Induction of Covalent DNA Adducts in Rodents by Tamoxifen

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Abstract

The antiestrogen tamoxifen, increasingly used as adjuvant treatment for breast cancer, has been found to covalently modify DNA of rodents. For instance, the liver DNA of female Sprague-Dawley rats treated with a single injection of tamoxifen contained two DNA adducts. Four additional DNA adducts were formed and adduct concentrations increased 5-7- and 10-15-fold after three and six tamoxifen injections, respectively, from levels observed after a single dose. The accumulation of DNA adducts with repeated administrations of tamoxifen to rodents may make this drug a poor choice for the chronic preventative treatment of breast cancer.

Introduction

The antiestrogen tamoxifen is increasingly being used as adjuvant treatment for breast cancer (1, 2). The drug has undergone testing mainly in Europe and is currently being evaluated in a long-term chemoprevention trial in the United States (3). Women at an elevated risk of contracting primary mammary carcinoma or of a recurrence of the disease are to receive chronic tamoxifen medication for many years as a cancer suppressing therapy. The effectiveness of tamoxifen to prevent estrogen-dependent tumor growth has been established in cells in culture and in rodent tumor models. For instance, tamoxifen decreases the growth of MCF-7 breast cancer cells (4). It inhibits the appearance of N-nitrosomethylurea- or dimethylbenzanthracene-induced rat mammary tumors and of estradiol-induced hamster kidney tumors (5-7). In these cell culture and animal models, effects of tamoxifen other than the desired tumoricidal action have not been reported. Likewise, in humans the drug is believed to be well tolerated and to be generally free of side effects (8, 9). This opinion on the safety of tamoxifen exists although detailed toxicological studies are not available.

The lack of toxicity data on this antiestrogen despite its increasingly widespread use in humans has prompted us to examine the genotoxicity of tamoxifen in female Sprague-Dawley rats and Syrian hamsters by 32P-postlabeling analysis, a sensitive technique established for the assay of covalent DNA adducts of carcinogens in animals and humans (10, 11).

Materials and Methods

Tamoxifen Treatment of Rats and Hamsters. Female Sprague-Dawley rats (3 animals/group, 7-8 weeks old) were purchased from Harlan Sprague-Dawley, Houston, TX. Rats received i.p. injections of 20 mg/kg/day tamoxifen citrate dissolved in ethanol:phosphate buffer (2:8, v/v) for 1, 3, or 6 days. Three control rats remained untreated. Female hamsters (3 animals/group, 7-8 weeks old; purchased from Harlan Sprague-Dawley), received single i.p. injections of 2, 5, or 10 mg/kg tamoxifen citrate. Four h after the injections, the animals were decapitated. Their livers and kidneys were removed, frozen on dry ice, and then stored at -80°C.

Inhibition of DNA Adduct Formation. Female Sprague-Dawley rats (3 animals/group) were treated with 20 mg/kg/day tamoxifen citrate for 3 days. Another group of 3 rats received this schedule of tamoxifen citrate plus 1.5% ascorbic acid and 0.5% sucrose in drinking water for 24 h prior to tamoxifen administration and then throughout the experiment. A third group of rats received this schedule of tamoxifen citrate plus 50 mg/kg N-acetylcysteine 30 min prior to and 4 h after each tamoxifen injection. The animals were killed 4 h after the last tamoxifen injection. Their liver tissues were removed, frozen on dry ice, and then stored at -80°C.

32P Postlabeling. DNA was isolated from 0.5-g tissue samples by solvent extraction combined with enzymatic digestion of protein and RNA. DNA adducts were analyzed by the nuclease P1, enhancement procedure as described previously (10, 11). Labeled DNA adducts were mapped by chromatography on polyethyleneimine cellulose sheets using the following solvents: D1, 1 M sodium phosphate, pH 6.8; D3, 4.25 M lithium formate-7.5 M urea, pH 3.5; D4, 0.7 M sodium phosphate-7 M urea, pH 6.5; D5, 1.7 M sodium phosphate, pH 6.0. Adducts were located by autoradiography with Du Pont Lightning Plus intensifying screens and Kodak X-Omat films exposed for 24-28 h at -80°C. DNA adduct spots were cut out and counted by Cerenkov assay for quantitative analysis.

Results

Postlabeling maps of liver DNA of rats treated with a single injection of 20 mg/kg tamoxifen contained two adducts (spots T1 and T2) (Fig. 1B) over the background radioactivity observed in control DNA (Fig. 1A). When antiestrogen injections were given once daily for 3 or 6 days, adduct intensities of spots T1 and T2 increased and new DNA adducts T3 through T6 appeared (Fig. 1, C and D, respectively). The locations of these DNA adducts on the chromatograms are in areas free of background radioactivity. Moreover, the chromatographic location, pattern, and number of adducts are unique for this drug and have not been observed previously with other chemicals or drugs administered to rodents. For these reasons, spots T1 through T6 are taken as evidence of covalent binding of tamoxifen or, more likely, its metabolite(s) to DNA. Adduct concentrations, determined by Cerenkov counting, increased 5-7-fold after 3 tamoxifen injections and 10-15-fold after 6 injections from levels observed after a single administration of the drug (Table I). The highest adduct concentrations occurred in livers of treated animals. In kidney DNA of these tamoxifen-treated rats, only one of these adducts (spot T1) was detectable at low intensity, after rats had received 6 injections (Fig. 1H). Kidney DNA of rats treated once or three times (Fig. 1, F and G) was indistinguishable from control DNA (Fig. 1E). DNA adducts could not be detected in uterine tissue of these animals (data not shown). Male rats, treated with one i.p. injection/day of 20 mg/kg tamoxifen for 3 days, showed only 1 of these DNA adducts (spot T2) at a much lower concentration (data not shown) than observed in female rats.

