REPRODUCTION STUDY OF CARTEOLOL HYDROCHLORIDE IN MICE. PART 2. PERI- AND POSTNATAL TOXICITY.

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Summary Carteolol was orally administered to mice once a day at doses of 3, 30 and 150 mg/kg/day during the perinatal and lactation periods and evaluated on its adverse effects on pregnant animals and their offspring. No appreciably abnormal findings related to the drug administration were revealed.

Therefore, it was concluded that carteolol have no serious toxic potential on parturition and lactation by mother animals, no adverse effects on growth and development, and behavioral and reproductive performance of offspring and no carcinogenic action through placental and milk transfer.

Key words : Carteolol, β -adrenergic blocking agent, peri- and postnatal study.

INTRODUCTION

Carteolol hydrochloride [5-(3-tert-butylamino-2-hydroxy)propoxy-3, 4-dihydrocarbo-
styryl hydrochloride] (carteolol), which was synthesized by Nakagawa et al. in 1974, has
a potent β -adrenergic blocking activity at a low dosage level (Takenaka et al., 1975;
Yabuuchi et al., 1974) and its clinical use has been recommended for the therapy of
arrhythmia and hypertension.

With respect to the teratogenicity of carteolol in experimental animals, Tanaka et
al. (1976 a, b, c) and Toyoshima et al. (1976) treated pregnant mice, rats and rabbits
during a period of organogenesis and observed the absence of its lethal and teratogenic
potential in their fetuses.

The purpose of the present investigation was to study the peri- and postnatal
toxicity of carteolol given to mice according to the guidelines issued by Japanese Ministry
of Health and Welfare (Yakushin No. 529).

MATERIALS AND METHODS

1. Test compound

Carteolol is a colorless and odorless crystalline powder. Its melting point is 275-

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279°C (decomposition) and its molecular weight is 328.84. The compound is extremely stable to heat and moisture.

2. Animals

Animals used were ICR-JCL mice obtained from Nippon Clea Co., Ltd., at 8 weeks of age and reared for 2-5 weeks thereafter at room temperature of $22\pm 2^{\circ}\text{C}$ and humidity of $55\pm 5\%$ on commercial solid food for reproduction (Nippon Haigo-Shiryo Co., Ltd., CA-1) for pregnant animals or solid food for rearing (Oriental Yeast Co., Ltd., MF) for other animals both with free access to tap water. In order to prepare pregnant animals nulliparous mice of 10-13 weeks of age were paired with males of the same strain and age overnight (approximately 16 hr) and those which showed formation of vaginal plug in the following morning (this day was taken as Day 0 of gestation) were separated. Eighty pregnant animals, 20 in each dosage group, were used in the experiments after confirmation of their pregnancy in terms of body weight gain.

3. Dosage and administration

For range finding determination, 2-week subacute toxicity test was initially conducted to select the highest dose for the present study by giving carteolol daily at graded doses of 10, 50, 150 and 450 mg/kg/day via a gastric tube to 10 adult female mice of the same strain at each dose level. In the group dosed at 450 mg/kg/day, sedation, depressed body weight gain, rough hair coat and diarrhea were observed, whereas no toxic symptoms other than sedation developed at 10-30 min after dosing were noted in other groups.

Therefore, the highest dose was set to be 150 mg/kg/day which was regarded to be near the maximum safety dose for adult mice, and then the doses of 30 and 3 mg/kg/day were selected. The high and low doses (150 and 3 mg/kg/day) correspond to 500 and 10 times the proposed human dose (0.3 mg/kg/day), respectively, and the high dose corresponds to 1/5 time the oral LD_{50} value in mice (810 mg/kg) obtained by an acute toxicity study (Tanaka et al., 1976 d).

The drug was dissolved in physiological saline at concentrations sufficient for dosing in a uniform volume dose of 10 ml/kg, and administered daily by gastric tube during a period from Day 15 of gestation to Day 21 postpartum. Control animals were given physiological saline alone in the same volume in the same manner.

4. Observations

A schedule of the study is shown in Fig. 1.

1) Mothers (*P*)

Body weight and food consumption were recorded daily from Day 0 of gestation to the end of the treatment (Day 21 postpartum), and parturition and nursing behaviors were observed.

For determination of drug effect on reproductive capability of mothers to conceive second litters, those of the control and the 30 and 150 mg/kg/day dose groups were

Peri- and Postnatal Toxicity of Carteolol

Fig. 1. Test schedule of peri- and postnatal study of carteolol in mice

mated again with healthy males of the same strain and age after one-week rest following weaning of newborns (*F1a*) on Day 21 postpartum, and the copulation and conception rates were calculated. Pregnant animals were laparotomized on Day 18 of gestation and growth and development of near-term fetuses (*F1b*) and major organs of the mothers were examined.

