Original Article

Toxicology and Toxicokinetics of Oral Pantoprazole in Neonatal and Juvenile Dogs

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BACKGROUND: Pantoprazole is an irreversible inhibitor of H^+/K^+ adenosine triphosphatase proton pump. This study encompassed the period of postnatal stomach development to determine whether immature animals are uniquely sensitive to progression of PPI-induced enterochromaffin-like cell hyperplasia. **METHODS:** Pantoprazole was administered to beagle dogs at 3, 10, or 30 mg/kg/day (10/sex/group) from PND 1 for 13 weeks, subsets of animals had a 13-week recovery period. Clinical signs, body weights, growth, clinical chemistry, and neurobehavioral endpoints were assessed. Selected organs were weighed and histologically examined. **RESULTS:** There were no effects on body weights, growth, landmarks of physical and reproductive development, or sensory and neurobehavioral function. Cholesterol and triglyceride levels were increased at 10 and 30 mg/kg/day, but resolved during the recovery period. Stomach weight was increased at all doses, but after recovery the differences in stomach weights resolved for females although male stomach weights remained slightly increased. Pantoprazole-related microscopic findings in the stomach consisted of increased mucosal height, glandular necrosis, and glandular dilation at all doses; and ECL cell hyperplasia, parietal cell vacuolation, and atrophy of chief cells are noted at 10 and/or 30 mg/kg/day. There was a partial recovery of these microscopic changes indicated by a decreased incidence and/or severity of increased mucosal height, glandular necrosis, ECL cell hyperplasia, and chief cell atrophy, and complete resolution of other microscopic observations. **CONCLUSION:** Pantoprazole administered to beagles from PND 1 for 13 weeks resulted in findings similar to those in adult dogs and juvenile dogs, which showed no increase in severity or progression of ECL hyperplasia. *Birth Defects Res (Part B)* 92:345–352, 2011. © 2011 Wiley-Liss, Inc.

Key words: *juvenile toxicity; postnatal development; dog; pantoprazole*

INTRODUCTION

Pantoprazole (Protonix®) is an irreversible inhibitor of H^+/K^+ adenosine triphosphatase, a member of a group of drugs commonly referred to as proton pump inhibitors (PPI). The principal use of PPIs is to reduce gastric acid production (Gibbons and Gold, 2003). The charged, active form of pantoprazole is produced in the highly acid environments of the intracellular canaliculi of actively secreting parietal cells, where it binds covalently to the H^+/K^+ ATPase enzyme, inactivating the enzyme (Gibbons and Gold, 2003).

Pantoprazole has been used to treat gastroesophageal reflux disease (GERD), erosive esophagitis, ulcer healing, and prevention of damage by nonsteroidal anti-inflammatory drugs and eradication of *Helicobacter pylori*. Pantoprazole is the only PPI that can be used intravenously and is employed for patients who cannot tolerate oral medications and/or who have Zollinger–Ellison syndrome (DeVault, 2007). Gastroesophageal reflux is a common condition that involves regurgitation, a passive return of gastric contents into the esophagus. GERD is considered to be reflux that results in pathological changes and may produce effects including anorexia, dysphagia, hematesis, anemia, and a general failure to thrive (Rudolph et al., 2001; Higginbotham, 2010). In infants, the condition typically peaks between 1 and 4 months of age, and usually resolves by 12 months of age (Higginbotham, 2010). Regurgitation has been reported in half of all infants, but is negligible by 1 year of age (Rudolph et al., 2001).

Concern has been voiced that prolonged PPI use may lead to enterochromaffin-like (ECL) cell hyperplasia, which could hypothetically result in carcinoid formation (Laine et al., 2000; Graham and Genta, 2008). Suppression of gastric acid secretion by PPIs and subsequent increase

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in intragastric pH can stimulate secretion of gastrin (Kuipers, 2006). Since gastrin is trophic for gastric mucosa, including ECL cells, hyperplasia of ECL cells may result. However, studies in adults have not shown carcinoids in patients treated with PPIs agents (Klinkenberg-Knol et al., 2000; Kuipers, 2006), indicating that ECL cell hyperplasia in the context of patients treated with long-term PPI therapy is a benign change. Consistent with the findings in adults, children receiving long-term PPI treatment continuously may develop minor degrees of ECL hyperplasia, but show no evidence of developing atrophic gastritis or carcinoid tumors (Tolia and Boyer, 2008; Hassall et al., 2011). In fact, one study reported a higher percentage of normal stomach biopsies following 48 months of PPI treatment than was noted after shorter term treatment, suggesting that earlier changes of parietal cell hyperplasia can normalize with ongoing treatment (Tolia and Boyer, 2008).

