EFFECTS OF A NEW PLATINUM COMPLEX ON MALE FERTILITY IN RATS

— COLLABORATIVE WORK TO DETERMINE
THE OPTIMAL PERIOD AND OPTIMAL PARAMETERS
TO DETECT EFFECTS ON MALE FERTILITY IN RATS —

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ABSTRACT — To assess the testicular toxicity induced by compound C, a new platinum complex being developed as an anti-cancer drug, the substance was intravenously administered to male rats at doses of 1, 3 and 10 mg/kg/day for 4 weeks and at doses of 0.3, 1 and 3 mg/kg/day for 9 weeks. Males were cohabited with non-treated females after these treatment periods and at sacrifice the genital organ weights and number of sperm in the testis were recorded and a histopathological examination performed. The females were sacrificed on day 13 of gestation and the numbers of corpora lutea, implantations and resorptions were counted. In the 4 weeks treatment 10 mg/kg/day group, the testis weight increased while the weights of the epididymides, seminal vesicles and prostate decreased significantly, and the number of sperm was significantly decreased. Extension of seminiferous tubules, vacuolization of spermatocytes, spermatids and Sertoli cells, and degeneration of spermatocytes and spermatids were observed by histopathological examination. Copulation and impregnation were not affected by treatment but the implantation rate was significantly decreased in the 10 mg/kg/day group. These results show that compound C has testicular toxicity like other platinum complexes and that organ weight, number of sperm, number of implantation and histopathological examination are useful for detection purposes. Treatment with compound C for 9 weeks did not affect male reproductive function in spite of severe general toxicity. This suggests that testicular toxicity should be detected after 4 weeks rather than 9 weeks treatment.

KEY WORDS : Platinum complex, Toxicity, Testis, Fertility, Rat.

INTRODUCTION

Generally, anti-cancer drugs, including platinum complexes such as cisplatin and carboplatin, have side effects on male reproductive function (Ettlin *et al.*, 1984 ; Seethalakshmi *et al.*, 1992 ; Kopf-Mainer, 1992). The mechanism of platinum complex-induced testicular toxicity is thought to be direct action on germ cells and secondary action caused by changes to hormonal circumstances (Kinkead *et al.*, 1992), or damage to Sertoli or Leydig cells (Ohara, 1988 ; Pogach *et al.*, 1989). The purpose of the present study was to assess the testicular toxicity induced by a new platinum complex being developed for anti cancer drug and to compare the ability to detect toxicity between 4 week and 9 week treatment studies.

MATERIALS AND METHODS

Animals : Sprague-Dawley rats (Slc : SD, Japan SLC, Inc., Shizuoka) were maintained in a room kept at $23 \pm 2^\circ\text{C}$ with a 14-hr light : 10-hr dark cycle and allowed free access to food (CE-2, Clea Japan, Inc., Tokyo) and water.

Test article : Compound C, a new platinum complex being developed by Chugai Pharmaceutical Co., Ltd., was dissolved in physiological saline. Treatment with compound C at dose of 10 mg/kg for 4 weeks increased the testis weight and caused decrease of spermatogenesis in a previous study. In this study, compound C was intravenously administered via the tail vein to 15 male rats at doses of 1, 3 and 10 mg/kg/day for 4 weeks in experiment 1, and to 10 male rats at doses of 0.3, 1 and 3 mg/kg/day for 9 weeks in experiment 2. The administration volume was 2 ml/kg body weight. For the control group, physiological saline was administered.

