COLLABORATIVE WORK TO EVALUATE TOXICITY ON MALE REPRODUCTIVE ORGANS BY REPEATED DOSE STUDIES IN RATS

11) TESTICULAR TOXICITY OF 2- OR 4-WEEK REPEATED OR SINGLE-DOSE ADMINISTRATION OF A NOVEL PLATINUM COMPLEX

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ABSTRACT — A novel platinum complex, compound C, developed as an antitumor agent, was intravenously administered at 10 mg/kg/day to 6-week-old and 8-week-old rats. After 4 weeks of administration to the former, testicular enlargement was observed at a similar incidence as testicular atrophy and dilatation or atrophy of the seminiferous tubules was observed. Degeneration/necrosis of the seminiferous epithelium, decrease of seminiferous epithelium, formation of multinucleated giant cells, and vacuolar degeneration of Sertoli cells were also seen. These lesions were more marked in seminiferous tubules with atrophy than in those without atrophy. After 2-week administration to 8-week-old rats, slight initial-phase findings such as dilatation of seminiferous tubules and degeneration/necrosis of the seminiferous epithelium were noted in all rats. In rats administered 20 mg/kg/day, for which the administration period was shortened to 1 week due to marked weight loss, very slight initial lesions were similarly observed. However, these lesions were not easy to detect in these rats. Following single administration of a sublethal dose (80 mg/kg), testicular lesions were clearly observed 14 days after administration, but the incidence and grade of lesions were very low at 7 days. In conclusion, testicular toxicity of compound C can be clearly detected after 2-week administration, although the progression of lesions differed from the case with 4-week administration.

KEY WORDS: Platinum complex, Toxicity, Testis, Rat

INTRODUCTION

Prior to first administration of candidate drugs to humans, results of repeated-dose toxicity studies must be evaluated. However, agreement concerning the period of repeated-dose studies has not been obtained in the ICH (ICH-M3 step IV guideline, 1997). Japan and the EU/US have respectively recommended 4 weeks and 2 weeks for this period. Evaluation of the effects on male reproduction are necessary before initial clinical trials in Japan but sufficient data indicating that effects on the male reproductive system may be adequately detected in 2-week studies have hitherto been lacking (ICH-M3 step IV guideline, 1997). The JPMA

and NIHS have therefore organized a validation study to determine the validity and limitations of 2-week repeated-dose toxicity studies for detecting effects on male reproductive organs in rats. As a part of this project, we administered compound C (an antitumor platinum complex developed by Chugai Pharmaceutical Co., Ltd.) for 2 weeks and compared the effects of this to those of 4-week administration. We also examined the testicular toxicity with single-dose administration.

MATERIALS AND METHODS

Test substance

Compound C is an antitumor platinum complex

developed by Chugai Pharmaceutical Co., Ltd., the dosage form of which is a white crystalline powder. Prior to use, it was dissolved in physiological saline to prepare 5 mg/mL and 2.5 mg/mL solutions.

Animals

Five- and 7-week-old male Slc:SD rats (Japan SLC, Inc., Shizuoka) were purchased. After three days of quarantine and acclimation, group allocation was conducted in such a fashion that there were no deviations in mean body weight among the groups. Compound C was administered starting at 6 (body weight range: 183.8 to 198.8 g) and 8 (body weight range: 258.4 to 290.3 g) weeks of age, respectively.

Animals were individually housed in stainless steel cages (W230 mm×D370 mm×H180 mm) in an animal room under the following conditions: temperature, $24 \pm 2^\circ\text{C}$; relative humidity, $55 \pm 10\%$; lighting time, 14 hr (5:00 a.m. - 7:00 p.m.); and ventilation, 14 to 16 times/hr. Solid food (CE-2, Clea Japan, Inc., Tokyo) and tap water were available *ad libitum*.

Experiment design

Compound C was administered into a tail vein once a day at a rate of approximately 2 mL/min. The days after administration were numbered, regarding the

date of the first administration as day 0.

