EFFECTS OF REPEATED DOSES OF COMPOUND E FOR 4 AND 9 WEEKS ON THE MALE REPRODUCTIVE ORGANS

Fumihiko OKADA, Nobuhiro NIWA, Satoru HOSOKAWA,
Takashi KAWAGUCHI, Yasuyuki OKUDA, Yoshio MATSUBARA,
Kiyomi YAMATSU and Toshiji IGARASHI*

*Department of Drug Safety Research, Eisai Co., Ltd.,
Takehaya, Kawashima-cho, Hashima-gun, Gifu 501-61, Japan*

**Department of R&D Documentation & Information Technology, Eisai Co., Ltd.,
4-6-10 Koishikawa, Bunkyo-ku Tokyo 112-88, Japan*

(Received February 28, 1995 ; Accepted June 30, 1995)

ABSTRACT — Results of two toxicity studies of Compound E, which is an anticancer drug, on male reproductive organs and fertility by oral repeated dosing at dose levels of 12.5, 25 and 50 mg/kg/day for 4 and 9 weeks in rats were compared. After repeated dosing, the male fertility was studied by mating with untreated female animals. At the dose of 50 mg/kg/day in the 4-week study, Compound E significantly decreased testes weight and number of epididymal sperm, caused histopathological changes in the testis and epididymis characterized by decreased germ cells, but did not affect fertility. The dose of 25 mg/kg/day in the 9-week study caused reduction in epididymal weight and number of epididymal sperm and histopathological changes in the testis. The dose of 50 mg/kg/day in the 9-week study was lethal and caused more prominent toxic effects in the reproductive organs and loss of fertility. The present studies suggest that the order of sensitivity of parameters on male reproductive organs is as follows ; histopathological examination=organ weight>number of sperm in epididymis>pregnancy index>copulation index. Further, 4-weeks repeated dosing is of sufficient duration to predict adverse effects of test compounds.

KEY WORDS : Repeated dose, Male fertility, Reproductive toxicity, Rat.

INTRODUCTION

In order to investigate what toxicity parameters in animal studies are most sensitive for detecting adverse effects of test drugs on male reproductive organs and fertility, as well as to examine how long test animals should be treated with repeated dosing, the results of two toxicity studies of a test compound given to rats by oral

repeated dosing for two different periods were compared.

For the present studies, the test compound (Compound E), which is an inhibitor of tubulin-polymerization (anticancer drug) and is still under development and undergoing clinical trials at Eisai R&D, was used as a positive drug because of its similar pharmacological and toxicological actions to those of vincristine. The compound was not positive in bacterial mutation tests but proved positive in chromosomal damage tests. The absolute bioavailability by oral administra-

Correspondence : Fumihiko OKADA at the above address.

tion in rats was more than 60%.

Before mating, sperm examination and histopathological studies, male rats were treated in 2 separate studies with an oral repeated dose for 4 weeks and 9 weeks, respectively. The former study was started in June 1991, and the latter in June 1993 at the Department of Drug Safety Research in Eisai Co., Ltd.

The test dose levels and parameters presented herein are limited to those common to both the 4- and 9-week studies. Other data have been deleted in making the comparisons.

MATERIALS AND METHODS

Test article

Compound E, a novel sulfonamide derivative, is not soluble in water but is readily soluble in dimethylformaldehyde. Test dose levels compared in the present paper were 12.5, 25 and 50 mg/kg/day by gavage. Compound E was suspended in 0.5% methyl cellulose solution in a volume of 0.5 ml/100 g body weight. Control animals were given the same amount of the vehicle solution.

Animals

SPF Sprague Dawley male rats were purchased from Japan SLC (Inasa Branch, Shizuoka). Rats for the 4-week study were 6 weeks old; those for the 9-week study were 3 weeks old. Age matched female rats were also prepared for both studies. In the fertility test, the animals were monitored for signs of ill health during a 2-week quarantine period and a subsequent one-week acclimation period. Two animals per cage (unless otherwise noted) were housed in a room maintained at 22–24°C and a relative humidity of 50–60% with artificial lighting from 7:00 to 19:00 and with 12 air changes per an hour. The animals had free access to a pellet diet for rodents (MF, diet for breeding, Oriental Yeast Co., Ltd., Tokyo) and to water sterilized by UV-radiation and supplied through an automatic watering system.

