TERATOGENIC EFFECTS OF CARBOPLATIN, AN ONCOSTATIC DRUG, ADMINISTERED DURING THE EARLY ORGANOGENETIC PERIOD IN RATS

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Abstract.....In the preceding study, no teratological effects against rat fetuses were observed when carboplatin, an oncostatic platinum coordination complex, was dosed to their dams from days 7 to 17 of gestation at a dose level of 4 mg/kg/day. However, there are a few reports which show the teratogenic action of carboplatin injected from days 6 to 15 of pregnancy at a dose level of 6 mg/kg/day. In the present study, carboplatin was administered intravenously to pregnant female Crj : CD (Sprague-Dawley) rats from days 6 to 9 or 7 to 10 of gestation at a dose level of 6 mg/kg/day in order to know the teratogenicity of this drug. Carboplatin were highly embryo-lethal in dams when dosed from days 6 to 9 of gestation, but not in animals when injected from days 7 to 10 of pregnancy. Carboplatin also produced external, internal and skeletal anomalies in fetuses such as gastroschisis, dilatation of cerebral ventricles, cleft sternum, fused ribs, malformed thoracic vertebra when administered from days 6 to 9 of gestation, but not in conceptuses when dosed from days 7 to 10 of pregnancy. However, the delayed ossification which was ascribed to the fetal growth retardation was observed in rats treated with this drug during both administration periods. These results suggest that carboplatin is embryotoxic, inducing intrauterine death and congenital malformations in rats, when injected during the early stages of gestation including day 6 of pregnancy.

Key words : Oncostatic drug, carboplatin, embryo-lethality, teratogenicity, intravenous administration, rats.

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INTRODUCTION

Carboplatin which was developed by Bristol-Myers Company, USA is one of cisplatin analogs, an oncostatic platinum coordination complex, and is used for cancer chemotherapy in foreign countries. This anticancer drug has been shown to have a potent antitumor activity against some experimental cancers and human neoplasmas (Bradner et al., 1980 ; Wolpert-DeFilippes, 1980 ; Evans et al., 1983 ; Smith et al., 1985 ; Basauri et al., 1986).

The authors have already reported the results on reproduction studies of carboplatin in rats (Kai et al., 1988a, b, c). The teratogenic action against rat fetuses was not observed when their dams were treated with carboplatin from days 7 to 17 of gestation (Kai et al., 1988b). In accordance with the toxicology report of Bristol-Myers Company, USA (unpublished), however, the visceral and skeletal malformations were produced when this drug was administered to pregnant female rats from days 6 to 15 of pregnancy. Furthermore, cisplatin, a platinum coordination complex like carboplatin, has been reported to be embryotoxic in rats (Keller and Aggarwal, 1983 ; Bajt and Aggarwal, 1985) and mice (Lazar et al., 1979 ; Keller and Aggarwal, 1983 ; Köpf-Maier et al., 1985) and teratogenic in mine (Lazar et al., 1979) and chicks (Narbaitz and Marino, 1988). The highest dose level of carboplatin used in our preceding studies was 4 mg/kg/day ; whereas the teratogenic dose level against rat fetuses was 6 mg/kg/day in the above-described Bristol-Myers' report. Therefore, it is considered that carboplatin may be teratogenic at the embryo-lethal dose and its teratogenic intensity may be dependent upon the first day of dosing. The present study was undertaken to know the teratogenicity of carboplatin at a dose level of 6 mg/kg/day which corresponded to the teratogenic dose in the above-described Bristol-Myers' report.

MATERIALS AND METHODS

1. Drug :

The chemical structure of carboplatin obtained from Johnson Matthey Service Ltd., UK is shown in Fig. 1. Its chemical name is cis-diammine (1, 1-cyclobutanedicarboxylato) platinum (II) and the molecular weight is 371.25. This

Fig. 1. Structural formula of carboplatin.

compound is a form of white powder and slightly insoluble in water and saline. Distilled water for injection used as a vehicle was supplied by Otsuka Pharmaceuticals Co., Ltd.

