Collaborative work on evaluation of ovarian toxicity
3) Effects of 2- or 4-week repeated-dose toxicity and fertility studies with tamoxifen in female rats

Shigeharu Tsujioka1, Yoshiki Ban1, L. David Wise2, Takayuki Tsuchiya1, Takahiro Sato1, Kenta Matsue1, Takanori Ikeda1, Minoru Sasaki1 and Masaru Nishikibe1

1Tsukuba Safety Assessment Laboratories, Banyu Pharmaceutical Co., Ltd. 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan
2Merck Research Laboratories, Safety Assessment, West Point, PA 19486, USA

ABSTRACT — To assess whether ovarian histopathological examination in repeated-dose rodent toxicity study could reliably anticipate toxic effects on female reproductive function and to assess whether ovarian change could be detected in a 2-week repeated-dose toxicity study, tamoxifen was administrated orally to female rats at 0.005, 0.03, or 0.2 mg/kg/day for 2 and 4 weeks in the repeated-dose toxicity studies, and for 2 weeks prior to cohabitation, during cohabitation, and through Gestation Day 7 in a female fertility study. The relationship between ovarian histopathological findings and fertility results was investigated. Findings at 0.03 and 0.2 mg/kg/day included decreases in body weight gains associated with decreases in food consumption, in 2- and 4-week repeated-dose toxicity studies and fertility study. The ovarian histopathological findings included increases in large atretic follicles, increases in interstitium cells and absence of newly-formed corpus lutea at 0.2 mg/kg/day in the 2-week study and at 0.03 and 0.2 mg/kg/day in the 4-week study. The treatment induced estrogenic and antiestrogenic reactions in the uterus, while mucinous degeneration was detected in the vagina. Effects on female fertility consisted primarily of disturbance of estrus cycle and decreases in numbers of pregnant rats which were considered to be related to ovarian histopathological changes. Based on these findings, ovarian histopathological evaluation in the repeated-dose toxicity study could anticipate the effects of tamoxifen on female fertility via ovarian dysfunction at slightly toxic doses, and 2-week treatment of tamoxifen at appropriate dose could be sufficient to detect ovarian toxicity by microscopic examination.

Key words: Female fertility, Ovarian toxicity, Rat, Tamoxifen

INTRODUCTION

As a part of the collaborative study on toxicity related to female fertility, we evaluated the effect of tamoxifen on female reproductive organs in rats when conducting 2- and 4-week repeated-dose toxicity studies and female fertility study with the same dosage levels.

Tamoxifen is one of selective estrogen receptor modulators clinically used for treatment of estrogen receptor-positive breast cancer (Brown et al., 1999; Kennel et al., 2003), and is known to have varied biological effects ranging from complete estrogen antagonism to pure estrogen agonism depending upon its concentration, the specific tissues and the target tissue (Kennel et al., 2003; Long et al., 2001). In human and rats, tamoxifen is predominantly antiestrogenic with residual estrogenic activities (Furr and Jordan, 1984). It has been reported that tamoxifen induces histomorphologic increased number of the cystic follicle and decrease in the corpus lutea in the ovaries of rats (Furr and Jordan, 1984; Matsuda et al., 1997; Brown et al., 1999; Cho et al., 2003).

The objectives of the present study were to investigate whether the ovarian histopathological examination in the repeated-dose rodent toxicity studies could reliably anticipate tamoxifen-induced effects on female fertility, and to evaluate whether 2-week repeated dose period is appropriate to detect the ovarian changes induced by tamoxifen.

