Pantoprazole: Investigation into Potential Effects on Thyroid Function After 2 Weeks of Treatment in Female Rats Using the Perchlorate Discharge Test (GTR-31719).

Testing Laboratory: [redacted]

Study Started: October 17, 1995

Study Completed: November 22, 1997

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were included.

Animals: Female Sprague Dawley rats were used in the study. At the start of treatment, animals were 9 to 10 weeks of age and the body weight range was 201 to 249 grams.

Drug Batch: Pantoprazole, batch number 0295250000
Phenobarbital, [redacted] batch number 122H0143
Propylthiouracil, [redacted] Batch number 65H2506

Methods: The effect of pantoprazole on thyroid gland function was assessed in female rats using the Perchlorate Discharge Test following a 2-week treatment period. Perchlorate blocks the uptake of iodine from the systemic circulation by follicular cells, which is the initial step in the biosynthesis of thyroid hormones. Effects of pantoprazole were compared with (1) propylthiouracil, an inhibitor of thyroid peroxidase, which disrupts the process of iodination to form iodothyronines, and (2) phenobarbital, a known hepatic enzyme inducer, which enhances the metabolism of thyroxine (T_4). There were 14 female rats per group. Group 1 served as controls and received deionized, distilled water at pH 10.5 by oral gavage at a dose volume of 10 mL/kg. Group 2 received phenobarbital in distilled water at 75 mg/kg/day by oral gavage at a dose volume of 5 mL/kg. Group 3 received propylthiouracil in a 0.5% methyl cellulose suspension at 200 mg/kg/day by oral gavage at a dose volume of 5 mL/kg. Group 4 received pantoprazole in distilled water (pH 10.5) at 200 mg/kg/day by oral gavage at a dose volume of 10 mL/kg. Animals were observed at least once daily for clinical signs of toxicity and morbidity/mortality. Body weight was measured twice weekly during the treatment period. Food consumption was measured weekly. Following the 2-week treatment period, 12 rats/group, at 24 hr after the last dose, received sodium ^{125}I (~1 μCi) in 0.5 mL of saline solution. Six hr later, 6 animals/group received potassium perchlorate at 10 mg/kg by the intraperitoneal route. Another, 6 animals/group received saline by the intraperitoneal route. At 2.5 min after treatment with perchlorate or saline, animals were anesthetized and 2 mL blood was collected by cardiac puncture. Following blood collection, animals were killed by cervical dislocation and the thyroid gland was removed, weighted, and analyzed for content of radioactivity. Spare animals were killed and discarded without further examination.

1. Observed Effects: Rats that received phenobarbital were observed with an unsteady gait that started immediately after dosing and continued through each treatment day. Salivation was observed in 9 of 14 rats that received propylthiouracil on day 2, immediately after treatment, and in all animals from day 4 to the end of the study period. Salivation was observed in 8 of 14 animals that received pantoprazole on day 1, immediately after dosing, and in all animals from day 2 onward.

2. Mortality: None.

3. Body Weight and Food Consumption: Body weight gain and food consumption were impaired for animals that received propylthiouracil. Body weights for female controls on days 1 and 15 were 222 and 247 g, respectively. Body weight gains for female rats that received either phenobarbital, propylthiouracil, or pantoprazole were 100.9, 32.3, and 110.5% of the control, respectively. Food consumption for rats that received propylthiouralcil was reduced to 83% of the control (336 g/animal during weeks 1 and 2).

4. Thyroid Weight, Radioactivity Concentrations in Blood and Thyroid Tissue, and the Thyroid/Blood Radioactivity Concentrations Following Treatment with Saline or Perchlorate: Treatment with propylthiouracil increased thyroid gland weight. Level of radioactivity in blood in propylthiouracil-treated rats following treatment with saline or perchlorate were increased to 160.4 and 157.5% of the control, respectively. The thyroid to blood radioactivity ratio in propylthiouracil-treated rats following treatment with saline or perchlorate was decreased to 9.8 and 6.0% of the control, respectively. The thyroid to blood radioactivity ratios in phenobarbital- and pantoprazole-treated rats were unchanged following saline treatment; however, ratios for phenobarbital- and pantoprazole-treated rats following treatment with perchlorate were increased to 203.8 and 171.4% of the control, respectively. The thyroid to blood ratios for radioactivity indicated that propylthiouracil inhibited ^{125}I uptake, while phenobarbital and pantoprazole enhanced ^{125}I uptake. For phenobarbital and pantoprazole-treated rats, there was an enhanced ability of the thyroid follicles to accumulate iodide under TSH stimulation. Perchlorate treatment did not lead to significant discharges of ^{125}I from phenobarbital and pantoprazole-treated rats suggesting that thyroid peroxidase activity and organification of iodide had not been effected.

Thyroid weight, radioactivity concentrations in blood and thyroid tissue, and the thyrcid/blood radioactivity concentrations following treatment with saline.

Treatment	Thyroid wt., g	Blood, dpm/g	Thyroid, dpm/g	Thyroid/Blood radioactivity ratio	Blood/Thyroid wt. ratio
Control	0.0142	6460	9,588,918	1487	483,100
Phenobarbital	0.0141	6601	16,213,098*	2451	470,947
Propylthiouracil	0.046*	10360*	1,558,548*	146*	226,604*
Pantoprazole	0.0176	7288	12,957,262*	1777	412,818

* $p < 0.05$

Thyroid weight, radioactivity concentrations in blood and thyroid tissue, and the thyroid/blood radioactivity concentrations following treatment with perchlorate.

Treatment	Thyroid wt., g	Blood, dpm/g	Thyroid, dpm/g	Thyroid/Blood radioactivity ratio	Blood/Thyroid wt. ratio
Control	0.0147	7471	7,451,226	1016	518,152
Phenobarbital	0.0166	6917	14,157,763*	2071*	425,126
Propylthiouracil	0.0346*	11766*	708,614*	61*	362,348
Pantoprazole	0.0175	8730	14,845,905*	1742*	510,352

* $p < 0.05$

5. Proportions of Radioactive Dose of ^{125}I-Iodide in the Thyroid Gland and Blood Following Treatment with Saline or Perchlorate: The % dose/g in blood for propylthiouracil-treated rats following treatment with saline or perchlorate was increased to 161.5 and 160% of the control, respectively. Similarly, the % dose in blood for propylthiouracil-treated rats following treatment with saline or perchlorate was increased to 153.5 and 141.5% of the control, respectively. The % dose/g in thyroid tissue for phenobarbital-treated rats following saline treatment was increased to 169% of control, while for propylthiouracil-treated rats, it was decreased to 16.2% of the control. The % dose/g in thyroid tissue for phenobarbital and propylthiouracil-treated rats following perchlorate treatment was increased to 190 and 199% of control, respectively, while for propylthiouracil-treated rats, it was decreased to 9.2% of the control. The % of dose in thyroid tissue for phenobarbital and propylthiouracil-treated rats following saline treatment was increased to 181.1 and 176.5% of control, respectively, while for propylthiouracil-treated rats, it was decreased to 55.8% of the control. The % of dose in thyroid tissue for phenobarbital and propylthiouracil-treated rats following perchlorate treatment was increased to 222.1 and 241.9% of control, respectively, while for propylthiouracil-treated rats, it was decreased to 23.7% of the control. The % dose/g or % dose in blood of propylthiouracil-treated rats was increased following treatment with saline or perchlorate indicating that propylthiouracil had inhibited organification of iodine. The % dose/g or % dose in thyroid gland tissue for phenobarbital- and pantoprazole-treated rats was increased, while it was decreased for propylthiouracil-treated rats. For phenobarbital and pantoprazole-treated rats, there was an enhanced ability of the thyroid follicles to accumulate iodide under TSH stimulation. Perchlorate treatment did not lead to significant discharges of ^{125}I from phenobarbital and pantoprazole-treated rats suggesting that thyroid peroxidase activity and organification of iodide had not been effected.