2. Animals and animal husbandry :

Eleven-week-old male and female Crj : CD (Sprague-Dawley) rats were purchased from Charles River Japan, Inc. They were housed in the air-conditioned room (room temperature, $23 \pm 2^{\circ}\text{C}$; relative humidity, $50 \pm 10\%$) with a 6 : 00 to 18 : 00 light : dark cycle. Males and females weighing 377 to 483g and 224 to 282g, respectively, were mated after one week acclimation. One female rat paired with one male rat overnight in the same cage and females with spermatozoa observed in the vaginal smears were considered on day 0 of gestation. The pregnant female rats were individually housed in wire bottom cages and provided with CRF-1 laboratory chow obtained from Oriental Yeast Industry Co., Ltd. and water *ad libitum*.

3. Dosage group description :

Based on the results of the preceding study in our laboratory (Kai et al., 1988b) and the Bristol-Myers' toxicology report (unpublished), each dosage group was determined as shown in Table 1.

Carboplatin was administered to pregnant female rats intravenously at a dose level of 6 mg/kg/day, which corresponded to the teratogenic dose in the above-described Bristol-Myers' report, from days 6 to 9 and 7 to 10 of gestation in Groups II and IV, respectively. Distilled water for injection was given to corresponding control dams (Groups I and III, respectively) with the same manner.

4. Measurements and observations :

Body weight changes in dams : Body weights in all dams were recorded daily from days 6 to 10 of gestation and every other day except for this period.

Sacrifice in dams at term : All pregnant female rats were sacrificed on day 20 of presumed gestation. The uterus of each dam was examined for the number of empty implantation sites, early or late resorption sites and live or dead fetuses. All live fetuses were examined for sexes and gross external anomalies. Furthermore, placental weights, fetal weights, crown-rump distances and tail lengths were recorded in these conceptuses.

Skeletal and visceral examination in fetuses : The one-half of the fetuses in each litter were preserved in 95% ethyl alcohol and the remaining one-half of the conceptuses in each litter were retained in Bouin's solution. Fetuses were assigned for two fixatives on an every-other-fetus basis. Fetuses fixed in 95% ethyl alcohol were prepared for the cleared skeletal specimens stained with alizarin red S by Dawson's method (Dawson, 1926) and examined for skeletal anomalies and variations, as well as states of ossification. On the other hand, conceptuses fixed in Bouin's solution were examined for visceral malformations and variations by Wilson's method (Wilson, 1965) and thoracic visceral malformations by microdissection method (Nishimura, 1974).

Table 1. Detailed description of the constitution of each individual group.

Groups	I (Control group for Group II)	II	III (Control group for Group IV)	IV
Drug and dose	Distilled water for injection	Carboplatin 6 mg/kg/day *	Distilled water for injection	Carboplatin 6 mg/kg/day *
Dosing period	Day 6 to 9 of gestation	Day 6 to 9 of gestation	Day 7 to 10 of gestation	Day 7 to 10 of gestation
No. of dams examined	9	10	9	10
No. of fetuses examined for external malformations	129	117**	133	144
No. of fetuses examined for internal malformations	62	50	65	71
No. of fetuses examined for skeletal malformations	67	54	68	73

* This dose level corresponds to the teratogenic dose against rat fetuses according to the toxicological report of Bristol-Myers Company, USA (unpublished).

