INTRAVENOUS REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES OF CIMADRONATE (YM175), A NOVEL BISPHOSPHONATE, IN RATS AND RABBITS

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ABSTRACT — Cimadronate (YM175) is a novel bisphosphonate with potent inhibitory activity on bone resorption under development for the treatment of tumor-induced hypercalcemia, metastatic bone disease and osteoporosis. We conducted intravenous reproductive toxicity and teratology studies (Segment I, II and III) of this compound in rats and teratology study in rabbits. The test compound was dissolved in physiological saline, which was also given as the vehicle control.

Rats were administered at a dosage of 0.06, 0.16 and 0.62 mg/kg/day in the male Segment I study. Dose levels in the other studies in rats including the female Segment I were 0.16, 0.31 and 0.62 mg/kg/day. In the Segment I study, no treatment-related abnormalities were observed in reproductive parameters or fetuses. In the Segment II study, slightly retarded fetal ossification was noted at 0.31 mg/kg/day or more, but the incidence of malformation did not increase. In the Segment III study, death of the dams and abnormal tooth growth of offspring were observed at 0.16 mg/kg/day or more. Further Segment III study showed that the no toxic effect level was 0.003 mg/kg/day.

In the rabbit teratology study, dose levels were 0.01, 0.025 or 0.05 mg/kg/day. No toxic effects on pregnant females or their litters were observed at up to 0.05 mg/kg/day.

KEY WORDS : Fertility, Teratology, Reproductive and developmental toxicity, Bisphosphonate, Cimadronate, YM175.

INTRODUCTION

Cimadronate (YM175), disodium cycloheptylaminomethylenediphosphonate monohydrate, is a novel bisphosphonate with potent inhibitory activity on bone resorption. This drug is expected to be useful in the treatment of hypercalcemia of malignancy, bone metastasis of cancer and osteoporosis (Kawamuki *et al.*, 1989; Kawashima, 1990). An intended route of administration in the clinical use of this drug in humans is intravenous.

This paper describes experiments performed to assess the effects of cimadronate on fertility, teratology and reproductive and developmental toxicity in rats and teratology in rabbits.

MATERIALS AND METHODS

1. Test compound and formulation

Cimadronate is an odorless, white crystalline powder easily soluble in water (Fig. 1). Lots H-8 and H-9 (Yamanouchi Pharmaceutical Co., Ltd.), with a purity by HPLC of 99.9% and 100.8%, respectively, were dissolved in physiological saline. pH of the solutions was 4.72–6.35 and osmolarity was 276–295 mOsm/kg·H₂O. Cimadronate was dosed intravenously at a constant volume of 5 (at low dose group of male Segment I study and additional Segment III study) or 10 ml/kg (other studies) for rats at a rate of 2 ml/min and 1 ml/kg for rabbits at a rate of 1 ml/min. Control animals received equivalent volumes of the vehicle.

Fig. 1.

2. Rationale for dose selection

In a 30-day repeated intravenous toxicity study in rats, mortality, renal and testicular toxicity and severe irritation at the injection site in the tail were seen at 1.25 mg/kg/day (Okazaki

et al. 1995). The high dose of 0.62 mg/kg/day was therefore selected for the Segment I, II and III studies in rats. Since testicular toxicity was observed in a 30-day toxicity study, the minimum dose level was selected to be 0.06 mg/kg in the male Segment I study, lower than female Segment I study, to confirm the no toxic dose level.

In a preliminary study in non-pregnant rabbits, mortality was observed at 0.3 mg/kg/day or more. Rabbits given 0.1 mg/kg/day showed decreased food intake and body weight gain. The high dose of 0.05 mg/kg/day was therefore selected for the main teratology study in rabbits.

3. Test animals

Sprague-Dawley (SD) rats and New Zealand White rabbits were used. All animals were housed individually in cages under controlled temperature, humidity and lighting conditions. A commercial pellet diet and municipal tap water were available *ad libitum*. All animals were acclimatized for at least one week and then randomly assigned to either the vehicle control or treatment groups. In the rat studies, the animal room environment was controlled at a temperature of 18 to 25°C, relative humidity of 30 to 70% and a 12-hr or 13-hr light cycle.

In the rabbit study, the animal room environment was controlled at a temperature of 15 to 23°C, relative humidity of 40 to 70% and a 12-hr light cycle.

4. Study design and observations

The administration period, number of animals examined and observation parameters were established in accordance with MHW Toxicity Guideline notification No. 24, issued in 1989.

