

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

209176Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 209176

Submission date: 6/16/16

Drug: edaravone

Applicant: Mitsubishi Tanabe Pharma Corp.

Indication: Amyotrophic Lateral Sclerosis

Reviewing Division: Division of Neurology Products

Discussion:

The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA and conclude that the information is sufficient to support approval of the application for the indication listed above.

Carcinogenicity studies are recommended by the division as post-marketing requirements.

Repeat dose toxicity studies showed central and peripheral nerve fiber degeneration in dogs and monkeys when edaravone was administered by continuous 24-hour intravenous infusion. This toxicity was not observed when the drug was administered by intravenous bolus or 1-2 infusions. Concern about this toxicity is diminished because the drug is not administered to humans by continuous infusion.

Conclusions:

I agree that the pharmacology/toxicology information is adequate to support approval of this application. It is reasonable to allow the carcinogenicity studies to be conducted post-marketing. Comments on labeling were provided to the division separately.

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/s/

PAUL C BROWN
05/01/2017

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 31, 2017

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 209-176 (edaravone; Radacava)

NDA 209-176 was submitted on September 8, 2016, by the sponsor (Mitsubishi Tanabe Pharma) for use of edaravone for the treatment of amyotrophic lateral sclerosis (ALS). NDA 209-176 is a 505(b)(1) application. Communications and meetings with the sponsor were conducted under Pre-IND 126396; no clinical protocol was submitted to initiate an IND.

The nonclinical studies conducted by the sponsor to support the NDA are as follows:

- Pharmacology
- Safety pharmacology (CNS, cardiovascular, and respiratory)
- PK/ADME/TK
- IV toxicology
 - acute: mouse, rat, and dog
 - repeat dose
 - 2-week, 30-day, and 26-week IV bolus in rat and dog
 - 28-day 2-hr IV infusion in dog
 - 28-day 24-hr continuous IV infusion in rat, dog, and monkey
 - investigative studies (neurotoxicity in dog; renal toxicity in rat)
- Reproductive and development toxicology
 - mating and early embryonic development in male and female rat
 - embryofetal development in rat and rabbit
 - peri- and postnatal development in rat (2 studies)
 - investigative study to assess effects on the estrus cycle
- Genetic toxicology
 - Ames assay, in vitro chromosomal aberration in CHL, in vivo IP micronucleus assay in mouse
- Other studies to assess impurities (acute and 14-day toxicity and genetic toxicology) and metabolites (pharmacology, acute toxicity)

During meetings conducted under Pre-IND 126396, the sponsor was told that the specification limit (NMR $\frac{(b)}{(4)}\%$) proposed for one impurity, $\frac{(b)}{(4)}$, and carcinogenicity study issues would be a matter of review. Regarding the assessment of carcinogenicity, the sponsor planned to submit published lifetime carcinogenicity studies in mouse and rat conducted by the National Cancer Institute and a request for waiver of the need for additional carcinogenicity assessment (Pre-NDA Meeting Minutes, dated January 7, 2016).

The nonclinical studies were reviewed in detail by Dr. Carbone (Pharmacology/Toxicology NDA Review and Evaluation, NDA 209176, David L. Carbone, Ph.D., March 27, 2017). Based on his review, Dr. Carbone has concluded that the nonclinical data are adequate to support approval of the NDA, with post-marketing requirements (PMR) for carcinogenicity studies.

This memo will briefly summarize the nonclinical data for edaravone, the adequacy of the data, and the need for PMRs.

Pharmacology

The sponsor conducted a series of studies to assess the primary, secondary, and safety pharmacology of edaravone, as well as studies in animal models of ALS.

Edaravone is characterized as a free radical scavenger, proposed to be therapeutic in ALS patients by protecting cells from oxidative tissue injury induced by lipid peroxidation. In *in vitro* studies using various tissue preparations (e.g., cultured brain homogenates or neurons), edaravone was converted to 2-oxo-3-(phenylhydrazono)-butanoic acid (OPB), its free radical ($\bullet\text{OH}$, $\text{LOO}\bullet$) reaction product, indicating interaction with free radicals. In *in vivo* studies in animal models of ALS (mutant SOD transgenic [H46R] rat) conducted by the sponsor demonstrated minimal, if any, efficacy at edaravone doses of 1.5-6 mg/kg IV (1-hr infusion). In the pivotal study, edaravone (0 or 3 mg/kg) was administered by 1-hr infusion for 2 days, followed by 2-day drug holiday, from 18 weeks of age until 39 weeks of age for behavioral endpoints or 28-29 week of age for histopathology. The only effect identified was a slight improvement on the inclined plane test in females (41.2 ± 1.6 vs 36.5 ± 1.2 degrees for C); there was no effect on the number of viable neurons (males: 31.8 ± 2.45 vs 32.2 ± 1.3 for C; females: 33.7 ± 2.0 vs 33.2 ± 1.2 for C).

However, published studies of edaravone in animal models of efficacy have reported beneficial effects. In a recent study in the wobbler mouse (Ikeda K, Iwasaki Y PloS One DOI-10.1371/journal.pone.0140316, 2015), edaravone was administered by intraperitoneal (IP) injection daily for 4 weeks, from onset of symptoms. Edaravone “attenuated muscle weakness, muscle contracture, denervation muscle atrophy in the forelimb, astrocyte proliferation and motor neuron degeneration of the cervical cord” at 10 (but not 1) mg/kg. In a mutant SOD1 transgenic mouse model of ALS, Ito *et al.* (Ito H. *et al. Exp Neurol* 213:448-455, 2008) reported beneficial effects at 50 (but not 5) mg/kg edaravone, administered daily by intraperitoneal injection. Because of the numerous differences (animal model of efficacy; route and dosing regimen) between the

sponsor's study and the published studies, the reasons for the discrepancy between the negative results of the sponsor's studies and the positive findings of the published studies is unknown.

The sulfate and glucuronide conjugate metabolites of edaravone exhibited no free radical scavenging activity in vitro.

Neither edaravone nor its metabolites exhibited notable binding affinity (i.e., $\geq 50\%$ at 10 μM) in an in vitro target binding panel.

PK/ADME

PK/ADME studies were conducted in ICR mouse, Sprague-Dawley and Wistar rat, Beagle dog, and cynomolgus monkey. PK parameters after a single IV bolus dose of edaravone or ^{14}C -edaravone are summarized in the following table.

SPECIES	V_d (L/kg)	Cl (L/hr/kg)	t_{1/2} (hr)
Wistar rat (^{14}C)	0.11-0.12	0.34-0.36	5.1-5.3
dog (^{14}C)	0.19	0.16	12.1
monkey (^{14}C)	0.4-1.2	0.08-0.10	6.6
monkey (cold)	0.58-0.77	1.29-1.57	1.2

Tissue distribution was assessed in Wistar rat and Beagle dog following single or multiple doses of ^{14}C -edaravone. The only tissues in which radioactivity levels exceeded plasma were aorta and kidney; after 21 daily doses, levels of radioactivity in the aorta at 196 hrs after the last dose were 44-65% of those at 5 min post dose. The sponsor conducted additional studies to assess distribution to aorta in rat and dog; the primary drug-related species was OPB, although edaravone was also detected (dog aorta).

In all species tested, including human, the major circulating metabolites formed following IV administration of edaravone were sulfate and glucuronide conjugates of edaravone. In human and rat, the sulfate conjugate was the most abundant drug-related compound identified in plasma, with levels ~ 10 times those of the parent compound. In dog plasma, the glucuronide conjugate was the most abundant drug-related species. Although the bridging TK studies conducted in rat and dog did not provide TK parameters for either metabolite, the plasma levels of the sulfate and glucuronide metabolites were similar or higher than those of edaravone at one hour post dose, suggesting adequate exposure in the pivotal nonclinical studies.

Toxicology

The pivotal toxicity studies were conducted in Wistar rat and Beagle dog; with IV bolus studies of up to 26 weeks' duration in both species. Additional 28-day studies were conducted using 24-hr continuous IV infusion in rat, dog, and cynomolgus monkey and using a 2-hr IV infusion in dog.

IV bolus/2-hr infusion

Rat: edaravone was assessed in 2-week, 30-day, and 26-week IV bolus toxicity studies in Wistar rat. Doses for the pivotal 26-week study were based on findings from the 2-week (non-GLP) and 30-day studies. Edaravone was tested at doses of 0, 200, 300, 400, and 500 mg/kg/day in the 2-week study and 0, 10, 30, 100, and 300 mg/kg/day in the 30-day (+14-day recovery) study. The NOAEL was 30 mg/kg/day in the 2-week study and 10 mg/kg/day in the 30-day study. The primary toxicities were consistent with CNS suppression (e.g., impaired righting reflex, staggered gait, sedation) and hemolytic anemia (e.g., decreased rbc ct, hgb, and hct; increased reticulocytes and total bilirubin). In the 26-week (+5-week recovery) study, edaravone was tested at doses of 0, 10, 30, and 100 mg/kg/day. There were no drug-related deaths. The primary toxicities observed were clinical signs (e.g., staggered gait), reduced body weight gain, and “changes in hepatic function” (increased urinary ketone bodies, increased PT, and reduced liver weight) but without histopathology correlates. A toxicokinetic (TK) analysis was not included in the study. The sponsor conducted a 7-day TK bridging study in Wistar rat at doses of 10 and 100 mg/kg/day (IV bolus). The Day 7 edaravone data are summarized in the following table.

DOSE (mg/kg)	MALE			FEMALE		
	C ₀ (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	t _{1/2} (hr)	C ₀ (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	t _{1/2} (hr)
10	17.8	2.14	1.26	19.3	2.22	0.66
100	287.8	53.1	4.80	218.9	70.2	5.57

TK parameters were not provided for the sulfate or glucuronide conjugates. The Day 7, 1-hr post dose concentrations (µg/mL) of these metabolites, in addition to corresponding edaravone concentrations, are provided in the following table (data for males-females).

DOSE (mg/kg)	EDARAVONE	SULFATE	GLUCURONIDE
10	86.0-78.9	1048-1146	156-47.7
100	2629-2898	14997-15463	2694-4932

Dog: edaravone was assessed in 2-week, 30-day, and 26-week IV bolus toxicity studies and in a 28-day 2-hr IV infusion study in Beagle dog. Doses for the pivotal 26-week IV bolus study were based on findings from the 2-week (non-GLP) and 30-day studies. Edaravone was tested at doses of 0, 100, 200, 300, and 400 mg/kg/day in the 2-week study and 0, 10, 30, 100, and 300 mg/kg/day in the 30-day study. The NOAEL was 100 mg/kg/day in the 2-week study and 30 mg/kg/day in the 30-day study. There was one death at 400 mg/kg/day. The primary toxicities were CNS suppression (e.g., hindlimb weakness) and regenerative anemia (decreased rbc ct, hgb, and hct; increased reticulocytes and total bilirubin). In the 26-week (+5-week recovery) study, edaravone was tested at doses of 0, 10, 30, and 100 mg/kg/day. There were no deaths. The primary toxicities observed were clinical signs (sedation, staggering gait, lethargy), reduced body weight gain, and regenerative anemia (decreased rbc ct, hgb, and hct; increased urinary bilirubin). The NOAEL was 30 mg/kg/day. TK analysis was not included in the 26-week

study; Day 7 data for edaravone from a TK bridging IV bolus study in dog are summarized in the following table.

DOSE (mg/kg)	MALE			FEMALE		
	C ₀ (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	t _{1/2} (hr)	C ₀ (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	t _{1/2} (hr)
10	20.2	3.87	3.96	23.3	3.69	8.81
30	46.0	15.8	10.3	54.9	15.1	10.7
100	210.2	77.0	6.46	208.5	67.3	6.9
300	412.1	280.8	7.33	444.2	255.2	6.95

PK parameters were not provided for the sulfate or glucuronide conjugates. The Day 7, 1-hr post dose concentrations (µg/mL) of these metabolites, in addition to corresponding edaravone concentrations, are provided in the following table (data for males-females).

DOSE (mg/kg)	EDARAVONE	SULFATE	GLUCURONIDE
10	0.29-0.23	7.59-46.8	1.95-19.9
30	1.56-1.39	49.1-53.2	30.7-18.4
100	9.01-6.62	65.3-130.3	181.7-130.3
300	84.6-70.6	150.8-123.4	272.2-356.4

In the 28-day 2-hr IV infusion study (non-GLP), edaravone was tested at doses of 0, 10, 30, and 100 mg/kg/day; no toxicity was observed. Day 7 edaravone data from a TK bridging 2-hr IV infusion study in male dogs are summarized in the following table.

DOSE (mg/kg)	MALE		
	C _{2 hr} (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	t _{1/2} (hr)
10	0.56	0.33	5.72
30	2.48	1.41	11.73
300	13.2	9.36	10.74

Continuous IV infusion

Rat: In the 28-day (+14-day recovery) continuous IV infusion study, edaravone was administered at doses of 0, 50, 100, 300, and 1000 mg/kg/day. Dose selection was based on data from dose-ranging studies at doses up to 1000 mg/kg; 100 mg/kg was identified as the NOAEL for a 24-hr continuous IV infusion.

In the 28-day study, death occurred at 300 and 1000 mg/kg, resulting from toxicity at the injection site; other findings at these doses were decreases in body weight gain and evidence of regenerative anemia. At the NOAEL of 100 mg/kg/day, plasma edaravone C_{ss} and AUC were 1.28-1.29 µg/mL and 29.14-29.46 µg*hr/mL, respectively.

Dog: In the 28-day (+14-day recovery) continuous IV infusion study, edaravone was administered at doses of 0, 30, 60, 120, and 200 mg/kg/day. Dose selection was based on data from 7- and 14-day dose-ranging studies. In the 7-day study (0, 100, 300, or 1000

mg/kg/day; 1/sex/group), evidence of regenerative anemia was observed at doses >100 mg/kg; there were no deaths or clinical signs. In the 14-day study (300 and 1000 mg/kg/day; 1M/group), one animal was sacrificed on Day 12, with loss of muscle tone and severely limited use of limbs. Microscopic evaluation revealed sciatic nerve fiber degeneration at both doses, accompanied by focal inflammation at 1000 mg/kg/day. Evidence of regenerative anemia was noted at both doses.

In the 28-day study (0, 30, 60, 120, or 200 mg/kg/day; 4/sex/group for main study; 0 or 120 mg/kg in 2/sex/group for recovery), a number of animals (1 M at 120 mg/kg/day and 4 M and 2 F at 200 mg/kg/day) were sacrificed on Days 22-25, with severely limited use of limbs. Limited limb movement was also noted at 60 mg/kg/day. Microscopic evaluation revealed peripheral (sciatic), spinal cord (dorsal funiculus), and auditory nerve fiber degeneration at >30 mg/kg/day, which remained at the end of the recovery period (120 mg/kg/day). Skeletal muscle atrophy, observed at >60 mg/kg/day, was considered secondary to the nerve fiber degeneration. Plasma edaravone exposures are summarized in the following table.

DOSE (mg/kg)	MALES		FEMALES	
	C _{ss} (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	C _{ss} (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)
30	0.13	3.46	0.13	3.72
60	0.28	7.19	0.31	7.92
120	0.62	15.24	0.82	20.72
200	1.67	40.45	1.61	39.64

A series of investigative studies (at doses up to 1000 mg/kg/day) were conducted in Beagle dog to further investigate the nerve fiber degeneration observed with continuous IV infusion. The results of these studies confirmed edaravone-induced central and peripheral nerve fiber degeneration (dose and duration-dependent) and resulting clinical manifestations (limb weakness and reduced reflexes). The peripheral nerve fiber degeneration in animals receiving 120 mg/kg/day for 2 weeks was reversible after a 13-week recovery period; nerve fiber degeneration in spinal cord was not reversible.

The sponsor proposed AChE inhibition or Vitamin B₆ deficiency as possible modes of action underlying the neurotoxicity. No effect on rbc and plasma AChE activity was detected at 300 or 1000 mg/kg/day after 8 days of dosing. Analysis of pyridoxal phosphate (PLP; active form of Vitamin B₆) in plasma from animals receiving a 5-day continuous IV infusion of edaravone (1000 mg/kg/day; 3 M/group; 3 M/group for recovery) indicated a decrease in PLP during the dosing period; however, the limited number of animals (3 M/group), the high variability in the data, and the lack of control groups preclude any conclusion regarding the role of Vitamin B₆ deficiency in the neurotoxicity produced by edaravone.

Monkey: In the 28-day continuous IV infusion study, edaravone was administered at doses of 0, 20, 100, and 1000 mg/kg/day. Dose selection was based on data from a 14-day dose-ranging study at doses up to 1000 mg/kg/day (2 M/group). Nerve fiber degeneration (“highly focal”) in lumbar spinal cord, lumbar dorsal root ganglion, lumbar

ventral nerve root, and vestibulocochlear nerve was detected in one HD animal. There was no effect on AChE activity.

In the 28-day study, nerve fiber degeneration, similar to that observed in dog, was detected in all (4/sex) HD animals, except that brain (medulla oblongata), as well as spinal cord and peripheral nerves, was affected in monkey. Accompanying clinical signs included limited limb use. (Recovery was not assessed.) TK parameters for edaravone and the sulfate and glucuronide metabolites are provided in the following table.

DOSE (mg/kg)	MALES		FEMALES	
	C _{ss} (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)	C _{ss} (µg/mL)	AUC _(0-24 hr) (µg*hr/mL)
EDARAVONE				
20	0.274	4.39	0.459	6.55
100	1.85	32.5	1.93	35.6
1000	33.4	492	35.5	558
EDARAVONE SULFATE				
10	2.17	52.1	2.40	57.5
100	10.8	265	8.16	198
1000	30.8	706	24.3	657
EDARAVONE GLUCURONIDE				
10	--	40.8	--	51.5
100	8.27	193	6.96	164
1000	--	1165	--	1068

Other Toxicology Studies

Metabolites: acute toxicity studies of edaravone sulfate (5/sex/group) and edaravone glucuronide (males only; 5/group) were conducted in ICR mouse. No toxicity was evident with the glucuronide conjugate at 2000 mg/kg IV. The sulfate conjugate produced decreased locomotor activity, abnormal gait, and prone posture at all doses tested (439-2000 mg/kg IV), tonic convulsions at 877 and 2000 mg/kg IV, and death at 2000 mg/kg IV.

Juvenile Animal Toxicology: The sponsor conducted a number of studies in juvenile animals (rat, dog); however, these data are not needed to support the NDA, which is only for an adult population.

Genetic Toxicology

A standard battery of in vitro (Ames and in vitro chromosomal aberration in Chinese Hamster Lung) and in vivo (mouse micronucleus) assays was conducted on edaravone. These assays were adequately conducted and negative for mutagenicity and clastogenicity.

Carcinogenicity

The sponsor submitted a published report of lifetime carcinogenicity studies in B6C3F1 mouse and Fischer 344 rat conducted by the National Cancer Institute (Technical Report No. 141, 1978). At this time, the data from these studies cannot be used to support the NDA, which was submitted under 505(b)(1). However, if the studies and the data needed to scientifically bridge to those studies are adequate, the sponsor could administratively change the NDA to a 505(b)(2) application.

In both studies, edaravone was administered in the diet (basal diet: Wayne Lab-Blox, Allied Mills, Inc., Chicago IL). The drug-diet admixture was prepared weekly and was stored in the dark at 4° C. Edaravone was tested at concentrations of 0, 7500, and 15000 ppm in mouse and 0, 2500, and 5000 ppm in rat. Both studies were negative for drug-induced tumors.

The number of animals per group and survival rates are summarized in the following table. In both studies, the number of control animals was inadequate.

SPECIES	MALES			FEMALES		
	C	LD	HD	C	LD	HD
mouse	16/20	40/50	43/50	18/20	38/50	34/50
rat	13/20	29/49	37/50	11/20	44/50	44/50

In mice, body weight (relative to C) was reduced at both doses (~8%) in males and at the HD in females (~16%); however, it is unclear if this was a direct drug effect or the result of unpalatability. There were no body weight effects in rats.

TK data were not collected in either study, which is particularly problematic because of the dietary route of administration used.

The sponsor conducted a 4-day TK bridging study in Sprague-Dawley rat (0, 2000, 6000, and 12000 ppm; estimated to be 0, 192-202, 547-601, and 630-1067 mg/kg/day); however, it was conducted only in males and in a different strain from that used in the NCI study. Edaravone was administered in the diet (MF, Oriental Yeast, Co. Ltd, lot #151104); the drug-diet admixture was “prepared once by the day before administration” and was stored at room temperature for up to 9 days. Plasma samples, collected at six time points over a 24-hr period (Day 3-Day 4), were analyzed for edaravone and its sulfate and glucuronide metabolites; however, TK parameters were not calculated. The data (range, in ng/mL) are summarized in the following table.

COMPOUND	LD	MD	HD
edaravone	158-226	643-1423	2610-4608
edaravone sulfate	2147-6610	8373-15590	11860-15590
edaravone glucuronide	595-1806	2882-10320	10870-21240

Because of the difference in diets and the strain of rat used, the relevance of these data to the NCI study is unclear.

Overall, the deficiencies in the conduct of the studies (for the purpose of the NDA) and in the bridging data submitted by the sponsor would preclude the use of these studies to support the NDA.

Reproductive and Developmental Toxicology

A battery of reproductive and developmental toxicology studies was conducted for edaravone. In all pivotal studies, edaravone was administered by IV bolus.

Fertility and early embryonic development: In the pivotal study, edaravone (0, 3, 20, and 200 mg/kg/day) was administered to male and female Wistar rats (26-30/sex/group). (Dose selection was based on a 2-week dose-ranging study [200-500 mg/kg/day]; reduced body weight gain was observed at doses ≥ 200 mg/kg/day.) Males were dosed for 63 days prior to mating, during the 2-week mating period, and up to either a confirmed copulation or the end of the mating period. Females were dosed for 15 days prior to mating, during the 2-week mating period, and up to gestation day (GD) 7; females were sacrificed on GD 21. Animals not successfully mating were paired with an untreated animal for a second mating period. There were no drug-related deaths. Clinical signs (ataxic gait) and reduced body weight (18 and 4%, relative to C, in males and females, respectively) were observed at 200 mg/kg/day. The primary findings were at the HD and consisted of prolonged/irregular estrus (10/29 HDF vs one affected F in each of the other groups) and reduced copulation index (1st mating period: 72.4% in HD vs 100% in C; overall: 82.8% in HD vs 100% in C). There were no effects on the fetus.

Follow-up studies (200 mg/kg/day for 2 weeks) in Wistar and Wistar Imamichi rats confirmed the effect of edaravone on the estrus cycle.

Embryofetal development: In Wistar rat, edaravone was administered at doses of 0, 3, 30, and 300 mg/kg/day on GDs 7-17 in the pivotal study; dams were either sacrificed on GD 21 (24/group) or allowed to deliver spontaneously (15/group). Dose selection was based on the results of two preliminary studies at doses of 0, 10, 30, 100, and 300 mg/kg/day. Clinical signs (staggered gait) and/or reduced body weight gain were observed in dams at doses of ≥ 30 mg/kg/day; reduced fetal body weight and delayed ossification were observed at 300 mg/kg/day but only in one of the preliminary studies.

In the pivotal study, 2 HDF died, accompanied by clinical signs (COD not determined). Clinical signs (staggering gait, prone position, and decreased activity) were observed at the HD. A decrease in fetal body weight ($\leq 7\%$) was observed at all doses, but there were no effects on other fetal parameters. In offspring, body weight was reduced at the HD ($\leq 12\%$ relative to control) during the lactation period.

In New Zealand White rabbit (13-18/group), edaravone was administered at doses of 0, 3, 20, and 100 mg/kg/day on GD 6-18 (sacrificed on GD 29) in the pivotal study. Dose selection was based on the results of a preliminary study at doses of 0, 0, 30, 100, and 300 mg/kg/day (2-6/group); edaravone was formulated using 1N, 3N, 0.3N, 1N, and 3N

NaOH), respectively. HD animals were sacrificed prematurely, and there was an increase in embryofetal death at 100 mg/kg/day.

In the pivotal study, clinical signs in dams (ataxia, paralysis, miosis, and hyperemia) and increased embryonic death (7.1, 14.6, 5.1, and 23.1% dead fetuses in C, LD, MD, and HD F, respectively) were evident at the HD. There were no deaths or effects on body weight in dams or effects identified upon fetal examination.

Peri- and postnatal development: Two pivotal studies were conducted, one in Wistar rat and the other in Wistar Imamichi rat. Dose selection for the first study was based on the results of a preliminary study conducted at doses of 0, 10, 30, 100, and 200 mg/kg/day, administered from GD 17 to postnatal day (PND) 21. Clinical signs (30-200 mg/kg) and “a tendency” to reduced body weight gain (200 mg/kg) were the primary findings.

In the first pivotal study, edaravone was administered during the same period at doses of 0, 3, 20, and 200 mg/kg/day. In dams, clinical signs (e.g., staggering gait, ptosis, and prone position) were observed at all but the LD; body weight gain was reduced (~46%), accompanied by reduced food consumption, at the HD during the dosing period. In offspring, survival on PND 4 was reduced at the HD but only because of total loss (13/13) in one litter. On the neurobehavioral parameters, the primary drug-related finding was an increase open field activity at the MD and HD. Physical development and reproductive performance in offspring was unaffected; however, pre-implantation loss was high in all groups (22-34%), including controls. Therefore, the sponsor conducted a second peri- and postnatal development study.