** Thirteen fetuses which had external anomalies were not examined for internal or skeletal malformations.

5. Statistical analyses :

When the data obtained from this study were analyzed statistically, fetal weights, crown-rump distances, tail lengths, placental weights and the number of ossified sternebrae, cervical vertebrae, lumbar vertebrae, sacrococcygeal vertebrae, metacarpi, distal phalanges of forelimbs and metatarsi were calculated in each litter. The incidence data including less than five frequencies were analyzed by Fisher exact test (Gad and Weil, 1982). Otherwise, chi-square test with Yates' correction (Gad and Weil, 1982) was employed. The measurement data were analyzed by the Student's t-test (Gad and Weil, 1982) when each treated group (Group II or IV) was compared with corresponding controls (Group I or III, respectively). On the other hand, the multiple comparisons among each individual group were made by Scheffe's test (Gad and Weil, 1982). The levels of significance were chosen as $P < 0.05$ and 0.01 .

RESULTS

1. *Boby weight changes in dams* (Fig. 2) :

Boby weight gains were statistically suppressed in Group II from days 7 to 20 of gestation as compared with Group I. However, the statistical suppression of body weight gains was found in Group IV only on day 12 of pregnancy in comparison with Group III. On the other hand, body weight gains were reduced in Group II on days 7, 18 and 20 of gestation compared with Group IV.

2. *Prenatal development in fetuses* (Table 2) :

There were no significant differences between each treated group and corresponding controls regarding the number of corpora lutea and implantations. In addition, the number of early or late resorption sites and dead fetuses in each treated group were not different from corresponding controls. Besides, these values in Group II were comparable for those in Group IV. However, the number of empty implantation sites and total deaths were statistically higher in Group II than those in Groups I and IV.

With regard to the number of live fetuses and their sex ratio, there were no differences in any statistical analyses. The placental weights, fetal weights, crown-rump distances and tail lengths in male and female fetuses were statistically reduced in Group II as compared with Group I. Moreover, these values in both sexes except tail lengths were decreased in Group IV in comparison with Group III. However, excluding tail lengths in male conceptuses, there were no significant differences between both the treated groups.

3. *External malformations in fetuses* (Fig. 3) :

Nine fetuses had the gastroschisis as a characteristic external malformation in Group II. In addition to this anomaly, the agnathia, gastroschisis accompanied by the agnathia or cleft lip and vestigial tail were independently observed for one fetus in this group. Therefore, eleven fetuses (9.4%) possessed the gastroschisis and there were thirteen fetuses (11.1%) malformed externally in Group II. However, no external anomalies were observed in the other groups. Statistical analyses of external malformations revealed significantly higher in the number of fetuses with the gastroschisis in Group II than those in Groups I and IV. Reflecting these changes, the number of deformed fetuses was statistically increased in the same group.

4. *Internal variations and malformations in fetuses* (Fig. 4) :

One fetus had the dilated third and lateral ventricles followed by the anophthalmia and microphthalmia of left and right eye, respectively, in Group II. Besides, two fetuses possessed the dilated lateral and third ventricles and three fetuses exhibited enlargement of the lateral ventricles in this group. In Group II, consequently, the dilated lateral ventricles were observed in six fetuses (12.0%). Further, one, two, one, one and one fetuses owned the hypoplasia of thyroid, thymic remnant in the neck, agenesis of left kidney and ureter, fused kidneys and hypoplasia of left uterus, respectively, in this group. While, one, two and one fetuses possessed the

Fig. 2. Body weight changes in pregnant female rats receiving carboplatin intravenously during the first one-third of organogenetic period. Groups I, II, III and IV: See Table 1 description. Vertical lines show standard deviations. * $P < 0.05$, ** $P < 0.01$: Significant difference from corresponding controls (Group I or III). # $P < 0.05$: Significant difference from Group IV.

Table 2. Effects of carboplatin on prenatal development in rat fetuses.

