Tamoxifen Induces Hepatic Aneuploidy and Mitotic Spindle Disruption after a Single in Vivo Administration to Female Sprague-Dawley Rats

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Abstract

Tamoxifen has found extensive use in the treatment of all stages of human breast cancer. The efficacy of tamoxifen treatment for the prevention of second primary tumors and its chemosuppressive action in animal models have led to initiation of clinical trials to test its efficacy for prevention of this disease in women. Recently, tamoxifen has been shown to induce hepatocellular carcinomas in rats. For determination of the mechanism of induction of these tumors and assessment of the possibility of risk of human cancer development from tamoxifen treatment, female Sprague-Dawley rats (five rats per treatment) were administered tamoxifen at doses ranging from 0.3 to 35 mg/kg. One day after treatment, the rats were sacrificed, and the hepatocytes were isolated and cultured for 50 h. Colcemid was added 3 h prior to harvest, and the hepatocytes were then prepared for karyotypic evaluation. One hundred metaphase spreads were examined per animal. Tamoxifen treatment resulted in the induction of aneuploidy in approximately 70% of the examined hepatocytes at the doses used. In addition, premature condensation (2–10%) and endoreduplication (5–10%) were observed in hepatocytes of rats treated with tamoxifen. Furthermore, exchanges between chromosomes as well as chromosome breakage were observed. Examination of the cultured hepatocytes from rats treated with tamoxifen by electron microscopy demonstrated both unipolar spindles and incompletely elongated spindles. Exposure of rats to a single in vivo dose of tamoxifen produced multiple changes in rat hepatocytes including clastogenic damage at doses comparable to that administered to humans. The occurrence of aneuploidy induction, premature condensation, chromosome breakage, and improper mitotic spindle formation indicates that risk versus benefit of tamoxifen treatment should be carefully evaluated.

Introduction

The antiestrogen, tamoxifen, has been used extensively to prevent the recurrence of human breast neoplasms, as well as the appearance of further primary lesions (1). The efficacy of tamoxifen treatment for all stages of breast cancer (1, 2) and the low incidence of acute side effects associated with its use (3) have led to the institution of large-scale clinical trials using tamoxifen as a chemopreventive agent in individuals with an increased risk of breast cancer (4, 5). This use of tamoxifen for nonmalignant indications has prompted a reconsideration of the toxicology of tamoxifen. While studies on the toxicity of tamoxifen have not shown significant mutagenicity in microbial systems (6), several experimental results suggest that tamoxifen may possess a carcinogenic potential. Recent results from chronic bioassays in rats have demonstrated an increased incidence of malignant liver neoplasms compared with that in untreated animals (7). Tamoxifen is an effective promoting agent in hepatocarcinogenesis in the rat (8). Mani and Kupfer (9) have demonstrated that tamoxifen can form a reactive intermediate capable of binding to protein in a cell-free system. In addition, 32P-postlabeled DNA adducts can be detected after repeated doses of tamoxifen (10–12). Further studies have demonstrated that tamoxifen can induce a G1 block in MCF-7 cells (13). Francavilla et al. (14) have demonstrated that tamoxifen can inhibit hepatic proliferation after a partial hepatectomy, suggesting that this agent also induces a G1 block in hepatocytes. Tamoxifen induces the formation of micronuclei in a metabolism-dependent lymphoblastoid cell line (11, 12). Because of the striking carcinogenic effect of tamoxifen on rat liver in vivo, its structural similarity to DES, and its ability to induce micronuclei, the effects of tamoxifen on hepatic ploidy and chromosomal integrity were investigated to determine whether either of these types of genetic damage might be integral to the carcinogenic action of tamoxifen.

Materials and Methods

In order to determine whether tamoxifen induced aneuploidy or chromosomal damage in rat liver in vivo, we administered tamoxifen citrate (Sigma Chemical Co., St. Louis, MO) to groups containing five female Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) in doses of 0.3, 3.0, and 35.0 mg/kg by gavage. Alternatively, the solvent, triocanolin (Pfalz and Bauer, Waterbury, CT), or the positive control for induction of aneuploidy (15), 35 mg TCOBOP, synthesized and provided as a gift by Dr. James Miller, McArdle Laboratory, was administered. Twenty-four hours after dosing, the rat livers were perfused with a collagenase solution as described previously (16). Hepatocytes were separated from litoral cells by a Percoll (Pharmacia, LKB Biotechnology, Piscataway, NJ) iodoside centrifugation and immediately plated in 75-cm collagen-coated flasks (16) at a density of 2.6 × 105 cells in 15 ml of serum-free "supplemented" Dulbecco's modified Eagle's medium/Ham's F-12 medium (17). The medium was supplemented with bovine serum albumin (0.2%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (18 mm), and sodium pyruvate (5 mm). The medium was changed after 3 h, and 10 ng epidermal growth factor (Collaborative Biomedical Products, Bedford, MA) was added per ml of medium to stimulate cell division. After 47 h of culture, 40 ng/ml Colcemid was added to each flask, and the cells were incubated for an additional 3 h. Parallel cultures were treated with 25 μM 5-bromo-2'-deoxyuridine (Sigma) for the last 6 h of culture to permit analysis of late replication banding (18). Cultures were randomly selected, and the cells were prepared for examination by electron microscopy by methods described previously (19) or harvested for karyotypic analysis. The hepatocytes were removed from the dish with 0.01% collagenase and harvested by hypotonic treatment (0.075 M KCl) for 8.5 min. Once removed from the flask, the hepatocytes were fixed with methanol:acetic acid (3:1, v/v), and slides were prepared as described by Sargent et al. (20). One hundred metaphases of good morphology were randomly selected from cultures of each rat.

