CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210864Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: March 21, 2019
From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 210-864 (Captisol-enabled (CE) fosphenytoin sodium)

NDA 210-864 was submitted on May 22, 2018, by Sedor Pharmaceuticals LLC to support marketing approval of CE-fosphenytoin sodium for the "treatment of generalized tonic-clonic status epilepticus and prevention and treatment of seizures occurring during neurosurgery" and "can also be substituted, as short-term use, for oral phenytoin," although it "should be used only when oral phenytoin administration is not possible." NDA 210-864 is a 505(b)(2) application, with Cerebyx (NDA 20-450) as the Listed Drug. Cerebyx is approved for the same indications. Clinical development was conducted under IND 74871, originally by CyDex Pharmaceuticals; the IND was transferred to Sedor Pharmaceuticals on February 17, 2006.

The primary safety concern during clinical development was the daily dose and rate of administration of the excipient, Captisol (sulfobutyl ether β-cyclodextrin), in the clinical formulation, as conveyed to the sponsor on multiple occasions (pIND Meeting Minutes, September 20, 2006; pre-NDA Meeting Minutes, September 21, 2008; Type C WRO, June 3, 2016; pre-NDA Meeting Minutes, July 25, 2017). Captisol (b) (4) fosphenytoin at room temperature and at a more physiological pH (7(b)(4)-8.2).

The sponsor (CyDex, Inc.) conducted a single nonclinical study of CE-fosphenytoin sodium, an intramuscular local toxicity study in Sprague-Dawley rat. All other nonclinical data were included by reference to **DMF #14364** for Captisol; a Letter of Authorization was provided by the DMF holder, CyDex, Inc. These studies have been reviewed by Dr. Fisher (Pharmacology/Toxicology Review and Evaluation, NDA 210-864, Ed Fisher, Ph.D., March 20, 2019). Dr. Fisher has concluded that the nonclinical data are adequate to support approval of CE-fosphenytoin in adults for the proposed indications but that they are inadequate to support approval in the pediatric population.

To treat status epilepticus (SE) in adults, CE-fosphenyltoin is to be administered intravenously as a loading dose, followed by up to two maintenance doses. In adults, the loading dose is 15-20 mg PE (phenytoin equivalents)/kg, administered at a rate of 100 to 150 mg PE/min. The maintenance dose is 4-6 mg PE/kg/day (in divided doses), administered at a rate no greater than 150 mg PE/min. The loading dose would result in a dose of Captisol of 40 mg/kg, administered at a rate

of 5 mg/kg/min (over a total of 8 min). On the first day of dosing (loading, followed by a maintenance dose), the total daily Captisol dose would be 52 mg/kg/day. Thereafter, the maintenance dose would result in a daily dose of Captisol of 12 mg/kg/day.

In pediatric SE patients (birth to <17 years of age), the loading dose is 15-20 mg PE/kg, administered at a rate of 2 mg PE/kg/min or 150 mg PE/min, whichever is slower. The initial maintenance dose (2-4 mg PE/kg or 100 mg PE/min, whichever is slower) is to be administered 12 hours after the loading dose, and every 12 hours (4-8 mg/PE/kg/day in divided doses, at a rate of 1-2 mg PE/kg/min or 100 mg PE/min, whichever is slower). The loading dose would result in a dose of 30-40 mg/kg, administered at a rate of 4 mg/kg/min. On the first day of dosing (loading, following by a maintenance dose), the total daily Captisol dose would be 48 mg/kg/day. Thereafter, the maintenance dose would result in a daily dose of Captisol of 16 mg/kg/day.

Of the nonclinical studies of Captisol in the DMF, the most relevant for the proposed indications are the subchronic IV bolus toxicity studies. No renal tubular necrosis was observed in those studies in Sprague-Dawley rat and beagle dog, at doses of up to 3000 mg/kg (over ~1 min) or 1500 mg/kg (rate not specified), respectively. (While the rate of administration was not specified for the 1-month study in dog, it was presumed be ~1500 mg/kg/min, based on previous Captisol studies in dog conducted by the same laboratory.) Based on body surface area, these doses are 9-16 times the total dose on the first day of administration and ≥100 times the maximum rate of administration in adult patients. Therefore, as Dr. Fisher concluded, the nonclinical data support administration of CE-fosphenytoin at the maximum rate and total daily dose of Captisol proposed for adults. They, however, do not substitute for human safety data.

The juvenile animal toxicology study of Captisol (**in the DMF**) is inadequate by design to support initiation of clinical studies or marketing approval for pediatric patients less than 12 years of age.

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/s/

LOIS M FREED
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	210-864
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	5/22/18
PRODUCT:	Captisol-enabled (CE) fosphenytoin sodium injection
INTENDED CLINICAL POPULATION:	epilepsy
SPONSOR:	Sedor Pharmaceuticals, LLC
REVIEW DIVISION:	Division of Neurology Products (HFD-120)
PHARM/TOX REVIEWER:	Ed Fisher
PHARM/TOX SUPERVISOR:	Lois Freed
DIVISION DIRECTOR:	Billy Dunn
PROJECT MANAGER:	Heather Bullock

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Note: All figures and tables in this review were excerpted from the sponsor's submission or DMF #14364. Except as specifically identified, all data and information discussed below are owned by Sedor or are data for which Sedor has obtained a written right of reference.

I. INTRODUCTION AND DRUG HISTORY

The sponsor is relying on the Agency's findings of safety and effectiveness for the fosphenytoin sodium injection Listed Drug (LD) Cerebyx (NDA 20450) to support the safety and efficacy of CE-fosphenytoin. The drug product is comprised of fosphenytoin sodium, USP, and a primary excipient sulfobutyl ether beta-cyclodextrin (SBECD; Captisol) (b) (4) While current fosphenytoin sodium injection products are labelled for storage under refrigerated conditions, (b) (4) room-temperature storage of the drug product at a more physiologic pH of 7(b) (4)–8.2. CE-Fosphenytoin is indicated for the treatment of generalized tonic-clonic status epilepticus (SE) and for the prevention and treatment of seizures occurring during neurosurgery. CE-fosphenytoin also can be substituted, with short-term use, for oral phenytoin. CE-fosphenytoin should be used only when oral phenytoin administration is not possible. CE-fosphenytoin is expected to be used as second-phase therapy upon arrival at the hospital emergency room or in the intensive care unit. CE-fosphenytoin is an intravenous and intramuscular injection drug product. The sponsor is seeking indications identical to those listed in the Cerebyx labeling, which include use in pediatric patients at ages down to birth.

The primary nonclinical safety issue concerning this formulation involves the use of the excipient, Captisol. The original sponsor (Cy-Dex) had adequately addressed questions relating to the potential effects of Captisol on drug tissue distribution and developmental toxicity, as discussed in the Pre-IND meeting (IND 74871 meeting minutes dated 9/20/06; Division email dated 7/10/07). According to the current sponsor, the following provide the nonclinical support for this 505(b)(2) application:

- For the API, by reference to the LD (Cerebyx).
- By the results of a single-dose intramuscular tolerance study in rats with CE-fosphenytoin.
- For the excipient Captisol, by reference to DMF #14364 for Captisol, for which a Letter of Authorization was provided.

The local tolerance study in rat and the most relevant toxicity information contained in the Captisol DMF are reviewed here.

II. TOXICOLOGY

A. Single dose local tolerance study of CE-fosphenytoin (submitted in NDA)

1. Intramuscular tolerance study of Captisol-enabled fosphenytoin sodium in albino rats ((b) (4) 640001, conducted by (b) (4), report dated 7/31/08, GLP)

Methods

Groups of Crl:CD(SD) rats (10/sex/group) received a single intramuscular (IM) injection (0.33 mL in males, 0.23 mL in females) of saline, Captisol, CE-fosphenytoin sodium (75 mg/mL), or Cerebyx (75 mg/mL). Following dose administration, all rats were euthanized following a 24-hour observation period.

Results

All animals survived to the scheduled necropsy. No treatment-related clinical observations were noted. The administration of either CE-fosphenytoin sodium or Cerebyx was associated with a low incidence of Grade 2 (females only) or 3 (males only) local tissue irritation (Table A.1.1). The histologic correlate for these grossly discernable grades of local tissue irritation was hemorrhage. Histologic alterations noted in the injection site tissue sections of CE-fosphenytoin- or Cerebyx-injected groups consisted of marginal increases in the incidence and/or severity of hemorrhage, characterized by focal to multifocal extravasations of red blood cells; mixed inflammatory cell infiltrates, characterized by focal to multifocal accumulations of mononuclear and polymorphonuclear leukocytes (including eosinophils); and muscle degeneration, characterized by irregular, hyalinized fibers being infiltrated by inflammatory cells. The local effects CE-fosphenytoin sodium and Cerebyx were very similar.

Table A.1.1.Summary of microscopic findings

----- MALE -----				
GROUP:	1	2	3	4
NUMBER OF ANIMALS IN DOSE GROUP	10	10	10	10
NUMBER OF ANIMALS EXAMINED	10	10	10	10
INJECTION SITE				
TOTAL NUMBER EXAMINED	10	10	10	10
EXAMINED, UNREMARKABLE	5	3	0	0
-DEGENERATION, MUSCLE	2	2	2	5
MINIMAL	2	2	2	5
-HEMORRHAGE	0	0	5	2
MINIMAL	NONE	NONE	1	1
MILD	NONE	NONE	3	1
MODERATE	NONE	NONE	1	NONE
-INFILTRATE, MIXED INFLAMMATORY CELL	5	7	10	9
MINIMAL	5	6	5	6
MILD	NONE	1	4	3
MODERATE	NONE	NONE	1	NONE

1- SALINE 2- VEHICLE 3-CE-FOSPHENYTOIN 4- CEREBYX

	----- FEMALE -----			
GROUP:	1	2	3	4
NUMBER OF ANIMALS IN DOSE GROUP	10	10	10	10
NUMBER OF ANIMALS EXAMINED	10	10	10	10
INJECTION SITE				
TOTAL NUMBER EXAMINED	10	10	10	10
EXAMINED, UNREMARKABLE	2	2	0	1
-DEGENERATION, MUSCLE	2	1	4	6
MINIMAL	2	1	2	3
MILD	NONE	NONE	2	2
MODERATE	NONE	NONE	NONE	1
-HEMORRHAGE	2	3	3	3
MINIMAL	2	3	NONE	1
MILD	NONE	NONE	3	2
-INFILTRATE, MIXED INFLAMMATORY CELL	7	6	10	9
MINIMAL	6	6	7	4
MILD	NONE	NONE	2	5
MODERATE	1	NONE	1	NONE

1- SALINE 2- VEHICLE 3-CE-FOSPHENYTION 4- CEREBYX

B. General toxicity studies of Captisol (CP-217,861-2, CAP, all studies from DMF #14364)

1. CP-217,861-2 - 14-day intravenous range-finding study in Sprague-Dawley rats (Study No. 93033, conducted by [redacted](b) (4), report dated 2/4/94, GLP)

Methods

CAP (0 (saline vehicle), 160, 240, 600, or 1500 mg/kg) was administered intravenously (5 mL/kg injected over 15 seconds) to S-D rats (5/sex/group) once daily for 14 days. Observations included mortality, clinical signs, BW, clinical pathology, and gross and microscopic pathology.

Results

There were no deaths, clinical signs, BW effects, hematological or clinical chemistry changes, and no signs of irritation at the injection sites. The only T-R findings reported were renal tubular vacuolation, which occurred with dose-related incidence and severity (Table B.1.1), and pulmonary foam cell foci at the three highest doses (Table B.1.2).

Table B.1.1.

Distribution and severity of renal tubular vacuolation

	Control		160 mg/kg		240 mg/kg		600 mg/kg		1500 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Grade 1	0	0	0	1	1	2	5	4	0	0
Grade 2	0	0	0	0	0	0	0	1	5	5
Total	0	0	0	1	1	2	5	5	5	5
At risk	5	5	5	5	5	5	5	5	5	5

Grade 1: less than 20% of cortical tubules affected. Epithelial cells contained predominantly small to medium-size vacuoles.
Grade 2: more than 50% of cortical tubules affected. Epithelial cells contained predominantly small to medium-size vacuoles.

Table B.1.2.