The mothers (*P*) of the 3 mg/kg/day dose group were sacrificed at weaning of offspring (*F1a*) to examine their major organs grossly.

2) Newborns (*F1a*)

Pregnant females (*P*) were allowed to deliver. The numbers of newborns (*F1a*), live and still born were counted, and body weight and external abnormalities were examined immediately after parturition. All litters (*F1a*) were reduced to 8 pups (4 males and 4 females) as a rule by random culling on Day 4 postpartum and lactated by dams until Day 21 postpartum. Body weight of newborns was recorded weekly, and general condition and developmental differentiation of external features and of oral cavity were observed.

3) Offspring (*F1a*) after weaning

Body weight and food consumption were checked weekly until 10 weeks of age, and external sexual differentiation was also observed.

The offspring culled to 4 of each sex per litter were randomly assigned to the following observations; two of each sex to behavioral study, one each to reproductive performance and the remaining one each of the 3 and 30 mg/kg/day dose groups to histopathologic observations of the reproductive organs and one each of the control and the 150 mg/kg/day dose groups to observations of carcinogenic signs.

With respect to the assessment of behavioral effects, spontaneous motor activity of the animals was recorded for 3 min once at 4-5 weeks of age by Animex (AB FARAD, Type X), and then numbers of ambulation, rearing and defecation were counted for

3 min once at 9–10 weeks of age using an improved model of an open field equipment prepared by Hall (1934). The animals were sacrificed at 26 weeks of age for the examination of gross abnormalities and organ weight of the brain, pituitary, submandibular gland, thymus, lungs, heart, liver, spleen, kidneys, adrenals, ovaries and testes. Any organs showing gross abnormalities were routinely fixed, sectioned and stained with hematoxylin-eosin for microscopical examination.

For determination of the reproductive capability of the offspring the animals were caged with those of different sex of the same group for 14 days at 10 weeks of age avoiding brother/sister mating. Copulation rates [(number of female offspring with vaginal plug/number of paired female offspring)×100] and conception rates [(number of pregnant females/number of copulated females)×100] were employed as reproductive indices. The pregnant animals were laparotomized on Day 18 of gestation and examined for major organs and development of near-term fetuses (*F2*).

One male and one female each of the 3 and 30 mg/kg/day dose groups were autopsied at 10 weeks of age, and the testes, epididymis and pituitary of the males and ovaries, uterus and pituitary of the females were removed and stained with hematoxylin-eosin for microscopical examination.

One male and one female each of the control and 150 mg/kg/day dose groups were bred until 74 weeks of age, measuring body weight every 4 weeks and observing general symptoms and mortality. The animals survived for 74 weeks were killed by ether anesthesia, and the brain, pituitary, submandibular gland, thymus, lungs, heart, liver, spleen, kidneys, adrenals, ovaries and testes were weighed. These organs and the pancreas, epididymis, uterus, mesentery lymph node and other organs with any abnormality were fixed in 10% formalin, embedded in paraffin, routinely sectioned and stained with hematoxylin-eosin for microscopical examination. Dead animals were autopsied immediately after detection and subjected to microscopical examination in such manner as described above.

4) Near-term fetuses (*F1b* and *F2*)

The animals (*P*) prepared to be pregnant for the determination of reproductive capability to conceive second litters and their pregnant offspring (*F1a*) prepared for the assessment of reproductive capability were sacrificed on Day 18 of gestation respectively, and by a laparotomy the uterus was examined for counting implantations, live fetuses, dead fetuses and resorptions, and for appearance of dead fetuses. Live fetuses were examined for body weight, sex ratio and external abnormalities including those of oral cavity. Half of live fetuses were fixed in 95% alcohol, and their skeleton was stained with alizarin red S according to Dawson's method (1926) and processed for the examination of any abnormalities and progress of ossification. The remaining half were fixed in Bouin's solution and examined for visceral abnormalities with a binocular microscope.

5) Statistical analysis

The incidence of death and external abnormalities of newborns (*F1a*), and the inci-

Peri-and Postnatal Toxicity of Carteolol

dence of death, external, visceral and skeletal abnormalities and sex ratio of near-term fetuses (*F1b* and *F2*) and tumor incidence of offspring (*F1a*) were statistically analysed by Chi-square test, and by Wilcoxon's rank sum test when necessary. Analysis of variance was used for other analyses. Statistical difference was regarded as significant at a probability of 5% or less.