In the second study, edaravone was administered to Wistar Imamichi rats (23-24/group); the doses and dosing period were the same as in the first study. As in the first study, clinical signs in dams were evident at all but the LD; a transient decrease in body weight gain was noted at the HD. On litter parameters, there was an increase in stillbirths (17 vs 0% in C) at the HD. In offspring, the Viability Index on PND 4 was reduced at the HD (79.1% vs 91.7% in C). The only apparent effect on physical development was a delay in vaginal opening at the HD. There were no clear effects on reproductive parameters. A neurobehavioral assessment was not included.

Impurities

In the nonclinical section, the sponsor discussed the presence of three impurities in the drug substance/drug product: (b) (4)

(b) (4).

(b) (4): The sponsor conducted no nonclinical studies to assess the toxicity of (b) (4), acknowledging that (b) (4) is considered to be a mutagenic carcinogen. During clinical development, the division stated that the specification limit for (b) (4) should be set to ensure a daily intake (based on up to 10 years of actual exposure) of not more than (NMT) 10 µg/day. The sponsor has proposed a

specification limit of NMT (b) (4), which would result in a daily intake of up to (b) (4) µg/day. Although the justification provided by the sponsor to support the higher daily intake of (b) (4) is not adequate, the CMC team has determined that the sponsor has made reasonable attempts to lower the specification limit to the extent feasible. The sponsor's limit is acceptable but only because of the seriousness of the indication.

(b) (4): The specification limits (NMT (b) (4)%) for these two degradants are below the qualification threshold for impurities; therefore, the only concern, from a safety standpoint, would be their mutagenic potential. Both were negative in adequate Ames assays conducted by the sponsor. Therefore, the proposed limits are acceptable.

Other Investigative Studies

Drug-Drug Interactions: The sponsor conducted a series of studies to investigate the potential for drug-induced renal toxicity. This was a concern raised by cases of serious renal toxicity, including renal failure, reported in humans following approval of edaravone in Japan, which the sponsor considered possibly related to edaravone. (Renal toxicity was not observed in the nonclinical studies.) The investigative studies identified a possible drug-drug interaction between edaravone and certain renal toxins (glycerol and cefalotin). However, the clinical team has concluded that the reported cases were confounded and has dismissed the concern.

Conclusions and Recommendations

The nonclinical studies conducted by the sponsor are adequate to support approval of edaravone for the treatment of ALS, with appropriate labeling and, as Dr. Carbone recommends, post-marketing requirements for carcinogenicity in two species (mouse and rat). Toxicokinetic data should be collected in one or both species in order to confirm adequate exposure to the sulfate conjugate, considering the toxicity observed in the acute IV bolus toxicity study in mouse. In addition, the studies should be conducted using a drug batch containing (b) (4) at levels relevant to the maximum daily human dose at the specification limit set for this impurity.

Central and peripheral nerve fiber degeneration was the most severe toxicity observed in animals (dog and monkey) and resulted from administration of edaravone as a 24-hour continuous IV infusion. Similar effects were not observed with IV bolus or 1-2 hour IV infusions in dog. It is notable that, at the same dose, the plasma edaravone AUCs were actually lower with the 24-hour continuous IV infusion than after IV bolus or shorter-duration IV infusions. However, these findings do not need to be included in labeling because, according to the clinical team, edaravone would never be administered to humans as a continuous IV infusion.

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/s/

LOIS M FREED
03/31/2017

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 209176
Supporting document/s: 1
Applicant's letter date: September 8, 2016
CDER stamp date: September 8, 2016
Product: Edaravone
Indication: Amyotrophic Lateral Sclerosis
Applicant: Mitsubishi Tanabe Pharma Corp.
Review Division: Neurology Products
Reviewer: David L. Carbone, Ph.D.
Supervisor: Lois M. Freed, Ph.D.
Division Director: Billy Dunn, M.D.
Project Manager: Jack Dan, R.Ph.

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1 Executive Summary

1.1 Introduction

Edaravone (RADICAVA™) is a free radical scavenger developed by Mitsubishi Tanabe Pharma Corp for treatment of amyotrophic lateral sclerosis (ALS). Edaravone is thought to reduce oxidative cell injury that may be associated with ALS.

1.2 Brief Discussion of Nonclinical Findings

In vitro pharmacology studies with edaravone demonstrated prevention of oxidative cell injury. However, administration of edaravone in SOD1 transgenic mice did not improve survival, reflex, or grip strength, but did marginally increase performance on an incline plane. No off target effects were predicted based on an *in vitro* panel of 79 receptors, ion channels, and transporters.

In safety pharmacology studies, *in vitro* concentrations of up to 1 mM edaravone did not inhibit the hERG current. CNS signs generally included increases in lacrimation and ptosis, and decreases in spontaneous movement in male mice and rats following a single IV dose of 30 or 100 mg/kg edaravone. Decreases in body temperature were observed in male mice administered a single 100 mg/kg IV injection of edaravone. In mongrel dogs, single IV administration of 30 or 100 mg/kg edaravone resulted in transient decreases in blood pressure and increases in carotid blood flow and heart rate. Single IV injection of up to 100 mg/kg edaravone in mongrel dogs did not affect respiratory rate; however, neither tidal or minute volumes nor hemoglobin oxygen saturation were evaluated.

Pharmacokinetic studies in mice, rats, dogs, and monkeys indicated a $t_{1/2}$ of 1 to 2 h following IV administration. IV administration of radiolabeled edaravone in rats and dogs indicated transport across the blood brain barrier, extensive accumulation in the aorta (likely due to protein binding), and extensive partitioning into the kidney. Primary metabolites in mice, rats, dogs, monkeys, and humans include sulfate and non-acyl glucuronide conjugates, neither of which were shown to have free radical scavenging activity. 24 h following IV administration, urinary excretion accounted for 61, 76, 83, and 90% of the administered dose in rats, dogs, monkeys, and humans, respectively, with excreted radioactivity existing primarily as the sulfate (rats and dogs) or glucuronide (humans) conjugate. Edaravone was 90, 83, 44, and 91% plasma protein bound in blood from mice, rats, dogs, and humans, respectively. In the same species, the sulfate conjugate was 90 to 99% plasma protein bound, while the glucuronide conjugate was 13 to 37% plasma protein bound. Administration of radiolabeled edaravone in pregnant rats resulted in milk and fetal plasma radioactivity concentrations up to 20 and 4%, respectively, of maternal plasma concentrations.

The toxicity of edaravone was evaluated by IV bolus or continuous infusion in Wistar rats, beagle dogs, and cynomolgus monkeys (continuous infusion only). Primary

toxicities following IV bolus or short (i.e., ≤ 4 h) infusion in male and female rats and dogs included transient CNS signs (e.g., sedation and hypoactivity) and decreases in weight gain at doses greater than 10 or 30 mg/kg, respectively. Continuous infusion in male and female rats and dogs was associated with hematologic signs of regenerative anemia at doses greater than 300 and 30 mg/kg, respectively. However, continuous infusion in dogs and monkeys also resulted in peripheral nerve degeneration. Additional studies in dogs indicated that nerve fiber degeneration was typically restricted to the axons and was accompanied by digestion chambers. These data suggest that the nerve fiber degeneration induced by edaravone may be reversible, although complete recovery was not observed after recovery periods up to 13 weeks. A mechanism explaining edaravone-induced nerve fiber degeneration was never defined, but additional studies suggest a role for vitamin B6 deficiency.

Renal toxicity was not seen in the toxicology studies. However, based on post-marketing surveillance in Japan, the sponsor conducted a battery of nonclinical studies to evaluate the potential for renal injury in patients receiving edaravone in combination with cephalosporin antibiotics. Mechanistic studies in rats suggested that edaravone may increase renal exposure to cephalosporin antibiotics, thus enhancing any potential renal toxicity associated with cephalosporin antibiotics.

No effects on fertility were seen in rats administered 0, 3, 20, or 200 mg/kg edaravone by IV injection. No malformations at IV doses up to 300 or 100 mg/kg were observed in rat or rabbit embryofetal development studies, respectively. However, IV doses greater than 3 mg/kg administered from GD 7 to 17 in rats resulted in decreases in fetal body weight and slight delays in markers of development. IV administration of 100 mg/kg in rabbits increased fetal death. Pre- and postnatal development studies in rats administered 0, 3, 20, or 200 mg/kg edaravone resulted in increased numbers of stillborn offspring at 200 mg/kg, and slight increases in open field activity and rearing behavior in offspring at 20 and 200 mg/kg.

Edaravone was negative in a complete battery of *in vitro* and *in vivo* genetic toxicology studies. Carcinogenicity studies of edaravone administered in diet, conducted by the National Cancer Institute in 1979, were referenced by the sponsor. There were no indicators of carcinogenicity, but issues including high mortality in a vehicle group and inconsistent dosing reduce the confidence in these studies. Adequately conducted studies should be conducted post marketing.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support approval of RADICAVA™.

1.3.2 Additional Non Clinical Recommendations

Carcinogenicity studies will be requested as a post marketing requirement.

5 Page(s) of Draft Labeling have been Withheld in Full as B4 (CCI/TS) immediately following this page

2 Drug Information

2.1 Drug

CAS Registry Number: 89-25-8

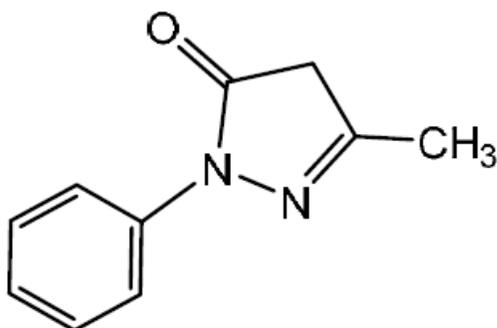
Generic Name: None

Code Name: MCI-186

Chemical Name: 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (IUPAC)

Molecular Formula/Molecular Weight: $H_{10}H_{10}N_2O$, 174.20 g/mol

Structure or Biochemical Description



Pharmacologic Class:
Free radical-scavenger

2.2 Relevant INDs, NDAs, BLAs, and DMFs

None

2.3 Drug Formulation

Edaravone Injection (30 mg/100 mL) is formulated with conventional excipients.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

The specification limit for [REDACTED] ^{(b) (4)} in the drug product is [REDACTED] ^{(b) (4)}%, resulting in a potential daily exposure of [REDACTED] ^{(b) (4)} μ g/day at the proposed dose of 60 mg/day.

However, based on discussion with the CMC team, it is unlikely that the sponsor can further reduce the levels of (b) (4). Impurities (b) (4), and (b) (4) are Ames-negative degradation products with specification limits below the qualification threshold

2.6 Proposed Clinical Population and Dosing Regimen

Edaravone is intended for use in adult patients with ALS. The proposed dose is 60 mg by IV infusion (1 h) once per day for 14 days, followed by a 2-week drug-free period. Subsequent cycles consist of 10 daily doses over 2 weeks followed by a 2-week drug-free period.

2.7 Regulatory Background

Edaravone was approved in Japan in 2001 for treatment of stroke, and in Japan and South Korea in 2015 for treatment of ALS.

The sponsor was advised at a pre-NDA meeting (December 9, 2015) that the acceptability of (b) (4) levels that exceed 10 µg/day will be a matter of review. The sponsor was informed that carcinogenicity studies would be required if edaravone is approved, but that the studies may be conducted post-approval.

3 Studies Submitted

3.1 Studies Reviewed

In vitro and *in vivo* primary pharmacology. Secondary pharmacology. Safety pharmacology. PK/ADME in mice rats, dogs, and monkeys. Single dose toxicology in mice, rats, and dogs. Repeat dose toxicology in rats, dogs, and monkeys. Fertility in rats. Embryofetal development in rats and rabbits. Pre- and postnatal development in rats. Juvenile animal toxicology in rats and dogs. *In vitro* (Ames, mammalian chromosomal aberration) and *in vivo* (mouse micronucleus) genotoxicity assays. Mechanistic studies for neurodegeneration (dogs) and renal toxicity (rats). Local and vascular irritation in rabbits. Studies to assess hemolysis, antigenicity, dependence, metabolite toxicity and impurities.

3.2 Studies Not Reviewed

Efficacy studies in animal models of ischemic injury. Toxicity studies for impurities not present in the proposed formulation.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

MCI-186 (edaravone) is thought to reduce cell injury through scavenging oxygen radicals. *In vitro* studies demonstrated prevention of linoleic acid oxidation by hydrogen peroxide and ferrous ions, reductions in lipid peroxidation in rat brain homogenate and isolated mitochondria, reduced oxidative injury to cultured endothelial cells, and reduced neuronal apoptosis in a model of excitotoxicity. However, IV administration of 3 or 6 mg/kg MCI-186 did not reduce neuronal cell death in a rat model of nerve avulsion. In a SOD-mutant transgenic rat model of ALS, a life-long regimen consisting of once-daily IV injection of 3 mg/kg MCI-186 for 2 days followed by a 2 day drug holiday did not increase lifespan or generally improve performance in a battery of reflex or strength tests, although a slight improvement was observed in the incline plane test.

4.2 Secondary Pharmacology

There were no significant interactions between MCI-186 or its sulfate or glucuronide conjugates in an *in vitro* panel of 79 receptors, ion channels, and transporters.

4.3 Safety Pharmacology

Study title: Safety Pharmacology Studies of MCI-186: Effects on hERG Current

Study no.:	B041335
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	January 11, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MCI-186, Lot J002ED, 99.9%

Findings

MCI-186 concentrations up to 1 mM did not inhibit hERG current.

Study title: General Pharmacological Studies of 3-methyl-1-phenyl-2-pyrazolin-5-one (MCI-186), a Novel Radical Scavenging Agent

Study no.: SP-1

Study report location: EDR

Conducting laboratory and location:

(b) (4)

Date of study initiation: Not provided

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: Not provided

Findings

The safety pharmacology battery included non-GLP evaluations of CNS, cardiovascular, respiratory, and gastrointestinal effects of MCI-186. The study report consisted of a publication in the journal Japanese Pharmacology & Therapeutics (Ando, Nishi et al., 1997).

Test	Species	Dose (mg/kg)	Route	Findings
CNS				
General Activity				
Irwin's Test	Male ddY mice (3/group)	0, 10, 30, 100	IV	lacrimation, ptosis, ↓ movement: MD, HD
	Male Wistar rats (3/group)	0, 10, 30, 100	IV	lacrimation, ptosis, ↓ movement: MD, HD
Spontaneous Activity				
Wheel Cage	Male ICR mice (7/group)	0, 10, 30, 100	IV	↓ movements: MD (but not HD)
Open Field	Male Wistar rats (8/group)	0, 3, 10, 30	IV	↓ movements: HD
Animex	Male Wistar (2/group)	0, 10, 30, 100	IV	No effect
Effects on pentobarbital-induced anesthesia				
pentobarbital	Male Wistar rats (8/group)	0, 10, 30, 100	IV	No effect
Anticonvulsive Effect				
Pentylenetetrazole	Male ddY mice (8/group)	0, 10, 30, 100	IV	No effect
Picrotoxin	Male ddY mice (8/group)	0, 10, 30, 100	IV	No effect
Maximum electroshock	Male ddY mice (8/group)	0, 10, 30, 100	IV	No effect

Test	Species	Dose (mg/kg)	Route	Findings
CNS (Continued)				
Convulsive Effect				
Pentylentetrazole	Male ddY mice (8/group)	0, 10, 30, 100	IV	No effect
Electroshock	Male ddY mice (8/group)	0, 10, 30, 100	IV	No effect
Analgesic				
Acetic acid-induced writhing	Male ICR mice (10/group)	0, 10, 20, 30	IV	↓writhing: LD (3/10), MD (6/10), HD (9/10)
Tail pinch	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
Heat stimulation	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
Body Temperature				
	Male ICR mice (8/group)	0, 3, 10, 30, 100	IV	↓2 deg C 60 min postdose: HD
	Male Wistar rats (7/group)	0, 10, 30, 100	IV	No effect
Hypothermia				
Reserpine	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
EEG				
	Male NZW rabbits (3/group)	0, 10, 30	IV	No effect
	Hybrid cats (3/group)	0, 10	IV	No effect
	Male Wistar rats (4/group)	0, 10, 30, 100	IV	No effect
Spinal Reflex	Male Wistar rats (5/group)	0, 3, 10, 30	IV	No effect
Motor Coordination	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
Conditioned Behavior	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
Cataleptic Effect	Male Wistar Rats (8/group)	0, 10, 30, 100	IV	No effect
Protection From Toxicity				
methamphetamine	Male ICR mice (10/group)	0, 10, 30, 100	IV	No effect
norepinephrine	Male ICR mice (10/group)	0, 10, 30, 100	IV	No effect
physostigmaine	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
Effects on Somatic System				
nerve-muscle junction	Male Wistar rats (3/group)	0, 10, 30, 100	IV	No effect
inclined screen	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
traction test	Male ICR mice (8/group)	0, 10, 30, 100	IV	No effect
Local anesthetic effect	Male NZW rabbits (3/group)	0.20%	Eye drop	No effect
Effects on Autonomic System				
isolated ileum contraction	Male guinea pigs	0, 10, 100, 1000 μM	perfusion	No effect
isolated uterus motility	Female Wistar rats	0, 10, 100, 1000 μM	perfusion	No effect
Cardiovascular and Respiratory				
CV/Respiratory				
BP	Mongrel dogs (5/group)	0, 1, 3, 10, 30, 100	IV	Transient ↓: ≥30 mg/kg
carotid blood flow				Transient ↑: ≥30 mg/kg
HR				Transient ↑: ≥30 mg/kg
ECG				No effect
respiratory rate				No effect

Test	Species	Dose (mg/kg)	Route	Findings
Cardiovascular and Respiratory (Continued)				
LV dP/dt_{max} contractility (isolated atria)	Male guinea pigs (6 total)	0, 10, 100, 1000 μ M	perfusion	Transient \uparrow followed by \downarrow : 100 mg/kg No effect
Digestive System				
intestinal transport	Mice (8/group); sex unknown	0, 10, 30, 100	IV	\downarrow transport: MD, HD
GI motility	Male JW rabbits (4/group)	0, 10, 30, 100	IV	\downarrow motility, MD, HD
gastric mucosa	Male Wistar rats (6/group)	0, 10, 30, 100	IV	No injury
Effects on Water and Electrolyte Metabolism				
urine volume	Male Wistar rats (6/group)	0, 10, 30, 100	IV	\uparrow output: LD, MD, HD
Effects on Blood System				
coagulation	Male Wistar rats (6-9/group)	0, 10, 30, 100	IV	No effect
platelet aggregation	JW rabbit blood	0, 0.1, 1, 10, 100 μ M	N/A	No effect
fasting blood glucose	Male Wistar rats (6/group)	0, 10, 30, 100	IV	\uparrow at HD
pododema	Male Wistar rats (9-10/group)	0, 10, 30, 100	IV	No effect

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Standard PK studies were conducted in CD1 mice, Wistar rats, and beagle dogs.

Route	Species	Dose (mg/kg)	C_{max} or C_0 (ng/mL)	T_{max} (h)	AUC (ng \times h/mL)	$t_{1/2}$ (h)	F (%)
IP	Mouse	1	183.10	0.083	74.57	0.39	N/A
		3	499.75	0.083	128.18	0.27	N/A
		10	857.37	0.083	347.21	0.94	N/A
	Rat (PND 7)	3	3385.20	0.083	997.05	1.33	N/A
		9	13414.33	0.083	3742.18	1.24	N/A
	Rat	1	223.14	0.083	82.42	0.25	N/A
		3	466.38	0.083	147.95	0.37	N/A
		10	2901.90	0.083	841.58	1.2	N/A
	IV	Mouse	2	2527.29	N/A	258.37	0.33
Rat		3	2177	N/A	1169	0.52	N/A
Dog		2	569	N/A	300	1.62	N/A
Human		0.2	223	N/A	201	1.49	N/A

Route (Cont.)	Species	Dose (mg/kg)	C _{max} or C ₀ (ng/mL)	T _{max} (h)	AUC (ng×h/mL)	t _{1/2} (h)	F (%)
Oral	Mouse	10	357.23	0.25	248.58	1.45	N/A
		30	1135.08	0.25	532.68	1.55	N/A
		100	12737.67	0.25	4925.45	1.51	N/A
	Rat	10	243.38	0.25	225.63	0.77	8.11
		30	1313.50	0.25	1097.59	2.46	13.85
		100	10998.68	0.25	8018.44	1.57	29.94
	Dog	5	132.52	0.25	84.08	1.26	16.9
		10	686.08	0.25	399.87	1.83	18.7
		30	9557.82	0.33	4946.17	2.59	75.8

IV administration of ¹⁴C MCI-186 in male and female Wistar rats revealed metabolism to sulfate (186S; 62%) or glucuronide (186G; 12%) conjugates within 5 minutes of dosing. With the exception of kidney, radioactivity did not extensively partition into tissues; brain concentrations of MCI-186 5 min after dosing were approximately 6% that of plasma. Approximately 90% of the radioactive dose was excreted in the urine. IV administration of radiolabeled MCI-186 in male beagle dogs and cynomolgus monkeys similarly revealed metabolism to 186S and 186G within 5 min of dosing. MCI-186 was predominantly excreted in the urine as 186S and 186G 24 h after IV bolus administration in rat, dog, monkey, and human. *In vitro* serum protein binding of MCI-186, 186S, and 186G was evaluated in mice, rats, dogs and humans.

Species	% Excretion in Urine			
	Unchanged	186S	186G	Total
Rat	1.59	51.19	8.25	61.04
Dog	2.82	64.47	9.15	76.44
Monkey	7.40	52.30	40.40	83.00
Human	0.68	6.58	83.17	90.43

Parent and metabolite profile in urine 24 h after IV bolus

Drug/ Metabolite	% Plasma Protein Binding			
	Mouse	Rat	Dog	Human
MCI-186	90	83.35	44.15	91.45
186S	90	97.5	97.9	99.3
186G	13	26.15	19.5	37.2

Single IV administration of ^{14}C MCI-186 in Wistar rats on GD 14 and 19 revealed fetoplacental transfer of parent, 186S, and 186G. Fetal radioactivity 5 minutes after dosing represented approximately 4 and 7% of the maternal plasma concentrations on GD 14 and 19, respectively.

Table II Concentrations of radioactivity, unchanged MCI-186, sulfate and glucuronide of MCI-186 in maternal plasma, placenta and fetus at 5 min after intravenous administration of ^{14}C -MCI-186 (2 mg/kg) to pregnant rats on day 19 of gestation

	Concentration, ng/g or ml (% of radioactivity)			
	Radioactivity	Unchanged MCI-186	MCI-186 sulfate	MCI-186 glucuronide
Maternal plasma	10157±1080(100)	1734±203(17.1)	6519±1373(64.2)	643±275(6.3)
Placenta	2177± 170(100)	440± 55(20.2)	1395± 177(64.1)	223± 36(10.2)
Fetus	775± 71(100)	N.D.	343± 46(44.3)	175± 43(22.6)

Radioactivity was converted to ng equivalent of MCI-186.

Each value represents the mean±S.D. of 4 animals.

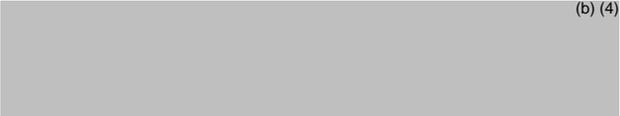
N.D. : Not detected.

(Sponsor's Table)

Single IV administration of 2 mg/kg ^{14}C MCI-186 in lactating rats (PND 11) revealed excretion of radioactive material into the milk, reaching a peak plasma/milk ratio of 0.2 15 min after dosing. Radioactive material in the milk consisted of MCI-186 (26.3%), 186S (36.1%), and 186G (17.0%). The remaining radioactive material was unidentified.

5.2 Toxicokinetics

Study title: A 7-Day Repeated Intravenous (Bolus) Dose Toxicokinetic Study of MCI-186 in Rats

Study no.: B031605
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: January 13, 2004
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot J001ED, 100.0%

Findings

These data support Study B-996 "Six-month chronic intravenous injection and one-month recovery study of MCI-186 in rats." 10 or 100 mg/kg MCI-186 was administered to male and female Wistar rats. Plasma concentrations of MCI-186 and the metabolites 186S and 186G were monitored on Days 1 and 7. C_0 and AUC were similar between males and females. Increases in C_0 were generally dose proportional, but increases in AUC were greater-than dose proportional. There were no effects of repeat dosing on plasma exposure. It was unclear why $t_{1/2}$ increased approximately 5-fold between 10

and 100 mg/kg, but according to the sponsor this might have been due to differences in detectable time points. TK parameters for the metabolites were not reported, but mean plasma concentrations of the sulfate and glucuronide conjugates 1 h after dosing with 10 mg/kg on Day 7 were 1048 and 156 ng/mL, respectively.

Table 5 Toxicokinetic parameters for MCI-186 following a 7-day repeated intravenous dose to rats

Dose (mg/kg)	Animal	Day	C ₀ (ng/mL)	No. of λ z (points)	t _{1/2} (hr)	AUC _{0-24hr} (ng·hr/mL)	AUC _{0-∞} (ng·hr/mL)
10	Male	Day 1	20203.72	3	0.66	2592.67	2578.81
		Day 7	17846.24	4	1.26	2140.46	2100.43
	Female	Day 1*	22832.58	3	0.61	2904.52	2893.16
		Day 7	19307.81	3	0.66	2219.75	2209.47
100	Male	Day 1	184187.33	3	5.03	53229.19	53300.86
		Day 7	287769.43	3	4.80	53118.42	53211.09
	Female	Day 1*	247644.06	3	4.98	67379.78	67459.44
		Day 7*	218882.99	3	5.57	70163.16	70312.04

Above parameters were calculated with the mean values of the plasma unchanged MCI-186 concentration shown in Tables 1 and 2 using WinNonlin Ver. 4.1. Non-compartmental analysis was carried out according to Linear trapezoidal method Model 201.