As shown in Fig. 1, our protocol comprised 4-week and 2-week repeated administration, as well as single-dose administration. In a previous study of compound C (Mizoguchi *et al.*, 1995), 4-week repeated administration of 10 mg/kg/day induced clear testicular toxicity. Doses were therefore set at 10 mg/kg/day for 4-week repeated administration, as well as 10 and 20 mg/kg/day for 2-week repeated administration. In addition, control groups receiving physiological saline were established for each administration period. Five male rats were assigned to each group. For the 4-week repeated administration, the first administration was given at 6 weeks of age, and for the 2-week repeated administration, administration was started at 8 weeks of age. For all animals, the age at autopsy was planned to be 10 weeks. However, in the 20 mg/kg/day group, weight loss was marked, and 2-week survival was considered unlikely. Autopsy was therefore performed 1 week after administration was started. For single-dose administration, doses were set at 40 and 80 mg/kg based on the findings that the 50% lethal dose is 107 mg/kg and that the minimal lethal dose is 87 mg/kg (Suzuki *et al.*, 1992). Administration was performed at 8 weeks of age. Five animals each in each group were autopsied 7 and 14 days after administration.

Fig. 1. Study design.

Clinical examination

Clinical signs such as physical appearance, behavior, and excretion were examined once a day until the day before administration, and once prior to administration daily during the administration period. Body weight was measured twice before administration was started, and twice a week before administration during the administration period.

Pathological examination

Under ether inhalation anesthesia, animals were euthanized by exsanguination through the abdominal aorta the day after the final administration in the groups undergoing repeated administration, and 7 or 14 days after administration in the groups receiving a single dose. Animals were fasted from the evening the day before autopsy. On autopsy, the testis and epididymis were weighed, and relative weights per 100 g body weight were calculated. For histopathological examination, the testis and epididymis were fixed in Bouin's fixative, and paraffin sections were prepared according to a routine procedures. Hematoxylin and eosin (HE) stained sections were examined under a light microscope.

Statistical analysis

For body weights and organ weights, the significance of differences in mean values between each treated group and the respective control group was tested using the multiple comparison method. Initially, homogeneity of variance was tested by the Bartlett method (Yoshimura, 1988). When homogeneity was observed, one-way analysis of variance (Yoshimura, 1988) was performed. When there was a significant difference between groups, the Dunnett multiple comparison test (Yoshimura, 1988) was used. When the Bartlett method revealed heterogeneity of variance, the Kruskal-Wallis H test (Yoshimura, 1988) was employed. When there was a significant difference between groups, the Dunnett multiple comparison test by rank conversion was performed. Values of $p < 0.05$ were considered significant.

RESULTS

Clinical signs and body weights

Data for changes in body weight are illustrated graphically in Figs. 2 and 3.

None of the animals in any group died.

1. Four-week administration

In the 10 mg/kg group, decrease in stools was

observed from 9 days after administration was started. Weight gain was markedly inhibited. Body weights were significantly decreased from 3 days after administration was started and final body weights (at autopsy after overnight fasting) were approximately 64% of those of the control.

2. Two-week administration

In the 10 mg/kg group, decrease in stools was observed from 9 days after administration was started. Body weights were significantly decreased from 3 days of the administration period and the final body weights were approximately 78% of those of the control. In the 20 mg/kg group, for which autopsy was performed 1 week after administration was started, decrease in stools was observed from 6 days after administration was started and diarrhea from 7 days. Weight loss was greater than in the 10 mg/kg group. The final body weights on day 7 of administration were approximately 73% of those of the control.

3. Single-dose administration

In the 40 mg/kg group, no overt clinical signs were observed. Weight gain was inhibited or slight weight loss was noted 3 days after administration. However, weight change subsequently reversed, with weight gain noted at 7 days after administration. In the 80 mg/kg group, a soiled perineal region and loose stools were noted 3 days after administration. Diarrhea occurred after 4 days. However, these signs gradually improved over time. Weight loss was noted until 3 or 7 days after administration, but gain was noted after 10 days. There were no abnormal findings after 10 days.

Pathological examination

1. Autopsy findings

Autopsy findings for the testis and epididymis are summarized in Tables 1 and 2. With the exception of 1 animal which exhibited a spontaneous node in the epididymis (spermatic granuloma), there were no abnormalities in any control animals.