RESULTS

1. Observations of mothers (*P*)

Mean body weight changes of mothers (*P*) are shown in Fig. 2, and parturition and nursing behaviors in Table 1. The weights of mothers in the 3 mg/kg/day dose group were significantly heavier on Days 17 and 18 postpartum than those in the control group. The values on other days for this treatment group and those of other dose groups showed similar courses of changes to those of the control group. Food consumption values were not significantly different between the treatment and control groups.

With respect to general symptoms most females dosed at 150 mg/kg/day developed sedation at 10–30 min after dosing, and one female had diarrhea and clonic convulsion on Day 13 postpartum followed by death.

Parturition was delayed in one animal of the 30 mg/kg/day dose group, and an autopsy, performed due to the absence of signs of parturition in the afternoon of Day 20 of gestation, revealed the presence of only one fetus with club foot.

Fig. 2. Mean body weight of mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum

Table 1. Delivery and lactation status of mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum

	Dosage (mg/kg)	Control (Saline)	3	30	150
No. of mothers (<i>P</i>)		20	20 ^{c)}	20 ^{d)}	20 ^{e)}
Mean length of gestation (days)		18.90 ± 0.31	19.00 ± 0.32	18.95 ± 0.23	19.05 ± 0.22
Offspring (<i>F_{1a}</i>)					
Total alive (Mean ± S.D.)		237(11.85 ± 2.41)	234(11.70 ± 1.87)	195(10.26 ± 3.40)	218(10.90 ± 2.15)
Total dead		1	9*	4	10*
Viability rate ^{a)}		95.2	87.8	97.9	94.2
Lactation rate ^{b)}		100	100	100	99.3
Mean body weight (g)					
at birth					
Male		1.65 ± 0.13	1.64 ± 0.12	1.70 ± 0.19	1.72 ± 0.13
Female		1.57 ± 0.14	1.55 ± 0.12	1.63 ± 0.20	1.63 ± 0.11
on day 4					
Male		3.26 ± 0.40	3.17 ± 0.47	3.19 ± 0.49	3.16 ± 0.39
Female		3.11 ± 0.35	3.10 ± 0.45	3.12 ± 0.52	3.06 ± 0.37
on day 7					
Male		5.37 ± 0.33	5.20 ± 0.57	5.18 ± 0.56	4.92 ± 0.39*
Female		5.28 ± 0.28	5.12 ± 0.54	5.05 ± 0.60	4.77 ± 0.35**
on day 14					
Male		8.60 ± 0.61	8.31 ± 1.01	8.27 ± 0.95	7.78 ± 0.74**
Female		8.48 ± 0.62	8.17 ± 0.92	8.19 ± 0.94	7.58 ± 0.76**
at weaning					
Male		13.8 ± 1.1	13.6 ± 1.6	13.3 ± 1.4	12.7 ± 1.6
Female		13.1 ± 1.1	13.0 ± 1.2	13.1 ± 1.3	12.3 ± 1.4
No. of offspring with anomaly (%)		2(0.84)	2(0.85)	3(1.54)	9*(4.13)
Open eyelid		2	2	1	6
Club foot		0	0	1	2
Curled tail		0	0	1	0
Cleft palate		0	0	0	1

a) (offspring alive on day 4 before culling/offspring born alive) × 100

b) (offspring alive on day 21/offspring alive on day 4 after culling) × 100

c) One mother killed her all offspring on day 1 postpartum

d) One mother was delayed to be delivered, and found to have only one fetus with club foot in the uterus

e) One mother died on day 13 postpartum

*; p < 0.05 compared with the control

**; p < 0.01 compared with the control

Cannibalism of all newborns was observed on Day 1 postpartum in one animal of the 3 mg/kg/day dose group.

Durations of gestation were not different between the treatment and control groups (Table 1).

There were no drug-related abnormalities in the mothers dosed the drug at 3 mg/kg/day when sacrificed at weaning.

2. Observations of newborns (*F1a*)

Numbers of still borns in the 3 and 150 mg/kg/day dose groups were significantly larger than that in the control group, while those of live borns were not.

There were no significant differences between the treatment and control groups in postnatal survival rate [(number of surviving newborns prior to the culling on Day 4 postpartum/number of newborns delivered) $\times$ 100] and nursing rate [(number of surviving newborns at weaning/number of surviving newborns after the culling on Day 4 postpartum) $\times$ 100].

Body weight gain of the surviving newborns of the 150 mg/kg/day dose group was significantly depressed at one and two weeks after birth, however, the weights for all treatment groups were not significantly different from the value for the control at 3 weeks.

Incidence of external abnormalities of newborns was significantly higher only in the 150 mg/kg/day dose group (4.13%) than that in the control group (0.84%). The abnormalities observed were open eyelids in 6 newborns, club foot in two cases and cleft palate in one case (Table 1). When 4 newborns with open eyelids were bred together with normal animals, eyelids became normal in the course of growth, and an autopsy at 26 weeks of age revealed no abnormalities. On the other hand, one newborn with club foot which was similarly bred was cannibalized by its mother on Day 4 postpartum.