* Indicated parameters were calculated except for the values shown in Table 2 with asterisk, i.e. the animal No. 50101, 50102 (female rats received at 10 mg/kg dosing on Day 1), 60101 (female rats received at 100 mg/kg dosing on Day 1), and 60119 (female rats received at 100 mg/kg dosing on Day 7) at 5 min after dosing (cf. Table 2 and Appendix 6-2).

(Sponsor's Table)

Study title: A Single-Dose Intravenous (2-Hour Infusion) Toxicokinetic Study of MCI-186 in Beagle Dogs

Study no.: B021679
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: January 10, 2003
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot 02001, 99.9%

Findings

These data support Study 7N085: "A 4-Week Intravenous Infusion Toxicity Study of MCI-186 in Beagle Dogs." Male beagle dogs were administered 10, 30, or 100 mg/kg by 2 h IV infusion. Increases in C_{max} and AUC were slightly greater than dose proportional.

Dose (mg/kg)	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-24h} (ng×h/mL)	t _{1/2} (h)
10	884.7	1.67	1692.6	2.12
30	1830	3.6508	7696.2	5.37
100	1670	14.3759	31472.5	5.22

Study title: A 7-Day Repeated Intravenous (Bolus) Dose Toxicokinetic Study of MCI-186 in Beagle Dogs

Study no.: B020845
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: January 17, 2002
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot 02001, 99.9%

Findings

These data support Study 916-119 “26-Week Chronic Toxicity Study in Beagle Dogs.” Male and female beagle dogs (3/sex/group) were administered 10, 30, 100, or 300 mg/kg MCI-186 by IV injection. High variability on Day 1 at the LD resulted in the calculation of an abnormally long t_{1/2}. C₀ and AUC_{0-24h} were consistent between males and females. Increases in C₀ were generally dose proportional. However, increases in AUC were greater than dose proportional and might have been due to saturation of metabolism. There was no effect of repeat dosing on C₀ or AUC.

Dose (mg/kg)	Sex	Day 1			Day 7		
		C ₀ (ug/mL)	AUC _{0-24h} (ug×h/mL)	t _{1/2} (h)	C ₀ (ug/mL)	AUC _{0-24h} (ug×h/mL)	t _{1/2} (h)
10	M	14.6711	2.7447	10.20*	20.166	3.8702	3.96
	F	14.8958	2.355	4.87	23.3138	3.6944	8.81
30	M	63.1525	12.4694	8.29	45.9908	15.7544	10.27
	F	68.3758	9.6711	9.45	54.8694	15.0751	10.72
100	M	100.3522	29.4016	6.37	210.177	77.0194	6.46
	F	111.0611	42.8157	6.65	208.4953	67.3359	6.9
300	M	458.6753	266.1863	6.04	412.0923	280.772	7.33
	F	385.4638	265.337	5.12	444.1847	255.1937	6.95

TK in beagle dogs (indicates high variability)*

6 General Toxicology

6.1 Single-Dose Toxicity

Rodent

Single-dose toxicity was evaluated in male and female ICR mice and Wistar rats following IV, oral, and SC dosing. There were mortalities at all doses, typically occurring within 3 h of dosing. COD was thought to be respiratory failure or acute cardiac failure. Gross findings in animals that died included red discoloration of the lungs, kidneys, or spleen. There were transient CNS signs at all doses in surviving animals including sedation, lacrimation, eye blinking, lack of righting reflex, and salivation. There was red/orange discoloration of the urine at all doses, which was thought to be due to a metabolite and was not considered adverse. Surviving animals were monitored for 14 days after dosing. Over the recovery period, there were no drug-related clinical signs, effects on weight gain, or additional deaths.

Mice (mg/kg)							Rats (mg/kg)						
IV Bolus (n=5/group)													
Sex	0	478	526	578	636	700	0	530	580	640	700	770	850
M	0	1	2	1	3	5	0	0	0	3	5	5	n/a
F	0	0	0	2	3	5	0	n/a	0	0	0	3	3
Oral (n=5/group)													
Sex	0	1000	1300	1690	2200	2860	0	1200	1560	2028	2636	3427	--
M	0	1	0	3	4	5	0	0	0	3	4	5	--
F	0	0	0	3	2	4	0	0	0	2	5	5	--
Subcutaneous (n=5/group)													
Sex	0	636	700	770	847	932	0	800	880	970	1070	1170	--
M	0	0	1	1	2	5	0	0	0	0	2	3	--
F	0	1	3	4	5	5	0	0	0	0	2	4	--

Single dose rodent mortality

Nonrodent

Male beagle dogs (8/group) were administered a single 0, 300, or 600 mg/kg IV bolus of MCI-186. All animals survived until scheduled necropsy (Days 4 and 15). Transient CNS signs including reduced activity, abnormal gait, incomplete eyelid opening, vomiting, and tachypnea were observed at the LD and HD on the day of dosing. Signs of regenerative anemia, including decreases from baseline in mean RBC (53%), hemoglobin (50%), and hematocrit (46%), and increases in reticulocytes (24×) were observed at the HD, and did not completely resolve by Day 15. Clinical chemistry revealed increases relative to baseline in mean fibrinogen (1.7×), AST (5×), ALT (1.8×), LDH (4.4×), ALP (1.6×), and total bilirubin (14×) at the HD, which resolved by Day 15. Transient elevations in plasma TNF- α and IL-6 levels were observed at the HD on Days 1 and 2, indicating an inflammatory response. Gross findings at the HD included dark

red discoloration and enlargement of the spleen and liver, accompanied by histologic signs of splenic congestion, splenic and hepatic erythrocytosis on Day 4, and brown pigment deposits in the Kupffer cells and renal proximal tubule epithelium, extramedullary hematopoiesis in the spleen, and increased hematopoiesis in the bone marrow on Day 15.

Male and female beagle dogs (1/sex/group) were administered 0, 400, 500, or 600 mg/kg MCI-186 by 4 h IV infusion, and monitored for 2 weeks prior to necropsy. The HDF died on study due to renal infarction, hemolytic anemia, and sustained hypotension; all other animals survived until scheduled necropsy. Clinical signs at all doses (not in control) included weakness, sedation, elevations in heart rate, incomplete eyelid closure, nictation, emesis, and staggering. Additional clinical signs at the HD included pale mucous membranes, cyanosis, accelerated respiration, and red discoloration of the urine. Reductions in RBC were observed at the MD and HD. Necropsy of the HDF revealed discoloration of the kidneys and spleen; blackish discoloration of the spleen was also observed at the LD and MD. Histopathology revealed hemosiderin deposits, renal infarction, and increased extramedullary hematopoiesis in the liver in the HDF. Increases in extramedullary hematopoiesis in the spleen were observed at the MD and in the HDM and LDF. The histology findings were consistent with hemolytic anemia.

Metabolites

The toxicity of the MCI-186 sulfate conjugate was evaluated in mice (5/sex/group) administered 0, 439, or 877 mg/kg 186S by IV bolus. There was no mortality. Transient CNS signs, including decreases in locomotor activity, abnormal gait, and abnormal breathing, were observed at both doses, and prone position, tonic convulsions, and lacrimation were observed at the HD. All clinical signs resolved within 2 h of dosing. There were no drug-related gross findings at necropsy. Histopathology was not evaluated.

The toxicity of the glucuronide conjugate was evaluated in male ICR mice (5/group) administered a single dose of 0 or 2000 mg/kg metabolite by IV injection. Animals were monitored for 4 h post injection, and then daily for 14 days. All animals survived until scheduled necropsy. There were no abnormal clinical signs or gross findings. Histopathology was not evaluated.

6.2 Repeat-Dose Toxicity

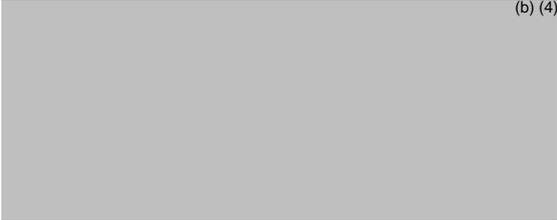
Study title: **Second screening study: 2-week toxicity test in rats**

Study no.: 1133
Study report location: EDR
Conducting laboratory and location: Not provided
Date of study initiation: January 24, 1986
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: Not provided

Findings

Male and female Wistar rats (4/sex/group control; 6/sex/group, drug) were administered 0, 3, 10, 30, or 100 mg/kg MCI-186 for 2 weeks by daily IV injection. There were no clinical signs at 3 or 10 mg/kg. Blinking, ptosis, and red discoloration of the urine were observed at 30 and 100 mg/kg; discoloration was not accompanied by occult blood. Mean body weights in HDM were approximately 6% lower than controls and correlated with slight reductions in food consumption. All animals survived until scheduled necropsy; there were no remarkable hematologic, clinical chemistry, gross pathology, or histological findings.

Study title: **Two-Week Intravenous Dose Range Finding Study of MY-7906 (MCI-186) in Rats**

Study no.: 1146-0
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: February 14, 1986
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: MCI-186, Lot EPE-7951, 100.3%

Findings

Male and female Wistar rats (6/sex/group control) were administered 0, 200, 300, 400, or 500 mg/kg MCI-186 for 2 weeks by daily IV injection. All animals survived until scheduled necropsy. Blinking, injection site irritation, and deficits in righting reflex were observed at all doses, but were more pronounced with increasing dose. Dose-dependent reductions in weight gain (up to 27% relative to control) occurred at all doses in males. Food consumption was decreased at all doses in males and at doses greater than 300 mg/kg in females. Dose-dependent decreases in RBC, hematocrit, and hemoglobin, and increase in WBC, platelets, and total bilirubin were observed in males

and females. Increases in reticulocytes were seen at 500 mg/kg in males and at doses greater than 300 mg/kg in females. Necropsy revealed enlargement and congestion of the spleen along with histological signs of extramedullary hematopoiesis, erythrophagocytosis, and deposition of yellow/brown pigment in males and females at doses greater than 300 mg/kg. There were no changes in bone marrow.

Study title: Maximum Tolerated Dosage Study by Continuous Infusion to CD Rats

Study no.: MUB088
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: April 17, 1998
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot P-RSV-64912R, 99.9%

Methods

Doses: 0, 100, 300, 1000 mg/kg/day
 Frequency of dosing: Continuous infusion for 7 days
 Route of administration: IV
 Dose volume: 4 mL/kg/h
 Formulation/Vehicle: Saline
 Species/Strain: CD rats
 Number/Sex/Group: 5/sex/group
 Age: 6 to 8 weeks at initiation
 Weight: 252 to 537 g (males), 207 to 301 g (females)
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: Two additional males were added to the HD group on Day 5 due to loss of patency.

Observations and Results

There were no drug-related mortalities. Clinical signs included orange or red discoloration of the cage tray paper, likely due to urine discoloration. Mean weight gain was decreased by 80% in HDM relative to control. Mean food consumption was decreased by 28% and 25% in HDM and MDM, respectively, relative to controls. Hematology and clinical chemistry evaluations on Day 7 revealed minimal decreases in RBC and hemoglobin and increases in prothrombin time. There were no drug-related gross findings.

Study title: A 14-Day Intravenous Infusion Toxicity Study of MCI-186 in the Albino Rat

Study no.: 56688
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: March 7, 2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot 99002, 99.96%

Methods

Doses: 0, 300, 1000 mg/kg/day
Frequency of dosing: Continuous infusion
Route of administration: IV
Dose volume: 48 mL/kg at 2 mL/kg/h
Formulation/Vehicle: Saline
Species/Strain: Male CD rats
Number/Sex/Group: 3/group
Age: 65 to 67 days at initiation
Weight: 298 to 336 g
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Observations and Results

There were no mortalities or drug-related clinical signs. Weight gain in HDM was decreased by 90% relative to control. There were no drug effects on food consumption. Hematology findings suggest regenerative anemia. Increases in BUN and cholesterol may have been drug-related. There were no abnormal gross findings.

Study title: A 1-Month Intravenous Dose Toxicity Study of MY-7906 in Rats

Study no.: 1146
Study report location: EDR
Conducting laboratory and location:  (b) (4)

Date of study initiation: April 30, 1986
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot EPE-7951, 100.3%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
Frequency of dosing: Once/day
Route of administration: IV injection
Dose volume: 3 mL/kg
Formulation/Vehicle: Saline
Species/Strain: Male and female Wistar rats
Number/Sex/Group: 12/sex/group (main); 6/sex/group (recovery)
Age: 6 weeks at initiation
Weight: 162.0 to 186.2 g (males), 115.2 to 136.0 g (females)
Satellite groups: 2 week recovery
Unique study design: None
Deviation from study protocol: A list of protocol deviations was not provided

Observations and Results

There were no mortalities. Clinical signs in males and females occurred immediately after dosing and included nictation and orange staining of the bedding at doses greater than 10 mg/kg, and incomplete eyelid closures and lacrimation at doses greater than 30 mg/kg. Decreases in righting reflex occurred at the HD immediately after administration, which transitioned within 5 min to unsteady gait. All clinical signs resolved within 24 h. Weight gain in HDM and HDF was reduced up to 16 and 12% relative to control. Reductions in weight gain up to 5% relative to control were also observed in males at 100 mg/kg. Weight gain in the affected groups returned to control levels over the 2 week recovery period. Food consumption at the HD was reduced by approximately 20% relative to controls, but resolved over the recovery period. Urinalysis revealed orange staining at doses greater than 10 mg/kg, and was thought to be due to excretion of MCI-186 or a metabolite, since there were no accompanying histologic changes. There were no drug-related ophthalmological findings. Decreases in RBC, HT, and Hb, and increases in reticulocytes and MCV suggested regenerative anemia at the HD. Signs of anemia generally resolved over the recovery period, although RBC remained decreased

by 8 and 4% in HDM and HDF, respectively. These findings correlated with increases in bone marrow erythroid series at the HD after the dosing period. Bone marrow changes resolved over the recovery period. Black splenic discoloration and increases in spleen weight at the HD were accompanied by histologic signs of congestion and extramedullary hematopoiesis, but generally resolved over the recovery period. The NOAEL for this study was 10 mg/kg.

Finding	Sex	Dose (mg/kg/day)				
		0	10	30	100	300
RBC ($10^4/\mu\text{L}$)	M	976	993	997	1006	853*
	F	857	856	856	857	765*
HT (%)	M	50.2	51.3	52.0	52.9	46.9*
	F	41.8	41.7	41.4	41.4	39.8*
HB (g/dL)	M	16.8	17.1	17.2	17.0	15.0*
	F	15.8	15.8	15.7	15.7	14.4*
Retic (%)	M	20.5	18.3	17.3	18.3	42.5*
	F	16.5	16.8	17.2	16.3	38.0*
MCV (pg)	M	51.4	51.6	52.2	52.6	55.1*
	F	48.8	48.8	48.4	48.4	52.0*

* indicates statistically significant difference ($p < 0.05$) from controls

Study title: A 28-Day Intravenous Infusion Toxicity Study of MCI-186 in the Albino Rat with a 14-Day Recovery Period

Study no.: 56539

Study report location: EDR

Conducting laboratory and location:



Date of study initiation: June 1, 2001

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: MCI-186, Lot 99002, 99.9%

Methods

Doses: 0, 50, 100, 300, 1000 mg/kg/day (main); 0, 300, 1000 mg/kg/day (recovery); 50, 100, 300, 1000 mg/kg/day (TK)

Frequency of dosing: Continuous infusion

Route of administration: IV

Dose volume: 1 to 2 mL/kg/h, 24 to 48 mL/kg/day

Formulation/Vehicle: Saline

Species/Strain: Male and female SD rats

Number/Sex/Group: 14/sex/group (main),
Age: 10 weeks at initiation

Weight: 273 to 372 g (males), 188 to 249 g (females)

Satellite groups: TK

Unique study design: None

Deviation from study protocol: There were no significant deviations.

Mortality

Animals were monitored for mortality or signs of morbidity twice daily. Single deaths occurred at 300 (female) and 1000 (male) mg/kg/day, but were thought to be procedure-related.

Clinical Signs

Detailed examinations were performed weekly over the dosing period; there were no drug-related clinical signs.

Body Weights and Food Consumption

Body weights and food consumption were measured weekly. Mean body weight gain relative to controls was decreased in HDM (36%), MDF (23%), and HDF (58%). Mean food consumption was reduced by approximately 13% in HD males and females relative to controls. Mean weight gain and food consumption at all doses were comparable to controls over the recovery period.

Ophthalmoscopy

Main and recovery groups were evaluated pretreatment and during Week 4 by indirect ophthalmoscopy and slit lamp biomicroscope; there were no drug effects.

ECG

Not evaluated

Hematology, Coagulation, Clinical Chemistry, and Urinalysis

Blood samples were collected prior to necropsy from fasted animals. Urine samples were collected towards the end of the dosing and recovery phases from individually housed animals (i.e., metabolic cages). Decreases in RBC, Hb, and Ht, and increases in reticulocytes and MCV suggest regenerative anemia at the HD, which generally resolved over the recovery period. Clinical chemistry findings included increases in urea and cholesterol at the HD relative to control; these findings resolved over the recovery period.

Finding (Dosing phase)	Sex	Dose (mg/kg)				
		0	50	100	300	1000
RBC ($10^6/\mu\text{L}$)	M	8.436	8.424	8.321	8.597	7.127*
	F	8.091	8.126	8.286	8.129	6.606*
HT (%)	M	45.08	46.28	45.6	47.25	44.25
	F	45.22	45.14	16.05	44.67	39.79*
HB (g/dL)	M	14.94	15.31	14.99	15.59*	14.2*
	F	15.07	15.00	15.23	14.99	12.91*
Retic (%)	M	2.21	2.44	2.22	2.19	5.28*
	F	2.44	2.34	2.51	2.03	5.74*
MCV ($\text{fL}/\mu\text{m}^3$)	M	53.44	54.99	54.78	54.98	62.14*
	F	55.96	55.61	55.66	54.94	60.38*
Urea (mg/dL)	M	19.21	18.99	21.55	20.39	24.81
	F	20.87	20.26	20.26	19.36	25.82*
Cholesterol (mg/dL)	M	54.6	56.6	54.7	56.1	77.2*
	F	63.7	57.9	62.0	64.1	77.4*

* indicates statistically significant difference ($p < 0.05$) from controls

Gross Pathology

Enlargement of the thyroid gland was noted in 2/15 HDM.

Organ Weights

Absolute thyroid weights were elevated by 65 and 29% relative to controls in HDM and HDF, respectively. Absolute spleen weights were elevated 40% relative to controls in HDF.

Histopathology

Adequate Battery: Yes

adrenal glands	heart	ovaries	spleen
aorta	ileum	pancreas	stomach
bone and bone marrow	infusion site	pituitary	testes
brain	jejunum	prostate	thymus
cecum	kidneys	rectum	thyroid lobes
colon	liver	salivary glands	tongue
duodenum	lungs	sciatic nerve	trachea
epididymides	mandibular LN	seminal vesicles	urinary bladder
esophagus	mesenteric LN	skeletal muscle	uterus
eyes	mammary gland	skin	vagina
Harderian glands	optic nerves	spinal cord	

Peer Review: Yes

Signed Pathology Report: Yes

Histological Findings: Drug-related histological findings were limited to the HD, and included follicular cell hypertrophy in the thyroid gland and increases in hematopoietic activity in the liver, spleen, and bone marrow. Histological findings generally resolved over the recovery period, although pigmentation was still visible in spleen and liver. Renal tubular basophilia was not noted by the sponsor but was observed in 5/15 HDM.

Finding	Sex	Main (mg/kg/day)					Recovery (mg/kg/day)		
		0 14M/14F	50 14M/14F	100 14M/14F	300 14M/15F	1000 15M/14F	0 7M/7F	300 7M/6F	1000 6M/7M
Bone Marrow									
hematopoiesis	M	0	0	0	0	1	0	0	1
	F	0	0	0	0	1	0	0	0
Kidney									
tubular basophilia	M	1	0	0	0	5	0	0	0
	F	1	0	0	0	0	0	0	0
Liver									
hematopoiesis	M	0	0	0	0	2	0	0	0
	F	0	0	0	0	1	0	0	0
pigment deposits	M	0	0	0	0	2	0	0	1
	F	0	0	0	0	2	0	0	3

Finding (cont.)	Sex	Main (mg/kg/day)					Recovery (mg/kg/day)			
		0 14M/14F	50 14M/14F	100 14M/14F	300 14M/15F	1000 15M/14F	0 7M/7F	300 7M/6F	1000 6M/7M	
Spleen hematopoiesis	M	1	3	4	2	15	1	6	3	
	F	0	1	1	2	13	1	0	1	
	pigment deposits	M	0	0	1	0	13	0	0	6
		F	1	0	1	0	13	5	3	7
Thyroid follicular hypertrophy	M	0	0	0	0	14	0	0	0	
	F	0	0	0	0	13	0	0	0	

Special Evaluation

None

Toxicokinetics

Steady-state was achieved 3 h after the initiation of dosing. Increases in C_{ss} and AUC_{1day} were generally dose proportional. There were no sex differences in plasma exposure.

Parameter (steady-state)	Sex	Dose (mg/kg/day)			
		50	100	300	1000
C_{ss} ($\mu\text{g/mL}$)	M	0.73	1.28	3.80	13.59
	F	0.54	1.29	4.43	16.23
AUC_{1day} ($\mu\text{g}\times\text{h/mL}$)	M	16.87	29.14	83.86	315.11
	F	12.52	29.46	102.75	387.33

Dosing Solution Analysis

All dosing solutions were within 10% of their respective target concentrations.

Study title: Chronic Toxicity Study in Rats Treated Intravenously with MCI-186 for 6 Months

Study no.: B-996
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: July 30, 1988
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot DCJ-1567, 100.4%

Methods

Doses: 0, 10, 30, 100 mg/kg (main); 0, 30, 100 mg/kg (recovery)
Frequency of dosing: Once daily
Route of administration: IV bolus
Dose volume: 3 mL/kg at 2 mL/min
Formulation/Vehicle: Saline
Species/Strain: SD rats
Number/Sex/Group: 22/sex/group (main), 8/sex/group (recovery)
Age: 6 weeks at initiation
Weight: 141 to 162 g (males), 116 to 135 g (females)
Satellite groups: 4-week recovery period.
Unique study design: None
Deviation from study protocol: A list of study deviations was not provided

Mortality and Clinical Signs

Animals were monitored for mortality, morbidity, and clinical signs 3 times per day during the dosing period. One MDM died after dosing on Day 170; necropsy revealed a ruptured liver, possibly caused by intrahepatic stasis through an unknown mechanism. One MDF died on Day 127, possibly due to heart failure. Neither death was thought to be drug-related.

Transient clinical signs were observed at the MD and HD, including eye blinking, head shaking, lacrimation, salivation, ptosis, preening, staggering gait, decreases in spontaneous movement, and orange staining in the urogenital area.

Body Weights

Animals were weighed prior to dosing, twice per week during Weeks 1 to 113, and once per week for the remainder of the study. Reductions in weight gain of 10 and 20% relative to control were observed in MD and HD, respectively, over the dosing period.

Weight gains over the recovery period were elevated relative to control by 61 and 86% in MDM and HDM, respectively, and 9 and 29% in MDF and HDF, respectively.

Food Consumption

Daily food consumption was estimated from 7-day cumulative consumption. Minimal decreases in food consumption relative to controls were observed at the HD during the dosing period. There were no drug effects on food consumption during the recovery period.

Ophthalmoscopy

Examinations were conducted with a fundus camera prior to dosing, during Months 1, 3, and 6, and during Week 4 of the recovery period. There were no drug-related findings.

ECG

Not evaluated

Hematology, Coagulation, Clinical Chemistry

Blood samples were collected from fasted animals at the time of necropsy.

Hematology findings included increases in PT (HDM), decreases in platelets (HDM, HDF), and a trend towards decreased RBC (MDF, HDF). Hematology findings resolved over the recovery period. No drug-related findings were observed on bone marrow smears. Clinical chemistry findings included increases in AP (HDF) and decreases in total cholesterol (HDM) and triglycerides (HDM and HDF). Decreases in platelets and total cholesterol were observed after recovery in MDM and HDM.

Finding	Sex	Main (mg/kg)				Recovery (mg/kg)		
		0	10	30	100	0	30	100
PT (sec)	M	13.8	12.9	14.0	15.9*	13.4	13.9	13.0
	F	11.3	11.1	11.2	11.1	11.1	11.3	11.4
Platelets (mg/dL)	M	116.7	113.7	112.4	94.5*	114.3	98.8*	99.5*
	F	173.5	168	162.8	144.9*	177.1	174.8	159.6
RBC ($\times 10^4/\mu\text{l}$)	M	1012.8	1023.1	1010.6	988.0	996.3	1006.9	1000.4
	F	875.7	870.0	851.2*	859.7	865.8	879.6	869.1
AP (U/L)	M	183.2	185.4	180.9	180.7	186	190.6	188
	F	135.3	133.7	149.4	162.6*	132.6	148.3	150
Tot. Cholesterol (mg/dL)	M	73.7	71.7	70.1	56*	72.4	59.5*	61.5*
	F	108.6	104	101.7	89.2	111.9	109.4	98.5
Triglycerides (mg/dL)	M	92.2	91.5	85.5	61.7*	87.1	76.3	65.9
	F	36.6	37.1	31.7	29.5*	37	37	35

* indicates statistically significant difference from control ($p < 0.05$)

Urinalysis

Urine from fasted animals was evaluated prior to dosing, during Months 1, 3, and 6, and during Week 4 of the recovery period. Increases in ketone bodies and bilirubin were observed at 1, 3, and 6 months, which were likely drug-related but resolved over the recovery period.