MATERIALS AND METHODS

Two- and four-week repeated-dose toxicity studies in female rats were conducted at Tsukuba Safety Assess-
The rats were approximately 6 weeks of age at the start of dosing in both 2- and 4-week studies. During a 1-week quarantine/acclimation period, rats were housed in 2 or 3 cage. After this period, they were transversally halved to observe maximum area of the ovary using light microscopy. Sections of tissues collected at necropsy from all rats were embedded in paraffin, sectioned and stained with hematoxylin and eosin, and examined microscopically. A morphological classification of the ovarian follicles was conducted according to the literature (Pedersen and Peters, 1968). In our study, type 1 to 3b, type 4 to 5a, and type 5b to 8 were defined as small follicle, medium follicle and large follicle, respectively. Additionally, immunohistochemistry of proliferating cell nuclear antigen (PCNA) was conducted in the ovaries from all rats with a view to easily detect and identify primordial follicles in ovaries. For immunohistochemical demonstration of PCNA, mouse monoclonal anti-PCNA (clone PC10; DAKO, Tokyo, Japan) at a dilution of 1:800 was used as the primary antibody. The procedure was performed according to the labeled streptavidin-biotin (LSAB) method with DAKO LSAB Kit (DAKO). Staining was developed with diaminobenzidine tetrachloride, and the sections counterstained with hematoxylin. For negative control, 100 mg/l mouse IgG (DAKO) replaced the primary antibody.

**Study design: female fertility study**

Female Sprague-Dawley Crl:CD(SD) rats were obtained from Charles River Laboratories (Raleigh, NC, USA). The rats were maintained at 22 ± 3°C and were housed in suspended, stainless-steel, wire-bottom cages in environmentally controlled, HEPA-filtered rooms with a 12-hr light/dark cycle. During a 1-week quarantine/acclimation period, rats were housed in 2 or 3 cage. After this period, they were housed individually. All rats had free access to Certified Rodent Diet #5002 (PMI Nutrition International, Inc., St. Louis, MO, USA) and water throughout the study. The rats were approximately 6 weeks of age at the start of dosing in both 2- and 4-week studies.

Groups of 10 female rats each were dosed orally once-daily at 0.005, 0.03, or 0.2 mg/kg/day of tamoxifen, or vehicle only, for either 14 or 28 days, at a dosing volume of 5 ml/kg body weight. Mortality and physical signs prior to and after dosing were observed daily on all rats. Body weight was recorded pretest (prior to the first dose on Study Day 1), once or twice per week on all rats. Food consumption was measured once per week on all rats during the treatment period.

Rats sacrificed at study termination were anesthetized with isoflurane and subsequently euthanized by exsanguination. Food was withdrawn overnight prior to terminal necropsy. Necropsies, including examination of the thoraco-abdominal viscera and collection of the following tissues, were done on all rats; ovaries, uterus, vagina, pituitary, adrenals, kidneys, and liver. The terminal body weights and following organ weights were recorded from all rats euthanized at scheduled necropsies; ovaries, uterus, vagina, pituitary, adrenals, kidneys and liver. The organ weight data were expressed as absolute weight and as percent of body weight. One rat in the control group of the 4-week study died accidently on Day 28, and the organ weight data were not collected from this dead rat. All collected tissues from all rats were fixed in 10% neutral buffered formalin. At dissection, the bilateral ovaries were transversally halved to observe maximum area of the ovary using light microscopy. Sections of tissues collected at necropsy from all rats were embedded in paraffin, sectioned and stained with hematoxylin and eosin, and examined microscopically. A morphological classification of the ovarian follicles was conducted according to the literature (Pedersen and Peters, 1968). In our study, type 1 to 3b, type 4 to 5a, and type 5b to 8 were defined as small follicle, medium follicle and large follicle, respectively. Additionally, immunohistochemistry of proliferating cell nuclear antigen (PCNA) was conducted in the ovaries from all rats with a view to easily detect and identify primordial follicles in ovaries. For immunohistochemical demonstration of PCNA, mouse monoclonal anti-PCNA (clone PC10; DAKO, Tokyo, Japan) at a dilution of 1:800 was used as the primary antibody. The procedure was performed according to the labeled streptavidin-biotin (LSAB) method with DAKO LSAB Kit (DAKO). Staining was developed with diaminobenzidine tetrachloride, and the sections counterstained with hematoxylin. For negative control, 100 mg/l mouse IgG (DAKO) replaced the primary antibody.
9 weeks of age at start of dosing.