Drug effects on external differentiation including sexual differentiation are shown in Table 2. There were no different findings in external differentiation until weaning between the treatment and control groups.

3. Observations of offspring (*F1a*) after weaning

Mean body weights of the offspring are indicated in Fig. 3 (males) and Fig. 4 (females). The values were not significantly different between the treatment and control groups.

Sexual differentiation was not significantly different between the treatment and control groups (Table 2).

The results of behavioral study are shown in Table 3. In the open field test, males from mothers dosed at 150 mg/kg/day showed low values in ambulation and rearing, and high in defecation, though the values were not significantly different from those in the control group.

Table 2. Effects of carteolol on postnatal external development of mouse offspring (F1a)
all findings represent mean $\pm$ S.D.

Dosage (mg/kg)	Control (Saline)					
	3		30		150	
Sex	Male	Female	Male	Female	Male	Female
External development						
No. of offspring	77	80	76	72	74	80
Detachment of auricles	3.35 $\pm$ 0.42	3.42 $\pm$ 0.46	3.54 $\pm$ 0.54	3.47 $\pm$ 0.40	3.61 $\pm$ 0.41	3.58 $\pm$ 0.46
Eruption of lower incisors	6.32 $\pm$ 0.39	6.39 $\pm$ 0.47	6.29 $\pm$ 0.37	6.34 $\pm$ 0.36	6.52 $\pm$ 0.46	6.48 $\pm$ 0.36
Emergence of abdominal hair	8.40 $\pm$ 0.46	8.51 $\pm$ 0.45	8.46 $\pm$ 0.66	8.58 $\pm$ 0.65	8.62 $\pm$ 0.53	8.59 $\pm$ 0.42
Separation of eyelids	13.10 $\pm$ 0.53	13.52 $\pm$ 0.49	13.13 $\pm$ 0.41	13.20 $\pm$ 0.45	13.16 $\pm$ 0.33	13.24 $\pm$ 0.34
Opening of vagina	—	25.33 $\pm$ 0.53	—	25.58 $\pm$ 0.62	—	25.62 $\pm$ 0.82
Descent of testis	21.00 $\pm$ 0.00	—	21.09 $\pm$ 0.15	—	21.07 $\pm$ 0.23	—

Peri-and Postnatal Toxicity of Carteolol

Fig. 3. Mean body weight of male offspring (*F1a*) from mother mice treated orally with carteolol from day 15 of gestation through day 21 postpartum

Fig. 4. Mean body weight of female offspring (*F1a*) from mother mice treated orally with carteolol from day 15 of gestation through day 21 postpartum

Table 3. Effects of carteolol on spontaneous motor activity and open field performance of mouse offspring (*F1a*) all findings represent mean $\pm$ S.D.

Dosage (mg/kg)	Sex	No. of offspring examined	Spontaneous motor activity ^{a)}	Ambulation ^{c)}	Open field test ^{b)} Defecation ^{d)}	Rearing ^{e)}
Control(saline)	Male	40	194.6 $\pm$ 20.0	94.3 $\pm$ 30.6	2.25 $\pm$ 1.68	21.4 $\pm$ 12.8
	Female	40	185.6 $\pm$ 27.2	91.2 $\pm$ 38.0	2.10 $\pm$ 1.47	17.7 $\pm$ 10.7
3	Male	38	184.2 $\pm$ 35.4	88.3 $\pm$ 38.7	2.24 $\pm$ 2.01	23.7 $\pm$ 14.2
	Female	38	180.1 $\pm$ 34.0	100.6 $\pm$ 30.8	2.66 $\pm$ 1.80	22.6 $\pm$ 11.4
30	Male	37	197.2 $\pm$ 24.8	93.3 $\pm$ 31.0	2.39 $\pm$ 1.65	23.7 $\pm$ 11.3
	Female	37	184.5 $\pm$ 19.0	108.3 $\pm$ 47.4	2.13 $\pm$ 1.74	25.4 $\pm$ 17.3
150	Male	38	175.1 $\pm$ 26.8	68.6 $\pm$ 25.1	2.47 $\pm$ 1.54	14.0 $\pm$ 8.2
	Female	38	170.5 $\pm$ 26.6	90.0 $\pm$ 25.4	2.68 $\pm$ 1.50	20.0 $\pm$ 11.8

a) Counts for 3 minutes per animal at 4-5 weeks after birth measured by Animex

b) Scores for 3 minutes per animal at 9-10 weeks after birth

c) Number of sections entered

d) Number of faecal boluses deposited

e) Number of actions

Table 4. Distribution of the death in offspring (*F1a*) from mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum

