Proliferative lesions of oviduct and uterus in CD-1 mice exposed prenatally to tamoxifen

BHALCHANDRA A. DIWAN, LUCY M. ANDERSON AND JERROLD M. WARD

Tamoxifen (TAM) is widely used as adjuvant breast cancer therapy after surgery and as a chemopreventive agent in women of child-bearing age. However, TAM therapy has been shown to result in an increased incidence of endometrial carcinoma in women. The present study was designed to investigate the effects of TAM (5 mg/kg and 7.5 mg/kg body wt) given i.g. to pregnant CD-1 mice (1x/day, days 12 through 18 of gestation) on their female offspring. Progressive proliferative hyperplasia of the oviduct was frequently seen in TAM-exposed offspring, reaching 100% incidence by 52 weeks in both treatment groups. These females also developed progressive proliferative uterine lesions, including moderate/severe cystic endometrial hyperplasia (34–50%) and polypoid adenomas (27–30%) between 53 and 78 weeks. Deciduomas (15%) occurred at young ages (12 and 24 weeks) while leiomyomas (14%), a malignant leiomyosarcoma, and ovarian granulosa cell tumors (14%), were found between 72 and 78 weeks. Our findings thus suggest a strong association between transplacental TAM and reproductive tract abnormalities in female CD-1 mice.

Tamoxifen (trans-[Z]-1-(4-[2-(dimethylamino)ethoxy]phenyl-1,2-diphenyl-1-butene) (TAM*) is widely used as adjuvant breast cancer therapy after surgery. This drug is effective in the treatment of primary breast cancer, in prevention of contralateral breast cancer and in healthy women who are at greater risk of developing the disease because of their family history (1–3). However, recent evidence suggests that TAM is a potent hepatocarcinogen in rats (reviewed in 4) and there may result in reproductive system abnormalities and neoplasia. In mice, which confirms that TAM exposure of female fetuses incision of vaginal adenocarcinoma (12). In view of these facts, and the long half-life of TAM (13,14), concern is raised about its potential as a transplacental carcinogen. We report here the results of a transplacental tumorigenesis trial of TAM in mice, which confirms that TAM exposure of female fetuses may result in reproductive system abnormalities and neoplasia.

Male and female CD-1 mice, 6–8 weeks old, were obtained from the Charles River Breeding Laboratory (Kingston, NY). Animal care was provided in accordance with the procedure outlined in the ‘Guide for Care and Use of Laboratory Animals’ (NIH Publication 86–23). Five females were placed overnight in one cage together with a male CD-1 mouse. Females were examined the next morning for a vaginal plug (day 1 of gestation) and pregnancy was confirmed by palpation 10 days later.

TAM (Sigma Chemical Co., St Louis, MO) was dissolved in tricaprylin (Sigma Chemical Co.) just before use. In a preliminary toxicity study, three pregnant mice received either 10 mg, 7.5 mg or 5 mg/kg body wt of TAM i.g. daily on days 12 through 18 of gestation. The highest dose was fetotoxic resulting in death of all fetuses either before birth (still born) or immediately after birth. The remaining two doses were well tolerated and used for long-term study. Equal numbers of pregnant females (10/group) received i.g. administration of either 5 mg/kg (group 1), 7.5 mg/kg (group 2) or an equal volume of tricaprylin (group 3). Pregnant mice were allowed to deliver naturally and to nurse their own offspring which were weaned and separated by sex at 3 weeks of age. Here, we report our observations for female offspring.

Ten mice per group were killed at 12 and 24 weeks of age, 15 per group were killed at 52 weeks of age, and the remainder were killed when moribund or at 78 weeks of age when the experiment was terminated. Complete necropsies were performed. Uterine masses were measured and their number and locations were recorded. Ovaries were weighed together with uterine horns. All gross lesions and representative samples from each major organ were embedded in paraffin, sectioned at 6 µm and stained with H&E for histological evaluation.