Distribution and severity of pulmonary foam cell foci

	Control		160 mg/kg		240 mg/kg		600 mg/kg		1500 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Grade 1	1	0	0	0	1	1	1	1	1	0
Grade 2	0	0	0	0	0	0	0	0	3	4
Grade 3	0	0	0	0	0	0	0	0	0	1
Total	1	0	0	0	1	1	1	1	4	5
At risk	5	5	5	5	5	5	5	5	5	5

Grade 1: less than 20 foci of foamy alveolar macrophages scattered throughout the routine section.
Grade 2: between 20 and 50 foci of foamy macrophages.
Grade 3: more than 50 foci of foamy macrophages.

Conclusions

Daily iv bolus administration of CAP at doses up to 1500 mg/kg/day and rates up to 6000 mg/kg/min to rats for 14 days resulted in vacuolar changes in the kidney at all doses and pulmonary foam cells at the 3 highest doses. At the LD, renal vacuolation was minimal, and there were no pulmonary foam cell foci. At the HD, most animals had both findings. There was no evidence of irritation at the injection sites and no hemolysis or other clinical pathology changes. The LD (160 mg/kg/day, 640 mg/kg/min) was considered the NOAEL.

2. CP-217,861-2: 14-day intravenous range-finding study in beagle dogs (Study No.: 93034, conducted by (b) (4), report dated 2/4/94, GLP)

Methods

CAP (0 (saline vehicle), 160, 240, or 750 mg/kg) was administered intravenously (64, 96, or 300 mg/mL; 2.5 mL/kg injected over 50 sec to 2 min; 375 mg/kg/min at HD) to beagle dogs (3/group) once daily for 14 days. Observations included mortality, clinical signs, BW, ECG, clinical pathology, and gross and microscopic pathology.

Results

There were no deaths, clinical signs, body weight differences, ECG effects, or clinical pathology changes, and no signs of irritation at the injection sites. Histopathological changes were confined to renal tissue. Vacuolation of the epithelial cells of the renal proximal convoluted tubules was seen with dose-related severity in all treated animals (Table B.2.1).

Table B.2.1.

Distribution and severity of renal tubular vacuolation

	Control		160 mg/kg		240 mg/kg		750 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1	0	0	1	1	0	1	0	0
Grade 2	0	0	0	1	1	0	1	1
Grade 3	0	0	0	0	1	0	1	0
Total	0	0	1	2	2	1	2	1
At risk	1	2	1	2	2	1	2	1

Grade 1 (minimal): vacuolar change characterized by cytoplasmic microvacuoles mainly located at the basal pole of epithelial cells of the proximal convoluted tubules. Less than 20% of cortical tubules were affected.
Grade 2 (mild): between 21 and 35% of cortical tubules were affected.
Grade 3 (moderate): vacuolar change characterized by small to medium-sized vacuoles. Many of these vacuoles contained a granular or punctiform material, PTAH and Weigert's fibrin negative. Between 36 and 50% of the tubules were affected.

Conclusions

Daily iv bolus administration of CAP to beagle dogs for 14 days at doses up to 750 mg/kg/day and rates up to 375 mg/kg/min resulted in renal tubular vacuolation in all treated dogs, with a dose-related severity. However, no necrosis or clinical chemistry correlates were reported. There were no signs of irritation at the injection sites.

3. A 14-Day toxicity study of Captisol administered by continuous intravenous infusion in cynomolgus monkeys (Study No.: 1145-166, conducted by (b) (4), (b) (4), report dated 2/12/03, GLP)

Methods

CAP (0 (saline vehicle), 0 (high sodium saline), or 5600 mg/kg/day) was administered to male cynomolgous monkeys (3/group) by continuous intravenous infusion (24 hours/day, 2.33 mL/kg/hr, 100 mg/mL CAP). Observations included mortality, clinical signs, BW, ECG, clinical pathology, and gross and microscopic pathology. Blood samples were collected for TK on Days 1, 7, and 15.

Results

There were no clinical signs, BW effects, ECG changes, or hematological effects. A slight but progressive decrease in cholesterol was observed in CAP-treated animals. Increased urinary sodium and/or urinary chloride concentrations were similar in CAP- and high concentration sodium chloride-treated control (secondary control) animals and represented secretion of the infusate. Macroscopic findings of pale kidneys, correlating with renal tubular vacuolation histologically, vacuolation of the urothelial cells of the renal pelvis and urinary bladder, vacuolated macrophages in multiple lymph nodes and in the red pulp of the spleen, and a slight increase in the number of pulmonary macrophages were seen in CAP-treated animals.

Steady-state concentrations of approximately 850 ug/mL were reached between 3-6 hours after start of infusion and remained constant throughout the continuous infusion period. The AUC (0-inf) for day 14 was 864 ug.h/mL, and exposure for the 14-day dosing period was 286000 ug.h/mL. The volume of distribution was approximately 2-fold higher than the central

volume, indicating that CAP was distributed outside the plasma and was equivalent to extracellular fluid in monkey. The α and β-half-lives were 0.139 and 0.942 h, respectively, indicating rapid distribution and elimination. Systemic clearance was 4.59 mL/min/kg (15.0 mL/min), which was approximately equivalent to the glomerular filtration rate in cynomolgus monkeys.

Conclusions

Continuous iv infusion of CAP to cynomolgus monkeys at a dose of 5600 mg/kg/day and rate of 233 mg/kg/hr produced the expected vacuolar changes in the kidney, urinary bladder, and spleen, but no evidence of functional effects was reported.

4. CP-217,861-2 - 1-month intravenous toxicity study, with 1-month reversibility, in Sprague-Dawley rats (Study No. 93082, conducted by (b) (4), report dated 6/24/94, GLP)

Methods

CAP (0 (saline vehicle), 160, 240, or 320 mg/kg) was administered intravenously (1.07 mL/kg injected over 10 seconds) to S-D rats (10/sex/group + 5/sex/grp recovery at 1 month) once daily for 28 days. Observations included mortality, clinical signs, BW, clinical pathology, and gross and microscopic pathology. Kidneys and lungs from C and HD groups were examined by electron microscopy.

Results

There were no deaths or T-R clinical signs, body weight effects, or signs of irritation at the injection sites. Clinical pathology parameters were not affected by the treatment. The main findings were renal tubular vacuolation and pulmonary foam cell foci. Renal tubular vacuolation occurred with a dose-related incidence and severity (Table B.4.1) and was characterized by an increased number and size of vacuoles derived from lysosomes. Pulmonary foam cell foci occurred at the mid and high doses (Table B.4.2) and were characterized by increased numbers and size of lysosomes. At the end of the reversibility period, renal tubular vacuolation was not completely resolved; grade 1 focal tubular vacuolation, characterized by the presence of macrovacuoles within epithelial cells of a few (less than 10/kidney) contiguous sections of a nephron, was observed in 1 LD male and 1 male and 4 females at the HD. Pulmonary foam cell foci was completely resolved after 1 month.

Table B.4.1.

Incidence and severity of renal tubular vacuolation

	Control		160 mg/kg		240 mg/kg		320 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1	0	0	1	1	2	5	6	4
Grade 2	0	0	0	0	2	0	3	5
Total	0	0	1	1	4	5	9	9
At risk	10	10	10	10	10	10	10	10

Grade 1: less than 20% of the tubules are affected.
Grade 2: more than 50% of the tubules are affected.
Tubular vacuolation affecting 20% to 50% of proximal cortical tubules was not observed in this study.

Table B.4.2.

Incidence of pulmonary foam cell foci

	Control		160 mg/kg		240 mg/kg		320 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1	1	1	0	1	3	5	5	7
Total	1	1	0	1	3	5	5	7
At risk	10	10	10	10	10	10	10	10

Grade 1: less than 20 foci of foamy alveolar macrophages scattered throughout the routine section.

Conclusions

Daily iv bolus administration of CAP to rats for 1 month at doses up to 320 mg/kg/day and rates up to 1920 mg/kg/min resulted in vacuolar changes in the kidney at all doses and pulmonary foam cells at the MD and HD. There was no evidence of irritation at the injection sites and no hemolysis or other clinical pathology changes. The histopathology changes were partially (kidney) or completely (lung) reversible after cessation of treatment. The LD (160 mg/kg/day, 960 mg/kg/min) was considered the NOAEL.

5. CP-217,861-02 - 1-month intravenous toxicity study in Sprague-Dawley rats with 2 and 5 month reversibility (Study No. 95107, conducted by (b) (4), report dated 12/19/96, GLP)

Methods

CAP (0 (saline vehicle), 300, 1000, or 3000 mg/kg) was administered intravenously (10 mL/kg injected over 60 to 70 seconds) to S-D rats (10/sex/group + 5/sex/grp recovery at 2 and 5 months) once daily for 28 days. Observations included mortality, clinical signs, BW,

ophthalmology, clinical pathology, and gross and microscopic pathology. Kidneys, liver, and lungs of selected animals from C and HD groups were examined by electron microscopy.

Results

Mortality, clinical signs, BW, ophthalmology

No treatment-related (T-R) mortality, clinical signs, BW effects, or ophthalmology changes were observed. A single HD female death was considered to have resulted from a dosing accident.

Clinical pathology

There was a mild statistically significant (SS) decrease in RBC parameters in HD males and females compared to C. Similar changes with a lower magnitude were observed in MD females. There was a minimal, but SS increase in platelet count in HD females. There were no T-R hematological differences among groups after the 2- and 5-month recovery periods.

At the end of the treatment period, increases in ALT (up to 4X) and AST (up to 3X) were seen at the HD. There were slight increases in urea (18 to 37%), creatinine (13-18%), and phosphates (6-13%, NS) in this group. Triglycerides decreased by about 40% in HD males. At the MD, the only difference between control and treated groups was a small (less than 20%) decrease in ALT in both sexes and a 30% increase in cholesterol in females. These changes were reversible.

Urinalysis showed a consistent increase in incidence of positive reactions to the qualitative test for hemoglobin at the HD. This effect was no longer seen after drug withdrawal. There was also a slight decrease in urine pH (of about 1 pH unit) at the HD and, to a lesser extent, at the MD, which was reversible in males but not in females.

Gross and microscopic pathology

T-R increases (20-40% compared to C) in kidney, liver, and spleen weights were present at the end of the 1-month treatment period mainly at the HD, although minimal, but SS increases in absolute and relative kidney weights (12 and 15%, respectively) were also observed in MD females. The changes were still present in the HD group after the 2-month recovery period and were observed in the kidneys and spleen after 5 months.

At the end of the treatment period, macroscopic observations of pale discoloration of the kidneys were noted in 7/19 HD and 2/20 MD rats, an enlarged kidney in 1/10 HD males, and pale discoloration of the lungs of 1/10 HD males. These changes were not present 2 and 5 months after cessation of treatment.

There was a dose-related increase in severity of the vacuolation of the epithelial cells of renal tubules in all treated groups (see Table B.5.1 below). Renal tubular vacuolation was confined to the cortical tubules in grades 1 and 2 and extended to the medulla in grades 3 and 4. It was characterized by an increased number and size of cytoplasmic vacuoles. Vacuoles were often empty or contained a faintly granular eosinophilic PAS-positive material. In grades 3 and 4, where large vacuoles were present, the cells were markedly swollen and obliterated the lumen of the tubules. There was no evidence of necrosis in treated animals, but at the HD tubular vacuolation was extensive (vacuolation of tubular segments in the renal medulla) and severe (occasional obliteration of the lumen of tubules), with evidence of occasional cell damage at the ultrastructural level (dissolution of organelles, loss of apical microvilli and dilation of mitochondrial cristae). Although renal vacuolation was reduced in severity, it was not completely reversed 5 months after the end of treatment.

Minimal vacuolation of the transitional epithelium in the renal pelvis was present in MD (13/20) and HD (15/20) animals at the end of the treatment period. Vacuolated cells were mainly located in the superficial layer of the epithelium. Two and 5 months after cessation of treatment, minimal vacuolation of transitional epithelium was still present at the MD (7/10 and 6/10 for 2 and 5 months, respectively) and HD (8/10 and 7/10 for 2 and 5 months, respectively), although vacuolated cells were less numerous than at the end of the treatment period. At the end of treatment, pyelonephritis characterized by a chronic mixed inflammation of the renal pelvis occurred in more than half of the HD females (8/10). After cessation of treatment, the incidence of pyelonephritis was similar among groups.

Table B.5.1.