Urinalysis Findings	Sex	Dose (mg/kg)			
		0 30M/30F	10 22M/22F	30 30M/30F	100 30M/30F
Month 1					
Ketone Bodies	M	4	1	7	12
	F	1	0	0	2
Bilirubin	M	4	3	4	15
	F	2	1	1	3
Month 3					
Ketone Bodies	M	11	11	17	22
	F	0	0	0	0
Bilirubin	M	9	8	7	19
	F	0	0	0	3
Month 6					
Ketone Bodies	M	17	16	24	26
	F	3	2	2	4
Bilirubin	M	5	6	14	19
	F	1	2	2	6
Recovery		8M/F	0M/0F	8M/8F	8M/8F
Ketone Bodies	M	4	--	2	1
	F	1	--	0	1
Bilirubin	M	3	--	1	1
	F	1	--	0	2

Gross Pathology

Orange/brown staining of the urogenital area was observed in 1/22 HDM, 5/22 MDF, and 22/22 HDF. Similar findings were observed after the recovery period in 3/8 HDM, 3/8 MDF, and 8/8 HDF.

Organ Weights

Slight decreases in liver/body weight ratios in MDF and HDF, and increases in adrenal/body weight ratios in HDM may have been drug-related, but were not observed following the recovery period.

Histopathology

Adequate Battery: Yes

(absolute organ weight): brain, pituitary, salivary gland (submandibular and sublingual glands), thymus, thyroid (including parathyroid), heart, lung (including bronchus), liver, kidney, spleen, adrenal, testis, ovary, prostate and uterus. Organ weight per 100 g body weight (relative weight) was calculated based on the absolute organ weight and body weight on the day of necropsy. Besides the above organs in which organ weight was measured, the following organs/tissues were fixed with phosphate buffered 10% formalin (however, the eyeball, optic nerve and Harderian gland were fixed with 4% glutaraldehyde), hematoxylin and eosin staining specimens were made by the routine methods and were subjected to histopathological examination: trachea, pancreas, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph node, urinary bladder, epididymis, seminal vesicle, vagina, sternum (including bone marrow), femur (including bone marrow), eyeball, optic nerve, Harderian gland, thoracic aorta, cervical lymph node, femoral muscle, spinal cord, sciatic nerve, skin, mammary gland and injection site. The animals found dead were examined to the extent of possible.

(Sponsor's Text)

Signed Pathology Report: Yes

Peer Review: No

Histological Findings: There were no drug-related findings.

Special Evaluation

None

Toxicokinetics

Supporting TK data were submitted in Study B031605: "A 7-Day Repeated Intravenous (Bolus) Dose Toxicokinetic Study of MCI-186 in Rats."

Dosing Solution Analysis

Test solutions were sent to the sponsor for analysis, but the results were not included in the study report.

Study title: Two-Week Intravenous Dose Range Finding Study of MY-7906 (MCI-186) in Beagle Dogs

Study no.: 1159-0
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: May 27, 1986
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: MCI-186, Lot EPE-795, 100.3%

Observations and Results

Male and female beagle dogs (1/sex/group) received 0, 100, 200, 300, or 400 mg/kg MCI-186 once daily for 14 days by IV bolus. Transient clinical signs including salivation, sedation, sneezing, ataxia, ptosis, and yellow/brown urine were observed at doses greater than 100 mg/kg. The HDF died 1 h after the sixth dose, likely due to anemia; prior to death, HDF body weight was reduced by 13% relative to baseline and was accompanied by an approximate 80% reduction in food consumption. At necropsy, enlarged, blackish spleens and decreases in RBC, hematocrit, and hemoglobin were observed at doses greater than 100 mg/kg. Increases in reticulocytes and platelet counts were observed at doses greater than 200 mg/kg. Histological changes included deposition of hemosiderin in the liver, spleen, bone marrow, and lymph nodes in the HDM and in females at doses ≥ 200 mg/kg. The NOAEL for this study is 100 mg/kg based on CNS signs and regenerative anemia.

Finding	Sex	Dose (mg/kg)				
		0	100	200	300	400
Spleen Weight (g)	M	17.9	26	37.4	38.2	64.3
	F	17.4	18.2	26.9	43.6	DOS
RBC ($10^4/\mu\text{L}$)	M	637	641	611	532	535
	F	730	668	621	546	DOS
HT (%)	M	41.2	41.4	38.2	38.1	40.4
	F	44.9	42.8	42.2	40.5	DOS
HB (g/dL)	M	14.5	14.4	12.7	12.1	12.20
	F	16.6	14.30	13.8	12.3	DOS
Retic (%)	M	17	7	19	45	25
	F	5	10	14	42	DOS
Platelets ($10^3/\mu\text{M}$)	M	327	292	258	418	471
	F	319	342	261	486	DOS

Study title: Maximum Tolerated Dosage by Continuous Infusion to Beagle Dogs

Study no.: MUB087
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: April 8, 1998
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, P-RSV-54912R, 99.9%

Observations and Results

Male and female beagle dogs (1/sex/group) received 100, 300, or 1000 mg/kg/day MCI-186 by continuous infusion for 7 days; there was no control group. All animals survived the study. There were no drug-related clinical signs or effects on weight gain or food consumption. Hematology on Day 8 revealed decreases in RBC, Hb, and Hct, and increases in reticulocyte counts and bilirubin at the MD and HD relative to baseline. Gross examination at necropsy revealed yellow discoloration of the gastric mucosa in the HDM, HDF, and MDF. Increases in AUC were greater-than dose proportional, but there were no sex differences.

Finding	Sex	Baseline			Day 8		
		100	300	1000	100	300	1000
RBC ($10^{12}/\mu\text{L}$)	M	5.65	5.80	6.49	5.65	5.60	4.59
	F	6.25	6.36	6.01	6.25	5.96	4.67
HT (%)	M	37.9	38.6	42.6	42.3	36.7	30.7
	F	42.9	39.9	39	40.8	37.8	32.0
Hb (g/dL)	M	12.2	12.9	14.4	14	12.3	10
	F	14.9	13.30	13	13.9	12.7	10.3
Retic (%)	M	0.2	1.7	0.3	0.2	2.3	2.0
	F	1	1.9	0.2	0.4	1.2	1.4
Bilirubin (md/dL)	M	0.1	0.1	0.1	0.1	0.1	0.4
	F	0.1	0.1	0.1	0.1	0.1	0.3

Parameter	Sex	Dose (mg/kg/day)		
		100	300	1000
AUC _{7 days} ($\mu\text{g}\times\text{h}/\text{mL}$)	M	3.3	23.1	49.1
	F	3.6	16.3	77.1

Study title: A 14-Day Intravenous Infusion Toxicity Study of MCI-186 in the Beagle Dog

Study no.: 56689
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: March 7, 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot 99002, 99.94%

Observations and Results

Male beagle dogs (1/group) were administered 300 or 1000 mg/kg/day MCI-186 by continuous IV infusion over 14 days; there was no control animal. Clinical signs appeared in the second week at both doses, and included slight reductions in food consumption, uncoordinated gait, tremors, decreased activity, and loss of muscle tone in the hindlimbs which progressed to include the forelimbs as well. Dosing was stopped on Day 10 at the HD; however clinical signs continued to worsen and the animal was euthanized on Day 12. Body weight on Day 14 was reduced by 7% relative to baseline; a terminal body weight was not recorded at the HD. Hematology was unremarkable at the LD; however, signs of anemia including reduced RBC counts, hemoglobin, and hematocrit, and increased reticulocytes and total bilirubin were observed at the HD. Increased ALT was also observed at the HD. Bilateral sciatic nerve degeneration with digestion chambers was observed at both doses; there were no other drug effects on histology. Based on CNS signs and sciatic nerve degeneration, there was no NOAEL.

Dose mg/kg/day	Day	RBC ×10 ⁶ μL	Hct %	Hb G/dL	Retic %	Tot. Bil mg/dL	ALT U/L
300	Pre	6.83	44.6	15.3	0.2	0.14	40
	Day 14	6.81	45.7	15.3	0.5	0.33	35
1000	Pre	6.43	42	14.5	0.4	0.07	30
	Day 12	4.77	34.6	11.2	4.6	0.38	156

Study title: A 1-Month Intravenous Dose Toxicity Study of MY-7906 (MCI-186) in Beagle Dogs

Study no.: 1159
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: March 23, 1986
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot EPE-7951, 100.3%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
Frequency of dosing: Once per day
Route of administration: IV bolus
Dose volume: 0.1 to 3 mL/kg; 10 mL/min
Formulation/Vehicle: Saline
Species/Strain: Beagle dogs
Number/Sex/Group: 3/sex/group
Age: 6 months
Weight: 7.4 to 9.7 kg (males), 6.6 to 8.5 kg (females)
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Mortality and Clinical Signs

Animals were monitored twice daily for mortality or signs of morbidity. All animals survived until scheduled necropsy. Detailed clinical observations were performed once per week. No line listing for clinical signs were provided. However, based on the sponsor's summary, clinical signs at doses greater than 30 mg/kg included sedation, rear limb weakness, nictation and sneezing, and salivation. Additional observations at the HD included incomplete eyelid closure, abnormal phonation, and lacrimation. There were no drug effects on respiratory rate, blood pressure, or body temperature.

Body Weights

Body weight and food consumption were recorded weekly; there were no drug effects.

Ophthalmoscopy

Examinations by slit lamp biomicroscope and fundus camera were performed prestudy and during Weeks 2 and 4; there were no drug effects.

ECG

Electrocardiograms were recorded prestudy and during Weeks 2 and 4; there were no drug effects.

Hematology, Coagulation, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected prestudy, and during Weeks 2 and 4. Hematology findings indicated anemia at the HD. Bone marrow smears revealed reductions in myeloid/erythroid ratio of approximately 50% relative to baseline at the HD. Clinical chemistry revealed an approximate 2-fold increase from baseline in total bilirubin at the HD. Brown/red discoloration of the urine was observed at the HD.

Dose mg/kg	Week	Males					Females				
		RBC ×10 ⁴ /μL	Hct %	Hb G/dL	Retic %	Platelets ×10 ³ /μL	RBC ×10 ⁴ /μL	Hct %	Hb G/dL	Retic %	Platelets ×10 ³ /μL
0	0	696.0	45.4	15.3	6.3	289.7	751.0	47.5	14.9	5.3	204.0
	2	631.7	39.8	13.8	2.3	350.3	705.3	44.0	15.2	5.0	268.3
	4	635.0	41.8	14.6	7.0	362.3	782.3	49.1	15.9	7.3	339.7
10	0	744.0	47.3	14.6	3.0	308.7	756.7	48.5	15.4	4.7	318.0
	2	697.0	43.5	14.6	3.3	324	748.7	46.8	16.3	9.3	336.0
	4	731.3	41.4	14.8	9.7	320	718.0	47.1	15.6	13.3	364.3
30	0	655.0	42.0	14.9	6.0	391	718.7	47.9	16.6	6.0	141.0
	2	675.0	41.9	13.8	6.7	396.3	711.7	45.4	15.7	5.3	280.0
	4	625.3	41.7	13.6	9.3	475.3*	745.3	51.6	16.1	12.0	249.3
100	0	665.3	43.2	13.9	6.3	253.7	726.0	45.4	15.6	5.7	240.3
	2	658.3	42.7	14	7.3	303	658.3	41.0	13.9	8.0	274.0
	4	677.0	44.6	14.2	13.3	340.7	680.0	46.3	14.8	13.0	321.0
300	0	677.3	44.0	14.5	4.0	324	656.7	43.3	14.7	5.0	228.7
	2	484.0	35.3	10.8	35.7	479.7*	454.3*	33.2*	9.9*	39.0*	439.7 ^a
	4	596.3	38.2	12.5	33	483.3*	554.7*	41.1	11.8*	37.3*	237.3

* Indicates statistically significant difference ($p < 0.05$) from baseline; ^a indicates high variability.

Gross Pathology

Spleens were enlarged in 1/3 MHDF, 2/3 HDM, and 3/3 HDF; blackish discoloration of the spleen was observed in 1/3 MHDF, 3/3 HDM, and 3/3 HDF.

Organ Weights

Mean absolute liver weight was increased by approximately 20% relative to control at the HD. Mean absolute spleen weight was increased by 80 and 154% relative to controls in HDM and HDF, respectively. Mean absolute prostate weights at all doses were reduced by 44 to 63% relative to control.

Histopathology

Adequate Battery: Yes

Cardiovascular system:	heart, aorta
Hematopoietic system:	bone marrow (sternum, femur), lymph nodes (submandibular, mesenteric, tonsil), spleen, thymus
Nervous system:	brain (cerebrum, brainstem, cerebellum, medulla oblongata), spinal cord, sciatic, nerve, eye (optic nerves)
Endocrine system:	pituitary, thyroid gland, parathyroid, adrenals, Langerhans` islands (included in pancreas)
Respiratory system:	lungs, trachea, bronchi (included in lungs)
Digestive system:	tongue, salivary gland (submandibular, parotid), liver, gallbladder, pancreas, esophagus, stomach (fundus, pylorus), small intestines (duodenum, jejunum, ileum), large intestine (colon)
Urinary system:	kidneys, urinary bladder
Genital system:	testes, epididymides, prostate, ovaries, uterus, vagina
Others:	skin, mammary glands, skeletal muscle, femur (caput, diaphysis), lacrimal gland, administration sites (forearm), gross lesions.

(Sponsor's Table)

Signed Pathology Report: Yes

Peer Review: No

Histological Findings: Splenic congestion was observed in 1/3 MHDF, 3/3 HDM, and 3/3 HDF. Splenic hemosiderin deposits were observed in 2/3 HDF. Increased hematopoiesis was observed in 2/3 HDM and 2/3 HDF. Hemosiderin deposits were observed in hepatic Kupffer cells in 2/3 HDM and 2/3 HDF.

Special Evaluation

None

Toxicokinetics

None

Dosing Solution Analysis

Not provided.

Study title: A 4-Week Intravenous Infusion Toxicity Study of MCI-186 in Beagle Dogs

Study no.: 7N085
Study report location: EDR
Conducting laboratory and location:  (b) (4)

Date of study initiation: December 25, 1987
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: MCI-186, Lot EPE-7951, 100.3%

Methods

Doses: 0, 10, 30, 100 mg/kg
Frequency of dosing: Once per day
Route of administration: 2 h IV infusion
Dose volume: 24 mL/kg
Formulation/Vehicle: Saline
Species/Strain: Male beagle dogs
Number/Sex/Group: 3/group
Age: 6 months
Weight: 7.8 to 9.3 kg
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Observations and Results**Mortality, Clinical Signs, Body weight, and Food Consumption**

Animals were monitored twice daily for mortality or clinical signs. All animals survived until scheduled necropsy. There were no drug-related clinical signs or effects on body weight, food consumption, body temperature, heart rate, or respiratory rate.

Ophthalmoscopy

Examinations by slit lamp biomicroscope and fundus camera were performed prestudy and during Weeks 2 and 4; there were no drug effects.

ECG

Evaluations were conducted prestudy and on Weeks 2 and 4; there were no drug-related findings.

Hematology, Coagulation, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected prestudy, and during Weeks 2 and 4. Hematology and clinical chemistry did not reveal any signs of anemia or other drug-related findings. Urinalysis did not reveal discoloration or other drug effects.

Gross Pathology and Organ Weights

There were no drug effects. Red discoloration was observed at the injection site in all groups, and was attributed to the dosing procedure.

Histopathology

Adequate Battery: Yes

Cardiovascular system:	heart, aorta
Hematopoietic system:	bone marrow (sternum, femur), lymph nodes (submandibular, mesenteric, tonsil), spleen, thymus
Nervous system:	brain (cerebrum, brainstem, cerebellum, medulla oblongata), spinal cord, sciatic nerve, eye (optic nerves)
Endocrine system:	pituitary, thyroid gland, parathyroid, adrenals, Langerhans' islands (included in pancreas)
Respiratory system:	lungs, trachea, bronchi (included in lungs)
Digestive system:	tongue, salivary gland (submandibular, parotid), liver, gallbladder, pancreas, esophagus, stomach (fundus, pylorus), small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon)
Urinary system:	kidneys, urinary bladder
Genital system:	testes, epididymides, prostate
Others:	skin, mammary gland, skeletal muscle, femur (caput, diaphysis), lacrimal gland, administration sites (forearm), gross lesions.

Sponsor's Table

Signed Pathology Report: No

Peer Review: No

Histological Findings: There were no drug-related findings.

Special Evaluation

None

Toxicokinetics

Supporting TK data were submitted in study B021679: A Single-Dose Intravenous (2-Hour Infusion) Toxicokinetic Study of MCI-186 in Beagle Dogs.”

Dosing Solution Analysis

Not provided.

Study title: A 28-Day Intravenous Infusion Toxicity Study of MCI-186 in the Beagle Dog with a 14-Day Recovery Period.

Study no.: 56562
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: June 1, 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot 99002, 99.92%

Methods

Doses: 0, 30, 60, 120, 200 mg/kg/day (main); 0, 120, 200 mg/kg/day (recovery)
 Frequency of dosing: Continuous infusion
 Route of administration: IV
 Dose volume: 12 to 48 mL/kg/day
 Formulation/Vehicle: Saline
 Species/Strain: Beagle dogs
 Number/Sex/Group: 4/sex/group (main); 2/sex/group (recovery)
 Age: 6 months
 Weight: 6.3 to 9.2 kg (males), 5.5 to 7.2 kg (females)
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: No HD animals were included in the recovery group due to excessive morbidity and unscheduled euthanasia. There were no other significant deviations from the protocol.

Mortality

Animals were monitored for mortality or signs of morbidity twice daily. One MHDM, 4 HDM, and 2 HDF were euthanized during the study due to severe deficits in limb use, dehydration, and weight loss (approximately 16% from baseline). The clinical signs leading to euthanasia were thought to be secondary to sciatic and spinal nerve fiber degeneration. An additional MHDM was found dead; however, the cause of death was determined to be acute pneumonia, and was not thought to be drug-related.

Group No./Treatment (mg/kg/day)	Animal No./Sex	Day of Unscheduled Euthanasia
4 (120)	4052♂	23
5 (200)	5031♂	23
	5042♂	22
	5052♂	25
	5062♂	22
	5511♀	23
	5531♀	23

Sponsor's Table

Clinical Signs

Detailed clinical examinations were performed daily. Progressive deficits in limb use were observed at the MHD and HD, typically affecting the hind limbs first, followed by the forelimbs 1 to 2 days later. Animals affected by limb use deficits also had signs of dehydration, thinness and coldness, and reddening of the eyeballs.

Body Weights

Body weights were measured weekly. Mean body weights on Day 28 were reduced by 6 and 16% relative to control in HDM and HDF, respectively.

Food Consumption

Food consumption was measured daily. Food consumption was reduced by up to 60% in MHD and HD animals with limited limb use.

Ophthalmoscopy

Examinations by indirect ophthalmoscopy and slit lamp biomicroscope were performed prestudy, during Week 4, and towards the end of the recovery period; there were no drug effects.

ECG

Electrocardiograms and blood pressure (using a tail cuff) were recorded prestudy, during Week 4, and towards the end of the recovery period; there were no drug effects.

Hematology, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected prestudy and on Weeks 4 and 7. There were no signs of anemia. RBC counts were increased up to 19% relative to baseline at the HD, but were thought to be due to dehydration. There were no other drug-related findings.

Gross Pathology

Dark discoloration of the thyroid was observed in 1 HDM and 1 HDF, but resolved during the recovery period.

Organ Weights

Absolute thyroid weights were increased by up to 100% in MHD and HD relative to controls, and did not completely resolve over the recovery period.

Histopathology

Adequate Battery: Yes

adrenals	kidneys	skeletal muscle
aorta	lachrymal gland	skin
bone and marrow	liver	spinal cord
brain	lungs	spleen
cecum	lymph nodes	stomach
colon	mammary gland	testes
duodenum	optic nerves	thymus
epididymides	ovaries	thyroid lobes
esophagus	pancreas	tongue
eyes	parathyroid glands	trachea
gall bladder	pituitary	urinary bladder
heart	prostate	uterus
ileum	rectum	vagina
infusion site	salivary gland	
jejunum	sciatic nerve	

Peer Review: No

Signed Pathology Report: Yes

Histological Findings: Histopathology revealed sciatic nerve, spinal cord, and skeletal muscle nerve fiber degeneration at doses greater than 30 mg/kg/day. Myofiber atrophy was observed at doses greater than 60 mg/kg/day, and was thought to be secondary to nerve fiber degeneration. Spinal and skeletal muscle nerve degeneration resolved to varying degrees over the recovery period; however, sciatic nerve degeneration at the HD did not resolve. Thyroid follicular cell distortion was observed at doses ≥ 60 mg/kg/day, but resolved over the recovery period.

Finding	Sex	Main					Recovery	
		0	30	60	120	200	0	120
<i>Animals/group</i>	M	4	4	4	5	6	2	1
	F	4	4	4	4	6	2	2
Skeletal muscle								
nerve fiber degeneration	M	0	0	0	3	6	0	1
	F	0	0	1	2	5	0	1
myofiber atrophy	M	0	0	0	1	2	0	0
	F	0	0	0	1	6	0	2
Sciatic nerve								
nerve fiber degeneration	M	0	0	1	5	6	0	1
	F	0	0	1	4	6	0	2
inflammation	M	0	0	1	3	4	0	1
	F	0	0	1	4	6	0	2
vascular necrosis	M	0	0	0	0	2	0	0
	F	0	0	0	0	1	0	0
Spinal Cord								
cervical fiber degeneration	M	0	1	1	1	2	1	0
	F	1	1	0	2	4	0	0
lumbar fiber degeneration	M	0	0	1	3	6	0	0
	F	0	0	1	4	6	0	2
thoracic fiber degeneration	M	0	0	0	0	4	0	0
	F	0	0	0	1	6	0	2
Thyroid								
follicular hyperplasia	M	0	0	1	3	6	0	0
	F	0	0	0	1	5	0	0

Special Evaluation

None

Toxicokinetics

Steady state was achieved 24 h after dosing on Day 1. Increases in C_{SS} and AUC were greater-than dose proportional. There were no sex differences in C_{SS} or AUC.

Dose level (mg/kg/day)	C _{ss} (µg/ml)		AUC _{1 day} (µg.h/ml)	
	Males	Females	Males	Females
30	0.13 (0.01)	0.13 (0.03)	3.46 (0.38)	3.72 (0.63)
60	0.28 (0.05)	0.31 (0.06)	7.19 (1.29)	7.92 (1.81)
120	0.62 (0.08)	0.82 (0.17)	15.24 (1.48)	20.72 (4.44)
200	1.67 (0.43)	1.61 (0.31)	40.45 (9.33)	39.64 (5.65)

(Sponsor's Table)

Dosing Solution Analysis

Dosing solutions were within 10% of their respective target concentrations.

Study title: 26-Week Chronic Study in Dogs

Study no.: 916-119
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: February 22, 1988
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot AWP 0041, 100%

Methods

Doses: 0, 10, 30, 100 (main), 0, 30, 100 (recovery)
 Frequency of dosing: Once daily for 26 weeks; recovery period was 5 weeks.
 Route of administration: IV bolus
 Dose volume: 0.1 to 1 mL/kg
 Formulation/Vehicle: Saline
 Species/Strain: Beagle dogs
 Number/Sex/Group: 4/sex/group (main), 2/sex/group (recovery)
 Age: 20-25 Weeks at initiation
 Weight: 5.9 to 8.7 kg (males), 5.2 to 7.1 kg (females)
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: No significant deviations

Mortality

Animals were observed twice daily for mortality or signs of morbidity. All animals survived until scheduled necropsy.

Clinical Signs

Detailed examinations were performed once daily after dosing. Drug-related effects at the HD included sporadic, transient limpness and decreases in activity, which usually resolved with 2 to 3 minutes of dosing.

Body Weights and Food Consumption

Body weights and food consumption were recorded once per week for Weeks 1-16, and once every 2 weeks for the remainder of the study. There were no drug effects on weight gain or food consumption.

Ophthalmoscopy

Animals were evaluated by indirect ophthalmoscopy on Weeks 5, 13, 26, and 31. There were no drug effects.

ECG

Evaluations were performed prior to dosing and on Weeks 4, 13, 26, and 31. There were no drug effects.

Hematology, Coagulation, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected from fasted dogs on Weeks 4, 13, 26, and 31. Decreases of approximately 10% from control values for RBC count, Hb, and Hct in the HDF was largely due to reduced values in two animals (Nos. 26046 and 26049). RBC parameters normalized over the recovery period. Myeloid/erythroid ratios were decreased in HDF (0.80) relative to control (1.33), but resolved over the recovery period. There were no drug effects on clinical chemistry. Dark yellow or brown discoloration of the urine was observed at the HD.