1) Four-week administration

In the 10 mg/kg group, bilateral testicular enlargement was observed in 2 rats, while unilateral enlargement was noted in 2. However, bilateral atrophy of the testis was also observed in 1 rat, and unilateral atrophy in 2. In the latter 2 rats with unilateral atrophy, the contralateral testis exhibited enlargement. In addition to atrophy of the testis, unilateral atrophy of the epididymis was noted in 2 rats. Furthermore, atrophy of the seminal vesicles and prostate, atrophy of the thy-

mus and spleen, hemorrhage of the glandular stomach, thinning of the small intestinal wall, and dark red foci in the lung were observed. A node thought to be a spontaneous lesion in the epididymis (spermatic granuloma) was observed in 1 rat.

2) Two-week administration

In the 10 mg/kg group, bilateral enlargement of the testis was observed in 3 rats. In addition, atrophy of the seminal vesicles and prostate, atrophy of the thymus and spleen, and dark red foci in the lung were seen. In the 20 mg/kg group, in which autopsy was performed 1 week after administration was started, no

Fig. 2. Body weight changes in male rats intravenously administered compound C for 4 weeks or 2 weeks. Values represent mean $\pm$ S.D.. 4-week study (●, ▲), 2-week study (○, △, □). Control (●, ○), 10 mg/kg (▲, △), 20 mg/kg (□). *, **: Significantly different from the control ($p < 0.05$ and $p < 0.01$, respectively).

Fig. 3. Body weight changes in male rats intravenously administered a single-dose of compound C. Values represent mean $\pm$ S.D.. Rats were sacrificed at 7 days (○, △, □) or 14 days (●, ▲, ■) after administration. Control (○, ●), 40 mg/kg (△, ▲), 80 mg/kg (□, ■). *, **: Significantly different from the control ($p < 0.05$ and $p < 0.01$, respectively).

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Table 1. Gross and histopathological findings in the testes and epididymides from male rats intravenously administered compound C for 4 weeks or 2 weeks.

Organ	Findings	Grade	4-week study				2-week study					
			Dose(mg/kg)				0		10		20 ^{a)}	
			Number of rats				5		5		5	
			Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Gross findings												
Testis												
	Enlargement	+	0 ^{b)}	0	2	4 ^{c)}	0	0	3	3	0	0
	Atrophy	+	0	0	3	1	0	0	0	0	0	0
Epididymidis												
	Atrophy	+	0	0	2	0	0	0	0	0	0	0
	Nodule	+	0	0	1	0	1	0	0	0	0	0
Histopathological findings												
Testis												
	Dilatation, seminiferous tubules	±	0	0	0	0	0	0	1	2	1	1
		+	0	0	0	0	0	0	4	3	0	0
		++	0	0	2	4 ^{d)}	0	0	0	0	0	0
	Atrophy, seminiferous tubules	+	0	0	1	0	0	0	0	0	0	0
		++	0	0	2	1	0	0	0	0	0	0
	Degeneration/necrosis, seminiferous epithelium	±	0	0	0	0	0	0	5	4	5	5
		+	0	0	2	4	0	0	0	0	0	0
		++	0	0	3	1	0	0	0	0	0	0
	Decrease, seminiferous epithelium	±	0	0	0	0	0	0	2	2	0	0
		+	0	0	2	4	0	0	3	3	0	0
		++	0	0	3	1	0	0	0	0	0	0
	Multinucleated giant cell formation	+	0	0	1	1	0	0	0	0	0	0
		++	0	0	3	1	0	0	0	0	0	0
	Vacuolar degeneration, Sertoli cells	+	0	0	5	5	0	0	0	0	0	0
Epididymidis												
Head												
	Atrophy, ducts	±	0	0	2	3	0	0	0	0	0	0
		+	0	0	3	1	0	0	0	0	0	0
	Decrease, sperm, ducts	±	0	0	1	1	0	0	0	0	0	0
		+	0	0	1	1	0	0	0	0	0	0
		++	0	0	1	0	0	0	0	0	0	0
		+++	0	0	2	1	0	0	0	0	0	0
Tail												
	Atrophy, ducts	±	0	0	2	3	0	0	0	0	0	0
		+	0	0	3	1	0	0	0	0	0	0
	Decrease, sperm, ducts	+	0	0	2	1	0	0	0	0	0	0
		++	0	0	1	0	0	0	0	0	0	0
	Granuloma, spermatic	P	0	0	1	0	1	0	0	0	0	0

±: Very slight, +: Slight, ++: Moderate, +++: Severe, P: Present.

a): Sacrificed at 1-week. b): Number of rats with lesions. c): Two animals showed atrophy of the right testis. d): Two animals showed atrophy of seminiferous tubules in the right testis.

changes were observed in the testis. However, atrophy of the seminal vesicles and prostate, atrophy of the thymus and spleen, hemorrhage of the glandular stomach, thinning of the small intestine, and dark red foci in the lung were observed.