Incidence and severity of renal tubular vacuolation throughout the study

	Treatment period							
	Control		300 mg/kg		1000 mg/kg		3000 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1			10	6				1*
Grade 2				2	4	7		
Grade 3					6	2	3	7
Grade 4							7	2
Total	0	0	10	8	10	9	10	10
At risk	10	10	10	10	10	10	10	10

*F802, found dead at day 5

Two months after treatment period

	Control		1000 mg/kg		3000 mg/kg	
	M	F	M	F	M	F
Grade 1			1		1	
Grade 2			2	4	2	
Grade 3			1	1	2	3
Grade 4						2
Total	0	0	4	5	5	5
At risk	5	5	5	5	5	5

Five months after treatment period

	Control		1000 mg/kg		3000 mg/kg	
	M	F	M	F	M	F
Grade 1			4	4	1	1
Grade 2				1	3	2
Grade 3					1	1
Grade 4						
Total	0	0	4	5	5	4
At risk	5	5	5	5	5	5

Grade 1: Less than 25% of cortical tubules affected.
Grade 2: More than 25% but less than 50% of cortical tubules affected.
Grade 3: More than 50% but less than 75% of cortical tubules affected.
Grade 4: More than 75% of cortical tubules affected.

At the end of the treatment period, a dose-related increase in minimal vacuolation of the transitional epithelium of the urinary bladder was observed in all treated groups (Table B.5.2). It was mainly located within the superficial layers of the epithelium (umbrella cells) and was seen multifocally along the mucosa. Two and 5 months after the end of treatment, vacuolation of the transitional epithelium was still present in MD (5/10 and 7/10) and HD (7/10 and 7/10) rats.

Table B.5.2.

Incidence of minimal vacuolation of the transitional epithelium of the urinary bladder

	Control		300 mg/kg		1000 mg/kg		3000 mg/kg	
	M	F	M	F	M	F	M	F
			4	2	6	7	10	7
At risk	10	10	10	10	10	10	10	10

Marked vacuolation of hepatocytes associated with increased liver weight was seen in HD animals. Ultrastructurally, vacuoles were of lysosomal origin, as in the kidney. This change

was only partially reversible. In some animals, vacuolated hepatocytes were accompanied by necrosis, which was thought to explain the increase in aminotransferases.

Foamy macrophages were observed in the sinusoids of the liver and in the alveoli of the lungs, as well as in the sinusoids of the lymph nodes, the red pulp of the spleen, the interstitium of the testis, ovaries, and uterus, in the valves of the heart and the vestigial lumen of the pituitary gland. The vacuolar content of these foamy macrophages was often PAS-positive and the lysosomal origin was confirmed ultrastructurally in the lungs and liver. Distribution of these activated vacuolated macrophages in the different organs varied with the dose level; only the lungs, testes, and pituitary gland were affected at all doses. After cessation of dosing, the number of foamy macrophages decreased with time and were absent in the ovaries, uterus, heart, and pituitary gland at the end of the reversibility period.

Conclusions

Daily bolus iv administration of CAP to rats for 1 month at doses up to 3000 mg/kg/day and rates up to approximately 2500-3000 mg/kg/min resulted in vacuolar changes in the kidney, urinary bladder, and liver, and foamy macrophages in multiple tissues at all doses. Evidence of hemolysis and other clinical pathology changes that correlated with the kidney and liver histopathology were seen at the HD. The histopathology changes were only partially reversible after cessation of dosing at the MD and HD.

6. 28-Day Continuous/Intermittent Intravenous Infusion Toxicity Study in Rats with a 4-Week Recovery Period (7802-113, conducted by (b) (4), report dated 3/13/09, GLP)

Methods

Vehicle (SBECD [lot # NC-04A-05023] in sterile water for injection) or drug in SBECD (Captisol) were administered to rats (Crl:CD(SD)) iv via a femoral vein catheter using a programmable syringe pump for 28 days according to the following protocol:

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/day) [redacted] CAP		Dose Concentration (mg/mL) [redacted] / CAP	Dose Volume[c] (mL/kg/day)	Dose Time[d] (hours/day)
Toxicity Animals							
1 (Control)	15	15	0	2500	0 / 250	10	24
2 (Low)	10	10	25	625	10 / 250	2.5	3
3 (Mid)	10	10	56	1400	10 / 250	5.6	6.7
4 (High)	15	15	100	2500	10 / 250	10	24
Toxicokinetic Animals							
5 (Control)	3	3	0	2500	0 / 250	10	24
6 (Low)	9	9	25	625	10 / 250	2.5	3
7 (Mid)	9	9	56	1400	10 / 250	5.6	6.7
8 (High)	9	9	100	2500	10 / 250	10	24

a. Group 1/5 received Captisol® (250 mg/mL) only.
b. Animals designated for recovery sacrifice (the last 5 animals/sex in Groups 1 and 4) underwent at least 4 weeks of recovery following dose administration.
c. The dose volume and dose time were varied to provide the test article at a constant concentration.
d. The dose rate for Groups 1/5 and 4/8 was 0.42 mL/kg/hour. The dose rate for Groups 2/6 and 3/7 was 0.84 mL/kg/hour.

Assessment of toxicity was based on mortality, clinical signs, ophthalmologic examinations, body weight (BW), food consumption, and clinical and anatomic pathology data. Blood was collected from toxicokinetic (TK) animals for plasma drug level determination. All tissues from animals in the control (C) and high-dose (HD) groups and from animals that died or were sacrificed at an unscheduled interval were processed and examined microscopically. Macroscopic lesions from animals in the low- and mid-dose (LD, MD) groups were examined microscopically.

The study was designed to give maximum exposure to the fixed ratio of Drug/SBECD (1:25) used in the clinical formulation.

Results

Mortality:

There were 5 deaths, but none was considered treatment-related (TR) in the study report. Two animals were found dead (C male B29521 on day 26 and HD female B29645 on day 11), 1 control animal was euthanized moribund (male B29523 on day 21), and 2 LD animals were removed from study as a result of catheter damage (male B29537 on day 21 and female B29618 on day 16). The cause of death for control male B29521 was undetermined, but acute pulmonary inflammation and edema were thought to be likely contributory. There were no TR clinical observations for this animal. HD female B29645 had no remarkable clinical observations except rough haircoat on days 8 and 11 and no cause of death was determined. C male B29523 was hypoactive, thin, and hunched with a rough coat; moribundity was attributed to septicemia. All other animals survived to the scheduled sacrifice.

Clinical signs and BW:

There were no TR clinical observations. Observations of alopecia, sores and scabs, and rough haircoat were noted in all groups with similar incidence and frequency and were associated with the use of the infusion harness. There were no TR ophthalmologic findings. There were small (92 to 94% of control; not statistically significant) body weight (BW) differences related to CAP dose.

Clinical pathology:

There were no apparent TR effects on clinical pathology parameters.

Necropsy:

There were no organ weight or macroscopic changes associated with treatment at the end of the dosing or recovery periods. Histological changes commonly noted in iv infusion studies at the catheterization and infusion sites (e.g., thrombus, endothelial hyperplasia, inflammation, mineralization, and pigment) were observed in all groups. Histological changes present in treated animals (vacuolation of renal tubule and transitional epithelium, and urinary bladder and prostate transitional epithelium; vacuolation of histiocytes present in mesenteric and mandibular lymph node sinuses; infiltrates of macrophages into heart valves and the interstitium of testes and epididymides; liver and lung infiltrates; lymphoid hyperplasia in spleen; Table B.6.1) were all considered related to the infusion of CAP. At the end of the 4-week recovery period, vacuolation of prostate transitional epithelium and mandibular lymph node sinus histiocytes, and infiltrates of macrophages into heart valves were no longer present. However, vacuolation of the renal tubule and transitional epithelium and urinary bladder transitional epithelium were still present at a similar incidence and severity (Table

B.6.2). According to the report, since these changes “were not associated with any correlating signs of toxicity,” they were not considered adverse.

Table B.6.1 Microscopic observations – Terminal sacrifice

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --				-- Animals Affected -- Females --			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
Tissues With Diagnoses	No. in group:	8	9	10	10	10	9	10	9
Brain	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Spinal Cord	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Adrenal, Cortex	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Abscess, with coccoid bacteria		0	0	0	0	0	0	0	0
Adrenal, Medulla	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Pituitary	Number examined:	8	0	0	10	10	0	0	8
	Unremarkable:	8	0	0	9	10	0	0	8
Vacuolation, Pars Distalis Cell		0	0	0	1	0	0	0	0
Nerve, Sciatic	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Trachea	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	9	0	0	9
Ectasia, Submucosal Gland		0	0	0	0	1	0	0	0
Esophagus	Number examined:	8	0	0	10	10	0	0	8
	Unremarkable:	8	0	0	10	10	0	0	8
Thyroid	Number examined:	8	0	0	10	10	0	0	8
	Unremarkable:	8	0	0	10	10	0	0	8
Parathyroid	Number examined:	7	0	0	8	8	0	0	6
	Unremarkable:	7	0	0	8	8	0	0	6
Heart	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	0	0	0	1	2	0	0	3
Infiltrate, Mononuclear		2	0	0	3	2	0	0	0
Infiltrate, Macrophage, Valvular		7	0	0	5	7	0	0	4
Hyperplasia, Epicardium		0	0	0	2	0	0	0	0
Inflammation, Epicardium		0	0	0	0	0	0	0	1
Infiltrate, Macrophage, Endothelial		0	0	0	0	1	0	0	0
Infiltrate, Neutrophils		0	0	0	0	0	0	0	1
Aorta	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Tongue	Number examined:	8	0	0	10	10	0	0	8
	Unremarkable:	8	0	0	10	10	0	0	8
Muscle, Bi Fem	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	8
Infiltrate, Mononuclear		0	0	0	0	0	0	0	1
Liver	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	0	0	0	0	0	0	0	0
Infiltrate, Mononuclear		8	0	0	10	10	0	0	9
Congestion		0	0	0	0	0	0	0	0
Hyperplasia, Hepatocellular		0	0	0	0	0	0	0	0
Hyperplasia, Kupffer Cell		0	0	0	0	0	0	0	0
Hematopoiesis, Extramedullary		0	0	0	0	0	0	0	0
Necrosis		0	0	0	0	0	0	0	0
Vacuolation, Hepatocyte, Midzonal		0	0	0	0	0	0	0	0
Vacuolation, Hepatocyte		0	0	0	0	0	0	0	0
Spleen	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	1	0	0	3	2	0	0	2
Hyperplasia, Plasma Cell		0	0	0	0	0	0	0	0
Hematopoiesis, Extramedullary, Increased		0	0	0	0	0	0	0	0
Hyperplasia, Lymphocytes		6	0	0	7	8	0	0	6
Depletion, Lymphocytes		1	0	0	0	0	0	0	1
Lung	Number examined:	8	2	0	10	10	0	1	9
	Unremarkable:	0	0	0	0	1	0	0	0
Infiltrate, Eosinophil, Perivascular		8	2	0	9	9	0	1	8
Infiltrate, Macrophage, Alveolar		4	0	0	3	3	0	0	4
Pneumonitis, Chronic Active		0	1	0	1	0	0	0	3
Pneumonitis, Acute		0	0	0	0	1	0	0	0
Thrombus		0	0	0	0	0	0	0	1
Mineralization		0	0	0	0	1	0	0	0
Thymus	Number examined:	8	0	0	10	10	0	0	9
	Unremarkable:	8	0	0	10	10	0	0	9
Necrosis, Lymphocytes		0	0	0	0	0	0	0	0

