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Evaluation of the safety in dogs of long-term, daily oral administration of capromorelin (ENTYCE®), a novel drug for stimulation of appetite

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The objective of the study was to evaluate the safety of capromorelin, a ghrelin agonist that stimulates appetite and causes increased body weight and the release of growth hormone (GH). Beagle dogs ($n = 32$) received either oral placebo or 0.3, 7, or 40 mg/kg capromorelin once daily for 12 consecutive months. Safety was evaluated by physical examinations, including ECG and ophthalmic examinations, and comprehensive clinical pathology. Serum levels of capromorelin, GH, and insulin-like growth factor 1 (IGF-1) were measured periodically. Necropsies and histopathological evaluations were performed at study termination. As expected, GH and IGF-1 levels were mildly increased in capromorelin-treated dogs. Adverse events were limited to mild emesis and loose stools in all groups and excess salivation among some dogs receiving higher capromorelin doses. Clinical pathology testing was generally normal, although blood lipids and alkaline phosphatase levels were moderately increased among dogs receiving capromorelin. Treated dogs had slightly longer post-treatment PR intervals seen on ECG, but with no changes in cardiac histopathology. Postmortem findings were normal. Drug-related increases in liver weight were linked to overall increases in body weight. Capromorelin was well tolerated in dogs at daily doses up to 40 mg/kg for 12 months, demonstrating a wide safety margin.

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This study was conducted at the Pfizer Central Research facility in Groton, CT, and has been used to support FDA approval of capromorelin oral solution in dogs.
These data were presented as an oral presentation at the annual meeting of the American College of Veterinary Internal Medicine, June 2015.

INTRODUCTION

Loss of appetite (anorexia) or reduced appetite (hyporexia) is associated with a host of veterinary conditions covering a wide range of pathogenesis (Delaney, 2006). The complex metabolic changes associated with disease and injury place dogs at risk of malnutrition and its associated negative consequences. Unlike the weight reduction that accompanies 'simple starvation' in healthy individuals, the 'stressed starvation' associated with anorexia caused by illness leads to the loss of lean body mass, negative protein balance, and other deleterious effects including reduced wound healing, immune deficiency, weakness, and poorer overall prognosis (Chan, 2004; Chan & Freeman, 2006; Freeman, 2012).

In addition to identifying and correcting the underlying illness, veterinarians have used various approaches to increase food intake. Feeding strategies can be used to improve appetite cues and increase food palatability, thereby increasing voluntary energy intake (Delaney, 2006). Enteral or parenteral nutrition can be used for nutritional support in animals that cannot or will not eat (Chan & Freeman, 2006). Research shows that such nutritional support can improve recovery and overall prognosis in hospitalized dogs and cats (Brunetto *et al.*, 2010).

A wide variety of drugs have been used in attempts to increase appetite in ill or injured cats and dogs, although none of these medications have been specifically approved by regulatory agencies for the stimulation of appetite. These include

anabolic steroids, glucocorticoids, benzodiazepines, megestrol acetate, propofol, mirtazapine, and cyproheptadine (Long & Greco, 2000; Agnew & Korman, 2014). Many of these drugs do not increase appetite reliably or predictably. In addition, their use is often not recommended (Chan & Freeman, 2006; Delaney, 2006) because they also can be associated with serious adverse effects (Agnew & Korman, 2014). For example, propofol is an anesthetic, and megestrol acetate is a progestational drug that can cause pyometra in dogs.