Proportions of radioactive dose of ^{125}I-Iodide in the thyroid gland and blood following treatment with saline.

Treatment	Blood, % Dose/g	Blood, % Dose	Thyroid, % Dose/g	Thyroid, % Dose
Control	0.26	4.28	390	5.23
Phenobarbital	0.27	4.34	659*	9.47*
Propylthiouracil	0.42*	6.57*	63*	2.92*
Pantoprazole	0.30	4.99	526	9.23*

* $p < 0.05$

Proportions of radioactive dose of ^{125}I-Iodide in the thyroid gland and blood following treatment with perchlorate.

Treatment	Blood, % Dose/g	Blood, % Dose	Thyroid, % Dose/g	Thyroid, % Dose
Control	0.30	5.26	303	4.39
Phenobarbital	0.28	4.85	575*	9.75*
Propylthiouracil	0.48*	7.46*	28*	1.04*
Pantoprazole	0.36	5.96	603*	10.62*

* $p < 0.05$

The effect of pantoprazole at 200 mg/kg/day on thyroid gland function was assessed in female rats using the Perchlorate Discharge Test following a 2-week treatment period. Perchlorate blocks the uptake of iodine from the systemic circulation by follicular cells, which is the initial step in the biosynthesis of thyroid hormones. Effects of pantoprazole were compared with (1) propylthiouracil at 200 mg/kg/day, an inhibitor of thyroid peroxidase, which disrupts the process of iodination to form iodothyronines, and (2) phenobarbital at 75 mg/kg/day, a known hepatic enzyme inducer, which enhances the metabolism of thyroxine (T_4). The thyroid to blood radioactivity ratios in phenobarbital- and pantoprazole-treated rats were increased indicating an enhanced ^{125}I uptake. However, a decreased thyroid to blood ratios for radioactivity for propylthiouracil-treated rats indicated an inhibition of ^{125}I uptake. For phenobarbital and pantoprazole-treated rats, there was an enhanced ability of the thyroid follicles to accumulate iodide under TSH stimulation. Perchlorate treatment did not lead to significant discharges of ^{125}I from phenobarbital and pantoprazole-treated rats suggesting that thyroid peroxidase activity and organification of iodide had not been effected. In contrast, propylthiouracil inhibited organification of iodine.

Effect on Eyes

Monkey

28 Day Intravenous (Bolus) Subchronic Electroretinographic Study in the Cynomolgus Monkeys (GTR-32005 and GTR-31188).

Testing Laboratory: [redacted]

Byk Gulden
Lomberg Chemische Fabrik GmbH
D-7750 Konstanz, Germany

Date Started: Unknown

Date Completed: June 5, 1998

GLP Compliance: A statement of compliance with GLP regulations and the quality assurance unit was included.

Animals: Cynomolgus monkeys (M. fascicularis) obtained from 4 different sources (Hong Kong, Russia, and the United Kingdom) were used in this study. Monkeys were 3 to 6 years of age and body weight ranges were 4.6-7.7 kg for male monkeys and 2.7-4.3 kg for female monkeys.

Drug Batch: Pantoprazole, batch number 0296 300 000

Methods: Cynomolgus monkeys received pantoprazole by the intravenous route of administration at doses of 0, 5, or 15 mg/kg/day for 28 days. Animals in the control group received the vehicle, 0.9% NaCl. There were 3 monkeys/sex/group. The dose volume for the control and 15 mg/kg/day groups was 3.75 mL/kg and the dose volume for the 5 mg/kg/day group was 1.25 mL/kg. Vehicle or pantoprazole was administered by the intravenous route into the vena brachialis by bolus injection over 1 to 2 min to non-fasting animals. Animals were monitored twice daily for morbidity/mortality. Animals were observed at least once daily for clinical signs of toxicity. Body weight was measured weekly. Food consumption was measured daily. Ophthalmic examinations were performed prior to the start of treatment and during week 5. Electroretinography was performed on all animals prior to the start of treatment and during weeks 2 and 5. One female at 5 mg/kg/day was allowed a 21-day recovery period and examined in weeks 6, 7, and 8. During electroretinography, the following responses were examined: scotopic ERG [seven measurements (white flash, rod response) with increasing flash intensities up to the maximal response to the standard flash (SF) of 2.6 cds/m^2], oscillatory potentials [measured at 0.4 log units above the SF], 30 Hz Flicker [immediately at onset of light adaptation and 10 min later], and Photopic ERG [three measurements (red flash, cone response) with increasing flash intensities; white flash cone response to the SF]. Cardiovascular examinations consisting of electrocardiograms time measurements [heart rate and RR, PR, QRS, and QT intervals in seconds] and voltage measurements [P, R, S, T in mV], and blood pressure measurements [systolic pressure, diastolic pressure, mean arterial pressure] were performed prior to treatment and during week 4 of treatment. Blood for determination of hematology and clinical chemistry parameter was collected prior to treatment and at termination of treatment. Blood for determination of serum levels of pantoprazole was collected on days 1 and 28 at predose and 10 min, 30 min, 1 hr, 2 hr, and 4 hr after dosing. Serum samples were assayed for pantoprazole by

Following the 4-week treatment period, all animals were sacrificed except for 1 female at 5 mg/kg/day. This female monkey was observed with decreased food consumption and body weight gain during the treatment period, and was allowed a 21 day recovery period. A full macroscopic examination of all tissues and organs was performed and all lesions were recorded. Absolute and relative weights were determined for the following organs: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, stomach, testes, and thyroid gland (with parathyroids). Tissues and organs were collected and preserved as follows: adrenal glands, aorta (arch thoracal and anterior abdominal), bone marrow, brain(visual cortex, thalamus, midbrain, medulla oblongata including nervus facialis, cerebellum, chiasma opticum)*, cecum, colon*, duodenum, epididymides, esophagus, eyes (and optic nerve)*, gall bladder, heart*, ileum, injection sites*, jejunum, kidneys*, liver*, lungs (with mainstem bronchi)*, lymph nodes (mandibular and mesenteric), ovaries, pancreas*, pituitary, prostate, rectum, salivary gland

(submandibular), sciatic nerve, seminal vesicle, skeletal muscle, skin and mammary gland, spinal cord (cervical), spleen, sternum, stomach*, testes*, thymus, thyroid gland (with parathyroids)*, tongue, trachea, urinary bladder, uterus, and all unusual lesions. Tissues and organs marked with an asterisk were processed, stained with hematoxylin and eosin, and subjected to microscopic examination.

Results:

1. **Observed Effects:** There were no treatment-related observed effects.

2. **Mortality:** None.

3. **Body Weight and Food Consumption:** There were no treatment-related effects on body weight and food consumption. Mean body weights for male controls at weeks 1 and 5 were identical at 6.0 kg. Body weight for the male 5 mg/kg/day group was unchanged, while body weight for the male 15 mg/kg/day group was increased by 1.7% from initial weight at week 1. Mean body weights for female controls at weeks 1 and 5 were identical at 3.5 kg. Body weight for the female 5 mg/kg/day group was decreased by 8.6% of initial weight at week 1; however, body weight for the female 15 mg/kg/day was increased by 3.0% of initial weight at week 1.