Cecum ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9
Jejunum ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9
Rectum ..... Number examined:	8	0	0	10	|	10	0	0	8
Unremarkable:	8	0	0	10	|	10	0	0	8
Inflammation, Adventitia	0	0	0	0	|	0	0	0	0
LN, Mesenteric ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	2	0	0	4	|	6	0	0	4
Vacuolation, Sinus Histiocytes (Macrophages)	6	0	0	6	|	4	0	0	5
Necrosis, Lymphocytes	0	0	0	0	|	0	0	0	0
Erythrocytosis, Sinus	0	0	0	0	|	0	0	0	0
LN, Mandibular ..... Number examined:	8	0	0	10	|	10	0	0	8
Unremarkable:	1	0	0	4	|	6	0	0	3
Vacuolation, Sinus Histiocytes	7	0	0	5	|	4	0	0	5
Erythrocytosis, Sinus	0	0	0	1	|	0	0	0	0
Necrosis, Lymphocytes	0	0	0	0	|	0	0	0	0
Gl, Mandib Saliv ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9
Pancreas ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	9	0	0	8
Infiltrate, Mononuclear	0	0	0	0	|	1	0	0	1
Nerve, Optic ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9
Eye ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	4	0	0	8
Dystrophy, Cornea	0	0	0	0	|	5	0	0	0
Keratopathy	0	0	0	0	|	0	0	0	1
Fibrosis, Cornea	0	0	0	0	|	1	0	0	0
Vascularization, Cornea	0	0	0	0	|	0	0	0	0
Kidney ..... Number examined:	8	0	1	10	|	10	0	0	9
Unremarkable:	0	0	0	0	|	0	0	0	0
Vacuolation, Tubule Cell	8	0	1	10	|	10	0	0	9
Vacuolation, Transitional Cell, Pelvis	7	0	0	10	|	10	0	0	9
Infiltrate, Mononuclear	3	0	0	5	|	2	0	0	3
Inflammation, Suppurative, with Coccoid Bacteria	0	0	0	0	|	0	0	0	0
Degeneration, Tubule Cell	6	0	1	7	|	5	0	0	5
Loss, Tubule with Stromal Collapse	1	0	0	0	|	1	0	0	0
Dilatation, Pelvis	0	0	0	0	|	0	0	0	0
Inflammation, Subacute, Tubulointerstitial/Pelvic	0	0	1	0	|	0	0	0	0
Mineralization	0	0	0	0	|	0	0	0	1
Basophilic Tubule	0	0	0	0	|	0	0	0	1
Inflammation, Chronic-Active, Tubulointerstitial/Pelvic	0	0	0	0	|	0	0	0	0
Hyperplasia, Transitional Cell	0	0	0	0	|	0	0	0	0
Regeneration, Tubule Cell	0	0	0	0	|	0	0	0	0
Urinary Bladder ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	0	0	0	0	|	0	0	0	0
Vacuolation, Transitional Cell	8	0	0	10	|	10	0	0	9
Inflammation, Acute	0	0	0	0	|	0	0	0	0
Inflammation, Chronic	0	0	0	0	|	0	0	0	0
Hyperplasia, Transitional Cell	0	0	0	0	|	0	0	0	0
Stomach, Gl ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	6	0	0	10	|	9	0	0	7
Dilation, Gastric Gland	2	0	0	0	|	0	0	0	0
Edema	0	0	0	0	|	1	0	0	2
Stomach, Nongl ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	7
Edema	0	0	0	0	|	0	0	0	2
Duodenum ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9
Ileum ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9
Colon ..... Number examined:	8	0	0	10	|	10	0	0	9
Unremarkable:	8	0	0	10	|	10	0	0	9

Skin/Subcutis ..... Number examined:	8	0	0	10		10	0	0	9
Unremarkable:	6	0	0	8		7	0	0	9
Ulcer	2	0	0	2		0	0	0	0
Inflammation, Chronic-Active, Dermis	1	0	0	0		0	0	0	0
Granulation Tissue	0	0	0	0		1	0	0	0
Abscess, Subcutis	0	0	0	1		0	0	0	0
Inflammation, Perivascular	0	0	0	0		1	0	0	0
Hyperkeratosis	0	0	0	0		1	0	0	0
Seminal Vesicle ..... Number examined:	8	0	0	10					
Unremarkable:	8	0	0	10					
Apoptosis, Epithelial	0	0	0	0					
Prostate ..... Number examined:	8	0	1	10					
Unremarkable:	6	0	0	8					
Vacuolation, Transitional Cell	2	0	0	2					
Inflammation, Suppurativc	1	0	0	0					
Dilatation, Urethra	0	0	0	0					
Inflammation, Suppurative, with Coccoid Bacteria	0	0	1	0					
Abscess	0	0	0	0					
Testis ..... Number examined:	8	0	0	10					
Unremarkable:	0	0	0	0					
Infiltrate, Macrophage	8	0	0	10					
Inflammation, Acute	0	0	0	0					
Degeneration, Seminiferous Tubule	0	0	0	1					
Epididymis ..... Number examined:	8	0	0	10					
Unremarkable:	0	0	0	0					
Infiltrate, Macrophage	8	0	0	10					
Mammary, Female ..... Number examined:						10	0	0	9
Unremarkable:						10	0	0	9
Ovary ..... Number examined:						10	0	0	9
Unremarkable:						10	0	0	9
Uterus ..... Number examined:						10	0	2	9
Unremarkable:						7	0	0	8
Dilatation						3	0	2	1
Cervix ..... Number examined:						10	0	0	9
Unremarkable:						9	0	0	9
Cyst, Keratinizing						1	0	0	0
Vagina ..... Number examined:						10	0	0	9
Unremarkable:						10	0	0	9
Bone, Femur ..... Number examined:	8	0	0	10		10	0	0	9
Unremarkable:	8	0	0	10		10	0	0	9
Marrow, Femur ..... Number examined:	8	0	0	10		10	0	0	9
Unremarkable:	8	0	0	10		10	0	0	9
Hypercellular	0	0	0	0		0	0	0	0
Bone, Sternum ..... Number examined:	8	0	0	10		10	0	0	9
Unremarkable:	8	0	0	10		10	0	0	9
Marrow, Sternum ..... Number examined:	8	0	0	10		10	0	0	9
Unremarkable:	8	0	0	10		10	0	0	9
Death Comment ..... Number examined:	8	0	0	10		10	0	0	9
Unremarkable:	0	0	0	0		0	0	0	0
Scheduled Sacrifice	8	0	0	10		10	0	0	9
Septicemia	0	0	0	0		0	0	0	0
Undetermined	0	0	0	0		0	0	0	0
Infusion Site ..... Number examined:	8	2	2	10		10	0	0	8
Unremarkable:	3	1	1	5		4	0	0	3
Hyperplasia, Endothelial	0	0	0	2		1	0	0	1
Thrombus	4	0	0	3		3	0	0	3
Abscess, with coccoid bacteria	1	1	1	0		0	0	0	0
Infiltrate, Macrophage, Perivascular	0	0	0	0		3	0	0	0
Inflammation, Chronic-Active	0	0	0	0		0	0	0	1
Mineralization	0	0	0	0		0	0	0	0
Pigment	0	0	0	0		0	0	0	0
Inflammation, Granulomatous, with suture material	0	0	0	0		0	0	0	0
Abscess, with suture material	0	0	0	0		0	0	0	0
Catheter Site ..... Number examined:	8	1	0	10		10	0	0	9
Unremarkable:	0	0	0	0		2	0	0	0
Infiltrate, Eosinophils	1	0	0	4		0	0	0	0
Inflammation, Granulomatous, Suture	3	0	0	4		3	0	0	4
Catheter Site ..... Number examined:	8	1	0	10		10	0	0	9
Unremarkable:	0	0	0	0		2	0	0	0
Thrombus	3	1	0	0		2	0	0	1
Atrophy, Fat	0	0	0	0		0	0	0	0
Infiltrate, Macrophage, Perivascular	1	0	0	0		0	0	0	0
Inflammation, Granulomatous	0	0	0	0		0	0	0	2
Fibrosis	0	0	0	2		3	0	0	2
Inflammation, Chronic-Active	0	0	0	0		0	0	0	0
Abscess	0	0	0	0		0	0	0	0
LN, Other ..... Number examined:	0	0	1	0		0	0	0	0
Unremarkable:	0	0	0	0		0	0	0	0
Hyperplasia, Lymphocytes	0	0	1	0		0	0	0	0
Hyperplasia, Plasma Cell	0	0	1	0		0	0	0	0

Table B.6.2 Microscopic observations – Recovery

Controls from group(s): 1 Tissues With Diagnoses	Animal sex: Dosage group: No. in group:	-- Animals Affected -- Males -- Ctls	4	-- Females -- Ctls	4
Brain	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Spinal Cord	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Adrenal, Cortex	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Abscess, with coccoid bacteria		0	0	0	0
Adrenal, Medulla	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Pituitary	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Vacuolation, Pars Distalis Cell		0	0	0	0
Nerve, Sciatic	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Trachea	Number examined:	5	5	5	5
	Unremarkable:	3	4	4	5
Ectasia, Submucosal Gland		2	1	1	0
Esophagus	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Thyroid	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Parathyroid	Number examined:	5	5	4	4
	Unremarkable:	5	5	4	4
Heart	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Infiltrate, Mononuclear		0	0	0	0
Infiltrate, Macrophage, Valvular		0	0	0	0
Hyperplasia, Epicardium		0	0	0	0
Inflammation, Epicardium		0	0	0	0
Infiltrate, Macrophage, Endothelial		0	0	0	0
Infiltrate, Neutrophils		0	0	0	0
Aorta	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Tongue	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Muscle, Bi Fem	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Infiltrate, Mononuclear		0	0	0	0
Liver	Number examined:	5	5	5	5
	Unremarkable:	0	0	0	0
Infiltrate, Mononuclear		5	5	5	5
Congestion		0	0	0	0
Hyperplasia, Hepatocellular		0	0	0	0
Hyperplasia, Kupffer Cell		0	0	0	0
Hematopoiesis, Extramedullary		0	0	0	0
Necrosis		1	0	0	0
Vacuolation, Hepatocyte, Midzonal		1	0	0	0
Vacuolation, Hepatocyte		0	1	0	1
Spleen	Number examined:	5	5	5	5
	Unremarkable:	2	1	1	0
Hyperplasia, Plasma Cell		0	0	0	0
Hematopoiesis, Extramedullary, Increased		0	0	0	0
Hyperplasia, Lymphocytes		3	4	4	5
Depletion, Lymphocytes		0	0	0	0
Lung	Number examined:	5	5	5	5
	Unremarkable:	4	5	4	5
Infiltrate, Eosinophil, Perivascular		0	0	1	0
Infiltrate, Macrophage, Alveolar		1	0	1	0
Pneumonitis, Chronic Active		0	0	0	0
Pneumonitis, Acute		0	0	0	0
Thrombus		0	0	0	0
Mineralization		0	0	0	0
Thymus	Number examined:	5	5	5	5
	Unremarkable:	5	5	5	5
Necrosis, Lymphocytes		0	0	0	0

Kidney	Number examined:	5	5	|	5	5
	Unremarkable:	0	0	|	0	0
Vacuolation, Tubule Cell		5	5	|	5	5
Vacuolation, Transitional Cell, Pelvis		3	4	|	4	2
Infiltrate, Mononuclear		2	3	|	3	4
Inflammation, Suppurative, with Coccoid Bacteria		0	0	|	0	0
Degeneration, Tubule Cell		5	4	|	4	3
Loss, Tubule with Stromal Collapse		0	0	|	1	0
Dilatation, Pelvis		0	0	|	0	0
Inflammation, Subacute, Tubulointerstitial/Pelvic		0	0	|	0	0
Mineralization		0	0	|	1	1
Basophilic Tubule		0	2	|	0	0
Inflammation, Chronic-Active, Tubulointerstitial/Pelvic		1	0	|	0	0
Hyperplasia, Transitional Cell		1	0	|	0	0
Regeneration, Tubule Cell		1	2	|	0	0
Urinary Bladder	Number examined:	5	5	|	5	5
	Unremarkable:	0	3	|	1	0
Vacuolation, Transitional Cell		4	2	|	4	5
Inflammation, Acute		0	0	|	0	0
Inflammation, Chronic		1	0	|	0	0
Hyperplasia, Transitional Cell		1	0	|	0	0
Stomach, Gl	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	3
Dilation, Gastric Gland		0	0	|	0	0
Edema		0	0	|	0	2
Stomach, Nongl	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Edema		0	0	|	0	0
Duodenum	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Ileum	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Colon	Number examined:	5	5	|	4	5
	Unremarkable:	5	5	|	4	5
Cecum	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Jejunum	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Rectum	Number examined:	5	5	|	5	5
	Unremarkable:	4	5	|	5	5
Inflammation, Adventitia		1	0	|	0	0
LN, Mesenteric	Number examined:	5	5	|	5	5
	Unremarkable:	4	4	|	4	5
Vacuolation, Sinus Histiocytes (Macrophages)		1	0	|	1	0
Necrosis, Lymphocytes		0	0	|	0	0
Erythrocytosis, Sinus		0	1	|	0	0
LN, Mandibular	Number examined:	5	4	|	5	5
	Unremarkable:	5	4	|	5	4
Vacuolation, Sinus Histiocytes		0	0	|	0	0
Erythrocytosis, Sinus		0	0	|	0	1
Necrosis, Lymphocytes		0	0	|	0	0
Gl, Mandib Saliv	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Pancreas	Number examined:	4	5	|	5	5
	Unremarkable:	4	5	|	5	5
Infiltrate, Mononuclear		0	0	|	0	0
Nerve, Optic	Number examined:	5	5	|	5	5
	Unremarkable:	5	5	|	5	5
Eye	Number examined:	5	5	|	5	5
	Unremarkable:	4	5	|	4	3
Dystrophy, Cornea		1	0	|	0	2
Keratopathy		0	0	|	0	0
Fibrosis, Cornea		0	0	|	0	0
Vascularization, Cornea		0	0	|	1	0