Based on the hypothetical concern that ECL hyperplasia might progress to a more serious condition in children, the United States Food and Drug Administration (FDA) issued a written request for nonclinical evaluations of pantoprazole, ''The Agency is concerned that pediatric patients may show progression of cellular changes beyond the proliferative changes in ECL cells observed in adults who have used pantoprazole sodium. Experimentally, PPI have been shown to be genotoxic (mutagenic, clastogenic) and carcinogenic. The experimental carcinogenicity was expressed not only by the development of carcinoids but also by the neoplastic growth of other gastrointestinal and systemic tumors.

To address this concern, the following studies must be performed with pantoprazole sodium:

- A 4-week repeated dose toxicity study in neonatal rats.
- A 90-day repeated dose toxicity study in neonatal dogs.

In these nonclinical studies, gastric ECL cell morphology must be specifically evaluated and toxicokinetic measurements must be performed. Special attention should be paid to the developmental parameters in these neonates. The study designs must also include 3-month recovery groups. These nonclinical studies must be performed before clinical pediatric studies in patients less than 1 year of age are conducted'' (Amended Written Request for pediatric studies for Protonix, December 2002).

In humans, the gastric pH of newborn infants is high and decreases significantly over time. It has been reported that by the time a child is 2 years old, the amount of hydrochloric acid secreted in the stomach is comparable to that of adults (Deren, 1971). The selection of a 4-week rat and a 13-week dog studies is consistent with the development of the gastrointestinal system, with rats showing adult level of gastric secretions by about 6 weeks of age and dogs by about 13 weeks of age (Walthall et al., 2005).

This article reports the design and findings from the 13-week toxicity study performed in juvenile beagle dogs with dose administration from postnatal day 1 that was performed to meet the FDA requirement.

MATERIALS AND METHODS

Animals

Gravid female beagles (*canis familiaris*) (CRP, VA), of 2 to 4 years of age and between 5 and 6 weeks of pregnancy were used to provide the pups for these studies. The dams were selected by veterinary staff based on their past reproductive performance. An acclimation period of approximately 3 weeks was allowed between receipt of dams and whelping in order to accustom the dams to the laboratory environment. The dams were provided with nesting boxes. Following parturition on day 0 post partum (pp), the pups were examined for malformations, sexed, and the numbers of live and dead recorded and weighed individually. The pups were gradually weaned over a period of 3 weeks, weaning being completed by the end of week 8. Subsequently, the pups were housed 2 or 3 per cage (as siblings). All animal housing and care procedures were in compliance with the guidelines of the Canadian Council of Animal Care and USA National Research Council. All procedures performed were approved by the Institutional Animal Care and Use Committee. The animal facility was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and the Canadian Council of Animal Care. The studies were conducted at CRL, Montreal, Canada.

The housing temperatures were $24 \pm 3^{\circ}C$ for the first 10 weeks and then were reduced to $21 \pm 3^{\circ}C$, humidity was 30 to 70% and the photoperiod was 12 hr light and 12 hr dark. All dams had access to pelleted feed (Eukanuba Premium Performance Formula). A ration of approximately 600 g/day was offered before whelping. The same diet was offered ad libitum after whelping. Pups were allowed to nurse from their respective dams and starting in Week 5 pp pups were offered the same feed as the dams ad libitum. For the pups, the diet was offered dry or mixed with water, as needed, to aid palatability. Water was freely available.

Between 7 and 8 weeks of age, all pups were vaccinated with a combined subcutaneous vaccine of distemper, adenovirus type 2, parainfluenza, parvovirus, and intranasal bordetella. A booster vaccination of distemper, adenovirus type 2, parainfluenza, and parvovirus was administered at 10 to 11 weeks of age.

