TERATOLOGICAL STUDIES OF HYDROXYPROPYLMETHYLCELLULOSE ACETATE SUCCINATE IN RATS

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Abstract.....A teratogenicity study was carried out in S1c : SD rats orally administered Hydroxypropylmethylcellulose acetate succinate (HPMCAS), a useful pharmaceutical excipient, at dose levels of 625, 1,250 and 2,500 mg/kg/day for a period of 11 days from day 7 to day 17 of gestation.

Two-thirds of the pregnant females in each group were sacrificed on Day 21 of gestation and their fetuses were examined. The remaining dams were allowed to litter naturally, and the postnatal development of the offsprings was observed.

The incidences of external, internal, and skeletal anomalies were not significantly increased in the fetuses of any treated groups. HPMCAS caused no effects on parturition, lactation, postnatal growth and reproductive ability of the male and female offspring.

Key words : HPMCAS, excipient, teratogenicity study, rat.

INTRODUCTION

The present paper deals with the effect of Hydroxypropylmethylcellulose acetate succinate (HPMCAS) administered orally during the period of organogenesis in rats. The study was carried out during the period from June and November, 1981, in accor-

dance with the "Guidelines for Reproduction Experiments to Evaluate the Safety of Drugs" issued by the Ministry of Health & Welfare, Japanese Government in March 1975.

MATERIALS AND METHODS

1. Materials

Hydroxypropylmethylcellulose acetate succinate (HPMCAS), a derivative of cellulose, is white and odorless powder and insoluble in water.

2. Methods

Animals and Experimental conditions ;

Sprague-Dawley rats of 10 weeks old of either sex were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals. They were maintained in an animal facility for at least 7 days prior to mating. Commercial chow (MF, Oriental Yeast Co. Ltd.) and fresh tap water were given to rats ad libitum. The rats were housed in a fully air-conditioned animal facility where the temperature ($24 \pm 2^\circ\text{C}$) and the relative humidity ($55 \pm 10\%$) were kept constant, and the room was ventilated in rate of 7 times per hour and was lighted for 12 hrs from 6 : 00 a. m. to 18 : 00 p. m.

Mating Procedure ;

Males were individually kept in a stainless steel wire cage ($26 \times 38 \times 20$ cm), and were paired with female from 17 : 00 p. m. on a 1 : 1 basis, with females. The mating was confirmed by the presence of vaginal plugs and the presence of sperm in vaginal smear on the next day. This day was determined to be day 0 of gestation. Pregnant rats (more than forty rats in each group) were individually kept in a polycarbonated cage ($27 \times 35 \times 16$ cm) bedded with White Flake (Chales River Japan).

Dosage ;

Based on the data of preliminary study in pregnant rats, dosage range was determined as follows ; the highest dosage level was set at 2,500 mg/kg which was a maximum dose to administer, and at the dose no significant toxic effects were observed. The dose levels of 1,250 mg/kg and 625 mg/kg were chosen as the medium and lowest level, respectively.

Preparation of test material and administration ;

HPMCAS was suspended in 0.25% carboxymethylcellulose (CMC) solution and prepared to 3.125, 6.25 and 12.5 w/v%. Each pregnant rat was dosed orally via a gastric tube once a day in a volume of 2 ml of the suspension per 100 g body weight during the period of organogenesis of 11 days from day 7 to day 17 of gestation. Control rats were dosed a similar volume of 0.25% CMC solution.

Observations ;

On day 21 of gestation, two-thirds pregnant females in each group (27 to 30 rats in each group) were anesthetized with ether and autopsied by Cesarean section. The number of corpora lutea, implantations, viable and dead fetuses, and resorbed embryos were counted and positions of implantations were also examined. Body weight of all

viable fetuses were measured individually and examined for external abnormalities and sex. Two-thirds of the viable fetuses per litter, randomly selected, were stained with the alizarin red S according to the modified method of Dawson (1926) and examined for skeletal anomalies and development. As an index of state of ossification, ossifying rate of first cervical vertebral centra, number of cervical vertebral centra, number of caudal vertebrae, lack of sternbrae, number of metacarpus, number of proximal diaphysis in forepaw, number of metatarsus, number of proximal diaphysis in hindpaw and number of distal diaphysis in hindpaw were observed. The remaining one-third fetuses were fixed with formalin-alcohol mixture, and visceral abnormalities were examined on the fetuses by the Wilson technique (1965).

One-third dams in each group were allowed to deliver spontaneously. The growth, development and reproductive ability of the offsprings were observed.

Maternal ;

- 1) All visible changes in behavior which the dams showed in responding to the oral treatment were recorded, in terms of details of type, severity, duration and time of onset.
- 2) Females were weighed every day during gestation and once a week after parturition.
- 3) Food consumption and water intake were measured twice a week during gestation, and once a week during nursing period.
- 4) Each animal was examined macroscopically for evidence of disease or adverse reaction by the treatment, and observed the development of mammary glands at the terminal period of gestation.

Fetuses ;

- 1) Number of died resorption, and its occurrence time were recorded.
- 2) All the viable fetuses were examined body weight placental weight, sex, and external anomalies. Two-thirds of the viable fetuses per litter were stained with the alizarin red S by the method of Dawson and examined for skeletal anomalies. Visceral abnormalities were examined on the remaining one-third fetuses by the Wilson technique.

Offsprings ;

- 1) Body weights of offsprings were measured on days 0, (at birth), 3, 7, 10, 14, 17 and 21 *post partum* , and at once a week thereafter.
- 2) Food consumption and water intake were recorded at once weekly.
- 3) On day 7 *post partum* numbers of offsprings were adjusted into ten per one litter.
- 4) The skeletal developments in F₁ rats at weaning time were examined.