Experimental design : Male rats were treated daily from 6 weeks of age, when they weighed 170–200 g. Clinical signs were noted daily and body weights were recorded twice per week. In experiment 1, 5 male rats per group were sacrificed at the end of the treatment period. The other male rats in experiment 1 and all male rats in experiment 2 were cohabited with non-treated proestrous female rats after the treatment period, and were sacrificed after copulation or the mating

period (maximum 3 days ; cohabitation with 3 separate females). Copulation was confirmed by observation of a vaginal plug or sperm in the vaginal smear. The day of confirmation of copulation was considered day 0 of gestation. All rats were killed by cervical dislocation. The genital organ weights (testis, epididymis, seminal vesicle and prostate) were recorded. One gram of tissue from the right testis was homogenized in 3% NaCl solution. The number of sperm in each homogenate were counted (sperm head counts). To count sperm, a hyper osmotic solution (3–4 % NaCl solution) is usually used for dilution (Oochi, 1973), so 3% NaCl solution was applied to homogenize testis tissue in this study. The organs were fixed in Bouin's solution, embedded in paraffin wax, processed to histological slides stained with HE and PAS, and examined histopathologically. Numbers of germ cells and Sertoli cells in seminiferous tubules of stages II-III, V, VII and XII were counted. The females were sacrificed at day 13 of gestation and the numbers of corpora lutea, implantations, resorptions and live embryos were counted.

Statistical analysis : All values are expressed as mean $\pm$ S.D. One-way analysis of variance was used to compare the means of data obtained from all treatment groups and Dunnett or Scheffe's multiple range tests were used to determine statistical difference between groups with regard to body and organ weights, number of sperm, cells in seminiferous tubules, corpora lutea, implantations and dead or live embryos. The Fisher's exact method was used for copulation index and pregnant index. The level of significance was taken as $P < 0.05$ or 0.01 .

RESULTS

Experiment 1 (4 weeks treatment) : No clinical signs were observed in any rats. Body weight gains were significantly decreased in the 3 and 10 mg/kg/day groups (Table 1). In the 10 mg/kg/day group, the testis weight increased while the weights of epididymides, seminal vesicles and prostate decreased significantly (Table 2). In the 3 mg/kg/day group, the epididymides weight significantly decreased but other organ weights did not change. In the 1 mg/kg/day group, there were no changes in absolute organ weights. In

Table 1. Body weight gains of male rats treated with compound C for 4 or 9 weeks.

Dose (mg/kg)	0	0.3	1	3	10
4 weeks treatment					
No. of males	15		15	15	15
Body weight gain(g)	154.2±16.6		147.3±13.3	127.4±12.7*	49.2±14.9**
9 weeks treatment					
No. of males	10	10	10	10	
Body weight gain(g)	220.3±18.4	216.4±39.7	208.4±39.3	156.5±30.6**	

Mean±S.D.

** : P<0.01 : Significantly different from control.

Table 2. Absolute organ weights (mg) for male rats treated with compound C for 4 or 9 weeks.

Dose (mg/kg)	0	0.3	1	3	10
4 weeks treatment					
No. of unmated males	5		5	5	5
Testes	3377±210		3265±165	3296±900	4864±639*
Epididymides	735±38		702±50	668±23*	598±39**
Seminal vesicles	805±156		777±212	751±72	396±68**
Prostate	355±25		306±56	333±51	172±17**
No. of mated males	10		10	10	10
Testes	3242±185		3154±165	3327±328	4492±1047*
Epididymides	702±37		687±77	676±94	552±92**
Seminal vesicles	505±77		497±95	505±127	342±80**
Prostate	213±36		203±48	217±37	153±27**
9 weeks treatment					
No. of mated males	10	10	10	9	
Testes	3143±158	3145±176	3159±223	3125±195	
Epididymides	982±48	1102±92	974±95	908±127	
Seminal vesicles	743±214	746±96	821±183	667±149	
Prostate	278±60	383±139	364±98	259±47	

Mean±S.D.

** : P<0.01 : Significantly different from control.

unmated animals of the 10 mg/kg day group, relative weights of the testes, epididymides and prostate were also changed as for absolute weights but there were no significant decrease in relative weights of epididymides and prostate for mated males (Table 3). There were no tendencies for differences in absolute weights of genital organs between unmated and copulated male rats. In the 1 and 3 mg/kg/day groups, there were no changes in relative organ weights.