Dosage (mg/kg)	Sex	Postnatal age (weeks)																										Mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
Control	Male					1C ^{a)}																1C		1C			1/77 ^{c)}	2/39 ^{d)}
	Female																										0/80	0/40
3	Male					1C											1C										1/76	1/38
	Female																		1L ^{b)}								0/72	1/38
30	Male																							1C			0/74	1/37
	Female																										0/70	0/37
150	Male																										0/75	0/38
	Female					1C																					1/71	0/38

a) C; Cannibalism

b) L; Leukemia

c) Mortality at 10 weeks old

d) Mortality at 10-26 weeks old

Mortality values of the offspring after weaning are shown in Table 4. The rates were obtained from the observation of all young after weaning until 10 weeks and the observation of two males and two females per litter from 10 to 26 weeks which were subjected to the behavioral study. There were no significant differences between the treatment and control groups in the mortalities at 10 and 26 weeks. One dead female animal in the 3 mg/kg/day dose group developed leukemia.

Organ weights of the offspring sacrificed at 26 weeks of age are shown in Fig. 5

(males) and Fig. 6 (females), and the histopathologic findings obtained at that time are given in Table 5. There were no significant differences in both absolute and relative weights between the treatment and control groups. Gross and histopathologic examinations revealed leukemia (Fig. 9 a-b) in two animals of the 3 mg/kg/day dose group, one animal of the 30 mg/kg/day dose group and four animals of the 150 mg/kg/day dose group. Lung adenoma (Fig. 10) was found in one animal in each group of the control and all treatment groups, atrophy of seminiferous tubules (Fig. 11) in one animal each in the control and 150 mg/kg/day dose groups, solitary cystic kidney in one animal of the 30 mg/kg/day dose group, monorchia in one animal of the 150 mg/kg/day dose group and cyst of pancreas in one animal of the 150 mg/kg/day dose group.

4. Determination of reproductive capability of offspring (*F1a*) and observations of their near-term fetuses (*F2*)

Fertility and reproductive performance of offspring (*F1a*) from treated mothers (*P*) and observations of their near-term fetuses (*F2*) are shown in Table 6.

There were no significant differences in copulation and conception rates between the treatment and control groups. One offspring from a mother dosed at 3 mg/kg/day showed the formation of vaginal plug but did not conceive, however, it made copulation and conceived in the second trial of mating conducted 3 weeks after the first pairing. One female in the 150 mg/kg/day dose group, which failed to form vaginal plug, was found to form the plug one month after the first pairing and then conceived.

Autopsy of pregnant animals performed on Day 18 of gestation gave normal results.

Fig. 5. Organ weight of male offspring (*F1a*) at 26 weeks from mother mice treated orally with carteolol from day 15 of gestation through day 21 postpartum

Fig. 6. Organ weight of female offspring (*F1a*) at 26 weeks from mother mice treated orally with carteolol from day 15 of gestation through day 21 postpartum

Table 5. Incidence of pathologic findings based on gross and microscopical investigation in offspring (*F1a*) at 26 weeks old from mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum

Dosage (mg/kg)	Control(Saline)		3		30		150	
Sex	M	F	M	F	M	F	M	F
No. of offspring	37	40	37	37	36	37	38	38
Findings								
Leukemia	0	0	0	2	0	1	1	3
Lung adenoma	1	0	1	0	1	0	0	1
Atrophy of testis	1	-	0	-	0	-	1	-
Follicular cyst of ovary	-	2	-	1	-	1	-	0
Dilatation of renal pelvis	1	0	0	0	0	0	1	0
Others	0	0	0	0	1 Solitary cystic kidney	0	1 Monorchia	0
							1 Cyst of pancreas	

The numbers of live fetuses, and dead fetuses and resorptions were not significantly different between the treatment and control groups. Incidence of external abnormalities in fetuses was significantly higher in the 150 mg/kg/day dose group than in the control group, and open eyelids were most frequently seen.