3) Single-dose administration

Seven days after administration, there were no changes in the testis in either the 40 mg/kg group or the 80 mg/kg group. In the 40 mg/kg group, atrophy of the thymus and spleen was seen. In the 80 mg/kg group, atrophy of the thymus and spleen, atrophy of the seminal vesicles and prostate, hemorrhage of the glandular

Table 2. Gross and histopathological findings in the testes and epididymides from male rats intravenously administered a single-dose of compound C.

administered a single-dose of compound C.																
Organ	Findings	Grade	Time of sacrifice		day 7				day 14							
			Dose(mg/kg)		0		40		80		0		40		80	
			Number of rats		5		5		5		5		5		5	
Gross findings																
Testis																
Enlargement	++	0 ^{a)}	0	0	0	0	0	0	0	0	0	0	2	2		
Atrophy	+	0	0	0	0	0	0	0	0	0	0	0	0	1		
Epididymidis																
Atrophy	+	0	0	0	0	0	0	0	0	0	0	0	0	1		
Nodule	+	0	0	0	0	0	0	0	0	0	0	1	0	0		
Histopathological findings																
Testis																
Dilatation, seminiferous tubules	±	0	0	0	0	2	1	0	0	1	2	1 ^{b)}	0			
	+	0	0	0	0	0	0	0	0	1	0	1	1			
	++	0	0	0	0	0	0	0	0	0	0	2	2			
Atrophy, seminiferous tubules	++	0	0	0	0	0	0	0	0	0	0	0	0	1		
Degeneration/necrosis, seminiferous epithelium	±	0	0	0	0	0	0	0	0	0	0	3	2			
	+	0	0	0	0	0	0	0	0	0	0	1	1			
	++	0	0	0	0	0	0	0	0	0	0	0	1			
Decrease, seminiferous epithelium	±	0	0	0	0	2	1	0	0	2	2	2	1			
	+	0	0	0	0	0	0	0	0	0	0	2	2			
	++	0	0	0	0	0	0	0	0	0	0	0	1			
Multinucleated giant cell formation	+	0	0	0	0	0	0	0	0	0	0	0	1			
Vacuolar degeneration, Sertoli cells	+	0	0	0	0	0	0	0	0	0	0	0	1			
Epididymidis																
Head																
Atrophy, ducts	+	0	0	0	0	0	0	0	0	0	0	0	0	1		
Decrease, sperm, ducts	+++	0	0	0	0	0	0	0	0	0	0	0	0	1		
Desquamated cell debris, ducts	+	0	0	0	0	0	0	0	0	0	0	0	0	1		
Tail																
Atrophy, ducts	+	0	0	0	0	0	0	0	0	0	0	0	0	1		
Decrease, sperm, ducts	++	0	0	0	0	0	0	0	0	0	0	0	0	1		
Desquamated cell debris, ducts	+++	0	0	0	0	0	0	0	0	0	0	0	0	1		
Granuloma, spermatic	P	0	0	0	0	0	0	0	0	0	1	0	0			

±: Very slight, +: Slight, ++: Moderate, +++: Severe, P: Present.

^{a)}: Number of rats with lesions. ^{b)}: This animal showed atrophy of seminiferous tubules in the left testis.

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stomach, thinning of the small intestinal wall, hemorrhage of the rectum, and red coloration of the mesenteric lymph nodes were observed. Fourteen days after administration, bilateral testicular enlargement was noted in 2 rats of the 80 mg/kg group. Unilateral atrophy of the testis and epididymis was also noted in 1 rat in this group. Furthermore, thymic atrophy was seen. A node thought to be a spontaneous lesion in the epididymis (spermatic granuloma) was observed in 1 rat in the 40 mg/kg group, autopsied 14 days after administration.