The numbers of testicular sperm were signifi-

cantly decreased in the 10 mg/kg/day group but not in the 1 and 3 mg/kg/day groups (Table 4). On histopathological examination, lesions in the seminiferous tubules of the testes were observed in all animals of the 10 mg/kg/day group (Table 5). In particular extension of seminiferous tubules, degeneration of spermatocytes and spermatids, vacuolization of spermatocytes and spermatids, and vacuolization of Sertoli cells were observed (Photos. 1–5). No changes in genital organs were observed on histopathological ex-

Table 3. Relative reproductive organ weights (mg/100 g body weight) for male rats treated with compound C for 4 or 9 weeks.

Dose (mg/kg)	0	0.3	1	3	10
4 weeks treatment					
No. of unmated males	5		5	5	5
Final B. W. (g)	348.5±24.7		349.3±5.5	321.5±19.3	239.2±16.6**
Testes	973±91		935±23	1029±80	2035±245*
Epididymides	212±13		201±14	209±17	251±17**
Seminal vesicles	231±41		222±60	234±26	167±38
Prostate	102±10		88±16	104±18	73±11*
No. of mated males	10		10	10	10
Final B. W. (g)	342.8±21.5		330.6±13.4	317.6±17.5	243.2±18.5**
Testes	947±46		955±51	1051±124	1836±375*
Epididymides	205±14		208±21	213±34	227±38
Seminal vesicles	147±21		150±26	159±37	141±32
Prostate	62±12		61±13	68±11	63±8
9 weeks treatment					
No. of mated males	10	10	10	9	
Final B. W. (g)	404.9±21.3	400.9±46.0	393.0±39.2	341.5±33.9*	
Testes	778±50	792±73	807±57	924±52**	
Epididymides	243±14	252±28	249±26	267±38	
Seminal vesicles	185±62	187±26	211±55	194±29	
Prostate	69±14	95±29	93±23	76±13	

Mean±S.D.

** : P<0.01 : Significantly different from control.

Table 4. Numbers of testicular sperm heads-in male rats treated with compound C for 4 or 9 weeks.

Dose (mg/kg)	0	0.3	1	3	10
4 weeks treatment					
No. of unmated males	5		5	5	5
(×10 ⁸ /g tissue)	0.834±0.123		0.855±0.056	0.891±0.070	0.266±0.073**
No. of mated males	7		7	7	7
(×10 ⁸ /g tissue)	0.836±0.073		0.845±0.083	0.817±0.099	0.357±0.127**
9 weeks treatment					
No. of mated males	10	10	10	10	
(×10 ⁸ /g tissue)	1.284±0.218	1.285±0.198	1.231±0.178	1.188±0.358	

Mean±S.D.

** : P<0.01 : Significantly different from control.

Table 5. Histopathological findings for male rats treated with compound C for 4 weeks.

Dose (mg/kg)	0	1	3	10
No. of males	15	15	15	15
Findings				
Extension of seminiferous tubules	0	0	0	14
Necrosis of seminiferous epithelium	0	0	0	1
Giant cell formation in seminiferous tubules	0	0	0	2
Vacuolization of nuclei of spermatocytes and spermatids	0	0	0	4
Degeneration of spermatocytes and spermatids	0	0	0	10
Vacuolization of spermatocytes and spermatids	0	0	0	15
Vacuolization of Sertoli cells	0	0	0	15

Table 6. Cell types and numbers in seminiferous tubules of male rats treated with compound C for 4 weeks.