Gross Pathology and Organ Weights

There were no drug effects on gross pathology or organ weights.

Histopathology

Adequate Battery: Yes

skin	prostate
lesions	ovaries
brain with brainstem (medulla/pons, cerebellar cortex, and cerebral cortex)	uterus with vagina and cervix
pituitary	mammary gland (females only)
thyroid (parathyroids)	skeletal muscle
thymus	esophagus
lung	stomach
trachea	duodenum, jejunum, and ileum
heart	colon, cecum, and rectum
sternum with bone marrow	urinary bladder
salivary glands (mandibular, parotid, and sublingual)	mesenteric lymph node
liver	mandibular lymph node
spleen	sciatic nerve
kidneys	cervical spinal cord
adrenals	mid-thoracic spinal cord
pancreas	lumbar spinal cord
injection site	eyes (both)
testes with epididymides	gallbladder
	aorta
	femur
	bone marrow smear - differential

(Sponsor's Table)

Peer Review: No

Signed Pathology Report: Yes

Histological Findings: No drug-related findings.

Special Evaluation

None

Toxicokinetics

Study B020845: "A 7-Day Repeated Intravenous (Bolus) Dose Toxicokinetic Study of MCI-186 in Beagle Dogs" was conducted to support this study."

Dosing Solution Analysis

Dosing solutions were within 6% of their respective target concentrations.

Study title: A 14-Day Intravenous Infusion Toxicity Study of MCI-186 in the Male Cynomolgus Monkey

Study no.: 57394
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: March 26, 2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot 99002, 99.9%

Methods

Doses: 0, 10, 100, 1000 mg/kg/day
Frequency of dosing: Continuous infusion
Route of administration: IV
Dose volume: 4.8 to 48 mL/kg
Formulation/Vehicle: Saline
Species/Strain: Male cynomolgus monkeys
Number/Sex/Group: 2/group
Age: 26 to 39 months
Weight: 2.2 to 2.8 kg
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Mortality

Animals were monitored twice daily for mortality or signs of morbidity. All animals survived until scheduled necropsy.

Clinical Signs

Detailed clinical examinations were performed daily. There were no drug-related clinical signs.

Body Weights and Food Consumption

Body weights were recorded on Days -1, 7, and 14. Food consumption was measured daily. There were no drug effects on weight gain or food consumption.

Ophthalmoscopy

Not evaluated

ECG

Not evaluated

Hematology and Clinical Chemistry

Blood samples were collected prestudy and on Day 1; there were no drug effects on hematology or clinical chemistry.

Gross Pathology and Organ weights

There were no drug-related gross findings or effects on organ weight.

Histopathology

Adequate Battery: Yes

Adrenals	Ileum	Salivary gland
Aorta	Infusion site	Seminal vesicle
Bone and marrow	Jejunum	Skeletal muscle
Brain	Kidneys	Skin
Cecum	Lacrimal gland	Spinal cord
Colon	Liver	Spinal ganglion
Diaphragm	Lungs	Spleen
Dorsal nerve roots	Lymph nodes	Stomach
Ventral nerve roots	Mammary gland	Testes
Duodenum	Optic nerves	Thymus
Epididymides	Pancreas	Thyroid lobes
Esophagus	Peripheral nerves	Tongue
Eyes	Pituitary	Trachea
Gallbladder	Prostate	Urinary bladder
Heart	Rectum	

Peer Review: Yes

Signed Pathology Report: Yes

Histological Findings: Peripheral nerve fiber degeneration occurred in 1/2 HDM and was described as follows:

Animal No. 401

Minimal, highly localized, focal nerve fiber degeneration, characterized by focal nerve fiber swelling and the presence of digestion chambers in the lumbar spinal cord at levels L1 (ventral funiculus) and L3 (dorsal funiculus). Focal, minimal nerve fiber degeneration was also noted in the lumbar dorsal root ganglion, the lumbar ventral nerve root at L5 (unilateral, left) and the vestibulocochlear nerve (unilateral, left).

Some minimal, focal gliosis in the dorsal funiculus of the cervical, thoracic and lumbar spinal cord and a minimal, perivascular inflammatory cuffing, primarily eosinophilic in character, was observed in the mid-brain and the grey matter of the spinal cord at C2 and L3 were noted. These changes were considered to be incidental or spontaneous in nature, because similar changes were not observed in animals given the same dose level in a 4-week study ((b) (4) Project No. 57395). The neuronal lesions described above may have resulted from this inflammation and gliosis, but this cannot be confirmed.

*Sponsor's Text***Special Evaluation**

There were no drug effects on plasma or RBC acetylcholinesterase activity.

Toxicokinetics

Increases in plasma C_{max} and AUC were less-than dose proportional. CSF concentrations of MCI-186 were typically lower than plasma levels, but increases were greater-than dose proportional.

Males								
Group	Dose Level	Animal	C_{max}	C_{24h}	C_{ss}^*	t_{last}	$AUC_{0-t_{last}}$	AUC_{24h}^a
No.	mg/kg/day	No.	($\mu\text{g/mL}$)	($\mu\text{g/mL}$)	($\mu\text{g/mL}$)	(h)	($\mu\text{g}\cdot\text{h/mL}$)	($\mu\text{g}\cdot\text{h/mL}$)
2	10	201	0.0997	0.0862	0.0930	48	3.89	1.94
		202	0.0698	0.0611	0.0654	48	2.97	1.48
Mean			0.0848	0.0736	0.0792	48	3.43	1.71
3	100	301	1.46	1.06	0.727	335.5	422	30.2
		302	1.97	0.858	1.03	335.5	507	36.2
Mean			1.71	0.958	0.878	336	465	33.2
4	1000	401	26.5	17.0	15.9	335.5	4827	345
		402	36.0	18.8	20.3	335.5	6843	489
Mean			31.2	17.9	18.1	336	5835	417

^a Calculated by $AUC_{0-t_{last}} / 14$ days to approximate the AUC in a 24 hour period.

* Mean of steady state values per animal.

(Sponsor's Table)

Dosing Solution Analysis

Dosing solutions were within 5% of their respective target concentrations.

Study title: A 28-Day Intravenous Infusion Toxicity Study of MCI-186 in the Cynomolgus Monkey

Study no.: 57395
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: August 23, 2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot 02001, 99.9%

Methods

Doses: 0, 20, 100, 1000 mg/kg
Frequency of dosing: Continuous infusion
Route of administration: IV
Dose volume: 9.6 to 48 mL/kg/h
Formulation/Vehicle: Saline
Species/Strain: Cynomolgus monkeys
Number/Sex/Group: 4/sex/group
Age: 22 to 26 months
Weight: 2.1 to 3.3 kg (males), 1.7 to 2.5 kg females
Satellite groups: None
Unique study design: None
Deviation from study protocol: A list of deviations was not provided. The sponsor included the following comment:
"Occasional minor deviations from the protocol occurred and were documented in the raw data. These deviations were considered not to have had impact on the outcome of the study or upon the interpretation of the results."

Mortality

Animals were observed twice daily for mortality or signs of morbidity. One HDM (No. 404) was euthanized on Day 22 due to reduced activity and muscle tone, limited usage of fore and hindlimbs, lack of pinch reflex, hypersensitivity, shallow breathing, and uncoordinated body movement. The clinical signs in this animal were thought to be drug-related and were correlated with histological signs of peripheral nerve degeneration. One HDF (No. 453) was euthanized on Day 24 due to impaired renal function; however, based on histopathology, this was thought to be secondary to ureteral stricture rather than due to the test article.

Clinical Signs

Drug-related clinical signs typically occurred towards the end of Week 4 and included reductions in muscle tone (1/3 HDM, 3/3 HDF), and reduced activity, absent pinch reflex, and limited fore and hindlimb use (3/3 HDF).

Body Weights

Animals were weighed weekly. There were no drug effects on weight gain.

Food Consumption

Qualitative food consumption was evaluated daily. There were no drug effects on food consumption.

Ophthalmoscopy

Examinations by direct and indirect ophthalmoscope and slit lamp biomicroscope were performed prestudy and during Week 4. There were no drug-related effects.

ECG

ECG recordings were taken prestudy and during Week 4; there were no drug-related findings.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected prestudy and on Day 27. Urine samples were collected prestudy and during Week 4. It was unclear if minimal decreases in RBC parameters and increases in reticulocytes were drug-related. Increases in mean PT were drug-related. Mean serum creatinine was increased 2 to 3-fold in HDM and HDF, respectively, but was likely due to elevations in single animals. There were no drug-related urinalysis findings.

Dose mg/kg	Day	Males					Females				
		RBC ×10 ⁶ /μL	Hct %	Hb G/dL	Retic ×10 ⁹ /μL	PT sec	RBC ×10 ⁶ /μL	Hct %	Hb G/dL	Retic ×10 ⁹ /μL	PT sec
0	Prestudy	5.335	39.48	12.75	38.95	10.45	5.120	38.75	12.3	38.43	10.23
	27	5.208	39.35	12.45	61.53	10.63	4.895	37.23	12.00	72.08	10.38
20	Prestudy	4.903	37.03	11.88	47.68	10.65	4.923	37.15	11.83	41.03	10.68
	27	5.068	38.80	12.20	66.33	10.5	5.353	41.03	12.88	47.13	10.75
100	Prestudy	5.013	38.00	11.78	36.95	10.83	5.318	39.20	12.35	29.10	10.7
	27	4.933	37.40	11.65	42.85	10.58	5.248	39.35	12.30	63.35	10.95
1000	Prestudy	5.260	39.50	12.55	31.55	10.73	5.338	39.18	12.13	42.93	10.75
	27	4.903	37.80	11.70	86.68	11.48*	4.913	37.03	11.08	85.33	15.28*

*Hematology findings; * indicates statistically significant difference relative to baseline (p ≤ 0.05)*

Gross Pathology

There were no drug-related findings.

Organ Weights

Mean thymus weights were decreased by approximately 50% at the HD. Mean spleen weights were increased nearly 3-fold in HDF, but there was no histological correlate.

Histopathology

Adequate Battery: Yes

abnormalities	ileum	salivary glands
animal identification	infusion site	seminal vesicles
adrenal glands	jejunum	skeletal muscle
aorta	kidneys	skin
bone marrow	lacrimal gland	spinal cord (cervical, thoracic, lumbar)
brain	liver	spinal ganglion (cervical, thoracic, lumbar)
cecum	lungs	spleen
cervix	lymph nodes	stomach
colon	mammary gland	testes
diaphragm	optic nerves	thymus
nerve roots (cervical, thoracic, lumbar)	ovaries	thyroid lobes
duodenum	pancreas	tongue
epididymides	peripheral nerves	trachea
esophagus	pituitary	urinary bladder
eyes	prostate	uterus
gallbladder	rectum	vagina
heart		

Signed Pathology Report: Yes

Peer Review: Yes

Nerve Fiber Degeneration

Minimal to marked nerve fiber degeneration was observed in the central and peripheral nervous systems of all HD animals. The affected axons were characterized by elliptical swelling/ballooning of the axon sheath, digestion chambers, and macrophages and other inflammatory infiltrate. Severe degeneration was more commonly observed in the larger peripheral nerves (i.e., radial, sciatic, tibial, and cauda equina). Degeneration of spinal nerves was more severe in the lumbar segments. Dorsal and ventral roots of the spinal nerves were minimally affected.

Nerve Fiber Degeneration	Males				Females			
	0	20	100	1000	0	20	100	1000
<i>Animals/group</i>	4	4	4	4	4	4	4	4
Spinocerebellar tract								
minimal	0	0	0	1	0	0	0	0
Fasiculus gracilis								
minimal	0	0	0	3	0	0	0	2
Cervical dorsal root ganglion								
minimal	0	0	0	2	0	0	0	1
Lumbar dorsal root								
minimal	0	0	0	3	0	0	0	0
slight	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Lumbar dorsal root ganglion								
minimal	0	0	0	1	0	0	0	1
slight	0	0	0	0	0	0	0	1
Lumbar ventral root								
minimal	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Intercostal								
minimal	0	0	0	1	0	0	0	1
slight	0	0	0	1	0	0	0	2
moderate	0	0	0	1	0	0	0	0
Phrenic								
minimal	0	0	0	2	0	0	0	2
Radial								
minimal	0	0	0	0	0	0	0	3
slight	0	0	0	1	0	0	0	0
moderate	0	0	0	2	0	0	0	0
marked	0	0	0	0	0	0	0	1
Sciatic								
minimal	0	0	0	1	0	0	0	0
moderate	0	0	0	3	0	0	0	1
marked	0	0	0	0	0	1	0	2
Sural								
minimal	0	0	0	1	0	0	0	0
slight	0	0	0	1	0	0	0	3
moderate	0	0	0	1	0	0	0	0

Nerve Fiber Degeneration (continued)	Males				Females			
	0	20	100	1000	0	20	100	1000
<i>Animals/group</i>	4	4	4	4	4	4	4	4
Tibial								
minimal	0	0	0	1	0	0	0	0
slight	0	0	0	0	0	0	0	1
moderate	0	0	0	3	0	0	0	1
marked	0	0	0	0	0	0	0	1
Vestibulo-Cochlear								
minimal	0	0	0	1	0	0	0	0
Spinal cord (cervical)								
minimal	0	0	0	3	0	0	0	3
slight	0	0	0	1	0	0	0	1
Spinal cord (lumbar)								
moderate	0	0	0	3				2
slight	0	0	0	0	0	0	0	1
Spinal cord (thoracic)								
minimal	0	0	0	3	0	0	0	2
slight	0	0	0	1	0	0	0	2

Other Histopathological Changes:

Moderate lymphoid atrophy was observed in the thymus (1/4 HDM and 4/4 HDF) and spleen (2/4 HDM and 1/4 HDF), and was thought to be secondary to nerve lesions.

Special Evaluation

None

Toxicokinetics

TK for MCI-186 and its sulfate and glucuronide conjugates were evaluated in plasma and CSF. Increases in plasma C_{max} and AUC were greater than dose-proportional for MCI-186 and less than dose proportional for the metabolites.

Dose mg/kg	Sex	MCI-186		Sulfate Conjugate		Glucuronide Conjugate	
		C_{max} µg/mL	AUC _{0-24h} µg×h/mL	C_{max} µg/mL	AUC _{0-24h} µg×h/mL	C_{max} µg/mL	AUC _{0-24h} µg×h/mL
20	M	0.274	4.39	2.56	52.1	3.04	40.8
	F	0.459	6.55	3.29	57.5	4.02	51.5
100	M	1.85	32.5	14.3	265	13.3	193
	F	1.93	35.6	9.95	198	11.2	164
1000	M	33.4	492	43.8	706	78.2	1165
	F	35.5	558	37.4	657	75.9	1068

Plasma/CSF ratios were approximately 4.7 for MCI-186. There was wide variability in CSF metabolite concentrations, but the following values were reported: Plasma/CSF ratios for the sulfate conjugate were 257 (LD), 324 (MD) and 39.6 (LD) in males and 959 (LD), (4613 (MD), and 11.5 (HD) for females. Plasma/CSF ratios for the glucuronide conjugate were 219 (LD), 381 (MD), and 54.6 (HD) in males and 231 (LD), 306 (MD), and 37.5 (HD) in females.

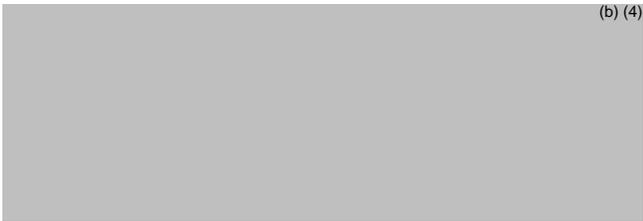
Dosing Solution Analysis

Dosing solutions were within 8% of their target concentrations.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Reverse Mutation Test of MY-7906 (MCI-186) in Bacteria

Study no.:	1145
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	February 12, 1986
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MCI-186, Lot EPE 7951, 100.3%

Methods

Strains:	TA100, TA1535, WP2uvrA, TA98, TA1537
Concentrations in definitive study:	10, 20, 50, 100, 200, 500, 1000, 2000, 5000 µg/plate
Basis of concentration selection:	Preliminary test (0.1, 1, 10, 100, 1000 µg/plate)
Negative control:	DMSO
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 h

		Base substitution mutation			Frameshift mutation	
		TA100	TA1535	WP2uvrA	TA98	TA1537
Direct method	Name	AF-2	ENNG	ENNG	4NQO	9AA
	Concentration (µg/plate)	0.01	5	2	0.2	80
Metabolic activation method	Name	B[a]P	2AA	2AA	B[a]P	B[a]P
	Concentration (µg/plate)	5	2	80	5	5

(Sponsor's Table)

Study Validity

This study was consistent with OECD guidelines. For a positive result, the number of revertant colonies per plate needed to exceed twice the negative control, and demonstrate a concentration-response.

Results

MCI-186 was negative in the Ames assay.

Study title: Bacterial Reverse Mutation Study of MCI-186

Study no.: 4L308
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: July 5, 1994
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot P-RSV-52409R, 100.0%

Methods

Strains: TA100, TA1535, WP2uvrA, TA98, TA1537
 Concentrations in definitive study: 156, 313, 625, 1250, 2500, 5000 µg/plate
 Basis of concentration selection: Preliminary test (same concentrations)
 Negative control: DMSO
 Positive control: See below
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 48 h

Strain	Without S9 mix ($\mu\text{g}/\text{plate}$)		With S9 mix ($\mu\text{g}/\text{plate}$)	
TA98	AF-2	0.1	2-AA	0.5
TA100	AF-2	0.01	2-AA	1
TA1535	NaN ₃	0.5	2-AA	2
TA1537	9-AA	80	2-AA	2
WP2 _{uvrA}	ENNG	2	2-AA	10

(Sponsor's Table)

Study Validity

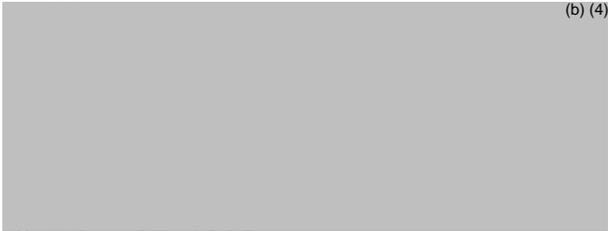
This study was consistent with OECD guidelines. For a positive result, the number of revertant colonies per plate needed to exceed twice the negative control, and demonstrate a concentration-response.

Results

MCI-186 was negative in the Ames assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Chromosomal Aberration Test of MCI-186 in Mammalian Cells

Study no.: 6N057
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)

Date of study initiation: October 27, 1986
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot EPE 7951, 100.3%

Methods

Cell line: Chinese hamster lung fibroblasts (CHL)
 Concentrations in definitive study: 50, 100, 200, 400 $\mu\text{g}/\text{mL}$ (-S9); 200, 400, 800, 1600 $\mu\text{g}/\text{mL}$ (+S9)
 Basis of concentration selection: Preliminary growth inhibition study; 218 $\mu\text{g}/\text{mL}$ resulted in 48% growth inhibition
 Negative control: DMSO
 Positive control: *N*-methyl-*N'*-nitro-*N*-nitroguanidine (-S9), benzo[a]pyrene (+S9)
 Formulation/Vehicle: DMSO

Incubation & sampling time: 24 and 48 h (-S9), 6 h followed by media change and an additional 8 h (+S9)

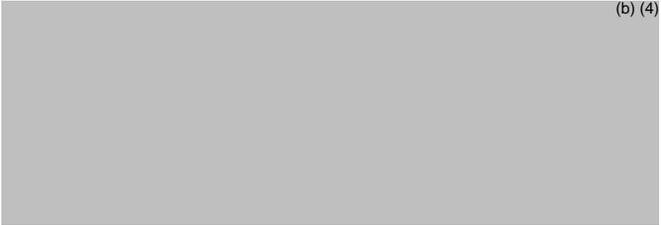
Study Validity

This study was consistent with OECD guidelines. Fewer than 5% aberrations was considered negative, between 5 and 10% was considered equivocal, and greater than 10% was considered positive.

Results

MCI-186 was negative in the chromosomal aberration assay.

Study title: Chromosomal Aberration Test of MCI-186 in Mammalian Cells

Study no.: 6N057-1
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: February 8, 1988
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot EPE 7951, 100.3%

Methods

Cell line: Chinese hamster lung fibroblasts (CHL)
Concentrations in definitive study: 200, 400, 800, 1600 µg/mL (-S9)
Basis of concentration selection: Study 6N057
Negative control: DMSO
Positive control: *N*-methyl-*N'*-nitro-*N*-nitroguanidine (-S9), benzo[*a*]pyrene (-S9)
Formulation/Vehicle: DMSO
Incubation & sampling time: 6 h incubation followed by media change and an additional 18 h incubation

Study Validity

This confirmatory study was consistent with OECD guidelines. Fewer than 5% aberrations was considered negative, between 5 and 10% was considered equivocal, and greater than 10% was considered positive.

Results

MCI-186 was negative in this confirmatory chromosomal aberration assay with metabolic activation.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Micronucleus test of MCI-186 in mice

Study no: 6K261
Study report location: EDR
Conducting laboratory and location:  (b) (4)

Date of study initiation: January 12, 1987
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot EPE 9751, 100.3%

Methods

Doses in definitive study: 0, 82.5, 165, 330 mg/kg
Frequency of dosing: Single dose
Route of administration: IP
Dose volume: 10 mL/kg
Formulation/Vehicle: Saline
Species/Strain: Male ICR mice
Number/Sex/Group: 6/group
Satellite groups: None
Basis of dose selection: Dose finding study (347 to 864 mg/kg IV)
Negative control: Saline
Positive control: Cyclophosphamide

Study Validity

This study was generally consistent with OECD guidelines; however, the use of IP rather than IV dosing was not justified.

Results

MCI-186 was negative in the *in vivo* mouse micronucleus assay.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A Study on Intravenous Administration of MCI-186 Prior to and in the Early Stages of Pregnancy in Rats

Study no.: 1167
Study report location: EDR
Conducting laboratory and location:  (b) (4)

Date of study initiation: July 21, 1986
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Lot No. EPE-7951, 100.3%

Methods

Doses: 0, 3, 20, 200 mg/kg
Frequency of dosing: Once per day
Dose volume: 3 mL/kg at 2 mL/min
Route of administration: IV bolus
Formulation/Vehicle: Saline
Species/Strain: Wistar rats
Number/Sex/Group: 26 or 30/sex/group
Satellite groups: None
Study design: Dosing in males began 63 days prior to pairing and continued until successful copulation. Dosing in females began 15 days prior to pairing and continued until GD 7. Estrous cycle was determined by daily vaginal smear for two weeks prior to dosing until the day of mating. Mating periods were 14 days (within study groups) or 7 days (MCI 186 with controls).

Deviation from study protocol: A summary of deviations was not provided

Mortality and Clinical Signs

All animals were observed daily for mortality or signs of morbidity. All animals survived until scheduled necropsy. Transient ataxic gait, blinking, lacrimation, incomplete eyelid closure, decreases in spontaneous movement, and crouching or leaning on the cage were observed at the HD. Blinking was also observed at the MD. Clinical signs typically resolved within 1 h of dosing. Prolonged diestrus (resulting in an estrous cycle of 7 or more days) was observed at control (1/25), MD (1/25), and HD (10/29). The copulation index was decreased at the HD (72.4%) relative to control (100%).

Body Weight

Body weight and food consumption were measured twice per week in males and every 3 days in females until pairing, after which only body weight was measured. Prior to pairing, mean body weight and mean food consumption decreased by approximately 18 and 12%, respectively, in HDM relative to controls and 18 and 12%, respectively, in HDF relative to controls.

Toxicokinetics

Not evaluated

Dosing Solution Analysis

Not provided

Necropsy

Males were necropsied after 1 or 2 mating periods; there were no drug effects on the testes, epididymides, or prostate, adrenal, or pituitary glands. Females were necropsied on GD21. There were no drug effects on mating, fertility index, numbers of corpora lutea, implantations, dead/live fetuses, sex ratio, or mean fetal and placental weights. External and skeletal examinations did not reveal any drug-related effects. Visceral examinations revealed thymic remnants in the neck of 6/95 C, 12/81 LD, 9/100 MD, and 19/91 HD fetuses.

Study title: Investigational Study of MCI-186 on Estrous Cycle and Copulation Index in Slc:Wistar Rats

Study no.:	8N051
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	August 26, 1988
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	MCI-186, Lot No EPE-7951, 100.3%

Findings

0 or 200 mg/kg MCI-186 was administered to female Wistar rats by daily IV injection for 2 weeks, at which time dosing was discontinued in the recovery arm, and animals in the mating group were paired with males. Dosing in the mating arm continued until GD 7. Animals in the recovery arm were monitored for an additional 4 weeks. Mean body

weight at Day 14 was reduced by 6% in the drug group relative to controls, and remained below controls for the duration of the study, corresponding with a decrease of up to 10% in mean food consumption. Estrous cycle was slightly prolonged by MCI-186 during dosing (5.1 days compared to 4.8 in controls), but there were no differences over the recovery period. There were no drug effects on mating. Cesarean sections did not reveal any drug effects on corpora lutea, implantation sites, resorptions or dead embryos, or live fetuses.

Study title: Effects of Intravenous Administration of MCI-186 on Estrous Cycle in Wistar Imamichi Rats

Study no.: 8N051-2
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: August 26, 1988
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: MCI-186, Lot No. EPE-7951, 100.3%

Findings

Daily IV injection of 200 mg/kg MCI-186 to female Wistar rats over 15 days resulted in a 7% decrease in mean weight and slight prolongation of the estrous cycle relative to controls.