For statistical analysis, Fisher’s exact test was used in pairwise comparison of incidence of oviduct and uterine lesions. Data on body wt and ratios of uterine horn (with ovaries) weights/body wts × 100 were examined by Dunnet’s $t$ test and Kruskal–Wallis nonparametric ANOVA test. In all cases, a probability level of $P < 0.05$ was considered to indicate a significant difference.

In the present study, transplacental treatment of TAM had no significant effect on the male reproductive system (data not shown). Similar exposure to TAM, on the other hand, induced progressive proliferative lesions of the reproductive tract in female mice. The most frequent observation was the occurrence of oviduct hyperplasia as early as 24 weeks of age (Table 1). Oviduct hyperplasia was classified as mild or moderate/severe. Mild oviduct hyperplasia comprised smaller lesions showing proliferation of normal (typical) oviduct epithelial cells while

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SHORT COMMUNICATION

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Table 1. Incidence of proliferative lesions in the oviducts and ovaries of 12–78 weeks old CD-1 mice treated prenatally with TAM

<table>
<thead>
<tr>
<th>Type of lesions</th>
<th>Age (weeks)</th>
<th>12 weeks</th>
<th>24 weeks</th>
<th>52 weeks</th>
<th>53–78 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAM (mg/kg)</td>
<td>(N = 10)</td>
<td>(N = 10)</td>
<td>(N = 10)</td>
<td>(N = 10)</td>
</tr>
<tr>
<td>Oviduct hyperplasia</td>
<td>Mild</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Moderate/severe</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ovary</td>
<td>Cysts</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Neoplasm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Compared to the control group.

*P < 0.03.
*P = 0.006.
*P = 0.0001.
*P = 0.04.
*P = 0.002.

moderate/severe oviduct lesions included enlarged oviducts with moderate to extensive papillary hyperplasia (Figure 1A–C). At 72 weeks, the incidence of moderate/severe oviductal hyperplasia reached 82% and 70% after the high dose and low dose TAM, respectively, while only 8% of the control mice showed moderate oviduct hyperplasia (P = 0.0001). These findings are consistent with a recent preliminary report in mice exposed to TAM as newborns (15). Intracytoplasmic eosinophilia was often found focally within hyperplastic oviduct epithelium (Figure 1D).

Non-neoplastic ovarian abnormalities were noted particularly in older animals and included mainly cystic and atrophied ovaries (Table I). Malignant ovarian granulosa cell tumors occurred in three (15%) high dose TAM offspring while no such lesions were found in other groups. The likely significance of this finding is indicated by the fact that the incidence of ovarian granulosa cell tumors in historical control mice of this strain was reported to be very low (0.8%) at 18 months of age (16).

TAM-exposed offspring also developed dose- and age-dependent proliferative lesions of endometrial epithelium. Histologically, uterine lesions were classified into endometrial hyperplasia and adenomatous polypos (17). Endometrial hyperplasias were subclassified into three categories on the basis of size and cellular atypia according to the criteria described by Nagaoka et al. (18). Briefly, slight/moderate endometrial hyperplasia referred to lesions that contained increased number of glands with slight to moderate atypical cells in focal and diffuse areas of the endometrium. Severe hyperplasias were composed of large cystic (Figure 2A) and/or dysplastic (Figure 2B) lesions with atypical cells which were lined with
Reproductive tract lesions induced by transplacental TAM

Fig. 1. Oviduct lesions in TAM-exposed CD-1 mice. (A) Oviduct of control mouse, 52 weeks old; H&E, x75. (B) Oviduct of low dose (5 mg/kg) TAM mouse, 52 weeks old, showing moderate papillary hyperplasia; H&E, x30. (C) Oviduct of high dose (7.5 mg/kg) TAM mouse, 52 weeks old, showing severe hyperplasia; H&E, x15. (D) Hyperplastic oviduct showing intracytoplasmic pale eosinophilic inclusions in a high dose (7.5 mg/kg) TAM mouse, 52 weeks old; H&E, x300.

hyperchromatic and pleomorphic epithelium and often obliterated the uterine lumen. Diagnosis of adenomatous polyps was based on a combination of gross and microscopic features.