4. **Hematology:** There were no treatment-related changes of hematology parameters.

5. **Blood Biochemistry:** There were no treatment-related changes of clinical chemistry parameters.

6. **Physical Examinations:** Cardiovascular and ophthalmic examinations revealed no treatment-related changes.

Cardiovascular Examination: Electrocardiograph and blood pressure measurements apparently revealed no biologically significant treatment-related changes.

Ophthalmic Examination: A number of changes were evident in electroretinographic measurements at week 2 for treatment groups; however, no changes were evident at week 5. Small differences observed between control and treatment groups with ophthalmic and electroretinographic examinations appeared to have no biological significance.

7. **Organ Weights:** There were no treatment-related changes of absolute or relative organ weights.

8. **Gross Pathology:** There were no treatment-related gross pathological changes.

9. **Histopathology:** Histopatholgical changes appear to have little relation to panteprazole treatment and may be common changes observed for cynomolgus monkeys of this age.

Histopathological changes for cynomolgus monkeys that received pantoprazole by the intravenous route of administration at doses of 0, 5, or 15 mg/kg/day for 4 weeks. N = 3 per group except for the female 5 mg/kg/day group, where N = 2 per group.

Organ/Tissue	0 mg/kg/day		5 mg/kg/day		15 mg/kg/day	
	Male	Female	Male	Female	Male	Female
Lungs						
-inflammatory cell foci	0	0	1	0	1	2
-pleuritis	1	0	2	1	2	1
Heart						
-inflammatory cell foci	0	1	2	1	1	0
Kidneys						
-basophilic tubules	0	0	0	0	1	1
Stomach						
-lymphoid hyperplasia	1	0	0	0	0	2

10. Serum Drug Levels: Serum AUC values for pantoprazole on days 1 were approximately proportional to dose. Serum AUC values for pantoprazole on day 28 were not proportional to dose. The AUC at 15 mg/kg/day is 4.92 times the value at 5 mg/kg/day; however, clearance at 5 mg/kg/day was approximately two times that observed at 15 mg/kg/day. Clearance was lower than hepatic (2.616 L/hr) or renal (1.66 L/hr) plasma flow. Further the volume of distribution at 5 mg/kg/day was 1.46 times that observed at 15 mg/kg/day. The volume of distribution exceeds blood volume (0.0734 L) suggesting distribution into tissues.

Parameter	Day 1		Day 28	
	5 mg/kg/day	15 mg/kg/day	5 mg/kg/day	15 mg/kg/day
AUC[A], mg*hr/L	11.99	47.98	7.71	37.94
C_{max}[B], mg/L	31.558	72.716	-	-
T½, hr	0.32	0.41	0.34	0.39
CL/kg, L/hr	0.450	0.452	0.951	0.469
Vdss/kg, L	0.198	0.226	0.355	0.244

A. On Day 1, AUC was 0 to ∞. On day 28, AUC was 0 to 24 hr.
B. The Cmax value was back extrapolated.

Cynomolgus monkeys received pantoprazole by the intravenous route of administration at doses of 0, 5, or 15 mg/kg/day for 4 weeks. The no effect dose was 15 mg/kg/day. There was no target organ of toxicity. Electrocardiograph and blood pressure measurements revealed no biologically significant treatment-related changes. Ophthalmic and electroretinographic examinations revealed no biologically significant treatment-related changes.

Gastrin Profile Studies

Serum Gastrin Levels in Rats after Oral Administration with Pantoprazole, Omeprazole, or Lansoprazole (GTR-32038).

Testing Laboratory: Byk Gulden
Lomberg Chemische Fabrik GmbH
D-7750 Konstanz
Germany

Date Started: March 19, 1996

Date Completed: January 6, 1998

GLP Compliance: Statements of compliance with GLP Regulations and the Quality Assurance Unit were included.

Animals: Female Sprague Dawley rats were used in this study. Animals were 6 weeks of age and had a body weight range of 137-187 g.

Drug Batch: Pantoprazole, batch numbers 0295250000 and 295220000
Omeprazole, batch number 4-003s
Lansoprazole, batch number Z1 19/027

Methods: The effects of proton pump inhibitors, pantoprazole, omeprazole, and lansoprazole, on serum gastrin levels in female rats were examined following a 4-week treatment period. Rats were treated with pantoprazole at doses of 50 and 200 mg/kg/day, which were identical to doses used in the carcinogenicity study with Sprague Dawley rats. The vehicle control rats for pantoprazole received water adjusted to pH 10 with NaOH. Rats were treated with omeprazole at doses of 45 and 138 mg/kg/day or lansoprazole at doses of 48 and 150 mg/kg/day. The vehicle control rats for omeprazole and lansoprazole received a 0.5% methocel suspension with sodium bicarbonate, pH 8-9. Omeprazole at 45 mg/kg/day and lansoprazole at 48 mg/kg/day were approximately equimolar to pantoprazole at 50 mg/kg/day. The high dose of each proton pump inhibitor used in the present study was identical to the high dose of each respective compound used in carcinogenicity studies with Sprague Dawley rats. The high dose group of each proton pump inhibitor was composed of 14 female rats. All other groups were composed of 10 female rats. Drugs were administered by the oral route at a dose volume of 10 mL/kg using a gastric tube. Rats were observed for clinical signs of toxicity at 0.5, 3, 6, and 24 hr after dosing. Animals were monitored daily for morbundity and mortality. Body weights were measured daily. Serum gastrin levels were measured on days 8/9 and 28/29 at 4 and 24 hr after dosing by [redacted] On day 30, 24 hr after the last dose, animals were sacrificed and subjected to a general macroscopic examination. Organ weights were determined for the liver, kidneys, thyroid gland, lungs, and stomach. Organs and tissues were collected, processed, and evaluated by microscopic examination as follows: liver, kidney, stomach, lungs, thyroid gland (including parathyroid gland), and any abnormalities.

Results:

1. **Observed Effects:** Dose-related clinical signs of toxicity for rats treated with pantoprazole at 50 and 200 mg/kg/day included hunched posture, ptosis in both eyes, and piloerection on various days throughout the treatment period. Additional observed effects for rats treated with pantoprazole at 200 mg/kg/day included reduced activity for 1 to 5 rats at various times throughout the treatment period, choking and gasping for 2 animals on day 8, and an increased respiratory rate for 2 animals on day 4. For rats treated with omeprazole at 45 or 138 mg/k/day, clinical signs included hunched posture and reduced activity; although, there were no dose response relationships. Additional observed effects for rats treated with omeprazole at 138 mg/kg/day included piloerection and ptosis in both eyes for 1 or 2 animals throughout the treatment period. Respiratory rate was increased for 1 animal on day 4. For rats treated with lansoprazole at 48 or 150 mg/k/day, clinical signs included hunched posture, reduced activity, piloerection, ptosis in both eyes, and hypersalivation; although, there were no dose response relationships. One animal at 48 mg/kg/day on day 1 was observed lying flat on its stomach with cyanosis and dyspnea. Additional observed effects for rats treated with lansoprazole at 150 mg/kg/day included an increased respiratory rate for 1 or 2 animals on 2 days.

2. **Mortality:** One or two rats per group died to due to errors related to gavage techniques or procedures related to blood sampling. Possible treatment-related deaths occurred for 1 rat in each of the high dose groups for pantoprazole and omeprazole. Errors in gavage technique were associated with the following histopathological changes: for the lungs, foreign body pneumonia, pleuritis, alveolar edema and congestion, and hemorrhage, and for the heart, peri/epicarditis. On day 29, a number of animals died during the blood sampling procedure at terminal sacrifice; although, this appeared to have no relation to treatment.