Groups of 20 rats each were dosed orally once-daily at 0.005, 0.03, or 0.2 mg/kg/day of tamoxifen, or vehicle only, for 2 weeks before cohabitation, during cohabitation, and through Gestation Day (GD) 7, at a dosing volume of 5 ml/kg body weight. Females were cohabited with untreated breeder male rats of the same strain in a 1:1 ratio for a maximum of 20 nights. Following 12 nights of cohabitation, apparently not bred females (1 female in the control group and 7 females in high-dose group) were rehoused with proven breeder male rats from the study (1:1 ratio). The presence of sperm in the vaginal lavage or a seminal plug in the vagina was considered evidence of a positive mating. The day of confirmed mating was considered GD 0.

All rats were observed daily for mortality and physical signs from initiation of treatment through sacrifice. Body weights were recorded on Premating Day (PMD) 1, 4, 8, 11 and 14, and on GD 0, 2, 4, 6, 8, 12 and 15. If there was no evidence of females having during cohabitation interval, additional weights were on PMD 22, 25, 29, 32, 36, 39, 43, 46 and 49. Food consumption was measured over the following 4-day intervals: PMD 1 to 5 and 8 to 12; GD 1 to 5 and 8 to 12.

On each day prior to and during the cohabitation interval the vaginal cytology of each female was evaluated and a stage of the estrous cycle was assigned (i.e., proestrus, estrus, diestrus or metestrus). Evaluation of estrous cyclicity was discontinued upon evidence of mating.

Rats with a confirmed mating were euthanized by carbon dioxide asphyxiation between GD 15 and GD 17. The uterus of each rat was examined to determine pregnancy status. If there was no gross evidence of pregnancy, uterus was stained with an ammonium sulfide solution to visualize any early implantation sites. Corpus lutea were counted and recorded as the total number per rat. Uterine implants were counted and each was classified as a live fetus, dead fetus or resorption. Fetuses were euthanized by rapid induction of hypothermia. Rats with no evidence of mating were euthanized on Post-cohabitation Day 15. The uterus of each rat was stained with an ammonium sulfide solution to visualize any early implantation sites, and the pregnancy status was recorded.

### Statistical analyses

For body weight gain, food consumption and organ weight parameters, a trend test was conducted between control and each dose groups (Tukey et al., 1985). The trend test was assessed using ordinal dose scale for an increase or decrease in the parameters with increasing dose of the test article. The statistical analysis for food consumption was conducted using mean values of all time points in individual rat.

For time to mating, mean length and number of days with anestrous stage, Dunnett’s multiple comparisons test was conducted between control and each dose groups (Dunnett, 1964). For number of corpus lutea per female, rate of peri-implantation loss, number of implantation per female, post-implantation loss and live fetus per pregnant female, student’s t-test was conducted between control and the 0.005 mg/kg/day group (Yoshimura, 1987). For the mating index, fecundity index, fertility index and number of anestrous females, the chi-square test was conducted between control and each dose groups (Yoshimura, 1987).

The significance level for all statistical tests was set at 0.05.

### RESULTS

#### Two- and four-week repeated-dose toxicity studies

### Mortality, physical sign, body weight and food consumption

There were no test article-related deaths or physical signs during the study. Body weight gain and food consumption data are summarized in Table 1. Test article-related significant (p < 0.05) decreases in mean body weight gains were noted from Study Weeks 1 to 2 at 0.03 and 0.2 mg/kg/day (17% and 35% below controls, respectively) in the 2-week study and from Study Weeks 1 to 4 at 0.03 and 0.2 mg/kg/day (21% and 36% below controls, respectively) in the 4-week study. Mean food consumption was significantly (p < 0.05) decreased throughout the study at 0.2 mg/kg/day in the 4-week studies (7% to 10% below controls).