The incidence of endometrial hyperplasia was low at 12 and 24 weeks of age (Table II) but by 52 weeks was significantly and dose-dependently greater in the TAM groups than in the controls. Between 53 and 78 weeks, 8/23 (34.3%) offspring in the low dose TAM group and 11/22 (50%) in the high dose TAM group developed moderate/severe hyperplasia, compared to 5/24 (21%) control animals ($P = 0.03$).

Uterine polypoid adenomas were seen in older mice, particularly in TAM groups (Table II). In controls, only one offspring (4%) developed a polypoid adenoma at 78 weeks of age, a value consistent with a low incidence (3.3%) of uterine polyps reported in historical control CD-1 mice at 18 months of age (16). After TAM exposure, however, 7/23 (30.4%, $P = 0.02$) and 6/22 (27.3%, $P = 0.04$) offspring had uterine polypoid adenomas between 53 and 78 weeks of age. Some of these lesions filled the uterine lumen (Figure 2C) and exhibited marked glandular proliferation of the endometrium embedded in stromal tissue (Figure 2D). At 24 weeks, one mouse exposed to a high dose of TAM showed extensive squamous metaplasia in endometrial epithelium lining the glands (Figure 2E). The incidence of squamous metaplasia increased to ~20% (two TAM groups combined) at 52 and 53–78 weeks of age while none of the control offspring showed such metaplasia. No endometrial carcinomas occurred in this study.

Prenatal TAM treatment also resulted in hyperplasia of the
myometrium and a low incidence of tumors of myometrial epithelium. Deciduomas (15%) occurred at an early age (Table II, Figure 2F). Deciduomas were characterized by a mass of large eosinophilic decidual cells. Spontaneous deciduomas are known to occur only rarely or not at all in the historical controls of this strain of mice (16). In addition, a small but significant number of older offspring (72–78 weeks) exposed to 7.5 mg/kg TAM also developed uterine leiomyomas and a leiomyosarcoma (4/22 vs 0/24 in the control group, $P = 0.05$). No vaginal or cervical tumors were seen.

The efficacy of brief prenatal exposure to TAM in causing permanent change in female reproductive tissues contrasts with results obtained from studies with adult rodents. In long-term studies involving exposure of mice to TAM for up to 24 months, no uterine tumors were found (19,20), although Tucker et al. (19) reported the occurrence of cystic hyperplasia. In a recent study (21), continuous administration of TAM to mice for 24 months produced hyperplasia of uterine endometrial proliferation and atrophy of the myometrium for the first 3 months, followed by atrophy of both the endometrium and myometrium for the remaining 21 months of the study. Mice were again found to be refractory to the development of uterine tumors over the 24 month treatment period. The results in the present study showed that limited prenatal TAM treatment resulted postnatally in progressive and persistent hyperplastic changes in the uterine endometrium and myometrial epithelium, and neoplastic or preneoplastic lesions of several types. Thus, in contrast to an adult mouse, a fetal mouse is extremely sensitive to induction by TAM of lasting perturbations of the female reproductive tract. This unique susceptibility of the developing mouse is reminiscent of the response to DES: treatment of mouse neonates with DES resulted in 90% uterine adenocarcinomas (22), but a similar short-treatment of adult mice failed to induce any adenocarcinomas.
In summary, our results provide the first evidence that prenatal administration of TAM in CD-1 mice induces reproductive tract abnormalities including progressive proliferative changes in oviduct and uterine epithelium. Although no endometrial carcinomas occurred in the present study, sequential changes such as severe hyperplasia of endometrium followed by the formation of polyps with cellular atypia, squamous metaplasia, and dysplasia were often seen, similar to alterations that precede endometrial carcinoma in women. The doses of TAM used in this study were ~6–9 times the maximally used human daily therapeutic dose [HDD = 0.8 mg/kg/day (23)] and were given for only 7 days during pregnancy. A strong association between transplacental TAM and reproductive tract abnormalities in female mice observed in the present study underscores concern about the possible increased risk of similar abnormalities in babies of women who become pregnant while using this drug.

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References

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