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 secondary 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 48 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 bio-

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3 The abbreviations used are: DES, diethylstilbestrol; TCBOPOB, 1,4-bis(2-(3,5-di-chloropyridylo))benzene.
Hepatocytes were isolated by perfusion administration at a dose of 35 mg/kg by gavage (23). Values for hepatocyte cultures from five rats receiving the solvent only or TCPOBOP are also given. TCPOBOP was administered at a dose of 35 mg/kg by gavage (23).

In the animals examined, approximately 70% of the hepatocytes were tetraploid (chromosome number is 84), and 30% were diploid (chromosome number is 42). A spread was considered aneuploid if it deviated from the established diploid or tetraploid number of chromosomes. The metaphases were analyzed blindly for ploidy, breakage, exchanges, premature condensation, and endoreduplication. The significance of differences between groups was determined by nonparametric statistics; evidence of a dose-response relationship was tested by regression analysis. The alpha level was adjusted to take into account the number of comparisons.

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 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-

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**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>100 ± 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 ± 5.0*</td>
<td>10.0 ± 2.0*</td>
</tr>
</tbody>
</table>

* Significant difference from the control treatment ($P < 0.05$); Tam, tamoxifen.

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Fig. 1. A hepatic metaphase spread prepared from a female Sprague-Dawley rat administered 35 mg/kg tamoxifen citrate and sacrificed 24 h later is shown. This metaphase spread demonstrates aneuploidy, exchanges, and endoreduplication. The two exchanges are indicated by double arrows, while the three chromosomes missing a homologue are indicated by single arrows. Hepatocytes were isolated by perfusion and Percoll isodensity centrifugation before culture and preparation for cytogenetic analysis. Further details on the methods are described in Table 1 and in "Materials and Methods."
The metaphase spread (A) has a spindle apparatus of normal length and contains two poles. The metaphase cell in (B) contains a monopolar spindle. 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 occurred 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 alteration 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 indicates 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 aneuploidy 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 appears 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 formation 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 and is required for proper formation of the spindle apparatus (31). Calmodulin-defective 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 hepatocytes, 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
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 micronuclei, 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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