The litters were randomly assigned to the treatment groups such that where possible, litters of at least 2 pups per sex were assigned to each group. Pups in poor health or with suspected abnormalities were excluded from the study. Litters were randomly assigned to dosage groups, with each pup in the litter receiving the same dose (termed a whole litter or between litter design). Each pup was uniquely identified on the day of delivery by its coat markings on its dorsum using a digital image and subsequently a BMDS identity chip was implanted subcutaneously into the lumbar region.

Pantoprazole Treatment

Pantoprazole (Protonix®), 5(difluoromethoxy)-2-[[(3,4-diethoxy-2-pyridinyl) methyl]sulfinyl]-1H-benzimidazole, monosodium salt, sesquihydrate (Wyeth Research, Chazy, NY), was adminstered by oral gavage using a rubber catheter attached to a plastic syringe. The dose volume was 5 ml/kg/day. After weaning, the gavage

tube was rinsed with 5 ml of water after dosing. Formulations were prepared once or twice weekly in the vehicle, 0.8% sodium carbonate, 0.2% sodium bicarbonate and 0.5% methylcellulose (4,000 cps). The pH was adjusted to a range of 10.5 to 10.6 with sodium hydroxide solution or hydrochloric acid. The formulations were stored, refrigerated, and protected from light. Analyses of these formulations were conducted at intervals during the studies and showed them to be within 11% of target.

Study Designs

13-Week tolerability study. Four groups of four male and four female pups were treated at dosages of 0, 3, 10, or 30 mg/kg/day from day 1 pp for 13 weeks, at a dosage volume of 5 ml/kg/day. Pup clinical condition, body weight (daily for the first 4 weeks then twice weekly), and growth measurements (height, to the shoulder and length, from the nose to the base of the tail) were collected. During weeks 4 and 13 of dosing, hematology and biochemistry were assessed and samples for toxicokinetic evaluation were collected at 0.5, 1, 2, 3, 4, 8, and 24 hr after dose. Following a minimum of 90 days dose administration, all pups were euthanized, given a gross examination, organ weights were recorded and lungs and stomach tissues were collected for histological examination.

13-Week definitive toxicity study with a 13-week recovery period. Four groups of up to 20 neonatal beagle pups, from five litters per group, were administered pantoprazole sodium by oral gavage at dosages of 0, 3, 10, or 30 mg/kg/day. Pups were treated for 13 weeks, from day 1 pp. Following the end of the treatment period, subsets of animals in each group were allowed a 13-week recovery (or post treatment development) period.

Pup evaluations were similar to those described above for the tolerability study for clinical condition, body weight, and growth measurements. Selection of a battery of physical development and sensory/neurobehavioral tests was based upon characteristics of physical development and the periods of dog behavior described by Fox (1966, 1971) as shown in Table 1. In the newborn period, nursing behavior and the day of development of eye opening were assessed, and in the transition period, tooth eruption was assessed. During the socialization period, auricular startle, pupil constriction and gait, and behavior were assessed. Also the day of development of vaginal opening and preputial separation was recorded. Hematology and biochemistry were assessed in weeks 12/13 and 26. Samples for toxicokinetic evaluation were collected in week 13 at 0.5, 1, 2, 4, 8 and 24 hr after dose. Following the completion of dosing, or the 90-day after dose recovery period, pups were euthanized, given a gross examination, organ weights were recorded, and tissues were collected for histopathology. All tissues were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin and, in addition for the stomach stained with Grimelius, and examined microscopically.

1-Week toxicokinetic study. As exposure assessments in the previous 13-week studies were limited to weeks 4 and 13, this study was performed to provide data covering the first week of dose administration. Groups of up to 17 neonatal beagle pups, from 2 to 3 litters per group, were administered pantoprazole by oral gavage at dosages of 3, 10, or 30 mg/kg/day. Pups were treated from day 1 pp for 7 days. Pup evaluations were similar to those described above for the tolerability study for clinical condition and body weight. Samples for toxicokinetic evaluation were collected at 0.5, 1, 2, 4, 8, and 24 hr after dose on day 7 pp.