The following studies were done:

Male fertility study (Segment I ♂) in rats: SD rats obtained from Charles River Laboratories, Inc. (Michigan, USA) were assigned to one control and three dose groups of 22 males and 22 females each. The test drug was intravenously administered to the male rats of the three groups beginning 64 days prior to mating and continued until euthanasia for a total of 94 or 95 days including the mating and post-mating periods at dosages of 0.06, 0.16 and 0.62 mg/kg/day. Male rats of the control group received the vehicle (saline) alone for the same period. The four groups of female rats remained untreated throughout the examination period.

On day 20 of gestation, the females were killed and examined macroscopically. Live fetuses were weighed, sexed and examined for external, visceral (Wilson, 1965) and skeletal (Dawson, 1926) abnormalities. Males were killed after 94 or 95 days of treatment and macroscopically examined. Major organs in the thoracic and abdominal cavities and reproductive organs were weighed and fixed in 10% formalin. This study was conducted by the International Research and Development Corp. (Michigan, USA) between September, 1990, and June, 1991.

Female fertility study (Segment I ♀) in rats : SD rats obtained from Iffa Credo (L Arbresle, France) were assigned to one control and three dose groups of 22 males and 22 females each. The test drug was intravenously administered to three groups of female rats from two weeks before mating with untreated males until day 7 of gestation at dosages of 0.16, 0.31 and 0.62 mg/kg/day. Female rats of the control group received the vehicle (saline) alone for the same period.

On day 20 of gestation, the females were killed and examined macroscopically. Live fetuses were weighed, sexed and examined for external, visceral (Wilson, 1965) and skeletal (Dawson, 1926) abnormalities.

This study was carried out at Hazleton France between July, 1990, and May, 1991.

Teratology study (Segment II) in rats : SD rats obtained from Iffa Credo (L Arbresle, France) were assigned to three groups of 36 mated rats each and given cimadronate by single daily intravenous injection from day 7 to day 17 of gestation at doses of 0.16, 0.31 and 0.62 mg/kg/day. A fourth group of rats given the vehicle (saline) over the same period served as a control group.

On day 20 of gestation, two-third of pregnant females in each group were killed and examined macroscopically. Live fetuses were weighed, sexed and examined for external, visceral (Wilson, 1965) and skeletal (Dawson, 1926) abnormalities.

One-third of pregnant females in each group were allowed to litter and rear their offspring to weaning. The size of each litter was adjusted to eight pups on day 4 after birth by eliminating extra pups by random selection to yield (where

possible) 4 male and 4 female pups per litter. Physical and functional development parameters examined in all pups during the lactation period were pinna unfolding, incisor eruption, eye opening, pain reflex (tail pinch test), surface righting reflex, gripping reflex and pupillary and auditory reflexes.

After weaning on day 22 of age, two male and female pups per litter (where possible) were selected for post-weaning behavioral tests, namely the rota-rod (week 4–5), water maze (week 5 to 6), pupillary reflex (week 6) and open field (week 7) tests. Of these, one male and one female per litter (where possible) were selected and mated at approximately 11 weeks of age to evaluate their reproductive capability.

F1 animals not selected after weaning were killed, and soft X-ray pictures of the skeletons were taken to detect any abnormalities. F2 pups were necropsied at weaning and preserved in ethanol.

This study was performed at Hazleton France between September, 1990, and March, 1991. Soft X-ray analysis of the skeleton was carried out at Yamanouchi Pharmaceutical Co., Ltd.

Peri- and postnatal study (Segment III) in rats : SD rats obtained from Charles River UK Ltd. (Kent, England) were divided into three groups of 22 pregnant rats each and given cimadronate during the peri- and post natal period. Cimadronate was administered intravenously from day 17 of gestation through day 21 after delivery at dose levels of 0.16, 0.31 and 0.62 mg/kg/day. Control rats received the vehicle (saline) throughout the same period.

All pregnant dams were allowed to deliver and rear their offspring to weaning. The duration of gestation and litter size were recorded. On day 4 of lactation, each litter was culled to eight pups to produce comparable litter sizes, whenever possible, four male and four female offspring in each litter. Physical and functional development parameters examined in all F1 offspring were pinna unfolding, dorsal hair growth, incisor eruption, eye opening, testicular descent, vaginal opening, auditory and visual function and pain reflex test. After weaning on day 22 of lactation, behavioral tests including physical activity (week 4), the water maze (week

4) and neuromuscular function (week 5, including traversing flat and round rods, rota-rod, air righting reflex, limb hanging and gripping ability) were conducted. At approximately 5 weeks of age, following completion of the behavioral and neuromuscular functional tests, one male and one female were selected from each litter (where possible) to form the F1 generation. These animals were mated at approximately 11 weeks of age to evaluate their reproductive capability.