### Pathology

Organ weight data are summarized in Table 2. In both 2- and 4-week studies, test article-related decreases in mean absolute/relative body weights of ovary and uterus were noted significantly (p < 0.05) at 0.2 mg/kg/day, and test article-related decreases in mean absolute/relative body weights of pituitary were noted significantly (p < 0.05) at 0.03 and 0.2 mg/kg/day.

In both 2- and 4-week studies, decreased sizes of ovaries and uterus were noted in the 0.2 mg/kg/day group at necropsy (ovary: 2/10 and 3/10, respectively; uterus: 3/10 and 3/10, respectively).

Test article-related histopathological findings were summarized in Table 3. Test article-related histopathological findings were noted in the ovary, uterus and vagina.
at 0.2 mg/kg/day in the 2-week study, and at 0.03 and 0.2 mg/kg/day in the 4-week study. Severity and features of these changes were similar between 2- and 4-week studies. In the ovary, an increase in the large atretic follicles was characterized by thin follicular walls filled with a large quantity of follicular fluid and occasionally follicular cyst-like appearance (Figs. 1A and B). An increase in the interstitial cells was characterized by increased number of interstitial cells with scanty and clear cytoplasm (Figs. 2A and B). These changes were accompanied by the absence of atretic follicles.
of newly formed corpus lutea. There were no noteworthy changes in the small and medium follicles from the ovarian sections including PCNA-immunostained sections in any groups. These histopathological ovarian changes were correlated with decreased weight of ovaries and

In the uterus, hypertrophy of the endometrial epithelium and atrophy of the endometrium and smooth muscle layer were noted. Atrophy of the uterus corresponded with a decreased weight of uterus and grossly noted decreased size of ovaries at 0.2 mg/kg/day. In the vagina, mucinous degeneration characterized by vacuolated and uncornified epithelium was noted, and the vaginal change was frequently associated with mucosal thickening of vaginal epithelium. There were no test article-related histopathological changes in the pituitary, adrenals, liver and kidneys.

### Fertility study

#### Mortality, physical sign, body weight and food consumption

There were no test article-related mortalities or physical signs during the study. Body weight and food consumption data are summarized in Table 4. There were test article-related significant (p < 0.05) decreases in mean body weight gains during the premating period at 0.03 and 0.2 mg/kg/day (34% and 89% below control, respectively). Commensurate with these body weight effects, there were test article-related significant (p < 0.05) decreases in mean food consumption on PMD 5 and 12 at 0.2 mg/kg/day (14% and 16% below control, respectively).

#### Estrous cyclicity, mating performance, fertility and litter parameters

Data of female fertility are summarized in Table 5. There were test article-related effects on estrous cycling in all tamoxifen-treated groups. Specifically there were significantly (p < 0.05) increases in the incidence of anestrus females, which was defined as any female with 7 or more consecutive days without an estrus stage. Most of the anestrus rats appeared to have a persistent proestrus stage of the estrous cycle. There were significantly (p < 0.05) decreases in the mean number of days with an estrus stage at 0.03 and 0.2 mg/kg/day. In addition there was a significantly (p < 0.05) increase in mean cycle length at 0.03 mg/kg/day, which was defined as the mean number of days between non-consecutive estrus stages. There was a test article-related decrease in the mating index and an increase in the mean time to mating

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>0.005</th>
<th>0.03</th>
<th>0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-week study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>Grade</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in large atretic follicle</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Absence of newly-formed corpus lutea</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increase in interstitial cell</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophy</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epithelial hypertrophy</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vagina</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous degeneration</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4-week study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of animals</td>
<td>Grade</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in large atretic follicle</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Absence of newly-formed corpus lutea</td>
<td>N/A</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Increase in interstitial cell</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophy</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Epithelial hypertrophy</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vagina</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous degeneration</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

N/A: Not applicable, +: slight
at 0.2 mg/kg/day; the former parameter statistically signif-

icant (p < 0.05) but the latter not statistically significant.