Following examinations were carried out between breeding and weaning period.

Physical development (Irwin 1974 and Fox 1965) ;

- 1) Righting reflex in air ; three weeks-old rats were left in air space at the height of 30–50 cm with rotation and then examined the sitting conditions on the sponge mat.
- 2) Corneal reflex ; three weeks-old rats were examined the winks when the eye was stimulated with a suture fiber.

3) Auditory function ; Auditory function was assessed using the startle response to a sudden sharp noise.

4) Pain response ; At 3 weeks old rat, whether or not to cry when pressed the tail with a forceps.

5) Swimming ability ; The swimming ability of 3 weeks-old rats was evaluated as four steps (0-3) determined from swimming behavior according to the method of Schapiro et al (1970).

6) Chinning test ; When 3 weeks-old rat was chinned in a wire (diameter 2 mm) at the height of 40 cm with fore legs alone, it was measured a time to drop.

Locomotor function ;

Open-field test was used for examination the locomotion function of offsprings ; The test apparatus had a square area of 50×50 cm in size. The floor was marked off into 25 sections at 10 cm intervals. The animal at 8 weeks of age was placed in the center of the field. Numbers of sections crossed, rearings, groomings and defecation were counted for a 3-minutes observation period.

Learning test ;

Reflex ; Examination of avoidance response against electric stimulus using 8-10 weeks-old rats. The experiment was done using Reflex Response Experimental Apparatus (shuttle box, Ohara medical industry K. K.). One session was 10-15 training per day. The experiments were successively carried out for 14 days at a rate of one session per a day.

$$\text{Percent of avoidance} = \frac{\text{No. of reflex}}{\text{No. of warning}} \times 100$$

Sex cycle ;

At 9 to 11 weeks-old rats, estrus cycle was determined as diestrus, proestrus, estrus-I and -II obtained by the microscopic examination for vaginal smears.

Reproductive ability ;

At 11 weeks of age, one male and female from each litter were mated within the same group but avoiding litter mates. The females were examined for the presence of vaginal plugs and spermatozoa in vaginal smears every morning. The females were sacrificed on day 21 of gestation and examined for reproductive status. The viable fetuses were weighed, sexed and examined for sex and gross abnormalities.

Organ weights ;

At 3 weeks of age, wet weight of each rats (F₁), of the following organs was weighed ; brain, thymus, heart, lung, liver, spleen, kidney, testis, ovary and uterus.

Statistical analysis ;

Data were analyzed statistically by student's t-test and Wilcoxon rank sum test.

RESULTS

1. Effect on dams

General condition and mortality ;

No death of dams has occurred on in the control group and the administered groups throughout the experiment. No abnormal behavior was also observed in the control and the administered groups. In spontaneous delivery groups, perturient abnormalities such as partus prematurus, abortus and partus difficilis were not observed.

Body weights ;

Changes of body weights in both the control group and the administered group were shown in Fig. 1 during the gestation period. Dose dependent changes of body weight were not seen in the administered groups.

Food consumption and water intake ;

Changes of the food and water consumption in both control group and the administered group were indicated in Table 1 during the gestation period. No significant difference in dose dependent changes was seen between the control group and the administered group, but the food consumption in the 2,500 mg/kg dosage group at late pregnancy was significantly decreased ($p < 0.01$).

2. Effect on fetuses

As seen in Table 2, no significant difference in the number of corpora lutea, implantations, resorptions, dead and viable fetuses, male/female (sex ratio) and litter weight was observed between the control group and administered groups. Placental weight in the 625 mg/kg or the 2,500 mg/kg dosage group was, however, significantly decreased compared to the control group ($p < 0.05$).

As shown in Table 3, club foot as external malformations was detected in both the

Fig. 1. Mean body weight of dams orally administered with HPMCAS on day 7-17 of gestation

control (Photo 1) and administered groups (Photo 2). An increase in incidence of club foot in dams treated with HPMCAS (8 in the 625 mg/kg, 9 in the 1,250 mg/kg and 16 in the 2,500 mg/kg groups) was observed dose-dependently, but this was not a statistically significant increase versus the control group. Short tail was also observed 2 cases in the 1,250 mg/kg dosage group, but its frequency was very low (0.58%) (Photo 3). Concerning internal malformations pyelectasis was observed to lower degrees in both control and administered groups (2 in the control, 1 in the 625 mg/kg, 1 in the 1,250 mg/kg and 3 in the 2,500 mg/kg groups), but no significant difference was seen between the control and the administered group.

Table 1 Water and food consumptions of dams orally administered HPMCAS on day 7-17 of gestation during gestation in rats

Dose (mg/kg/day)		Control gestation n=45	625 gestation n=43	1,250 gestation n=41	2,500 gestation n=43
Day ^{a)}					
Water (ml)	2-3	27.6 ^{b)} ±0.9 ^{c)}	28.7 ±1.0	26.1 ±0.9	26.5 ±0.8
	6-7	29.4 ±0.7	28.9 ±0.7	28.3 ±0.8	27.0 ±0.7
	9-10	28.2 ±0.9	30.5 ±1.0	27.7 ±0.9	27.3 ±0.8
	13-14	31.1 ±0.8	31.5 ±0.8	33.4 ±0.9	30.3 ±0.8
	16-17	36.2 ±0.8	36.0 ±0.7	34.3 ±1.0	33.2* ±1.0
	20-21	27.2 ±1.2	30.6* ±1.2	27.2 ±1.2	26.5 ±1.4
Food (g)	2-3	14.5 ±0.3	14.5 ±0.3	14.1 ±0.3	14.9 ±0.3
	6-7	15.5 ±0.3	15.9 ±0.3	15.8 ±0.4	15.6 ±0.3
	9-10	14.8 ±0.4	15.5 ±0.3	14.7 ±0.4	14.4 ±0.3
	13-14	16.8 ±0.4	16.3 ±0.4	15.9 ±0.3	15.9 ±0.3
	16-17	18.0 ±0.4	17.9 ±0.4	17.7 ±0.4	17.6 ±0.4
	20-21	12.0 ±0.7	12.3 ±0.8	12.3 ±1.0	9.0** ±0.7

Each value represents mean (b) and standard error (c). Day of gestation (a).