Cervix	Number examined:				5	5
	Unremarkable:				5	5
Cyst, Keratinizing					0	0
Vagina	Number examined:				5	5
	Unremarkable:				5	5
Bone, Femur	Number examined:	5	5		5	5
	Unremarkable:	5	5		5	5
Marrow, Femur	Number examined:	5	5		5	5
	Unremarkable:	4	5		5	5
Hypercellular		1	0		0	0
Bone, Sternum	Number examined:	5	5		5	5
	Unremarkable:	5	5		5	5
Marrow, Sternum	Number examined:	5	5		5	5
	Unremarkable:	5	5		5	5
Death Comment	Number examined:	5	5		5	5
	Unremarkable:	0	0		0	0
Scheduled Sacrifice		5	5		5	5
Septicemia		0	0		0	0
Undetermined		0	0		0	0
Infusion Site	Number examined:	5	5		5	4
	Unremarkable:	2	4		2	0
Hyperplasia, Endothelial		0	0		0	0
Thrombus		2	0		0	0
Abscess, with coccoid bacteria		0	0		0	0
Infiltrate, Macrophage, Perivascular		0	1		1	2
Inflammation, Chronic-Active		1	0		0	0
Mineralization		0	0		1	0
Pigment		0	0		1	0
Inflammation, Granulomatous, with suture material		0	0		1	1
Abscess, with suture material		0	0		0	1
Catheter Site	Number examined:	5	5		5	5
	Unremarkable:	0	1		1	3
Infiltrate, Eosinophils		0	0		0	0
Inflammation, Granulomatous, Suture		3	3		3	1
Catheter Site	Number examined:	5	5		5	5
	Unremarkable:	0	1		1	3
Thrombus		0	1		1	0
Atrophy, Fat		0	0		0	0
Infiltrate, Macrophage, Perivascular		0	0		0	0
Inflammation, Granulomatous		1	0		0	0
Fibrosis		0	1		0	0
Inflammation, Chronic-Active		1	0		0	0
Abscess		0	0		0	1
LN, Other	Number examined:	0	0		0	0
	Unremarkable:	0	0		0	0

Conclusions

Administration of the CAP vehicle to rats for 28 days at doses of up to 2500 mg/kg/day and rates of up to 105 mg/kg/hr (1.75 mg/kg/min) was associated with histopathological changes (vacuolization of renal tubule and transitional epithelium and urinary bladder and prostate transitional epithelium; vacuolation of histiocytes in lymph node sinuses; infiltrates of macrophages into heart valves, testes, and epididymides; liver and lung infiltrates; lymphoid hyperplasia in spleen) that were only partially reversible after 4 week. There were no clinical pathology correlates for these findings.

7. CP-217,861-02: 1-month intravenous toxicity study in beagle dogs with 2 and 5 months reversibility (Study No. 95106, conducted by [redacted] (b) (4), report dated 3/28/97, GLP)

Methods

CAP (0 (saline vehicle), 300, 750, or 1500 mg/kg) was administered intravenously (5 mL/kg; rate not stated, previous dog studies by the same lab used 50-60 sec) to beagle dogs (3/sex/group + 1/sex/C, MD, HD recovery at 2 and 5 months) once daily for 28 days. Observations included mortality, clinical signs, BW, ophthalmology, clinical pathology, and gross and microscopic pathology. Kidneys and liver of selected animals from C and HD groups were examined by electron microscopy. The high dose (1500 mg/kg) was limited by

the solubility of the compound (300 mg/mL) and the maximal acceptable volume of administration in the dog (5 ml/kg).

Results

Mortality, clinical signs, BW, ophthalmology, clinical pathology

No mortality, clinical signs, BW effects, ophthalmology changes, or clinical pathology differences were observed.

Gross and microscopic pathology

T-R increases in relative (females: 18%, males: 23%) and absolute (females: 27%, males: 33%) liver weights were seen at the HD. There were no differences between control and treated animals at the end of the 2 and 5-month recovery periods.

No macroscopic changes were reported.

Vacuolation of the epithelial cells of renal tubules was seen in all CAP-dosed animals, with a dose-related increase in severity (Table B.7.1). According to the report, vacuolation was confined to the cortical tubules in grades 1 and 2 and extended to the medulla in grades 3 and 4. It was characterized by an increased number and size of cytoplasmic vacuoles. There was no evidence of necrosis in treated animals. Ultrastructurally, vacuolated epithelial cells were observed in proximal tubules and Henle's loop and were characterized by variably-sized membrane-bound vacuoles interpreted to be of lysosomal origin. Their content was finely granular with some electron-dense membranous profiles. Large vacuoles compressed the nucleus, which was displaced peripherally. In Henle's loop, only small-sized vacuoles were present. The distal tubules were normal. After 2- and 5-month recovery periods, renal tubular vacuolation was still present in most MD and HD animals, although the severity appeared somewhat decreased.

Minimal vacuolation of transitional epithelium in the renal pelvis was present at all doses and was still present at the MD and HD after 2 and 5 months.

At the end of the treatment period, minimal to moderate vacuolation of the transitional epithelium of the urinary bladder was observed in all treated groups. It was mainly located within the superficial layers of the epithelium (umbrella cells) and was seen multifocally along the mucosa. This was still present in the recovery groups with a similar incidence and severity to that of the HD at the end of treatment.

Vacuolation of hepatocytes consisted of relatively large, predominantly clear vacuoles. Grade 1 represented a few scattered vacuoles throughout the sections, while in grade 3 the distribution tended to involve nearly all of the mid-zonal areas, grade 2 fell in between these two. Ultrastructurally, variably-sized membrane-bound vacuoles of lysosomal origin containing finely granular material were seen in hepatocytes; large ones filled most of the cytoplasm displacing the nucleus peripherally. The presence of numerous slightly dilated lysosomal profiles was also noted In Kupffer cells and sinusoidal cells. In addition to the granular content, there were some lamellar bodies in these lysosomes. The severity of liver vacuolation was reduced 5 months after cessation of treatment.

At the end of treatment, all MD and HD animals and 1/6 LD animals had numerous enlarged (hypertrophic), vacuolated (foamy) macrophages in the liver and lymph nodes, which were decreased 2 and 5 months after cessation of dosing.

Table B.7.1.

Incidence (I) and average severity grades (S) of treatment-related changes

		Doses (mg/kg)							
		Control	300	750			1500		
Time in study		ALL	EOT[1]	EOT	2 mo[2]	5 mo[2]	EOT	2 mo	5 mo
Vacuolation									
Renal tubular epithelium	I	0/10	6/6	6/6	2/2	0/2	6/6	2/2	2/2
	S	0	1.2	1.3	1.0	0.0	2.8	1.5	1.0
Renal pelvic epithelium	I	0/10	2/6	5/6	2/2	1/2	5/6	2/2	1/2
	S	0	0.3	0.8	1.0	0.5	0.8	1.0	0.5
Urinary bladder epithelium	I	0/10	4/6	6/6	2/2	2/2	6/6	2/2	2/2
	S	0	0.6	1.4	2.0	2.0	1.7	2.0	2.0
Hepatocytes	I	0/10	3/6	6/6	1/2	1/2	6/6	2/2	2/2
	S	0	0.5	1.5	0.5	0.5	1.8	2.0	1.0
Foamy macrophages									
Liver	I	0/10	1/6	6/6	1/2	1/2	6/6	2/2	2/2
	S	0	0.15	1.0	0.5	0.5	1.5	1.0	1.0
Cervical lymph node	I	0/10	2/6	5/6	2/2	0/2	6/6	1/2	1/2
	S	0	0.7	1.5	1.5	0.0	2.2	1.0	0.5
Mesenteric lymph node	I	0/10	2/6	5/6	2/2	1/2	5/6	1/2	1/2
	S	0	0.3	0.8	1.0	0.5	1.5	0.5	0.5

[1] End of treatment
[2] 2-month or 5-month recovery period

Conclusions

Daily bolus iv administration of CAP to dogs for 1 month at doses up to 1500 mg/kg/day (rate not provided) resulted in vacuolar changes in the kidney, urinary bladder, and liver and foamy macrophages in multiple tissues at all doses. The histopathology changes were only partially reversible after cessation of treatment at the MD and HD. No functional abnormalities in these organs were reported. There was no evidence of irritation at the injection sites.

8. 28-Day Continuous/Intermittent Intravenous Infusion Toxicity Study in Dogs with a 4-Week Recovery Period (7802-114, conducted by [redacted](b) (4), report dated 3/13/09, GLP)

Methods

Vehicle (SBECD [lot # NC-04A-05023] in sterile water for injection) or drug in SBECD (Captisol [CAP]; 10 mg/mL Drug:250 mg/mL CAP) were administered to beagle dogs intravenously via a jugular vein catheter for 28 days using a programmable pump, according to the following protocol:

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/day) [redacted]	CAP	Dose Concentration (mg/mL) [redacted]	CAP	Dose Volume[c] (mL/kg/day)	Dose Time[d] (hours/day)
1 (Control)	5	5	0	2500	0	250	10	24
2 (Low)	3	3	25	625	10	250	2.5	3
3 (Mid)	3	3	56	1400	10	250	5.6	6.7
4 (High)	5	5	100	2500	10	250	10	24

a Group 1 received the control article at a constant dose rate of 0.42 mL/kg/hour as a continuous infusion.
b Animals designated for recovery sacrifice (the last 2 animals/sex in Groups 1 and 4) underwent at least 4 weeks of recovery following dose administration.
c The dose volume and dose time (24, 3, 6.7 and 24 hours/day, respectively) were varied to provide the test article at a constant concentration.
d The dose rate for Groups 1 and 4 was 0.42 mL/kg/hour. For Groups 2 and 3 the dose rate was 0.84 mL/kg/hour.

Assessments consisted of mortality, clinical signs, ophthalmologic and electrocardiographic examinations, BW and food consumption data, and clinical and anatomic pathology. Blood was collected from all dogs on days 1, 8, 15, 22, and 28/29 for plasma drug level determination and TK evaluations. All tissues from all animals were examined microscopically.

According to the report, the study was designed to give maximum exposure to the Drug/CAP(1:25) combination used in the clinical formulation.

Results

Mortality:

There were no T-R deaths during the study. A single HD (100/2500 mg/kg/day) male (H47459) was euthanized on day 29 due to weight loss, inappetence, and clinical signs of hypoactivity, nonformed feces, labored respiration, pale gums, and appearance of dehydration, but this dog's condition was attributed to mechanical catheter-related injury based on microscopic findings of localized inflammation.

Clinical signs and BW:

There were no other TR clinical observations noted during the dosing and recovery phases other than those associated with infusion procedures. No visible lesions were noted at ophthalomologic examination. There were no TR differences in electrocardiographic parameters and no body weight (BW) differences related to CAP dose.

Clinical pathology:

No TR effects on clinical pathology parameters were apparent. High neutrophil counts were noted in several C and treated dogs were attributed to inflammation at the catheter site.

Gross and microscopic pathology:

There were no changes in body or organ weight that were considered TR. Small, but SS increases in relative liver (27% above C) and kidney weights (11%) in HD females were considered in the report to be incidental in the absence of a histological correlate. There were no differences after the recovery period.

No TR macroscopic or microscopic observations were noted. According to the pathology report, infusion of this formulation showed no evidence of venous irritation. Renal tubule

vacuolization, which was present in both C and drug-treated groups with an incidence ranging from 67 to 100%, was attributed to administration of CAP (Table B.8.1). This finding was most pronounced in the C and HD animals, which received the highest dose of CAP. In the LD group, with the lowest dose of CAP, only one male had evidence of renal tubular vacuolization. Renal tubule vacuolization was absent in both C and HD recovery females but was still present in 1/2 C and 2/2 HD recovery males (Table B.8.2). Infiltrate of vacuolated macrophages in the mandibular lymph node was also noted in C and HD males and in females in all groups and was still present at a lower incidence after the recovery period. There was no evidence of vacuolation of the transitional epithelium of the urinary bladder at any dose.

