COLLABORATIVE WORK TO EVALUATE TOXICITY ON MALE REPRODUCTIVE ORGANS BY REPEATED DOSE STUDIES IN RATS  
18) COMPARATIVE 4 AND 2 WEEKS ORAL REPEATED DOSING STUDIES ON MALE REPRODUCTIVE ORGANS IN RATS TREATED WITH 5-FUOROURACIL

Akira INOMATA, Hirotaka MATSUMOTO and Ikuo HIRAI

Department of Preclinical Science, Nippon Roche Research Center,  
200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan

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ABSTRACT — Oral repeated toxicity studies were conducted to compare the effects of 5-fluorouracil (5-FU) administered for 4 or 2 weeks on male reproductive organs of Sprague-Dawley (SD) rats.

In both studies, decrease of feces, abnormal fur and emaciation were observed. On gross autopsy examination, softening/atrophy of the testis as well as atrophy of accessory reproductive organs were noted, and absolute organ weights of male reproductive organs were almost all reduced in both studies. Histopathologically, degeneration of the seminiferous epithelium in the testis and desquamated cell debris in the epididymal ducts were apparent after both time periods. In the 2-week study, furthermore, exfoliation of the seminiferous epithelium, formation of multinucleated giant cells and vacuolation of Sertoli cells in the seminiferous tubules in the testis, and decrease of sperms in the epididymal ducts were observed.

The present results indicated that a 2-week study is sufficient to detect effects on the male reproductive organs of 5-FU treatment. In our study, however, changes in associated parameters after 2-week treatment of 8-week-old rats were greater than those after 4 weeks in 6-week-old rats. Thus, for detection of 5-FU-induced male reproductive toxicity, we can evaluate more accurately by using maturated rats of the same age.

KEY WORDS: 5-Fluorouracil, Sprague-Dawley Rats, 2-Week study, 4-Week study, Male reproductive organs

INTRODUCTION

The present study was conducted as part of a collaborative project of JPMA and NIH to obtain information on the validity and limitations of the 2-week repeated dosing to detect effects on male reproductive organs in rats. In terms of the administration period for assessment prior to entry-into-human trials, this was not sufficiently harmonized in ICH (Ulbrich and Palmer, 1995); Japan recommends 4-week studies instead of the 2-week study recommended by the EU/US, it being considered that those have not been enough data indicating that the shorter period is sufficient to detect possible effects on male reproductive organs (Takayama et al., 1995). As a part of this assessment, we administered 5-fluorouracil (5-FU) for 2 weeks and compared the effects with those observed after 4-week administration.

5-FU continues to be one of the most frequently prescribed cytostatics for the adjuvant treatment of common types of cancer, such as breast and colorectal tumors. Palliative therapy with 5-FU, however, also causes cytotoxic effects on those cells that have high proliferative activity, such as the gastrointestinal and hematopoietic cells. With respect to male reproductive organs in rats, it has been reported that hypospermogenesis was observed in the testes of Wistar rats in a 4-week oral repeated dose study (Miyazaki et al., 1974),

Correspondence : Akira INOMATA
with decrease of organ weights in the seminal vesicles
and prostate, and atrophic change in testes accompa-
nied by diminution of sperm also noted after 5-week
repeated oral treatment of SD rats (Horii et al., 1985).

In the present study, we compared the results of 4-
and 2-week oral repeated dose studies for with a 5-FU
dosage expected to exert toxic effects on the male repro-
ductive organs.

MATERIALS AND METHODS

Animals

A total of 25, 5-week-old male Sprague-Dawley
(SD-slc) rats obtained from Japan SLC Inc. (Shizuoka,
Japan) were used. Twenty and 13 rats were employed
in the 4- and 2-week studies, respectively. After 1 or 3
weeks acclimatization, 10 healthy rats (5 rats/group;
body weights 187 to 214 g for the 4-week study, 291-
334 g for the 2-week study) were selected for each
study. They were housed individually in metal cages in
the animal facility and maintained on a 12L-12D cycle
(light on from 07 : 00 to 19 : 00) under conditions of
constant temperature and humidity (22 ± 2 °C, 55 ±
10%). They were given food (CRF-1: Charles River
Japan, Inc.) and tap water ad libitum. Each animal was
identified with a picric acid label.

