Uterine carcinoma in mice treated neonatally with Tamoxifen

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The induction of preneoplastic and neoplastic lesions by the widely used antiestrogen Tamoxifen was studied in female mice. Outbred CD-1 mice were treated with Tamoxifen (1, 2, 5, 10, 25 or 50 µg/pup/day) for the first 5 days after birth. At 14–17 months, reproductive tract tissues were examined for pathological changes. In the ovary, corpora lutea were lacking while cysts were quite common in Tamoxifen-exposed mice at all doses; cystadenomas were seen in two mice. Structural malformations and epithelial hyperplasia of the oviduct were seen in 100% of the treated mice. Malformations of the uterus, cervix, and vagina were also seen. Excessive vaginal keratinization was not a common feature although vaginal adenosis was observed more often after Tamoxifen treatment than previously reported after similar treatment with diethylstilbestrol (DES). The most striking histological features, however, were seen in the uterus. One hundred percent of the Tamoxifen-treated mice at all doses exhibited uterine hyperplasia with focal areas of basal cell hyperplasia in the lining endometrium. Progressive cellular atypias were seen in the lining endometrium ranging from atypical hyperplasia to uterine adenocarcinoma; the highest incidence of uterine adenocarcinoma was 7/14 (50%) observed in the Tamoxifen 10 µg/pup/day dose group. No similar tumors were observed in corresponding control mice. The induction of atypical uterine hyperplasia and adenocarcinoma combined with other abnormalities observed in genital tract structure following neonatal treatment with Tamoxifen suggests the developing reproductive tract is exquisitely sensitive to perturbation by compounds with hormonal activity. These studies provide the basis for future investigation into the mechanisms of Tamoxifen’s carcinogenic effects in experimental animals, and to the risk benefit analysis for the prophylactic use of Tamoxifen in healthy women who are at risk of developing breast cancer.

Introduction

Tamoxifen is a triphenylethylene antiestrogen widely used for the treatment of a number of benign and malignant abnormalities (1). It is estimated that there have been over 7 million patient-years of treatment with Tamoxifen since it was first approved for use in 1973 (1). The use of Tamoxifen has been growing steadily since the 1980’s, when it was reported to help prevent the recurrence of breast cancer (2). In fact, use of Tamoxifen has been considered to be a medical breakthrough by many researchers and clinicians for the treatment of breast cancer in post menopausal women (3,4). The drug is reported to bind to the estrogen receptor in tumors that are estrogen receptor positive, thereby blocking the growth stimulus that estrogen provides (5). The success of the drug in cancer treatment led to the establishment of chemopreventative trials with Tamoxifen designed to enlist disease-free women who are at high risk for developing breast cancer (6). These chemopreventative trials have been ongoing in over 250 cancer treatment centers throughout the world since 1991. However, from the time of their initiation, concerns have existed of the unknown risks to the patient from long term Tamoxifen exposure as well as the developmental risks for the fetus treatment, should these women become pregnant while using Tamoxifen (7,8).

Tamoxifen is structurally and functionally similar to diethylstilbestrol (DES*), a potent synthetic estrogen that is well known for its teratogenic and carcinogenic effects on the reproductive tract (for review, 9). Tamoxifen, although reported to be an estrogen antagonist in some tissues depending on the species, doses, timing, and tissue compartment studied (10), exerts estrogenic effects on the female reproductive tract, particularly if exposure occurs during development (11). Many reproductive tract lesions observed in rats (12–17), mice (18–21), and guinea pigs (22) after perinatal administration of Tamoxifen or related triphenylethylene derivatives are similar to lesions induced by DES (23–27). In humans, Tamoxifen has been reported to inhibit the formation of the fetal uterus (28). Apparently, the uterus is a primary target for the adverse effects of this compound, although other tissues may be affected (29–31). While recognizing the beneficial effects of Tamoxifen in cancer therapy, it was recently classified as a carcinogen by IARC because patients undergoing Tamoxifen therapy for breast cancer are at increased risk of developing endometrial carcinoma (1). Although uterine neoplasia has not been reproduced experimentally in Tamoxifen-treated animals, it has been shown that neonatal exposure of mice to a number of estrogenic compounds like DES is associated with a high incidence of uterine adenocarcinoma (25). The neonatal mouse model has been shown to be a relevant study of human exposure since similar perinatal developmental events in the reproductive tract occur in the last trimester in humans but in early neonatal life for mice (25). The current study was undertaken specifically to examine potential risks of developmental exposure to Tamoxifen. Uterine lesions have been described in a preliminary report (21).