Treatment	Accidental Deaths	Treatment-Related Deaths or Unknown	Total Deaths
Water/0	0	0	0
Methocel/0	1	0	1
Panto/50 mg/kg/day	1	0	1
Panto/200 mg/kg/day	2	1	3
Ome/45 mg/kg/day	2	0	2
Ome/138 mg/kg/day	2	1	3
Lanso/48 mg/kg/day	1	0	1
Lanso/150 mg/kg/day	3	0	3

3. **Body Weight:** There were no treatment-related effects on body weight gain with any proton pump inhibitor. Body weights for the vehicle control group for pantoprazole on days 1 and 28 were 156 and 228 g, respectively. Body weight gains for rats treated with pantoprazole at 50 or 200 mg/kg/day were 86.7 and 105.6% of the control, respectively. Body weights for the vehicle control group for omeprazole and lansoprazole on days 1 and 28 were 154 and 228 g, respectively. Body weight gains for rats treated with omeprazole at 45 or 138 mg/kg/day were 92.6 and 97.85%, respectively. Body weight gains for rats treated with lansoprazole at 48 and 150 mg/kg/day were 86.7 and 92.5% of the control, respectively.

4. **Serum Gastrin Levels:** Gastrin levels were elevated on days 8/9 and 28/29 at 4 and 24 hr after dosing with pantoprazole, omeprazole, or lansoprazole; however, there were no findings to suggest dose response relationships. Levels at 4 hr after dosing were higher than levels at 24 hr after dosing. Values on days 8/9 and 28/29 were comparable.

Serum gastrin levels (ng/L) in female rats on days 8/9 and 28/29 at 4 and 24 hr after treatment with pantoprazole at 50 or 200 mg/kg/day, omeprazole at 45 or 138 mg/kg/day, or lansoprazole at 48 and 150 mg/kg/day. Values in parentheses represent percent of vehicle control.

Day	Post-Dosing	Water/0	Pantoprazole, mg/kg/day		Meth/0	Omeprazole, mg/kg/day		Lansoprazole, mg/kg/day	
			50	200		45	138	48	150
8/9	4 hr	167	916 (548%)	1230 (737%)	150	702 (468%)	1316 (877%)	961 (641%)	959 (639%)
	24 hr	73	294 (403%)	609 (834%)	79	356 (451%)	448 (567%)	175 (222%)	451 (571%)
28/29	4 hr	155	1089 (703%)	1002 (646%)	139	1035 (745%)	1039 (747%)	1316 (947%)	1236 (889%)
	24 hr	61	387 (634%)	421 (690%)	72	290 (403%)	405 (562%)	313 (435%)	647 (899%)

5. **Organ Weights:** Organ weight changes were observed for the stomach, liver, thyroid gland, and lungs that appeared to be correlated with histopathological observations. Changes were also observed for kidney weight; although, there were no corresponding histopathological changes.

Stomach: Absolute stomach weights for rats that received pantoprazole at 50 and 200 mg/kg/day were increased to 106 and 110.8% of the control (1.66 g), respectively. Relative stomach weights for rats that received pantoprazole at 50 and 200 mg/kg/day were increased to 111 and 112.3% of the control (0.73%), respectively. Absolute stomach weights for rats that received omeprazole at 45 and 138 mg/kg/day were increased 106.1 and 110.4% of the control (1.63 g), respectively. Relative stomach weights for rats that received omeprazole at 50 and 200 mg/kg/day were increased to 108.3 and 113.9% of the control (0.72%), respectively. Absolute stomach weights for rats that received lansoprazole at 48 and 150 mg/kg/day were increased to 107.4 and 110.4% of the control (1.63 g), respectively. Relative stomach weights for rats that received lansoprazole at 48 and 150 mg/kg/day were increased to 109.7 and 112.5% of the control (0.73%), respectively.

Liver: Absolute and relative liver weights for rats that received pantoprazole at 200 mg/kg/day were increased to 116.8 and 116.7% of the control (10.45 g and 4.61%), respectively. Absolute liver weights for rats that received lansoprazole at 48 and 150 mg/kg/day were increased to 107.6 and 114.8% of the control (10.20 g), respectively. Relative liver weights for rats that received omeprazole at 45 and 138 mg/kg/day were increased to 111.4 and 113% of the control (4.47%), respectively. Relative liver weights for rats that received lansoprazole at 48 and 150 mg/kg/day were increased 109.6 and 119.5% of the control (4.47%), respectively.

Thyroid: Absolute thyroid weight for rats that received pantoprazole at 200 mg/kg/day was increased to 125% of the control (0.012 g).

Lungs: Absolute lung weights for rats that received omeprazole at 138 mg/kg/day or lansoprazole at 150 mg/kg/day were increased to 106.5 and 112.1% of the control (1.24 g), respectively. Relative lung weights for rats that received omeprazole at 138 mg/kg/day or lansoprazole at 150 mg/kg/day were increased to 109.1 and 114.5% of the control (0.55%), respectively.

6. **Gross Pathology:** There were no treatment-related gross pathological findings.

7. **Histopathology:** The stomach, liver, thyroid gland, and lungs were the target organs of toxicity. For the stomach in drug treatment groups, a dose-related increased incidence in the vacuolization of the fundic parietal cells was found. Chief cell hyperplasia was observed for animals that received omeprazole at 45 mg/kg/day, lansoprazole at 150 mg/kg/day, and pantoprazole at 200 mg/kg/day. The sponsor reported that semi-quantitative evaluation of enterochromaffin-like (ECL) cells in fundus found an increase of this cell type in all drug treatment groups. Dose-related increases in the number of these cells were found in omeprazole and lansoprazole groups, but the increase in pantoprazole groups was relatively flat (i.e., the incidence in groups that received pantoprazole at 50 or 200 mg/kg/day were relatively similar). A hyperplasia of the antral mucosa was observed for animals that received pantoprazole at 200 mg/kg/day, omeprazole at 138 mg/kg/day, and lansoprazole at 48 and 150 mg/kg/day. For the liver, an increased incidence of centrilobular hypertrophy was found in the high dose groups for all 3 compounds. For the thyroid gland, follicular cuboidal epithelium (i.e., activation of the thyroid gland) was observed for high dose groups that received pantoprazole or omeprazole, but not lansoprazole. For the lungs, an increased incidence of emphysema was observed in treatment groups; although, this change was not test article-specific and dose response relationships were not present for all drugs. Hemorrhage in the lungs was attributed to errors in gavage technique.

Histopathological changes for female rats that received pantoprazole at 50 or 200 mg/kg/day, omeprazole at 45 or 138 mg/kg/day, or lansoprazole at 48 or 150 mg/kg/day for 4 weeks. Includes animal found dead during the treatment period, died due to procedural errors during the treatment period, and animals killed at terminal sacrifice.