A class of drugs, ghrelin agonists (also known as growth hormone secretagogues; GHS), enhances appetite through mimicking ghrelin, the peptide hormone produced by endocrine cells in the stomach. Ghrelin is secreted in response to fasting and has marked appetite-stimulating effects (Hersch & Merriam, 2008; White *et al.*, 2009; Garcia *et al.*, 2013). In addition to its appetite-stimulating activity, ghrelin agonists are known as growth hormone secretagogues, because they stimulate the release of growth hormone (GH), a pituitary hormone that plays an important metabolic role for both the growing and adult animal. Ghrelin and ghrelin agonists have activity at both the hypothalamic and pituitary levels to enhance the production of GH, which in turn stimulates the production of insulin-like growth factor I (IGF-I) by the liver (Hersch & Merriam, 2008). The IGF-1 serves as negative feedback to GH secretion, thereby regulating its level. Ghrelin and ghrelin agonists have been shown to increase appetite and weight gain in laboratory animals (Holubova *et al.*, 2013), healthy older people (White *et al.*, 2009), and human patients suffering from cancer cachexia (Garcia *et al.*, 2013).

Many peptide and small-molecule growth hormone secretagogue drugs have been developed for use in human medicine (Smith, 2005). The small-molecule ghrelin agonists have the advantage of being absorbed through the gut, so are amenable to oral administration (Hersch & Merriam, 2008). One such small-molecule ghrelin agonist developed for human use is capromorelin (Carpino *et al.*, 2003) (note: capromorelin is also referred to as AT-002 and CP-424,391). Capromorelin (ENTYCE®) has been approved by the FDA for the stimulation of appetite in dogs. Safety data in support of capromorelin human development included toxicological testing in dogs. This study reports the results from a dog dose ranging safety study that was carried out in support of human drug development. The original objective of this study was to evaluate the safety of capromorelin in dogs, as potentially predictive of human safety. Because of the original study intent, the duration of the study was longer (12 months) than is generally required for the development of veterinary drugs; the doses used were higher than the 5× the dose required by the FDA for veterinary drug testing; and ECGs and ophthalmic examinations were required. Hence, this study also serves to provide extensive safety information about the compound in dogs and was used in support of FDA approval for capromorelin for the stimulation of appetite in dogs.

Previously published data have shown that oral capromorelin given daily for 7 days caused statistically significant increases in food intake and body weight in laboratory Beagle dogs (Zollers *et al.*, 2014). In addition, in a masked, placebo-controlled, randomized clinical pilot study of client-owned dogs with reduced appetite from a number of clinical conditions, capromorelin, given once a day orally, was shown to cause a statistically significant increase in appetite and body weight (Zollers & Rhodes, 2014).

MATERIALS AND METHODS

Thirty-two (16 male and 16 female) healthy Beagle dogs were randomly assigned to receive either deionized water or capromorelin L-(+)-tartrate salt dissolved in water, at doses of 0.3, 7, or 40 mg/kg as equivalent doses of free acid daily for 12 consecutive months. Each group consisted of four male and four female dogs.

The study was conducted at the Pfizer Central Research facility (Groton, CT, USA), which is accredited by the American Association for Accreditation of Laboratory Animal Care. Unless otherwise indicated, study procedures and analyses were conducted at this facility. All procedures and analyses were in compliance with regulations pertaining to Good Laboratory Practice for Nonclinical Laboratory Studies (as set forth in the Code of Federal Regulations: 21 CFR part 58), with the exception of plasma analyses for GH and alkaline phosphatase isoenzymes. This study was approved by the Animal Care and Use Committee at the test facility.

Animals

Beagle dogs were purchased from Marshall Farms (North Rose, NY, USA) and acclimatized to the test facility for approximately 4 weeks prior to study initiation. Health was determined by veterinarian physical examinations, clinical pathology, and records of routine health monitoring.

At the treatment initiation, dogs were approximately 11–12 months of age, with individual body weights ranging from 9.4 to 11.7 kg for males and 6.3 to 9.8 kg for females.

Dogs were housed individually in 4 × 6 foot stainless steel cages in a single room dedicated to the study. Ambient conditions were maintained at a relative humidity of 45% (±10%), a temperature of 70 °F (±5 °F), and a 12-h light/dark cycle. Drinking water was provided *ad libitum* except during urine collection. For urine collection, the dogs were removed from the runs and placed in stainless steel metabolism cages until sufficient urine was collected (approximately 5 h). If a sufficient urine sample was not collected within the 5-h period, the animals were kept in the metabolism cages until a sample was collected or the animal was retested on another day.