Study title: Effect of Restricted Feeding on Estrous Cycle in Slc:Wistar Rats

Study no.: 8N051-1
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: August 26, 1988
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: N/A

Findings

MCI-186 was not administered in this study. 50 and 70% dietary restriction over 2 weeks resulted in prolonged estrous cycles in Wistar rats. Although such findings support the sponsor's suggestion that drug-related decreases in food consumption

underlie disturbed estrous cycle in rats administered 200 mg/kg MCI-186 for 14 days, these results are correlative and do not constitute proof of such a mechanism. This hypothesis would have been better addressed with calorically-matched control and MCI-186 groups.

9.2 Embryofetal Development

Study title: Preliminary teratology study of MY-7906 (MCI-186) in rats

Study no.:	1138
Study report location:	EDR
Conducting laboratory and location:	Not provided
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Not provided

Observations and Results

Wistar rats (8 to 11 per group) were administered 0, 10, 30, 100, or 300 mg/kg MCI-186 by IV injection once daily from GD 7 to 17. All animals survived until scheduled necropsy. Clinical signs included red discoloration of the urine at all doses, blinking after drug administration (>10 mg/kg), ptosis and reductions in spontaneous activity (>30 mg/kg), and ataxia and recumbence at 300 mg/kg. Mean body weights relative to control were decreased up to 7% from GD 8 to 18 at the HD and up to 3% on GD 14 to 18 at the MHD. Mean food consumption was decreased sporadically throughout dosing at the HD. Gross findings included slight enlargement of the adrenals (6/9 HD), slight thymic involution (6/9 HD), and slight discoloration of the liver (1/9 HD).

Litter Data

There were no drug effects on numbers of corpora lutea, implantations, live fetuses, or sex ratio. Mean fetal body weights were decreased by 5 to 6% relative to control at doses >30 mg/kg. There were no drug-related external or visceral findings; however, ossification of the phalanges of the hind limb was significantly delayed at the HD. According to the sponsor, there were no signs of teratogenicity.

Study title: Preliminary Teratological Study in Rats Treated Intravenously with MCI-186

Study no.: R-216
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: February 15, 1989
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot DCJ-1567, 100.4%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
Frequency of dosing: Once daily
Dose volume: 2 mL/min
Route of administration: IV bolus
Formulation/Vehicle: Water
Species/Strain: Female Wistar rats, 11 weeks of age at initiation
Number/Sex/Group: 8 females/group
Satellite groups: None
Study design: Females were paired 1:1 with males. MCI-186 was administered from GD 7 to 17, and animals were necropsied on GD 21.
Deviation from study protocol: No significant deviations

Mortality and Clinical Signs

All animals survived until scheduled necropsy. Clinical signs included transient eye blinking, preening, head shaking, and staggering gait were observed at doses >10 mg/kg, ptosis at doses >30 mg/kg, and prone position at 300 mg/kg.

Body Weight and Food Consumption

Mean body weights were decreased by 3 and 5% relative to control at the MHD and HD. There were no drug effects on food consumption.

Toxicokinetics

Not evaluated

Dosing Solution Analysis

Dosing solutions ranged from 97.6 to 106.0% of their target concentrations.

Necropsy

There were no drug-related gross findings.

Cesarean Section Data

There were no drug-effects on the numbers of corpora lutea, implantations, pre-implantation loss, resorptions, or live or dead fetuses.

Offspring

There were no drug-effects on placental or offspring weights, or sex ratio. There were no drug-related external, visceral, or skeletal malformations.

Study title: Teratology Study in Rats Treated Intravenously with MCI-186

Study no.: R-217
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: March 16, 1990
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, lot No. DCJ-1567, 100.4%

Methods

Doses: 0, 3, 30, 300 mg/kg
Frequency of dosing: Once per day
Dose volume: 2 mL/min
Route of administration: IV bolus
Formulation/Vehicle: Water
Species/Strain: Female Wistar rats
Number/Sex/Group: 24 to 26/group (cesarean), 15/group (spontaneous delivery)
Satellite groups: Spontaneous delivery
Study design: Mating was initiated at 12 weeks of age. MCI-186 was administered from GD 7 to 17. Animals in the cesarean section groups were necropsied on Day 21.

In the Delivery groups, nursing behavior was monitored until weaning of the offspring at PND 21, at which time the dams were necropsied. In the offspring, open field and multiple T-maze behaviors were evaluated at 4 and 5 weeks of age, respectively, and mating performance was

evaluated between Weeks 12 and 14. Males were necropsied after the mating period. In successfully mated females, cesarean sections followed by necropsy were performed on GD 21. Dosing solutions ranged from 98.9 to 103.5% of target concentrations. TK were not evaluated.

Deviation from study protocol: No significant deviations

Results

Cesarean Group:

F0 animals were observed 3 times/day during the dosing phase for mortality or signs of morbidity and twice daily during non-dosing phases. There were 2 acute deaths at the HD:

- Animal 4106 died after showing tonic convulsions immediately after dosing on GD 13. However, no abnormalities were observed following necropsy and histopathological evaluation. COD was not determined.
- Animal 4123 died after showing eye blinking, prone position, decreased spontaneous movement, and ptosis immediately after dosing on GD 17. Necropsy revealed light red pleural fluid, and histopathology revealed mild myocarditis. COD was thought to be "mild carditis associated with excessive burden by administration of the test article."

Clinical signs in all animals at doses >3 mg/kg included transient eye blinking, head shaking, and grooming immediately after dosing. Additional clinical signs in all HD animals included transient prone position, staggering gait, decreased spontaneous movement, lacrimation, and ptosis immediately after dosing. Mean body weight gain at the HD relative to control was reduced by 42% during the dosing stage and 15% from GD 17-21; however, weight gain at the HD was slightly higher than control during the lactation period. Decreases in weight gain at the HD were accompanied by decreases in food consumption of up to 16% relative to control. There were no drug-related findings at necropsy. Cesarean section did not reveal any drug-related effects on numbers of corpora lutea or implantations, pre- or post-implantation loss, number of live fetuses, or placental weight. There were no drug-effects on sex ratio; however, decreases in fetus weight relative to control were observed in LDM (3%), MDM and MDF (2%), and HDM and HDF (7%). There were no external malformations. Visceral and skeletal effects were not evaluated in the cesarean group.

Delivery Group:

There were no drug effects on delivery index, length of gestation, number of implantations, or stillbirths or live births. There were no effects on male/female ratio or drug-related external malformations. Poor nursing and nesting behavior was observed in control (1/15), LD (1/15), MD (2/15) and HD (1/15), but was not thought to be drug-

related. There were no drug-related visceral or skeletal abnormalities or effects on ossification. Body weight in the F1 generation up to PND 70 was reduced by approximately 15% in HDM and 4% in HDF relative to controls. There were no drug-effects on offspring viability; however, delays in pinna detachment, eruption of lower incisors, opening of eyelids, and testicular descent were observed in HD offspring. There were no drug effects on righting reflex, pupillary reflex, pinna reflex, corneal reflex, or auditory startle, or open field or multiple T-maze behavior. There were no drug effects on insemination or fertility indices or body weight of the F1 dams. Cesarean section of the mated females did not reveal any drug-related findings. Necropsy of F1 males and females after mating did not reveal any drug-related findings.

Summary:

The NOAEL for general toxicity was 3 mg/kg, based on CNS signs. The NOAEL for the F1 generation was 30 mg/kg based on reductions in body weight and delayed development at the HD. No TK data were provided.

Study title: Dose Range Finding Test of Teratogenicity in NZW Rabbits with MY-7906 (MCI-186)

Study no.:	1149-0
Study report location:	EDR
Conducting laboratory and location:	Not provided
Date of study initiation:	Not provided; test period was April 1, 1986 to July 31, 1986.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	MCI-186

Results

0, 30, or 100, or 300 mg/kg MCI-186 was administered once daily by IV injection to pregnant NZW rabbits (at least 5/group) from GD 6 to 18. The 30, 100, and 300 mg/kg dosing solutions were reconstituted in 0.3, 1, or 3N NaOH, respectively, and vehicle controls consisted of 1 or 3N NaOH. Severe injection site toxicity at the HD and its corresponding control group was thought to be due to the 3N NaOH vehicle, and resulted in early suspension of dosing in these groups. Clinical signs at the MD included urine discoloration, lacrimation, respiratory distress, lateral position, sedation, pupil contraction, and ataxia. Cesarean section on GD 29 did not reveal any drug effects on corporal lutea, implantation sites, sex ratio, fetal body weight, placental weight, or body length; however, fetal deaths were increased at the MD. The NOAEL for this study was 30 mg/kg.

Dose (mg/kg)	Control (1N NaOH)	Control (Saline)	30 mg/kg	100 mg/kg
No. of				
Litters examined	2	6	4	5
Corpora lutea	12.0±1.41	10.2±1.72	9.5±3.11	12.6±1.52
total	(24)	(61)	(38)	(63)
Implantation	11.0±2.83	8.8±1.94	9.0±3.56	12.0±2.35
total	(22)	(53)	(36)	(60)
Dead fetuses (%)	3 (13.6)	6 (11.3)	6 (16.7)	27 (45.0)*
early	1	1	1	21
late	2	5	5	6
Live fetuses	9.5±2.12	7.8±1.47	7.5±4.12	6.6±2.51
total	(19)	(47)	(30)	(33)
Sex ratio	0.73	1.04	0.76	0.65
(male/female)	(8/11)	(24/23)	(13/17)	(13/20)
Mean fetal weight (g)	38.5±1.73	39.5±4.01	37.7±8.83	34.7±9.47
Placental weight (g)	4.41±0.12	4.62±1.21	4.15±1.26	4.11±0.70
Body length (cm)	8.69±0.14	8.81±0.36	8.68±0.78	8.38±0.77

Values are mean ± standard deviation.

(Sponsor's Table)

Study title: A Study on Intravenous Administration of MY-7906 (MCI-186) during the Period of Organogenesis in Rabbits

Study no.: 1149

Study report location: EDR

Conducting laboratory and location:



Date of study initiation: February 19, 1986

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: MCI-186, lot EPE-7951, 100.3%

Methods

Doses: 0, 3, 20, or 100 mg/kg

Frequency of dosing: Once daily

Dose volume: 1 mL/kg at 2 mL/min

Route of administration: IV bolus

Formulation/Vehicle: 1N NaOH, diluted to 10% or less with saline

Species/Strain: NZW rabbits

Number/Sex/Group: 17/group (0, 3, 20 mg/kg), 19/group (100 mg/kg)

Satellite groups: None

Study design: Pregnant NZW rabbits were administered MCI-186 or vehicle from GD6 to 18. Cesarean sections were performed on GD 29.

Deviation from study protocol: No significant deviations

Clinical Signs

Animals were monitored daily for clinical signs. Red discoloration of the urine was observed at the MD and HD. Abnormal respiration, ataxic gait, hind limb paralysis, lacrimation, miosis, and hyperemia of the eyes were observed at the HD. Two HD animals were removed from the study due to injection site toxicity.

Body Weight and Food Consumption

There were no drug-effects on body weight or food consumption.

Toxicokinetics

Not evaluated

Dosing Solution Analysis

Not provided

Necropsy

Edema, discoloration, inflammation, and necrosis were observed at the injection sites in all groups, and were thought to be related to the NaOH vehicle. There were no drug-related findings.

Cesarean Section Data

There were no drug effects on litter size, sex ratio, or the numbers corpora lutea or implantations. There were no drug effects on fetal weight or body length, or degree of ossification. Increased fetal death was observed at the HD (23.1%), which was outside the historical range of 2.7 to 11.3%, and was therefore thought to be drug-related.

Offspring

There were no drug effects on external, visceral, or skeletal malformations.

9.3 Prenatal and Postnatal Development

Study title: Preliminary Peri- and Post-Natal study in Rats Treated Intravenously with MCI-186

Study no.: R-283
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: May 22, 1990
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot EPE-7951, 99.9%

Methods

Doses: 0, 10, 30, 100, 200 mg/kg
Frequency of dosing: Once daily
Dose volume: 3 mL/kg at 2 mL/min
Route of administration: IV bolus
Formulation/Vehicle: For the 200 mg/kg dose, MCI-186 was initially dissolved in 1N NaOH, which was then diluted to 20% with water. Solutions for the lower doses were created by further dilution with water.
Species/Strain: Wistar rats
Number/Sex/Group: 8/group (0 to 100 mg/kg), 12/group (200 mg/kg)
Satellite groups: None
Study design: Pregnant rats were administered MCI-186 from GD 17 to PND 21.
Deviation from study protocol: No significant deviations

F₀ Dams

Survival:	All animals survived until scheduled necropsy
Clinical signs:	Clinical signs were monitored 2 to 3 times daily. Transient head shaking, eye blinking, and preening were observed in all animals at doses >10 mg/kg. Lacrimation was observed at the MHD (3/8) and HD (all). Additional clinical signs at the HD included staggering gait (all), prone position (5/12) and decreased spontaneous movement (1/12).
Body weight:	Mean body weight relative to control was reduced by approximately 4% at 10, 100, and 200 mg/kg on GD21. Mean body weight at the HD was reduced over the lactation period by up to 6% relative to control.
Food consumption:	Mean food consumption at the HD was reduced relative to control by up to 20% during gestation and up to 8% during lactation.
Uterine content:	There were no abnormal deliveries in any group. There were no drug effects on gestation index or length, number of implantation sites, stillbirth index, number of liveborn pups, liveborn index, or sex ratio. There were no drug-related external malformations or effects on nursing behavior.
Necropsy observation:	No drug-related findings
Toxicokinetics:	Not evaluated
Dosing Solution Analysis:	Concentrations ranged from 98.7 to 103.0% of the nominal concentrations.
Other:	N/A

F₁ Generation

Survival:	No drug effects
Clinical signs:	No drug effects
Body weight:	No drug effects
Food consumption:	N/A
Physical development:	N/A
Neurological assessment:	N/A
Reproduction:	N/A
Other:	N/A

Study title: Peri- and Post-Natal Study in Rats Treated Intravenously with MCI-186

Study no.: R-334
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: June 10, 1991
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, lot DCQ0467, 99.9%

Methods

Doses: 0, 3, 20, 200 mg/kg
Frequency of dosing: Once per day
Dose volume: 2 mL/min
Route of administration: IV bolus
Formulation/Vehicle: The HD dosing solution was dissolved in 1N NaOH and then diluted with water. Dosing solutions for MD and LD groups were created by additional dilutions.
Species/Strain: Female Wistar SPF rats
Number/Sex/Group: 24/group
Satellite groups: None
Study design: Pregnant rats were administered MCI-186 by IV injection from GD 17 to PND 21.
Deviation from study protocol: No significant deviations

F₀ Dams

- Survival: All animals survived until scheduled necropsy.
- Clinical signs: All MD and HD animals showed eye blinking, head shaking, and preening after dosing. All HD animals also showed staggering gait, lacrimation, and prone position. Decreased spontaneous movement was observed in 18/24 HDF.
- Body weight: Mean body weight on GD 21 was decreased by 3% relative to control at the HD, and remained decreased by approximately 5% throughout the lactation period (until PND 21).
- Food consumption: Mean food consumption was decreased on GD 19 and 21 by approximately 10% at the HD relative to controls, and remained decreased by approximately 11% throughout the lactation period.
- Uterine content: One HDF did not deliver by GD 24; uterine contents included retention of dark red fluid, 2 resorbed embryos, 1 placental remnant, and 2 dead fetuses. There were no other abnormalities in delivery or uterine contents among all groups. There were no effects on gestation length.
- Necropsy observation: No drug-related gross findings.
- Toxicokinetics: Not evaluated
- Dosing Solution Analysis: Dosing solution concentrations ranged from 92.9 to 98.7% of the nominal concentrations.
- Other: N/A

F₁ Generation

Survival: No drug effects
 Clinical signs: No drug-related effects
 Body weight: No drug effects
 Food consumption: No drug effects
 Physical development: There were no drug effects on pinna detachment, appearance of abdominal hair, eruption of lower incisor, opening of eyelid, descent of testis, or opening of vagina.
 Neurological assessment: Functional evaluations were performed on PND 21, and open field and water-filled multiple T-maze evaluations were performed during postnatal weeks 4 and 5, respectively.

All offspring showed normal righting, pupillary, pinna, corneal, and acoustic startle reflexes.

Open field testing revealed increases in ambulation on both the first and second tests at the MDM and HDM relative to control. Rearing behavior in MDM and HDM was increased relative to control on the first test.

Dose (mg/kg)	Day 1		Day 2
	Ambulation (±SD)	Rearing (± SD)	Ambulation (± SD)
0	17.5 (20.7)	5.5 (3.9)	17.3 (26.5)
3	27.2 (19.9)	5.7 (3.4)	22.8 (22.3)
20	38.1 (23.2)*	8.1 (4.3)*	40.1 (33.0)*
200	45.1 (20.8)*	9.7 (3.5)*	38.3 (31.7)*

** indicates statistically significant difference (p<0.05) from control*

Water-filled T-maze revealed significant prolongations in elapsed time and increases in error count in the first trial at the HD, but testing on subsequent days revealed a shortening of elapsed time and reductions in error count in all groups, so it was unclear if such behavior was drug-related.

Reproduction: There were no differences among groups on copulation, insemination, or fertility indexes. Following Cesarean section, there were no drug effects on numbers of corpora lutea or implantation, pre- or post-implantation index, number of live fetuses, or placental weight. There were no drug effects on sex ratio or body weight. There were no drug-related external malformations. Preimplantation loss ranged from 22 to 34% across control and active groups; the sponsor stated that such a

rate was higher than expected, though the reason for the finding remains unexplained. Given this unexplained finding, this study was repeated (Study R-779).

Other: There were no gross findings in animals used for behavioral testing.

Study title: Peri- and Post-Natal Study in Rats Treated With MCI-186 (Additional Study)

Study no.: R-779
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: October 25, 1999
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot 99002, 99.95%

Methods: Study conducted due to higher preimplantation loss, and lower insemination and fertility in all F1 groups, including control in study R-334.

Doses: 0, 3, 20, 200 mg/kg
Frequency of dosing: Once daily
Dose volume: 2 mL/min, 3 mL/kg
Route of administration: IV bolus
Formulation/Vehicle: The HD dosing solution was dissolved in 1N NaOH and then diluted with water. Dosing solutions for MD and LD groups were created by additional dilutions.
Species/Strain: Female Wistar Imamichi
Number/Sex/Group: 24/group
Satellite groups: None
Study design: Pregnant rats were administered MCI-186 or vehicle once daily from GD 17 to PND 21.
Deviation from study protocol: No significant deviations

Observations and Results

F₀ Dams

- Survival: There was one death at the LD during delivery on GD 22, and one HDF was found dead on lactation Day 1. The sponsor did not consider the LD death to be drug-related; however, the HD death was preceded by staggering gait and decreased spontaneous movement and might have been caused by the test article.
- Clinical signs: Transient clinical signs after dosing included eye blinking in all animals at the MD and HD. Additional findings at the HD include staggering gait and decreased spontaneous movement in all animals, and ptosis in 9 animals. Poor nesting and lactating behavior was observed at the MD (1/23) and HD (3/24).
- Body weight: Mean body weight gain relative to control was decreased by approximately 24% over gestation at the HD, and mean body weight remained decreased by approximately 5% throughout lactation.
- Food consumption: Mean food consumption at all doses was generally similar to controls, with the exception of a 20% decrease at the HD on GD 20.
- Uterine content: With the exception of the LD death on GD 22, there were no drug-related findings during delivery. There were 17/389 stillborn pups at the HD, compared to 0/354, 0/368, and 1/383 at C, LD, and MD, respectively. Gestation length was increased at the HD by approximately 0.3 days (7.2 h) relative to control. There were no drug effects on sex ratio, or drug-related external malformations.
- Necropsy observation: No drug-related gross findings.
- Toxicokinetics: N/A
- Dosing Solution Analysis: Dosing solution concentrations ranged from 97.3 to 103.0% of the nominal concentrations.
- Other: N/A

F₁ Generation

Survival: Viability at the HD was decreased by 11% relative to controls between PND 0 and 4 due to poor nursing behavior by 3 dams. However, there were no drug-effects on viability from GD 4 to 70.

Clinical signs: No drug-effects

Body weight: No drug-effects

Food consumption: Not evaluated

Physical development: Pinna detachment, lower incisor eruption, and eyelid opening began occurring 1 to 2 days earlier at all doses relative to control. There was no drug-effect on testis descent; however, vaginal opening was slightly delayed at the HD relative to control. There were no abnormal findings in any group at the time of weaning.

Neurological assessment: Not evaluated

Reproduction: There were no drug-effects on estrus cycle, insemination or fertility indices, or body weight over gestation. There was a slight, dose-dependent increase in resorptions ranging from 4.9% in control to 11.8% at the HD. There were no drug effects on the mean number of live fetuses.

Other: N/A

F₂ Generation

Survival: No drug-effects

Body weight: No drug-effects

External evaluation: No drug-effects

Male/Female ratio: No drug-effects

Other: N/A

Study title: A 2 Week Intravenous Toxicity Study of MCI-186 in Juvenile Rats (Dose Range Finding Study)

Study no.: B-5668

Study report location: EDR

Conducting laboratory and location:  (b) (4)

Date of study initiation: August 29, 2005

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: MCI-186, Lot M008ED, 99.9%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
 Frequency of dosing: Once per day
 Route of administration: IV bolus
 Dose volume: 2 mL/min, 3 mL/kg
 Formulation/Vehicle: Water
 Species/Strain: SD Rats
 Number/Sex/Group: 6/sex/group
 Age: PND 22 at initiation of dosing
 Weight: At Initiation of dosing: 65.4 to 72.9 g (males),
 58.6 to 67.3 g (females)
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: No significant deviations

Results

All animals survived until scheduled necropsy. General observations made immediately before and after dosing, and approximately 1h after dosing revealed CNS signs at doses >10 mg/kg. Decreases in mean body weight relative to control were observed in MHDM (10%), HDM (17%), and HDF (8%). Mean food consumption was decreased relative to controls by up to 15% in MLDM and MHDM, 17% in HDM, 7% in MHDF, and 14% in HDF. There were no drug-related hematological or clinical chemistry findings. There were no drug-related gross findings. Drug-related decreases in absolute thymus weight relative to controls were observed in HDM (39%). Dosing solutions were within 97.6 to 103.0% of their respective target concentrations.

Clinical Signs	Sex	Dose (mg/kg); n=6/group				
		0	10	30	100	300
Incomplete eyelid opening	M	0	0	6	6	6
	F	0	0	6	6	6
Salivation	M	0	0	6	6	6
	F	0	0	6	6	2
Iacrimation	M	0	0	1	5	2
	F	0	0	3	5	5
Staggering gait	M	0	0	0	6	6
	F	0	0	0	6	6
Crouching position	M	0	0	0	6	6
	F	0	0	0	6	6
Blinking	M	0	0	0	6	6
	F	0	0	0	6	6
Prone position	M	0	0	0	0	6
	F	0	0	0	0	6

Study title: A 2 Week Intravenous Toxicity Study of MCI-186 in Juvenile Dogs (Dose Range Finding Study)

Study no.: B-5669
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: September 14, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot M008ED, 99.9%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
Frequency of dosing: Once per day
Route of administration: IV bolus
Dose volume: 5 mL/min, 3 mL/kg
Formulation/Vehicle: Water
Species/Strain: Beagle dogs
Number/Sex/Group: 2/sex/group
Age: 10-11 weeks at initiation of dosing
Weight: At Initiation of dosing: 4.0 to 4.6 kg (males), 3.3 to 4.1 kg (females)
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Results

All animals survived until scheduled necropsy. Drug-related clinical signs consisting of salivation, staggering gait, adynamia, incomplete eyelid opening, red discoloration of the eyes and oral mucosa, prone or lateral position, and sneezing were observed in all animals at the HD. There were no drug effects on body weight, food consumption, or clinical chemistry. Hematology revealed decreases in erythrocyte count, hemoglobin concentration, and hematocrit, and increases in MCV, MCH, reticulocytes, and platelets at the HD. Drug-related gross findings occurred at the HD and included dark discoloration of the spleen and excess fluid in the abdominal cavity in all animals, and were accompanied by increases in absolute mean spleen weight of 2-3 fold relative to control. Dosing solutions were within 98.8 to 101.0% of their respective target concentrations.