Unselected F1 animals were killed at approximately eight weeks of age and F2 animals were killed after weaning.

This study was performed at Life Science Research Ltd. (Suffolk, England) between December, 1990, and May, 1991.

Additional Segment III study: Since death of the dams and abnormal tooth growth of offspring were observed at 0.16 mg/kg or more in the Segment III study, further Segment III study at lower dose levels was conducted to examine the no effect dose level.

SD rats obtained from Charles River Japan Ltd. (Kanagawa, Japan) were divided into three groups of 21 or 22 pregnant rats each and given cimadronate during the peri- and post natal period. Cimadronate was administered intravenously from day 17 of gestation through day 21 after delivery at dose levels of 0.0003, 0.001 and 0.003 mg/kg/day to examine the effect on dams and on the incisors of their offspring (F1) up to Week 11 after birth. Control rats received the vehicle (saline) throughout the same period.

This study was performed at Yamanouchi Pharmaceutical Co., Ltd. between March, 1992, and June, 1992.

Teratology study in rabbits: New Zealand White rabbits were obtained from Froxfield SPF Rabbits Ltd., Hampshire, England. Females were artificially inseminated with pooled semen from the same strain bucks aged at least 12 months and their fertility was confirmed. Following insemination, each female was injected intravenously with 25 i.u. of luteinizing hormone to ensure successful ovulation. The day of insemination was designated as day 0 of pregnancy.

Pregnant rabbits were dosed intravenously with cimadronate once daily from day 6 through day 18 of gestation at a dose of 0.01, 0.025 and 0.05 mg/kg/day. Control animals received the

saline vehicle for the same period. Pregnant rabbits were killed by intravenous injection of sodium pentobarbitone on day 29 of gestation. After cesarean section, all live fetuses were weighed and examined for external, visceral and skeletal abnormalities. This study was conducted at Life Science Research Ltd. between January, 1991, and August, 1991.

5. Statistical analysis

In rat studies, mean values and standard deviations were calculated using the litter as the analytical unit. The data were analyzed using the χ^2 -test, Fisher's Exact probability test, the Mann-Whitney U-test or the *t*-test.

In the rabbit study, data were analyzed using the Mann-Whitney U-test, *t*-test or Fisher's Exact probability test.

Reproduction-related data were evaluated by the following equations:

Copulation index = No. of animals with successful copulation / No. of mated animals $\times 100$

Fertility index = No. of pregnant animals / No. of animals with successful copulation $\times 100$

Gestation index = No. of females with live offspring / No. of pregnant females $\times 100$

Birth index = No. of offspring born alive / No. of implantations $\times 100$

Viability index on day 4 = No. of offspring alive on day 4 after birth / No. of offspring born alive $\times 100$

Weaning index = No. of offspring alive at weaning / No. of offspring alive on day 4 after birth after culling $\times 100$

RESULTS

1. Fertility study (Segment I) in rats

Effect on males (Table 1): No drug-related changes in clinical sign were seen among controls or any treated rats administered doses of 0.16 mg/kg/day or less. At 0.62 mg/kg/day one male died on day 40 of dosing. The cause of death was severe pulmonary congestion of unknown etiology. There was no drug-related change in body weight and food consumption in any rats. At necropsy, organ weights and necropsy findings in the treated groups were comparable with those in the control group. Reproductive parameters, including copulatory and fertility indices and copulatory interval, were comparable between

Table 1. Summary of fertility study in male rats treated with cimadronate (Segment I ♂).

Generation	Observations		Dose level mg/kg/day			
			0 (control)	0.06	0.16	0.62
F0 ♂	No. of animals paired		22	22	22	22
	No. of deaths		0	0	0	1
	Clinical signs		NAD	NAD	NAD	NAD
	Body weight (g)	Week 0	298±13	293±12	295±14	294±14
		Week 9	495±46	489±41	492±43	477±45
	Food consumption		NAD	NAD	NAD	NAD
	Copulation index (%)		100	100	96	100
	Fertility index (%)		86	86	91	91
F0	No. of corpora lutea		15.5±2.0	16.4±2.9	16.8±2.2	17.8±3.2
♀	No. of implantations		13.8±2.0	14.9±1.8	14.8±1.0	15.3±1.3*
	Pre-implantation loss (%)		10.8	8.7	11.9	13.8
	Post-implantation loss (%)		10.3	9.9	11.7	10.9
F1	No. of live fetuses		12.4±2.6	13.5±2.3	13.1±2.7	13.7±2.1
	No. of dead fetuses		0	0	0	0
	Sex ratio, ♂ / ♀		1.23	1.06	1.06	0.88
	Fetal body weight g	♂	3.7±0.4	3.6±0.2	3.7±0.3	3.5±0.2
		♀	3.5±0.2	3.4±0.3	3.6±0.3	3.4±0.2
	External abnormality (%)		0.4	0	0	0
	Visceral abnormality (%)		0	1.6	0	0
	Skeletal abnormality (%)		0.8	0.8	0.9	0