Statistical Analysis

For quantitative results obtained from the pups, mean, standard deviation, and sample size for each treatment group were calculated at each time point for each sex and/or both sexes combined according to the statistical results that were obtained using the method described below. The significance level was set at 0.05 for each statistical test. Quantitative results obtained from the pups were analyzed using a two-factor nested analysis of variance (ANOVA). Factors in the ANOVA model included sex, group, and their interaction, and the ANOVA model took into account the fact that the pups are nested within litters. If the ANOVA results indicated that there was no significant interaction effect ($p > 0.05$), the overall *F*-test results for the main effects of the sex and the group factors were used to determine their significance. Otherwise, the sex effect was tested for each group using *t*-tests on the least-squares means and a contrast was used to test the significance of the overall group differences within each sex. Whenever the considered group effect was significant ($p < 0.05$), the pairwise comparisons of the control group with each treated group were performed for the respective data set

Table 1
Stages of Dog Behavioral Development

Stage	Weeks	Behavior
Newborn	0 to 2	Pups spend most of their time sleeping, eating and or (neonatal) growing
Transition	2 to 3	Pups learn to walk and develop the ability to drink. Also they develop the ability to urinate and defecate for themselves
Socialization	3 to 12	Divided into three phases
Phase 1	3 to 5	Pups learn to play and start reacting to sound
Phase 2	5 to 8	Pups begin to use facial expressions and ear movements. They become more coordinated, form a dominance A hierarchy in the litter and are weaned
Phase 3	8 to 12	Pups start to experience fear and learn quickly
Juvenile	12 to 26	Pups become increasingly independent and chewing behaviors develop
Adolescent	26 to 52–78	The pups develop sexual maturity and stronger chewing behaviors

Based on publications by Fox (1966, 1971).

using *t*-tests on the least-squares means. Levene's test was performed on the residuals from the ANOVA to test for the homogeneity of sex-group variances. Whenever Levene's test was significant ($p < 0.05$), the statistical analysis was repeated using an ANOVA model allowing for heterogeneous sex-group variances and the previous ANOVA results were discarded unless the heterogeneous ANOVA model could not be applied. The heterogeneous ANOVA model was defined by specifying sex-group heterogeneity in the covariance structure of the residuals and by using the Kenward and Roger's method when computing the denominator degrees of freedom for the tests involving the fixed effects (Dunn, 1964). For each pairwise group comparison of interest, significance was reported at the 0.05, 0.01, and 0.001 levels.

RESULTS

Tolerability Study

Data from the tolerability study are not provided. There was no compound-related mortality. Incidental clinical signs included dehydration and reduced activity; this was typically seen during the period immediately postpartum. There were no treatment-related effects noted on body weight or growth parameters. There were slight to mild decreases in red cell parameters (red blood cell count, hemoglobin, and hematocrit) in males and females and mean corpuscular volume in males at the 30-mg/kg/day dose level and a slight increase in absolute neutrophil count in females at dosages $\geq$10 mg/kg/day when compared with controls (data not shown). There was an increase in cholesterol in males and females at dosages $\geq$10 mg/kg/day when compared with controls at weeks 4 and 13. There were no pantoprazole related gross necropsy findings. A treatment-related increase in absolute and relative weight was seen in the stomachs of males and females at 3, 10, and 30 mg/kg/day and was considered to be related to observations of parietal-cell hypertrophy and foveolar (neck cells) hypertrophy and are consistent with the pharmacologic effects of pantoprazole. Results of the tolerability study indicated the suitability of these doses for the definitive study.

13-Week Study

There was no pantoprazole-related mortality or clinical signs. Incidental clinical signs included dehydration and reduced activity, typically seen during the immediate pp period. There were no pantoprazole-related effects on body weight (data not presented), growth (Figs. 1 and 2), landmarks of physical development (eye opening and tooth eruption; Table 2), or landmarks of reproductive development (vaginal opening and preputial separation; Table 2). Sensory and neurobehavioral assessments (nursing behavior auricular startle, pupil constriction, and gait/behavior) similarly showed no effects (data not presented).

At the end of the treatment period, there was a slight decrease (7% to 10%; data not presented by gender) in red cell mass parameters (red blood cell count, hemoglobin, and hematocrit) in males given 30 mg/kg/day, which was not apparent in females or when reported for the genders combined (Table 3). There was an increase in cholesterol (36 and 83% at 10 and 30 mg/kg/day, respectively) and triglycerides (30 and 46% at 10 and 30 mg/kg/day, respectively) at the end of the treatment period (Table 3). These changes in clinical pathology parameters had resolved at the end of the 13-week recovery phase (Table 3).