Materials and methods

Pregnant outbred female CD-1 [Crl:CD-1(ICR)/BR] mice near term were obtained from the breeding colony at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC. Mice were individually housed in plastic cages under a 12-h light and 12-h dark schedule in a

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removed, fixed in 10% neutral buffered formalin, embedded in paraffin, and killed, animals were weighed and reproductive tract tissues were quickly

Tam-1, 16 Tam-2, 11 Tam-5, 14 Tam-10, 11 Tam-25, and 11 Tam-50. Once the number of animals examined at each dose was as follows: 12 Control, 21 housed 4/cage, and maintained until killed at 14–17 months of age. The day. Control and Tamoxifen-treated mice were weaned on day 21 of age, ad libitum fresh water temperature (21–22°C) controlled room and fed NIH 31 mouse chow and
care guidelines. At delivery, litters were adjusted to 8 female pups/dam. Pups were given daily s.c. injections with Tamoxifen (Sigma Chemical Co., St Louis, MO; CAS# 10540–29–1) in corn oil or corn oil alone (as a control)
given daily s.c. injections with Tamoxifen (Sigma Chemical Co., St

Table I. Reproductive tract abnormalities in female mice (14–17 months of age) exposed neonatally to Tamoxifen*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>Ovary</th>
<th>Oviduct</th>
<th>Uterus/cervix</th>
<th>Vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>12/12 CL. (100)&lt;sup&gt;b, c&lt;/sup&gt;</td>
<td>1/12 PPL (8)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0/12 Hypoplastic (0)</td>
<td>0/10 Adenosis (0)</td>
</tr>
<tr>
<td>Tam-1</td>
<td>21</td>
<td>0/17 CL. (0)</td>
<td>17/17 PPL (100)</td>
<td>2/12 CEH (17)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0/21 Adenosis (0)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tam-2</td>
<td>16</td>
<td>3/15 CL. (20)</td>
<td>14/15 PPL (93)</td>
<td>16/16 Hypoplastic (100)</td>
<td>2/15 Adenosis (13)</td>
</tr>
<tr>
<td>Tam-3</td>
<td>11</td>
<td>1/11 CL. (9)</td>
<td>9/9 PPL (100)</td>
<td>11/11 Hypoplastic (100)</td>
<td>1/11 Adenosis (9)</td>
</tr>
<tr>
<td>Tam-5</td>
<td>14</td>
<td>0/12 CL. (0)</td>
<td>11/11 PPL (100)</td>
<td>14/14 Hypoplastic (100)</td>
<td>5/14 Adenosis (36)</td>
</tr>
<tr>
<td>Tam-25</td>
<td>11</td>
<td>0/9 CL. (0)</td>
<td>11/11 PPL (100)</td>
<td>11/11 Hypoplastic (100)</td>
<td>1/11 Adenosis (9)</td>
</tr>
<tr>
<td>Tam-50</td>
<td>11</td>
<td>0/11 CL. (0)</td>
<td>10/10 PPL (100)</td>
<td>11/11 Hypoplastic (100)</td>
<td>1/11 Adenosis (9)</td>
</tr>
</tbody>
</table>

*Neonatal exposure was by subcutaneous injection on days 1–5 of life at 1, 2, 5, 10, 25, or 50 µg/pup/day. (Appropriate tissue sections were not available to score each lesion for all animals in each group.)

**CL = corpora lutea.

<sup>b</sup>Number in parenthesis is %; <sup>d</sup>PPL = progressive proliferative lesion as previously described (32,33).

<sup>e</sup>CEH = cystic endometrial hyperplasia.

<sup>f</sup>The number of animals with adenosis may not reflect the occurrence of this disease because only five sections per vagina were screened in this study compared to data obtained from serial sections through the entire vagina as previously published (24).