There were test article-related decreases in the fecundi-

ty and fertility indices in all tamoxifen-treated groups,

although the decreases were not statistically significance
at 0.005 mg/kg/day.

There were no pregnant females in the 0.2 mg/kg/
day group, and one pregnant female in the 0.03 mg/kg/
day group had all resorptions. There was a significant
(p < 0.05), test article-related, increase in percent of peri-
implantation loss at 0.005 mg/kg/day, which resulted in
significant (p < 0.05) decreases in mean implants and live
fetuses per pregnant female.

DISCUSSION

It has been widely documented that treatment with
estrogenic compounds affect the estrous cycle, serum hor-
mones concentrations, and ovarian function (Rodriguez et
al., 1993). Tamoxifen is one of selective estrogen recep-

Fig. 1. A: Ovary from control rat, showing normal appearance. HE staining. × 32.
B: Ovary from a rat treated with tamoxifen at 0.2 mg/kg/day for 2 weeks. Increases in large atretic follicle were noted. Newly-formed corpus lutea was not observed in the section. HE staining. × 32.

Fig. 2. A: Ovary from control rat, showing normal appearance. HE staining. × 200.
B: Ovary from a rat treated with tamoxifen at 0.2 mg/kg/day for 2 weeks. Increases in interstitial cells were noted. HE staining. × 200.
Table 4. Body weight gains and food consumption for female fertility study rats treated with tamoxifen

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>0.005</th>
<th>0.03</th>
<th>0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Body weight gains (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premating Day 1 to 14</td>
<td>17.9 ± 8.6</td>
<td>19.3 ± 6.5</td>
<td>11.9 ± 7.1*</td>
<td>2.05 ± 6.12**</td>
</tr>
<tr>
<td>Food consumption (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premating Day 5</td>
<td>20.8 ± 2.9</td>
<td>21.0 ± 1.7</td>
<td>20.2 ± 2.2</td>
<td>17.8 ± 1.8**</td>
</tr>
<tr>
<td>Premating Day 12</td>
<td>20.8 ± 2.8</td>
<td>20.2 ± 2.0</td>
<td>19.4 ± 1.8</td>
<td>17.4 ± 2.1**</td>
</tr>
</tbody>
</table>

Data are mean ± S.D. values.
Significantly different from control, *: p < 0.05; **: p < 0.01

Table 5. Summary of female fertility for rats treated with tamoxifen

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>0.005</th>
<th>0.03</th>
<th>0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals examined</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. of females cohabited</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>No. of mated females</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>No. of pregnant females</td>
<td>19</td>
<td>17</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Time to mating (4-day period ± S.D.)</td>
<td>1.20 ± 0.70</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.67 ± 1.29</td>
</tr>
<tr>
<td>Mating index (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>75*</td>
</tr>
<tr>
<td>Fecundity index (%)</td>
<td>95</td>
<td>85</td>
<td>5**</td>
<td>0**</td>
</tr>
<tr>
<td>Fertility index (%)</td>
<td>95</td>
<td>85</td>
<td>5**</td>
<td>0**</td>
</tr>
<tr>
<td>No. of anestrus females</td>
<td>3</td>
<td>10*</td>
<td>16**</td>
<td>20**</td>
</tr>
<tr>
<td>Mean cycle length</td>
<td>4.0</td>
<td>4.2</td>
<td>5.1**</td>
<td>NE</td>
</tr>
<tr>
<td>Mean days with an estrous stage</td>
<td>3.4</td>
<td>2.7</td>
<td>1.6**</td>
<td>0.8**</td>
</tr>
<tr>
<td>No. of Corpora lutea/pregnant females</td>
<td>15.5 ± 2.3</td>
<td>15.5 ± 1.9</td>
<td>10 NE</td>
<td></td>
</tr>
<tr>
<td>Peri-implantation loss (%)</td>
<td>3.1 ± 5.8</td>
<td>33.7 ± 34.6**</td>
<td>50.0 NE</td>
<td></td>
</tr>
<tr>
<td>No. of Implants/pregnant females</td>
<td>15.0 ± 2.2</td>
<td>10.5 ± 6.0**</td>
<td>5 NE</td>
<td></td>
</tr>
<tr>
<td>Resorptions/implants (%)</td>
<td>6.8 ± 6.6</td>
<td>11.1 ± 23.9</td>
<td>100 NE</td>
<td></td>
</tr>
<tr>
<td>Dead fetuses/implants (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NE</td>
</tr>
<tr>
<td>Post-implantation loss (%)</td>
<td>6.8 ± 6.6</td>
<td>11.1 ± 23.9</td>
<td>100 NE</td>
<td></td>
</tr>
<tr>
<td>Live fetuses/pregnant female</td>
<td>14.0 ± 2.4</td>
<td>9.7 ± 5.5**</td>
<td>0 NE</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± S.D. values.
Significantly different from control, *: p < 0.05; **: p < 0.01. NE: Not examined.
1): Anestrus was defined as 7 or more consecutive days without estrous stage.
2): (No. of mated females/no. of females cohabited) x 100.
3): (No. of pregnant females/no. of mated females) x 100.
4): (No. of pregnant females/no. of females cohabited) x 100.