NAD : No abnormality detected. Values are mean±S.D. * : P<0.05.

the control and treatment groups. No treatment-related fetal malformations or developmental variations were observed. Thus, the no toxic effect dose was 0.62 mg/kg/day for male fertility and F1 fetuses.

Effect on females (Table 2): No notable changes in clinical sign, body weight or food consumption were observed at doses up to 0.62 mg/kg/day. Most females had a stable estrus cycle before mating. Reproductive parameters, including copulatory and fertility indices and copulatory interval, were not affected by treatment. At necropsy, no pathological findings related to the test compound were found. The mean number of corpora lutea, implantation sites and live fetuses showed no differences between the control and the treated groups and no treatment-related fetal abnormalities were found. The no toxic effect level was therefore 0.62 mg/kg/day for female fertility and F1 fetuses.

2. Teratology study (Segment II) in rats

Effect on F0 dams (Table 3): One parent female in the 0.62 mg/kg/day dose group was found dead of possible test compound-related causes on day 2 of lactation after showing reduced activity, piloerection and a pale appearance on day 1 after parturition. Post-mortem examination revealed the presence of one dead fetus retained in utero. With the exception of this animal, there were no significant treatment-related changes in clinical sign, body weight and food consumption in any treatment group. At necropsy of F0 dams on day 20 of gestation and day 22 of lactation, no treatment-related pathological findings were observed in any treatment group.

The no toxic effect level was 0.31 mg/kg/day for F0 dams.

Effect on F1 fetuses (Tables 3 and 4): Cesarean data, including pre- and post-implantation losses, fetal sex ratio and fetal and placental

Table 2. Summary of fertility study in female rats treated with cimadronate (Segment I ♀).

Generation	Observations			Dose level mg/kg/day			
				0 (control)	0.16	0.31	0.62
F0 ♀	No. of animals pregnant/paired			22/22	19/22	22/22	21/22
	Clinical signs			NAD	NAD	NAD	NAD
	Body weight (g)	Premating	Week 0	229±9	225±10	225±9	223±12
			Week 2	254±16	251±14	253±18	251±18
	Gestation	Day 0	256±13	255±15	256±16	256±16	
		Day 20	400±29	406±38	399±27	403±32	
	Food consumption			NAD	NAD	NAD	NAD
	Copulation index (%)			100	100	100	100
	Fertility index (%)			100	86	100	96
	No. of corpora lutea			17.6±2.3	17.1±3.3	17.3±2.4	16.9±3.0
No. of implantations			14.7±2.0	14.8±3.0	14.7±2.3	14.8±2.3	
F1	Pre-implantation loss (%)			15.5	12.0	13.6	11.6
	Post-implantation loss (%)			11.3	6.5	4.7	3.2
	No. of live fetuses			13.0±3.2	14.1±3.6	14.0±2.0	14.3±2.2
	No. of dead fetuses			0	0	0	0
	Sex ratio, ♂ / ♀			1.13	1.00	1.04	0.89
	Fetal body weight (g)	♂		4.2±0.2	4.3±0.4	4.1±0.3	4.3±0.2
		♀		3.9±0.3	4.1±0.4	3.9±0.4	4.1±0.2
	External abnormality (%)			0	0	0.6	0
	Visceral abnormality (%)			0	0	0	0
	Skeletal abnormality (%)			0	0	0	0
Ossification			NAD	NAD	NAD	NAD	

NAD : No abnormality detected. Values are mean±S.D.

weights were not obviously affected by treatment. No external fetal abnormalities were found.

One fetus from a dam given 0.16 mg/kg/day was found to have displaced kidneys at visceral examination ; this finding was not considered to be of biological significance. All other visceral abnormalities found were minor and/or incidental in nature.