All dogs were fed a fixed amount of dry commercial diet once daily. The ration was initially provided at 300 g/day, and this was increased to 325 g/day after several months.

Dosing

Test materials were prepared weekly by diluting the appropriate amount of capromorelin with deionized water to achieve

the concentrations of 0.3, 7, and 40 mg/mL. The stability of the compound for a minimum of 7 days was confirmed prior to the study and the compound was stable over a concentration range of 0.3–40 mg/mL as determined by HPLC. Also, concentrations of the dosing solutions were verified and homogeneity was determined during dose weeks 1, 4, 24, 31, 40, and 52, and all dose solutions were within an acceptable range (91–105%) of the intended concentrations and were considered homogeneous (Note: on Day 1, the males dosed in the low-dose group received 1 mg/kg, but all subsequent doses in this group were 0.3 mg/kg.)

Upon study initiation, fasted animals were gavaged daily with 1 mL/kg of the appropriate solution, with controls receiving only deionized water. Gavage tubes were rinsed with water following each dose. To acclimatize dogs to the procedure, all received daily gavage of water for 1 week prior to initial dosing.

Safety assessment

All dogs were on a schedule (Table 1) of physical and laboratory assessments that included cage-side observations; physical examinations; electrocardiograms (ECGs); ophthalmological examinations; clinical pathology evaluations including hematology, serum chemistry, and urinalysis; and measurements of body weight and blood pressure (BP). Food consumption was estimated by study personnel observations to help assist dog's overall health, but this study was not designed to measure the food intake effects of treatment. Serum chemistry included the measurement of alkaline phosphatase isoenzymes at weeks 51–52, and these analyses were performed at the University of Illinois College of Veterinary Medicine (Urbana, IL, USA).

Ophthalmoscopic examinations were performed by a veterinarian after the instillation of a mydriatic (1% tropicamide). The ECGs (leads I, II, and III) were conducted on conscious dogs and analyzed by a veterinarian using a physiologic data acquisition and analysis software platform (PO-NE-MAH® Digital Acquisition System; Data Sciences, Inc., St. Paul, MN, USA). Indirect systolic BP was measured using a pulse transducer (BIOCOM® 1010; UFI, Morro Bay, CA, USA) connected to an infant compression cuff and a mean of three measurements was considered the indirect systolic blood pressure value. ECGs and BP measurements were recorded once prior to treatment administration and during weeks 8–9, 17–18, 24–25, 32–33, 40–41, and 49–50 prior to dosing and at approximately 1–2 h postdose.

Toxicokinetic and hormone measurements

Capromorelin concentrations in plasma were measured on Days 90, 181, and 349, with heparinized blood samples taken at 0.5, 2, 4, and 8 h after dosing. An additional predose sample was added on Day 349. All plasma samples were stored frozen and shipped on dry ice to Phoenix International Life Sciences, Inc. (Montreal, QC, Canada), for analysis conducted using a validated HPLC-MS/MS method for dog plasma (Phoenix Project No. 962713/GET) with a dynamic range of 1–500 ng/mL. Plasma standards of capromorelin were prepared by serial dilution of fortified control dog plasma. A 150-mL aliquot of sample plasma was added to a 16 × 100-mm culture tube and vortex-mixed with 50 mL of an internal standard solution (619.2 ng/mL of CP-395,477 in water), 50 mL of a 1.0 M sodium hydroxide solution, and 500 mL of water. After the addition of 7.0 mL of ethyl acetate, the samples were vortexed (10 min) and centrifuged (3200 rpm, 10 min at 10 °C) and the organic layer was transferred to clean 13 × 100-mm borosilicate culture tubes. After the solvent was evaporated to dryness under a stream of nitrogen at 40 °C, the residue was reconstituted in 50 mL of methanol. Samples (10 mL) were injected onto a Hypersil 4.6 × 50 mm 3 mm C18 column at ambient temperature with a run time of 3.0 min. A mobile phase of 90% methanol and 10% 25 mM ammonium acetate flowed through the column at 1.00 mL/min. The entire column effluent entered the heated nebulizer source (480 °C) of a PE-Sciex API 300 triple quadrupole mass spectrometer. Drug and internal standard were measured using multiple reaction monitoring (m/z = 506–244 and 529–267, respectively) at approximate retention times of 0.70 and 0.67 min, respectively. Area ratios of drug over internal standard were fit using least-squares linear regression analysis with 1/x weighting. Samples thought to contain CP-424,391 at concentrations above 500 ng/mL were diluted with control dog plasma and assayed as above.