Table 6. Fertility and reproductive performance of offspring (*F1a*) from mother mice (*P*) treated orally with carteolol and the findings of their litter at term

Dosage (mg/kg)	Control(Saline)			30	150
No. of offspring					
Male	20	19	19	19	18
Female	20	19	19	19	18
No. of females with vaginal plug (Mating rate, %)					
pregnant (Conception rate, %)					
Fetuses (<i>F2</i>)					
Total implants (Mean $\pm$ S.D.)	293(14.65 $\pm$ 1.90)	245(13.61 $\pm$ 2.28)	284(14.95 $\pm$ 1.43)	233(13.71 $\pm$ 2.08)	216(12.71 $\pm$ 1.65)
Total alive (Mean $\pm$ S.D.)	268(13.40 $\pm$ 1.98)	223(12.39 $\pm$ 2.28)	267(14.05 $\pm$ 1.51)	216(12.71 $\pm$ 1.65)	17(7.30)
Total dead (%)	25(8.53)	22(8.98)	17(5.99)	12	10
early resorption	17	17	12	3	6
late resorption	7	3	2	1	1
dead fetus	1	2			
Alive fetuses					
Sex ratio (M/F)					
Mean body weight (g)					
Male	137/131(1.05)	127/96*(1.32)	130/137(0.95)	112/104(1.08)	
Female	1.40 $\pm$ 0.07	1.41 $\pm$ 0.07	1.36 $\pm$ 0.11	1.42 $\pm$ 0.07	
No. of fetuses with anomaly (%)	1.35 $\pm$ 0.08	1.35 $\pm$ 0.07	1.31 $\pm$ 0.09	1.37 $\pm$ 0.10	
Open eyelid	1(0.37)	2(0.90)	4(1.50)	11*(5.09)	
Club foot	0	1	1	8	
Curled tail	1	0	2	3	
Cleft tail	0	0	1	0	
Cleft palate	0	1	0	0	
Skeletal examination					
No. of fetuses examined	140	113	138	111	
Anomaly (%)					
Fusion between exoccipital bone and cervical arch	0	0	0	1(0.90)	
Fusion between adjacent cervical arches	0	1(0.88)	0	0	
Variation (%)					
Incomplete fusion of supraoccipital bone	1(0.71)	0	0	0	
Bifurcation or split of cervical arch	24(17.14)	19(16.81)	34(24.64)	34(30.63)	
Cervical rib	12(8.57)	12(10.62)	16(11.59)	20(18.02)	
Split of thoracic arch	0	0	0	1(0.90)	
14th rib	42(30.00)	45(39.82)	50(36.23)	50(45.05)	
Extra sternbrae	20(14.29)	15(13.27)	11(7.97)	12(10.81)	
Visceral examination					
No. of fetuses examined	128	110	129	105	
Hydrocephaly (%)	0	0	1(0.78)	1(0.95)	

* ; $p < 0.05$ compared with the control** ; $p < 0.01$ compared with the control

Skeletal abnormalities observed were fusion between the 5th and 6th cervical arches (Fig. 12) in the 3 mg/kg/day dose group and fusion between exoccipital bone and the first cervical arch (Fig. 13) in the 150 mg/kg/day dose group, however, there were no significant differences in the incidence of abnormalities between the treatment and control groups.

The examination of the internal organs showed no visceral abnormalities except hydrocephaly in one animal each of the 30 and 150 mg/kg/day dose groups.

No abnormal histopathologic changes of reproductive organs were observed in the young after weaning in the 3 and 30 mg/kg/day dose groups at 10 weeks of age.

5. Determination of reproductive performance of mothers (*P*) to yield second litters and observations of their near-term fetuses (*F1b*)

The second fertility and reproductive performance of mothers (*P*) and their near-term fetuses (*F1b*) were observed as shown in Table 7.

Copulation and conception rates were not significantly different between the treatment and control groups.

The autopsy of the mothers on Day 18 of gestation gave normal findings.

Numbers of live fetuses, and dead fetuses and resorptions, sex ratio and incidence of external abnormalities were not significantly different between the treatment and control groups. The number of implantations in the 30 mg/kg/day dose group was significantly smaller.

The observations of the skeletal system showed split and fusion of vertebral bodies (Fig. 14) in one animal in the 150 mg/kg/day dose group. The incidences of skeletal abnormalities were not significantly different between the treatment and control groups.

6. Determination of carcinogenic action in offspring (*F1a*) through the placental or milk transfer

Time courses of mean body weight changes of the offspring from 10 to 74 weeks of age are shown in Fig. 7. There were no significant differences in the course between the control and 150 mg/kg/day dose groups.

Time of death and type of tumors in dead animals are given in Fig. 8. Mortality and overall tumor incidence were not significantly different between the control and 150 mg/kg/day dose groups, and the tumors in the control group tended to be manifested earlier than those in the 150 mg/kg/day dose group. The tumors observed were leukemia in all cases except one female of the 150 mg/kg/day dose group which developed lung adenoma.

Absolute organ weights measured at autopsy of surviving offspring at 74 weeks of age are shown in Table 8. The values were not significantly different between the control and 150 mg/kg/day dose groups.