*: p<0.05 vs control **: p<0.01 vs control

Skeletal malformations such as fission of thoracic vertebral centra, synostosis of sternbrae and shortening of ribs were observed as follows (Table 3); as to fission of thoracic vertebral centra 1 in the 625 mg/kg dosage group (0.45%) (Photo 4), as to synostosis of sternbrae 1 in the 625 mg/kg dosage group (0.45%) (Photo 5), as to shortening of ribs one in the control group (0.44%) (Photo 6) and one in the 1,250 mg/kg dosage group (0.45%) (Photo 7).

Numbers of fetuses having skeletal variations were 5 in the control group, 5 in the 625 mg/kg dosage group, 11 in the 1,250 mg/kg dosage group and 7 in the 2,500 mg/kg dosage group. As shown in Table 4, the lists of skeletal variations were deformation of thoracic vertebral centra, asymmetry of sternbrae, fission of sternbrae and formation

Table 2 Some aspects of fetuses born of rats after oral administration of HPMCAS on day 7-17 of gestation

Dose (mg/kg/day)	Control	625	1,250	2,500
No. of mothers	30	29	27	29
Maternal mortality	0	0	0	0
Abortion	0	0	0	0
Total mother of all resorption	0	0	0	0
Nature delivery	0	0	0	0
Total number of corpora luteum (Mean±S. E.)	421 (14.0±0.3)	406 (14.0±0.3)	377 (14.0±0.3)	411 (14.2±0.3)
Total implantation (Mean±S. E.)	410 (13.7±0.3)	398 (13.7±0.3)	366 (13.6±0.3)	407 (14.0±0.3)
Total resorption (%)	33 (8.0)	22 (5.5)	19 (5.2)	21 (5.2)
Early	32	22	18	21
Late	1	0	1	0
Total dead (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total live (%) (Mean±S. E.)	377 (92.0) (12.6±0.3)	376 (94.5) (13.0±0.3)	347 (94.8) (12.9±0.4)	386 (94.8) (13.3±0.3)
Male	201 (53.3)	190 (50.5)	160 (46.1)	218 (56.5)
Sex (%) Female	176 (46.7)	186 (49.5)	187 (53.9)	168 (43.5)
Male/female	1.1	1.0	0.9	1.3
Mean litter weight (g)	59.88±1.47	62.64±1.39	61.16±1.33	62.36±1.25
Mean body weight (g)				
Male	4.90±0.05	4.99±0.06	4.90±0.06	4.83±0.06
Female	4.66±0.05	4.71±0.05	4.70±0.05	4.52±0.06
Mean placental weight (g)	0.53±0.01	0.50±0.01*	0.51±0.01	0.50±0.01*
Loss of pre-implatation (%)	2.6	2.0	2.9	1.0
Loss of post-implatation (%)	8.0	5.5	5.2	5.2

*: $p < 0.05$ vs control

Table 3 Malformations of fetuses (F₁) born of rats administered HPMCAS in the teratogenicity study

Dose (mg/kg/day)	Control	625	1,250	2,500
<u>Malformations</u>				
External (%)	3/377(0.80) ^{a)}	8/376(2.13) ^{a)}	11/347(3.17)	16/386(4.15)
Club foot	3	8	9	16
Short tail	0	0	2	0
Internal (%)	2/136(1.47)	1/134(0.75)	1/123(0.81)	3/138(2.17)
Pyelectasis	2	1	1	3
Skeletal (%)	1/225(0.44)	2/223(0.90)	1/224(0.45)	0.248(0.00)
Fission of thoracic vertebral centra	0	1	0	0
Synostosis of sternebrae	0	1	0	0
Shortening of ribs	1	0	1	0

a) : Loss of specimen during preparation (16 in the control and 19 in the 625).¹

Photo 1. Control
Clubfoot and normal

Photo 2. HPMCAS 2,500 mg/kg
Clubfoot

Photo 3. HPMCAS 1,250 mg/kg
Short tail

Photo 4. HPMCAS 625 mg/kg
Fission of thoracic vertebral centra

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Photo 5. HPMCAS 625 mg/kg
Synostosis and asymmetry
of sternebrae

Photo 6. Control
Littleness ribs (Bilateral)

Photo 7. HPMCAS 1,250 mg/kg
Littleness rib (Right)

Photo 8. Control (Female)
Littleness rib (Right)

Photo 9. HPMCAS 2,500 mg/kg
Fission of thoracic vertebral centra

Table 4 Effects of HPMCAS on skeletal development in rat fetuses (F₁)
(Rat Segment 2)