4 weeks treatment				
Dose (mg/kg)	Control	1	3	10
No. of males	5	5	5	5
Stages II-III				
Sertoli cells	18.60 ± 2.05	17.10 ± 1.76	16.30 ± 1.94	10.93 ± 2.89**
Spermatogonia	22.23 ± 3.64	20.70 ± 4.09	16.73 ± 7.83	16.13 ± 3.53
Pachytene spermatocytes	52.30 ± 2.37	48.97 ± 8.52	52.63 ± 6.17	40.67 ± 4.10*
Round spermatids	149.27 ± 11.38	135.33 ± 19.22	129.53 ± 16.00	100.80 ± 10.80**
Elongate spermatids	147.37 ± 5.55	148.93 ± 24.67	141.40 ± 23.66	94.80 ± 31.54
Stage V				
Sertoli cells	15.24 ± 1.68	15.53 ± 1.38	14.90 ± 2.60	9.67 ± 0.87**
Type A spermatogonia	2.27 ± 0.25	1.47 ± 0.49	1.67 ± 0.59	1.60 ± 1.21
Type B spermatogonia	32.73 ± 4.49	27.40 ± 3.50	28.73 ± 7.72	22.70 ± 6.56
Pachytene spermatocytes	55.53 ± 2.58	52.77 ± 2.67	53.03 ± 7.20	40.43 ± 5.96**
Round spermatids	151.87 ± 15.33	135.73 ± 5.40	137.60 ± 14.86	98.40 ± 20.02**
Elongate spermatids	163.43 ± 16.02	152.43 ± 13.87	146.23 ± 18.28	95.80 ± 36.21**
Stage VII				
Sertoli cells	16.53 ± 1.25	15.33 ± 2.84	16.17 ± 1.40	11.33 ± 2.08**
Spermatogonia	2.50 ± 0.51	1.80 ± 0.30	1.77 ± 0.73	1.33 ± 0.76
Preleptotene spermatocytes	40.63 ± 2.67	35.67 ± 4.45	37.63 ± 3.50	28.37 ± 4.02**
Pachytene spermatocytes	61.36 ± 2.56	60.60 ± 2.57	60.90 ± 6.79	44.23 ± 5.45**
Round spermatids	144.17 ± 13.07	127.00 ± 5.99	136.23 ± 16.21	91.07 ± 12.30**
Elongate spermatids	154.13 ± 10.19	145.10 ± 9.92	152.30 ± 17.07	97.37 ± 18.05**
Stage XII				
Sertoli cells	18.83 ± 1.70	18.67 ± 1.99	18.47 ± 0.86	14.63 ± 1.87**
Spermatogonia	3.83 ± 0.62	3.50 ± 0.89	3.40 ± 0.93	2.60 ± 0.90
Zygotene spermatocytes	48.27 ± 3.58	44.13 ± 3.60	45.70 ± 3.62	34.13 ± 3.42**
Pachytene spermatocytes	68.40 ± 4.15	62.60 ± 2.77	60.70 ± 6.14*	44.23 ± 4.29**
Elongate spermatids	174.70 ± 17.40	161.03 ± 15.85	162.03 ± 12.84	100.50 ± 21.80**

Mean ± S.D.

* : P < 0.05, ** P < 0.01 : Significantly different from control.

Photo. 1. Testicular histology of a control rat.
HE stain, $\times 100$.

Photo. 2. Testis from a rat exposed to 10 mg/kg compound C for 4 weeks.
Note extension of seminiferous tubules and atrophy of Leydig cells.
HE stain, $\times 100$.

Photo. 3. Testicular histology of a control rat.
Seminiferous tubule in stage XII.
HE stain, $\times 400$.

Photo. 4. Testis from a rat exposed to 10 mg/kg compound C for 4 weeks.
Note vacuolization of Sertoli cells, spermatocytes and spermatids in stage XII of the seminiferous tubule. HE stain, $\times 400$.

Photo. 5. Testis from a rat exposed to 10 mg/kg compound C for 4 weeks.
Note degeneration and necrosis of seminal epithelium, giant cell formation and vacuolization of nuclei of spermatids. HE stain, $\times 400$.

amination in the 1 and 3 mg/kg/day groups.