Test/control articles and treatment

5-fluorouracil: 5-FU, MW 130.1 (Fig. 1) was pro-
vided by Nippon Roche K.K. It was weighed and dis-
solved in sterile distilled water and test compound and
control (distilled water) solutions were administered
to the rats by daily oral intubation at doses of 0 and 20
mg/kg/day for the 4-week study, and 20 and 30
mg/kg/day for the 2-week study with an administration
volume of 1 ml/100 g body weight. Volumes for
administration were calculated based on the most
recent body weights.

Dose selection rationale

In the 5-week study of rats orally administered 5-
FU (Horii et al., 1985), almost all animals given 40
mg/kg/day died on day 7-19 after the start of the
administration. In the 20 mg/kg/day group, no mortal-
ity was encountered; however, decrease of food con-
sumption, inhibition of body weight gain, and slight
atrophic changes in the testis were observed. Based on
these results, the dose of 20 mg/kg/day was set for both
4- and 2-week studies to induce changes in the male
reproductive organs. The 30 mg/kg/day was also set as
dose expected to induce remarkable changes in the
testis within a shorter period.

Experimental design

Starting age in each experiment: After acclimatization,
6- and 8-week-old rats were supplied for the 4- and 2-
week studies, respectively.

Observation of clinical signs and measurement of body
weight and food consumption: Clinical signs were
observed daily, and body weights and food consump-
tion were measured twice a week (Fig. 2).

1. Gross autopsy and organ weights

After rats were euthanized with CO2 gas, gross
findings were noted, weights of the male reproductive
organs (testis, epididymis, seminal vesicle and prostate)
were measured, and relative organ weights (per 100 g
body weight) were calculated.
Four and 2 weeks comparative studies of 5-FU in rats.

2. Histopathological examination
Testes and epididymides were fixed in Bouin's solution, embedded in paraffin and 2-3 μm transverse sections were prepared according to routine protocols and stained with hematoxylin and eosin (HE).

Statistical analysis
The data for body and organ weights for the treated and control groups were evaluated statistically by the F test before application of the Student's or Aspin-Welch's t-tests (the t-test with F-test for homogeneity of variance, JIS (1965)).

RESULTS
Mortality, clinical signs, body weight, and food consumption
No mortalities were encountered in either study.
In the 4-week study, decrease of feces, abnormal fur and emaciation were observed only in 1/5 animals of the 20 mg/kg group. In the 2-week study, decrease of feces, abnormal fur and loose stool were observed in 1-3/5 animals of the 20 mg/kg group; furthermore, deposits of a blood-like substance in the visible mucosa and soiling of the lower belly were observed in 1-5/5 animals of the 30 mg/kg group. In the 2-week study, these changes were observed dose-dependently at earlier stages and were more remarkable than in the 4-weeks study.

In the 4-week study, the inhibition of body weight gain was observed in the 20 mg/kg group from day 7 (Fig. 3). In the 2-week study, drastic decrease in body weight was observed in the 20 and 30 mg/kg groups dose-dependently from day 3. Food consumption in both studies changed in a pattern similar to the changes in body weight.

Gross autopsy and organ weights
On gross autopsy examination, softening of testis and a decrease in the size of the testes, epididymides, seminal vesicles and prostate were observed in all treated groups in both studies. These changes were more remarkable in the 2-week study than 4-week study.
Most of absolute weights of male reproductive organs were decreased in both studies (Fig. 4), again most remarkably in the 2-week study. Whereas the relative weights of the seminal vesicles and prostate were decreased, the weights of the testes was increased in both studies.

Histopathological examination
Degeneration of the seminiferous epithelium in

Fig. 3. Body weights of rats in 4- and 2-week studies.
Data are expressed as mean values. Underlined numbers on the horizontal axis show days in the 2-week study.
Statistical significance: differences between controls and each treatment group found with p<0.05(*), p<0.01(**).
the testes (especially pachytene spermatocytes) and desquamated cell debris in ducts of the epididymides were observed in the treated groups of both studies. The incidences of these changes in the 2-week study were higher than those in the 4-week study. In addition, exfoliation of the seminiferous epithelium, formation of multinucleated giant cells and vacuolation of Sertoli cells in the seminiferous tubules in the testis, desquamated cell debris and decrease of sperms in the epididymal ducts were observed in 2 animals of the 20 mg/kg group in the 2-weeks study (Table 1, Photos. 1-4).