Table 1. Safety assessment schedule

		Week (Range)									
Activity	Prestudy	1–4	8/9	13	16–18	24–26	32/33	38/39	40/41	49/50	51/52
Clinical observations (daily)											
Food consumption (daily)											
Body weight (1×/week)											
Ophthalmology examinations	X			X		X				X	
Physical examinations (including BP & ECG)	X		X		X	X	X		X	X	
Clinical pathology (hematology, serum chemistry, urinalysis)	X				X	X		X			X
Capromorelin plasma concentrations				X		X				X	
GH plasma concentrations		Day 1				X				X	
IGF-1 plasma concentrations	X	X	X		X	X				X	

Blood was collected for the determination of plasma GH on Days 1, 170, and 351, with samples taken at 0, 0.5, 1, 2, 4, and 8 h after dosing. Blood was also collected for the determination of IGF-I plasma levels 1–2 days prior to the initial dose and on Days 1, 7, 14, 21, 28, 62, 120, 170, and 351. Blood for IGF-I measurement was collected prior to dosing on Day 1, at 8 and 24 h after dosing on Days 7–170, and at 0, 8, and 24 h after dosing on Day 351. All plasma samples for hormone analysis were stored frozen and shipped on dry ice to the Diagnostic Laboratory at the New York State Veterinary College (Cornell University, Ithaca, NY, USA) for analysis using the methods validated for canine GH and canine IGF-1. Canine GH and antisera developed by Dr. A Parlow in conjunction with the NIH were used in a conventional double-antibody radioimmunoassay. For IGF-1, the samples were extracted with acid ethanol to dissociate IGF-binding proteins from the IGF-1; then, the analysis of IGF-1 in the soluble extract was accomplished by a double-antibody radioimmunoassay using a commercial assay kit that was validated for dog IGF-1.

Postmortem assessment

At the end of the 12-month dosing period, all dogs were humanely euthanized (via exsanguination under pentobarbital anesthesia) and necropsied. Gross examination of internal organs was conducted, and a comprehensive set of tissues were collected for microscopic examination. Selected organs (kidneys, liver, testes, adrenals, pituitary, ovaries, brain, and heart) were weighed, with absolute and relative (to body or brain weight) weights recorded.

Statistical analysis

Clinical pathology parameters, body weights, vital signs, and organ weights were statistically analyzed using a commercial statistical package (SAS Institute Inc., 2011) (PROC MIXED, SAS, version 9.3.1; SAS Institute Inc., Cary, NC, USA). Statistical significance was set at the level of $P \leq 0.05$.

Endpoints measured once during the study were analyzed using a mixed-model analysis of covariance (ANCOVA), with the baseline value as a covariate and the classification variables dose level, sex, and dose level by sex interaction as fixed effects. Endpoints measured multiple times were analyzed using a mixed-model, repeated-measures ANCOVA, with the baseline value included as a covariate. The models included fixed effects of dose level, time, and sex, with interaction terms for dose level by time, dose level by sex, and dose level by time by sex. Several covariance structures were explored for the repeated-measures models, and the one providing the smallest Akaike's Information Criterion was used. If the dose-level effect of any model was statistically significant, then pairwise comparisons to control were constructed using linear contrast statements.