Table B.8.1 Histopathology findings - terminal sacrifice

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males				-- Animals Affected -- Females			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
Tissues With Diagnoses	No. in group:	3	3	3	2	3	3	3	3
Bone, Femur	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Marrow, Femur	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	2
Hypocellular		0	0	0	0	0	0	0	1
Hyperplasia, Myeloid		0	0	0	0	0	0	0	0
Bone, Sternum	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Marrow, Sternum	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Hyperplasia, Myeloid		0	0	0	0	0	0	0	0
Spinal Cord	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Nerve, Sciatic	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Pituitary	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	1	2	1	1	3	2	2	2
Cyst		2	1	2	1	0	1	1	1
Adrenal, Cortex	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	2	3
Cyst		0	0	0	0	0	0	1	0
Adrenal, Medulla	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Aorta	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Trachea	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	3	3	2	3	3	3	3
Thyroid	Number examined:	3	3	3	2	3	3	3	3
	Unremarkable:	3	2	3	2	3	3	3	3
Cyst, Ultimobranchial		0	1	0	0	0	0	0	0
Thymus, Ectopic		0	1	0	0	0	0	0	0

Thyroid ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	2	3	2	|	3	3	3	3
Hyperplasia, C-cell	0	0	0	0	|	0	0	0	0
Parathyroid ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	2
Cyst	0	0	0	0	|	0	0	0	1
Lung ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	2	2	3	1	|	2	1	1	3
Inflammation, Chronic-Active	1	0	0	1	|	1	1	2	0
Inflammation, Acute	0	1	0	0	|	0	1	0	0
Emphysema	0	0	0	0	|	0	0	0	0
Fibrosis, Pleural/Subpleural	0	0	0	0	|	0	0	0	0
Hemorrhage	0	0	0	0	|	0	0	0	0
Thrombus	0	0	0	0	|	0	1	0	0
Liver ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	2	1	0	2	|	3	3	0	3
Inflammation, Acute	1	0	0	0	|	0	0	0	0
Vacuolation, Hepatocyte, Centrilobular	0	2	3	0	|	0	0	1	0
Infiltrate, Lymphocytes/Macrophages	0	0	0	0	|	0	0	1	0
Vacuolation, Hepatocyte, Periportal	0	0	0	0	|	0	0	1	0
Gallbladder ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Vacuoles, Epithelium	0	0	0	0	|	0	0	0	0
Kidney ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	0	2	0	0	|	1	3	1	0
Pyelonephritis	0	0	0	0	|	0	0	0	0
Vacuolation, Tubule Cell	3	1	3	2	|	2	0	2	3
Urinary Bladder ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Esophagus ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Uterus ..... Number examined:					|	3	3	3	3
Unremarkable:					|	3	3	3	3
Vagina ..... Number examined:					|	3	3	3	3
Unremarkable:					|	3	3	3	3
Cervix ..... Number examined:					|	3	3	3	3
Unremarkable:					|	3	3	3	3
Eye ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	1	3	3	2	|	3	3	3	3
Cyst, Retina	2	0	0	0	|	0	0	0	0
Nerve, Optic ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Gl, Lacrimal ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Heart ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Brain ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	3	3	3	2	|	3	3	3	3
Infiltrate, Lymphocytes	0	0	0	0	|	0	0	0	0
Death Comment ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	0	0	0	0	|	0	0	0	0
Scheduled Sacrifice	3	3	3	2	|	3	3	3	3
Moribund Sacrifice	0	0	0	0	|	0	0	0	0
Infusion Site ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	0	0	0	1	|	1	0	0	0
Hyperplasia, intima	3	3	3	1	|	2	3	3	2
Inflammation, Chronic-Active	0	1	1	0	|	0	0	0	0
thrombus	0	0	0	0	|	0	1	2	2
Catheter Site ..... Number examined:	3	3	3	2	|	3	3	3	3
Unremarkable:	0	0	0	1	|	0	0	0	0
Inflammation, Chronic-Active	2	1	0	0	|	0	2	1	0
Fibrosis	3	3	3	1	|	3	3	3	3

Table B.8.2 Microscopic observations – Recovery

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --				-- Animals Affected -- Females --			
Tissues With Diagnoses	Dosage group: / No. in group:	Ctls 2	2 0	3 0	4 2	Ctls 2	2 0	3 0	4 2
Bone, Femur	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Marrow, Femur	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Hypocellular		0	0	0	0	0	0	0	0
Hyperplasia, Myeloid		0	0	0	0	0	0	0	0
Bone, Sternum	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Marrow, Sternum	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Hyperplasia, Myeloid		0	0	0	0	0	0	0	0
Spinal Cord	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Nerve, Sciatic	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Pituitary	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Cyst		0	0	0	0	0	0	0	0
Adrenal, Cortex	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Cyst		0	0	0	0	0	0	0	0
Adrenal, Medulla	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Aorta	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Trachea	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Thyroid	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	1	0	0	2	2	0	0	2
Cyst, Ultimobranchial		0	0	0	0	0	0	0	0
Thymus, Ectopic		0	0	0	0	0	0	0	0
Thyroid	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	1	0	0	2	2	0	0	2
Hyperplasia, C-cell		1	0	0	0	0	0	0	0
Parathyroid	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Cyst		0	0	0	0	0	0	0	0
Lung	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	1	1	0	0	0
Inflammation, Chronic-Active		0	0	0	1	1	0	0	1
Inflammation, Acute		0	0	0	0	0	0	0	0
Emphysema		0	0	0	0	1	0	0	0
Fibrosis, Pleural/Subpleural		0	0	0	0	1	0	0	1
Hemorrhage		0	0	0	0	0	0	0	0
Thrombus		0	0	0	0	0	0	0	0
Liver	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Inflammation, Acute		0	0	0	0	0	0	0	0
Vacuolation, Hepatocyte, Centrilobular		0	0	0	0	0	0	0	0
Infiltrate, Lymphocytes/Macrophages		0	0	0	0	0	0	0	0
Vacuolation, Hepatocyte, Periportal		0	0	0	0	0	0	0	0
Gallbladder	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Vacuoles, Epithelium		0	0	0	0	0	0	0	0
Kidney	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	1	0	0	0	2	0	0	2
Pyelonephritis		0	0	0	0	0	0	0	0
Vacuolation, Tubule Cell		1	0	0	2	0	0	0	0
Urinary Bladder	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2
Esophagus	Number examined:	2	0	0	2	2	0	0	2
	Unremarkable:	2	0	0	2	2	0	0	2

Uterus ..... Number examined:						2	0	0	2
Unremarkable:						2	0	0	2
Vagina ..... Number examined:						2	0	0	2
Unremarkable:						2	0	0	2
Cervix ..... Number examined:						2	0	0	2
Unremarkable:						2	0	0	2
Eye ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	2	0	0	2		2	0	0	2
Cyst, Retina	0	0	0	0		0	0	0	0
Nerve, Optic ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	2	0	0	2		2	0	0	2
Gl, Lacrimal ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	2	0	0	2		2	0	0	2
Heart ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	2	0	0	2		2	0	0	2
Brain ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	2	0	0	2		2	0	0	1
Infiltrate, Lymphocytes	0	0	0	0		0	0	0	1
Death Comment ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	0	0	0	0		0	0	0	0
Scheduled Sacrifice	2	0	0	2		2	0	0	2
Moribund Sacrifice	0	0	0	0		0	0	0	0
Infusion Site ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	0	0	0	2		1	0	0	0
Hyperplasia, intima	2	0	0	0		1	0	0	2
Inflammation, Chronic-Active	0	0	0	0		0	0	0	0
thrombus	0	0	0	0		0	0	0	0
Catheter Site ..... Number examined:	2	0	0	2		2	0	0	2
Unremarkable:	0	0	0	0		0	0	0	0
Inflammation, Chronic-Active	0	0	0	0		0	0	0	0
Fibrosis	2	0	0	2		2	0	0	2

Conclusions

Continuous (HD) or intermittent (LD and MD) iv infusion of Drug/CAP (CAP doses up to 2500 mg/kg/day and rates up to 210 mg/kg/hr) to dogs for 28 days did not result in any apparent drug-related toxicity and produced no evidence of venous irritation. Renal tubular vacuolization and infiltration of vacuolated macrophages in the mandibular lymph node seen in all groups was attributed to the CAP vehicle. These CAP-induced histopathological findings showed only partial reversal after a 4-week recovery period.

9. CP-217,861-2 - 6-month intravenous toxicity study in Sprague-Dawley rats (Study No. 94-28-21, conducted by [redacted] (b) (4), report dated 1/8/96, GLP)

Methods

CAP (0 (saline vehicle), 200, 320, or 600 mg/kg) was administered intravenously (1.82 mL/kg injected at a rate of 3 mL/min; concentrations of 110, 178, or 330 mg/mL) to S-D rats (20/sex/group) once daily for 6 months. Observations included mortality, clinical signs, BW, ophthalmology, clinical pathology, and gross and microscopic pathology (all animals).

Results

There were no deaths or clinical signs and no T-R effects on BW or ophthalmology exams.

After 6 months, there were slight (5-6% compared to C) but SS decreases in RBC parameters. T-R increases (SS) in ALT (up to 70% at month 6 in HD males) and AST were seen in both sexes, and there was a dose-related decrease in urinary pH throughout the treatment period in all treatment groups compared to C.

Dose-dependent increases in absolute and relative kidney weights were found in males and females (relative weights 6, 10 and 22% in males and 8, 15 and 29% in females at LD, MD, and HD, respectively; SS increases in absolute and relative spleen weights were observed in MD (absolute, 10%; relative, 13%) and HD females (17% for both absolute and relative). A slight increase in relative liver weight was noted in HD females (7%).

Bilateral discoloration of the kidneys was observed in 18, 27 and 39 rats at the LD, MD, and HD, respectively. There was a dose-related increase in the incidence and severity of the vacuolation of the renal tubular epithelial cells in all treated groups (Table B.9.1). Renal tubular vacuolation was characterized by an increased number and size of cytoplasmic vacuoles within the epithelial cells. Small to medium-sized vacuoles were observed in all grades. In addition, large vacuoles, occasionally increasing the usual size of cells, were observed at grades 3 and 4. Renal tubular vacuolation was limited to the renal cortex· (grades 1 and 2) or extended to the medulla (grades 3 and 4). These vacuoles sometimes contained a small amount of granular or punctiform, eosinophilic, and PAS-positive material, but no acicular pseudocrystals. Tubular degeneration or necrosis were not observed.

Table B.9.1.

Distribution and severity of renal tubular vacuolation

	Control		200 mg/kg		320 mg/kg		600 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1			2	1				
Grade 2			16	16	1	6	2	
Grade 3			2	2	19	14	17	14
Grade 4							1	6
Total	0/20	0/20	20/20	19/20	20/20	20/20	20/20	20/20

Grade 1: Less than 25% of cortical tubules affected.
Grade 2: More than 25% but less than 50% of cortical tubules affected.
Grade 3: More than 50% but less than 75% of cortical tubules affected.
Grade 4: More than 75% of cortical tubules affected.

Slight vacuolation of transitional epithelium of the renal pelvis was observed in most treated rats (Table B.9.2).

Table B.9.2

Distribution of transitional epithelium vacuolation of the renal pelvis

Control		200 mg/kg		320 mg/kg		600 mg/kg	
M	F	M	F	M	F	M	F
		16/20	19/20	20/20	20/20	20/20	18/20

Pulmonary foam cell foci (alveolar histiocytosis, grade 1) was increased in incidence in males at all doses and in HD females (Table B.9.3).

Table B.9.3.

Distribution of pulmonary foam cell foci

Control		200 mg/kg		320 mg/kg		600 mg/kg	
M	F	M	F	M	F	M	F
2/20	3/20	9/20	4/20	6/20	4/20	17/20	11/20

Hypertrophic macrophages were observed with a dose-related incidence in all treated males and in HD females (Table B.9.4). Changes were characterized as scattered hypertrophic macrophages mostly in portal areas. Material in the macrophages was PAS positive.

Table B.9.4.

Distribution of hypertrophic macrophages

Control		200 mg/kg		320 mg/kg		600 mg/kg	
M	F	M	F	M	F	M	F
0/20	0/20	3/20	0/20	6/20	0/20	18/20	3/20

Cytoplasmic vacuolation of urinary bladder epithelium was observed in all treated groups (Table B.9.5).

Table B.9.5.