2. Organ weights

Data for testicular and epididymal weights are summarized in Tables 3 and 4.

1) Four-week administration

The mean absolute testicular weight was increased from $3,300 \pm 162$ mg (control group) to $3,558 \pm 863$

mg by the intravenous administration of 10 mg/kg/day for 4 weeks. The mean relative testicular weight was significantly increased from $1,040 \pm 43$ mg (control group) to $1,735 \pm 330$ mg by the intravenous administration of 10 mg/kg/day for 4 weeks. The standard deviation for testicular weight in the administered group was large compared to that for the control group, as indicated by autopsy findings including not only swelling but also atrophy of the testis. The mean absolute epididymal weight was significantly decreased in the administered group.

2) Two-week administration

The mean absolute and relative testicular weights were significantly increased from $3,201 \pm 189$ mg and $1,012 \pm 83$ mg (control group) to $3,706 \pm 257$ mg and $1,507 \pm 135$ mg by the intravenous administration of 10 mg/kg/day for 2 weeks. The mean absolute epididymal

Table 3. Organ weights for male rats intravenously administered compound C for 4 weeks or 2 weeks.

Dose (mg/kg)		4-week study		2-week study		
		0	10	0	10	20 ^{a)}
Number of rats		5	5	5	5	5
Body weight at necropsy (g)		317.9 ± 20.5	$203.3 \pm 14.0^{**}$	317.2 ± 18.4	$246.4 \pm 9.3^{**}$	230.7 ± 24.0
Testes (mg)	Absolute	3300 ± 162	3558 ± 863	3201 ± 189	$3706 \pm 257^{**}$	3039 ± 202
	Relative ^{b)}	1040 ± 43	$1735 \pm 330^{**}$	1012 ± 83	$1507 \pm 135^{**}$	1321 ± 61
Epididymides (mg)	Absolute	735 ± 14	$458 \pm 49^{**}$	771 ± 68	$678 \pm 58^{*}$	549 ± 55
	Relative	232 ± 15	225 ± 15	243 ± 13	$275 \pm 25^{*}$	238 ± 17

Values represent mean $\pm$ S.D.

* : Significantly different from the controls ($p < 0.05$), ** : Significantly different from the controls ($p < 0.01$).

^{a)} : Sacrificed at 1-week. No comparison was conducted.

^{b)} : Absolute/100 g body weight.

Table 4. Organ weights for male rats intravenously administered a single-dose of compound C.

Time of sacrifice		day 7			day 14		
Dose (mg/kg)		0	40	80	0	40	80
Number of rats		5	5	5	5	5	5
Body weight at necropsy (g)		285.8 ± 6.6	268.7 ± 10.7	$219.7 \pm 28.1^{**}$	317.7 ± 13.9	315.7 ± 11.0	$261.9 \pm 25.9^{**}$
Testes (mg)	Absolute	3138 ± 95	3113 ± 128	2982 ± 248	3253 ± 212	3399 ± 187	3580 ± 943
	Relative ^{a)}	1098 ± 44	1159 ± 30	$1368 \pm 138^{**}$	1027 ± 97	1078 ± 80	1365 ± 337
Epididymides (mg)	Absolute	615 ± 74	572 ± 82	526 ± 96	773 ± 70	773 ± 54	703 ± 97
	Relative	215 ± 26	214 ± 39	238 ± 23	244 ± 29	245 ± 21	269 ± 32

Values represent mean $\pm$ S.D.

*: Significantly different from the controls ($p < 0.05$), **: Significantly different from the controls ($p < 0.01$).

^{a)}: Absolute/100 g body weight.

weight was significantly decreased, while the mean relative epididymal weight was increased. However, the degree of change was small. In the 20 mg/kg group, mean absolute testicular and epididymal weights were slightly decreased, although statistical analysis could not be performed for these parameters.

3) Single-dose administration

The mean relative testicular weight was significantly increased in rats in the 80 mg/kg group that were autopsied 7 days after the administration.

3. Histopathological findings

Tables 1 and 2 summarize data for lesions of the testis and epididymis.

1) Four-week administration

In the 10 mg/kg group, testicular lesions were detected in all animals. Bilateral and unilateral dilatation of the seminiferous tubules, consistent with enlargement of the testis on gross observation, was observed in 2 rats each (Photo 1B), as compared with the control group (Photo 1A).