Dose (mg/kg/day)	Control	625	1,250	2,500
No. of fetuses examined	225	223	224	248
No. of fetuses with skeletal variations (%)	5 (2.22)	5 (2.24)	11 (4.91)	7 (2.82)
Deformation of thoracic vertebral centra	0	1	3	1
Asymmetry of sternebrae	1	1	2	2
Fission of sternebrae	1	0	1	1
Formation of cervical ribs	2	0	1	1
Formation of lumbar ribs	1	3	4	3
State of ossification				
Ossifying rate of 1st cervical vertebral centra (%) (Mean±S. E.)	45.52±5.36	51.62±5.16	38.46±5.08	36.92±3.56
No. of cervical vertebral centra (Mean±S. E.)	5.02±0.19	4.77±0.13	5.29±0.16	4.55±0.13*
No. of caudal vertebrae (Mean±S. E.)	7.41±0.14	7.30±0.12	7.31±0.12	7.13±0.11
Lack of sternebrae	22	19	17	18
No. of metacarpus (Mean±S. E.)	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00
No. of proximal diaphysis in forepaw (Mean±S. E.)	7.41±0.18	7.47±0.14	7.36±0.16	7.41±0.13
No. of distal diaphysis in forepaw (Mean±S. E.)	9.98±0.01	10.00±0.00	10.00±0.00	10.00±0.00
No. of metatarsus (Mean±S. E.)	9.93±0.07	10.00±0.00	9.99±0.01	10.00±0.00
No. of proximal diaphysis in hindpaw (Mean±S. E.)	3.21±0.47	2.79±0.49	2.61±0.48	2.29±0.41
No. of distal diaphysis in hindpaw (Mean±S. E.)	10.00±0.00	10.00±0.00	9.96±0.04	10.00±0.00

*: p<0.05 vs control

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Table 5 Some aspects of newborn born of rats after oral administration of HPMCAS on day 7-17 of gestation

Dose (mg/kg/day)		Control	625	1,250	2,500
No. of dams (F ₀)		15	14	14	14
Gestation period	Mean	21.6	22.1**	21.8	21.8
	S. E.	0.1	0.1	0.1	0.2
Implantation	Total	201	202	185	189
	Mean	13.4	14.4	13.2	13.5
	S. E.	0.5	0.4	0.5	0.5
Total born	Total	192	185	178	176
	Male	103	93	87	88
	Female	89	92	91	88
Stillborn	Total	5	9	1	4
	Male	2	5	0	4
	Female	3	4	1	0
Liveborn	Total	187	176	177	172
	Male	101	88	87	84
	Female	86	88	90	88
Sex ratio (Male/Female)		1.17	1.00	0.97	0.96
0 day	Male	101	88	87	84
	Female	86	88	90	88
3 day	Male	92 (9) ^{a)}	80 (9)	87	79 (5)
	Female	81 (5)	77 (11)	88 (2)	81 (7)
7 day	Male	92	79 (1)	85 (2)	78 (1)
	Female	80 (1)	75 (2)	86 (2)	79 (2)
Selected	Male	72	61	68	67
	Female	66	59	72	73
10 day	Male	72	61	68	67
	Female	66	59	72	73
14 day	Male	72	61	68	64 (3)
	Female	66	58 (1)	72	70 (2)
17 day	Male	72	61	68	61 (3)
	Female	66	58	72	68 (2)
21 day	Male	72	61	68	61
	Female	66	58	72	68
Delivery rate (%)	Mean	95.32	91.65	95.92	93.40
	S. E.	1.97	3.61	1.59	2.43
Viability index at birth (%)	Mean	96.97	93.21	99.49	97.46
	S. E.	2.05	6.33	0.51	1.99
Nursing rate (%)	7th Mean	92.59	82.92	97.04	90.68
	S. E.	6.65	9.47	1.73	4.49
	21th Mean	100.00	99.17	100.00	92.14
	S. E.	0.00	0.83	0.00	7.12
External anomaly		0/192	0/185	0/178	0/176

a) : Each value in parenthesis represents the number of dead newborn

** : p<0.01 vs control

of cervical ribs and lumbar ribs. Frequency of each skeletal variation in all groups was 2.22% to 4.91% (Table 4). Number of cervical vertebral centra was significantly ($p < 0.05$) decreased in the 2,500 mg/kg dosage group and there was no significant difference in other index of the state of ossification among the groups.

3. Effect on delivery and nursing

Average gestation period was significantly ($p < 0.01$) longer in the 625 mg/kg dosage group, but this prolongation was within a normal range of gestation period (Table 5). Delivery rate and viable offsprings rate were 91.65% to 95.92% and 93.21% to 99.49%, respectively. No external anomalies was also observed.

Table 6 Effects of HPMCAS on postnatal development of youngs (F_1) from dams orally administered HPMCAS on day 7-17 of gestation

Dose (mg/kg/day)		Control		625		1,250		2,500	
Item	Sex	Male	Female	Male	Female	Male	Female	Male	Female
Body weight of youngs (g)	at birth	5.05 ^{a)} $\pm 0.11^b$	4.82 ± 0.11	5.00 ± 0.08	4.75 ± 0.12	5.11 ± 0.11	4.81 ± 0.12	4.91 ± 0.11	4.70 ± 0.13
	3 day	6.82 ± 0.21	6.48 ± 0.19	6.92 ± 0.22	6.53 ± 0.22	7.12 ± 0.29	6.74 ± 0.24	6.56 ± 0.24	6.26 ± 0.25
	7 day	11.14 ± 0.25	10.58 ± 0.32	11.34 ± 0.32	10.76 ± 0.39	11.57 ± 0.45	10.92 ± 0.35	10.85 ± 0.47	10.39 ± 0.35
	10 day	15.87 ± 0.21	15.10 ± 0.33	16.15 ± 0.62	15.64 ± 0.55	16.59 ± 0.46	15.52 ± 0.32	15.24 ± 0.60	14.75 ± 0.46
	14 day	22.47 ± 0.27	21.54 ± 0.39	22.36 ± 0.73	22.10 ± 0.67	23.04 ± 0.66	21.80 ± 0.55	21.64 ± 0.70	21.22 ± 0.57
	17 day	27.11 ± 0.40	26.03 ± 0.50	27.21 ± 0.91	26.97 ± 0.67	27.87 ± 0.78	26.30 ± 0.61	26.67 ± 0.72	26.09 ± 0.58
	21 day	36.27 ± 0.57	35.11 ± 0.69	36.42 ± 1.32	36.12 ± 1.03	37.58 ± 1.24	35.83 ± 1.03	35.65 ± 1.08	35.10 ± 0.85
Separation of ear auricula (days)		3.69 ± 0.08		3.65 ± 0.09		3.74 ± 0.13		3.81 ± 0.13	
Emerged of abdominal hair (days)		9.34 ± 0.13		9.38 ± 0.09		9.29 ± 0.16		9.58 ± 0.16	
Eruption of lower insisors (days)		11.74 ± 0.11		11.82 ± 0.09		11.99 ± 0.13		12.09 ± 0.14	
Separation of eyelids (days)		16.32 ± 0.14		16.26 ± 0.22		16.29 ± 0.14		16.25 ± 0.13	
Descent of testis (days)		30.36 ± 0.13		30.39 ± 0.11		30.36 ± 0.10		30.51 ± 0.13	
Opening of vagina (days)		36.24 ± 0.24		35.44 ± 0.34		35.48 ± 0.31		35.38* ± 0.30	
No. of young with skeletal anomaly	Male								
	Female	0/15 1/12		0/13 0/10		0/13 0/15		0/9 0/16	