Table 9 shows tumor incidence in live and dead offspring. Leukemia (Fig. 15 a-b) and lung adenoma (Fig. 16) were frequently seen in the control and 150 mg/kg/day

Table 7. Fertility and reproductive performance of mother mice (P) after weaned first litters, and the findings of their second litter at term

Dosage (mg/kg)	Control (Saline)	
	30	150
No. of females (P)		
examined	20	15
with vaginal plug (Mating rate, %)	20(100)	15(100)
pregnant	16(80.00)	14(93.33)
Fetuses (F _{1b})		
Total implants (Mean ± S. D.)	225(14.06 ± 1.39)	193(13.79 ± 2.04)
Total alive (Mean ± S. D.)	208(13.00 ± 1.75)	178(12.71 ± 2.46)
Total dead (%)	17(7.56)	15(7.77)
early resorption	7	4
late resorption	10	9
dead fetus	0	2
Alive fetuses		
Sex ratio (M/F)	104/104(1.00)	100/78(1.28)
Mean body weight (g)		
Male	1.43 ± 0.08	1.42 ± 0.10
Female	1.37 ± 0.07	1.35 ± 0.09
No. of fetuses with anomaly (%)	12(5.77)	6(3.37)
Open eyelid	7	2
Club foot	4	2
Curled tail	1	1
Cleft palate	0	1
Cranioschisis	0	0
Skeletal examination		
No. of fetuses examined	102	90
Anomaly (%)		
Split and fusion of vertebral bodies	0	1(1.11)
Variation (%)		
Bifurcation or split of cervical arch	16(15.69)	15(16.67)
Cervical rib	9(8.82)	9(10.00)
14th rib	27(26.47)	36(40.00)
Extra sternebrae	7(6.86)	7(7.77)

a) One fetus is the complex anomaly of Cranioschisis and Open eyelid

*; p < 0.05 compared with the control

Fig. 7. Mean body weight of offspring (*F1a*) investigated for carcinogenicity from mother mice treated orally with carteolol from day 15 of gestation through day 21 postpartum

Fig. 8. Distribution of the death in offspring (*F1a*) investigated for carcinogenicity from mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum.

dose groups. Overall tumor incidence and the incidence for each tumor were not significantly different between the two groups. Nodular hyperplasia in the liver (Fig. 17) was demonstrated in one and three males of the control and 150 mg/kg/day dose groups, respectively, but the incidences of this abnormality were not significantly different.

Peri-and Postnatal Toxicity of Carteolol

Table 8. Body and absolute organ weight on offspring (*F1a*) investigated for carcinogenicity from mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum

Dosage (mg/kg)	Sex	No. of offspring		Terminal body weight (g)	Brain (g)	Subman-bibular glands (g)	Thymus (mg)	Lung (g)	Heart (g)	Liver (g)	Spleen (g)	Kidneys (g)	Adrenals (mg)	Testes (g)	Ovaries (mg)
Control (Saline)	Male	11	Mean	49.10	0.53	0.29	28.1	0.25	0.23	2.07	0.16	0.90	4.3	0.30	—
			S. D.	6.61	0.043	0.046	13.2	0.062	0.047	0.262	0.174	0.122	1.6	0.052	—
	Female	12	Mean	40.17	0.54	0.14	62.0	0.24	0.19	2.04	0.24	0.60	6.9	—	14.7 ^a
			S. D.	8.47	0.079	0.040	44.9	0.060	0.032	0.778	0.323	0.119	1.4	—	11.5
	Male	13	Mean	48.27	0.53	0.30	21.4	0.27	0.23	2.19	0.16	0.89	4.8	0.29	—
			S. D.	6.66	0.022	0.039	10.9	0.069	0.036	0.336	0.185	0.116	0.9	0.047	—
150	Female	15	Mean	39.71	0.54	0.16	51.6	0.24	0.18	1.82	0.29	0.57	7.3	—	22.7
			S. D.	5.50	0.048	0.034	33.6	0.051	0.036	0.370	0.315	0.084	1.6	—	37.5

a) One offspring was excluded for extreme follicular cyst of ovary

Table 9. Summary of neoplasm incidence in offspring (*F1a*) investigated for carcinogenicity from mother mice (*P*) treated orally with carteolol from day 15 of gestation through day 21 postpartum

Dosage (mg/kg)	Control (Saline)		150	
Sex	Male	Female	Male	Female
No. of offspring	16	20	18	17
Findings				
Leukemia	5	6	4	4
Lung adenoma	4	4	4	2

DISCUSSION

Newborns (*F1a*) of the 150 mg/kg/day dose group gained body weight significantly less than the control group during the initial two weeks after birth, but their body weights returned to normal level at three weeks of age followed by normal increase. Hence, the depression of weight gain in an early stage of growth was interpreted temporal.