Organ/Tissue	Water/0	Pantoprazole, mg/kg/day		Meth/0	Omeprazole, mg/kg/day		Lansoprazole, mg/kg/day	
		50	200		45	138	48	150
n =	10	10	14	10	10	14	10	14
Stomach, fundus								
-parietal cell vacuolization	0	3	9	0	3	10	0	9
-chief cell hyperplasia	0	0	2	0	1	0	0	8
-eosinophilic chief cells	0	0	0	0	0	0	0	1
Stomach, antrum								
-mucosal hyperplasia	0	0	11	0	0	4	1	5
Liver								
-centrilobular swelling	5	4	11	2	6	10	7	10
-centrilobular necrosis	0	0	0	0	0	0	0	1
Thyroid gland								
-follicular epithelium, cuboidal	0	0	8	0	1	6	0	0
-bronhiogenic, cyst	0	2	2	3	5	3	5	2
-ectopic thymus	0	0	0	1	0	3	1	1
Lungs								
-emphysema	2	2	4	1	5	3	4	6
-hemorrhage	0	4	0	3	0	0	2	2
-BAL activation	2	5	7	8	2	3	3	0

The effects of proton pump inhibitors, pantoprazole, omeprazole, and lansoprazole, on serum gastrin levels in female rats were examined following a 4-week treatment period. Rats were treated with pantoprazole at doses of 50 and 200 mg/kg/day, which were identical to doses used in the carcinogenicity study with Sprague Dawley rats. Rats were treated with omeprazole at doses of 45 and 138 mg/kg/day or lansoprazole at doses of 48 and 150 mg/kg/day. Omeprazole at 45 mg/kg/day and lansoprazole at 48 mg/kg/day were approximately equimolar to pantoprazole at 50 mg/kg/day. The high dose of each proton pump inhibitor used in the present study was identical to the high dose of each respective compound used in carcinogenicity studies with Sprague Dawley rats. Possible treatment-related deaths occurred for 1 rat in each of the high dose groups for pantoprazole and omeprazole. Gastrin levels were elevated on days 8/9 and 28/29 at 4 and 24 hr after dosing with pantoprazole, omeprazole, or lansoprazole; however, there were no findings to suggest dose response relationships. Levels at 4 hr after dosing were higher than levels at 24 hr after dosing. Values on days 8/9 and 28/29 were comparable. The stomach, liver, thyroid gland, and lungs were the target organs of toxicity. For the stomach in drug treatment groups, a dose-related increased incidence in the vacuolization of the fundic parietal cells was found. Chief cell hyperplasia was observed for animals that received omeprazole at 45 mg/kg/day, lansoprazole at 150 mg/kg/day, and pantoprazole at 200 mg/kg/day. The sponsor reported that semi-quantitative evaluation of enterochromaffin-like (ECL) cells in fundus found an increase of this cell type in all drug treatment groups. Dose-related increases in the number of these cells were found in omeprazole and lansoprazole groups, but the increase in pantoprazole groups was relatively flat (i.e., the incidence in groups that received pantoprazole at 50 or 200 mg/kg/day were relatively similar). A

hyperplasia of the antral mucosa was observed for animals that received pantoprazole at 200 mg/kg/day, omeprazole at 138 mg/kg/day, and lansoprazole at 48 and 150 mg/kg/day. For the liver, an increased incidence of centrilobular hypertrophy was found in the high dose groups for all 3 compounds. For the thyroid gland, follicular cuboidal epithelium (i.e., activation of the thyroid gland) was observed for high dose groups that received pantoprazole or omeprazole, but not lansoprazole. For the lungs, an increased incidence of emphysema was observed in treatment groups; although, this change was not test article-specific and dose response relationships were not present for all drugs.

Gastrin Profile Study in Rats (GTR-31277).

Testing Laboratory: [redacted]

Byk Gulden
Konstanz, Germany

Date Started: February 20, 1989

Date Completed: May 20, 1998 (Stamp Date)

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were not included.

Animals: Wistar and Sprague Dawley rats were used in this study. Body weight ranges were 203-360 g for Wistar rats and 228-420 g for Sprague Dawley rats.

Drug Batch: Pantoprazole, batch number 589085

Methods: Plasma gastrin profiles were examined in Wistar and Sprague Dawley rats that received pantoprazole by oral gavage at doses of 0, 0.5, 1, and 3 mg/kg/day for 30 days. There were 5 rats/sex/group. Control groups received the vehicle, water at pH 10.8. The dose volume was 10 mL/kg. Blood for determination of plasma gastrin levels was collected on days 15 and 30 at 4, 8, 12, 16, 20, and 24 hr after dosing. Gastrin was quantified by [redacted]. Animals were sacrificed on day 31, and stomach were collected and preserved.

Results: Drug-related increases in plasma gastrin levels were observed in both Wistar and Sprague Dawley rats; however, increases were only significant at a dose of 3 mg/kg. A dose response relationship was weak. Generally, the maximum plasma gastrin levels were observed at 4 hr after dosing and declined thereafter. The sponsor did not sample at time points prior to 4 hr. The degree and duration of elevated gastrin levels displayed a dose-dependency. A dose of 0.5 mg/kg produced marginal increases (≤1.8-fold) between 4 and 8 hr after dosing. A dose of 1 mg/kg produced increases (≤ 3.2-fold) for up to 16 hr after dosing. A dose of 3 mg/kg produced increases (≤ 10.2-fold) for up to 24 hr after dosing.

Plasma gastrin levels (pg/mL) on days 15 and 30 in Wistar rats that received pantoprazole by oral gavage at doses of 0, 0.5, 1, and 3 mg/kg/day.

Dose mg/kg/day	Day 15				Day 30			
	4 hr P.A.		24 hr P.A.		4 hr P.A.		24 hr P.A.	
	Male	Female	Male	Female	Male	Female	Male	Female
0	250	116	135	91	161	78	110	63
0.5	224	248	150	158	145	142	98	74
1	163	251	119	164	227	246	96	93
3	681	790	302	290	523	795	220	206

Plasma gastrin levels (pg/mL) on days 15 and 30 in Sprague Dawley rats that received pantoprazole by oral gavage at doses of 0, 0.5, 1, and 3 mg/kg/day.

Dose mg/kg/day	Day 15				Day 30			
	4 hr P.A.		24 hr P.A.		4 hr P.A.		24 hr P.A.	
	Male	Female	Male	Female	Male	Female	Male	Female
0	176	276	98	188	98	192	68	138
0.5	248	202	89	127	109	210	71	106
1	319	443	102	139	233	204	78	104
3	721	737	257	196	483	660	229	206

Drug-related increases in plasma gastrin levels were observed on days 15 and 30 in both Wistar and Sprague Dawley rats that received pantoprazole by oral gavage at doses of 0.5, 1, and 3 mg/kg/day; however, increases were only significant at a dose of 3 mg/kg. The degree and duration of elevated gastrin levels displayed a dose-dependency.

Comparison of the Effects of Lansoprazole, Pantoprazole, and Omeprazole on Plasma Gastrin Levels and Gastrin-Dependent Parameters in the Rat Stomach (GTR-32030).

Testing Laboratory: [redacted]

Byk Gulden
Konstanz, Germany

Date Started: January 1992

Date Completed: May 20, 1998 (Stamp Date)

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were not included.

Animals: Female Sprague Dawley rats were used in this study. At the start of treatment, animals were 10 weeks old and had a mean body weight of 200 g.

