COLLABORATIVE WORK TO EVALUATE TOXICITY ON MALE REPRODUCTIVE ORGANS BY REPEATED DOSE STUDIES IN RATS 4) FADROZOLE HYDROCHLORIDE: AN ORAL 2/4-WEEK MALE REPRODUCTIVE ORGAN TOXICITY STUDY

Hiroto KAWASHITA, Kazuyuki HIRATSUKA, Junji KURODA, Yoshiiito ASADA, Tadanori SUZUKI, Yukimi MUGURUMA, Satoko TOMIOKA, Mizuno TANI, Masahide KONDO, Hiroshi MINESHIMA and Yusuke NAGAE

Toxicology/Pathology, Tsukuba Research Institute, Novartis Pharma K. K., 8 Okubo, Tsukuba, Ibaraki 300-2611, Japan

(Received July 21, 2000; Accepted July 25, 2000)

ABSTRACT — Doses of 0, 30 and 60 mg/kg/day of fadrozole hydrochloride (Afem®; non-steroidal aromatase inhibitor, antitumor agent) were given perorally by gavage to Hanlbm WIST male rats from 6 or 8 weeks of age for 2 weeks, and from 6 weeks of age for 4 weeks. In all treatment groups, reduced weights of seminal vesicle, prostate and epididymis, and degeneration/necrosis of the pachytene spermatocytes in stages VII or VIII seminiferous tubules, were dose-relatedly observed. Effects could also be assessed quantitatively by staging analysis with the result of a reduction in the numbers of stage VII pachytene spermatocytes at 30 and 60 mg/kg/day. Epididymal sperm examination revealed no treatment-related changes in any groups.

The effects of 4-week treatment on male reproductive organs were similar to those of 2-week treatment at the same dose levels, except for the weights of seminal vesicle and prostate, which were more reduced by 4-week treatment than by 2-week treatment. There was no notable difference in detectability of toxicity in male reproductive organs between 2-week treatment from 6 weeks of age and 2-week treatment from 8 weeks of age.

It was concluded that the changes observed in the rat male reproductive organs following 4 weeks of treatment with fadrozole hydrochloride could be detected also with 2 weeks of treatment.

KEY WORDS: Fadrozole, Aromatase inhibitor, 2-Week treatment, Male reproductive organ toxicity, Rat

INTRODUCTION

Before first administration of drug candidates to human, results of repeated dose toxicity studies must be evaluated. However, the period of repeated dose study was not harmonized in ICH (1997). Japan and EU/US recommend 4 weeks and 2 weeks, respectively. This was because evaluation of the effects on male reproduction was necessary before first clinical trials in Japan and there were not enough data indicating that 2-week studies can reliably detect effects on male reproductive organs. Therefore, the JPMA and NIHS organized a validation study to obtain information on the validity and the limitations of 2-week repeated dose toxicity study to detect effects on male reproductive organs in rats. As a part of this study, we administered fadrozole hydrochloride for 2 weeks and compared the effects with those of 4-week administration.

Fadrozole hydrochloride is a potent, selective and non-steroidal aromatase inhibitor (Dowsett et al., 1990; Steele et al., 1987; Santen et al., 1991), which suppresses the estrogen biosynthesis from androgen and is clinically used for the treatment of breast cancer. The purpose of this study was to investigate whether any
toxic effect on rat male reproductive organs could be detected following 2 weeks of treatment with fadrozole hydrochloride. In this study, doses of 0, 30 and 60 mg/kg/day of the test article were given perorally by gavage to HanIbm:WIST (Wistar) male rats. The animals were allocated to 9 groups, and were treated from 6 or 8 weeks of age for 2 weeks, or from 6 weeks of age for 4 weeks. All animals were sacrificed at the end of the 2- or 4-week treatment period, and changes in reproductive organs were compared among the groups.

MATERIALS AND METHODS

Animals

HanIbm:WIST (Wistar) male rats (4 weeks old) were obtained from RCC Ltd., Fuellinsdorf, Switzerland. The animals were individually housed in suspended stainless steel cages in a room with a temperature of 23±2°C, a relative humidity of 55±15%, a 12-hr lighting cycle (fluorescent lighting on from 7:00 a.m. to 7:00 p.m.) and filtered ventilation of 13 to 20 times per hour. After a 7-day quarantine period, healthy animals were applied for the study. Tap water via an automatic watering system and irradiated certified rodent diet #5002 C33 (PMI Feeds Inc.) were offered ad libitum.