Tamoxifen as well as other SERMs have been known to act as full estrogen agonists for food intake, body weight and energy balance (Hoyt et al., 1998). Thus, the dose selection of these studies was considered appropriate to evaluate ovarian histopathology and female fertility.

Histopathological examinations of the ovaries in the present study revealed increases in the large atretic follicles, increases in the interstitial cells, and the absence of the newly formed corpus lutea at 0.2 mg/kg/day in the 2-week study, and at 0.03 and 0.2 mg/kg/day in the 4-week study. Decreases in the ovary weights were noted at 0.2 mg/kg/day in both studies. There were no note-
worthy changes in the small and medium follicles in any dose groups. These changes of ovaries observed in the present study were similar to those of female rats following oral administration of tamoxifen for 2 or 4 weeks in the previous reports (Matsuda et al., 1997; Cho et al., 2003; Kennel et al., 2003). The mechanism of tamoxifen-induced ovarian change has been explained as an antago-
nistic effect associated with an inhibition of the positive feedback effect of estrogens on the hypothalamic-pitu-
tary axis (Furr and Jordan, 1984). However, the effects of tamoxifen on the ovary are likely due to estrogen recep-
tor (ER) agonism at hypothalamus-pituitary axis rather than ER antagonism. Pure anti-estrogen has been known to induce a secondary increase in pituitary gonadotrophin secretion, reflected by an increase in ovarian weight and circulating basal estrogen levels (Ferland et al., 1976). The compound known as ZM 182780, which is pure estrogen antagonist without estrogenic activation, has inhibited not only the positive feedback but also negative feedback effects of estrogens on the hypothalamic-pitu-
tary axis, causing block of the preovulatory luteinizing hormone (LH) surge due to a sustained increase in basal LH level (Donath et al., 1998). In contrast, tamoxifen decreased both the basal and preovulatory surge of LH levels suggesting that tamoxifen inhibits only the positive feedback effect of estrogens for the gonadotropin surges, but does not act as antagonist on the negative feedbacks of estrogens (Tucker et al., 1984; Yuan, 1991; Donath et al., 1998). Tamoxifen has been reported to down-regulate production and release of follicle-stimulating hormone (FSH) and LH from the pituitary after preventing release of GnRH by directly acting as agonist to the hypothala-
mus (Yuan, 1991; Donath et al., 1998). These were con-
sidered to result in interruption of follicular development with gonadotrophin-dependency and inhibition of ovula-
tion, which corresponded to microscopic evidence of ovarian malfunction observed in the present study. Inter-
stitial cells are well known to arise from thecal cells of atretic follicles (Davis et al., 1999). The increase in the interstitial cells observed in the present study would result from the increase in the large atretic follicles. Dose-
dependent decreases in the pituitary weights were noted at 0.03 and 0.2 mg/kg/day in both 2- and 4-week studies. The decrease in the pituitary weight seemed to be related to decreases of basal LH and FSH levels although these hormonal levels were not examined in our study. Histol-
ogical uterine change observed in the present study was characterized by atrophy of endometrium and smooth muscle layer and hypertrophy of the endometrial epithe-
lum. Interestingly, the histological uterine changes had concomitant estrogenic and antiestrogenic activi-
ty, although tamoxifen showed an antiestrogenic effect on uterine growth overall since there was decrease in uterus weight. These compartmentalized estrogenic and antiestro-
genic effects of tamoxifen in the rat uterus have been previously reported (Nephew et al., 2000; Kennel et al., 2003). Mucinous degeneration of the vagina was noted in rats at 0.03 and 0.2 mg/kg/day. This finding may sug-
gest that tamoxifen administration results in reproductive cycle arrest in the proestrus stage in rats at 0.03 and 0.2 mg/kg/day.