There were no fetuses with skeletal malformations in any group. Skeletal examination indicated slightly retarded fetal ossification in the 0.62 mg/kg/day group. A similar but equivocal effect was also seen in the 0.31 mg/kg/day group. This finding was observed in sacral vertebrae, caudal vertebrae, thoracic vertebrae, sternebrae, ischia and pubic bones. On day 22 of lactation there was no difference in the state of ossification in F1 offspring between the control and treated groups.

The no toxic effect level was 0.16 mg/kg/day for F1 fetuses.

Effect on F1 offspring (Table 3) : Twelve parental generation females gave birth to live pups in each group. Gestation length was similar for all groups. Pup data, including litter size, viability, clinical sign, body weight, sex ratio and physical development, were not affected by treatment of the parental females.

Post-weaning developmental and behavioral tests (rota-rod, water maze and open field) did not show any treatment-related differences.

Necropsy findings of pups killed on day 4 after birth, after weaning or after completion of the post-weaning behavioral tests showed no effect of treatment of the parental females.

All F1 males and females survived to terminal necropsy. Clinical sign and body weight of the F1 males and females were similar in all

Table 3. Summary of teratology and general reproduction toxicity study of cimadronate in rats (Segment II).

Generation	Observations	Dose levels mg/kg/day			
		0 (control)	0.16	0.31	0.62
F0	No. of animals, Dead/Pregnant	0/23 ^a ±12 ^b	0/18 ^a ±12 ^b	0/21 ^a ±12 ^b	1/21 ^a ±12 ^b
	Clinical signs	NAD	NAD	NAD	DLA, PIE ^c
	Body weight gain	NAD	NAD	NAD	NAD
	Food consumption	NAD	NAD	NAD	NAD
	Gestation period (day)	22.2±0.4	22.2±0.4	22.1±0.3	22.1±0.3
	No. of corpora lutea	14.9±2.4	15.9±2.0	15.4±2.9	15.9±1.9
	No. of implantations	12.6±4.2	13.3±4.4	12.7±4.3	14.5±2.7
F1 fetus	Post-implantation loss (%)	11.6	8.4	11.8	11.2
	No. of live fetuses	11.3	12.1	11.6	12.9
	No. of dead fetuses	0	0	0	0
	Sex ratio, ♂/♀	1.08	1.08	0.96	0.96
	Fetal body weight g, ♂	4.2±0.3	4.1±0.3	4.3±0.5	4.1±0.6
	♀	4.1±0.3	3.9±0.6	4.0±0.5	3.9±0.6
	External malformation (%)	0	0	0	0.0
	Visceral malformation (%)	0	1.3	0	0.0
	Skeletal malformation (%)	0	0	0	0.0
	Ossification	NAD	NAD	delayed	delayed
F1 pup	Gestation index (%)	100	100	100	100
	Birth index (%)	91	88	88	92
	Viability index on day 4 (%)	99	99	98	90
	Weaning index (%)	100	100	99	99
	External abnormality	NAD	NAD	NAD	NAD
	Body weight/Growth	NAD	NAD	NAD	NAD
	Development-Behavior	NAD	NAD	NAD	NAD
	Copulation index (%)	100	100	92	100
	Fertility index (%)	92	83	91	100
F2 pup	Gestation index (%)	100	100	100	100
	Birth index (%)	95	94	87	91
	Viability index on day 4 (%)	97	97	96	100
	Weaning index (%)	100	100	99	100
	Body weight/Growth	NAD	NAD	NAD	NAD
	Development	NAD	NAD	NAD	NAD

NAD : No abnormality detected, DLA : Decrease in locomotor activity, PIE : piloerection

Values are mean±S.D.

^a : killed on day 20 of gestation.^b : allowed to spontaneously deliver.^c : only one dead animal had toxic symptom.

Table 4. Results of external, visceral and skeletal examination in F1 fetuses of rats (Segment II).

Observations	Dose levels mg/kg/day			
	0 (control)	0.16	0.31	0.62
No. of litters examined	23	18	20	21
No. of fetuses examined for external changes	260	217	243	270
Head : hematoma	0	0	0	1
No. of fetuses examined for visceral changes	90	76	84	92
Kidney (s) : displaced	0	1	0	0
No. of fetuses examined for skeletal changes	248	235	227	250

groups during all phases of the study. Mating performance, pre-coital interval and fertility of the F1 generation were not obviously affected by treatment of the parental females. Gestation length was similar in all groups.

The no toxic effect level was 0.62 mg/kg/day for the F1.

Effect on F2 offspring (Table 3) : Pup data, including litter size, viability, clinical sign, body weight, sex ratio and physical development, were comparable in all groups. No treatment-related changes were found at necropsy of the F1 adults or F2 pups.