Experimental design

Fig. 1 shows the experimental design of this study. At 6 weeks of age, 54 healthy animals were randomly allocated to 3 control groups and 6 treatment groups (6 animals/group) based on their body weights (136 to 167 g). Fadrozole hydrochloride was orally administered to animals at dose levels of 0, 30 and 60 mg/kg/day. The animals in Groups 1, 2 and 3 were treated for 2 weeks from 6 weeks of age, Groups 4, 5 and 6 for 2 weeks from 8 weeks of age, and Groups 7, 8 and 9 for 4 weeks from 6 weeks of age.

Test article

The chemical structure of fadrozole hydrochloride (Afema®) is shown in fig. 2. It was dissolved in dis-

![Fig. 1. Experimental design.](image1)

![Fig. 2. Chemical structure of fadrozole hydrochloride.](image2)
tilled water to the requisite concentrations for dosing (6 and 12 mg/mL), and orally administered once daily at doses of 30 and 60 mg/kg with a dose volume of 5 mL/kg. The vehicle, distilled water, was administered to the control groups (Groups 1, 4 and 7) in the same manner.

**Dose justification**

Dosage levels in this study were determined on the basis of the results of the preliminary study in which male rats were treated orally with fadrozole hydrochloride at doses of 3, 10 and 30 mg/kg/day for 4 weeks. In testicular histopathology, degeneration/necrosis of pachytene spermatocytes in stages VII or VIII seminiferous tubules was observed at 10 and 30 mg/kg/day. Since this change was very slight at 10 mg/kg/day, 30 mg/kg/day was selected as a low dose which would certainly affect testes after 4 weeks of treatment in this study. Additionally, in order to investigate a possible dose-related change, 60 mg/kg/day was set as a high dose.

**In-life examinations**

All animals were observed for clinical signs twice daily (predosing and approximately 1 hr postdosing), and body weight and food consumption were determined once weekly during the treatment period.

**Gross evaluation**

All animals were sacrificed under deep ether anesthesia by exsanguination on the scheduled termination days (on the 15th or 29th day from the start of treatment). The animals were subjected to a detailed necropsy, and were examined macroscopically for organ changes. Testis (right/left), epididymis (right/left), seminal vesicle and prostate were weighed.

**Sperm examinations**

1. **Sperm head count**

In all animals, the caudal part was cut off from left epididymis at the site of the vas deferens, weighed and kept frozen in a 50 mL tube until analysis of the sperm head count. After thawing the sample at room temperature, 30 mL of distilled water was added to the tube. The sample was homogenized at 25,000 rpm for approximately 1 min using a homogenizer (Polytron homogenizer, PT-10-35, KINEMATICA AG) and ultrasonicated for approximately 2 min using an ultrasonicator (Ultrasonic processor, Model: GE-50T, Biomic). Epididymal sperm heads were counted manually under a microscope using a hemocytometer (Improved Neubauer hematocytometer, Erma Tokyo) at ×400 magnification, and values were calculated as numbers per gram of caudal epididymal tissue.

2. **Sperm motility**

Before necropsy, sperm samples in all animals were taken from the caudal epididymis by cutting just before the vas deferens. One small drop of spermatozoa suspension was transferred to 5 mL of medium (0.5% BSA / 0.42% HEPES / PBS / pH 7.3 ~ 7.4) and incubated at 37°C for approximately 15 min prior to videotaping of sperms under a phase-contrast microscope. The total number of sperm and the number of non-motile sperm were manually counted on the playback, up to a total number of 200, whenever possible.

3. **Sperm morphology**

A drop of sperm-suspended medium prepared for assessment of sperm motility was smeared on a slide glass and dried in air. The sperm smears from all animals were fixed with ethanol for approximately 1 min and stained with 1% Eosin Y / 1% acetic acid for approximately 1 hr. For each slide, 200 sperm were examined for abnormalities in shape under a microscope at ×400 magnification, and the number of morphologically abnormal sperm to the total of 200 examined sperm was recorded.

**Histopathology**

Testes of all animals were fixed in Bouin's solution for 1 day and then in 10% phosphate buffered formalin. These fixed testes were dehydrated through a graded ethanol series, embedded in paraffin, sectioned, stained with hematoxylin/eosin (HE) and with periodic acid-schiff (PAS), and examined histopathologically.