Hematology	Sex	Prestudy					Week 2				
		0	10	30	100	300	0	10	30	100	300
Erythrocyte ($\times 10^4/\mu\text{L}$)	M	562	554	598	593	559	582	614	610	592	437
	F	598	596	522	569	555	561	629	592	609	381
Hemoglobin (g/dL)	M	12.4	12.2	13	12.7	12.1	12.8	13.3	13.1	12.7	10.6
	F	12.4	13.2	11.9	12	12.2	12.1	13.8	13.4	12.8	9.3
Hematocrit (%)	M	38	37	40	40	37	39	41	40	39	33
	F	39	40	37	37	38	37	42	41	40	30
MCV (fL)	M	67.2	66.8	65.7	66.4	66.1	66	65.6	65.1	65.7	74.1
	F	67.7	67.2	69.6	64.8	68.6	66.5	66.1	68.2	64.5	48.9
MCH (pg)	M	22.1	22	21.8	21.4	21.6	65.1	21.7	21.5	21.4	24.1
	F	21.8	22.1	22.8	21	22.1	21.5	22	22.6	21.1	24.3
Reticulocyte ($\times 10^4/\mu\text{L}$)	M	5	2	2	4	2	2	3	2	3	16
	F	7	8	5	2	4	3	5	2	4	32
Platelets ($\times 10^4/\mu\text{L}$)	M	54.6	39.6	37.1	38.4	42.6	46.1	36	36.2	35.5	75.3
	F	54.5	48.6	43.6	44.3	46.1	42.7	42.2	43	43.4	61.8

Study title: A 4 Week Intravenous Toxicity Study of MCI-186 in Juvenile Rats

Study no.: B-5767
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: November 14, 2005
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: MCI-186, Lot M008ED, 99.9%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
 Frequency of dosing: Once per day
 Route of administration: IV bolus
 Dose volume: 2 mL/min, 3 mL/kg
 Formulation/Vehicle: Water
 Species/Strain: SD Rats
 Number/Sex/Group: 10/sex/group (main), 20/sex/group (TK)
 Age: PND 22 at initiation of dosing
 Weight: At initiation of dosing: 53.4 to 74.3 g (males),
 53.7 to 70.5 g (females)
 Satellite groups: TK
 Unique study design: None
 Deviation from study protocol: No significant deviations

Mortality

Animals were monitored once daily for mortality or signs of morbidity. There were no drug-related deaths.

Clinical Signs

Clinical signs were evaluated immediately before and after dosing, and 1 h postdose. CNS signs at doses >10 mg/kg included incomplete eyelid opening, salivation, lacrimation, crouching position, staggering gait, and blinking.

Finding	Sex	Dose (mg/kg); n=10/group				
		0	10	30	100	300
Incomplete eyelid opening	M	0	0	10	10	10
	F	0	0	10	10	10
Salivation	M	0	0	3	10	7
	F	0	0	10	10	7
lacrimation	M	0	0	9	6	7
	F	0	0	5	7	5
Staggering gait	M	0	0	0	10	10
	F	0	0	0	10	10
Crouching position	M	0	0	1	10	10
	F	0	0	0	10	10
Blinking	M	0	0	0	10	10
	F	0	0	0	10	10
Prone Position	M	0	0	0	0	10
	F	0	0	0	0	10

Body Weights and Food Consumption

Body weight gain was reduced by 25 and 18% relative to control in HDM and HDF, respectively. Food consumption was reduced by 16% in HDM relative to controls.

Ophthalmoscopy

Animals were evaluated prestudy and during Week 4 by slit lamp biomicroscopy and indirect ophthalmoscopy; there were no drug-related findings.

ECG

Not evaluated

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected at the time of necropsy. Urine samples were collected during Week 4. Hematology revealed increases in reticulocytes (HD) and decreases in

platelets (HDF). Clinical chemistry revealed minimal elevations in bilirubin, potassium, and A/G ratios, and decreases in total protein and percentages of α 1- and β -globulin at the HD. There were no drug-related urinalysis findings.

Findings	Sex	Dose (mg/kg)				
		0	10	30	100	300
Erythrocyte ($\times 10^4/\mu\text{L}$)	M	732	725	729	733	704
	F	720	741	724	735	695
Hemoglobin (g/dL)	M	15.9	15.9	15.9	15.8	15.4
	F	15.9	16.1	15.9	16.2	15.2
Hematocrit (%)	M	46	46	46	46	45
	F	45	45	45	45	43
MCV (fL)	M	63.3	63.2	63.7	62.9	64.4
	F	61.7	61.2	61.7	61.5	62
MCH (pg)	M	21.7	21.9	21.8	21.6	21.8
	F	22.1	21.8	22	22	21.9
Reticulocyte ($\times 10^4/\mu\text{L}$)	M	2985	2865	2890	2862	3820*
	F	1711	1649	1885	1715	2997*
Platelets ($\times 10^4/\mu\text{L}$)	M	122.2	122.6	126.1	123.4	118.5
	F	123.3	123.5	121.1	125.1	108.5*

*Hematology findings; * indicates statistically significant difference relative to baseline ($p \leq 0.05$)*

Findings	Sex	Dose (mg/kg)				
		0	10	30	100	300
Bilirubin (mg/dL)	M	0	0.1	0.1	0.1	0.1
	F	0.0	0.0	0.0	0.0	0.1
Potassium (nM)	M	4.8	4.7	5	5	5.4*
	F	4.7	4.7	4.7	4.6	5.1
A/G	M	0.92	0.92	0.95	0.98	1.07*
	F	0.94	0.94	0.94	0.98	1.05*
Total Protein (g/dL)	M	5.6	5.6	5.6	5.5	5.3*
	F	5.6	5.6	5.8	5.6	5.2*
α 1 globulin (%)	M	21.3	21.5	20.9	19.9	18.3*
	F	20.2	20.5	19.9	19.3	17.9*
β -globulin (%)	M	17.1	16.9	16.5	16.6	16.1*
	F	16.8	16.6	16.5	16.6	15.8*

*Clinical chemistry findings; * indicates statistically significant difference relative to baseline ($p \leq 0.05$)*

Gross Pathology and Organ weights

There were no drug-related gross findings. Mean absolute thymus weight relative to control was decreased by 41 and 31% in HDM and HDF, respectively.

Histopathology

Adequate Battery: Yes

Cerebrum	Thoracic Aorta	Kidney
Cerebellum	Trachea	Urinary Bladder
Spinal Cord	Lung	Testis/Ovary
Sciatic Nerve	Tongue	Epididymis/Uterus
Eyes	Esophagus	Prostate/Vagina
Optic Nerve	Stomach	Seminal Vesicle
Harderian Gland	Duodenum	Mammary Gland
Pituitary	Jejunum	Sternum
Thyroid	Ileum	Femur
Parathyroid	Cecum	Femoral Skeletal Muscle
Adrenal	Colon	Skin
Thymus	Rectum	Injection Site
Spleen	Submandibular Gland	Larynx
Mandibular Lymph Node	Sublingual Gland	Nasal Cavity
Mesenteric Lymph Node	Liver	
Heart	Pancreas	

Peer Review: *No*

Signed Pathology Report: No

Histological Findings: Findings in the spleen included minimal atrophy of the white pulp (4/10 HDM), minimal or mild pigmentation (8/10 HDM, 10/10 HDF), minimal increase in extramedullary hematopoiesis (7/10 HDF), and minimal or mild congestion (10/10 HDM, 10/10 HDF).

Special Evaluation

None

Toxicokinetics

TK were evaluated on Day 1 and during Week 4. Increases in C_0 were generally dose dependent, but increases in AUC were greater-than dose dependent. There were no sex differences. Repeat dosing did not affect C_0 or AUC. MCI-186 was rapidly metabolized to sulfate and glucuronide conjugates. Plasma concentrations of the sulfate conjugate were higher than the glucuronide conjugate at doses up to 100 mg/kg, after

which concentrations of the glucuronide conjugate were higher, suggesting saturation of the sulfation pathway.

Parameter	Male (mg/kg)				Female (mg/kg)			
	10	30	100	300	10	30	100	300
Day 1								
C ₀ (µg/mL)	12.5	59.5	183	421	13.2	64	151	479
t _{1/2} (h)	0.659	1.43	4.91	3.83	0.605	1.39	4.74	3.58
AUC _{0-24h} (µg×h/mL)	1.96	9.33	59.7	470	1.91	10.2	58.6	453
AUC _{0-∞} (µg×h/mL)	1.94	9.25	59.8	470	1.9	10.1	58.7	453
Week 4								
C ₀ (µg/mL)	12.7	65.5	198	520	25.8	62.5	238	554
t _{1/2} (h)	0.91	6.53	4.86	3.95	1.87	6.02	3.96	3.51
AUC _{0-24h} (µg×h/mL)	2.13	10.4	67.7	351	3.14	10.5	71.3	440
AUC _{0-∞} (µg×h/mL)	2.09	10.5	67.8	351	3.1	10.6	71.5	441

[Plasma] at 1 h (µg/mL)	Male (mg/kg)				Female (mg/kg)			
	10	30	100	300	10	30	100	300
Day 1								
MCI-186	0.0771	0.397	2.32	144	0.0867	0.443	2.81	156
MCI-186 sulfate	1.16	3.26	13.1	25.3	1.21	3.7	17.3	40.4
MCI-186 glucuronide	0.178	0.476	6.65	128	0.186	0.605	7.64	127
Day 28								
MCI-186	0.115	0.7998	3.73	58.9	0.098	0.537	2.67	85.8
MCI-186 sulfate	1.78	5.42	26.9	47.6	1.74	3.67	17.7	54.8
MCI-186 glucuronide	0.114	1.67	5.49	71.4	0.174	0.959	3.28	118

Dosing Solution Analysis

Dosing solutions were within 96.4 to 103.0% of their respective target concentrations.

Study title: A 4 Week Intravenous Toxicity Study of MCI-186 in Juvenile Dogs

Study no.: B-5768
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: December 1, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MCI-186, Lot M008ED, 99.9%

Methods

Doses: 0, 10, 30, 100, 300 mg/kg
Frequency of dosing: Once per day
Route of administration: IV bolus
Dose volume: 5 mL/min, 3 mL/kg
Formulation/Vehicle: Water
Species/Strain: Beagle dogs
Number/Sex/Group: 3/sex/group
Age: 10 to 11 weeks at initiation of dosing
Weight: At initiation of dosing: 3.4 to 4.6 kg (males), 3.1 to 5.2 kg (females)
Satellite groups: None
Unique study design: None
Deviation from study protocol: No significant deviations

Mortality and Clinical Signs

Clinical signs were evaluated immediately before and after dosing, and 1 h postdose. All animals survived until scheduled necropsy. CNS signs included salivation, staggering gait, reduced strength, incomplete eyelid opening, sneezing, redness of the conjunctiva, oral mucosa, or pinna, and prone or lateral position were observed in all animals at the HD. There were no drug-related clinical signs at lower doses.

Body Weights and Food Consumption

Body weights and food consumption were monitored daily; there were no drug effects.

Ophthalmoscopy

Animals were evaluated prestudy and during Week 4 by slit lamp biomicroscopy and indirect ophthalmoscopy; there were no drug-related findings.

ECG

Electrocardiography was performed prestudy and during Week 4; there were no drug effects.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected following a 16 h fasting period. Urine samples were collected over 22 h periods, during which the animals had free access to food and water. Hematology indicated regenerative anemia at the HD. Bilirubin was slightly elevated in HDM (0.2 mg/dL) and HDF (0.1 md/dL) relative to baseline (0.0 mg/dL). Brown urine was observed in 1 MHDM at Week 2, and all animals at the HD on Week 4.

Findings	Sex	Baseline					Week 2					Week 4				
		0	10	30	100	300	0	10	30	100	300	0	10	30	100	300
Erythrocyte ($\times 10^4/\mu\text{L}$)	M	531	551	546	536	542	530	539	518	547	403*	576	558	564	570	448*
	F	524	567	525	545	574	577	586	545	563	385*	613	595	563	583	468*
Hemoglobin (g/dL)	M	12	12.4	12.5	12	12.3	11.9	12.2	11.7	12.1	9.8*	13	12.4	12.5	12.3	10.8*
	F	11.7	12.7	12.4	12.1	13.4	13	13.3	12.6	12.6	9.8	13.6	13.2	12.9	13	11.6
Hematocrit (%)	M	36	37	37	36	37	36	36	35	37	29*	38	37	37	38	33*
	F	35	39	37	37	40	39	39	38	38	29*	40	40	39	39	35*
MCV (fL)	M	67.8	67.2	67.5	67	58.2	67.2	66.9	67.1	66.6	72.4*	66.6	67.1	66.5	66.4	73.7*
	F	67.4	67.8	70.1	67.4	69.6	67.1	67.4	69.4	67.1	76.2*	66	67.1	68.8	66.9	74.5*
MCH (pg)	M	22.5	22.6	22.8	22.4	22.7	22.5	22.6	22.5	22.2	24.4	22.5	22.2	22.1	21.7	24.2*
	F	22.4	22.5	23.6	22.3	23.4	22.6	22.7	23.1	22.3	25.4*	22.2	22.2	23	22.2	24.7*
Reticulocyte ($\times 10^4/\mu\text{L}$)	M	3.2	2.8	3.5	1.6	7.5*	2.1	2.3	2.6	2.1	135.4	1.2	1.7	2.1	2.7	74.5*
	F	3.4	3.9	3.4	5.1	5.8	3.9	2.5	4.6	3.2	147.6	2.2	2.1	2.6	4.2	66.4
Platelets ($\times 10^4/\mu\text{L}$)	M	47.3	48.5	49.8	43.6	40.7	47.9	41.2	45.4	40.5	65.8*	38.9	36.3	40.7	42.1	50.8*
	F	36.3	43.1	53.2	37.5	45.5	35.2	39.2	44.2	34.1	61.6*	33.9	32.1	40.4	36.3	57.3*

*Hematology findings; * indicates statistically significant difference relative to baseline ($p \leq 0.05$)*

Gross Pathology and Organ weights

Spleen and liver weights at the HD were elevated approximately 1.2 and 2-fold relative to control. Dark discoloration of the spleen was observed in all animals at the HD, and enlargement of the spleen was observed in 1/3 HDM. Excess abdominal fluid was observed in 1/3 MHDM, 2/3 HDM, and 1/3 HDF.

Histopathology

Adequate Battery: Yes

Cerebrum	Heart	Parotid gland
Cerebellum	Aorta	Liver
Medulla oblongata	Trachea	Gallbladder
Spinal cord	Lung	Pancreas
Optic nerves	Tongue	Kidney
Sciatic nerves	Esophagus	Urinary bladder
Eyeballs	Stomach	Testis/Ovary
Lacrimal gland	Duodenum	Epididymis/Uterus
Pituitary	Jejunum	Prostate/Vagina
Thyroid gland	Ileum	Mammary gland
Parathyroid	Cecum	Sternum
Adrenal	Colon	Femur
Thymus	Rectum	Femoral skeletal muscle
Spleen	Submandibular gland	Skin
Submandibular lymph node	Sublingual gland	Injection site
Mesenteric lymph node		

Peer Review: No

Signed Pathology Report: No

Histological Findings: Drug-related histology findings were limited to the HD, and included splenic congestion (3/3M, 3/3F), pigmentation (3/3M, 3/3F), and extramedullary hematopoiesis (2/3M, 2/3F), hepatic Kupffer cell pigmentation (3/3M, 3/3F) and extramedullary hematopoiesis (1/3F), pigmentation of the proximal tubular epithelium (1/3F), and pigmentation and increased hematopoiesis in the bone marrow (3/3M, 3/3F).

Special Evaluation

None

Toxicokinetics

TK were evaluated on Days 1 and 28. Increases in C_0 and AUC were greater-than dose proportional. There were no sex differences. Repeat dosing did not affect C_0 or AUC. MCI-186 was rapidly metabolized to sulfate and glucuronide conjugates. PK parameters were not calculated for the metabolites. However, plasma concentrations the sulfate conjugate were higher than the glucuronide conjugate at doses up to 30 mg/kg, after which concentrations of the glucuronide conjugate were higher, suggesting saturation of the sulfation pathway.

MCI-186	Male (mg/kg)				Female (mg/kg)			
	10	30	100	300	10	30	100	300
Day 1								
C ₀ (µg/mL)	8.3	26.2	141	416	8.92	60.5	160	444
t _{1/2} (h)	1.35	1.19	4.59	4.09	0.66	1.11	3.38	3.82
AUC _{0-24h} (µg×h/mL)	1.85	7.2	41.9	292	1.83	12.3	52.9	353
AUC _{0-∞} (µg×h/mL)	1.81	7.07	41.9	292	1.81	12.2	52.7	353
Day 28								
C ₀ (µg/mL)	12.2	30.2	158	591	8.85	34	170	516
t _{1/2} (h)	2.28	4.3	6.01	2.07	1.65	7.81	5.63	3.49
AUC _{0-24h} (µg×h/mL)	2.46	8.41	54	393	1.95	8.4	52.2	295
AUC _{0-∞} (µg×h/mL)	2.43	8.47	54.2	393	1.9	8.51	52.5	295

[Plasma] at 1 h (µg/mL)	Male (mg/kg)				Female (mg/kg)			
	10	30	100	300	10	30	100	300
Day 1								
MCI-186	0.132	1.09	2.92	53.6	0.133	1.11	3.29	72.1
MCI-186 sulfate	17.6	31.2	57.1	104	17.9	27.9	64.4	119
MCI-186 glucuronide	5.72	16.2	81.6	306	5.23	17.4	94.5	306
Day 28								
MCI-186	0.177	1.2	3.89	76.5	0.156	1.13	3.73	52.9
MCI-186 sulfate	19.5	31.6	58.6	100	20.1	32.6	66.6	80.7
MCI-186 glucuronide	11.8	23.7	113	380	4.89	20.6	124	310

Dosing Solution Analysis

Dosing solutions were within 97.3 to 102.0% of their respective target concentrations.

10 Special Toxicology Studies

Neurodegeneration:

Continuous infusion of 0 or 1000 mg/kg/day MCI-186 in male beagle dogs (3/group) over a 2-week period resulted in drug-related fore and hindlimb paralysis by Day 9; this finding correlated with histopathological signs of sciatic, tibial, radial, and lumbar spinal cord fiber degeneration. An additional study in which 0, 300, or 1000 mg/kg/day MCI-186 was continuously administered to male beagle dogs (3/group) also resulted in neurodegeneration of peripheral nerves and lumbar spinal cord fibers on Day 10 (HD) and Day 13 (LD). Deficits in patellar and triceps reflexes were observed prior to visible reductions in limb use, suggesting that neurodegeneration was initially affecting the reflex arc. All animals at the HD and 1/3 at the LD were euthanized on Day 10 and 13,

respectively, due to limited fore and hindlimb use. Histopathology revealed that degeneration of the peripheral nerves and spinal cord fibers was accompanied by leukocyte infiltration and Schwann cell proliferation, and electron microscopy revealed degeneration of the axoplasmic components.

The time from initiation of dosing until the onset of symptoms was evaluated in male and female beagle dogs administered 0, 60, 120, or 300 mg/kg/day MCI-186 by continuous infusion, with sacrifices (4/sex/group) on Days 6, 11, and 15. No clinical or histologic signs of neurodegeneration were observed at the LD. Signs of fore- and hindlimb weakness at the MD and HD did not manifest until Day 12, but histopathology performed on animals necropsied on Day 11 revealed signs of peripheral nerve and spinal cord fiber degeneration at the HD. Histopathology performed after Day 15 revealed neurodegeneration at the MD and HD. Neurological evaluation did not reveal signs of cranial nerve pathology at any dose.

Nerve Degeneration (4/sex/group)	Sex	Day 6		Day 11		Day 15	
		120	300	120	300	120	300
Intercostal	M	0	0	0	2	1	4
	F	0	0	0	1	0	4
Tibial	M	0	0	0	2	1	3
	F	0	0	0	0	0	4
Radial	M	0	0	0	2	1	4
	F	0	0	0	1	0	4
Sciatic	M	0	0	0	3	2	4
	F	0	0	0	3	0	4
Spinal cord (dorsal funiculus)	M	0	0	0	0	0	2
	F	0	0	0	0	0	2

To determine whether the peripheral and spinal nerve degeneration was reversible, male beagle dogs (3/group) were administered 1000 mg/kg/day MCI-186 by continuous IV infusion for 5 days, followed by a 2-week recovery period. Necropsy following the recovery period revealed that degeneration of the sciatic nerve was characterized by axonal swelling and digestions chambers, indicating the potential for regeneration. It was unclear if similar findings accompanied the degeneration of the lumbar spinal cord fibers. Continuous dosing with 0, 60, 120, 180, or 300 mg/kg/day MCI-186 for 5 days similarly induced peripheral nerve and spinal cord fiber degeneration at doses >120 mg/kg/day that did not resolve over a 4-week recovery period. In a final study evaluating the reversibility of drug-mediated neurodegeneration, male dogs were administered 0 or 120 mg/kg/day MCI-186 by continuous infusion for 2 weeks. Six dogs/group were necropsied after dosing, while an additional 6 dogs/group were maintained for a 13-week recovery period. Spinal cord (1/6) and peripheral nerve (2/6) fiber degeneration was observed by the end of 2 weeks. Peripheral nerve findings resolved over 13 weeks and were accompanied by phagocytes, possibly indicating a repair process, but lumbar spinal nerve degeneration was more prevalent (3/6 animals) and of equal severity compared to that observed after the 2-week dosing period.

A mechanism underlying MCI-186-mediated peripheral and spinal nerve fiber degeneration was not defined. However, limited evidence was provided suggesting that drug effects on vitamin B6 concentrations may play a role. Based on apparent similarities between MCI-186-induced nerve degeneration and that mediated by vitamin B6 deficiency, the sponsor measured vitamin B6 levels in male beagle dogs (3/group) administered 1000 mg/kg/day MCI-186 by continuous infusion for 5 days followed by a 2 week recovery period or for 11 days with no recovery. This study indicated a decrease in vitamin B6 of approximately 90% relative to predose levels during drug administration, with restoration of vitamin B6 levels to approximately 75% of predose levels over the 2-week recovery period. A similar restoration of vitamin B6 was not observed in non-recovery group. However, although decreases in vitamin B6 appeared to be drug-related, the lack of a vehicle control limits confidence in this finding. In a second study, 2 mg/kg/day vitamin B6 was co-administered by IV or SC injection with continuous infusion of 300 mg/kg MCI-186 in male beagle dogs (3/group) for 14 days. All animals that received MCI-186 developed sciatic nerve and lumbar spinal cord degeneration; however, the severity was reduced in groups that were co-administered vitamin B.

Dose (mg/kg/day)	Male		Female	
	C _{ss} (ng/mL)	AUC _{24h} (µg×h/mL)	C _{ss} (ng/mL)	AUC _{24h} (µg×h/mL)
Study 1R256: 24h continuous infusion (no NOAEL)				
300	4416.76	86.74	N/A	N/A
1000	12725.89	313.14	N/A	N/A
Study 4R246: 24h continuous infusion (NOAEL = 120 mg/kg/day)				
60	477.3	7.6507	379.8	7.0692
120	816.5	16.1004	752.5	17.645
180	1473.6	28.2155	1499.4	29.3695
300	2671.1	58.3057	2671.4	54.9373

TK from neurotoxicity mechanism studies

Renal Toxicity

The *in vitro* cytotoxicity of MCI-186 and sulfate and glucuronide conjugates (186S and 186G, respectively) was tested in human renal proximal tubule epithelial cells. Cell viability was determined 24 and 48 h after administration of 1 to 1000 µM drug or metabolite. After 48 h, decreases in cell viability were observed at 1000 µM MCI-186 (66.4%), 186S (82.5%), and 186G (96.0%). In isolated human renal cortical epithelial cells and hepatocytes, administration of 0.1, 1, 10, 100, and 1000 µM MCI-186, 186S, or 186G resulted in reduced mitochondrial function by MCI-186 and 186S at 1000 µM in renal epithelial cells. Single IV administration of 200 mg/kg MCI-186 in rats did not affect renal cortical or medullar blood flow, nor did it potentiate proximal tubule degeneration when co-administered with the diuretic furosemide. There were no adverse effects on the kidneys following BID IV administration of 200 mg/kg MCI-186; however, simultaneous co-administration of 200 mg/kg (BID) MCI-186 potentiated the proximal tubule degeneration and necrosis induced by a single administration of glycerol (1 g/kg)

and the antibiotic cephalothin (CET; 2000 mg/kg). This effect was enhanced in water-deprived rats.

TEXT TABLE 1: Incidence of treatment-related microscopic findings

Water condition	Supplied		Deprived		Supplied				Deprived				
Dose of Glycerol (g/kg)	0		0		1				1				
Dose of Cefalotin (mg/kg)	0		0		2000				2000				
Dose of MCI-186 (mg/kg, bid)	0	200	0	200	0	20	100	200	0	20	100	200	
Organs/tissues	No. examined												
Findings	10	10	10	10	10	10	10	10	10	10	10	9	
Kidneys													
Degeneration/necrosis of proximal tubular epithelium in cortex	+	0	0	0	0	0	0	1	8	5	5	7	3
	++	0	0	0	0	0	0	0	0	0	0	3	4
	+++	0	0	0	0	0	0	0	0	0	0	0	1
Dilatation of distal tubule	+	0	0	0	1	0	0	0	2	0	0	1	5
	++	0	0	0	0	0	0	0	0	0	0	0	1
Cast, hyaline*	+	0	1	1	1	2	2	0	5	0	1	4	4
	++	0	0	0	0	0	0	0	0	0	0	0	1
Cast, granular	+	0	0	0	0	0	0	0	1	0	0	0	1
	++	0	0	0	0	0	0	0	0	0	0	0	1

+: Slight, ++: Moderate, +++: Severe.

*: Moderate lesion (++) was judged as the treatment-related change.

