MINIREVIEW

Tamoxifen Experimental Carcinogenicity Studies: Implications for Human Effects

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Tamoxifen is an effective antiestrogen in the treatment of breast cancer and is considered highly safe (1). In recent years, several trials have been initiated in women to evaluate its potential for the prevention of breast cancer (2). Such long-term administration of a medication to healthy people requires a substantial degree of safety. This review examines experimental carcinogenicity and mechanistic studies on tamoxifen and the implications for human effects.

Findings

Carcinogenicity Studies. Tamoxifen has been demonstrated to be hepatocarcinogenic in rats in four independent studies (3–6). In a 1-year study, Hard *et al.* (6) reported a 100% incidence of liver cancer in female Sprague-Dawley strain rats given 22.6 mg/kg/day by gavage and a 44% incidence of liver cancer in animals given 11.3 mg/kg/day compared with none in controls. At 22.6 mg/kg/day, tamoxifen also induced a substantial incidence of liver preneoplastic lesions by 3 months. In a 2-year study, Greaves *et al.* (5) found in Wistar strain-derived rats given 5 mg/kg/day, a 16% incidence of liver cancer in males compared with 1% in controls and a 12% incidence in females compared with none in controls. Thus far, in a lifetime study, a noncarcinogenic dose of tamoxifen in rats has not been reported.

In addition to the liver findings, in rats a low incidence of granulosa cell tumors of the ovary was reported by Hard *et al.* (6).

Carcinogenicity studies in the Alderly Park strain mouse were reported by Tucker *et al.* (7). At doses of up to 50 mg/kg/day, the only tumor increase was interstitial cell tumors of the testes.

These carcinogenicity studies were, of course, done at administered doses higher than the therapeutic dose (about 0.3 mg/kg), as is customary in such studies. This is appropriate because rodents generally biotransform xenobiotics more rapidly than humans and thereby achieve a lower internal dose at the same administered dose (8). In fact, Hard *et al.* (6) found that the blood level of tamoxifen at the dose of 11.3 mg/kg/day in rats was about 146 ng/ml, which is approximately the human blood level at the therapeutic dose (9). Likewise, Greaves *et al.* (5) reported a mean serum tamoxifen concentration of 166 ng/ml in rats given 5 mg/kg/day. Thus, the doses used in the carcinogenicity studies are quite appropriate to assessment of human safety.

Recently, Vancutsem *et al.* (10) reported that the tamoxifen-induced rat liver tumors from the study of Hard *et al.* (6) were characterized by a high frequency of mutations in the p53 tumor suppressor gene. These mutations were all clustered in either codon 231 (75%) or codon 294 (33%), indicating a high degree of specificity.

Mechanistic Considerations. In different species and in different tissues, tamoxifen has variable estrogen agonist and antagonist properties (11). To assess tamoxifen's hepatocarcinogenicity in rats, these effects must be considered. In rat liver, tamoxifen produces both estrogen-like and antiestrogenic effects (12). The estrogenicity of tamoxifen, however, is unlikely to be the sole basis for hepatocarcinogenicity since the related antiestrogen, toremifene, which has estrogenic effects in rat liver comparable to those produced by carcinogenic doses of tamoxifen (12), does

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not produce liver tumors at a dose approximately 4-fold that of a carcinogenic dose of tamoxifen (6). Moreover, tamoxifen, produces other effects that could be the basis for carcinogenicity.

Tamoxifen was first reported by Han and Liehr (13) to produce covalent DNA adducts in rat liver. In female Sprague-Dawley rats given 20 mg/kg/day for 6 days, they found five adducts totaling 127 adducts per 10^9 bases. Tamoxifen at a single injection of 5 mg/kg also produced adducts in female Syrian hamsters. White *et al.* (14) reported that tamoxifen at 45 mg/kg for 7 days produced seven adducts. With 4 days of exposure, the total adducts were 116 per 10^8 nucleotides. These findings have been confirmed (6, 15) and, importantly, human liver microsomes have also been shown to mediate DNA adduct formation by tamoxifen (16). These demonstrations of DNA-reactivity of tamoxifen in liver or mediated by liver microsomes are supported by studies showing cytochrome P450-mediated activation of tamoxifen to a reactive species (17). It has been hypothesized that tamoxifen can be activated by formation of an epoxide at the ethylene double bond (6) or by α-oxidation of the ethyl side chain (18). Tamoxifen 1,2-epoxide reacts with DNA to yield adducts similar to those found in DNA from livers of tamoxifen-exposed rats (19). The major adduct was with guanine.

Many chemicals are established to be carcinogenic as a consequence of reaction with DNA and production of mutations (20). At present, this appears to be the most likely explanation for the carcinogenicity of tamoxifen. This is supported by the rapid induction of preneoplastic liver lesions by tamoxifen.

Conclusion

Tamoxifen is clearly DNA-reactive and carcinogenic in rat liver. Since most human carcinogens are DNA-reactive carcinogens in rodents (20), such findings with tamoxifen raise grave concern. The observations that tamoxifen is also DNA-reactive in hamster liver and is activated by human liver microsomes to form DNA adducts provide evidence that its toxicity is not confined to the rat. Accordingly, tamoxifen must be presumed to be a human cancer hazard, unless some mechanistic basis for nonsusceptibility of humans is discovered. The presumption of hazard is reinforced by the finding of p53 mutations in the tamoxifen-induced liver tumors. The p53 gene is the most commonly mutated gene in human neoplasms (21, 22).