Two-way and three-way interactions were included for model completeness as part of regulatory compliance. However, analyses by sex or study day showed no consistent or clinically important patterns, so only main-effect treatment terms were reported.

RESULTS

All but one dog survived the 12-month dosing period. One dog in the 40 mg/kg group died immediately after receiving its Day 68 dose, because of accidental delivery of test material into the respiratory tract during gavage.

Most dogs were estimated to eat 76–100% of their daily food ration. Two dogs in the placebo group consumed less food on particular days, with one dog consuming less than 75% on many days. There was no significant treatment effect for body weight ($P = 0.14$), but by the end of the study treated dogs tended to have heavier mean weights than did controls.

Clinical observations

All dogs appeared healthy throughout the study, with no treatment-related effects noted on physical examinations. Reddened/swollen paws occurred in all treatment groups, but were more common in the 7 and 40 mg/kg groups, observed approximately 20–40 days among approximately half of the dogs in these two groups.

Single dogs in the 7 and 40 mg/kg groups had isolated instances of ataxia, lacrimation, or pale skin. The more common adverse events (AE) included emesis and loose stools, which were distributed across all groups without an apparent pattern. However, increased salivation was more common in the 7 and 40 mg/kg dose groups and may have been related to treatment (Table 2).

There were no significant treatment-related effects on vital signs. Ophthalmological examinations were normal, with no changes related to treatment. There were similarly no

Table 2. Percentage of dogs (range of days affected)* showing common adverse events over 12 months on study

	0 mg/kg ($n = 8$)	0.3 mg/kg ($n = 8$)	7 mg/kg ($n = 8$)	40 mg/kg ($n = 8^{\dagger}$)
Emesis	75% (1–3)	63% (1–2)	63% (1–4)	75% (1–6)
Loose stools	100% (1–41)	100% (5–32)	100% (2–220)	100% (1–46)
Excess salivation	13% (4)	0	88% (1–104)	100% (34–354)

*Dogs were observed multiple times per day, but the results are reported as the numbers of days in which adverse events were observed, not the total number of observations.
†One dog died on Day 68.

treatment-related changes in BP. ECG lengthening of PR intervals was observed in the 7 and 40 mg/kg treatment groups at 1–2 h after dosing. In the 7 mg/kg group, the PR interval was approximately 4–22% greater than that of controls. In the 40 mg/kg group, this increase was approximately 13–24% greater than among controls.

Clinical pathology

Clinical pathology results that showed statistically significant changes ($P \leq 0.05$) compared to controls are given in Table 3. Decreases that were not statistically significant were noted in red blood cells, hemoglobin, and hematocrit related to treatment in the 40 mg/kg dose group. Among the ANCOVA models, there were significant treatment effects for several relative (percent) blood cell parameters, but not for absolute measures of these variables. There was a statistically significant treatment effect observed for mean activated partial thromboplastin time in capromorelin-treated dogs with the time slightly lengthened compared to controls but within normal limits.

Serum chemistry parameters were not affected by treatment in a manner that would indicate any clinical significance. Aspartate aminotransferase levels were significantly lower among those receiving capromorelin, and bile acid levels were significantly higher, but neither exhibited a dose-dependent relationship. Cholesterol, high-density lipoprotein, and alkaline phosphatase appeared to be significantly increased in a dose-related manner. The elevation in alkaline phosphatase in the 40 mg/kg group was due to the liver-specific isoenzyme.

There were no statistically significant differences in capromorelin- and placebo-treated dogs for the urinary parameters such as pH and specific gravity.

