SHORT COMMUNICATION

Tamoxifen does not form detectable DNA adducts in white blood cells of breast cancer patients

David H. Phillips1, Alan Hewer, Philip L. Grover, Grace K. Poon2,3 and Paul L. Carmichael

Haddow Laboratories and 1CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, UK
2Present address: SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR, UK
3To whom correspondence should be addressed

DNA from white blood cells of seven women receiving tamoxifen as adjuvant therapy for breast cancer and of three women who served as healthy controls was analysed for the presence of tamoxifen-DNA adducts using 32P-postlabelling with a limit of detection of 8 adducts/1010 nucleotides. No postlabelled adducts with the chromatographic properties of known tamoxifen-DNA adducts were detected in any of the samples. It is concluded that at therapeutic levels of exposure there is no significant formation of DNA adducts by tamoxifen or its metabolites in circulating white blood cells.

Tamoxifen is an anti-oestrogen that is effective in the treatment of breast cancer and which is being evaluated for its ability to prevent breast cancer in high risk women with a family history of the disease. Concerns about the safety of tamoxifen for cancer prevention stem from the fact that it is a potent hepatocarcinogen in rats (1-3) and that its use is associated with an increased incidence of endometrial cancer among breast cancer patients (4-7). An increased risk of gastrointestinal cancers, which requires confirmation, has also been reported (7). An understanding of the mechanism(s) of tamoxifen carcinogenicity in humans and rats is clearly essential if an accurate assessment of the risks associated with the use of tamoxifen in cancer chemoprevention is to be made (8).

In the rat recent evidence indicates that tamoxifen is a genotoxic carcinogen that is metabolized by hepatocytes to α-hydroxytamoxifen, which is further activated to a product that binds principally to the exocyclic amino group of guanine residues in DNA (9-12). The same adduct pattern is formed in mouse hepatocytes treated with tamoxifen or α-hydroxytamoxifen and in human hepatocytes treated with α-hydroxytamoxifen (13). Tamoxifen at low doses also causes aneuploidy in hepatocytes (14) and induces micronuclei in a human cell line that expresses a battery of drug metabolizing enzymes (15,16).

Tamoxifen and several of its metabolites are readily detected in the blood of patients taking tamoxifen (17,18). It was reported recently that low levels of DNA adducts were formed in human lymphocytes treated in vitro with tamoxifen (19). In the present study we have analysed, by 32P-postlabelling, white blood cell DNA from women with breast cancer in order to determine whether therapeutic doses of the drug form detectable levels of tamoxifen-DNA adducts in blood cells in vivo.

Blood samples (5 ml) were obtained from seven breast cancer patients who were receiving tamoxifen adjuvant therapy and from three healthy control women (Table I). All the women were non-smokers. All donors gave informed consent and the study received approval from the local Ethics Committee. The blood samples were drawn into heparinized tubes, frozen and transported on the same day to the Institute of Cancer Research. Control samples were provided by research staff. The samples were thawed and DNA was isolated from the cell pellet using a phenol/chloroform extraction procedure described previously (20). Yields of 32-68 μg were obtained. The concentration of tamoxifen in the serum was determined by an LC-MS method described elsewhere (13).

32P-Postlabelling analysis was carried out using the nuclease P1 digestion method of sensitivity enhancement and the TLC solvent system described previously (13). Each sample was analysed three times, twice using 4 μg DNA and once using 20 μg. Autoradiography of the thin layer chromatograms was carried out for 3 days at -85°C in order to visualize very faint adduct spots that may have been present. Based on the ability to detect adduct spots containing 30 c.p.m. above background, the limit of detection of the assay was estimated to be 4 adducts/109 nucleotides for analysis of 4 μg DNA and 8 adducts/1010 nucleotides for analysis of 20 μg DNA.

As a positive control a sample of DNA containing tamoxifen-DNA adducts, isolated from mouse hepatocytes that had been treated with 10 μM tamoxifen (13) was used.

In order to verify patient compliance with their tamoxifen therapy, serum samples from each subject were analysed for the presence of tamoxifen (Table I). Concentrations ranged from 34 to 178 ng/ml (110 ± 45) in the seven breast cancer patients and tamoxifen was not detected in any of the three control samples.

The pattern of 32P-postlabelled adducts obtained with DNA from tamoxifen-treated mouse hepatocytes (Figure 1A) is the same as that obtained from rat hepatocytes treated similarly (13) and also from rat liver after treatment in vivo by gavage (12). The three adducts formed (spots 1-3) all derive from intermediate formation of α-hydroxytamoxifen (13) and are also produced by reaction of the synthetic derivative α-acetoxytamoxifen with DNA, the principal product of this reaction detected in digested DNA being (E)-α-(N2-deoxyguanosinyl)tamoxifen (12). The mouse hepatocyte 32P-postlabelling adduct pattern was compared with the patterns obtained with DNA isolated from the blood cells of women taking tamoxifen and of the control subjects. None of the 10 human samples showed any evidence of radioactive spots that cochromatographed with tamoxifen-DNA adducts and no differences were noted in the chromatograms of the seven tamoxifen-exposed samples (Figure 1B-H) compared with the three controls (Figure 1I-K). It should be noted that the chromatography conditions used are very similar to those used by Hemminki et al. (19). The pattern of other adducts detected in both the tamoxifen-exposed and the control women is similar to that seen in other studies of blood cell DNA and may be a consequence either of as yet unidentified environmental genotoxic agents or of endogenous DNA dam-
Fig. 1. Autoradiographs of the TLC maps of $^{32}$P-postlabelled digests of DNA. (A) A positive control sample of DNA isolated from a primary culture of mouse hepatocyte treated with 10 μM tamoxifen for 18 h (20). The three tamoxifen-DNA adducts are indicated by arrows. (B-H) Blood cell DNA from breast cancer patients P1-P7 respectively. (I-K) Blood cell DNA from control subjects C1-C3 respectively. Autoradiography was for 3 days at -80°C.