**Staging analysis**

Quantitative evaluation of spermatogenic cells of the seminiferous tubules in the testis was conducted for four spermatogenic stages (stages II-III, IV, VII and X II). One tubule per animal was randomly selected for each spermatogenic stage and the numbers of seminiferous epithelial cells were counted. The cell types distinguished were spermatogonia, preleptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, round spermatids and Sertoli cells. Then, the ratios of spermatogenic cells to Sertoli cells in each stage were calculated.

**Statistical analysis**

The data obtained are expressed as mean±S.D.
Statistical tests were performed with SAS® (Version 6.12). The difference of means between the control and treatment groups was first analyzed for homogeneity of variance by Bartlett’s test (Bartlett, 1937). When the difference between groups was significant, the Kruskal-Wallis test (Kruskal and Wallis, 1952) followed by the Steel’s test (Steel, 1959) was used for heterogeneous data. When there was no difference between groups, the data were assessed using analysis of variance (ANOVA) (Snedecor and Cochran, 1967) followed by the Dunnett’s test (Dunnett, 1955). p-values less than 0.05 were considered to be statistically significant.

**RESULTS**

**In-life examinations**

Neither mortality nor clinical signs were observed in any groups.

Reduced body weight gain was apparent in the 30 and 60 mg/kg/day groups (Table 1), though these changes were not statistically significant except for the body weight gain between days 8 to 15 of treatment in Group 3 (60 mg/kg/day). The extent of the reduced body weight gain in the 30 mg/kg/day groups was similar to that in the 60 mg/kg/day groups.

There were no remarkable changes in food consumption in any group.

**Macroscopic observations**

Seminal vesicle was reduced in size in all treated groups (30 and 60 mg/kg/day). This change was dose-relatedly apparent in most animals in the 60 mg/kg/day groups (Table 2). Decrease in prostate size was seen in all treated groups except for Group 3.

There were no differences in the degrees of these macroscopic findings related to either treatment duration (2 and 4 weeks of treatment) or treatment start age (6 and 8 weeks old).

**Organ weights**

Fig. 3 shows the relative weights of testes, epididymides, seminal vesicles and prostate to body

| Table 1. Body weights, body weight gain and food consumption of rats treated with 0, 30 and 60 mg/kg/day of fadrozole hydrochloride for 2 and 4 weeks. |
|-------------|--------------|-------------|-------------|---------------|-------------|
| Group       | 2-week treatment (from 6 weeks of age) | 2-week treatment (from 8 weeks of age) | 4-week treatment (from 6 weeks of age) |
| Dose [mg/kg/day] | 0 (Control) | 0 (Control) | 0 (Control) |
| Numbers of animals | 6 | 6 | 6 |

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Body weights [g]</th>
<th>Body weight gain [g]</th>
<th>Food consumption [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>168.8 ± 11.9</td>
<td>262.3 ± 17.4</td>
<td>171.7 ± 9.8</td>
</tr>
<tr>
<td>8</td>
<td>207.3 ± 17.2</td>
<td>292.2 ± 21.7</td>
<td>213.5 ± 16.7</td>
</tr>
<tr>
<td>15</td>
<td>249.7 ± 22.0</td>
<td>316.7 ± 24.2</td>
<td>253.3 ± 20.9</td>
</tr>
<tr>
<td>22</td>
<td>287.0 ± 24.5</td>
<td>328.6 ± 24.3</td>
<td>287.0 ± 24.5</td>
</tr>
<tr>
<td>29</td>
<td>321.2 ± 26.6</td>
<td>317.5 ± 8.7</td>
<td>317.5 ± 8.7</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.

*: Significantly different from the control value (p<0.05).
Effects of fadrozole on male reproductive organs.

There were no differences in the relative testes weights between the treated groups (30 and 60 mg /kg/day) and the control groups.

Epididymides weights were dose-relatedly decreased in both the 2-week and 4-week treated groups. There were no differences related to either treatment duration (2 and 4 weeks of treatment) or treatment start age (6 and 8 weeks old).

Seminal vesicles weights were dose-relatedly decreased in both the 2-week and 4-week treated groups. The weight of seminal vesicles in the 4-week treated groups (Groups 8 and 9) was more reduced than in the 2-week treated groups from 8 weeks of age (Groups 5 and 6) at the same dose levels. Among the 2-week treated groups, the seminal vesicle weights in the groups treated from 6 weeks of age (Group 2 and 3) was more reduced than in the groups treated from 8 weeks of age (Groups 5 and 6).