Each value represents mean (a) and standard error (b)

*: $p < 0.05$ vs control

Nursing rate was 82.92% to 97.04% at the 7th day of birth and 92.14% to 100% at the 21st day of birth among the groups.

Body weight gains and postnatal development of young are shown in Table 6. There was no significant difference among the groups.

4. Effect on young during weaning period

Body weight gains, water intake and food consumption from the 3rd week up to the 10th week were shown in Fig. 2, Fig. 3, and Fig. 4, respectively. No difference were observed between the control and the administered groups.

Absolute and relative organ weight in young rats at 3-weeks of age were indicated in Table 7 and Table 8, respectively. As to absolute organ weight, heart weight of males was significantly ($p < 0.05$) decreased in the 2,500 mg/kg dosage group and ovary weight of females was significantly ($p < 0.05$) increased in 1,250 mg/kg dosage group. With regard to relative organ weight, in males, heart and testis weights were significantly ($p < 0.05$) decreased in the 625 mg/kg dosage group and heart weight was significantly ($p < 0.05$) decreased in the 2,500 mg/kg dosage group. In females, heart, lung and liver weight were significantly ($p < 0.05$) decreased in the 625 mg/kg dosage group and ovary weight was significantly ($p < 0.05$) increased in the 1,250 mg/kg dosage group. These changes were not dependent on the dosage of HPMCAS.

Table 9 showed the results of skeletal development of 3 weeks old rats. As to skeletal malformations there was one littleness ribs in the control group (3.70%) (Photo 8). As to state of ossification, number of caudal vertebrae was within the range of 27.78 and 27.86 and no variations were detected.

Fig. 2. Growth curves of male and female rats (F₁) in the teratological study

Fig. 3. Water consumption of F₁ rats born of the HPMCAS-treated dams (F₀)

Fig. 4. Food consumption of F₁ rats born of the HPMCAS treated dams (F₀)

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Table 7 Absolute organ weights in F₁ males and females at weaning time

		Male				Female				Mean S. E.
Dose (mg/kg/day)		Control n=15	625 n=12	1,250 n=13	2,500 n=13	Control n=12	625 n=12	1,250 n=15	2,500 n=13	
Brain (g)		1.433 ^{a)} ±0.020 ^{b)}	1.367 ±0.026	1.422 ±0.020	1.387 ±0.014	1.393 ^{a)} ±0.018 ^{b)}	1.362 ±0.022	1.386 ±0.015	1.362 ±0.011	
Thymus (mg)		149.3 ±9.5	151.7 ±14.5	164.6 ±9.9	154.6 ±10.9	162.5 ±9.2	153.3 ±13.8	173.3 ±8.5	163.8 ±8.7	
Heart (mg)		230.0 ±5.2	215.0 ±10.3	235.4 ±6.0	210.0* ±7.1	216.7 ±8.0	207.5 ±7.0	221.3 ±6.3	206.9 ±7.5	
Lung (mg)		366.7 ±10.5	363.3 ±18.6	364.6 ±15.3	359.2 ±11.9	354.2 ±13.3	340.0 ±16.7	362.7 ±8.1	358.5 ±8.4	
Liver (g)		1.965 ±0.078	2.020 ±0.144	2.093 ±0.051	2.020 ±0.082	1.998 ±0.063	1.965 ±0.066	2.028 ±0.044	2.035 ±0.059	
Spleen (mg)		188.7 ±9.5	176.7 ±15.3	191.5 ±8.3	180.8 ±8.4	175.8 ±11.6	182.5 ±10.9	186.0 ±6.2	180.0 ±6.5	
Kidney (mg)	Total	544.7 ±14.2	544.3 ±30.1	575.4 ±16.3	534.6 ±17.9	537.5 ±18.7	540.0 ±21.8	569.3 ±14.1	540.8 ±16.3	
	Right	270.7 ±7.1	275.8 ±15.5	289.2 ±9.0	266.9 ±8.9	270.0 ±9.1	273.3 ±11.0	288.0 ±8.2	269.2 ±8.5	
	Left	274.0 ±7.3	267.5 ±14.7	286.2 ±7.8	267.7 ±9.3	267.5 ±9.9	266.7 ±11.3	281.3 ±6.2	271.5 ±8.0	
Testis (M) Ovary (F) (mg)	Total	242.7 ±9.0	230.8 ±15.9	270.8 ±14.4	233.1 ±10.6	26.08 ±1.01	24.75 ±1.40	29.73* ±1.13	26.92 ±1.07	
	Right	120.0 ±4.8	115.8 ±8.3	136.2 ±7.0	116.2 ±5.6	13.33 ±0.59	12.50 ±0.75	14.33 ±0.51	13.46 ±0.51	
	Left	122.7 ±4.4	115.0 ±7.7	134.6 ±7.5	116.9 ±5.2	12.75 ±0.55	12.25 ±0.71	15.40* ±0.74	13.46 ±0.64	
Uterus (mg)						33.67 ±1.74	32.25 ±3.05	34.47 ±1.17	30.54 ±1.54	