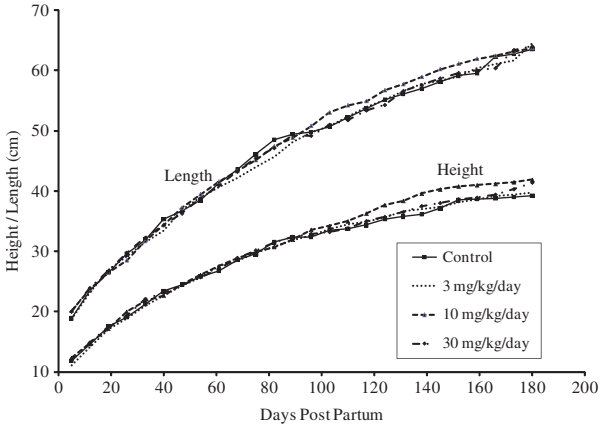

Fig. 1. Height and length measurements of male dog pups during the treatment and recovery periods.

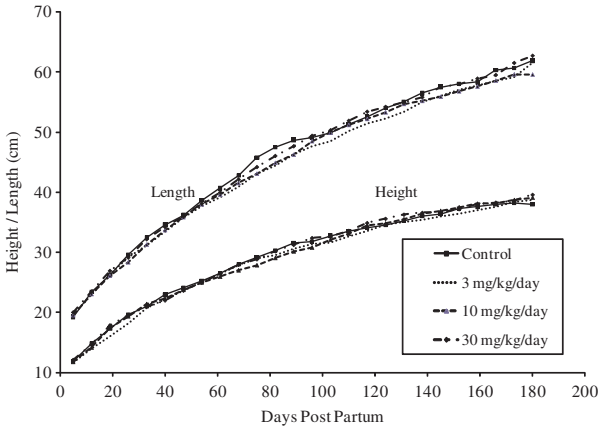

Fig. 2. Height and length measurements of female dog pups during the treatment and recovery periods.

There were no macroscopic changes associated with pantoprazole administration. There was an increase in mean stomach weight in treated males (27, 36, and 62% at 3, 10 and 30 mg/kg/day, respectively) and females (20, 13, and 21% at 3, 10 and 30 mg/kg/day, respectively) (Table 4). After a recovery period of 13 weeks, there were no statistically significant differences in stomach weights for males or females, although male stomach weights were increased by 33, 23, and 34% at 3, 10 and 30 mg/kg/day, respectively, indicating a partial return to normal while stomach weights of females were comparable to control at the end of the recovery period. Pantoprazole-related microscopic findings in the stomach of males and females were characterized by a combination of increased mucosal height, glandular necrosis, and glandular dilation noted at all doses; and ECL cell hyperplasia, parietal cell vacuolation, and atrophy of chief cells noted at dosages of 10 and/or 30 mg/kg/day (Table 5). After a recovery period of 13 weeks, there was a partial recovery of these microscopic

Table 2
Age at Demonstration of Physical and Reproductive Landmarks of Development

Group		Eye opening		Vaginal opening	Preputial separation
		Male	Female		
Control	Mean	13.13	11.91	21.82	26.13
	SD	1.13	1.45	4.64	4.67
	N	8	11	11	8
3 mg/kg/day	Mean	12.9	13.2	22.8	29
	SD	1.2	1.23	2.74	5.12
	N	10	10	10	10
10 mg/kg/day	Mean	13	12.82	23.6	25.3
	SD	2.52	1.99	3.41	5.1
	N	12	11	10	10
30 mg/kg/day	Mean	13.5	12	20.7	31.2
	SD	1.78	1.94	2.98	5.27
	N	10	10	10	10

Data presented as mean age (days) at demonstration of landmark. No statistically significant differences.