Prostate weights were decreased in both the 2-week and 4-week treated groups. The weights of prostate in the 4-week treated groups (Groups 8 and 9) were more reduced than in the 2-week treated groups (Groups 2, 3, 5 and 6) at the same dose levels. Among

<table>
<thead>
<tr>
<th>Table 2. Macroscopic findings in rats treated with 0, 30 and 60 mg/kg/day of fadrozole hydrochloride for 2 and 4 weeks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Dose [mg/kg/day]</td>
</tr>
<tr>
<td>Numbers of animals</td>
</tr>
</tbody>
</table>

| Seminal vesicle | Small | 0 | 4 | 6 | 0 | 1 | 5 | 0 | 3 | 5 |
| Prostate | Small | 0 | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 3 |

Fig. 3. Relative organ weights of rats treated with 0, 30 and 60 mg/kg/day of fadrozole hydrochloride for 2 and 4 weeks (testes, epididymides, seminal vesicles and prostate). Values are mean ± S.D.
1−3, 4−6 and 7−9 are 2-week treated groups from 6 weeks of age, 2-week treated groups from 8 weeks of age and 4-week treated groups, respectively.
*, ** and #: Significantly different from the control value (p<0.05, p<0.01 and p<0.001 respectively).
the 2-week treated groups, the prostate weights in the groups treated from 8 weeks of age (Groups 5 and 6) were more reduced than in the groups treated from 6 weeks of age (Groups 2 and 3).

Among the reproductive organs, seminal vesicle was the most affected. The order of organ weight susceptibility to treatment was: seminal vesicle > prostate

> epididymis > testis.

Sperm examinations

Fig. 4 shows the results of sperm examinations.

1. Sperm head count

The sperm head counts of the treated groups (30 and 60 mg/kg/day) were comparable to those of their corresponding controls. The sperm head counts of the 2-week treated groups from 6 weeks of age including the control group (Groups 1-3) were smaller than those of the other groups (Groups 4-9). These lower sperm head counts would be related to the 2 weeks younger age of Groups 1-3 at sperm examination.

2. Sperm motility

The sperm motilities of the treated groups were comparable to those of their corresponding controls.

3. Sperm morphology

The rates for morphologically normal sperm of the treated groups were comparable to those of their corresponding controls.

Histopathology

Table 3 summarizes the results of testicular histopathology.

Degeneration/necrosis of pachytene spermatocytes in seminiferous tubules at stages VII or VIII of spermatogenesis was observed at the single-cell level in all treated groups (30 and 60 mg/kg/day) (Photo 1). The affected cells were spherically shrunk with pyknosis. This change was dose-relatedly observed as follows: 0/6 (0 mg/kg), 3/6 (30 mg/kg) and 5/6 (60 mg/kg) animals in the 2-week treated groups from 6 weeks of age; 0/6 (0 mg/kg), 3/6 (30 mg/kg) and 5/6 (60 mg/kg) animals in the 2-week treated groups from 6 weeks of age; and 0/6 (0 mg/kg), 2/6 (30 mg/kg) and 4/6 (60 mg/kg) animals in the 4-week treated groups. However, since the numbers of the affected pachytene spermatocytes were few, this change could be considered slight. The rates were almost the same at the same dose levels in both the 2-week and 4-week treated groups.

Atrophy of seminiferous tubulus was observed in Groups 6, 7, 8 and 9. There were no spermatids and spermatocytes in these atrophic tubules, which were lined mainly with Sertoli cells. Since this change was not bilateral, occurred infrequently and also observed in control animals, it was not considered to be treatment-related.
Effects of fadrozole on male reproductive organs.

Table 3. Testicular histopathology in rats treated with 0, 30 and 60 mg/kg/day of fadrozole hydrochloride for 2 and 4 weeks.

<table>
<thead>
<tr>
<th></th>
<th>2-week treatment (from 6 weeks of age)</th>
<th>2-week treatment (from 8 weeks of age)</th>
<th>4-week treatment (from 6 weeks of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Dose [mg/kg/day]</td>
<td>0</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Numbers of animals</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Degeneration/necrosis of pachytene spermatocytes at stage VII or VIII</td>
<td>0 (±: 2, +: 1) (±: 3, +: 2)</td>
<td>0 (±: 3) (±: 5)</td>
<td>0 (±: 2) (±: 3, +: 1)</td>
</tr>
<tr>
<td>Atrophy of seminiferous tubules</td>
<td>0 (±: 1)</td>
<td>0 (±: 1)</td>
<td>1 (±: 1)</td>
</tr>
</tbody>
</table>

Grading of degeneration/necrosis of pachytene spermatocytes at stages VII or VIII.
±: very slight (affected pachytene spermatocytes were observed in less than 20% of the stages VII or VIII tubules).
+: slight (affected pachytene spermatocytes were observed in 20% to 50% of the stages VII or VIII tubules).