Numbers of Sertoli cells and all types of germ cells other than spermatogonia in seminiferous tubules decreased at all stages examined in the 10 mg/kg/day group (Table 6). Numbers of spermatogonia tended to decrease but this was not significant. In the 1 and 3 mg/kg/day groups, numbers of Sertoli cells and germ cells were not changed by the treatment.

Reproductive performance including ability to copulate and impregnate was not affected by the treatment (Table 8). The implantation rate

was significantly lowered in the 10 mg/kg/day group, but there was no significant difference in mortality after implantation. In the 1 and 3 mg/kg/day groups, the implantation rate did not change.

Experiment 2 (9 weeks treatment) : No clinical signs were observed in any but one rat, which died after treatment for 6 weeks in the 3 mg/kg/day group. Body weight gain was significantly lowered in the 3 mg/kg/day but not in the 0.3 and 1 mg/kg/day groups (Table 1). There were no significant changes in the absolute weights of

Table 7. Cell types and numbers in seminiferous tubules of male rats treated with compound C for 9 weeks.

9 weeks treatment				
Dose (mg/kg)	Control	0.3	1	3
No. of males	5	5	5	5
Stages II-III				
Sertoli cells	19.67 ± 3.00	20.43 ± 2.75	19.67 ± 2.45	19.43 ± 1.36
Spermatogonia	18.50 ± 3.24	18.63 ± 4.94	17.47 ± 3.64	16.33 ± 3.19
Pachytene spermatocytes	55.70 ± 4.47	52.20 ± 5.54	52.14 ± 5.97	52.37 ± 3.63
Round spermatids	147.53 ± 8.03	137.07 ± 9.13	133.00 ± 11.87	138.20 ± 9.96
Elongate spermatids	164.60 ± 15.54	154.63 ± 11.74	148.20 ± 16.28	149.56 ± 19.05
Stage V				
Sertoli cells	19.57 ± 3.65	19.80 ± 2.26	18.27 ± 3.42	19.73 ± 1.50
Type A spermatogonia	2.17 ± 0.58	2.13 ± 0.76	1.73 ± 0.76	1.50 ± 0.33
Type B spermatogonia	36.13 ± 7.16	35.40 ± 3.81	33.27 ± 7.98	32.60 ± 4.83
Pachytene spermatocytes	58.17 ± 6.25	56.67 ± 4.94	56.80 ± 7.38	57.87 ± 6.91
Round spermatids	152.33 ± 18.88	145.77 ± 8.95	142.33 ± 14.33	149.17 ± 19.62
Elongate spermatids	167.83 ± 16.35	170.17 ± 7.66	159.73 ± 21.71	163.73 ± 24.38
Stage VII				
Sertoli cells	18.86 ± 1.52	17.73 ± 1.24	17.03 ± 2.28	17.80 ± 1.62
Spermatogonia	2.40 ± 0.44	2.43 ± 0.56	3.17 ± 0.91	2.10 ± 0.38
Preleptotene spermatocytes	41.77 ± 3.50	41.50 ± 2.56	39.13 ± 3.85	40.80 ± 3.97
Pachytene spermatocytes	63.90 ± 2.82	58.86 ± 2.99	61.50 ± 5.42	61.00 ± 6.86
Round spermatids	145.10 ± 11.00	138.07 ± 14.73	136.40 ± 14.51	146.60 ± 12.43
Elongate spermatids	174.33 ± 2.48	166.20 ± 11.55	153.73 ± 10.13**	166.00 ± 10.77
Stage XII				
Sertoli cells	20.60 ± 2.52	19.97 ± 1.49	19.76 ± 1.68	21.27 ± 1.17
Spermatogonia	4.16 ± 0.24	4.83 ± 1.54	4.27 ± 1.12	3.13 ± 1.04
Zygotene spermatocytes	53.13 ± 3.94	50.20 ± 4.83	50.97 ± 4.91	50.97 ± 5.58
Pachytene spermatocytes	69.17 ± 4.66	64.83 ± 3.98	63.93 ± 4.59	64.93 ± 7.49
Elongate spermatids	180.46 ± 27.31	160.67 ± 14.27	175.17 ± 17.17	178.77 ± 26.80

Mean ± S.D.