In the female fertility parameters, there were dose-
dependent effects on estrous cyclicity and fertility in all tamoxifen-treated groups. Most females at 0.03 and 0.2 mg/kg/day showed anestrus and did not become a preg-
nancy. Anestrus and effects on female fertility at 0.03 and 0.2 mg/kg/day might be related to lack of pre-ovulatory LH and FSH surges associated with an inhibition of the positive feedback effect of estrogens on the hypotha-
lamic-pituitary-gonadal axes. The irregular estrous cycle characterized by prolonged proestrus periods at 0.005 mg/kg/day were most likely associated with an impair-
ment or delay in ovulation. These effects of tamoxifen on estrous cyclicity were similar to previous reports for tamoxifen as well as other SERMs (Harper and Walpole, 1967; Hoyt et al., 1998; Treinen et al., 1998). An increase in peri-implantation loss and a decrease in fertility index were noted at 0.005 mg/kg/day. Female rats treated with tamoxifen on GD 1 to 3 demonstrated that embryonic loss with/without preventing of pregnancy and absorp-
tion of embryos (Harper and Walpole, 1967; Dao et al., 1996; Kaplan-Kraicer et al., 1996). The mechanism of tamoxifen-induced peri-implantation loss in rats has been explained as an acceleration of embryo transport through oviduct induced by agonistic effect of tamoxifen, result-
ing in the arrival of premature embryos into the uterus that reduces significantly implantation in rate due to failure of the synchronization between the development stage of the embryo and uterine receptivity (Itskovitz and Hodgen, 1988; Kaplan-Kraicer et al., 1996). Thus, the decrease in fertility at 0.005 mg/kg/day was considered to be related directly to an estrogenic effect of tamoxifen on the oviduct for early pregnancy period rather than changes via ovarian dysfunction.

The effects of tamoxifen on female fertility consisted primarily of disturbance of estrus cycle, decreases in number of pregnant rats, and increased peri-implantation loss. The effect on peri-implantation was considered to be related directly to an estrogenic effect on the oviduct in the early pregnancy period rather than changes via ovar-
ian dysfunction. The effects on estrous cyclicity and preg-
nancy were considered to result from hormonal distur-
Ovarian toxicity of tamoxifen in rats

bance associated with inhibition of the positive feedback effect of estrogens on the hypothalamic-pituitary axes via ovarian dysfunction corresponding to ovarian histopathological changes in the 4-week study. The ovarian histopathological evaluation in 4-week repeated-dose toxicity study could anticipate the effect on female fertility via ovarian dysfunction when the dosage levels of these studies included general toxic effects. The histopathological changes in the ovary induced by tamoxifen were noted in both 2- and 4-week studies at doses where slight general toxicity induced, and the ovarian changes in the 2-week study had similar features and severity to those in the 4-week study. Therefore, 2-week treatment of tamoxifen at appropriate dose could be sufficient to detect ovarian toxicity by microscopic examination.

REFERENCES