Each value represents mean (a) and standard error (b)

*: p<0.05 vs control

Table 8 Relative organ weights in F₁ males and females at weaning time

		Male				Female				Mean S. E.
Dose (mg/kg/day)		Control	625	1,250	2,500	Control	625	1,250	2,500	
Brain (g/100 g B. W.)		4.134 ^{a)} ±0.092 ^{b)}	3.918 ±0.207	3.871 ±0.133	4.092 ±0.131	4.088 ^{a)} ±0.122 ^{b)}	3.841 ±0.124	3.871 ±0.101	3.935 ±0.106	
Thymus (mg/100 g B. W.)		424.6 ±21.5	410.9 ±30.6	446.9 ±31.4	444.6 ±21.5	470.7 ±18.8	423.3 ±26.1	478.8 ±19.2	467.8 ±19.4	
Heart (mg/100 g B. W.)		662.3 ±16.1	601.8 ±15.3*	638.2 ±21.1	614.0 ±13.8*	630.0 ±14.8	581.5 ±14.8*	614.5 ±15.7	593.7 ±16.9	
Lung (mg/100 g B. W.)		1,053.3 ±25.4	1,018.7 ±34.2	982.3 ±27.6	1,050.5 ±25.2	1,030.9 ±26.3	946.4 ±19.9*	1,008.5 ±20.9	1,031.8 ±26.2	
Liver (g/100 g B. W.)		5.609 ±0.125	5.530 ±0.205	5.654 ±0.101	5.881 ±0.122	5.815 ±0.095	5.498 ±0.096*	5.635 ±0.102	5.847 ±0.148	
Spleen (mg/100 g B. W.)		537.9 ±18.7	478.9 ±29.3	518.9 ±25.2	525.6 ±13.8	509.8 ±26.4	507.7 ±19.3	514.8 ±10.5	517.6 ±18.4	
Kidney (mg/100 g B. W.)	Total	1,561.1 ±41.2	1,508.2 ±21.9	1,554.5 ±35.8	1,560.5 ±27.5	1,565.8 ±38.7	1,514.8 ±56.2	1,580.3 ±29.2	1,552.4 ±37.2	
	Right	778.6 ±20.2	765.1 ±12.1	780.6 ±18.2	779.1 ±12.9	787.5 ±21.4	767.3 ±29.8	798.3 ±15.2	773.0 ±20.3	
	Left	787.7 ±19.1	742.8 ±10.6	773.7 ±19.6	781.2 ±16.2	778.3 ±19.1	747.5 ±27.6	781.9 ±15.5	779.4 ±17.4	
Testis (M) Ovary (F) (mg/100 g B. W.)	Total	696.4 ±23.0	635.6 ±20.6	735.5 ±50.1	676.3 ±14.8	76.26 ±3.12	69.40 ±3.53	82.58 ±2.81	77.32 ±2.72	
	Right	344.1 ±11.9	318.4 ±10.8	369.5 ±23.7	336.3 ±8.3	38.98 ±1.81	34.91 ±1.65	39.73 ±1.10	38.68 ±1.37	
	Left	352.4 ±11.8	317.1 ±10.7*	366.1 ±26.5	339.9 ±8.2	37.28 ±1.70	34.46 ±2.08	42.87 ±1.96*	38.61 ±1.60	
Uterus (mg/100 g B. W.)						99.90 ±7.91	90.13 ±7.55	96.05 ±3.70	87.80 ±4.12	

Each value represents mean (a) and standard error (b)

*: p<0.05 vs control

Table 9 Effects of HPMCAS on skeletal development in F₁ rats at weaning time

Dose (mg/kg/day)		Control	625	1,250	2,500
No. of rats examined	Total	27	23	28	25
	Male	15	13	13	9
	Female	12	10	15	16
Skeletal malformation (%)	Total	1/27(3.70)	0/23(0.00)	0/28(0.00)	0/25(0.00)
	Male	0/15(0.00)	0/13(0.00)	0/13(0.00)	0/9(0.00)
	Female	1/12(8.33)	0/10(0.00)	0/15(0.00)	0/16(0.00)
Shortening of ribs	Total	1	0	0	0
	Male	0	0	0	0
	Female	1	0	0	0
State of ossi- fication No. of caudal vertebrae (Mean±S. E.)	Total	27.85±0.17	27.78±0.17	27.86±0.17	27.80±0.14
	Male	28.00±0.24	27.69±0.24	28.08±0.24	27.78±0.22
	Female	27.67±0.22	27.90±0.23	27.67±0.23	27.81±0.19

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Table 10 Results of function test in F₁ rats