Study Designs

Fig. 1 shows the study design.

4-Week study: Fifteen male rats per group were randomly allocated using a computer system. After the 4-week treatment period, five rats per group were sacrificed by decapitation/

exsanguination under anesthesia and organ weight determinations, sperm examination and histopathological studies on the reproductive organs were conducted. Testis weights were measured as paired weights, and epididymis weights were of the left organs. The remaining 10 rats were cohabited with non-treated female rats. After an 8-week recovery period, the animals were sacrificed to conduct organ weight, sperm and histopathological examinations.

9-Week study: Twenty-four male rats were randomly allocated using a computer system. After a 9-week treatment period, all male rats were cohabited with untreated female rats. The animals were sacrificed to conduct organ weight, sperm and histopathological examinations after the 9-week treatment period and a 2-week mating period. Testis and epididymis weights were both measured as paired weights.

Mating

After repeated dosing for 4 or 9 weeks, 10 of 15 male rats per group in the 4-week study and all 24 male rats per group in the 9-week study were cohabited with untreated female rats of 11 weeks old. In the 4-week study, each male animal cohabited with two female rats for up to 7 days, and in the 9-week study, each male animal cohabited with one female rat for up to 14 days. The male rats were treated with a daily dose of Compound E throughout the mating period. Copulation was confirmed by the presence of a vaginal plug and/or sperm in the vagina in both studies. The day of confirmation of the copulation was counted as Day 0 of gestation.

Sperm examination

Number of sperm and abnormality in shape were examined according to the method described by Wyrobek and Bruce (1975) and a modified method (Fukazawa and Kotosai, 1987). In brief, the right epididymis was weighed, and a sperm suspension was prepared by mincing the tissue in saline solution using a pair of scissors. The suspension was made up to 5 ml with saline, and filtered through tetron-mesh T150 (pore size 114 micrometer) to remove tissue fragments. Then 0.1 ml of fluid was gain diluted with 5 ml saline and the number of sperm measured using a hemacytometer. A portion of the sperm suspension was mixed with 1% Eosin Y (H₂O) and a smear allowed to dry in air on a slide glass. For

Fig. 1. Study design :

- 1) 4-week study : Fifteen male rats per dose were treated with 12.5, 25 or 50 mg/kg/day for 4 weeks. At the end of treatment, five were sacrificed to conduct organ weight, sperm and histopathological examinations. The remaining 10 rats were cohabited with non-treated female rats at the age of 11 weeks, and the animals were then sacrificed to conduct organ weight, sperm and histopathological examinations at the end of an 8-week recovery period.
- 2) 9-week study : Twenty-four male rats per dose were treated with 12.5, 25 or 50 mg/kg/day for 9 weeks. After the treatment period, all rats were cohabited with untreated female rats aged 11 weeks, and the animals were then sacrificed to conduct organ weight, sperm and histopathological examinations.
- 3) Cesarean section : Female rats, which were not treated with Compound E but mated with the treated males, were subjected to cesarean sections on Day 20 of gestation to count the numbers of corpora lutea, implantations, resorptions and dead and live fetuses.

each slide, 200 sperm were examined at 1000-fold magnification for abnormalities in shape.

Histopathology

The testes and epididymides were fixed in Bouin's solution, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin or by the periodic acid-Schiff's reaction. Histopathological examination was carried out considering testicular characteristics and the spermatogenetic stages. In addition, to confirm the presence or absence of testicular changes, quantitative analysis of stage VII seminiferous tubules was made for dose levels of 25 mg/kg/day in the 4-week study and 12.5 mg/kg/day in the 9-week study, which did not cause any overt histopathological changes. Quantitative analysis of seminiferous tubules was carried out on 5 animals (3 tubules per animal) from each group and the results compared with those for controls. For

quantitative analysis, numbers of germ cells in a tubule were counted and expressed relative to Sertoli cells.

Cesarean section

Female rats which were not treated with Compound E but mated with the treated male rats were sacrificed by cervical dislocation at Day 20 of gestation. The numbers of corpora lutea, implantations, resorptions and dead and live fetuses were counted, weighed, and examined for external anomalies.