Groups		I	II	III	IV
No. of dams examined		9	10	9	10
No. of corpora lutea	Total	139	160	148	162
	Mean $\pm$ S.D. per litter	15.4 $\pm$ 2.2	16.0 $\pm$ 1.4	16.4 $\pm$ 1.4	16.2 $\pm$ 1.8
No. of implantations	Total	133	145	142	154
	Mean $\pm$ S.D. per litter	14.8 $\pm$ 1.6	14.5 $\pm$ 2.6	15.8 $\pm$ 1.6	15.4 $\pm$ 1.5
No. of empty implantation sites (%)		3 (2.3)	22 (15.2) **, #	9 (6.3)	10 (6.5)
No. of early resorption sites (%)		1 (0.8)	4 (2.8)	0	0
No. of late resorption sites (%)		0	1 (0.7)	0	0
No. of dead fetuses (%)		0	1 (0.7)	0	0
No. of total deaths (%)		4 (3.0)	28 (19.3) **, ##	9 (6.3)	10 (6.5)
Live fetuses	Total	129	117	133	144
	Mean $\pm$ S.D. per litter	14.3 $\pm$ 1.4	11.7 $\pm$ 3.9	14.8 $\pm$ 1.8	14.4 $\pm$ 1.6
	Sex ratio (males/females)	1.22 (71/58)	1.13 (62/55)	1.05 (68/65)	0.76 (62/82)
	Placental weight $\pm$ S.D., g	Male 0.50 $\pm$ 0.03	0.39 $\pm$ 0.04 **	0.49 $\pm$ 0.02	0.42 $\pm$ 0.03 **
		Female 0.48 $\pm$ 0.05	0.38 $\pm$ 0.04 **	0.49 $\pm$ 0.04	0.40 $\pm$ 0.04 **
	Fetal weight $\pm$ S.D., g	Male 3.93 $\pm$ 0.12	3.05 $\pm$ 0.45 **	3.81 $\pm$ 0.27	3.46 $\pm$ 0.29 *
		Female 3.69 $\pm$ 0.18	2.98 $\pm$ 0.42 **	3.57 $\pm$ 0.23	3.24 $\pm$ 0.24 **
	Crown-rump distance $\pm$ S.D., cm	Male 3.83 $\pm$ 0.10	3.39 $\pm$ 0.25 **	3.78 $\pm$ 0.12	3.60 $\pm$ 0.18 *
		Female 3.70 $\pm$ 0.09	3.37 $\pm$ 0.21 **	3.65 $\pm$ 0.12	3.47 $\pm$ 0.14 **
	Tail length $\pm$ S.D., cm	Male 1.49 $\pm$ 0.04	1.37 $\pm$ 0.07 **, ##	1.49 $\pm$ 0.05	1.46 $\pm$ 0.04
		Female 1.46 $\pm$ 0.04	1.37 $\pm$ 0.06 **	1.46 $\pm$ 0.05	1.42 $\pm$ 0.04

Groups I, II, III and IV: See Table 1 description.

*P<0.05, **P<0.01: Significant difference from corresponding controls (Group I or III).

#P<0.05, ##P<0.01: Significant difference from Group IV.

Fig. 3. Effects of carboplatin on external malformations in rat fetuses. Groups I, II, III, and IV : See Table 1 description. ** $P < 0.01$: Significant difference from corresponding controls (Group I or III). ## $P < 0.01$: Significant difference from Group IV.

agenesis of thyroid, thymic remnant in the neck and agenesis of left kidney and ureter, respectively, in Group IV. Two and one fetuses had the hydroureter in Groups I and III, respectively. Therefore, the number of fetuses which showed the internal variations and malformations were two (3.2%), eleven (22.0%), one (1.5%) and four (5.6%) in Groups I, II, III and IV, respectively. The number of fetuses with the dilated lateral ventricles observed were particularly high for Group II as compared with Groups I and IV. Reflecting these changes, the incidence of abnormal fetuses was significantly increased in the same group.