Dose (mg/kg/day)	Control		625		1,250		2,500		
Item	Sex	Male	Female	Male	Female	Male	Female	Male	Female
<u>Swimming test</u> (21 day)									
No. of rats		15	12	12	12	13	15	13	13
Negative		0	0	0	0	0	0	0	0
Complete		15	12	12	12	13	15	13	13
<u>Chinning test</u>									
Sec.		155.6	166.3	114.5	158.6	131.5	126.8	190.0	106.9
S. E.		23.6	33.7	22.0	27.3	27.2	22.3	52.0	23.0
<u>Open field test</u>									
No. of rats		14	14	12	12	14	14	13	13
Mean Passing		17.8	19.0	18.8	16.6	21.7	20.2	21.3	21.3
S. E.		1.5	1.4	1.0	1.9	1.4	1.2	1.6	1.2
Rearing		4.9	4.2	4.9	3.7	4.6	4.9	4.6	4.2
		0.5	0.5	0.6	0.4	0.6	0.4	0.6	0.7
Grooming		0.2	0.1	0.3	0.2	0.4	0.5	0.3	0.2
		0.1	0.1	0.2	0.1	0.3	0.2	0.1	0.1
Defecation		0.2	0.1	0.0	0.0	0.1	0.1	0.2	0.1
		0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1
<u>Reflex test</u> (21 day)									
No. of rats		72	66	61	58	68	72	61	68
Righting		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eye-lid		„	„	„	„	„	„	„	„
Pain response		„	„	„	„	„	„	„	„
Auditory		„	„	„	„	„	„	„	„
<u>Reflex test</u> (70 day)									
No. of rats		42	42	36	36	42	42	39	39
Auditory		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Light		„	„	„	„	„	„	„	„

Results of function test such as reflex, swimming, chinning and open field were shown in Table 10. No abnormalities were observed with the regard to righting reflex, eye-lid reflex, pain response and auditory. As to swimming test the condition of swimming was completely normal in all rats and they showed swimming in coordination. No difference in chinning time was observed among the groups. Since in the open field test numbers of passing, grooming, rearing and defecation were not different among the groups, it assumed that there was not influence of HPMCAS on locomotion functions. The results of learning test of rats in shuttle box were shown in Fig. 5. After the training period of 14 days, number of rats having no avoidance response against electric stimulation were as follows : one in the 1,250 and the 2,500 mg/kg dosage groups (10%) in males, each two in the control and the 625 mg/kg dosage groups (20%), each one in the 1,250 and the 2,500 mg/kg dosage groups (10%) in females. There was no significant difference in a time course of development of learning ability rate among the groups.

Sex cycle and reproductive ability were shown in Table 11. Sex cycle was normal (4.04–4.17 days) in all groups. Copulation rate and pregnancy rate were 100% and 85.0–100.0%, respectively. Body weight gains, food consumption and water intake of dams (F_0) during the gestation period were shown in Fig. 6 and 7, respectively. There

Fig. 5. Conditioned avoidance response of F_1 rats born of the HPMCAS-treated dams (F_0)

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Fig. 6. Body weight gain of dams (F₁) born of the HPMCAS-treated dams (F₀) during gestation period

Fig. 7. Water and food consumption of dams (F₁) born of the HPMCAS-treated dams (F₀) during gestation period

Table 11 Reproductive ability of female F₁ rats

Dose (mg/kg/day)		Control	625	1,250	2,500
No. of females		28	23	28	26
Mean sex cycle	Days	4.07	4.17	4.09	4.04
	S. E.	0.03	0.07	0.04	0.04
No. of females paired with males		20	20	20	20
No. of females copulated	1st	16	20	15	18
	2nd	4	0	5	2
	Total	20	20	20	20
Copulation rate (%)		100.0	100.0	100.0	100.0
No. of pregnant females		19	18	20	17
Pregnancy rate (%)		95.0	90.0	100.0	85.0

Table 12 Reproductivity tests of F₁ offsprings born of the HPMCAS treated dams (F₀)

Dose (mg/kg/day)		Control	625	1,250	2,500
No. of F ₁ mothers		18	18	20	17
Maternal mortality		0	0	0	0
Abortion		0	0	0	0
Total mother of all resorption		0	0	0	0
Nature delivery		0	0	0	0
Total number of corpora luteum (Mean±S. E.)		216 (12.0±0.5)	223 (12.4±0.7)	231 (11.6±0.6)	232 (13.6±0.4)*
Total implantation (Mean±S. E.)		204 (11.3±0.7)	212 (11.8±0.9)	210 (10.5±0.8)	226 (13.3±0.4)*
Total resorption (%)		9 (4.4)	12 (5.7)	9 (4.3)	15 (6.6)
	Early	9	12	7	13
	Late	0	0	2	2
Total dead (%)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total live (F ₂) (%) (Mean±S. E.)		195 (95.6) (10.8±0.6)	200 (94.3) (11.1±0.9)	201 (95.7) (10.1±0.7)	211 (93.4) (12.4±0.5)
Sex (%) (F ₂)	Male	98 (50.3)	90 (45.0)	104 (51.7)	94 (44.5)
	Female	97 (49.7)	110 (55.0)	97 (48.3)	117 (55.5)
	Male/Female	1.0	0.8	1.1	0.8
Mean litter weight (F ₂) (g)		53.4±2.7	54.2±4.1	50.0±3.5	61.1±2.4*
Mean fetal weight (F ₂) (g)	Male	5.12±0.08	5.11±0.08	5.17±0.07	5.08±0.04
	Female	4.80±0.07	4.79±0.05	4.86±0.07	4.81±0.04
Mean placental weight (g)		0.50±0.02	0.56±0.02*	0.59±0.02**	0.53±0.02
Loss of pre-implantation (%)		5.6	4.9	9.1	2.6
Loss of post-implantation (%)		4.4	5.7	4.3	6.6

*: p<0.05 vs control

**: p<0.01 vs control

was no significant difference in mean body weight changes among the groups. Food consumption and water intake were significantly decreased in the administered groups, but these variations were not dose dependent.

Some aspects of fetuses (F_2) at late pregnancy were shown in Table 12. In the 2,500 mg/kg group number of corpora lutea significantly increased ($p < 0.05$). As to number of corpora lutea, 12.0 ± 0.5 in control 13.6 ± 0.4 in the 2,500 mg/kg dosage group, but this change was not dose-dependent.