(Sponsor's Table)

Further studies in rats co-administered MCI-186, CET, and glycerol revealed that potentiation occurred only if CET/glycerol were administered up to 2 h prior to MCI-186 administration; administration of CET/glycerol 2-4 h after MCI-186 did not result in increased renal toxicity. MCI-186/CET/glycerol regimens that enhanced renal toxicity did not affect systemic exposure to MCI-186 or edaravone. However, such regimens were shown to increase kidney concentrations of CET by 3.7-fold. Gene array studies were performed on rat kidney tissue following administration of the MCI-186/CET/glycerol combination, indicating potential induction of a large number of genes thought to be involved in cell stress/injury; however, in the absence of confirmation by more quantitative methods, such studies add little to any understanding of the mechanism of toxicity. An additional mechanistic study in isolated dog leukocytes indicated possible increases in IL-6 and TNF- α secretion, but it was unclear what concentrations of MCI-186 the cells were exposed to, and the inflammatory response by the cells (i.e., cytokine secretion) was inconsistent between repetitions.

Antigenicity:

No antidrug antibodies were detected in rabbits or guinea pigs administered MCI-186 by SC or IV injection, or in mice following IP injection. Intradermal administration resulted in a delayed skin allergic reaction in guinea pigs previously sensitized by IV injection with MCI-186 and FCA; however, a follow-up study did not indicate sensitization of guinea pigs by IV administration of up to 100 mg/kg MCI-186 (without FCA) for 5 days.

Hemolytic Potential

Incubation of rabbit RBC with 0, 0.75 mg/mL, or 1.4 mg/mL MCI-186 did not result in hemolysis. Moreover, 30 minute incubation of 0.5, 1, or 2 mg/mL MCI-186 with human blood did not reveal signs of hemolysis. Repeat daily IV administration of 0, 300, or 500 MCI-186 in male SD rats revealed a 25% decrease in RBC at the HD. A Coombs test did not reveal lysis of human RC by complement activation.

Local Tolerance

Single IV or SC injection of 100 mg MCI-186 in male rabbits did not result in injection site irritation beyond that observed in saline-injected controls. Repeat IV or SC injection of 100 mg over 4 days MCI-186 in male rabbits resulted in severe hemolysis and local vascular irritation. However, no vascular irritation was observed at the same dose but with a 5-fold dilution of the dosing solution.

Impurities

Specification limits for the degradation products (b) (4) were (b) (4)%. (b) (4) is present at less than (b) (4)% in the drug product. The sponsor evaluated (b) (4), and (b) (4) “according to the ICH guideline Q3 (R2)”.

(b) (4)

Single and Repeat Dose Toxicology

Single IV administration of 0, 267, 400, and 600 mg/kg of the MCI-186 degradation product (b) (4) was evaluated in male and female ICR mice (5/sex/group). Clinical signs were monitored daily until necropsy on Day 15. There were no (b) (4)-related changes in body weight, or effects on gross pathology. Clinical signs were similar to MCI-186. Repeat IV administration of 0, 0.1, or 1 mg/kg for 2 weeks in rats (10/sex/group) did not result in any drug-related mortality or clinical signs, or effects on body weight, food or water consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, gross findings, or histopathology.

Finding	Sex	Dose (mg/kg); n=5/group			
		0	267	400	600
Mortality	M	0	0	0	2
	F	0	0	0	1
Tonic convulsion	M	0	0	2	5
	F	0	0	0	5
Decreased locomotor activity	M	0	0	2	5
	F	0	0	0	5
Dyspnea	M	0	0	2	5
	F	0	0	0	5
Abnormal gait	M	0	0	2	1
	F	0	0	0	3

Clinical signs after single administration of (b) (4)

Genetic Toxicology

(b) (4) was negative in the Ames assay; however, while generally consistent with OECD guidelines; however, the S9 mix should have been validated with another control in addition to 2-aminoanthracene. (b) (4) was evaluated in an *in vitro* chromosomal aberration assay in Chinese hamster lung (CHL) fibroblasts. (b) (4) was negative after 6 h in the presence or absence of S9. However, dose-dependent increases in chromatid breaks and chromatid exchanges were observed after 24 h (-S9). (b) (4) was therefore positive in an OECD-compliant *in vitro* chromosomal aberration assay. (b) (4) was negative in an

OECD-compliant mouse micronucleus assay in which male ICR mice (6/group) were administered 0, 60, 100, 160, or 250 mg/kg (b) (4) once daily by IV injection for 2 days, with bone marrow sampling occurring 24 h after the second dose.

(b) (4)

Repeat Dose Toxicology

Repeat IV administration of 0, 0.1, or 1 mg/kg (b) (4) for 2 weeks in rats (10/sex/group) did not result in any drug-related mortality or clinical signs, or effects on body weight, food or water consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, gross findings, or histopathology.

Genetic Toxicology

(b) (4) was negative in the Ames assay; however, while generally consistent with OECD guidelines, a positive control in addition to 2-aminoanthracene should have been used for the +S9 arm. (b) (4) did not induce structural or numerical chromosome aberrations in an OECD-compliant mammalian cell chromosomal aberration assay in CHL cells. (b) (4) was negative in an OECD-compliant mouse micronucleus assay in which male ICR mice (6/group) were administered 0, 250, 400, 600, or 1000 mg/kg (b) (4) once daily by IV injection for 2 days, with bone marrow sampling occurring 24 h after the second dose.

(b) (4)

Repeat Dose Toxicology

Beagle dogs (3/sex/group) were administered 0, 0.3, 1.0, or 3.0 mg/kg (b) (4) once daily by IV injection for 28 days. There were no drug-related deaths or clinical signs, or effects on body weight, food or water consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights, gross findings, or histopathology.

Genetic Toxicology

(b) (4) was negative in the Ames assay; however, while generally consistent with OECD guidelines; however, the S9 mix should have been validated with another control in addition to 2-aminoanthracene. (b) (4) was evaluated in an *in vitro* chromosomal aberration assay in CHO cells. Dose-dependent increases in chromosomal structural aberrations were observed with and without S9 after a 4 h exposure followed by a 16 h recovery period. (b) (4) was therefore positive in an OECD-compliant *in vitro* chromosomal aberration assay. (b) (4) was negative in an OECD-compliant mouse micronucleus assay in which male ICR mice (5/group) were administered 0, 43.75, 87.5, or 175 mg/kg (b) (4) once daily by IV injection for 2 days, with bone marrow sampling occurring 24 h after the second dose.

11 Integrated Summary and Safety Evaluation

Introduction

Edaravone (RADICAVA™) is a free radical scavenger developed by Mitsubishi Tanabe Pharma Corp for treating amyotrophic lateral sclerosis (ALS). The proposed dose is 60 mg by 1 h IV infusion once per day for 14 days, followed by a 2-week drug-free period. Subsequent cycles consist of 10 treatments over 2 weeks followed by a 2-week drug-free period.

Pharmacology

Edaravone is thought to suppress redox imbalances that may potentiate neuronal degeneration in ALS. *In vitro* studies with edaravone demonstrated prevention of linoleic acid oxidation by hydrogen peroxide and ferrous ions, reduction of lipid peroxidation in rat brain homogenate and isolated mitochondria, reduced oxidative injury to cultured endothelial cells, and reduced neuronal apoptosis in a model of excitotoxicity. IV administration of 3 or 6 mg/kg MCI-186 did not reduce neuronal cell death in a rat model of nerve avulsion. In an SOD-mutant transgenic rat model of ALS, a regimen of once-daily IV injection of 3 mg/kg MCI-186 for 2 days followed by a 2-day drug holiday for the life of the animals did not increase life span or generally improve performance in a battery of reflex or strength tests, although a slight improvement was observed in the incline plane test. No potential off target effects were predicted by an *in vitro* panel of 76 receptors, ion channels, and transporters.

The safety pharmacology battery evaluated the effects of edaravone on CNS, cardiovascular, and respiratory systems. CNS safety pharmacology was evaluated in mice, rats, rabbits, and cats. CNS signs typically included increases in lacrimation and ptosis, and decreases in spontaneous movement in male mice and rats following a single IV dose of 30 or 100 mg/kg edaravone. Decreases in body temperature were observed in male mice administered a single 100 mg/kg IV injection of edaravone. In cardiovascular safety pharmacology studies, concentrations of up to 1 mM edaravone did not inhibit hERG current. In mongrel dogs, single IV administration of 30 or 100 mg/kg edaravone resulted in transient decreases in blood pressure and increases in carotid blood flow and heart rate. Single IV injection of up to 100 mg/kg edaravone in mongrel dogs did not affect respiratory rate; however, neither tidal or minute volumes, nor hemoglobin oxygen saturation were evaluated.

PK/ADME

Standard PK studies conducted in mice, rats, dogs, and monkeys indicated rapid elimination from the body. IV bolus administration of edaravone in mice, rats, dogs, and monkeys revealed $t_{1/2}$ values ranging from 0.3 to 1.6 h. By comparison, IV administration in humans resulted in plasma $t_{1/2}$ of 4.5 to 6 h according to the clinical pharmacology review (Office of Clinical Pharmacology Review, NDA 209176, 1/25/2017). Distribution studies using ^{14}C -edaravone administered by IV injection indicated accumulation of radiation in the kidney and aorta, with the latter thought to be due to endothelial cell binding. Penetration of the blood brain barrier was limited, with only 6% of plasma radioactivity levels found in the CSF 5 min after dosing in rats.

Edaravone metabolites consisted of sulfate and non-acyl glucuronide conjugates in mice, rats, dogs, monkeys, and humans. 24 h after IV administration of edaravone, urinary excretion accounted for 61, 76, 83, and 90% of the administered dose in rats, dogs, monkeys, and humans, respectively. Unchanged drug accounted for 0.68 to 1.59% of the excreted drug-related compound in all species. Differences in metabolism were attributed to species differences in expression of sulfotransferases and glucuronide transferases. Administration of radiolabeled edaravone to pregnant rats resulted in milk and fetal plasma radioactivity concentrations up to 20 and 4%, respectively, of maternal plasma concentrations. Edaravone was 90, 83, 44, and 91% plasma protein-bound in blood from mice, rats, dogs, and humans, respectively, while the sulfate conjugate was 90 to 99% plasma protein bound and the glucuronide conjugate was 13 to 37% plasma protein bound.

Single and Repeat Dose Toxicology

Studies in juvenile animals (Wistar rats and beagle dogs) were conducted, but are not relevant to this indication. General toxicology was adequately evaluated by single dose studies in CD-1 mice, Wistar rats, and beagle dogs, and repeat dose studies in Wistar rats, beagle dogs, and cynomolgus monkeys. Single IV bolus or short infusion (i.e., ≤ 4 h) of edaravone in mice, rats, and dogs was generally well-tolerated. Primary toxicities in the single dose mouse and rat studies included signs of CNS depression followed by mortality due to ensuing cardiovascular or respiratory suppression. Single IV bolus or short infusion in dogs similarly resulted in CNS depression, but was also accompanied by signs of hemolytic anemia, resulting in death due to renal infarction in one female. Repeat IV bolus or short infusion in Wistar rats and beagle dogs for up to 6 months resulted in CNS depression. Although not consistent with the proposed clinical dosing regimen, continuous IV infusion was also evaluated in Wistar rats, beagle dogs, and cynomolgus monkeys, resulting in hemolytic anemia in rats and peripheral and spinal nerve fiber degeneration in dogs and monkeys.

Single Dose

Rodents

Single IV injection in CD-1 mice resulted in mortality and CNS signs (sedation, lacrimation, loss of reflex, salivation) at doses ranging from 478 to 700 mg/kg. Single IV bolus in Wistar rats resulted in similar CNS signs at doses ranging from 530 to 850 mg/kg, and death at doses ranging from 640 to 850 mg/kg. Death in mice and rats was thought to be due to acute respiratory failure or acute cardiac failure secondary to CNS depression. Red/orange discoloration of the urine was observed at all doses, which was thought to be due to the presence of an edaravone metabolite and was not considered adverse.

Nonrodents

The toxicity of single IV bolus or 4 h infusion was evaluated in beagle dogs. Administration of 300 or 600 mg/kg edaravone by IV bolus in male beagle dogs resulted in CNS signs including decreases in activity, abnormal gait, incomplete eyelid opening, vomiting, and tachypnea, and hematologic and histologic signs of regenerative

hemolytic anemia. There were minimal increases in AST, ALT, LDH, and ALP, suggesting possible hepatotoxicity, and increases in plasma TNF- α and IL-6 suggesting an inflammatory response to edaravone. A single 4 h IV infusion of 0, 400, 500, or 600 mg/kg edaravone in male and female beagle dogs (1/sex/group) resulted in death of the HDF 7 days after dosing; the COD was thought to involve renal infarction, likely secondary to hemolytic anemia and sustained hypotension. Clinical signs at all doses included weakness, sedation, incomplete eyelid closure, nictation, emesis, staggering gait, and heart rate elevations.

Repeat Dose

Rats

Primary toxicities in Wistar rats administered edaravone by repeat IV injection or continuous infusion included transient CNS signs (sedation, loss of righting reflex, lacrimation, salivation), reductions in body weight gain during the dosing period, and hematologic signs of regenerative anemia (i.e., reductions in RBC parameters and increases in reticulocytes). All toxicities generally resolved over 2-week recovery periods. Hematologic or CNS toxicity in rats was dependent on whether edaravone was administered by bolus/short infusion or 24 h continuous infusion. Daily 3 h IV infusion of 0, 10, 30, or 100 mg/kg edaravone for 6 months in male and female rats resulted in transient CNS signs at the MD and HD, along with reductions in mean body weight gain (-20% relative to control), elevations in urinary ketone bodies and bilirubin, and a trend towards reductions in RBC and platelets, though no clear hematologic or histologic signs of anemia occurred. TK analysis was not conducted in this study; however supporting TK from a bridging study with a similar dosing paradigm indicated systemic exposure of $C_0 = 18,577$ ng/mL and $AUC_{0-24h} = 2,155$ ng \times h/mL at the NOAEL of 10 mg/kg. In contrast, no CNS signs were seen in male and female rats administered 0, 50, 100, 300, or 1000 mg/kg/day edaravone for 28 days by continuous infusion. Relative to controls, there were reductions in mean weight gain at 300 and 1000 mg/kg/day, decreases in mean food consumption at the HD, and hematologic signs of regenerative anemia at the HD. The NOAEL based on signs of anemia was 300 mg/kg/day ($C_{ss} = 4,115$ ng/mL, $AUC_{0-24h} = 93,305$ ng \times h/mL).

Dogs

As with rats, toxicity in beagle dogs was a function of the IV dosing paradigm. Repeat IV injection of 0, 10, 30, or 100 mg/kg edaravone for 26 weeks in male and female beagle dogs resulted in transient CNS signs (salivation, sedation, sneezing, ataxia, ptosis) and minimal hematologic signs of anemia at the HD. No TK evaluation was performed in this study, but a supporting TK study indicated that exposure at the NOAEL of 30 mg/kg was likely in the range of $C_0 = 50,400$ ng/mL and $AUC_{0-24h} = 15,415$ ng \times h/mL. By comparison, continuous IV infusion of 0, 30, 60, 120, or 200 mg/kg/day edaravone in male and female beagle dogs resulted in decreases in body weight gain and food consumption, and progressive deficits in fore and hindlimb use leading to euthanasia (between Days 22 and 25) at 120 and 200 mg/kg/day. Histology revealed degeneration of muscle fiber nerves, sciatic nerve, and cervical and lumbar spinal cord fibers at doses greater than 30 mg/kg/day, with incomplete recovery at the HD over a

2-week recovery period. The NOAEL for continuous infusion in beagle dogs was therefore 30 mg/kg/day ($C_{ss} = 130$ ng/mL, $AUC_{0-24h} = 3590$ ng×h/mL).

Additional studies were conducted to better characterize the axonopathy associated with continuous infusion of edaravone in beagle dogs. Administration of up to 300 mg/kg/day resulted in the appearance of fore and hindlimb weakness by Day 12; however, interim histologic analyses revealed signs of intercostal, tibial, radial, sciatic, and spinal cord fiber degeneration by Day 11, suggesting that toxicity was occurring prior to the manifestation of clinical signs. Detailed examination of the sciatic nerve after a 2-week recovery period in a separate study revealed axonal degeneration and swelling characterized by digestion chambers, indicating a potential for regeneration. However, although peripheral nerve degeneration was no longer present after a 13-week recovery period in beagle dogs administered 120 mg/kg/day for 2 weeks, degeneration of the lumbar spinal nerves was unresolved. A mechanism underlying edaravone-induced neurodegeneration was not determined; however, according to the sponsor, vitamin B6 deficits result in a similar pattern of neuropathy. To support such a link, a study in beagle dogs demonstrating a correlation between continuous edaravone infusion and decreasing plasma levels of vitamin B6 was conducted, followed by a second study in which co-administration of vitamin B6 limited the sciatic nerve degeneration induced by continuous edaravone infusion. While these studies suggest that vitamin B6 deficits may play a role in edaravone-induced axonopathy, additional studies are necessary to prove such a mechanism.

Monkeys

Continuous infusion of 0, 10, 100, or 1000 mg/kg/day edaravone for 14 days in male cynomolgus monkeys did not induce visible CNS signs. However, histologic findings in 1 HDM revealed the presence of peripheral nerve fiber degeneration characterized by fiber swelling and the presence of digestion chambers. Continuous administration of 0, 20, 100, or 1000 mg/kg/day (4/sex/group) for 28 days led to unscheduled euthanasia of 1 HDM due to reductions in activity and muscle tone, limited usage of fore and hindlimbs, lack of pinch reflex, hypersensitivity, shallow breathing, and uncoordinated body movement. Clinical signs suggestive of peripheral nerve degeneration (i.e., reductions in muscle tone) appeared by Week 4 in the remaining animals at the HD. There were no signs of anemia, but histopathology revealed peripheral and spinal nerve degeneration at the HD. Based on these findings, the NOAEL for continuous edaravone infusion in monkeys was 100 mg/kg/day ($C_{max} = 1,890$ ng/mL, $AUC_{0-24h} = 34,050$ ng×h/mL).

Genetic Toxicology

Genetic toxicology was adequately evaluated by Ames, *in vitro* chromosomal aberration, and mouse *in vivo* micronucleus assays; edaravone was negative in these assays.

Reproductive Toxicology

Reproductive and developmental toxicology was adequately evaluated by a battery of fertility, embryofetal, and peri- and postnatal development studies in Wistar rats and New Zealand White rabbits (embryofetal development only).

Fertility and embryonic development was evaluated in Wistar rats administered 0, 3, 20, or 200 mg/kg edaravone by IV injection for 63 days prior to mating (males) or from 15 days prior to mating until GD 7 (females). Transient CNS signs including decreases in movement, ataxic gait, lacrimation, and incomplete eyelid closure were seen at the HD. There was a 28% decrease in copulation index at the HD. A slight (0.3 day) increase in estrous cycle length was also observed at the HD, but was thought to be related to decreases in food consumption.

IV injection of 0, 3, 30, or 300 mg/kg edaravone in Wistar rats from GD 7 to 17 resulted in transient eye blinking and head shaking at the MD and HD, and sedation, prone position, staggering gait, and reductions in body weight at the HD. One fatality at the HD was likely caused by mild carditis and was thought to be drug-related. No malformations were identified following cesarean section on GD 21; however, body weights of the F1 generation were decreased in HD male (15%) and female (4%) offspring relative to controls. There were also slight delays in pinna detachment, eruption of the lower incisors, opening of the eyelids, and testicular descent at the HD. The NOAELs were 3 mg/kg based on maternal toxicity, and 30 mg/kg for the offspring based on reductions in body weight and developmental delays.

In New Zealand White rabbits, IV injection of 0, 3, 20, or 100 mg/kg edaravone from GD 6 to 18 resulted in abnormal respiration, ataxic gait, hind limb paralysis, lacrimation, miosis, and hyperemia of the eyes at the HD. There were no external, visceral, or skeletal malformations. However, fetal death at the HD was 23.1%, which exceeded the sponsor's historical range of 2.7 to 11.3%, and was therefore thought to be drug-related. The NOAEL for maternal and embryofetal toxicity in rabbits was 30 mg/kg.

Peri- and postnatal toxicity was evaluated by two studies in Wistar rats administered 0, 3, 20, or 200 mg/kg edaravone by daily IV injection from GD 17 to PND 21. In the first study (R-334), all animals survived until scheduled necropsy. Transient clinical signs in the F0 generation included eye blinking, head shaking, and preening at the MD and HD, and staggering gait, lacrimation, and prone position at the HD. There were slight reductions in body weight accompanied by reductions in food consumption at the HD. After birth, the sponsor reported "poor maternal behavior, such as nesting and lactating" at the MD and HD. Behavioral evaluation of the offspring revealed increased ambulatory movement and rearing behavior by MDM and HDM in open field tests. There were no additional drug effects on the F1 generation; however, a marked and unexplained increase in preimplantation loss at in all groups, including controls, was seen after mating. Over concerns that this anomaly might confound analyses of fertility in the F1 generation, a second study was conducted (R-779), which resulted in expected rates of preimplantation loss. Study R-779 also revealed an increased number of stillborn pups at the HD (17/389) relative to control (0/354), LD (0/368), or MD (1/383). The greater

number of stillbirths at the HD was attributed by the sponsor to increased litter size; however, such a conclusion is questionable given that the MD had a similarly high number of implants/litter, but without an accompanying increase in stillborn pups. Additional findings in the F0 generation included slightly longer gestation length (7 h) at the HD, likely due to increased numbers of stillborn pups.

Other Studies

Metabolites

Edaravone is conjugated to sulfate (186S) and glucuronide (186G) in rats, dogs, monkeys, and humans. A single-dose toxicity study with 186S in ICR mice revealed transient CNS signs including abnormal gait and breathing, and reduced activity at 439 and 877 mg/kg, and tonic convulsions and lacrimation at 877 mg/kg. No toxicity was associated with single IV injections of up to 2000 mg/kg 186G in ICR mice.

Metabolite TK parameters were generally not available in the clinical and nonclinical study reports. However, the mean plasma concentrations of 186S 1 h after IV bolus administration of 10 mg/kg edaravone in Wistar rats was 1048 ng/mL, which approaches the C_{max} of 1727 ng/mL for parent drug in humans that received 1 mg/kg edaravone over a 40 min IV infusion in clinical study csr-mci-186-01. Given that the C_{max} for parent drug in Wistar rats administered 10 mg/kg also exceeds the C_{max} in humans by over 10-fold, the data suggest adequate coverage of 186S by the rat repeat dose and reproductive and developmental toxicity studies. Although the data do not suggest similar coverage for 186G, concern over human exposure to this metabolite is minimal given that non-acyl glucuronide conjugates are generally not of toxicologic concern.

Renal Toxicity

Renal toxicity was not seen in the toxicology studies. However, based on post-marketing surveillance in Japan, the sponsor conducted a battery of nonclinical studies to evaluate the potential for renal injury in patients receiving edaravone in combination with cephalosporin antibiotics. The *in vitro* toxicity of edaravone and its sulfate and glucuronide conjugates was evaluated in human proximal tubule epithelial cells, renal cortical cells, and hepatocytes, but effects on cell viability were only observed at doses of 1 mM, suggesting minimal direct toxicity. There were also no effects of single IV administration of up to 200 mg/kg edaravone on renal cortical or medullar blood flow in Wistar rats. Given that acute renal failure in humans was generally limited to patients receiving cephalosporin-class antibiotics in combination with edaravone, an additional series of studies evaluating renal toxicity secondary to such a combination was conducted in Wistar rats, revealing potentiation of cephalothin-induced renal toxicity when administered approximately 2 h prior to edaravone. Although TK evaluation did not reveal changes in systemic exposure to cephalothin or edaravone, an analysis of tissue drug levels indicated a 3.7-fold increase in kidney cephalothin levels after administration of the cephalothin/edaravone combination. It was therefore suggested by the sponsor that the renal failure encountered in patients may be due to edaravone-mediated increases in kidney tissue levels of cephalosporin antibiotics.

Antigenicity and Hemolytic Potential

No anti-drug antibodies were detected following SC or IV injection of edaravone in guinea pigs and rabbits, or IP injection in mice. SC administration in guinea pigs resulted in a delayed skin allergic reaction; however, no such reaction occurred following IV administration, which is the clinical route. Hemolytic anemia was frequently seen in the toxicity studies, but incubation of human or rabbit blood with edaravone did not result in direct hemolysis, nor did a Coomb's test using human red blood cells indicate activation of complement. The mechanism behind edaravone-mediated anemia in the nonclinical studies therefore remains unclear.

Impurities

(b) (4) is a genotoxic carcinogen, and is an immediate precursor and degradation product of edaravone. Discussion with CMC indicates that the sponsor has made a reasonable effort to minimize hydrolysis of edaravone. The maximum potential dose of (b) (4) is (b) (4) µg/day, which exceeds recommendations for genotoxic impurities; however, given the seriousness of the indication, the specification limit of (b) (4) % for (b) (4) is acceptable. Additional degradation products evaluated by the sponsor are (b) (4). The specification limits for these impurities are below the qualification threshold, and all three were negative in Ames assays.

Carcinogenicity

Carcinogenicity studies of edaravone administered in diet were conducted by the National Cancer Institute in 1979, and were referenced by the sponsor. There was no evidence of carcinogenicity, but issues including high mortality in a vehicle group and inconsistent dosing reduce the confidence in these studies. Adequately conducted studies should be conducted post marketing.

Conclusions

The nonclinical studies submitted in support of edaravone were adequate. Primary toxicities following repeat IV bolus or short infusion in rats and dogs for up to 6 months included transient sedation, decreases in weight gain, and regenerative anemia. However, concern over such toxicity is minimal given the transient and reversible nature of the findings. The nonclinical data support approval of RADICAVA™.

References

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/s/

DAVID L CARBONE
03/27/2017

LOIS M FREED
03/27/2017