Statistical analysis

Statistical comparisons were made between the control and respective treated groups. Probability values of 5% or less were deemed significant. Litter data were compared using litter mean values as the sample unit.

Test items such as body weight, food consumption, number of corpora lutea, implanta-

tions, live fetuses, and fetal weight were examined for uniformity of variance among groups by Bartlett's method. When the heterogeneity of variance among groups was not significant, differences in group mean values were tested by Dunnett's *t*-test unless sample sizes were unequal, in which case Scheffé's method was applied. When the heterogeneity of variance among groups was significant, the group mean ranks were compared using Dunnett's test for non-parametric data unless sample sizes were unequal, in which case Scheffé's method was employed. Student's *t*-test was used to assess proportions of each cell type relative to Sertoli cells. Non-parametric data such as incidences of sperm abnormality, copulation and pregnant indices were tested using Fisher's exact test.

RESULTS

Systemic toxicity

There were no findings of systemic toxicity in animals treated with the repeated dose of Compound E at 12.5 and 25 mg/kg/day for 4 weeks. The highest dose of 50 mg/kg/day slightly reduced (statistically not significantly) the weight gain during the 4 weeks of repeated dosing. There were no changes in clinical signs, food consumption during the treatment nor any macroscopic findings at autopsy.

In the 9-week study, the highest dose of 50 mg/kg/day was lethal, but there were no changes in clinical signs or food consumption in the animals treated with repeated doses of Compound E at 12.5 and 25 mg/kg/day for 9 weeks. Findings in animals treated with 50 mg/kg/day included soiled hair, emaciation, hypoactivity and suppression of food consumption and body weight gain. Out of 24 male rats in the group, 9 died and 3 were sacrificed because of their moribund condition during the treatment period (one died during the mating period). The cause of death was not confirmed in the present study. However, a previous repeated dose toxicity study in rats revealed that severe gastrointestinal toxicity and/or secondary infection due to severe depletion of myeloid cells were main causes of death (unpublished data). Histopathological findings of the testes and epididymides in the animals which died or were killed were similar to

those apparent at sacrifice at the end of the 9+2 week treatment period.

Macroscopic findings and weights of male reproductive organs

There were no significant differences in body weights at autopsy in either study. Macroscopic findings were atrophy of testes and epididymis in some treated animals. Testis and epididymis organ weights measured at autopsy are summarized in Table 1.

A significantly reduced testis organ weight was found in animals treated with 50 mg/kg/day at the end of repeated dosing for 4 weeks. Although there were no significant changes in weights of the epididymis (left) at this time point, statistically significant reduction in epididymis weight of animals in the 50 mg/kg/day group was evident at the end of the 8-week recovery period. The reduction in testes and epididymides weights of animals treated with 50 mg/kg/day for 9 weeks was more remarkable. The relative epididymis weight in animals treated with 25 mg/kg/day for 9 weeks was also significantly reduced.

Sperm examination findings

Data on the number of sperm in the freshly removed cauda epididymis and indices of shape abnormalities are summarized in Table 2. There were no significant changes in the numbers of sperm from animals treated with 12.5 and 25 mg/kg/day in the 4- or 9-week studies. The number of sperm in animals treated with 50 mg/kg/day for 4 weeks was significantly decreased to 58% of the control level. Even after the 8-week recovery period, the number of sperm in the 50 mg/kg/day group was only 67% of the controls, although the difference was not significant. The number of sperm from animals treated with 50 mg/kg/day for 9 weeks was extremely low (2% of the controls). There was no obvious difference in the incidence of abnormal sperm among groups.

Histopathological findings

Histopathological findings in the testes and epididymides are summarized in Table 3. No histopathological changes were observed in the prostate, coagulating gland and seminal vesicles in any group.