Table 13 External, internal and skeletal malformations observed in the rat fetuses (F_2) born of the F_1 offsprings in the teratogenicity study

Dose (mg/kg/day)	Control	625	1,250	2,500
<u>Malformations</u>				
External (%)	1/195 (0.51)	0/200 (0.00)	0/201 (0.00)	1/211 (0.47)
Club foot	1	0	0	1
Internal (%)	0/71 (0.00)	2/71 (2.82)	0/73 (0.00)	0/74 (0.00)
Pyelectasis	0	2	0	0
Skeletal (%)	0/122 (0.00)	1/128 (0.78)	0/127 (0.00)	1/136 (0.74)
Fission of thoracic vertebral centra	0	1	0	1

Malformations of fetuses (F_2) were shown in Table 13. As to internal malformations, pyelectasis was observed in two cases in the 625 mg/kg dosage group (2.82%). Club foot was observed in one case of the control group (0.51%), one in the 2,500 mg/kg dosage group (0.47%). Numbers of fetuses having skeletal variations were 5 in the control group, one in 625 mg/kg dosage group, 11 in the 1,250 mg/kg dosage group and 5 in the 2,500 mg/kg dosage group.

As to skeletal malformations, fission of thoracic vertebral centra was one in observed in one case of the 625 mg/kg dosage group (0.78%), one of the 2,500 mg/kg dosage group (0.74%). Photo 9 showed fission of thoracic vertebral centra of the 2,500 mg/kg dosage group.

The skeletal variations observed were formation of cervical ribs and lumbar ribs, deformation of thoracic vertebral centra and fission of sternbrae in all groups, but the frequency of each skeletal variation was not significantly different between the control group and the dosage groups (Table 14); 4.10% in the control group, 0.78% in the 625 mg/kg dosage group, 8.66% in the 1,250 mg/kg dosage group, 3.68% in the 2,500 mg/kg dosage group. As to the state of ossification, ossifying rate of first cervical vertebral centra was decreased in the 625 mg/kg dosage group ($p < 0.05$), but the state of other ossification was not significantly different among the groups.

Table 14 Effects of HPMCAS on skeletal development in F₂ rat fetuses born of F₁ dams

Dose (mg/kg/day)	Control	625	1,250	2,500
No. of fetuses examined	122	128	127	136
No. of fetuses with skeletal variations (%)	5 (4.10)	1 (0.78)	11 (8.66)	5 (3.68)
Formation of cervical ribs	2	0	1	0
Formation of lumbar ribs	1	1	5	1
Deformation of thoracic vertebral centra	2	0	2	4
Fission of sternebrae	0	0	3	0
State of ossification				
Ossifying rate of 1st cervical vertebral centra (%) (Mean±S. E.)	57.24±6.62	35.94±6.24*	54.25±7.34	72.18±5.61
No. of cervical vertebral centra (Mean±S. E.)	4.59±0.19	4.49±0.19	4.24±0.19	4.52±0.20
No. of caudal vertebrae per fetus (Mean±S. E.)	8.03±0.16	7.79±0.17	7.89±0.23	8.04±0.18
Lack of sternebrae	7	2	6	4
No. of metacarpus (Mean±S. E.)	7.99±0.01	8.05±0.05	8.00±0.00	8.00±0.00
No. of proximal phalanges in forepaw (Mean±S. E.)	7.62±0.17	7.57±0.11	7.69±0.19	7.78±0.10
No. of distal phalanges in forepaw (Mean±S. E.)	9.89±0.07	9.96±0.02	9.81±0.09	9.99±0.01
No. of metatarsus (Mean±S. E.)	9.96±0.02	9.96±0.03	9.97±0.02	9.97±0.02
No. of proximal phalanges in hindpaw (Mean±S. E.)	4.35±0.54	3.09±0.48	3.38±0.48	4.75±0.58
No. of distal phalanges in hindpaw (Mean±S. E.)	9.95±0.03	9.95±0.04	9.94±0.03	10.00±0.00

*: p<0.05 vs control

DISCUSSION

The present study was carried out to examine HPMCAS possesses teratogenic actions in Sprague-Dawley rats. The substance was administered orally at 625, 1,250 and 2,500 mg/kg/day between Day 7 and 17 of gestation.

No death of dams was observed in the control group and the administered groups. No remarkable changes in gestation period, delivery and nursing periods were also seen in both the control group and the administered groups.

The results of fetuses up to two generations indicate a lack of fetal toxicity of HPMCAS; the frequency of external, internal and skeletal variations was extremely low and there changes were not dose-dependent except for the incidence of club foot. The test compound tended to increase in incidence of club foot dose-dependently, but this was not a statistically significant increase. Since an oral dose of HPMCAS was poorly absorbed (less than 5%) and was excreted in the faeces predominantly during the first 48 hr following dosing (unpublished data), it is tempting to speculate that there appears to be no significant dose-related finding attributable to the administration of HPMCAS.

There was no noticeable variation in the body weight of newborns. There was also no significant difference in reflex and auditory functions between the control group and administered groups.

Further, no abnormalities in chinning test or swimming test was detected among the groups. No variations in locomoter condition was also observed in all groups. No dose-dependent changes in locomoter functions and learning ability were seen.

In the present study, no toxic effect, death in fetuses, delay in delivery and nursing of newborns or abnormality in general behavior or reproductive ability of F₁ offsprings were observed.

From the results mentioned above, it might be considered that HPMCAS has none of fetal toxicity and teratogenicity, and also shown no effect on development of skeleton and reproductive ability of F₁ offsprings when orally administered in doses of 625, 1,250 and 2,500 mg/kg under the present experimental condition.

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