IN ORDER to examine whether or not long-term tamoxifen treatment was associated with any changes in the plasma or endometrial IGF-system and whether or not these changes were associated with tamoxifen-related uterine and endometrial side-effects, the expression of mRNA for IGF-I, IGF-II and IGFBPs 1–6 in the endometrium (dot and Northern blot techniques) and plasma IGF-I, IGFBP-1 and IGFBP-3 concentrations were determined in postmenopausal breast cancer patients with and without tamoxifen treatment.

The most important finding in our study was that the plasma IGFBP-1 concentrations were significantly increased in tamoxifen patients compared to controls. In contrast to previous reports [2], there were no significant differences in the mean plasma concentrations of IGF-I and IGFBP-3 between the groups. The cross-sectional nature of the study may be an explanation on why we did not find significantly decreased IGF-I levels in patients receiving long-term tamoxifen treatment.

We found a significant correlation between the plasma IGF-I concentration and the volume of the uterus in the tamoxifen ($r = 0.34$, $P = 0.037$), but not in the control group ($r = 0.07$, NS). Plasma IGF-I and IGFBP-1 concentrations in the tamoxifen group were significantly higher in women with a proliferative endometrium than in women with an atrophic endometrium.

IGF-I mRNA was detectable in all endometrial samples of the postmenopausal breast cancer patients with no significant quantitative difference between the tamoxifen-treated and the control patients. IGF-II mRNA expression was not detected in the endometrium. Of the 6 IGFBPs, the mRNA of IGFBP-2, -3, 4 and -6 were detected in all endometrial specimens. In contrast, IGFBP-1 mRNA was not detected in any of the samples. The expression of IGFBP-2 and -4 mRNA predominated in the endometrium of the tamoxifen-treated patients. A statistically significant difference was found between the tamoxifen-treated and the control patients in IGFBP-2 detection. The highest levels of IGFBP-2 and -3 mRNA expression were detected in a tamoxifen-treated patient with grade 1 endometrial adenocarcinoma. The biological significance of this difference between tamoxifen-treated and control patients remains unknown.

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I.6 Tamoxifen Metabolism and Activation: the Reason for Interspecies Differences

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Tamoxifen induces liver tumours in rats by a genotoxic mechanism. Activation to DNA binding products involves, firstly, cytochrome P450-mediated hydroxylation at the α-ethyl position. This occurs in both rats and humans. α-Hydroxytamoxifen is then further metabolised to a sulphate ester in rat hepatocytes by hydroxysteroid sulphotransferase (hST). This activation occurs in bacterial and mammalian cells expressing rat hST, but not in cells expressing human hST. It is proposed that the activation pathway in rats does not occur to a significant extent in humans and thus may not account for the increase in endometrial cancer among women taking tamoxifen.

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TAMOXIFEN IS a human and animal carcinogen [1]; it causes endometrial cancer in women and is a potent inducer of liver tumours in rats. In order to assess fully the long-term risks of tamoxifen therapy or prophylaxis it is important to understand its mechanism of tumour induction and to what extent extrapolations between species can be made.

Tamoxifen has many properties of a genotoxic carcinogen. It forms covalent tamoxifen-DNA adducts in rat liver *in vivo* and in cultures of rat hepatocytes, but not in human hepatocytes [2]. The metabolite α-hydroxytamoxifen forms the same pattern of adducts in rat liver cells, but at a 50-fold higher level, indicating that it is an intermediate in the metabolic pathway that activates tamoxifen [3].

However, DNA binding by α-hydroxytamoxifen is 300-fold lower in human hepatocytes than in rat hepatocytes [2]. When the synthetic derivative α-acetoxytamoxifen is reacted with DNA, its adducts are chromatographically indistinguishable from those formed by tamoxifen and α-hydroxytamoxifen in cells, and the major adduct formed is (E)-α-(N^2-deoxyguanosinyl)tamoxifen [4]; minor adducts include the *cis*-isomer of this and analogous *cis*- and *trans*-substitution at the N^6-position of adenine residues in DNA [5]. It is not yet clear whether or not tamoxifen forms DNA-adducts in human endometrium [6, 7]. Thus it is apparent that the carcinogenicity of tamoxifen in rats is mediated by a genotoxic mechanism, but it is less clear what the mechanism of tumour formation in human endometrium is, or whether there are additional long-term risks to other organs from treatment with tamoxifen.

Our recent studies suggest reasons for the apparent interspecies differences. The further activation of α-hydroxytamoxifen to DNA binding products in rat hepatocytes is highly dependent on sulphate [8]. DNA binding of both tamoxifen (10 μM) and α-hydroxytamoxifen (1 μM) was 10-fold higher with medium containing 10 μM sulphate than in sulphate-free medium, and the level of DNA adduct formation was directly proportional to the concentration of sulphate in the medium. Furthermore, inhibition of hydroxysteroid sulphotransferase (hST) by dehydroisoandrosterone-3-sulphate (0.1 mM) reduced DNA binding of both compounds by up to 80%. It is concluded that the activation of tamoxifen in rat liver cells proceeds predominantly through hydroxylation followed by sulphate ester formation at the α-position of the ethyl group.

Studies of the metabolism and DNA binding of tamoxifen and α-hydroxytamoxifen in bacteria and mammalian cells genetically engineered to express specific enzymes confirm these findings and indicate that tamoxifen is metabolised to α-hydroxytamoxifen by CYP3A4, which is further activated by hST. However α-hydroxytamoxifen is a much better substrate for the rat isoform of the sulphotransferase than for the human form. Thus, adducts are formed and mutant colonies are generated in *S. typhimurium* TA1538 and in V79 cells expressing rat hST treated with α-hydroxytamoxifen, but not in cells expressing human hST.

While these results account for the metabolic activation of tamoxifen in rat liver, they do not exclude the possibility of other pathways in other species or organs. It remains to be established whether or not other pathways contribute to the carcinogenicity of tamoxifen.

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