The no toxic effect level was 0.62 mg/kg/day for the F2 pups.

3. Peri- and postnatal study (Segment III) in rats

Effect on F0 dams (Table 5) : Five females receiving 0.62 mg/kg/day, four receiving 0.31 mg/kg/day and six receiving 0.16 mg/kg/day were found dead, or were killed in moribund condition close to the expected time of or during parturition. Terminal signs included a red secretion in the corners of the eyes and on the nose, closed eyes, salivation, erratic or labored respiration, cyanosis, coldness to touch and piloerection. Another female receiving 0.62 mg/kg/day was killed in moribund condition on day 15 after delivery after exhibiting terminal signs of an encrusted, swollen, reddened and weeping tail. In surviving females there were occasional signs related to administration into the tail vein in all groups, but otherwise the clinical sign was unaffected by treatment. Body weight change during the treatment phase of gestation for females receiving 0.62 mg/kg/day was slightly but not statistically significantly lower than that of the controls. Females at lower doses were unaffected and the pattern of weight change during

the lactation period was similar in all groups. Food intake during the gestation and lactation periods were unaffected by treatment. Gestation length of surviving females was unaffected by treatment. At weaning, necropsy of F0 females revealed no drug-related macroscopic abnormalities.

Effect on pups (F1) (Table 5) : Birth index for surviving females, litter size, offspring survival and sex ratio were unaffected by treatment. Body weight gain of offspring at 0.62 mg/kg/day was slightly lower than that of the controls up to weaning, but subsequent weight gain to 8 weeks of age was similar in all groups.

A dose-dependent incidence of abnormal tooth growth, as indicated by malocclusion and uneven growth of the lower incisors, was recorded around the time of weaning for offspring from all treated groups, and the onset and completion of tooth eruption was delayed for offspring at 0.62 mg/kg/day. Female offspring at 0.62 mg/kg/day showed a slightly higher failure rate in the rota-rod test, but functional and behavioral responses were otherwise unaffected. Necropsy of F1 offspring that died before termination confirmed abnormal tooth growth in eleven offspring at 0.62 mg/kg/day and one at 0.16 mg/kg/day. Apart from two offspring at 0.31 mg/kg/day with abnormal tooth growth of the incisors, necropsy findings of offspring (8 weeks of age) not selected for the F1 generation revealed no treatment-related abnormalities.

The clinical sign of animals of the F1 generation was similar in all groups. Tooth abnormalities were recorded in one female at 0.16 mg/kg/day, one male and one female at 0.31 mg/kg/day, and two males and three females at 0.62 mg/kg/day. One control female was found dead on day

Table 5. Summary of peri- and postnatal study of cimadronate in rats (Segment III).

Generation	Observations	Dose levels mg/kg/day			
		0 (control)	0.16	0.31	0.62
F0	No. of animals used	22	22	22	22
	No. of dead/killed animals	0	6	4	6
	No. of live litters born	22	16	18	17
	Clinical signs	NAD	CDR, PIE, PTS, SAL, BDR (seen in dead or killed animals only)		
	Body weight (g)				
	Gestation day 17	367±26	367±21	369±23	362±23
	day 20	414±30	412±22	415±27	406±27
	Lactation day 1	321±25	319±22	324±20	311±19
	day 22	339±23	339±23	344±24	336±29
	Food consumption	NAD	NAD	NAD	NAD
F1 pup	Gestation length	NAD	NAD	NAD	NAD
	No. of implantations	15.7±1.8	15.4±1.8	15.8±1.7	15.6±2.2
	Gestation index (%) A	100	73*	82	82
	B	100	100	100	100
	Birth index (%) A	90	63	67	66
	B	90	88	87	87
	No. of pups born alive	14.2±1.3	13.4±2.2	13.7±2.3	13.4±1.8
	Sex Ratio	1.11	1.05	1.20	1.03
	Viability index on day 4 (%)	97	95	98	98
	Weaning index (%)	96	98	99	98
	Body weight 1st day (♂/♀)	6.5/6.1	6.7/6.3	6.6/6.2	6.5/6.1
	(g) 3rd week (♂/♀)	55.7/52.0	55.7/52.9	52.6/51.1	49.1**/46.7**
	10th week (♂/♀)	436/275	436/275	434/277	426/261
	Development	NAD	NAD	NAD	tooth eruption delayed
	Abnormal tooth growth				
	No. of pups (litter)	0	15(4)	33(7)	70(12)
	Behavior, rotarod treadmill	NAD	NAD	NAD	♀, failing ^{a)}
	Copulation index (%)	100	100	100	100
	Fertility index (%)	100	95	95	100
F2 pup	Gestation index (%)	100	100	100	95
	Birth index (%)	83	90	87	92
	No. of pups born alive	13.1±2.8	14.2±2.0	13.9±3.4	14.0±3.4
	Sex ratio	1.16	1.10	0.81	1.01
	Viability index on day 4 (%)	98	99	100	98
	Weaning index (%)	99	100	100	99
	Body weight 1st day (♂/♀)	6.7/6.4	6.6/6.1	6.8/6.3	6.5/6.1
	3rd week (♂/♀)	66.0/63.9	67.2/62.3	68.3/65.2	66.1/63.5
	External abnormality	NAD	NAD	NAD	NAD