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The abbreviations used are: DES, diethylstilbestrol; TCOBOP, 1,4-bis(2-(3,5-di-chloropyridylo))benzene.
Results

Karyotypic analysis of metaphase spreads from Sprague-Dawley female rats was performed to determine the effect of a single tamoxifen treatment on the number and integrity of hepatic chromosomes. Only a low level of breakage and aneuploidy similar to that reported previously for control untreated rats was observed in the solvent-treated rats (Table 1). TCPOBOP, which has previously been shown to induce aneuploidy in the rat liver in vivo (15), was used as a positive control to demonstrate that aneuploidy could be detected with this model. The present study showed that 35 mg TCPOBOP/kg body weight resulted in substantial aneuploidy (Table 1). Tamoxifen also induced a marked level of aneuploidy compared with the solvent control ($P < 0.05$). The level of aneuploidy was extremely high compared with the control. Aneuploidy was significantly increased for all doses of tamoxifen tested ($P < 0.05$). Since the level of aneuploidy noted was independent of the dose of tamoxifen used, doses lower than 0.3 mg/kg may be necessary to demonstrate a dose-dependent effect for induction of aneuploidy by tamoxifen. Tamoxifen treatment induced a dose-related increase in premature condensation and chromosome breakage (Table 1) that was significantly greater than that observed in solvent-treated rats ($P < 0.05$). Additionally, a dose-dependent induction of endoreduplication was observed with tamoxifen treatment. Furthermore, a significant level ($P < 0.05$) of aneuploidy, chromosomal exchanges, and endoreduplication were observed for all doses of tamoxifen administered (Table 1).

Fig. 1 is a photomicrograph of a metaphase of a type typically observed in rats treated with 35 mg tamoxifen citrate/kg. The spread is aneuploid. The presence of diplochromosomes (paired homologous chromosomes) indicates that the spread has undergone endoreduplication. In addition, chromosomes without a homologue can be observed (Fig. 1, arrow). Also, two exchanges are shown in this metaphase spread (Fig. 1, double arrows). All of the observed exchanges were between homologous chromosomes, indicating that the exchanges occurred during S synthesis (21).

Since the mechanisms of induction of aneuploidy and endoreduplication can be quite varied (22), two factors potentially responsible for these effects were examined. In order to determine whether the tamoxifen-induced aneuploidy and endoreduplication are associated with an inhibition of chromosome synthesis, we examined metaphase spreads by late replication banding (18). In these studies, all metaphase spreads investigated showed a normal banding pattern with a light-staining, late-replicating X chromosome (data not shown), indi-

### Table 1 Effect of a single oral dose of tamoxifen on rat hepatic chromosomal number and integrity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aneuploidy</th>
<th>Breakage</th>
<th>Premature Condensation</th>
<th>Exchanges</th>
<th>Endoreduplication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent control</td>
<td>3 ± 3.0</td>
<td>5.0 ± 2.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>35 mg TCPOBOP/kg</td>
<td>65 ± 6.5*</td>
<td>10.0 ± 5.0*</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>0.3 mg Tam/kg</td>
<td>71 ± 8.0*</td>
<td>8.0 ± 2.0</td>
<td>2.0 ± 1.0*</td>
<td>8.0 ± 3.0*</td>
<td>5.0 ± 1.0*</td>
</tr>
<tr>
<td>3 mg Tam/kg</td>
<td>70 ± 5.0*</td>
<td>9.4 ± 3.0</td>
<td>4.0 ± 2.5*</td>
<td>5.0 ± 2.0*</td>
<td>6.0 ± 2.0*</td>
</tr>
<tr>
<td>35 mg Tam/kg</td>
<td>85 ± 7.0*</td>
<td>23.0 ± 4.0*</td>
<td>10.0 ± 5.0*</td>
<td>10.0 ± 2.0*</td>
<td>10.0 ± 2.0*</td>
</tr>
</tbody>
</table>

* Significant difference from the control treatment ($P < 0.05$); Tam, tamoxifen.
cating that the observed aneuploidy and endoreduplication were not
due to a block in S synthesis.