Results

A comparison of reproductive tract abnormalities observed in control and neonatally Tamoxifen-exposed mice (days 1–5) at different doses is summarized in Table I. At 14–17 months, cystic ovaries were commonly seen at all Tamoxifen doses; corpora lutea were absent in all of the Tamoxifen-exposed mice except 3/15 (20%) in the Tam-2 and 1/11 (9%) in the Tam-5 group. Cystadenoma of the ovary was seen in 1/19 (5%) of the Tam-1 and 1/12 (8%) in the Tam-10 group; hemangioma of the ovary was seen in 2/11 (18%) of the Tam-5 group. In the control animals, corpora lutea were observed in 100% of the ovaries; no tumors were observed.

The oviducts of the Tamoxifen-treated mice were hypoplastic (developmentally-arrested) as previously described with DES treatment (32) although the severity of the lesion was less with Tamoxifen than with DES. Although the oviductal structure was hypoplastic, extreme epithelial hyperplasia was a common observation in Tamoxifen-treated mice at all doses examined; enlarged cells with pink cytoplasm were frequently seen (Figure 1) but ciliated cells were not. As described previously with DES treatment (32), the pattern of tubal plications of mucosa was distorted. Distortion of mucosal folds was manifested as failure to plicate or as irregularities in size and shape of the mucosal folds relative to the control animals. The mucosal folds had an adenomatous (gland-like) appearance but maintained connection with the oviductal lumen. In some animals, the mucosal folds were observed to extend through the muscle wall to the serosal surface (Figure 1). This abnormality in proliferation was termed progressive proliferative lesion (PPL) in DES-treated animals (33) since it was not demonstrated to spread along the serosal surface or to metastasize. The degree of extension and the area of involvement of the oviductal mucosa increased with increasingly higher doses of Tamoxifen. Similar to the developmentally-arrested structure resulting from DES-treatment, the epithelial changes in the oviduct following Tamoxifen treatment were never as severe as with DES.

The range of abnormalities in the uteri of mice exposed neonatally to Tamoxifen was more striking than those observed in the oviduct. One hundred percent of the uterine, in treated mice regardless of dose, were hypoplastic and under-
Tamoxifen induced uterine cancer

Fig. 1. Cross section through the isthmic region of an oviduct from a 17 month old mouse treated neonatally with Tamoxifen (10 µg/pup/day) on days 1–5. Note irregularities in size and shape of the mucosal folds. Secretory cells of the mucosa can be seen to extend to the serosal surface of the oviduct. Enlarged cells with pink cytoplasm are shown bulging through to the serosal surface (↑). (H&E, ×50)

Fig. 2. Well differentiated invasive uterine adenocarcinoma in a 17 month old mouse neonatally treated with Tamoxifen (10 µg/pup/day) on days 1–5. Note irregularly shaped glands that extend through the myometrium. (H&E, ×25).

developed. All cellular compartments were affected. The uterine stroma from Tamoxifen-treated mice was more collagenous than the stroma from the corresponding control mice and there were fewer stromal cells present. The muscle layer was also underdeveloped. Gland development was markedly inhibited in the uterine horns while the oviductal and cervical regions, which do not normally demonstrate gland development, contained areas of ‘glandular structures’. Although the uterine horns were hypoplastic, the lining epithelium in the caudal region near the cervix often had areas of basal cell hyperplasia. Focal areas of squamous metaplasia were observed along the entire uterine horn; there was no apparent association with squamous metaplasia and other abnormalities in the uterus. In addition to the uterine lesions described above, more severe uterine lesions were seen after neonatal Tamoxifen treatments. Atypical hyperplasia was seen in 4/21 (19%) in Tam-1, 4/16 (25%) of the Tam-2, 2/14 (14%) of the Tam-10, and 2/11 (18%) of the Tam-25 groups. Some animals demonstrated cellular alterations which had progressed to uterine adenocarcinoma (Figure 2): 4/21 (19%) in Tam-1, 3/16 (19%) in Tam-2, 7/14 (50%) in Tam-10, and 1/11 (9%) in Tam-25. The uterine tumors were often composed of multiple types of neoplastic epithelial cells forming well-defined glandular patterns to areas with a mixed population of neoplastic squamous, cuboidal, and columnar cells (Figure 3); hobnail cells showing luminal protrusion of the nucleus and scant apical cytoplasm were occasionally seen in some lesions (Figure 4). The Tam-10 dose group demonstrated the highest incidence of uterine tumors over the dose range of Tamoxifen tested in this study (from 1–50 µg/pup/day on days 1–5 of neonatal life). Unlike the oviduct, in the uterus the amount of organ involvement and the degree of cellular changes in the tumors were more severe than those previously reported after DES treatment (24,25,34). Uterine polyps (Figure 5) suggesting...
A progression of cellular atypia was seen in the uterine lesions ranging from cystic endometrial hyperplasia, atypical hyperplasia, well-differentiated uterine adenocarcinoma to less well-differentiated uterine neoplasia. Many of the uterine tumors demonstrated areas of squamous differentiation. The association of squamous metaplasia in the uterus with these areas of squamous differentiation in the uterine tumors is unknown. In general, the Tamoxifen-induced tumors appeared to be a higher grade of malignancy than those previously reported DES tumors (25), as determined by cellular atypias and invasion of the underlying stroma compartments. It was interesting to note in this study that excessive vaginal keratinization, a marker of acyclicity and constant estrogen levels, was not a common finding and was not associated with the malignant uterine lesions. In contrast, the uterine tumors seen after neonatal DES treatment were always associated with excessive vaginal keratinization. The differences in uterine cellular atypias and the lack of association of persistent estrogen stimulation following Tamoxifen verses DES treatment suggests that the mechanisms involved in the induction of uterine adenocarcinoma may be different between the two compounds. Furthermore, the fact that the Tamoxifen-induced lesions were mainly found in the lower region of the uterus where the endometrium changes to the cervical lining epithelium was another factor suggesting differences between the two compounds; DES-associated tumors were usually found in the body of the uterine horn. Additional studies are underway to compare DES-and Tamoxifen-induced uterine tumors.