DISCUSSION

Four and 2-week oral repeated toxicity studies in SD rats were conducted to compare these two periods for ability to detect effects of 5-FU on male reproductive organs.

Changes related to male reproductive organs treated with 5-FU were observed in both studies as follows; Softening of the testis, atrophy of the testis, epididymis, seminal vesicle and prostate, and an almost consistent reduction in male reproductive organ weights on gross autopsy examination. On histopathological examination, degeneration of the seminiferous epithelium (especially pachytene spermatocytes) in the testis and desquamated cell debris in the epididymal ducts were observed in the 4-week study. In the 2-week study, in addition, exfoliation, formation of multinucleated giant cells and vacuolation of Sertoli cells in the seminiferous tubules of the testis, and decrease of sperm in the epididymal ducts were apparent. In a previous study, atrophy and diminution of sperm in the testis and epididymis were observed histologically, the difference from our results perhaps being due to the longer experiment term (Horii et al., 1985).

There are two major pathways by which 5-FU is metabolized in cells. In one pathway, it is converted to 5-FdUMP, which binds to thymidylate synthase and inhibits DNA synthesis (Waxman et al., 1990); in the other, it is converted to 5-FUTP, which depresses RNA synthesis directly by blocking the incorporation of uracil and orotic acid (Carter et al., 1977). Recent experiments have reported that the effects of 5-FU on mRNA might be tightly related to cytotoxicity in vivo (Dolnick and Wu, 1993). Although 5-FU primarily inhibits DNA synthesis, the histopathological changes in the present study could be attributed to effects on RNA synthesis leading to the decreased production of proteins. RNA synthesis increases rapidly and peaks in middle or late pachytene spermatocytes in mice (Monesi, 1965). Furthermore, androgen binding protein (ABP) (Hall, 1990), inhibin and ABP (Griswold, 1988), which are necessary for spermatogenesis, are

Fig. 4. Organ weights of male reproductive organs in 4- and 2-week studies. Data are expressed as mean ± S.D. Statistical significance; differences between controls and each treatment group found with p<0.05(*), p<0.01(**).
Four and 2 weeks comparative studies of 5-FU in rats.

Table 1. Histopathological examination results in 4- and 2-week studies.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Number of animals examined</th>
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<th>2-week study 20</th>
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<td>Formation of multinucleated giant cell in seminiferous tubules</td>
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<td>Decrease of sperms in ducts</td>
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Grade 1: slight, Grade 2: moderate, Grade 3: severe.

Photo 1. Degeneration of seminiferous epithelium of the testis treated with 20 mg/kg of 5-FU in the 4-week study. HE stain. ×70.
Photo 2. Degeneration of seminiferous epithelial cells with multinucleated giant cell formation, exfoliation of seminiferous epithelial cells, vacuolation of Sertoli cells in seminiferous tubules of the testis treated with 20 mg/kg of 5-FU in the 2-week study. HE stain. ×70.

Photo 3. Desquamated cell debris in ducts of the epididymis treated with 20 mg/kg of 5-FU in the 4-week study. HE stain. ×70.
Four and 2 weeks comparative studies of 5-FU in rats.

synthesized and secreted in Sertoli cells.

The present results demonstrated that a 2-week study is sufficient to detect effects of 5-FU treatment on male reproductive organs. In our study, however, changes in the 2-week study starting with 8-week-old rats were greater than those in the 4-week study with 6-week-old rats. This may be related to a difference in susceptibility to 5-FU depending on the starting age. Similar results showing an importance of the starting age for testicular toxicity were reported for experiments using compounds such as 1,3-dinitrobenzene (Brown et al., 1994) and doxorubicin (Bechter et al., 1987): immature rats were tolerant, but they became susceptible as they aged. Thus, to detect 5-FU-induced male reproductive toxicity, we can obtain a more accurate evaluation by using a 2-week study with more mature rats.

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REFERENCES


Photo 4. Desquamated cell debris and decrease of sperms in ducts of the epididymis treated with 20 mg/kg of 5-FU in the 2-week study. HE stain. X70.
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