The induction of DNA adducts by tamoxifen was also observed in female Syrian hamsters (Fig. 2). This species was used...
to determine the lowest dose necessary for the detection of DNA adducts by 32P-postlabeling analysis. Adducts were clearly visible in liver DNA of hamsters treated with a single injection of 5 or 10 mg/kg tamoxifen (spots 1 and 2 in Fig. 2, C and D). At a dose of 2 mg/kg, spots 1 and 2 were barely detectable (Fig. 2B) over background radioactivity (Fig. 2A). These data demonstrate that single doses of tamoxifen in the low mg/kg range induce detectable DNA adducts in livers of treated rodents.

In a third experiment, it was attempted to inhibit the DNA adduct formation by tamoxifen using previously established inhibitors of DNA adduction such as ascorbic acid or N-acetylcycteine (12, 13). Tamoxifen was injected for 3 days (20 mg/kg/day) to induce DNA adduct levels (Fig. 3A) comparable to those shown in Fig. 1C. The treatment of animals with ascorbic acid for 1 day prior to the first tamoxifen injection and then throughout the experiment had no noticeable effect on tamoxifen-induced adduct levels in their hepatic DNA (Fig. 3B). Likewise, the administration of N-acetylcycteine prior to and after each of the three tamoxifen injections did not protect the rats from induction of hepatic DNA damage (Fig. 3C). Thus, strategies which demonstrably decrease DNA adduction by other chemicals did not protect rats from the tamoxifen-induced DNA adducts shown above.

### Discussion

The data demonstrate that tamoxifen produces DNA adducts in two rodent species and that DNA damage accumulates with repeated administration of this antiestrogen. Moreover, levels of tamoxifen-induced DNA adducts cannot be modulated by coadministration of ascorbic acid or N-acetylcycteine, substances known to inhibit carcinogenesis and DNA adduction (12-15). The structures of the DNA adducts reported here and their mechanism of formation are not yet known and require additional studies. Nevertheless, levels of covalent DNA modification by carcinogens have been correlated with the incidence of cancers in humans (16, 17). The reported DNA damage by this antiestrogen may lead to other genotoxic end points, i.e., point mutations, deletions, etc., which must be examined as part of a safety evaluation of this drug.

The long-term administration of tamoxifen to women has
Fig. 2. \(^{32}\)P-postlabeling analysis of liver DNA of female Syrian hamsters untreated (A) or treated with single i.p. injections of 2 (B), 5 (C), or 10 (D) mg/kg tamoxifen. Hamster liver DNA was analyzed as described in the legend to Fig. 1.

Fig. 3. \(^{32}\)P-postlabeling analysis of liver DNA of rats treated with 20 mg/kg/day tamoxifen citrate for 3 days (A), with 20 mg/kg/day tamoxifen for 3 days plus ascorbic acid in drinking water (B), or with 20 mg/kg/day tamoxifen for 3 days plus \(N\)-acetylcysteine (C). Three female rats were treated with tamoxifen for 3 days as described in the legend to Fig. 1. A second group of 3 rats were given 1.5% ascorbic acid plus 0.5% sucrose in drinking water for 24 h prior to tamoxifen treatment and then throughout the experiment until animals were decapitated (B). A third group of rats received i.p. injections of 50 mg/kg \(N\)-acetylcysteine 30 min prior to and 4 h after each tamoxifen treatment. The animals were killed and their tissues were excised and analyzed as described in the legend to Fig. 1. Adducts are lettered at the top of each spot.
been shown in a number of clinical trials to increase the risk of endometrial cancer [reviewed by Gusberg (18)]. Tamoxifen-induced DNA adducts have not been detected in the uterus, but mainly in liver and, to a minor extent, in kidney of rats. This occurrence of higher concentrations of covalent DNA modifications in livers of rats correlates well with the binding of tamoxifen metabolites to hepatic protein (19) and with tamoxifen-induced liver cancer in rats (20). It is possible that in humans tamoxifen may generate DNA damage in uterus and that these covalent DNA modifications initiate the development of uterine cancer. In addition, endogenous estrogen or the partial agonist activity reported for tamoxifen (21) may be required for cell transformation and endometrial tumor growth. Further studies are required to examine this hypothesis by measuring DNA adduct levels in tissues of tamoxifen-treated women.

References

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