Table 3
Selected Clinical Pathology Parameters

	Control	3 mg/kg	10 mg/kg	30 mg/kg
Measured at the end of the 13-week treatment period				
Red blood cells (10^6/μL)	5.60±0.12	5.62±0.12	5.72±0.12	5.40±0.12
Hemoglobin (g/dL)	12.1±0.2	11.7±0.2	11.4±0.2	11.3±0.2
Hematocrit (%)	36.8±0.8	36.4±0.8	35.6±0.8	34.9±0.8
Mean corpuscular volume (fL)	65.8±0.8	64.7±0.8	62.2±0.8**	64.7±0.8
Neutrophils (10^3/μL)	58.05±2.32	57.81±2.36	55.71±2.22	54.41±2.43
Cholesterol (mg/dL)	170.9±14.0	187.2±13.8	231.7±13.8**	313.3±13.8**
Triglycerides (mg/dL)	33.3±2.9	35.6±2.8	43.3±2.8*	48.7±2.8**
Recovery—measured at the end of the 13-week recovery period				
Red blood cells (10^6/μL)	6.84±0.17	6.88±0.18	6.56±0.18	6.64±0.18
Hemoglobin (g/dL)	15.7±0.3	15.4±0.3	14.9±0.3	15.5±0.5
Hematocrit (%)	46.1±1.1	46.5±1.2	44.5±1.2	45.0±1.2
Mean corpuscular volume (fL)	67.5±0.9	67.8±0.9	67.6±0.9	67.9±0.9
Neutrophils (10^3/μL)	52.94±1.84	52.90±1.89	51.95±1.89	52.77±1.89
Cholesterol (mg/dL)	219.8±10.4	228.2±10.2	223.9±10.2	234.1±10.2
Triglycerides (mg/dL)	39.4±3.6	40.6±3.6	43.0±3.6	37.3±3.6

Data presented as mean±standard error of the mean. $N = 10$/sex/group except for control group from the main treatment phase that had $N = 19$ (8 males and 11 females).
*$p \leq 0.05$; **$p \leq 0.01$ (*t*-test).

Table 4
Organ Weights

		End of 13-week treatment		End of recovery	
		Male	Female	Male	Female
Stomach	0	59.94±5.93	57.46±6.47	71.87±1206	68.64±15.05
	3	76.19±7.90	68.88±8.88	95.57±11.37	76.03±7.94
	10	81.53±9.88*	65.17±13.20	88.51±8.74	76.03±17.80
	30	97.36±14.13**	69.51±10.36	96.25±15.52	73.01±12.60
Liver	0	187.72±27.96	186.20±45.77	295.24±7.26	250.53±32.92
	3	199.32±29.51	174.53±28.00	327.77±74.06	271.11±34.33
	10	185.28±18.08	148.90±10.09	303.76±43.91	249.44±21.57
	30	240.42±42.45	183.09±17.42	307.32±56.75	264.27±32.98
Brain	0	62.46±6.83	66.27±4.88	73.74±7.76	70.78±8.24
	3	63.85±6.14	66.51±3.48	74.41±3.59	71.37±3.26
	10	66.31±5.95	60.17±6.22	75.04±2.81	69.24±5.84
	30	66.94±2.75	61.72±2.051	75.61±4.16	70.12±3.39

Data presented as mean (g)±standard deviation. *$p \leq 0.05$; **$p \leq 0.05$ (Dunnett's test). For the 13-week treatment period, $N = 5$/group except for control males where ($N = 4$). For the recovery phase, $N = 5$/group except for control males ($N = 4$) and females ($N = 6$).

Table 5
Microscopic Observations in the Stomachs of Neonatal/Juvenile Dogs Following 13 Weeks of Treatment

	Male				Female			
	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Number examined	4	5	5	5	6	5	5	5
Hyperplasia: ECL-cell								
Group incidence	0	0	0	1	0	0	0	2
Average severity[a]	–	–	–	1	–	–	–	1.0
Increased mucosal height								
Group incidence	0	1	2	3	0	1	2	3
Average severity[a]		1	1.0	1.0		1	1.0	1.0
Vacuolation: parietal-cell								
Group incidence	0	0	4	2	0	0	4	4
Average severity[a]	–	–	1.0	1.5	–	–	1.0	1.3
Dilatation: Glandular								
Group incidence	0	1	4	5	0	1	4	5
Average severity[a]	–	1	1.0	1.2	–	1	1.0	1.0
Atrophy: chief-cell								
Group incidence	0	0	3	3	0	0	2	1
Average severity[a]	–	–	1.0	1.5	–	–	1.0	1.5

For treatment groups with more than one animal with a finding, the average severity score is provided; if only one animal is presented with a finding, the severity score for that animal is presented.
[a]Severity graded on a scale of 1–5 with 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe.