Table 3. Least squares mean values of selected* serum chemistry, hematology and urinalysis variables

		Capromorelin dose				
Variables	Reference limits†	0 mg/kg	0.3 mg/kg	7 mg/kg	40 mg/kg§	*P* value
Neutrophils (%)	60–70‡	–	–	–	–	–
Least-squares mean	–	60.46	55.70	60.73	57.60	0.0382
Difference from control value	–	–	−4.76	0.27	−2.86	–
P value for pairwise comparisons with control value	–	–	0.0175	0.8903	0.1783	–
Reticulocytes (%)	0–1.5‡	–	–	–	–	–
Least-squares mean	–	0.55	0.35	0.60	0.40	0.0002
Difference from control value	–	–	−0.19	0.06	−0.15	–
P value for pairwise comparisons with control value	–	–	0.0018	0.3545	0.0215	–
Activated partial thromboplastin time (sec)	9.9–17.6	–	–	–	–	–
Least-squares mean	–	11.25	12.06	12.16	12.37	0.0099
Difference from control value	–	–	0.82	0.92	1.12	–
P value for pairwise comparisons with control value	–	–	0.0283	0.0094	0.0017	–
Aspartate Aminotransferase (U/L)	21–49	–	–	–	–	–
Least-squares mean	–	30.59	27.02	29.71	25.64	0.0271
Difference from control value	–	–	−3.57	−0.88	−4.95	–
P value for pairwise comparisons with control value	–	–	0.0483	0.6173	0.0083	–
Alkaline Phosphatase (U/L)	40–218	–	–	–	–	–
Least-squares mean	–	54.42	52.60	67.53	109.41	<0.0001
Difference from control value	–	–	−1.82	13.11	54.99	–
P value for pairwise comparisons with control value	–	–	0.8932	0.3175	0.0001	–
Bile acids	5.1–11.5	–	–	–	–	–
Least-squares mean	–	10.60	10.51	11.60	11.44	0.0446
Difference from control value	–	–	−0.10	1.00	0.83	–
P value for pairwise comparisons with control value	–	–	0.8333	0.0392	0.0828	–
Cholesterol (mg/dL)	103–264	–	–	–	–	–
Least-squares mean	–	164.04	162.20	181.28	212.21	0.0026
Difference from control value	–	–	−1.84	17.24	48.17	–
P value for pairwise comparisons with control value	–	–	0.8927	0.2023	0.0009	–
High-density lipoproteins (mg/dL)	98–202	–	–	–	–	–
Least-squares mean	–	132.54	129.31	141.54	162.77	0.0098
Difference from control value	–	–	−3.22	9.00	30.23	–
P value for pairwise comparisons with control value	–	–	0.7444	0.3530	0.0038	–

n/a, not applicable; values are generally compared to control dog values.

*$P \leq 0.05$.

†Reference limits were based on historical values from male and female control dogs housed at the test facility.

‡Merck Veterinary Manual (Eighth Edition, 1998).

§One dog died on Day 68.

GH and IGF-1

Capromorelin treatment produced a significant increase in plasma GH levels compared to controls ($P < 0.0001$). The highest levels were after the first dose with mean levels peaked at 0.5 h after Day 1 treatment with mean (±SD) in control dogs of 1.82 ± 0.65 ng/mL, and means in capromorelin- treated dogs ranging from 90.06 (±36.50) to 134.08 (±68.89) ng/mL. Over the first few hours, GH levels were approximately 50- to 100-fold higher than among controls. This effect was noted in all treatment groups. These high levels of GH decreased significantly by 8 h after treatment, with controls dogs having mean plasma GH concentrations of 0.86 ± 0.44 ng/mL and capromorelin-treated dogs having mean concentrations ranging from 1.65 (±0.57) to 5.03 (±3.18) ng/mL. On Day 170 and Day 351, the GH increases were markedly reduced following capromorelin treatment compared to Day 1, with GH levels in treated animals at 30 min after dosing having mean concentrations ranging from 3.27 (±1.78) to 5.59 (±3.41) on Day 170, and from 3.33 (±2.12) to 6.39 (±2.57) on Day 351, while by 8 h postdosing capromorelin-treated dogs had GH levels comparable to controls, with GH ranging from 0.92 (±0.38) to 0.96 (±0.51) compared to 1.03 (±0.45) ng/mL in control dogs.