5. *Skeletal ossification processes and variations in fetuses* (Table 3) :

Regarding the degree of ossification, the number of fetuses whose sternebrae except the first to fourth ones in Group IV did not ossify was significantly increased in both the treated groups. Reflecting these changes, the average number of ossified sternebrae was significantly lower in the same groups. Also, the average number of ossified cervical vertebral centers was lower in both the treated groups than that in corresponding controls. Further, the average numbers of ossified sacrococcygeal vertebrae and metacarpi were reduced in Group II. The numbers of fetuses whose hyoid bodies and the fifth sternebrae ossified poorly were decreased in each treated group as compared with corresponding controls. The number of fetuses whose first to fourth sternebrae and thoracic vertebral centers ossified poorly was increased in

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Fig. 4. Effects of carboplatin on internal variations and malformations in rat fetuses. Groups I, II, III and IV : See Table 1 description. ** $P < 0.01$: Significant difference from corresponding controls (Group I or III). ## $P < 0.01$: Significant difference from Group IV.

both the treated groups. In addition, the number of fetuses whose thoracic vertebral centers did not ossify and pubis ossified poorly was augmented in Groups II and IV, respectively. On the other hand, the number of fetuses whose sternbrae except the sixth ones did not ossify was higher in Group II than that in Group IV.

With respect to the skeletal variations, the number of fetuses whose thoracic and lumbar vertebral centers shaped like a dumbbell and thoracic vertebral centers were bipartite was increased in Group II. Furthermore, the number of fetuses which had the dumbbell-shaped centers of lumbar vertebra was higher in Group II than Group

Table 3. Effects of carboplatin on skeletal ossification processes and variations in rat fetuses.

Groups		I	II	III	IV
No. of dams		9	10	9	10
No. of fetuses examined		67	54	68	73
Skeletal ossification processes:					
Supraoccipital	Poorly ossified (%)	4 (6.0)	0	2 (2.9)	0
Hyoid body	Poorly ossified (%)	10 (14.9)	2 (3.7) *	9 (13.2)	0 **
Sternebra	Av. no. of ossified sternebrae ± S. D.	5.91±0.11	4.66±0.72 **	5.77±0.23	5.17±0.49 **
	Unossified	0	7 (13.0) **, #	0	1 (1.4)
	1st-4th (%)	6 (9.0)	44 (81.5) **, ##	12 (17.6)	42 (57.5) **
	5th (%)	0	19 (35.2) **	3 (4.4)	19 (26.0) **
	6th (%)	3 (4.5)	22 (40.7) **	3 (4.4)	19 (26.0) **
	1st-4th (%)	44 (65.7)	10 (18.5) **	45 (66.2)	25 (34.2) **
	5th (%)	51 (76.1)	34 (63.0)	46 (67.6)	49 (67.1)
	6th (%)	0.88±0.51	0.17±0.28 **	1.04±0.69	0.47±0.26 *
Cervical vertebra	Av. no. of ossified centers ± S. D.	1 (1.5)	4 (7.4)	0	1 (1.4)
	Poorly ossified arches (%)	0	4 (7.4) *	0	3 (4.1)
Thoracic vertebra	Unossified centers (%)	0	6 (11.1) **	0	5 (6.8) *
	Poorly ossified centers (%)	0	1 (1.9)	0	1 (1.4)
Lumbar vertebra	Poorly ossified centers (%)	0	7.65±0.39 **	7.99±0.39	7.81±0.33
Sacrococcygeal vertebra	Av. no. of ossified vertebrae ± S. D.	8.20±0.26	1 (1.9)	0	5 (6.8) *
Pubis	Poorly ossified (%)	2 (3.0)	3.33±0.39 **	3.72±0.30	3.54±0.32
Forelimbs	Av. no. of ossified metacarpus ± S. D.	3.83±0.20	3.31±0.39 **	3.69±0.34	3.49±0.32
	Av. no. of ossified distal phalanges ± S. D.	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
	Right	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Hindlimbs	Av. no. of ossified metatarsus ± S. D.	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
	Right	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
	Left	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Skeletal variations:					
Sternebra	Asymmetric (%)	0	1 (1.9)	0	1 (1.4)
	Bipartite (%)	0	1 (1.9)	0	0
Ribs	Cervical ribs	0	2 (3.7)	0	2 (2.7)
	Right (%)	0	1 (1.9)	0	2 (2.7)
	Left (%)	0	3 (5.6)	1 (1.5)	6 (8.2)
	Lumbar ribs	1 (1.5)	6 (11.1)	3 (4.4)	8 (11.0)
	Right (%)	6 (9.0)	6 (11.1) **	0	2 (2.7)
	Left (%)	0	6 (11.1) **	0	2 (2.7)
Thoracic vertebra	Bipartite centers (%)	33 (49.3)	27 (50.0)	30 (44.1)	23 (31.5)
	Dumbbell-shaped centers (%)	6.00±0.00	5.95±0.16	6.00±0.00	6.00±0.00
Lumbar vertebra	Av. no. of ossified vertebrae ± S. D.	0	2 (3.7)	0	0
	Bipartite centers (%)	0	7 (13.0) **, #	0	0
	Dumbbell-shaped centers (%)	0	0	0	0