Accordingly, we have recommended that women being given tamoxifen should be carefully monitored for carcinogenic effects (23–25). It is important in this regard to recognize that the target site for human carcinogens is not always the same as in rodents (20).

1. Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic or immune therapy: 133 Randomized trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Lancet **339**:71–85, 1992.
2. Powles TJ. The case for clinical trials of tamoxifen for prevention of breast cancer. Lancet **340**:1145–1147, 1992.
3. Williams GM, Iatropoulos MJ, Djordjevic MV, Kaltenberg OP. The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. Carcinogenesis **14**:315–317, 1993.
4. Hirsimäki P, Hirsimäki Y, Nieminen L, Payne BJ. Tamoxifen induces hepatocellular carcinoma in rat liver: A 1-year study with two antiestrogens. Arch Toxicol **67**:49–54, 1993.
5. Greaves P, Goonetilleke R, Nunn G, Topham J, Orton T. Two-year carcinogenicity study of tamoxifen in Alderley park Wistar-derived rats. Cancer Res **53**:3919–3924, 1993.
6. Hard GC, Iatropoulos MJ, Jordan K, Radi L, Kaltenberg OP, Imondi AR, Williams GM. Major difference in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Crl:CD(BR) rats. Cancer Res **53**:4534–4541, 1993.
7. Tucker MJ, Adam HK, Patterson JS. Tamoxifen. In: Laurence DR, McLean DEM, Wetherall M, Eds. Safety Testing of New Drugs: Laboratory Predictions and Clinical Performance. London: Academic Press, pp125–161, 1984.
8. Iatropoulos M. Comparative histokinetic and xenodynamic considerations in toxicity. In: Welling PG, de la Iglesia FA, Eds. Drug Toxicokinetics. New York: Marcel Dekker, pp245–266, 1993.
9. Robinson SP, Langan-Fahey L, Johnson DA, Jordan VC. Metabolites, pharmacodynamics and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab Dispos **19**:36–43, 1991.
10. Vancutsem PM, Lazarus P, Williams GM. Frequent and specific mutations of the rat p53 gene in hepatocarcinomas induced by tamoxifen. Cancer Res **54**:3864–3867, 1994.
11. Kangas L. Agonistic and antagonistic effects of antiestrogens in different target organs. Acta Oncol **31**:143–146, 1992.
12. Kendell ME, Rose DP. The effects of diethylstilbestrol, tamoxifen, and toremifene on estrogen-inducible hepatic proteins and estrogen receptor proteins in female rats. Toxicol Appl Pharmacol. **114**:127–131, 1992.
13. Han X, Liehr JG. Induction of covalent DNA adducts in rodents by tamoxifen. Cancer Res **52**:1360–1363, 1992.
14. White INH, de Matteis F, Davies A, Smith LL, Crofton-Sleigh C, Venitt S, Hewer A, Phillips DH. Genotoxic potential of tamoxifen and analogues in female Fischer F344/n rats, DBA/2 and C57BL/6 mice and in human MCL-5 cells. Carcinogenesis **13**:2197–2203, 1992.
15. Montandon F, Williams GM. Comparison of DNA reactivity of the polyphenylethylene hormonal agents diethylstilbestrol, tamoxifen and toremifene in rat and hamster liver. Arch Toxicol **68**:272–275, 1994.
16. Pathak DN, Bodell WJ. DNA adduct formation by tamoxifen with rat and human liver microsomal activation systems. Carcinogenesis **15**:529–532, 1994.
17. Mani C, Kupfer D. Cytochrome P450 mediated activation and irreversible binding of the antiestrogen tamoxifen to proteins in rat and human liver: Possible involvement of flavin-containing monoxygenases in tamoxifen activation. Cancer Res **51**:6052–6058, 1991.
18. Potter GA, McCague R, Jarman M. A mechanistic hypothesis for DNA adduct formation by tamoxifen following hepatic oxidative metabolism. Carcinogenesis **15**:439–442, 1994.
19. Phillips DH, Hewer A, White INH, Farmer PB. Co-chromatography of a tamoxifen epoxide-deoxyguanylic acid adduct with a

major DNA adduct formed in the livers of tamoxifen-treated rats. Carcinogenesis **15**:793–795, 1994.
20. Williams GM, Weisburger JH. Chemical carcinogenesis. In: Amdur MO, Doull J, Klaassen CD, Eds. Casarett and Doull's Toxicology The Basic Sciences of Poisons (4th ed). New York: Pergamon Press, p127, 1991.
21. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science **253**:49–53, 1991.
22. Lane DP. p53, guardian of the genome. Nature **358**:15–16, 1992.
23. Williams GM, Iatropoulos MJ, Hard GC. Long-term prophylactic use of tamoxifen: Is it safe? Eur J Cancer Prev **1**:386–387, 1992.
24. Hard HC, Williams GM, Iatropoulos MJ. Tamoxifen and liver cancer. Lancet **342**:444–445, 1993.
25. Williams GM, Hard GC. Correspondence re: R.R. Love the national surgical adjuvant breast project (NSABP) breast cancer prevention trial revisited. Cancer Epidemiol Biomark Prev **2**:403–407, 1993, **3**:185–186, 1994.