Table 6
Microscopic Observations in the Stomachs of Neonatal/Juvenile Dogs After Recovery (13 Weeks)

	Male				Female			
Finding	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Number examined	4	5	5	5	5	5	5	5
Hyperplasia: ECL-cell								
Group incidence	0	0	0	1	0	0	0	0
Average severity[a]	–	–	–	1	–	–	–	–
Increased mucosal height								
Group incidence	0	2	0	2	0	1	2	1
Average severity[a]	–	1.0	–	1.0	–	1	1.0	1
Necrosis: glandular								
Group incidence	0	0	1	1	0	1	0	0
Average severity[a]	–	–	1	1	–	1	–	–
Vacuolation: parietal-cell								
Group incidence	0	0	0	0	0	0	0	0
Average severity[a]	–	–	–	–	–	–	–	–
Dilatation: glandular								
Group incidence	0	0	0	0	0	0	0	0
Average severity[a]	–	–	–	–	–	–	–	–
Atrophy: chief-cell								
Group incidence	0	0	1	0	0	0	0	0
Average severity[a]	–	–	1	–	–	–	–	–

For treatment groups with more than one animal with a finding, the average severity score is provided; if only one animal is presented with a finding, the severity score for that animal is presented.
[a]Severity graded on a scale of 1 to 5 with 1 = minimal, 2 = slight, 3 = moderate, 4 = marked, 5 = severe.

changes indicated by a decreased incidence and/or severity of increased mucosal height, glandular necrosis, ECL cell hyperplasia, and chief cell atrophy (Table 6), and complete resolution of other microscopic observations evident at the end of treatment.

In the liver, there was an increase (28%) in mean liver weight in males treated at 30 mg/kg/day as compared with controls, but not in females (Table 4). There were no microscopic changes noted. After a recovery period of 13 weeks, mean liver weights were comparable between control and treated males, indicating recovery (Table 4).

Toxicokinetics

Following 1 week of dosing, the apparent terminal half-life values were short and ranged from 0.5 to 2.1 hr (Table 7). Comparison of the dose-normalized AUC_{0-24} values showed no sex- or dose-related effects. The week

Table 7
Pantoprazole Toxicokinetics in Juvenile Beagle Dogs

	3 mg/kg		10 mg/kg		30 mg/kg	
Parameter	M	F	M	F	M	F
Day 7						
AUC_{0-24} (ng hr/mL)	7,320	5,502	12,500	27,702	62,532	51,467
C_{max} (ng/mL)	3,301	3,487	5,247	9,497	24,389	17,111
T_{max} (hr)	0.9	0.8	0.8	0.5	1.0	1.4
Week 4						
AUC_{0-24} (ng hr/mL)	3,814	3,656	11,813	12,021	23,811	74,059
C_{max} (ng/mL)	2,356	2,342	8,891	7,524	11,323	35,390
T_{max} (hr)	0.5	0.5	0.5	0.7	1.1	0.7
Week 13						
AUC_{0-24} (ng hr/mL)	369	540	2,315	2,342	9,950	10,885
C_{max} (ng/mL)	333	561	3,121	2,205	6,583	9,424
T_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5

4 data were taken from the tolerability study where pantoprazole was rapidly absorbed and eliminated by the male and female pups. For the week 13 data, pantoprazole was rapidly absorbed and eliminated by juvenile male and female dogs. Exposures increased with dosage and were similar in males and females. Although the exposures determined in this study were highly variable, comparisons of the dosage-normalized AUC values suggest that the change in exposure to pantoprazole was slightly greater than dose–proportional. Pantoprazole blood levels, as measured by C_{max} and AUC, decreased slightly between weeks 1 and 4, with more pronounced marked reductions between weeks 4 and 13. With these age-associated decreases, blood levels at 13 weeks of age were lower when compared with adult dogs. A comparison of exposure data from the neonatal/juvenile dog (at 30 mg/kg/day) with data from the 1-month toxicity study in adult dogs indicated that exposure to pantoprazole was relatively similar in the 1-week old dog (AUC approximately 50–63 μg hr/mL) and the mature dog (AUC = 65.6 μg hr/mL), but higher in the adult dog compared with the 13-week old juvenile dog (AUC approximately 10 μg hr/mL).