Grading of atrophy of seminiferous tubules
±: very slight (a single tubule was affected in one cross section of testis).
+: slight (several tubules were affected in one cross section of testis).

Photo 1. Seminiferous tubule at stage VII in a rat treated with 30 mg/kg/day of fadrozole hydrochloride for 2 weeks. Degeneration/necrosis of pachytene spermatocytes (arrows). HE stain. ×400.
Staging analysis

Fig. 5 shows the results of staging analysis. Effects on spermatogenic cells were observed in pachytene spermatocytes at stages VII and XII.

When compared to the controls, the numbers of stage VII pachytene spermatocytes in the 30 and 60 mg/kg/day groups were reduced. The reduction in the number, which appeared to be dose-related, was statistically significant in Group 6 (60 mg/kg/day for 2 weeks). There were no differences related to either treatment duration (2 and 4 weeks of treatment) or treatment start age (6 and 8 weeks old), similar changes being seen at the same dose levels.

A reduction in the numbers of pachytene spermatocytes in stage XII was observed in the 4-week treated groups at 30 and 60 mg/kg/day, though they were not statistically significant. As this change was not observed in the 2-week treated groups in stage XII, it could be considered that the effect on the pachytene spermatocyte in stage VII carried over to stage XII due to the 2-week longer treatment duration and was expressed as reduced numbers of the stage XII pachytene spermatocyte only in the 4-week treated groups.

DISCUSSION

Based on the results of this study, toxic effects on rat male reproductive organs could be detected following both 4-week and 2-week oral treatment with fadrozole hydrochloride at doses of 30 and 60 mg/kg/day. These effects, which consisted of reduced weights of seminal vesicle, prostate and epididymis, and degeneration/necrosis of pachytene spermatocytes in stages VII or VIII seminiferous tubules, were dose-related. Regarding treatment duration, these effects of 4-week treatment on male reproductive organs were similar to those of 2-week treatment at the same dose levels, except for the weights of seminal vesicle and prostate, which were more reduced by 4-week treatment than by 2-week treatment. Concerning the age at which treatment started, there was no notable difference in detectability of toxicity in male reproductive organs between 2-week treatment from 6 weeks of age and 2-week treatment from 8 weeks of age.

Fadrozole hydrochloride is a potent, selective and non-steroidal aromatase inhibitor (Dowsett et al., 1990; Steele et al., 1987; Santen et al., 1991) which suppresses the biosynthesis of estrogen from androgen. In males, estrogen is present in low concentration in the blood, and a suppression of plasma estradiol has been seen in male rats administered with fadrozole hydrochloride for 14 days (Bhatnagar et al., 1992).

In the developing and mature rodent, estrogen receptors have been known to be expressed in testis, epididymis, seminal vesicle and prostate (Cooke et al., 1991; Danzo et al., 1983; Greco et al., 1993; Hess et al., 1997; Schleicher et al., 1984; Stumpf and Sar, 1976; Tilley et al., 1989; West and Brenner, 1990). It becomes more evident that estrogen would have profound effects on differentiation, growth and maintenance of male reproductive organs. As evidence of this, estrogen has been known to have synergistic effect with androgen on the growth of male accessory reproductive organs. Estrogen enhances the proliferating effects of androgen observed as an increase in the weights of prostate and seminal vesicle in castrated rats (Grayhack, 1965; Jackson et al., 1977; Yamanaka et al., 1977) and young castrated dogs (DeKlerk et al., 1979; Isaacs and Coffey, 1981). Benign hyperplasia of canine prostate evoked by androgen is accelerated by the addition of estrogen (DeKlerk et al., 1979; Isaacs and Coffey, 1981; Merk et al., 1986). Taking these facts into consideration, the decrease in weights of epididymis, prostate and seminal vesicles observed in this study, was considered to be due to organ growth suppression caused by lower estrogen level as a result of the pharmacological potential of fadrozole hydrochloride.