Aging agents (21). Maximum levels of these adducts were 1.5 adducts/10⁸ nucleotides in the present study. For comparison, the tamoxifen-DNA adducts depicted in Figure 1A represent a 10-fold higher level of DNA modification (15 adducts/10⁸ nucleotides). (The patterns shown in Figure 1 are from analyses of 4 μg samples of DNA. No additional adduct spots were detected in experiments with 20 μg DNA samples.)

Typical published concentrations of tamoxifen in the serum of women receiving therapeutic doses (generally 20 mg/day, equivalent to 0.29 mg/kg for a woman weighing 70 kg) are 80–300 ng/ml (0.22–0.8 μM) (17,22). The values reported here (34–178 ng/ml) are compatible with these values. In experiments with human lymphocytes treated with tamoxifen in vitro DNA adducts were detectable at drug concentrations of 10 μg/ml (27 μM) and above, but were not observed at 5 μg/ml (13.5 μM) or at lower concentrations (19). Thus the doses at which adducts are detectable in vitro are substantially higher than those likely to be achieved in vivo in adjuvant therapy or prophylactic use. With some breast cancer patients with advanced disease substantially higher doses of tamoxifen have been administered (23). In some of these patients, many of whom suffered severe neurological side effects, plasma concentrations as high as 9 μM were reported. This is close to the concentration of tamoxifen at which DNA adducts are reported to be detectable in in vitro experiments (19); it is therefore conceivable that patients receiving high doses of tamoxifen might have detectable tamoxifen-DNA adducts in their blood (this presupposes that isolated lymphocytes have a similar capacity to activate tamoxifen in vitro as do the cells in vivo, where metabolism by other tissues is also occurring). However, the doses involved, up to 520 mg/m²/day (~830 mg/day) are up to 40 times higher than the usual dose (20 mg/day) for adjuvant therapy and chemoprevention trials.

In the present study adducts characteristic of tamoxifen-DNA adducts formed in rodent cells were not detected in any of the women shown by independent LC-MS assay to be taking tamoxifen. Tamoxifen is a potent hepatocarcinogen in rats (1–3) and tamoxifen-DNA adducts have been readily
detected by $^{32}$P-postlabelling in the livers of rats given tamoxifen by gavage at doses of 5–45 mg/kg/day (24). However, in these same animals tamoxifen–DNA adducts were not detected in the peripheral lymphocytes nor in the kidney, lung, spleen and uterus (24). Thus, in a species in which tamoxifen is clearly a genotoxic carcinogen, DNA adduct formation is largely confined to the target organ and circulating white blood cells are not a suitable surrogate tissue in which to observe genotoxicity.

From the point of view of DNA adduct formation, studies conducted so far indicate that human cells have a lower capacity to activate tamoxifen than rodent cells. DNA adducts were not detected in human hepatocytes incubated with tamoxifen, although a low level of adducts was formed by $\alpha$-hydroxytamoxifen, the putative proximate carcinogenic metabolite (13). Also, in a small study of seven liver samples from tamoxifen-treated women adduct levels were apparently no higher than in seven liver samples from untreated women (25), although adduct levels were reported to be much higher in both groups (26 adducts/10$^7$ nucleotides), but with no clear adduct spots in the thin layer chromatograms, than reported here for blood cells. Nevertheless, in our own study of explants of human endometrium we did not find evidence for tamoxifen–DNA adduct formation when the explants were incubated with tamoxifen, although the metabolite $\alpha$-hydroxytamoxifen did form adducts (26). More significantly, tamoxifen–DNA adducts were not detected in endometrial tissue from 18 women who had been taking tamoxifen (10–40 mg/day) for from 3 months to 9 years (26).

Acknowledgements

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References


Table I. Details of breast cancer patients and controls

<table>
<thead>
<tr>
<th>Identification number</th>
<th>Age</th>
<th>Dose of tamoxifen citrate (mg/day)</th>
<th>Duration of treatment of tamoxifen</th>
<th>Serum concentration (ng/ml)</th>
<th>Other concurrent or recent medication</th>
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<tr>
<td>P1</td>
<td>54</td>
<td>20</td>
<td>10 months</td>
<td>101</td>
<td>Prempak-C</td>
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<td>P2</td>
<td>53</td>
<td>20</td>
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<td>40</td>
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<td>108</td>
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Post-labelling of DNA from women taking tamoxifen.


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