Drug Batch: Pantoprazole, batch number 500205; Lansoprazole, batch number 512/91 [redacted]; and Omeprazole, batch number art.no 25-314-16 [redacted]

Methods: The effects of pantoprazole on plasma gastrin levels and activation/proliferation of stomach enterochromaffin-like (ECL) cells in the rat were compared with lansoprazole and omeprazole. Female rats received vehicle, pantoprazole at 77 mg/kg/day, lansoprazole at 50 mg/kg/day, or omeprazole at 138 mg/kg/day by oral gavage for either 5 or 7 days/week for a total of 10 weeks. Controls received the vehicle, aqueous carbonate-buffered 0.5%hydroxypropylmethylcellulose suspension, pH 9. There were 10 female rats/group. The dose volume was 5 mL/kg. In rats treated for 5 or 7 days/week, plasma gastrin levels were measured at 2 hr after dosing at weeks 1, 4, 8, and 10. Plasma gastrin levels were also measured at 24 hr after dosing in rats treated for 7 days/weeks or at 72 hr after dosing in rats treated 5 days/week. Gastrin was quantified by ———————. Rats were sacrificed 2 hr after the last dose. Stomachs were processed for determination of ECL cell density in the oxyntic gland area and determinations of gastrin and somatostatin cell densities in the antrum. Histidine decarboxylase activity and the histamine concentration were also measured in the oxyntic mucosa.

Results: Plasma gastrin levels at 2 hr after dosing with pantoprazole, omeprazole, or lansoprazole were elevated to 7 times the control (145 pg/mL) at week 1 and 11 times the control at weeks 4, 8, and 10. Results were similar for rats treated for 5 or 7 days/week. Plasma gastrin levels at 24 hr after dosing were elevated to approximately 1300 pg/mL for the 3 agents, and at 72 hr after dosing were elevated to approximately 350 pg/mL for the 3 agents. Stomach weights were unchanged for all treatment groups; however, oxyntic mucosal weights were approximately 1.5 times the control (200 mg) for all 3 agents. Histidine decarboxylase activities were elevated to 5-8 times the control (50 pmol CO_2/mg wet weight and hr) for all 3 agents. Histamine concentrations in the oxyntic mucosa were elevated to approximately 3-4 times the control (30 μg/g wet weight) for all 3 agents. ECL cell (histadine decarboxylase or histamine) densities in the oxyntic mucosa with the 3 agents were approximately twice the control (125 or 200/visual field, respectively). Gastrin cells in the antral mucosa with the 3 agents were approximately twice the control (50/visual field); however, somatostatin cell densities were reduced to 50% of the control (~27 visual field).

The effects of pantoprazole on plasma gastrin levels and activation/proliferation of stomach enterochromaffin-like (ECL) cells in the rat were compared with lansoprazole and omeprazole. Female rats received vehicle, pantoprazole at 77 mg/kg/day, lansoprazole at 50 mg/kg/day, or omeprazole at 138 mg/kg/day by oral gavage for either 5 or 7 days/week for a total of 10 weeks. Results were similar with the 5 or 7 day/week treatment schedule. Increases of gastrin levels, oxyntic mucosa weights, histadine decarboxylase activities in the oxyntic mucosa, histamine concentrations in the oxyntic mucosa, ECL cell (histadine decarboxylase or histamine) density, and gastrin cell density in the antral mucosa were observed for all 3 agents. Decreases of somatostatin cell densities in the antral mucosa were observed for all 3 agents.

Serum Cholesterol Study

Rat

Effects of Pantoprazole on Serum Cholesterol in Rats (GTR-32025).

Testing Laboratory: [redacted]

Byk Gulden
Konstanz, Germany

Date Started: June 7, 1991

Date Completed: October 22, 1992 (Stamp Date of January 8, 1998)

GLP Compliance: Statements of compliance with GLP regulations and the Quality Assurance Unit were not included.

Animals: Male Sprague Dawley rats with a mean weight of 300 g at the start of treatment.

Drug Batch: Pantoprazole, Batch No. 399175

Methods: In the1-year chronic oral toxicity study with rats, serum cholesterol levels were increased dose-dependently. Serum cholesterol levels in short term toxicity studies were not monitored. In the present study sponsor assessed the time course of the increase in total serum cholesterol in rats after drug administration. Groups of rats (n=12 males) were given vehicle (purified water) or 300 mg/kg/day of pantoprazole (pH 10.5) by oral gavage for 60 days. Serum cholesterol levels were monitored on days 15, 30 and 60 of the study.

Results: The serum cholesterol levels were increased by 51%, 54% and 66% on days 15, 30 and 60 of the study respectively, when compared with their corresponding control values.

Addendum:

Serum cholesterol levels (mg/dL) in rats that received pantoprazole by oral gavage at doses of 0 or 300 mg/kg/day for 60 days.

Dose, mg/kg/day	Day 15	Day 30	Day 60
0	66.5	69.8	74.2
300	100.3	107.7	123.2

Male rats received pantoprazole by oral gavage at doses of 0 or 300 mg/kg/day for 60 days. Cholesterol levels were significantly elevated on days 15, 30, and 60 in rats that received pantoprazole at 300 mg/kg/day.

Mitogenesis

Study of the Possible Mitogenic Action of Pantoprazole on Rat Liver After Oral Administration for 7 and 14 Days (GTR-31334).

Testing Laboratories: [redacted]

Dates Study Started and Completed: May 18, 1993 and July 9, 1993

Animals: Sprague-Dawley rats (7 weeks old, males = 231-272 g and females = 180-204 g).

Drug Batch No.: 500205

Methods: Groups of rats (4/sex/group) were given orally (gavage) pantoprazole at daily doses of 200, 500 and 700 mg/kg/day for 14 days. The control group animals received the vehicle (water) in similar fashion. The volume of administration was 5 mL/kg and pH of drug solution was not indicated. Two additional groups were included in the study, one received cyproterone (130 mg/kg/day for 7 days) and other group received phenobarbital (dose not mentioned clearly). Two rats/sex/group were sacrificed on day 7 of the study and the remaining rats were sacrificed on day 14 of the study. Liver samples were collected and liver DNA was determined by the [redacted] methods.

Results: One female of high dose group was killed on day 3 due to poor condition. At the end of 14-day treatment period, mean hepatic DNA (mg/100 g body wt.) levels were increased by 66% and 47% in high dose treated males and females respectively, when compared to control values. The comparator (cyproterone: 130 mg/ kg/day x 7 days) produced increases of 68% and 81% in hepatic DNA of male and female rats respectively. In female rats, phenobarbital (150 mg/kg/day for 3 day then 120 mg/kg/day for 11 days) produced 32% increase in hepatic DNA while phenobarbital (150 mg/kg/day x 14 days) had no significant effect on male hepatic DNA levels. Overall data indicated that high dose of pantoprazole (i.e. 700 mg/kg/day) had some mitogenic activity in rat's liver. However, a dose of 200 mg/kg/day of pantoprazole (which was the top dose in SD rat carcinogenicity study) had no significant mitogenic activity in rat's liver.

Pantoprazole administered by oral gavage at a dose of 700 mg/kg/day for 14 day had a mitogenic action in male and female rats as reflected by increased hepatic DNA levels. Dose of 200 and 500 mg/kg/day did not produce statistically significant increases in hepatic DNA levels. Positive controls-produced expected increases in hepatic DNA levels.

Antigenicity/Sensitization Studies

Guinea Pig

Maximization Tests in Guinea Pigs (GTR-31995).

Testing Laboratory: Byk Gulden Pharmaceuticals

Date of the Study: Nov. 2, 1987 - February 1988.

Methods: Pantoprazole was evaluated in the guinea pig maximization test according to Magnusson and Kligman. Female Pirbright white guinea pigs (n= 11) were pretreated with a 10% pantoprazole solution twice (induction) in order to induce hypersensitivity. The compound was administered intracutaneously with Freund's adjuvant on week one and topically on week 2. In week 4, the animals were challenged topically.

Results: Pantorpazole did not show any sensitizing properties in the test.

Pantoprazole was negative in the guinea pig maximization test.