4-week study: In the testes, atrophy of seminiferous tubules, mainly characterized by decrease of round spermatids (Photo. 1), was recog-

Table 1. Weights of male reproductive organs (testis and epididymis).

a) 4-week study (after 4-weeks treatment)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of animals	5	5	5	5
Testes (g)	3.30±0.05	3.19±0.09	3.05±0.18	2.40±0.24 [#]
per B.W. (g%)	0.81±0.00	0.81±0.04	0.74±0.04	0.62±0.06 [#]
Epididymis (g)	0.52±0.03	0.49±0.02	0.57±0.13	0.54±0.04
per B.W. (g%)	0.13±0.00	0.13±0.00	0.14±0.03	0.14±0.00

b) 4-week study (after 8-weeks recovery)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of males	10	10	10	10
Testes (g)	3.41±0.08	3.25±0.13	3.30±0.07	3.07±0.15
per B.W. (g%)	0.69±0.02	0.62±0.02	0.65±0.02	0.60±0.03
Epididymis (g)	0.67±0.02	0.61±0.03	0.62±0.02	0.56±0.02 ^{**}
per B.W. (g%)	0.14±0.00	0.12±0.02 [*]	0.12±0.02	0.11±0.02 ^{**}

c) 9-week study (after 9-weeks treatment)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of males	24	24	24	12
Testes (g)	3.37±0.17	3.36±0.21	3.26±0.31	1.47±0.34 ^{# #}
per B.W. (g%)	0.69±0.04	0.68±0.05	0.65±0.07	0.31±0.07 ^{# #}
Epididymis (g)	1.13±0.07	1.11±0.12	1.06±0.11	0.83±0.11 ^{# #}
per B.W. (g%)	0.23±0.02	0.22±0.03	0.21±0.03 ^{# #}	0.17±0.03 ^{# #}

Data represent mean±S.D.

Significant difference from Controls : * P<0.05, ** P<0.01 by the Dunnett test and [#]P<0.05, ^{# #}P<0.01 by the Dunnett-ranking test.

nized in all animals treated with 50 mg/kg/day. Such decrease of round spermatids was not stage specific, but in stage XIV seminiferous tubules, germ cells in meiotic phase cell division were frequently necrotic (Photo. 2). Although most other types of germ cells were well preserved, some seminiferous tubules showed the disappearance of spermatogonia, spermatocytes and spermatids, which resulted in focal loss of germ cells in single tubules (Photo. 3). In moderately atrophied seminiferous tubules, decrease of many kinds of germ cells was evident. Necrotic spermatogonia and spermatocytes were sometimes recognized in various stages of seminiferous tubules. In addition to the above-described seminiferous changes, reactive Leydig cell hyperplasia was also recognized in some animals. In

the epididymides of these animals, desquamated seminiferous epithelia were observed in the epididymal ducts. No histopathological changes were recognized in the animals treated with 25 mg/kg/day. No differences between numbers of germ cells in stage VII seminiferous tubules were found between control and 25 mg/kg/day group animals (Figs. 2 and 3). Although the above-described testicular and epididymal changes were still detectable after the 8-week recovery period, the grade of testicular changes was appreciably milder, indicating a degree of reversibility.

9-week study: Testicular and epididymal changes were also recognized in the 9-week study. Moreover, these changes were detectable in animals treated with both 25 and 50 mg/kg/day. The testicular changes in 9-week study were

Table 2. Sperm examination: numbers of sperm ($\times 10^6$ /cauda) and abnormalities (ratio of abnormal sperm/200 sperm examined in %).

a) 4-week study (after 4-weeks treatment)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of animals	5	5	5	5
No. of sperm	62 ± 4	63 ± 12	67 ± 13	$36 \pm 19^*$
Abnormalities	0.1	0.0	0.1	0.7

b) 4-week study (after 8-weeks recovery)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of animals	10	10	10	10
No. of sperm	104 ± 13	138 ± 33	134 ± 45	70 ± 37
Abnormalities	0.1	0.0	0.3	0.2

c) 9-week study (after 9-weeks treatment)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of animals	24	24	24	12
No. of sperm	56 ± 33	47 ± 20	44 ± 44	$1 \pm 1^{##}$
Abnormalities	0.1	0.2	0.2	n.d.

Data represent mean $\pm$ S.D.Significant difference from controls : * $P < 0.05$ by the Dunnett test and $^{##}P < 0.01$ by the Dunnett-ranking test.

n.d. : not determined because of markedly decreased numbers of sperm.