Although the uterus appeared to be a primary target for Tamoxifen’s carcinogenic effects, other tissues of the reproductive tract were also affected by neonatal exposure to Tamoxifen. The ovaries were lacking corpora lutea suggesting that the animals were not cycling even at the lowest Tamoxifen doses tested. Ovarian tumors were demonstrated although the incidence was not greatly increased, but cysts in the ovary were a common finding. Multiovular follicles in the ovaries were not seen in this study as previously reported in young animals treated with estrogenic compounds during neonatal life (36); in fact, the ovaries in this study had very few oocytes remaining. Studies are underway to determine if the oocytes never developed or if they degenerated at a particular stage of development. The oviduct was also permanently affected by Tamoxifen-treatment; the oviductal epithelium was greatly hyperplastic while the oviduct structure was under-developed. Uterine polyps were identified in some mice but they were not quantitated because serial sections were not examined on all animals. Further studies are underway to specifically address Tamoxifen’s effects on the stromal compartment and its potential association with uterine polyps and fibroids. In the vagina, adenosis was occasionally seen but the small number of sections screened suggested that the lesion was more extensive and probably occurred more frequently than with DES treatment. Adenosis following DES treatment was such a rare finding that a study specifically designed to determine the incidence of the lesion could only be done by examining serial sections through the entire vagina (24).

The molecular mechanisms of developmentally-induced carcinogenesis are unknown. Tamoxifen treatment has been reported by Hemminki et al. (37) to be associated with DNA-adducts in the uterus of both experimental rodents and women, however, others have not shown similar findings indicating DNA damage (38). Due to its hormonal activity, Tamoxifen may have multiple carcinogenic mechanisms. Tamoxifen may
be acting on the developing uterus through the estrogen receptor (ER) pathway. The ontogeny of the ER and estrogen responsive protein markers like lactoferrin (LF) have been studied in the rodent genital tract. ER was demonstrated in stromal cells of the developing uterus in many studies. In addition, reports exist on the ontogeny of the ER in uterine epithelial cells in early neonatal life and two reports have demonstrated immunolocalization of the ER in uterine epithelial cells in prenatatal life (38,39). Furthermore, Tamoxifen has been shown to induce ER expression in uterine epithelial cells 24 h after the first neonatal injection (40). Thus, it is possible that Tamoxifen mediates its effects through the ER in both stroma and epithelial cells thereby resulting in abnormalities in multiple tissue compartments.

In summary, the induction of uterine carcinogenesis described in this report may provide new insights into the pathogenesis and molecular basis of the cellular changes resulting from developmental exposure to Tamoxifen and other hormonal disruptors.

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References


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