Examination of hepatic metaphase spreads from solvent- and
tamoxifen-treated rats by electron microscopy shows some interesting
differences in mitotic spindle integrity (Fig. 2). Bipolar spindles were
noted in control cultures (Fig. 2A), while in a significant number of
cultures from tamoxifen-treated rats, monopolar spindles (Fig. 2B)
were observed. Of the 36 informative spindles examined, 9 were
monopolar. In addition, the organelles were not excluded from the
spindle in a number of these spreads. In some tamoxifen-treated
hepatocytes (data not shown), a normal bipolar spindle appeared to
have formed, but during anaphase the spindle elongation was much
less than in untreated hepatocytes. Thus, while tamoxifen treatment
does not appear to affect S phase DNA synthesis, there is clearly an
effect of the in vivo administration of this agent on the structure of the
mitotic apparatus that could lead to aneuploidy (22).

Discussion

The administration of tamoxifen induces premature condensation of
the hepatic chromosomes, chromosome exchanges, and chromosome
breaks. Compounds structurally related to tamoxifen can block cell
cycle progression (23). In addition, another structural analogue of
tamoxifen, DES, induces mitotic arrest (24). Tamoxifen induces a G1
block in MCF-7 cells (13) and possibly in hepatocytes in vivo (14).
Since the blockage of cell cycle progression can result in premature
condensation, this action of tamoxifen should be explored to uncover
the mechanism by which tamoxifen causes premature condensation.
The presence of tamoxifen-induced DNA adducts (10—12) may result
in premature condensation, exchanges between chromosomes, and
chromosomal breakage. Since the exchanges were observed in this
study only between homologous chromosomes, the exchanges oc-
curred during S (21). Thus, tamoxifen-induced DNA damage results
in changes in cell cycle progression and further karyotypic instability.

Tamoxifen administration in vivo results in aneuploidy, an alter-
ation in the number of chromosomes, and endoreduplication, which
is the result of two or more synthesis cycles in the absence of an
intervening mitosis. A block in DNA synthesis or the inhibition or
delay of spindle formation can result in endocycles and in aneuploidy.
The presence of a light-staining, late-replicating X chromosome indi-
cates that the endoreduplication and aneuploidy that occurred after
tamoxifen treatment were not due to a block in S synthesis (18).
The presence of monopolar spindles and incompletely elongated spindles
from which the organelles were not excluded suggests that the aneu-
ployidy and endoreduplication observed with tamoxifen treatment may
be due to the induction of spindle aberrations. A structural analogue
of tamoxifen, DES, has also been shown to induce micronuclei (25),
aneuploidy (26), and cellular transformation (27). In addition, DES-
treated cells have been shown to undergo mitotic arrest (24) and to
contain monopolar spindles (28). Therefore, this class of agents ap-
pears to have multiple effects on the cell that result in the disruption
of the normally tightly regulated coordination of spindle formation
and chromosomal integrity.

Multiple factors are involved in the maintenance and proper for-
mation of the mitotic spindle apparatus. Although the mechanism by
which tamoxifen induces aneuploidy and karyotypic instability is
unknown, tamoxifen is known to alter cellular calcium levels (29) and
acts as a calmodulin antagonist (30). Although the role of calmodulin
in spindle assembly is not known, calmodulin is associated with the
spindle pole body and is required for proper formation of the spindle
apparatus (31). Calmodulin-defective mutants in yeast contain a single
spindle pole body, monopolar spindles, and shortened spindles that
appear not to have elongated (31). The phenotypes associated with
these calmodulin mutants include aneuploidy in some of the cells, as
well as a low percentage of cells with endoreduplication (32). Since
these phenotypes are similar to the observed effects of tamoxifen, the
aneuploidy and endoreduplication observed in the rat may be due to
inhibition of the action of calmodulin. Furthermore, yeast mutants
that have improper spindle pole body formation also contain monopolar
spindles (31, 32), as was observed in the present study in rat hepa-
tocytes, further supporting the possibility that tamoxifen may alter both
the number and integrity of chromosomes through its inhibitory action
on calmodulin or one of the spindle pole body proteins.

The effectiveness of tamoxifen as a clastogenic agent indicates
that tamoxifen induces changes in both the number and structural
integrity of the chromosomes and may increase the progression of
cells toward frank neoplasia. Aneuploidy and endoreduplication

![Figure 2](https://example.com/figure2.jpg)
are often associated with malignant conversion (33). In addition, the hallmark of the stage of progression is one of an evolving karyotypic instability, including an increase in aneuploidy and chromosome breakage with gene deletions, rearrangements, and duplications (34). Since tamoxifen possesses clastogenic action as evidenced by the induction of microcurei, aneuploidy, and chromosomal breakage, its carcinogenic action may include effectiveness during the stage of progression. This supposition is supported by the induction of transformation of Syrian hamster embryo cells by tamoxifen (35). Retrospective studies have found an increased incidence of endometrial cancers in tamoxifen-treated women (36). The present study performed in rat hepatocytes indicates that tamoxifen alters the number of chromosomes, increases the incidence of chromosomal aberrations, and may disrupt mitotic spindle integrity. The carcinogenic action of tamoxifen for the rat liver, coupled with the occurrence of aggressive breast and uterine tumors, albeit at a low incidence in women treated with tamoxifen, suggests that a careful assessment of the risk as well as the benefit of chronic tamoxifen administration should be considered in the nonmalignant use of this drug.

References


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