Antigenicity Study of DZ-2352a (Pantoprazole) (GTR-32029).

Testing Laboratories:

Study Started: January 26, 1994

Study Completed: May 24, 1994

GLP Requirements: A Statement of Compliance with the Japan GLP regulations was included.

Drug Batch No.: 293140

Animals: Male Hartley guinea pigs.

Part 1-Active Systemic Anaphylaxis -(ASA) Test in Guinea Pigs:

Methods: Groups of guinea pigs (5-10/group) were sensitized three times in 2 week period by s.c. administration of vehicle (0.9% saline = negative control) mixed with FCA, pantoprazole (10 or 100 mg/kg) mixed with FCA, pantoprazole (0.2 mg/kg) + 2 mg/kg ovalbumin mixed with FCA or 2 mg/kg of ovalbumin (positive control) mixed with FCA. Fourteen days after the last sensitization all animals were challenged by intravenous administration of respective antigens [pantoprazole (10 mg/kg), pantoprazole (10 mg/kg) plus BSA (5 mg/kg), or ovalbumin (5 mg/kg)].

Results: No anaphylactic symptoms were seen in pantoprazole treated animals when challenged with pantoprazole or pantoprazole plus BSA. All positive control animals showed moderate to severe anaphylactic reactions. Thus, pantoprazole did not induce antigenicity in guinea pigs.

Part 2-Homologous Passive Cutaneous Anaphylaxis (Homo-PCA) Test in Guinea Pigs:

Methods: In the above experiment (ASA test), two weeks after the last sensitization dose, blood samples were collected from each animal and serum samples were obtained for PCA test. Serum sample after appropriate dilution was injected intracutaneously on the back of recipient guinea pigs. Four hours after the passive sensitization, animals were challenged with the respective antigens (pantoprazole, pantoprazole + BSA and OVA [as positive control]) mixed with 1% Evan's blue by I.V. injection. After 30 min animals were killed and examined for dyed extravasation in the back. Sites having dye spot of >_5 mm is considered positive.

Results: No PCA reaction was seen in guinea pigs. Positive control group animals had PCA reaction. Thus, pantoprazole did not elicit sensitization activity in passive cutaneous anaphylaxis test in guinea pigs.

Pantoprazole was negative in both the active systemic anaphylaxis and passive cutaneous anaphylaxis tests with guinea pigs.

Test for Sensitizing Properties of the Thiol Metabolite (B 8401-026) in the Guinea Pig (GTR-32259).

Testing Laboratories: Byk Gulden
Inst. for Pathology and Toxicology
Hamburg, Germany

Dates Study Started and Completed: August 19, 1991 and February 25, 1992.

GLP Requirements: A Statement of Compliance with DE GLP regulations was included.

Animals: Hairless Female Guinea Pigs (6 weeks old, 342-428 g).

Methods: The thiol metabolite of pantoprazole (B8401-026) was evaluated in the guinea pig maximization test according to Magnusson and Kligman. Ten female guinea pigs were given intracutaneous injection of the thiol metabolite of pantoprazole, B 8401-026, (0.2 ml of 0.5% = 1 μg) with Freund's adjuvant (1st induction). On day 9, animals received B 8401-026 in propylene glycol (0.015 ml of 10% solution = 1.5 μg) topically (2nd induction). The animals of the control group (n=5) received similar treatment except drug (B 8401-026) was replaced with placebo (propylene glycol). On day 23, all animals (including controls) were challenged by applying 0.015 ml of 10% (1.5 μg) solution of B 8401-026 in propylene glycol topically. The skin reaction was observed at 24, 48 and 72 hours after the challenge.

Results: No delayed hypersensitivity reaction was seen in B 8401-026 treated guinea pigs.

No delayed hypersensitivity reaction was observed in guinea pigs treated with the thiol metabolite of pantoprazole (B 8401-026).

Local Tolerance Studies

Rats

The Local Toxicity of Pantoprazole After A Single Intramuscular Administration in the Rat (GTR-31991 and GTR-31998).

Testing Laboratory: Byk Gulden
Konstanz, Germany

Date Started: December 7, 1987
March 21, 1986

Date Completed: June 29, 1988 (Stamp Date of December 22, 1997)
February 5, 1988 (Stamp Date of January 6, 1998)

GLP Compliance: There were no statements of compliance with GLP regulations or the Quality Assurance Unit.

Animals: Male Sprague Dawley rats were used in this study. At the start of treatment, animals were 6 weeks of age and had a body weight range of 397-443 g.

Drug Batch: Pantoprazole, Batch 4F

Methods: The local tolerance of pantoprazole after a single intramucular injection of 0.1 mL of a 0.4% or 6% solution was examined. Pantoprazole or placebo (PEG 300 in physiological saline) was administered into m. quadriceps. There were 6 animals for each pantoprazole concentration. Drug was administered on one side and placebo was administered on the contralateral side. At 48 hr after injection, the muscles were removed and evaluated for necrosis.

Results: No differences in incidences or severity of necrosis or local signs of intolerance were found following I.M. injection of either the placebo or pantoprazole (0.1 mL of 0.4% or 6% solution).

No differences in incidences or severity of necrosis or local signs of intolerance were found following I.M. injection of either the placebo or pantoprazole (0.1 mL of 0.4% or 6% solution).

The Local Toxicity of B8610-023 (Lyophilisate) After a Single I.M. Injection in Rats (GTR-32024).

Testing Laboratories: Byk Gulden
Inst. for Pathology and Toxicology
Hamburg, Germany

Study Started: October 28, 1992

Study Completed: November 27, 1992

GLP Requirements: A Statement of Compliance with EC GLP regulations was included.

Animals: Sprague Dawley (Crl:CD®(SD)BR) Male Rats.

Drug-Batch No.: 013927

Methods: Sprague Dawley rats (n=6) were given 0.1 ml of 0.4% pantoprazole (free acid) intramuscularly into the quadriceps muscle of one hind leg of each rat. The other hind leg of each rat was given vehicle (0.9% saline) in a similar fashion. All rats were killed at 48 hr after the injection and muscle was examined microscopically.

Results: Macroscopic examinations revealed discolored area at the injection sites in 2/6 drug-treated rats and none in the control. Peripheral hemorrhages in muscles were seen in 4/6 treated and 1/6 control rats. Histological examinations of muscle slices revealed minimal to marked mesenchymal reaction in 5/6 treated rats and muscle fiber degeneration in 3/6 treated rats. No abnormalities were seen in muscle treated with control solution. Hence, the drug produced local reactions at the injection sites when given by i.m. route.

Administration of 0.1 ml of 0.4% pantoprazole (free acid) intramuscularly into the quadriceps muscle of the hind leg of rats produced local reactions at the injection sites.

The Local Toxicity of Pantoprazole Lyophilisate After a Single I.M. Injection in Rats (GTR-32028).

Testing Laboratories: Byk Gulden
Inst. of Pathology and Toxicology
Hamburg, Germany

Study Started: December 14, 1994

Study Completed: March 30, 1995

GLP Requirements: A Statement of Compliance with EC GLP regulations was included.

Animals: Sprague Dawley [Crl:CD (R) (SD) BR] Male rats (323 - 358 g).

Drug Batch No.: 425-623

Methods: Sprague Dawley rats (n=6) were given 0.1 ml of 0.4% pantoprazole lyophilisate (without saccharose) intramuscularly into the quadriceps muscle of one hind leg of each rat. The other hind leg of each rat was given vehicle (0.9% saline) in similar fashion. All rats were killed at 48 hr after the injection and muscle was examined microscopically.