** : P < 0.01 : Significantly different from control.

Table 8. Caesarean section data for females mated with males treated with compound C for 4 or 9 weeks.

Dose (mg/kg)	0	0.3	1	3	10
4 weeks treatment					
No. of pairs	10		10	10	10
Copulation rate (%)	100.0		100.0	100.0	90.0
(Copulated/mated)	(10/10)		(10/10)	(10/10)	(9/10)
Fertility rate (%)	100.0		100.0	90.0	77.8
(Pregnant/copulated)	(10/10)		(10/10)	(9/10)	(7/9)
No. of corpora lutea	13.9 ± 1.3		14.0 ± 1.2	13.2 ± 1.2	12.9 ± 1.8
No. of implantations	13.5 ± 1.3		13.4 ± 1.1	12.4 ± 1.7	5.0 ± 3.4**
Implantation rate (%) ^{a)}	97.2 ± 4.7		95.9 ± 6.2	94.1 ± 10.0	37.8 ± 21.9**
No. of resorptions	0.6 ± 0.7		0.4 ± 0.7	0.8 ± 0.4	0.7 ± 1.3
Mortality (%) ^{b)}	4.7 ± 5.9		3.1 ± 5.4	6.6 ± 3.9	9.7 ± 19.0
No. of live embryos	12.9 ± 1.7		13.0 ± 1.4	11.6 ± 1.4	4.3 ± 2.8**
9 weeks treatment					
No. of pairs	10	10	10	9	
Copulation rate (%)	90.0	100.0	80.0	100.0	
(Copulated/mated)	(9/10)	(10/10)	(8/10)	(9/9)	
Fertility rate (%)	100.0	100.0	100.0	88.9	
(Pregnant/copulated)	(9/9)	(10/10)	(8/8)	(8/9)	
No. of corpora lutea	13.9 ± 2.0	14.1 ± 1.3	13.9 ± 1.1	13.9 ± 1.6	
No. of implantations	13.4 ± 2.4	12.6 ± 3.1	13.4 ± 1.1	13.3 ± 1.5	
Implantation rate (%) ^{a)}	96.3 ± 6.7	88.4 ± 17.6	96.5 ± 5.0	95.7 ± 5.5	
No. of resorptions	0.4 ± 0.5	1.2 ± 1.1	0.3 ± 0.5	1.3 ± 0.5	
Mortality (%) ^{b)}	3.7 ± 4.7	12.6 ± 18.1	1.8 ± 3.3	9.7 ± 4.2	
No. of live embryos	13.0 ± 2.5	11.4 ± 3.7	13.1 ± 1.0	12.0 ± 1.7	

a) : (No. of implantations / No. of corpora lutea) × 100.

b) : (No. of resorptions / No. of implantations) × 100.

Mean ± S.D.

* : P < 0.05, ** P < 0.01 : Significantly different from control.

genital organs but the relative testis weight was significantly decreased in the 3 mg/kg/day group (Tables 2, 3). In the 0.3 and 1 mg/kg/day groups, there were no significant changes in the absolute or relative weights of genital organs. Numbers of sperm in the testis were not changed by the treatment (Table 4). On histopathological examination, no changes in genital organs were observed with any dose. Numbers of Sertoli cells and all types of germ cells in the seminiferous tubules were not altered (Table 7). Reproductive performance and implantation rate were not affected (Table 8).

DISCUSSION

Treatment with compound C at 10 mg/kg/day for 4 weeks in the present study caused toxic effects on male reproductive function with general side effects as evidenced by severe decrease in body weight gain. The weights of epididymides, seminal vesicles and prostate were decreased while testis weight was increased. Changes in organ weight were not affected by mating, but weights of epididymides, seminal vesicles and prostate were decreased with ejaculation, and this is a complicating factor with mating.

