Breast, uterine or colon tissue is removed, and DNA extracted analysed for [^{14}C]. Initial data indicate that although tamoxifen is reaching the uterine tissue, no DNA adducts can be detected. A further study with an increased specific activity is being undertaken.

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VI.5 Tamoxifen-DNA Adducts in Breast Cancer Patients

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TAMOXIFEN IS an important anticancer agent used in a long-term adjuvant therapy of breast cancer. A side-effect of treatment is the risk of secondary cancer in uterine endometrium. An estimate of 10% (100 patients/year) of endometrial cancer is diagnosed in Sweden in patients who have received tamoxifen earlier in their life. We have recently developed a ^{32}P-postlabelling method, applying high-performance liquid chromatography (HPLC) and radioactivity detection for a sensitive and reproducible measurement of tamoxifen adducts in humans [1]. Using the method we demonstrated DNA adducts of tamoxifen in total white blood cell and endometrial cell DNA in blinded studies [2, 3]. The measured levels of adducts were 5/10^9 nucleotides in white blood cells and one-half in endometrial DNA. There have been further methods development and further analysis from other human and animal tissues. Additionally, attempts have been made to identify specific adducts with the help of standard compounds.

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VI.6 Detection of DNA Adducts in the Human Endometrium: a Lack of Evidence

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A study exploring DNA adducts in human endometria, utilising TLC-^{32}P-postlabelling, found no evidence for adducts in tamoxifen-treated women, however a subsequent study utilising HPLC-^{32}P-postlabelling suggested that very low levels could be detected. We have sought to confirm or dispute these findings by reproducing the HPLC methodology at two centres, analysing endometrial DNA from 20 patients treated with 20 mg/day tamoxifen for between 22 and 65 months. Liver DNA isolated from tamoxifen-treated rats was used as a positive control. We found no convincing evidence for tamoxifen-derived DNA adducts in human endometrium. HPLC elution profiles were indistinguishable from those obtained from untreated women and from women taking the analogue toremifene.

THE ASSOCIATION of the drug tamoxifen with human endometrial cancer has been the cause of considerable controversy. Debate has centred upon whether tamoxifen is genotoxic to human tissues, as is the case in the rat where it is metabolised to a reactive intermediate (α-hydroxytamoxifen; the proximate carcinogen) which gives rise to high levels of DNA adducts in the liver (a genotoxic event). A study exploring DNA adduct formation in human endometria, utilising thin layer chromatography (TLC)-^{32}P-postlabelling, found no evidence for such adducts in women treated with the drug [1]. Furthermore, in this study it was demonstrated, using endometrial explant cultures, that the minimum concentration of α-hydroxytamoxifen required to give detectable adducts in the cultures was 10,000 times greater than the concentration measured as a circulating metabolite in patients receiving tamoxifen treatment. However, a subsequent study utilising high performance liquid chromatography (HPLC)-^{32}P-postlabelling suggested that very low levels of adducts could be detected in 5 out of 7 endometrial samples from 6 patients treated with 20 or 40 mg/day tamoxifen [2].

Through a joint centre approach, we have sought to confirm or dispute these findings by reproducing the HPLC methodology at both Imperial College and the Institute of Cancer Research, analysing endometrial DNA from 20 patients treated with 20 mg/day tamoxifen for periods ranging from 22 to 65 months. In initial analyses, directly reproducing the published HPLC method [2], we compared HPLC elution profiles of the human DNA with those of liver DNA from tamoxifen-treated rats and found no evidence for the presence of tamoxifen-derived DNA adducts in any of the human samples. Furthermore, we found no evidence for DNA adducts induced by the tamoxifen analogue, toremifene in endometria from 8 patients treated with 60 mg/day for 6 or 12 months. In subsequent analyses we altered the primary and secondary digestions of DNA samples, increasing the concentrations of micrococcal nuclease, spleen phosphodiesterase and nuclease P1 (by 2.5 times) and increased the ^{32}P-ATP concentration (by 3.6 times). Under these conditions we increased the detection of the rat liver tamoxifen adduct standard by approximately 4-fold. Utilising the improved protocol we re-analysed DNA from 14 control patients, 14 tamoxifen-treated patients and 6 toremifene-treated patients. With these conditions we found that we could detect radioactive peaks at, or very close to, the retention time of the tamoxifen adduct standard. However, peaks with very similar retention times to the standard were detected in 5/14 control patients (peak height range 2–18 times background), 7/14 tamoxifen patients (peak height range 2–14 times background) and 3/6 toremifene patients (peak height range 2–5 times background). Hence, we have demonstrated that the putative 'tamoxifen adducts' that are detectable using HPLC-^{32}P-postlabelling in the DNA of tamoxifen-treated patients, are also present in a similar proportion of control patients and toremifene-treated patients. Thus, these 'adducts' are probably endogenous species or artefacts detectable by the highly sensitive ^{32}P-postlabelling procedure. On the basis of this evidence and our previous studies, it would appear that neither tamoxifen nor toremifene is metabolised in women to electrophiles that bind to DNA in endometrium in sufficient quantity to implicate a genotoxic mechanism for carcinogenicity. However, the possibility that tamoxifen and toremifene may have other effects on human endometrial tissue cannot be ruled out.

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