DISCUSSION

Pantoprazole (Protonix®) is a proton pump inhibitor used to treat GERD and other conditions associated with gastric acid production. This toxicity study in juvenile dogs was performed at the request of the US FDA to address a hypothetical concern that immature animals may show progression of cellular changes in ECL cells beyond those noted in adult animals. The findings from this juvenile toxicity study in beagle dogs produced minimal ECL hyperplasia at the highest dose tested, similar to what was noted in adult animals, but showed no differences in severity and no evidence for progression of the hyperplasia to a more serious condition. In addition, the decreased incidence of ECL hyperplasia noted following a 14-week recovery period in conjunction with the normalization of stomach weights, indicates that the pantoprazole-induced changes in stomach morphology are transient pharmacologically mediated effects that resolve following cessation of treatment. Taken together, these results indicate that the stomach of juvenile dogs is no more sensitive to PPI than that of adult dogs.

The toxicokinetic data from the three studies taken together showed age-related change in pantoprazole exposure. Pantoprazole blood levels, as measured by C_{max} and AUC, decreased slightly between weeks 1 and 4, with more pronounced marked reductions between weeks 4 and 13. However, even with these age-associated decreases, blood levels at 13 weeks of age were similar to those seen in adult dogs. Similar to other PPI's, pantoprazole undergoes metabolism in the liver by CYP isoenzymes, primarily CYP2C19 and to a lesser extent CYP3A4 (Kearns and Winter, 2003). The age-related differences in exposure noted in this study may be attributed to the changes in the activity of metabolic enzymes during this stage of development (Tanaka et al., 1998; Tibbitts, 2003). Overall, the data indicated that the juvenile dogs had slightly higher exposure early in the study but end the 13-week study with exposures similar to adult dogs. Despite the slight elevation in exposure early in the study, the target organs identified and magnitude of effects were similar to those observed in adult dogs.

The concern over increased sensitivity to progression of ECL hyperplasia in immature animals is apparently based on the postnatal development of the stomach. Postnatal development of the stomach involves thickening of the glandular region of the stomach and the maturation of chief cells (Deren, 1971). The volume of pepsin and hydrochloric acid secretions increases gradually over the first months after birth. At birth, human gastric pH of newborn infants is high but decrease as a result of increased gastric acid secretion, which reaches adult levels by age 2 (Deren, 1971). For comparison, rats show adult level of gastric secretions by about 6 weeks of age and dogs by about 13 weeks of age (Walthall et al., 2005). Therefore, based on the postnatal maturation processes occurring in the stomach, the juvenile dog or rat represent appropriate models for understanding potential effects of PPIs on the immature human stomach.

In addition to increased stomach weight and corresponding histopathological changes, pantoprazole

treatment was associated with decreased red cell mass parameters, and increased cholesterol and triglyceride levels. Similar changes were seen in the adult toxicity studies (data not presented) and the effects noted in juvenile animals were resolved during the 13-week recovery period. Increased liver weights were noted for males but there were no accompanying pathological changes noted; similar increases were previously noted in adult dogs administered pantoprazole (data not presented).

Although the primary purpose of this study was to evaluate the potential for increased sensitivity to progression of ECL cell hyperplasia in juvenile dogs, the study also incorporated endpoints for growth, physical and reproductive development, and behavior; pantoprazole had no effects on any of these processes. This study was designed and conducted before the promulgation of FDA and EMA guidance for conduct of nonclinical studies to support pediatric drug development (FDA, 2006; EMEA, 2008). A study design was developed that, while addressing the stated concern, also included endpoints for postnatally maturing organ systems not of direct concern based on the pharmacology or toxicity profiles in adult animals (i.e. reproductive maturation and behavior function). Additionally, the study also incorporated all the endpoints in a traditional general toxicity screening study. In hindsight, given the precise objective of this study, a targeted study design approach may have been more appropriate (Cappon et al., 2009). In addition, it is probable that the stated objectives could have been obtained with a single species. The stomach findings were consistent for both the rat and dog and a single juvenile study, preferably in the rat, would have sufficed to address the concerns.

CONCLUSION

Overall, when pantoprazole was given to beagle pups from postnatal day 1 for 13 weeks, findings were generally similar to those in adult dogs and juvenile dogs showed no increased susceptibility to severity or progression of ECL hyperplasia that is commonly associated with long-term PPI exposure.

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