Results: Unlike the previous study (GTR-32024), no signs of discoloration or necrosis were seen at the site of injection. Petechial hemorrhages were seen in 4/6 and 6/6 control and treated rats, respectively. Histopathological examinations of the muscle slices revealed slightly greater degree of focal monocytic infiltration in treated rats than in control rats. Hence, new formulation of pantoprazole (i.e. without saccharose) was well tolerated in rats when given via I.M. route.

Administration of 0.1 ml of 0.4% pantoprazole (new formulation without saccharose) intramuscularly into the quadriceps muscle of the hind leg of rats produced no local reactions at the injection sites.

Rabbits

Local Toxicity of Pantoprazole in the Rabbit After a Single Intravenous Injection (GTR-31990 and GTR-31997).

Testing Laboratory: Byk Gulden
Konstanz, Germany

Date Started: March 23, 1988
Unknown for GTR-31997

Date Completed: June 29, 1988 (Stamp Date of February 10, 1998)
February 5, 1988 (Stamp Date of January 6, 1998)

GLP Compliance: GTR-31997 contained a statement of compliance with the Quality Assurance Unit.

Animals: Female New Zealand White rabbits were used. For studies with a 0.4% solution of pantoprazole, animals had a mean body weight of 2.7 kg. For studies with a 6% solution, animals had a body weight range of 3.0-3.4 kg.

Drug Batch: Pantoprazole, batch numbers 3 and 4F.

Methods: The local tolerance of pantoprazole following a single intravenous administration was examined in rabbits. Pantoprazole as a 0.4 or 6% solution was administered in a volume of 1 mL into the lateral ear vein. The vehicle, PEG 300 diluted with physiological saline, was administered on the contralateral side. There were 3 rabbits for each concentration of pantoprazole. Local reactions were observed at 0.5, 1, 3, and 6 hr on the first day and once daily from days 2 to 9. Histological changes were also evaluated.

Results: No signs of local intolerance were observed with rabbits that received a single intravenous injection of a 0.4% pantoprazole solution. However, more extensive local reactions were observed with a 6% solution. Perivascular mesenchymal reactions were observed with the 6% solution as well as the placebo.

No signs of local intolerance were observed with rabbits that received a single intravenous injection of a 0.4% pantoprazole solution; however, more extensive local reactions were observed with a 6% solution.

Local Toxicity of Pantoprazole after a Single Paravenous Injection (GTR-31988).

Testing Laboratory: Byk Gulden
Konstanz, Germany

Date Started: March 23, 1988

Date Completed: June 29, 1988 (Stamp Date of December 22, 1997)

GLP Compliance: There were no statements of compliance with GLP regulations or the Quality Assurance Unit.

Animals: Female New Zealand White rabbits were used. Rabbits had a body weight range of 2.5 to 2.9 kg.

Drug Batch: Pantoprazole, batch number 4F

Methods: The local tolerance of pantoprazole was examined following a single paravenous injection medial to the lateral ear vein. Pantoprazole as a 0.4% solution was administered in a volume of 1 mL. The vehicle, PEG 300 diluted with physiological saline (1 to 30), was administered on the contralateral side. Three rabbits were used in this study. Local reactions were observed at 0.5, 1, 3, and 6 hr on the first day and once daily from days 2 to 9.

Results: A single paravenous administration of a 0.4% solution of pantoprazole did produce evidence of irritation.

A single paravenous administration of a 0.4% solution of pantoprazole did produce evidence of irritation.

Local Toxicity of Pantoprazole in the Rabbit After a Single Intraarterial Injection (GTR-31989 and GTR-31996).

Testing Laboratory: Byk Gulden
Konstanz, Germany

Date Started: March 23, 1988
December 10, 1987

Date Completed: June 29, 1988 (Stamp Date of December 22, 1997)
February 5, 1988 (Stamp Date of January 5, 1988)

GLP Compliance: There were no statements of compliance with GLP regulations or the Quality Assurance Unit.

Animals: Female New Zealand White rabbits were used. Body weight ranges were 2.5 to 3.0 kg and 3.0 to 3.8 kg.

Drug Batch: Pantoprazole, batch number 3 and 4F, lot 1.

Methods: Local tolerance of pantoprazole was examined in rabbits following a single intraarterial administration. Pantoprazole as a 0.4 or 6% solution was administered in a volume of 1 mL into the central ear artery. The vehicle, PEG 300 diluted with physiological saline (1 to 30), was administered on the contralateral side. There were 3 rabbits for each concentration of pantoprazole. Local reactions were observed at 0.5, 1, 3, and 6 hr on the first day and once daily from days 2 to 9. Histological changes were also evaluated.

Results: No signs of local intolerance were observed after intraarterial administration of a 0.4% solution of pantoprazole. However, a 6% solution of pantoprazole produced discoloration, swelling, and scabs at the injection site. Microscopic evaluation found hemorrhagic necrotizing tissue changes in the area around the central ear artery and clot formation (i.e., thrombus in organization or thrombotic residue) within the artery itself.

No signs of local intolerance were observed after intraarterial administration of a 0.4% solution of pantoprazole. However, a 6% solution of pantoprazole produced discoloration, swelling, and scabs at the injection site. Microscopic evaluation found hemorrhagic necrotizing tissue changes in the area around the central ear artery and clot formation (i.e., thrombus in organization or thrombotic residue) within the artery itself. These changes are presumed to occur due to occlusive vascular processes, which may have caused by the pH of solution at 9 to 11. The sponsor stated that the maximum therapeutic concentration for parenteral use should be 2.56%.

Local Toxicity of Pantoprazole Lyophilisate After a Single Intravenous, Paravenous, or Intra-Arterial Injection in the Rabbit (GTR-32027).

Testing Laboratories: Byk Gulden
Inst. for Pathology and Toxicology
Hamburg, Germany

Study Started: November 28, 1994

Study Completed: March 10, 1995

GLP Requirements: A Statement of Compliance with EC GLP regulations was included.

Animals: New Zealand White male rabbits (2.2-2.8 kg).

Drug Batch No.: 425623

Methods: Rabbits (3/group) were given a single I.V., paravenous (P.V.) or intra-arterial (I.A.) injection of 0.4% pantoprazole lyophilisate (without saccharose) in the ear. The volumes of injection were 1, 0.5 and 0.5 mL for I.V., P.V. and I.A. routes, respectively. The other ear of rabbits received vehicle (0.9% saline) in a similar fashion. All animals were observed for 9 days and then sacrificed and all ears (injection sites) were examined microscopically,

Results: No treatment-related local irritation was evident when the drug was given via the I.V., P.V., or I.A. routes. No histological abnormalities were seen at the injection sites when drug was given via I.V. or I.A. routes. A minimal cellular infiltration was seen in 2 out of 3 ears treated with a paravenous injection of pantoprazole lyophilisate. This finding was considered to be a sporadic finding because no local irritation was evident in similar study conducted earlier (GTR-32039).

A single I.V., paravenous (P.V.), or I.A. injection of 0.4% pantoprazole (free acid) into the ear did not produce local irritation in rabbits.

Local Toxicity of B8610-023 (Lyophilisate) After a Single I.V., Paravenous, or I.A. Injection in Rabbits (GTR-32039).

Testing Laboratories: Byk Gulden
Inst. for Pathology and Toxicology
Hamburg, Germany

Study Started: October 19, 1992

Study Completed: November 27, 1992

GLP Requirements: A Statement of Compliance with EC GLP regulations was included.