Groups I, II, III and IV: See Table 1 description.

* P < 0.05, ** P < 0.01: Significant difference from corresponding controls (Group I or III).

P < 0.05, ## P < 0.01: Significant difference from Group IV.

IV.

6. *Skeletal malformations in fetuses* (Fig. 5) :

In Group II, six (11.4%), eight (14.8%), four (7.4%) and five (9.3%) fetuses had the cleft sternum, fused ribs, nodulated ribs and malformation of thoracic vertebra, respectively. In addition to these deformities, the fused cervical vertebral arches and agenesis of lumbar vertebra were observed in each one fetus in this group. In Group IV, one, one, two and one exhibited the cleft sternum, hypoplasia of ribs, defect of thoracic vertebral centers and defect of lumbar vertebral centers, respectively. Furthermore, two and one fetuses possessed the nodulated and wavy ribs, respectively.

Fig 5. Effects of carboplatin on skeletal malformations in rat fetuses. Groups I, II, III and IV : See Table 1 description. * $P < 0.05$, ** $P < 0.01$: Significant difference from corresponding controls (Group I or III). # $P < 0.05$, ## $P < 0.01$: Significant difference from Group IV.

ly, in Group I. However, no skeletal anomalies were observed in Group III. Since most of these malformed fetuses had two or more skeletal anomalies, the number of deformed fetuses were two (3.0%), eleven (20.4%) and three (4.1%) in Groups I, II and IV, respectively. Statistical analyses of skeletal anomalies revealed significantly higher in the number of fetuses with the cleft sternum, fused ribs and malformation of thoracic vertebra in Group II than those in Group I. In addition to these skeletal deformities, the number of fetuses with nodulated ribs were more in Group II than that in Group IV. Reflecting these changes, the incidence of malformed fetuses was statistically increased in the same group.

DISCUSSION

Carboplatin administered during the early organogenetic period did not affect maternal ovulation and implantation. However, an increase of fetal mortality was shown in dams treated with carboplatin from days 6 to 9 of gestation and fetal development was delayed in surviving conceptuses whose dams were given this drug from both days 6 to 9 and 7 to 10 of pregnancy. In the preceding studies, carboplatin achieved the embryo-lethal effects when administered before the organogenetic period (Kai et al., 1988a); whereas this drug retarded fetal development but revealed no embryo-lethality when dosed during the organogenetic period (Kai et al., 1988b). Since cisplatin, one of platinum coordination complexes, is a bifunctional agent which causes interstrand/intrastrand DNA crosslinking in mammalian cells (Mansy et al., 1973; Munchausen, 1974; Pascoe and Roberts, 1974; Munchausen and Rahn, 1975; Van Den Berg and Roberts, 1976), its major mechanism of action has been proposed to be the inhibition of DNA replication due to these crosslinking effects (Roberts and Pascoe, 1972; Roos, 1977; Zwelling et al., 1979; Zwelling and Kohn, 1980). The mechanism of action of carboplatin seems to be similar to that of cisplatin because of the resemblance in the chemical structure between these two compounds. Further, it has been shown that cisplatin-induced embryo-lethality varies with the stage of gestation (Keller and Aggarwal, 1983; Köpf-Maier et al., 1985). These results suggest that carboplatin administered to pregnant rats during the pregnancy may induce fetal death until day 6 of gestation and fetal growth retardation thereafter. Though elevations in the number of dead fetuses during the early gestation period were considered to be predominantly due to the direct effects of carboplatin, secondary effects through dams could not be denied because maternal weight gains were significantly suppressed when this drug was injected from days 6 to 9 of pregnancy.