Fig. 2. Numbers of cells in seminiferous tubules in rats treated with Compound E at spermatogenic stage VII. Sperma., spermatogonia; Prelep., preleptotene spermatocytes; Pachy., pachytene spermatocytes; Round., round spermatids; Sertoli., Sertoli cells. **Fig. 3.** Cell types and numbers in seminiferous tubules in rats treated with Compound E at spermatogenic stage VII. Sperma., spermatogonia; Prelep., preleptotene spermatocytes; Pachy., pachytene spermatocytes; Round., round spermatids; Sertoli., Sertoli cells.

Table 3. Histopathological findings in male reproductive organs (testis and epididymis).

a) 4-week study (after 4-weeks treatment)

Group	Control			12.5 mg/kg			25 mg/kg			50 mg/kg		
	(-)	(±)	(+)	(++)	(-)	(±)	(+)	(++)	(-)	(±)	(+)	(++)
<i>Testis</i>												
Atrophy of seminiferous tubules	5	0	0	0	5	0	0	0	5	0	0	0
Leydig cell hyperplasia	5	0	0	0	5	0	0	0	5	0	0	0
<i>Epididymis</i>												
Atrophy of epididymis	5	0	0	0	5	0	0	0	5	0	0	0
Decreased number of sperm	5	0	0	0	5	0	0	0	5	0	0	0
Desquamated seminiferous epithelia	5	0	0	0	5	0	0	0	5	0	0	0

b) 4-week study (after 8-weeks recovery)

Group	Control			12.5 mg/kg			25 mg/kg			50 mg/kg		
	(-)	(±)	(+)	(++)	(-)	(±)	(+)	(++)	(-)	(±)	(+)	(++)
<i>Testis</i>												
Atrophy of seminiferous tubules	10	0	0	0	10	0	0	0	10	0	0	0
Leydig cell hyperplasia	10	0	0	0	10	0	0	0	10	0	0	0
<i>Epididymis</i>												
Atrophy of epididymis	10	0	0	0	10	0	0	0	10	0	0	0
Decreased number of sperm	10	0	0	0	10	0	0	0	10	0	0	0
Desquamated seminiferous epithelia	10	0	0	0	10	0	0	0	10	0	0	0

c) 9-week study (after 9-weeks treatment)

Group	Control			12.5 mg/kg			25 mg/kg			50 mg/kg		
	(-)	(±)	(+)	(++)	(-)	(±)	(+)	(++)	(-)	(±)	(+)	(++)
<i>Testis</i>												
Atrophy of seminiferous tubules	24	0	0	0	24	0	0	0	18	5	1	0
Leydig cell hyperplasia	24	0	0	0	24	0	0	0	24	0	0	0
<i>Epididymis</i>												
Decreased number of sperm	24	0	0	0	24	0	0	0	23	0	1	0
Desquamated seminiferous epithelia	24	0	0	0	24	0	0	0	17	6	1	0

Grade of pathological change, (-) : normal, (±) : slight, (+) : moderate and (++) : advanced.
 Data represent number of rats in the category.

Photo. 1. Stage VII seminiferous tubule 4 weeks after treatment with 50 mg/kg/day. Almost all round spermatids have disappeared. The other types of germ cells are intact. HE stain, $\times 220$.

Photo. 2. Stage XIV seminiferous tubule 4 weeks after treatment with 50 mg/kg/day. Necrotic germ cells in meiotic phase (arrows) can be seen. HE stain, $\times 220$.

Photo. 3. Stage IV seminiferous tubule 9 weeks after treatment with 25 mg/kg/day. Focal damage (arrow) and disappearance of spermatogonia and spermatocytes can be seen within a single seminiferous tubule. PAS stain, $\times 220$.

Photo. 4. Seminiferous tubules 9 weeks after treatment with 50 mg/kg/day. Severe depletion of germ cells is evident with only Sertoli cells remaining. Leydig cell hyperplasia can also be seen in the interstitium. HE stain, $\times 110$.

similar to those of the 4-week study, but were more remarkable. Seminiferous tubules were severely depleted of germ cells with accompanying Leydig cell hyperplasia. Some animals showed complete loss of germ cells resulting in "Sertoli-only syndrome" (Photo. 4). No histo-

pathological changes were recognized in the animals treated with 12.5 mg/kg/day. No differences in germ cell counts of stage VII seminiferous tubules were found between control and 12.5 mg/kg/day group animals (Figs. 2 and 3).