Distribution of vacuolation of urinary bladder epithelium

Control		200 mg/kg		320 mg/kg		600 mg/kg	
M	F	M	F	M	F	M	F
0/20	0/20	18/20	20/20	18/20	19/20	19/20	17/20

Conclusions

Daily iv bolus administration of CAP at doses up to 600 mg/kg/day and rates up to 990 mg/min to rats for 6 months produced tubular vacuolation in the kidney and pulmonary foam cell foci as well as hypertrophic macrophages in the liver and epithelial vacuolation in the urinary bladder and renal pelvis. There were no signs of irritation at the injection sites. Since the renal histopathology findings were not associated with any evidence of functional changes, they were considered to be of no toxicological importance in the study report.

10. CP-217,861-2 - 6-month intravenous toxicity study in beagle dogs (Study No. 94-28-22, conducted by [redacted](b) (4), report dated 1/8/96, GLP)

Methods

CAP (0 (saline vehicle), 150, 300, or 600 mg/kg) was administered intravenously (1.82 mL/kg injected at a rate of 5 mL/min; concentrations of 82.5, 165, or 330 mg/mL) to beagle dogs (4/sex/group) once daily for 6 months. Observations included mortality, clinical signs, BW, ophthalmology, ECG, clinical pathology, and gross and microscopic pathology (all animals).

Results

Mortality, clinical signs, BW, ophthalmology, ECG, clinical pathology.

There were no deaths or clinical signs and no T-R effects on BW, ophthalmology exams, ECG, or clinical pathology, with the exception of a 3-4-fold increase in ALT in 2/8 HD dogs (females F3 l and F32) at 6 months.

Gross and microscopic pathology

There were no organ weight changes related to treatment. Bilateral pale discoloration of the kidneys was observed in 4/4 males and 2/4 females at the HD.

There were dose-related increases in the severity of the vacuolation of the renal tubular epithelium in treated groups (Table B.10.1). According to the report, “the changes occurred mainly in the proximal tubule and were characterized by unstained cytoplasmic microvacuoles, although in some dogs with grade 3 findings, the affected cells had macrovacuoles which caused cell enlargement.” No necrosis or other degenerative changes were reported.

Table B.10.1.

Distribution and severity of renal tubular vacuolation

	Control		150 mg/kg		300 mg/kg		600 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1			4	4	1	3	1	1
Grade 2					3	1	2	1
Grade 3							1	2
Total	0/4	0/4	4/4	4/4	4/4	4/4	4/4	4/4

Grade 1 : Less than 25% of cortical tubules affected.
Grade 2 : More than 25% but less than 50% of cortical tubules affected.
Grade 3 : More than 50% but less than 75% of cortical tubules affected.

Vacuolation of transitional epithelium of the renal pelvis was observed in most treated animals (Table B.10.2).

Table B.10.2

Distribution and severity of transitional epithelium vacuolation of the renal pelvis

	Control		150 mg/kg		300 mg/kg		600 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1			3	4	3	4	2	4
Grade 2							2	
Total	0/4	0/4	3/4	4/4	3/4	4/4	4/4	4/4

Grade 1 : Changes characterized by cytoplasmic microvacuolation.
Grade 2 : Changes characterized by cytoplasmic macrovacuolation causing cell enlargement.

Cytoplasmic vacuolation of urinary bladder epithelium was observed in most treated dogs. (Table B.10.3).

Table B.10.3.

Distribution and severity of vacuolation of urinary bladder epithelium

	Control		150 mg/kg		300 mg/kg		600 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1			3	3	3	3	2	3
Grade 2							1	1
Total	0/4	0/4	3/4	3/4	3/4	3/4	3/4	4/4

Grade 1 : Changes characterized by cytoplasmic microvacuolation.
Grade 2 : Changes characterized by cytoplasmic macrovacuolation causing cell enlargement.

In the liver, slight vacuolation of hepatocytes occurred in 2/8 HD dogs. Hypertrophic macrophages were observed at the HD, mainly in the portal area of the liver (Table B.10.4). As described in the report, “their cytoplasm was foamy in appearance and often contained an accumulation of pigment and amorphous material, some of which was PAS positive.”

Table B.10.4.

Distribution of hypertrophic macrophages

	Control		150 mg/kg		300 mg/kg		600 mg/kg	
	M	F	M	F	M	F	M	F
Grade 1			1/4	1/4			3/4	2/4

Grade 1 : Changes characterized by scattered hypertrophic macrophages mostly in portal area.

Conclusions

Daily iv bolus administration of CAP doses up to 600 mg/kg/day and rates up to 1650 mg/min to dogs for 6 months produced an increase in ALT (HD); epithelial vacuolation of the renal tubules, renal pelvis, and urinary bladder (all doses); and vacuolation of hepatocytes and hypertrophic macrophages in the liver (HD). There were no signs of irritation at the injection sites. Because these findings were not associated with any evidence of functional changes, other than the ALT increase at the HD, they were considered (in the study report) to have no toxicological consequences.

C. Developmental toxicity studies of Captisol (from DMF #14364)

1. CP-217,861-02 Reproductive Study 1: Fertility and early embryonic development in Sprague-Dawley rats (Study Number: 96-28-31, conducted by (b) (4) (b) (4), report dated 8/26/97, GLP)

Methods

S-D rats (20/sex/group) were administered CAP (0 (saline vehicle), 100, 400, or 1500 mg/kg/day) by iv injection (rate not given) for 14 days prior to mating and were continuously treated during the mating period and through GD 7. All sperm-positive females were sacrificed on gestation day 14 for examination of reproductive and fetal parameters. All males were sacrificed and necropsied beginning on study day 64. Preparations of caudal epididymal sperm were examined for assessment of sperm count and motility. Histopathological examination was performed on the testes of all males and the prostate and seminal vesicles of the control and high dose males.

Results

No deaths or CAP-related clinical signs were noted in any group. BW gain was decreased (SS) in HD females during early gestation (GD 0-8) and food consumption was decreased (SS) in HD males and females. Fertility, reproductive parameters, sperm counts, and sperm motility were not affected by treatment. Scattered foamy interstitial macrophages were observed in the testes of 18/20, 20/20 and 20/20 males in the LD, MD, and HD groups, respectively.

Conclusions

Administration of CAP by iv infusion at doses up to 1500 mg/kg to male and female rats prior to and during mating through GD 7 in females produced no evidence of CAP-related reproductive toxicity.

2. CP-217,861-02 Teratology study (Reproductive Study III) in Sprague-Dawley rats (Study Number: 95-28-52, conducted by (b) (4) report dated 2/25/97, GLP)

Methods

CAP ((0 (saline vehicle), 100, 600, or 3000 mg/kg/day) was administered intravenously (rate not given) to pregnant rats (20/group) throughout organogenesis (GDs 6-17). All dams were sacrificed on day 21 of gestation for examination of reproductive and fetal parameters. Half of the fetuses of each litter were examined for visceral anomalies and the remaining fetuses for skeletal anomalies and degree of ossification.

Results

No deaths or drug-related clinical signs were noted in any of the treated dams. Maternal body weight gain and food consumption were decreased (SS) at the HD. There were no T-R effects on reproductive or developmental parameters.

Conclusions

Administration of CAP by iv infusion at doses up to 3000 mg/kg to pregnant rats throughout organogenesis produced no adverse effects on embryofetal development.

3. A continuous infusion embryo-fetal development study in the rat (Study no. 901954, conducted by [redacted] (b) (4), report dated 3/9/10, GLP)

Methods

Pregnant S-D rats were administered saline, vehicle (CAP [lot # NC-04A-08048] in sterile water for injection) or the drug in CAP (10 mg/mL Drug:250 mg/mL CAP) by iv infusion (0.375 mL/kg/hr) on days GD 6 to 17 according to the following protocol:

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Group Number Identification	[redacted] Dose Level (mg/kg/day)	Captisol Dose Level (mg/kg/day)	Dose Rate (mL/kg/hr)	Main Study Number of Females
1/ Saline Control	0	0	0.375	22
2/ Captisol	0	2250	0.375	22
3/ High [redacted]	90	2250	0.375	22
4/ Mid [redacted]	45	1125	0.375*	22
5/ Low [redacted]	22.5	562.5	0.375**	22

* Animals were infused for 12 hours daily.

** Animals were infused for 6 hours daily.

Detailed examinations of dams and measurement of BW and food consumption were performed and blood was sampled for TK on GD 13 (2 hours following the daily syringe change for groups 1, 2 and 3, and 15 minutes prior to the end of infusion for groups 4 and 5). On GD 21, a gross pathological examination was performed, the gravid uterus was weighed, the uterine contents were examined, and the number and position of live and dead fetuses and resorptions were recorded. Each fetus was weighed, given a detailed external examination and the sex recorded. An internal examination was performed on approximately one-half the fetuses in each litter using a dissecting microscope. The heads of these fetuses were removed and placed in Bouin's fluid for examination by the Wilson technique. A skeletal examination was performed on the remaining one-half of the fetuses in each litter after they were eviscerated and stained with alizarin red S.

According to the report, doses were based on existing rat oral embryofetal development studies of the drug, TK data comparing the oral and iv routes, the rat iv infusion toxicity study, and the rate limits for iv infusion.

Results:

Maternal toxicity:
Excessive toxicity was noted following less than 5 hours of administration for 5 animals at 360 mg/kg (original HD group 5) and after 1 day of administration for 5 animals at 180 mg/kg (original MD group 4). Clinical signs at these doses included decreased activity, labored

breathing, increased respiratory rate, coldness to the touch, lying on side, and decreased muscle tone. At 360 mg/kg the animals were further described as unconscious. These animals were euthanized due to poor condition and replaced to obtain the full group size at adjusted doses of 45 and 22.5 mg/kg/day for Groups 4 and 5, respectively. There were no TR clinical signs at 22.5, 45, or 90 mg/kg/day.

There was no TR effect on BW, corrected BW (BW on GD 21 minus the gravid uterus weight), or corrected BW gain. BW and BW gain, uncorrected and corrected, were comparable between saline and CAP controls.

Uterine findings:

Uterine parameters (live fetuses and resorptions; placenta and gravid uterus weights) were comparable between the saline and CAP control groups (Table C.3.1).

Table C.3.1 Group Mean Uterine Findings

Group 1 - Saline Control
Group 2 - Captisol
Group 3 - [redacted] 90 mg/kg/day
Group 4 - [redacted] 45 mg/kg/day
Group 5 - 22.5 mg/kg/day

Group	Animal Number	Live Fetuses	Dead Fetuses	Early Resorptions	Middle Resorptions	Late Resorptions
1	Mean	13.2	0.0	0.6	0.0	0.05
	SD	1.3	0.0	0.7	0.0	0.21
	N	22	22	22	22	22
2	Mean	13.0	0.0	0.6	0.0	0.0
	SD	2.1	0.0	0.7	0.0	0.0
	N	22	22	22	22	22
3	Mean	12.4	0.0	0.7	0.05	0.0
	SD	2.6	0.0	1.0	0.21	0.0
	N	22	22	22	22	22
4	Mean	13.9	0.0	0.9	0.0	0.0
	SD	3.0	0.0	1.7	0.0	0.0
	N	22	22	22	22	22
5	Mean	12.9	0.0	0.6	0.0	0.0
	SD	1.5	0.0	0.7	0.0	0.0
	N	22	22	22	22	22

Significantly different from control group (group 2) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ ([redacted] (b) (4))

Group	Animal Number	Sum of Resorptions	Preimplantation Loss (%)	Post implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	0.6	9.91	4.50	108.1
	SD	0.7	6.74	5.06	10.1
	N	22	22	22	22
2	Mean	0.6	13.45	4.50	107.0
	SD	0.7	11.13	4.99	17.7
	N	22	22	22	22
3	Mean	0.8	13.27	6.02	98.8
	SD	1.0	12.13	8.66	18.5
	N	22	22	22	22
4	Mean	0.9	11.54	5.76	108.3
	SD	1.7	9.95	11.56	21.6
	N	22	22	22	22
5	Mean	0.6	12.15	4.60	104.1
	SD	0.7	9.96	5.25	12.9
	N	22	22	22	22

Significantly different from control group (group 2) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (b) (4)

Fetal findings:

There were no CAP-related effects on fetal weights or malformation incidences (Table C.3.2). Malformations consisted of conjoined twins (Dam No. 1515/fetus 10) in 1 saline control litter; omphalocele (Dam No. 2507/fetus 6) and anophthalmia, ectrodactyly, abnormal flexure of the hindlimbs, absent fibula, and severe dilatation of the lateral ventricles of the brain (Dam No. 2512/fetus 15) in the CAP control group; and situs inversus (Dam No. 5509/fetus 1) and anasarca, gastroschisis, and opened eye (Dam No. 5519/fetus 1) in the LD group.