* : $P < 0.05$, ** : $P < 0.01$, NAD : No abnormality detected. Values are mean $\pm$ S.D.

Gestation index % A : calculated for all pregnant animals including dead or killed animals.

Gestation index % B : calculated for all pregnant animals that survived parturition.

Birth index % A : calculated for all females with implantation.

Birth index % B : calculated for all females that survived parturition.

CDR : chromodacryorrhea, PIE : piloerection, SAL : salivation, BDP : bradypnea, PTS : ptosis

^{a)} : F1 female in 0.62 mg/kg/day dose group showed an increased failure ratio on the rotarod.

22 of gestation following parturition. Body weight gain of F1 animals showed some variation, but no consistent effect of treatment was apparent.

Mating performance, fertility, parturition and litter responses of F1 animals were unaffected by treatment of F0 females.

Necropsy of F1 adults and F2 offspring revealed no adverse effects of treatment.

Additional Segment III study (Table 6) : Further study at the lower dose levels of 0, 0.003, 0.001 and 0.003 mg/kg/day was conducted. Results are summarized in Table 6. The no toxic effect level was 0.003 mg/kg/day for F0 animals and F1 pups.

4. Teratology study in rabbits

Effect on F0 dams (Table 7) : The clinical sign of control and treated dams was essentially similar throughout the study. One female each from the 0.025 and 0.05 mg/kg/day groups was killed following suspected accidental spinal injury, and cimadronate was not implicated in these deaths. Maternal body weight and food intake were similar in all groups and unaffected by treatment.

Effect on fetuses (Table 8) : Fetal growth and survival in utero was unaffected by treatment.

A slight increase in cranial abnormalities was observed at a dose of 0.05 mg/kg/day although the involvement of cimadronate was considered unlikely.

The no toxic dose was 0.05 mg/kg/day for F0 animals and F1 fetuses.

DISCUSSION

In the Segment I study, cimadronate had no adverse effect on reproduction and fertility of male and female parents or the growth and development of fetuses up to 0.62 mg/kg/day.

In the Segment II study in rats, retardation of fetal ossification was found. Similar findings were observed in a Segment II study of pamidronate in rats (Graepel *et al.*, 1992). The pups at 22 days old did not show any retardation of ossification.

Some malformations were observed in the control and treated groups in the rat and rabbit teratology studies. These malformations are commonly seen in historical data for fetuses of these rat and rabbit strains (Kameyama *et al.*, 1980 ; Morita *et al.*, 1987) and were considered

Table 6. Supplementary pre- and postnatal study of cimadronate in rats (Segment III).

Generation	Observations	Dose levels mg/kg/day			
		0 (control)	0.0003	0.001	0.003
F0	No. of animals, Dead/used	0/22	0/22	0/22	0/22
	No. of dams with live pups	22	21	21	22
	Clinical signs	NAD	NAD	NAD	NAD
	Body weight				
	Gestation day 17	396±6	390±7	404±6	379±5
	day 20	439±6	426±6	445±7	415±6
	Lactation day 0	324±6	320±5	323±5	314±6
	day 21	334±4	336±4	335±3	328±5
	Food consumption	NAD	NAD	NAD	NAD
	Gestation period	NAD	NAD	NAD	NAD
F1 pup	Gestation index (%)	100	100	100	95.5
	No. of pups born alive	14.7±0.6	14.1±0.6	16.0±0.6	12.5±1.0*
	Sex ratio ♂/♀	1.03	0.90	1.05	1.25
	Viability index on day 4 (%)	96	98	95	98
	Weaning index (%)	99	99	99	100
	External abnormality (%)	0	0	0	0.68 ^{a)}

NAD : No abnormality detected. Values are mean±S.E. * : P<0.05

^{a)} : Edema, vestigial tail.