Table 4. Copulation ratio (number of animals mated per paired) and fertility (number of pregnant animals per mated). Treated male rats were each paired with two untreated female rats in the 4-week study, while with one untreated female rat in the 9-week study.

a) 4-week study (after 4-weeks treatment)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Copulation ratio : males	9/10	10/10	9/10	8/10
Copulation ratio : females	15/20	20/20	15/20	15/20
Fertility	15/15	20/20	15/15	15/15

b) 9-week study (after 9-weeks treatment)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
Copulation ratio	22/24	23/24	22/24	12/13
Fertility	21/22	20/23	20/22	0/12**

Significant difference from Controls : ** $P < 0.01$ by the Fisher's exact test.

Table 5. Cesarean section data.

a) 4-week study (mated with males treated for 4 weeks)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of corpora lutea	14.8±0.4	14.3±0.3	14.0±0.3	14.4±0.6
No. of implantations	13.8±0.9	13.9±0.3	13.1±0.4	13.2±0.8
Implantation ratio (%)	92.3	97.3	94.0	91.3
Resorption and death (%)	2.2	4.6	3.3	2.9
No. of live fetuses	13.5±0.8	13.2±0.3	12.7±0.4	12.8±0.7
Fetal weight (g)	3.70±0.14	3.51±0.04	3.70±0.11	3.56±0.05
No. of external anomalies	0	0	1 (see@1)	1 (see@2)

b) 9-week study (mated with males treated for 9 weeks)

Group	Control	12.5 mg/kg	25 mg/kg	50 mg/kg
No. of corpora lutea	15.1±1.8	15.1±1.8	14.1±1.8	—\$
No. of implantations	14.5±1.9	14.6±1.9	12.7±3.1	—
Implantation ratio (%)	96.0	96.6	88.9*	—
Resorption and death (%)	4.9	9.7*	7.8	—
No. of live fetuses	13.7±1.9	13.2±2.3	11.9±3.4*	—
Fetal weight (g)	3.45±0.17	3.61±0.16	3.50±0.24	—
No. of external anomalies	0	1 (see@3)	0	—

Data represent mean ± S.D.

Significant difference from Controls : * $P < 0.05$, ** $P < 0.01$ by Wilcoxon-rank sum test.

\$: not examined because there were no pregnant females.

Type of anomaly :

@1 : edema+brachyury

@2 : anal atresia+genital organ hypoplasia

@3 : exencephaly+microphthalmia+protruding tongue

Effects on fertility

Table 4 summarizes the results of mating after 4 weeks and 9 weeks, repeated dosing with Compound E. The copulation index was not affected by the treatment with any test dose, and the fertility index was reduced only by the repeated dosing with 50 mg/kg/day for 9 weeks.

Cesarean section findings

Table 5 summarizes results of the cesarean section examination of pregnant rats. There were no remarkable changes in numbers or incidences of corpora lutea, implantations, resorptions, dead/live fetuses and fetal external anomalies. There was also no change in fetal weights.

Although there was a significant reduction in the implantation index in the group given 25 mg/kg/day and the number of live fetuses in the group given 25 mg/kg/day in the 9-week study, the findings were within the normal ranges of our historical background data. There were no notable fetal external anomalies.

DISCUSSION

Compound E caused histopathological changes in the testis and epididymis or reduction in the epididymis weight, respectively, at a dose of 50 mg/kg/day in the 4-week study or 25 mg/kg/day in the 9-week study. With the other parameters, reproductive toxicity could not be detected at these dose levels. Therefore, the most sensitive parameters were reproductive organ weights and histopathological features. Histopathologically, the most characteristic testicular changes were decrease or disappearance of round spermatids, and subsequent focal or complete loss of germ cells, including spermatogonia and spermatocytes with secondary Leydig cell hyperplasia. Compound E is an inhibitor of microtubule polymerization, and has been revealed to induce mitotic arrest in the cells of many different organs. Such mitotic arrest leads to apoptotic cell death (unpublished data). It is well known that microtubule polymerization inhibitors such as vinca alkaloids and colchicine have testicular toxicity, and cause a sloughing of cells in the seminiferous epithelium in the early stages of spermatogenesis (Söderström and Røyt-tä, 1986). Hodel *et al.* (1984) have demonstrated that vincristine affects meiosis less than