In the seminiferous tubules demonstrating dilatation, degeneration/necrosis of the seminiferous epitheli-

Photo 1A. Normal seminiferous tubules in a control rat. HE stain. $\times 60$.

Photo 1B. Seminiferous tubules in a rat treated with 10 mg/kg/day for 4 weeks, showing dilatation of the tubules and decrease of seminiferous epithelium. HE stain. $\times 60$.

Photo 1C. Seminiferous tubule in a rat treated with 10 mg/kg/day for 4 weeks, showing dilatation of the tubule, degeneration and necrosis of seminiferous epithelium, decrease of seminiferous epithelium, and vacuolation of Sertoli cells. HE stain. $\times 240$.

Photo 1D. Seminiferous tubules in a rat treated with 10 mg/kg/day for 4 weeks, showing atrophy of the tubules, degeneration and necrosis of seminiferous epithelium, decrease of seminiferous epithelium, and multinucleated giant cell formation. HE stain. $\times 60$.

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um, decrease of seminiferous epithelium, and vacuolar degeneration of Sertoli cells were observed. The grade of these changes was slight (Photo 1C). Formation of multinucleated giant cells was only observed in 1 rat.

Bilateral and unilateral atrophy of the seminiferous tubules, consistent with atrophy of the testis on gross observation, was seen in 1 and 2 rats, respectively. In these cases, degeneration/necrosis and decrease of the seminiferous epithelium were more marked than in seminiferous tubules with dilatation. Formation of multinucleated giant cells was often observed (Photo 1D). In testes with dilatation of the seminiferous tubules, changes in the epididymis were less marked than in testes with atrophy of the seminiferous tubules. Atrophy of the efferent ductules and ducts of the epididymis was slight or very slight. Decrease of sperm was noted solely in the head of the epididymis. However, in testes with atrophy of the seminiferous tubules, changes in the epididymis were more marked. In particular, decrease of sperm was marked in the head, and was noted in the tail of the epididymis as well.

2) Two-week administration

In the 10 mg/kg group, testicular lesions with dilatation of the seminiferous tubules were detected in all animals. In 3 rats, dilatation was slight bilaterally. In 2 rats, dilatation was slight on one side and very slight on the other side (Photo 2A). Degeneration/necrosis of the seminiferous epithelium and decrease of the seminiferous epithelium were slight or very slight (Photo

2B). In the 20 mg/kg group, for which autopsies were performed on day seven of administration, bilateral dilatation of the seminiferous tubules was observed in 1 rat, although the degree of dilatation was very slight. In addition, very slight degeneration/necrosis of the seminiferous epithelial cells was noted in all rats. There were no abnormalities in the epididymis.

3) Single-dose administration

Seven days after administration, there were no abnormalities in the 40 mg/kg group. However, in the 80 mg/kg group, bilateral and unilateral dilatation of the seminiferous tubules, with decrease of seminiferous epithelium, was noted in 1 rat each, although these findings were very slight. There were no abnormalities in the epididymis even in the 80 mg/kg group. Fourteen days after administration, bilateral dilatation of the seminiferous tubules, with decrease of seminiferous epithelium, was noted in 2 rats of the 40 mg/kg group, although in each case the severity of these changes was slight or very slight. In the 80 mg/kg group, moderate bilateral dilatation of the seminiferous tubules was detected in 2 rats. Very slight or slight bilateral and unilateral dilatation of the seminiferous tubules was detected in 1 rat each. In addition, unilateral atrophy of the seminiferous tubules, consistent with autopsy findings, was detected in 1 rat. In rats with dilatation of the seminiferous tubules, very slight or slight degeneration/necrosis of the seminiferous epithelium and decrease of seminiferous epithelium were observed (Photo 3A). However, in rats with atrophy of the semi-

Photo 2A. Seminiferous tubules in a rat treated with 10 mg/kg/day for 2 weeks, showing dilatation of the tubules and decrease of seminiferous epithelium. HE stain. $\times 60$.