Table C.3.2 Group Incidence of Fetal External, Visceral and Skeletal Findings - Major Malformations and Minor Anomalies

Group 1 - Saline Control
Group 2 - Captisol
Group 3 - [redacted] 90 mg/kg/day
Group 4 - [redacted] 45 mg/kg/day
Group 5 - [redacted] 22.5 mg/kg/day

	Group 1		2		3		4		5	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	22	290	22	287	22	273	22	306	22	283
Visceral (VIS)	22	147	22	143	22	138	22	154	22	141
Skeletal (SKE)	22	144	22	145	22	135	22	152	22	142
Technique of Wilson (WT)	22	146	22	143	22	138	22	154	22	141
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Major Malformations (Total)	1	1	2	2	0	0	0	0	2	2
Minor External and Visceral Anomalies (Total)	2	2	1	1	1	4	1	1	3	3
Minor Skeletal Anomalies (Total)	18	49***	21	84	21	61*	22	70*	17	48***

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 2) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Conclusions

Administration of Drug/CAP(1:25) by iv infusion (1.56 mg/kg/min) to pregnant rats during organogenesis produced no evidence of CAP-related developmental toxicity at doses of up to 2250 mg/kg/day.

4. CP-217,861-02 Reproductive Study II: Prenatal and postnatal development in Sprague-Dawley rats intravenous route (Study Number: 96-28-61, conducted by (b) (4) (b) (4), report dated 7/17/97, GLP)

Methods

CAP (0 (saline vehicle), 100, 600, or 3000 mg/kg/day) was administered intravenously (rate not given) to female S-D rats (23/grp) from implantation through weaning (GD6 - PND21). Dams were observed for mortality, clinical signs, BW, and food consumption and were allowed to litter and to raise their offspring until lactation day 21. Postnatal development indices and reflex behaviors; FOB, motor activity, and learning and memory (Cincinnati maze and passive avoidance); and reproductive performance were evaluated in the offspring.

Results

Maternal body weight gain was decreased during gestation and the first week of lactation at the HD. Food consumption was also reduced during gestation in this group.

The number of stillbirths was significantly increased in the HD group compared to C (Table C.4.1). Three litters contained more than 5 stillbirths in this group (#646; 6 stillbirths, #663; 8 stillbirths, #665; 6 stillbirths). The number of litters that had at least one stillbirth was also increased (2, 4, 5 and 11 litters for C, LD, MD, and HD groups, respectively). The mean number of pups was decreased at birth (NS) and at PND 4 (SS) in HD litters.

Table C.4.1

REPRODUCTIVE DATA OF LITTERING F0 RATS AND F1 PUP VIABILITY

			Control	100 mg/kg	600 mg/kg	3000 mg/kg
No. of pregnant females			20	21	21 [a)]	22
No. of females with viable litters			20	21	20	21
Length of gestation, days		Mean ± S.D.	21.4 ± 0.5	21.4 ± 0.6	21.5 ± 0.6	21.7 ± 0.7
No. of implantation sites		Mean ± S.D.	15.1 ± 3.4	14.4 ± 3.4	14.1 ± 4.7	14.1 ± 4.6
No. of resorptions		Mean ± S.D.	1.7 ± 1.7	1.1 ± 1.5	1.4 ± 1.5	1.2 ± 1.5
No. of stillbirths		Mean ± S.D.	0.1 ± 0.3	0.3 ± 0.7	0.4 ± 0.9	1.4 ± 2.3 *
No. of F1 pups						
At birth	No. of litters		20	21	21	22
	Total	Mean ± S.D.	13.3 ± 3.0	13.0 ± 3.2	12.3 ± 4.4	11.5 ± 4.8
	Male	Mean ± S.D.	6.2 ± 2.6	6.3 ± 2.1	5.8 ± 2.6	6.1 ± 3.2
	Female	Mean ± S.D.	7.1 ± 3.1	6.7 ± 2.8	6.5 ± 2.4	5.4 ± 2.9
Day 4	No. of litters		20	21	20	21
	Total	Mean ± S.D.	13.1 ± 2.8	11.9 ± 3.9	11.1 ± 4.4	9.7 ± 5.0 **
Day 21	No. of litters		17	19	15	16
	Total	Mean ± S.D.	8.0 ± 0.0	7.9 ± 0.3	7.9 ± 0.4	7.9 ± 0.3
Day 56	No. of litters		17	19	15	16
	Total	Mean ± S.D.	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
No. of F1 pups with						
External malformations			0	0	0	0
Visceral malformations			1 [b)]	1 [b)]	3 (2 [b)], 1 [c)])	1 [b)]

* P < 0.05, ** P < 0.01

a) Data from one dam(# 633) sacrificed on GD 26 because of no sign of parturition, was excluded only for calculations of mean length of gestation.

b) Diaphragmatic hernia

c) Hydronephrosis and hydroureter

BWs were decreased in HD pups beginning on postnatal day 1 and continuing throughout the lactation period. Postweaning BWs were comparable across groups. There were no CAP-related effects on pre- and postweaning developmental indices (including sexual maturation), the FOB, motor activity, sensory function, passive avoidance and Cincinnati water maze tests of learning and memory, or reproductive performance.

Conclusions

Administration of CAP by iv infusion at doses up to 3000 mg/kg to female rats throughout pregnancy and lactation produced adverse developmental effects (increased mortality and decreased BW in offspring) at the HD, which was also maternally toxic. The developmental NOAEL was the MD (600 mg/kg/day) based on the increased number of stillbirths, decreased number of pups on PND 4, and lower body weight during the pre-weaning period in the HD group.

III. SUMMARY AND EVALUATION

To support the safety of a 505(b)(2) application for a new formulation of fosphenytoin to be used on a short-term basis in epilepsy, the sponsor conducted a local toxicity study in rats and **provided an LoA from Cy-Dex to the MF for Captisol (sulfobutyl ether beta-cyclodextrin; CAP)**, the primary excipient used in the new formulation. Based on the results of the intramuscular local toxicity study in the rat using the clinical formulation, there was no evidence of excipient-related local toxicity. The toxicity of intravenous (iv) bolus and continuous infusion of CAP has been characterized in rat, dog, and monkey studies conducted by the Captisol manufacturer (Cy-Dex).

Rat studies of CAP at daily iv bolus doses of up to 1500 mg/kg (injected over 15 sec) for 14 days, 3000 mg/kg (injected over 60-70 seconds) for 1 month, and 600 mg/kg (990 mg/min) for 6 months and continuous infusion doses of up 2500 mg/kg/day (105 mg/kg/hr) for 1 month showed vacuolar changes in the kidney, urinary bladder, and liver and foamy macrophages in multiple tissues at bolus doses as low as 320 mg/k/day and for durations as short as 14 days. The LD (160 mg/kg/day, 640 mg/kg/min) was considered the NOEL in the 14-day rat study. Evidence of hemolysis and other clinical pathology changes (elevated ALT, ALP, urea, creatinine, phosphates) that correlated with the kidney and liver histopathology were seen at the highest dose (3000 mg/kg/day) in the 1 month iv bolus study. The renal histopathology findings were not associated with any evidence of functional changes in the other rat studies, but decreases in RBC parameters and transaminase elevations were seen in the 6-month rat study. There was no evidence of renal cell necrosis or degeneration in the iv bolus studies, although some hepatocyte necrosis was seen at the highest doses. The histopathology changes were only partially reversible after recovery periods as long as 5 months. In the 1-month continuous infusion study in rats, similar histopathology changes were observed, but renal tubule cell degeneration was also seen at the 2 highest doses (1/20 at 1400 and 12/19 at 2500 mg/kg/day), which had not reversed after 1 month. The LD (625 mg/kg/day, 210 mg/kg/hr) was a NOEL for vacuolar changes in this study.

In dog studies of CAP conducted by the manufacturer, iv bolus administration of CAP to dogs at doses of up to 750 mg/kg (375 mg/kg/min) for 14 days, 1500 mg/kg (injected over 50-60 sec) for 1 month, and 600 mg/kg/day (1650 mg/min) for 6 months and continuous infusion doses of up 2500 mg/kg/day (105 mg/kg/hr) induced vacuolation of the urinary tract epithelium (renal tubules, renal pelvis and urinary bladder) and hepatocytes, and foamy macrophages in the liver and mesenteric and cervical lymph nodes. Vacuolar changes were seen at the lowest doses tested in the 14-day iv bolus study (160 mg/kg/day, administered over 50 seconds) and 1-month continuous infusion study (625 mg/kg/day, 210 mg/kg/hr), but with the exception of vacuolation of the transitional epithelium of the urinary bladder, all changes were dose-related in incidence and/or severity and diminished in severity after cessation of treatment. There did not appear to be any progression of the histopathology in the 6-month iv bolus study. The clinical pathology data did not indicate any functional abnormalities and there was no evidence of cell degeneration or necrosis.

Continuous iv infusion of CAP to cynomolgus monkeys at a dose of 5600 mg/kg/day and rate of 233 mg/kg/hr produced the expected vacuolar changes in the kidney, urinary bladder, and spleen, but no evidence of cell degeneration or necrosis or renal or hepatic functional toxicity was reported. Steady-state CAP concentrations of approximately 850 ug/mL were measured in this study. The AUC (0-inf) for day 14 was 864 ug.h/mL, and exposure for the 14-day dosing period was 286000 ug.h/mL.

The histopathology findings seen in the current iv infusion studies of CAP in the rat, dog, and monkey are consistent with those reported in the literature (reviewed by Stella and He, Toxicologic Pathology, 36:30-42, 2008 and Luke et al., J Pharm Sci, 99:3291-301, 2010). These consist primarily of renal tubule, urinary bladder, and hepatocyte vacuolization and macrophage

infiltration of various organs, which show evidence of reversibility, albeit very gradual in some cases. Little or no evidence of inflammatory responses, cell death, cell degeneration, or functional changes has been reported.

The results of the rat development toxicity studies indicate that administration of CAP alone (iv bolus doses up to 3000 mg/kg/day) or in a Drug/CAP formulation (continuous infusion doses up to up to 2250 mg/kg/day) produced no effects on embryofetal development. However, in the rat pre- and postnatal development study of CAP, effects on offspring survival and body weight were seen at the HD (iv bolus dose of 3000 mg/kg/day). The developmental NOAEL in this study was the MD (600 mg/kg/day). There were no apparent effects on fertility or reproductive performance in rats at iv bolus doses of up to 1500 mg/kg/day.

In an adult patient with SE, the loading dose would deliver up to 2400 mg of CAP at up to 300 mg/min (5 mg/kg/min), and the maintenance dose would deliver up to 720 mg of CAP. Administration of a loading dose followed by the maintenance dose on the same day would result in a total dose of up to 3120 mg (52 mg/kg or 1924 mg/m2). This dose is approximately 1/4 of the lowest CAP dose (in mg/m2) at which minimal renal tubular epithelial cell degeneration was observed in the 1-month continuous infusion study in rats and only 1/2 the NOAEL for tubule cell degeneration in this study. However, in rats and dogs given 9-16X higher iv bolus doses and higher rates of iv administration for 14 days or 1 month, degenerative changes were not reported. Theses iv bolus studies would appear to be more relevant to the clinical use of this fosphenytoin product; for example, CAP would be administered at a rapid rate (maximum rate of 300 mg/min) for SE.

The sponsor is seeking indications identical to those listed in the Cerebyx labeling, which include use in pediatric patients at ages down to birth; however, there are no nonclinical data to support iv CAP use in children. The only nonclinical study in juvenile rats provided in the CAP DMF is a rat study in which subcutaneous doses of a drug in a CAP vehicle (2400 mg/kg/day, equivalent to 14,400 mg/m2/day) were administered every three hours from postnatal days 3 through 12. No unique toxicity and no effects on renal development related to the CAP vehicle were reported in this study, but the route and limited dosing period (equivalent to human neonatal period) make this study inadequate to support the proposed indication. (Nephrogenesis is complete at birth in humans, but renal functional maturation continues through 2 years of age.)

The nonclinical data provided in the current application and available in the CAP DMF, for which a LOA was provided, are considered adequate to establish the safety of the new fosphenytoin formulation for short-term use in adults.

IV. RECOMMENDATIONS

The application is approvable from a pharmacology/toxicology standpoint for adult indications.

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