In the testis, degeneration/necrosis of stages VII or VIII pachytene spermatocytes was observed at doses of 30 and 60 mg/kg/day although there were no changes in either macroscopic examination or organ weight measurement. This effect was also expressed quantitatively by staging analysis as a reduction in the numbers of stage VII pachytene spermatocytes.

Degeneration/necrosis of stages VII or VIII pachytene spermatocytes was seen at the single-cell level and characterized by spherical shrinkage with pyknosis which is a morphological feature of apoptosis. An increase in apoptosis in spermatogenic cells has been reported in aromatase gene knockout mice (Robertson et al., 1999). Apoptosis occurs in cells subjected to growth factor or hormone deficiency (Kyprianou and Isaacs, 1988; Tapanainen et al., 1993). Developing germ cells are controlled by nutritional and hormonal factors from Leydig cells and Sertoli cells. Fadrozole hydrochloride suppresses the biosynthesis of estrogen from androgen. Hence, the alteration seen in pachytene spermatocytes following treatment with fadrozole hydrochloride is probably apoptosis, although investigation of DNA ladder patterns by DNA gel electrophoresis, light-microscopic features by
Fig. 5. Staging analysis of rats treated with 0, 30 and 60 mg/kg/day of fadrozole hydrochloride for 2 and 4 weeks (number of spermatogenic cells per Sertoli cell per seminiferous tubule). Values are mean ± S.D. 1 - 3, 4 - 6 and 7 - 9 are 2-week treated groups from 6 weeks of age, 2-week treated groups from 8 weeks of age and 4-week treated groups, respectively. *: Significantly different from the control value (p<0.05).
TUNEL staining and electron-microscopic observation are needed to confirm apoptosis.

Recently, a decrease in number of germ cells has been reported in aromatase gene knockout mice (Robertson et al., 1999). Furthermore, disruption of spermatogenesis, degeneration of seminiferous tubules and reduced numbers of sperm are observed in estrogen receptor α gene knockout mice (Eddy et al., 1996). These facts suggest that estrogen play an important role in spermatogenesis.

Aromatase and estrogen receptor are known to be present in germ cells including pachyteme spermatocytes as well as Leydig cells and Sertoli cells. It is reported that the mRNA for cytochrome P450 aromatase (P450arom) is synthesized in testicular germ cells in adult mice (Nitta et al., 1993) and rats (Carreau and Levallet, 1997; Carreau et al., 1998; Janulis et al., 1996). Immunoreactive aromatase using an anti-P450arom antibody and biologically active P450arom revealed by the tritiated water assay, are detected in pachyteme spermatocytes, round spermatids and elongating spermatids in adult rats (Carreau and Levallet, 1997; Carreau et al., 1998; Levallet et al., 1998; Janulis et al., 1998). Estrogen receptor-beta (ER beta) is also reported to be expressed in adult rat spermatogenic cells by immunohistochemistry and in situ hybridization (Enmark et al., 1997; Saunders et al., 1997 and 1998; van Pelt et al., 1999). ER β mRNA and ER beta protein have been found in stages VII-XIV pachyteme spermatocytes, stages I-VIII round spermatids and all type A spermatagonia. (van Pelt et al., 1999).

The evidence suggest that estrogen is synthesized in germ cells as well as Leydig cells and Sertoli cells, and that estrogen is essential for spermatogenesis and may directly act on germ cells in a paracrine and/or autocrine fashions. Therefore, the degeneration/necrosis observed in pachyteme spermatocytes in this study might be attributable to suppression of estrogenic paracrine and/or autocrine functions essential for spermatogenesis. However, an indirect action of fadrozole hydrochloride on the endocrine system via the pituitary gland is also conceivable. Further studies are needed to elucidate the mechanism of action.

In conclusion, the changes observed in the rat male reproductive organs following 4 weeks of treatment with fadrozole hydrochloride could be detected also with 2 weeks of treatment.

ACKNOWLEDGMENT

The authors would like to express special thanks to Dr. Kunitoshi Mitsumori (Division of Pathology, National Institute of Health Sciences), Dr. Kazuo Yasuhara (Division of Pathology, National Institute of Health Sciences) and Dr. Michihito Takahashi (Pharmaceutical Sciences, Showa University) for their kind advice on histopathological evaluation. The authors are also grateful to the chairman of this collaborative work, Dr. Yasuo Ohno (Division of Pharmacology, National Institute of Health Sciences) for his guidance.

REFERENCES

Effects of fadrozole on male reproductive organs.