The skeletal ossification was delayed in fetuses treated with carboplatin during both administration periods in this study and the similar effects had already been observed in our preceding study (Kai et al., 1988b). It is well-known that the retarded ossification is intimately involved in the low fetal body weights (Knight and

Roe, 1978 ; John et al., 1979 ; Ariyuki et al., 1980, 1982 ; Kanoh et al., 1982). Therefore, it was considered that the delayed ossification was attributable to the fetal growth retardation produced by carboplatin.

Carboplatin induced various abnormalities such as external malformations, visceral variations or anomalies and skeletal deformities when administered to pregnant female rats at 6 mg/kg/day from days 6 to 9 of gestation. Especially, the incidences of gastroschisis, dilatation of cerebral ventricles, cleft sternum, fused ribs and malformed thoracic vertebra were higher in these fetuses than corresponding controls and conceptuses treated from days 7 to 10 of pregnancy. Further, the frequency of nodulated ribs was higher in fetuses injected from days 6 to 9 of gestation than that of conceptuses dosed from days 7 to 10 of pregnancy. Reflecting these changes, the number of aberrant fetuses were increased in animals administered from days 6 to 9 of gestation as compared with the other two groups. According to the toxicology report of Bristol-Myers Company (unpublished), a very high number of visceral and skeletal abnormalities were observed consisting of an enlargement of the lateral ventricles of the brain, abdominal hernia (celiosoma), extrasternal heart and skeletal malformations when this drug was dosed to pregnant rats at 6 mg/kg/day from days 6 to 15 of gestation. These results are consistent with the data obtained from dams treated from days 6 to 9 of gestation in this study. However, carboplatin has been already reported to achieve no teratological effects against rat fetuses when administered at 4 mg/kg/day from days 7 to 17 of gestation and these results agree with the data acquired from pregnant rats given from days 7 to 10 of pregnancy in the present study. Therefore, it appears that carboplatin reveals the teratogenic action against rat conceptuses when dosed at 6 mg/kg/day until day 6 but not after day 7 of gestation. This finding was supported by the fact that a significant elevation in the number of empty implantation sites was revealed when carboplatin was injected to pregnant rats at 6 mg/kg/day from days 6 to 9 of gestation. Many alkylating agents, which crosslink DNA like cisplatin, have been shown to produce developmental defects in the chick (Murphy et al., 1957), mouse (Danforth and Center, 1954) and rat (Haskins, 1948 ; Murphy et al., 1958 ; Chaube et al., 1967 ; Greenaway et al., 1982 ; Padmanabhan and Singh, 1984 ; Slott and Hales, 1986). Moreover, cisplatin has been also reported to be teratogenic in chicks (Narbaitz and Marino, 1988) and mice (Lazar et al., 1979). Consequently, carboplatin seems to have teratological effects in rats when administered until day 6 of gestation, because its mechanism of action is probably identical with that for cisplatin as above described.

In conclusion, it is considered that carboplatin is embryotoxic, inducing intrauterine death and congenital malformations in rats, when administered during the early gestation period including day 6 of pregnancy.

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