Table 7. Summary of teratology study of cimadronate in rabbits.

Generation	Observations	Dose levels mg/kg/day			
		0 (control)	0.01	0.025	0.05
F0	No. of animals, Dead/used	0/16	0/16	1 ^a /16	1 ^a /17
	Clinical signs, Abortion	NAD	NAD	NAD	NAD
	Body weight gain	NAD	NAD	NAD	NAD
	Food consumption	NAD	NAD	NAD	NAD
	Corpora lutea	12.4±1.8	13.1±2.4	11.8±2.5	12.1±2.0
	Implantations	10.8±2.1	12.0±2.7	8.9±2.9	11.0±2.2
	Necropsy	NAD	NAD	NAD	NAD
F1	Post-implantation loss (%)	15.0	18.8	12.6	9.1
fetus	Live fetuses	9.2±1.8	9.8±2.7	7.8±2.9	10.0±2.4
	Dead fetuses	0.3±0.6	0.8±0.0	0.2±0.0	0.3±0.0
	Sex ratio, ♂/♀	1.04	1.04	1.08	0.82
	Mean fetal body weight g ♂	42.1±3.5	40.0±3.2	43.9±2.6	39.9±2.4
	♀	40.8±2.4	41.4±2.7	44.3±2.5	39.1±3.0
	External abnormality (%)	0.7	1.2	0.8	2.4
	Visceral abnormality (%)	0.7	0.6	0.0	0.6
	Skeletal abnormality (%)	1.0	0.9	2.4	0.6 ^b , 1.7 ^c

NAD : No abnormality detected. Values are mean±S.D.

^a) : accidental death during restraint.

^b) : abnormality observed in head (2/3 of live fetuses were examined).

^c) : abnormality observed in thoracic and abdominal portion (All live fetuses were examined).

spontaneous in origin.

In the Segment III study, mortality of some dams and abnormal tooth growth in offspring were observed at 0.16 mg/kg/day or more. Mortality was also observed in pregnant rats at 0.01 mg/kg/day (data not shown). Death of pregnant dams have been observed with other bisphosphonates (Graepel *et al.*, 1992, Minsker *et al.*, 1993). Cimadronate is known to reduce bone turnover and to lower blood calcium levels in hypercalcemic cancer patients (Fukumoto *et al.*, 1994). Further, cimadronate reduced blood calcium levels in experimental animals approximately 10 % in general toxicity studies (Okazaki *et al.*, 1994). In pregnant animals, calcium demand rises sharply near parturition because ossification of the fetal skeleton is at its most rapid rate at this time (Minsker *et al.*, 1993 ; Garner *et al.*, 1988). This demand can normally be met by mobilizing calcium from the dam's skeleton (Halloran *et al.*, 1979). This mechanism, however, is blocked or made ineffective by cimadronate through its inhibition of bone resorption. This

could lead to marked hypocalcemia and to the observed distress syndrome with possible lethal consequences before or during parturition. However, there were no adverse effects of treatment with cimadronate on body weight change and reproductive performance of females which delivered live pups. The number of pups born or weaned in treated litters was comparable to that in the control. More extensive studies on the abnormal tooth growth are currently in progress.

We conclude that the maximum no toxic effect level of cimadronate on parent rats and offspring is 0.003 mg/kg/day, and that on rabbit dams and their offspring is 0.05 mg/kg/day. Cimadronate showed no evidence of any teratogenic potential in rats or rabbits at any dose level tested.

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Table 8. Results of external, visceral and skeletal examination in F1 fetuses of rabbits.

Observations	Dose levels mg/kg/day			
	0 (control)	0.01	0.025	0.05
No. of litters examined	16	16	15	16
No. of fetuses examined for external changes	147	156	125	170
	cyclopia reduced eye	open eyes, cervical oede- ma bilateral hind- limb flexure otocephaly absence of eyes and jaws	otocephaly	acephaly cleft palate microphthalmia
Total fetal external abnormalities	1	2	1	3
No. of fetuses examined for visceral changes	147	156	125	170
	gastroschisis	encephalocele		encephalocele
Total fetal visceral abnormalities	1	1	0	1
No. of fetuses examined for skeletal changes	Head	99	107	84
	Other	147	156	125
	anomalous thoracic and lumbar vertebrae	anomalous lumbar vertebrae	anomalous thoracic vertebrae anomalous rib cage and vertebral column	fusion of frontal bone fusion of frontal and parietal bones fused ribs
Total fetal skeletal abnormalities	1	1	2	3

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