Capromorelin treatment resulted in an increase in plasma IGF-1 levels, although this effect was not observed until Day 7. From Days 7 to 170, the IGF-I plasma concentrations among dogs receiving 7 and 40 mg/kg capromorelin were approximately 2- to 3-fold greater than among dogs in the control group. Concentrations of IGF-1 for dogs receiving 0.3 mg/kg were generally in between those observed in controls and the higher doses. Capromorelin-treated dogs had mean values at 8 h after the Day 170 dose ranging from 83.13 (±26.55) to 101.56 (±49.97) ng/mL compared to mean values in controls of 53.89 (±22.38) ng/mL. On Day 351, this difference had been reduced to capromorelin-treated dogs having approximately 50% greater sustained IGF-1 levels than control dogs with mean levels in control dogs at 8 h after dosing of 80.16 (±26.74) ng/mL compared to a range of 89.34 (±31.00) to 118.94 (±48.69) ng/mL concentrations in the capromorelin-treated dogs.

Pharmacokinetics

At Day 90, mean values for maximum capromorelin concentration (C_{max}) were 1428 ng/mL in the 7 mg/kg group and 8957 ng/mL in the 40 mg/kg group. These peak concentrations were achieved by 30–60 min after dose administration. Corresponding 8-h area under the curve (AUC_{0-8}) values were 3375 and 25 571 ng/mL, respectively. Concentrations on Days 181 and 349 were comparable to those observed on Day 90, demonstrating that capromorelin did not accumulate in plasma over time.

Postmortem findings

There were no drug treatment-related changes seen on gross pathology. There was a dark-red discoloration on the lung of the one dog in the 40 mg/kg group that died following treatment on Day 68, which is consistent with death caused by a misplaced gavage tube.

The ANCOVA results showed significant organ weight treatment effects for the adrenal glands, heart, and liver, although a true exposure–response relationship was demonstrated only for the liver. Liver weights were increased in the 7 and 40 mg/kg treatment groups, likely related to an increased body weight. There were no significant treatment effects for relative organ-to-body-weight ratios (P values ranged from 0.11 to 0.55), suggesting that absolute weight increases in these organs were related to the increases in body weight.

All tissues were examined histologically. Microscopic findings were normal, with changes considered to be incidental or spontaneous in nature and unrelated to drug administration. Cardiac tissues were histopathologically normal. There was a slight increase in hepatocellular cytoplasmic vacuolation among treated animals. Periodic acid–schiff (PAS) stain confirmed the presence of glycogen in vacuolated hepatocytes, which is consistent with a slight treatment-related trend in absolute liver weight. There were no treatment-related changes in bone, but one dog in the 0.3 mg/kg group and one dog in the 7 mg/kg group had fracture and/or tissue reaction consistent with chronic damage to the sternum. These latter findings were considered to be traumatic in origin and not drug related.

DISCUSSION

Capromorelin was well tolerated in dogs at daily oral doses up to 40 mg/kg for 12 months. The toxicokinetic measurements demonstrated that oral administration resulted in a significant exposure to capromorelin and rapidly produced peak blood concentrations of approximately 1000–9000 ng/mL, with AUC_{0-8} exposures in excess of 25 000 ng/mL in the 40 mg/kg dose group demonstrating high exposure of treated dogs.

Capromorelin has been approved by the FDA for the stimulation of appetite in dogs. In this study, designed as a toxicity study to support human drug development (and used here for support of use in dogs), significant increases in food intake were not seen, but this is likely due to the fact that the dogs were not fed *ad libitum*. Most dogs ate all of the food offered, and capromorelin dogs may have consumed additional food had it been available. This also may explain why dogs in the capromorelin-treated groups did not gain a significant body weight compared to controls. Studies have been conducted that clearly demonstrate dogs treated with capromorelin daily have increased food intake and body weights (Zollers & Rhodes, 2014; Zollers *et al.*, 2014, 2016; FOI summary, CVM website).