mitosis in the rat testis. Compound E influences mitotic cell division due to its pharmacological effects on microtubules, but inhibitory effects were also exerted on meiotic phase germ cells as evidenced by their frequent necrosis observed in Stage XIV tubules. In the seminiferous tubules only slightly affected by Compound E, spermatogonia and spermatocytes were better preserved than round spermatids. These findings suggest that the meiotic phase is more affected by Compound E than the mitotic phase. It has been reported that the testicular changes induced by vincristine are irreversible (Takahashi *et al.*, 1986). However, in the present study, the testicular lesions induced by Compound E were clearly demonstrated to be reversible to some degree. We therefore speculate that the target cells, i.e. the type of spermatogonia, of these two microtubule polymerization inhibitors might be different.

At a dose of 25 mg/kg/day, the testis weight was unchanged despite the histopathological changes, while the epididymis weight revealed a slight decrease. We consider that this reflects histopathological changes such as decreased sperm in the epididymal ducts rather than a direct action of Compound E on the epididymis.

The epididymal sperm count was decreased only in the 50 mg/kg/day group animals in both the 4- and 9-week studies. No effects on fertility were detected in the 50 mg/kg/day group of the 4-week study although the sperm count decreased to 58% of the control. However, infertility was induced in the 50 mg/kg/day group of the 9-week study when severe depletion (2% of the control) of the epididymal sperm was observed. The copulation index was not affected by Compound E in either study. Although it has been reported that vincristine (Hodel *et al.*, 1984) and vinblastine (Bruce and Heddle, 1979) affect sperm morphology in mice, no increase in the incidence of abnormal sperm was induced by Compound E. Meistrich (1989) reported that infertility occurs when the sperm count falls below 10% of that in normal animals. Therefore, it can be concluded that the infertility of the 50 mg/kg/day group in the 9-week study was due to the markedly lowered epididymal sperm count.

The present studies suggest that the sensitivity of parameters used to detect toxic effects of

this type of drugs on male reproductive organs is in the following order ; histopathological examination=organ weight>number of sperm in epididymis>pregnancy index>copulation index. With regard to the duration of the dosing period, although testicular toxicity was detected in both 4-week and 9-week studies, a higher dose was needed to detect testicular toxicity in the 4-week case. Therefore, there is a possible risk of false negative findings for reproductive toxicity when the high-dose level used to detect reproductive toxic potential in fertility studies is different from that used to detect general toxic potential in 4-week repeated dose toxicity studies. It is concluded that 4-weeks repeated dosing is of sufficient duration to predict the testicular adverse effects of test compounds if appropriate dosage design and adequate morphological evaluation, i.e. testicular fixation and histopathology taking characteristic testicular morphology into consideration, are achieved.

REFERENCES

- Bruce, W. R. and Heddle, J. A. (1979): The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays. *Can. J. Genet. Cytol.*, **21**, 319-334.
- Fukazawa, H. and Kotosai, K. (1987): Investigation of the sperm examination method and the properties of sperm in rats. *J. Tokyo Vet. Anim. Sci.*, **35**, 1-6.
- Hodel, C., Ettlin, R. A. and Zschauer, A. (1984): Morphological changes produced in rats by anti cancer drugs. *Arch. Toxicol. Suppl.*, **7**, 147-150.
- Meistrich, M. L. (1989): Evaluation of reproductive toxicity by testicular sperm head counts. *J. Amer. Coll. Toxicol.*, **8**, 551-567.
- Söderström, K-O. and Røyttä, M. (1986): Short-time effects of taxol on the seminiferous epithelium of the rat. *Cell Tissue Res.*, **245**, 591-598.
- Takahashi, N., Kadota, T., Kawano, S., Ohta, K., Ishikawa, K., Kuroyanagi, K., Hamajima, Y., Ohta, S., Kai, S. and Kohmura, H. (1986): Toxicity studies of VP 16-213 (II) Oral one-month subacute toxicity in rats. *J. Toxicol. Sci.*, **11**, Suppl. I, 89-122.
- Wyrobek, A. J. and Bruce, W. R. (1975): Chemical induction of sperm abnormalities in mice. *Proc. Nat. Acad. Sci. USA*, **72**, 4425-4429.