Viability of newborns and nursing rate of the mothers (*P*) appeared normal in all

the treatment groups, and hence carteolol was considered to exhibit no undesirable effects on nursing and lactation of the mothers.

External abnormalities occurred in significantly higher incidence in newborns (*F1a*) from the mothers (*P*) treated at 150 mg/kg/day, and fetuses (*F2*) from the offspring (*F1a*) of the same dosage group also had high incidence of external abnormalities. These abnormalities were, however, not statistically significant by Wilcoxon's rank sum test.

Of the abnormalities observed, open eyelids were predominant. This abnormality is reported spontaneous by Takano et al. (1965), and there were no specific external abnormalities. The comparable incidence of external abnormalities in second litters (*F1b*) from the mothers (*P*) of the control group seems to support the assumption that the abnormalities encountered in this study are not drug-induced but sporadic.

In the study of fertility and reproductive capability of the offspring (*F1a*) after weaning, one non-fertile animal from a mother dosed at 3 mg/kg/day and one non-copulated animal from a mother dosed at 150 mg/kg/day were observed. However, the copulation and conception rates were within the range of normal variations in all treatment groups.

The examination of reproductive performance of the mothers (*P*) to conceive second litters (*F1b*) revealed normal status of copulation and conception. The number of implantations in the 30 mg/kg/day dose group (11.77 ± 3.17) was significantly smaller than that in the control (14.06 ± 1.39), however, the drug effect on the implantation was not dose-dependent.

During the observation of offspring (*F1a*) after weaning leukemia was found in one animal from a mother dosed at 3 mg/kg/day which was dead at 121 days of age, and by autopsy performed at age of 26 weeks the tumor was also noted in two offspring of the 3 mg/kg/day dose group, one of the 30 mg/kg/day dose group and four of the 150 mg/kg/day dose group. The incidences of leukemia in the offspring at 26 weeks of age were 3/76 (4.0%), 1/74 (1.4%), and 4/76 (5.3%) respectively for the experimental groups in the order of increasing dosage levels. Nomura et al. (1972) reported in their study on transplacental carcinogenicity of urethan (ethyl carbamate) using ICR-JCL mice that the incidence of lymphosarcoma at autopsy of 32 weeks of age was 2.3% for the offspring from mothers of the control group. According to Percy et al. (1971), spontaneous lymphoreticular tumors in CD^(R)-1 HaM/ICR mice occurred earlier than 4 months of age and with the incidence of 6.6% in the animals aged at 4-8 months. The time of occurrence and the incidence of the leukemia observed in this study were not apparently different from those reported in these literature so that the leukemia thus observed was considered spontaneous in nature.

In the assessment of carcinogenic potential of the drug in offspring of the control and 150 mg/kg/day dose groups bred until 74 weeks of age, leukemia was encountered earlier in the control group rather than in the treatment group and the incidences were not significantly different between the two groups.

Therefore, carteolol was considered to elicit no carcinogenic action through the

placental or milk transfer to the fetus or newborn.

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Peri-and Postnatal Toxicity of Carteolol

Fig. 9-a

Fig. 9-b

Fig. 9 Leukemia in a male offspring (F_{1a}) in the 150 mg/kg/day dose group autopsied at 26 weeks of age.

a) Liver. H & E, $\times 107$

b) Brain. H & E, $\times 107$

Fig. 10 Lung adenoma in a male offspring (F_{1a}) in the control group autopsied at 26 weeks of age. H & E, $\times 214$

Fig. 11 Atrophy of seminiferous tubules in a offspring (F_{1a}) in the 150 mg/kg/day dose group autopsied at 26 weeks of age. H & E, $\times 107$

Fig. 12 Fusion between the 5th and 6th cervical arches in a fetus (F_2) from offspring (F_{1a}) in the 3 mg/kg/day dose group.

Fig. 13 Fusion between exoccipital bone and the first cervical arch in a fetus (F_2) from offspring (F_{1a}) in the 150 mg/kg/day dose group.

Fig. 14 Split and fusion of vertebral bodies in a fetus (F_{1b}) from mother (P) dosed at 150 mg/kg/day.

Fig. 15-a

Fig. 15-b

Fig. 15 Leukemia in a female offspring (F_{1a}) in the control group autopsied at 74 weeks of age.
a) Liver. H & E, $\times 107$
b) Kidney. H & E, $\times 214$

Fig. 16 Lung adenoma in a male offspring (F_{1a}) in the 150 mg/kg/day dose group autopsied at 74 weeks of age. H & E, $\times 107$

Fig. 17 Nodular hyperplasia in the liver in a male offspring (F_{1a}) in the control group autopsied at 74 weeks of age. H & E, $\times 107$