoscopic examinations were conducted prior to treatment, and during week 25 of treatment. Hematology and blood chemistry studies were conducted from blood

samples collected during treatment weeks 13 and 26 and recovery week 4. Urinalysis was conducted from metabolic cages on samples collected prior to treatment and during weeks 12 and 25 of treatment. Blood samples for toxicokinetic studies were collected on day 1 and week 26 at 0, 1, 2, 4, 6 and 8 hours after dosing. Three males and three females were sampled at each timepoint. During the treatment period three rats in the following groups died or were found dead: control, 1 female (rupture at bifurcation of liver lobes, blood in abdomen), middle dose, 1 male (died during blood collection, week 13 with no histologic findings) and high dose, 1 male (stomach and duodenum contained drug, no microscopic findings). During blood collection, five rats were killed for humane reasons due to eye damage (control 1 male, 1 female, low dose, 2 females, high dose, 1 female). No clinical signs were seen in the surviving rats during the study or recovery period. Bodyweight gain was decreased in a dose related manner compared with that of controls. Food consumption, urinalysis and ophthalmoscopy appeared not affected by drug treatment. Hematologic findings included decreased lymphocyte counts in all treated groups at week 26 (not seen at week 13). Following recovery, lymphocyte counts of treated rats were recovered to control levels. Blood chemistry effects included elevated ALT in the middle and high dose males during week 26 and elevated glucose in high dose males. Organ weights were unaffected by treatment. Histopathology findings included decreased incidence of pancreatic islet fibrosis. One high dose male had malignant lymphoma. There were no histopathologic findings after recovery.

Pharmacokinetic findings included

Dose (mg/kg/day)	C _{max} (ng/ml)				AUC(ng h/ml)			
	Day 1		week 26		Day 1		week 39	
	Males	females	males	females	Males	females	males	females
50	—	—	—	—	59.2	42.1	na	Na
150	—	—	—	—	100.1	84.6	na	Na
300	—	—	—	—	137.9	115.5	na	Na

Na= not available, samples below limit of detection

Exposure increased with dose from the 50 to the 150 mg/kg dose but was not increased proportionately at the 300 mg/kg dose, probably indicating a saturation of the absorptive capacity of the orally administered drug.

Due to the lymphocyte decreases at all doses, a NOAEL was not achieved in this study, however it was not seen at 13 weeks of treatment and was reversible following recovery.

PHARMACOKINETIC STUDY

Rifaximin The effect on selected hepatic and intestinal drug metabolizing enzymes in the CD rat after oral administration at 0 and 300 mg/kg/day for 26 weeks followed by a 4-week recovery period,

Study no — J37/992890 (N2236), performed by —
8 November 1999, GLP

Liver and jejunum specimens were collected from 5/sex randomly selected rats in the control and 300 mg/kg groups at both the 26 week and post recovery necropsies. Microsomes from each tissue were prepared for cytochrome P450 quantification and measurement of 7-ethoxyresorufin O-deethylase, lauric acid hydroxylase, p-nitrophenol UDP-glucuronyltransferase and testosterone metabolizing activity. Microsomal protein was decreased in recovery females, but hepatic cytochrome P450 concentrations were not significantly different between treated and untreated rats at either timepoint. Jejunal cytochrome P450 was below the limits of detection in all samples. The activity of 7-ethoxyresorufin O-deethylase and p-nitrophenol UDP-glucuronyltransferase were unaffected by drug treatment. In males, testosterone metabolizing activity was increased as demonstrated by increased androstenedione production following recovery. Treated males also had increased production of the lauric acid metabolite 11-hydroxylauric acid. The sponsor cites the activity as markers for P450 subtypes but human liver preparation would be of greater utility.

Steven C Kunder, Ph D
Reviewing Pharmacologist

concurrences

HFD-590/DeputyDir/RAIbrecht
HFD-590/PharmTL/KHastings
Steven C Kunder/Pharm/

disk

HFD-590/KHastings

cc

HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/JPowers
HFD-590/NSchmuff
HFD-590/SBala
HFD-590/DWillard

Histopathology Inventory for IND #52980/032

Study	
Species	
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	X
Harderian gland	X
Heart	X*
Hyphophysis	
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	X
Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
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	X
Seminal vesicles	X*
Skeletal muscle	X
Skin	
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	

* organ weight obtained

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature**

/s/

Steve Kunder
2/4/03 03 31 49 PM
PHARMACOLOGIST

Kenneth Hastings
2/5/03 06 49 31 AM
PHARMACOLOGIST

Renata Albrecht
2/11/03 09 31 30 AM
MEDICAL OFFICER