Photo 2B. Seminiferous tubule in a rat treated with 10 mg/kg/day for 2 weeks, showing dilatation of the tubule, degeneration and necrosis of seminiferous epithelium, and decrease of seminiferous epithelium. HE stain. $\times 240$.

niferous tubules, these lesions were more marked. Formation of multinucleated giant cells and vacuolar degeneration of Sertoli cells were also observed (Photo 3B). In the epididymis, atrophy of the efferent ductules and duct of epididymis, decrease of sperm, and desquamated cell debris were observed in the ducts of the head and tail regions.

DISCUSSION

Compound C is a novel platinum complex, developed as an antitumor agent, which inhibits DNA and RNA synthesis (Endoh *et al.*, 1989). Representative platinum complex agents such as cisplatin and carboplatin are known to damage the seminiferous epithelial cells and Sertoli cells, and thus to exhibit testicular toxicity (Huang *et al.*, 1990; Kopf-Mainer, 1992; Pogach *et al.*, 1989; Seethalakshmi *et al.*, 1992). Compound C has also been demonstrated to have toxic effects on the testis (Mizoguchi *et al.*, 1995). In this study, we used a histopathological approach to evaluate the effect of administration period on the testicular toxicity of this agent in relation to determining the shortest administration period for rodents in repeated-dose toxicity studies before first clinical trials, described in the "Non-clinical Safety Studies for The Conduct of Human Clinical Trials" (ICH-M3 step IV guideline, 1997). Briefly, we examined whether the effects of 4-week administration on male genital organs could be detected after 2 weeks of administration. We also examined whether testicular

toxicity could be detected for single-dose administration.

In the 10 mg/kg group treated for 4 weeks, testicular toxicity was observed, as previously reported (Mizoguchi *et al.*, 1995). Histopathologically, dilatation or atrophy of the seminiferous tubules, which are characteristic testicular toxicities of this compound, were observed with similar incidence. Degeneration/necrosis of the seminiferous epithelium, decrease of seminiferous epithelium, formation of multinucleated giant cells, and vacuolar degeneration of Sertoli cells were observed in the seminiferous tubules. These lesions were more marked in atrophic seminiferous tubules than in dilated tubules. Dilatation of the seminiferous tubules may occur earlier than atrophy following compound C administration, and may be associated with retention of a large volume of seminiferous fluid. It has been reported that cisplatin affects Sertoli cells, decreasing seminiferous fluid potassium levels but increasing sodium levels (Pogach *et al.*, 1989). However, dilatation of the seminiferous tubules following cisplatin administration has not been reported. The pathogenesis of the dilatation of the seminiferous tubules including the relation to vacuolar degeneration of Sertoli cells remains to be clarified.

Concerning shortening of the administration period from 4 weeks to 2 weeks, the first issue considered by this study, testicular lesions were detected by administration of 10 mg/kg with both periods. In all rats, slight dilatation of the seminiferous tubules was

Photo 3A. Seminiferous tubules in a rat 2 weeks after a single dose of 80 mg/kg, showing dilatation of the tubules and decrease of seminiferous epithelium. HE stain. $\times 60$.

Photo 3B. Seminiferous tubules in a rat 2 weeks after a single dose of 80 mg/kg, showing atrophy of the tubules, degeneration and necrosis of seminiferous epithelium, and multinucleated giant cell formation. HE stain. $\times 60$.

observed. Very slight or slight degeneration/necrosis of the seminiferous epithelium and decrease of seminiferous epithelium were also noted. In the 20 mg/kg group, in which autopsy was performed 1 week after administration was started, bilateral dilatation of the seminiferous tubules was observed in 1 rat. In addition, degeneration/necrosis of the seminiferous epithelium was noted in all rats. However, these changes were less marked than that in 4-week or 2-week administration. If we had not obtained information regarding the marked changes observed in the testis after 4-week or 2-week administration, it would have been difficult to detect these lesions.

Following single administration of sublethal doses, lesions were marked, as after 2-week repeated administration, in animals that were autopsied 14 days after administration. However, in animals that were autopsied 7 days after administration, both the incidence and grade of testicular lesions were very low. About 2 weeks are thus required to detect the testicular toxicity of compound C.

In conclusion, the testicular toxicity of compound C, a platinum complex compound, can be accurately detected by 2-week administration, although the progression of lesions differed from that with 4-week administration.

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