The observed increase in testis weight is an interesting response to compound C because

other platinum complexes caused decreased testis weights in previous studies (Huang *et al.*, 1990 ; Seethalakshmi *et al.*, 1992). The increase of testis weight might be due to accumulation of tissue fluid because extension of seminiferous tubules was observed on histopathological examination. Cisplatin affects on Sertoli cell secretory function such as increased sodium and decreased potassium concentrations in seminiferous tubular fluid and damage of tight junctions between Sertoli cells have been reported (Pogach *et al.*, 1989). Carboplatin disrupts the blood/testis barrier (Kopf-Mainer, 1992). In this study, vacuolization of Sertoli cells was observed suggesting cellular damage. Accumulation of tissue fluid in seminiferous tubules can result from abnormal junctions between Sertoli cells or between Sertoli cells and germ cells, or lesions of Sertoli cell secretory function causing ion or osmotic imbalances.

Histopathological examination revealed degeneration of spermatocytes and spermatids, vacuolization of spermatocytes and spermatids, and vacuolization of Sertoli cells in most animals of the 10 mg/kg/day group. The same changes have been observed with cisplatin (Kopf-Mainer, 1992) and carboplatin (Seethalakshmi *et al.*, 1992).

The observed effects on germ cells are presumably due to a direct action of compound C, which inhibits mitosis by combining with DNA like other platinum complexes. Cisplatin and carboplatin change the concentrations of several hormones such as testosterone, LH, FSH and androgen binding protein (Kinkead *et al.*, 1992 ; Huang *et al.*, 1990 ; Pogach *et al.*, 1989 ; Seethalakshmi *et al.*, 1992), and therefore an endocrinological study is also necessary to explain the testicular toxicity induced by compound C.

In the present study, there were no changes in spermatogonia evident on light microscopic examination and numbers of spermatogonia did not decrease at any stage. However, all germ cells in spermatogenesis after spermatocytes demonstrated morphological changes and their numbers decreased. Moreover, numbers of sperm (sperm head counts) in testis homogenates also decreased. From these results, it is obvious that compound C affects spermatogenesis.

Counting of germ cells in seminiferous

tubules at specific stages of spermatogenesis is an appropriate method to detect testicular toxicity (Takahashi and Matsui, 1993) and this study provided supportive evidence of the utility of this approach. The problem is that treatment of compound C for 4 weeks damaged all types of germ cells except spermatogonia and Sertoli cells in seminiferous tubules at all stages. Therefore for routine fertility or repeated dose studies, counts of germ cells at specific stages is not so informative. Single dosing or short treatment study is needed to find the first affected cells.

The fact that the implantation rate was decreased by compound C is in line with earlier findings from a mating study with cisplatin (Seethalakshmi *et al.*, 1992). Decrease of the implantation rate is thought to be caused not only by decrease sperm in number but also by decreased ability to fertilize and disruption of normal embryonic development before implantation by damage to DNA.

Several parameters were used to detect the toxicity by compound C in this study. Organ weights, number of testicular sperm, number of implantations in the mating test and histopathological examination proved useful to detect the changes by compound C. However sensitivity could not be compared because they were all able to detect toxicity only at the 10 mg/kg dose in the 4 week treatment study. Copulation and impregnation were not good indices to detect testicular toxicity by compound C because they were not affected. Nevertheless, observation of copulation is necessary because toxicity caused by damage to the nerve system or changes in hormonal circumstance may be apparent which can not be detected in terms of organ weights, histopathological examination or sperm number.

Treatment with compound C for 9 weeks did not affect male reproductive function. The dose setting of 3 mg/kg/day was considered appropriate for a routine fertility study, because obvious decrease in body weight gain was seen and moreover one animal died at this dose. With long treatment periods, doses must be lower to avoid death or severe general side effects if test substances, like anti-cancer drugs, have strong toxicity. The results suggest that toxicity can better be detected after 4 weeks rather than 9 weeks treatment.

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