Because capromorelin is a GH secretagogue compound, it was expected that it would stimulate GH release in dogs. This study demonstrates this effect, with superphysiologic plasma levels of GH measured in blood after the first dose. This high level of GH causes the liver to produce IGF-1, which serves as negative feedback to further GH release. Therefore, because of

this increase in plasma IGF-1, subsequent doses of capromorelin do not cause such a dramatic stimulation of GH, and after chronic dosing GH levels are slightly above baseline levels after dosing and return to baseline by 8 h postdose. GH levels in capromorelin-treated dogs, although higher than control dogs, are only slightly elevated, even though the doses of capromorelin were high, as demonstrated in this study. This negative feedback effect is rapid; in another study in laboratory dogs, the increase in IGF-1 and the lessening of the GH stimulation were seen after the fourth day of treatment (Zollers *et al.*, 2014). The clinical relevance of this is that daily dosing of capromorelin assures that the GH release is small, and therefore unlikely to cause clinical signs of excessive GH. Capromorelin causes a sustained increase in serum IGF-1, which likely also alters IGF-1 binding protein levels, but in this study no measurements of IGF-1 binding protein were conducted, so the amounts of unbound and active IGF-1 are not known. IGF-1 is known to potentially alter glucose metabolism (Reusch, 2004), although no changes in serum or urinary glucose were seen in capromorelin-treated dogs in this study.

Emesis and loose stools were common across all groups, including controls, suggesting a possible influence of general study stressors such as daily gavage dosing and repeated handling/testing. Excessive salivation was possibly caused by a central mechanism given that the smell or taste of food stimulates areas of the brain that influence both the salivary nuclei and appetite center, such that the stimulation of appetite may lead to saliva production (Hall, 2011).

There is no known mechanism to account for the increased prevalence of redness and swelling in the paws of the 7 and 40 mg/kg groups, although it is not uncommon for dogs kept in stainless steel cages with mesh floors to show these clinical signs. This is unlikely to be related to an untoward systemic treatment effect in that other skin areas were not affected and histopathology of the affected skin was normal.

The minor lengthening of the PR interval associated with the higher doses is not clinically important and was likely a chance finding. There was no evidence of behavioral changes or altered cardiac histopathology that might have suggested a cardiotoxic effect of capromorelin. This finding is unlikely to be related to altered vagal tone given that such an effect should have slowed the heart rate, which was not observed at the higher doses.

In general, hematology, serum chemistry, and urinalysis parameters were considered to be unaffected by treatment because they were within normal ranges or lacked any consistent dose–time relationship. Increases in cholesterol and high-density lipoproteins in the highest dose group were likely associated with GH-accelerated lipolysis (Reusch, 2004).

This comprehensive safety assessment evaluated multiple body systems over an extended period of time, producing a large number of statistical tests. Therefore, some of the significant results that were observed are likely to be chance findings due to multiple comparisons.

As in all laboratory safety studies, the limitation of this study is that the relatively small number of dogs per treatment group precludes drawing conclusions about rare or idiosyncratic events. Such a determination awaits pharmacovigilance on a larger population of treated animals.

Clinical studies of capromorelin administered as a flavored liquid in healthy laboratory dogs and dogs presenting at veterinary clinics with the loss of appetite have demonstrated that a 3 mg/kg daily oral dose causes an increase in food intake and appetite (Zollers & Rhodes, 2014; Zollers *et al.*, 2014). A large, multisite, placebo-controlled, masked effectiveness study in client-owned dogs has been completed using this liquid formulation and has demonstrated statistically significant increases in appetite and body weight in dogs dosed for 4 days with capromorelin at a dose of 3 mg/kg (FOI summary, CVM website).

A comparative dog pharmacokinetic study was completed comparing the flavored liquid formulation with the formulation in water used in this 12-month safety study (FOI summary, CVM website). When adjusted for the differences in formulation between the formulation used in the current study (water) and the formulation developed for commercial use (flavored liquid), the exposure at the 40 mg/kg dose in this study is approximately 17.5 times the proposed clinical dose, which represents a wide safety margin for capromorelin in dogs.

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