GENOTOXICITY

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Cumulative exposure to tamoxifen: DNA adducts and liver cancer in the rat

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Abstract Tamoxifen is a potent rat liver carcinogen, currently being used as a long-term chemopreventative for breast cancer in healthy women. The mechanism by which tamoxifen causes liver cancer in rats is known to be associated with the accumulation of tamoxifen DNA adducts in this organ. We have examined the dose-response relationship of tamoxifen-induced DNA adducts in the liver and the subsequent increase in the development of liver cancer, with and without phenobarbital promotion. Female Wistar (Han) rats were fed 420 ppm tamoxifen in the diet for 0, 1, 4, 8 or 12 weeks after which time rats were either examined immediately for hepatic tamoxifen-induced DNA damage using the $^{32}$P-postlabelling assay, or left for lifetime for tumour assessment. A proportion of rats left for lifetime study were given phenobarbital in their drinking water. There was a clear dose-response relationship with respect to duration of tamoxifen exposure for both accumulation of DNA adducts and lifetime risk of liver cancer. In the absence of phenobarbital promotion there was a threshold value for tamoxifen-induced DNA adducts (180 adducts/10⁶ nucleotides) and the subsequent induction of liver cancer. This study demonstrates the relationship between the accumulation of hepatic tamoxifen-induced DNA adducts and the development of liver cancer and establishes the threshold for hepatocarcinogenesis in terms of DNA adduct formation. These data could provide useful information in interpreting the relevance of low levels of DNA adducts in humans.

Keywords Liver cancer · Tamoxifen · Phenobarbital · DNA adduct

Introduction

A major concern with the chemopreventative use of tamoxifen in women has been the risk of cancers developing by a genotoxic mechanism, similar to that by which tamoxifen causes liver cancer in rats (Greaves et al. 1993; Hirsimaki et al. 1993; Williams et al. 1993; Carthew et al. 1995a, 1995b). In rats there is a causal relationship between tamoxifen-induced DNA damage and liver tumour formation (Carthew et al. 1995a). Tamoxifen requires metabolic activation before it can react with DNA to form adducts that can be visualised and quantitated by $^{32}$P-postlabelling with HPLC separation (Martin et al. 1995; Brown et al. 1999). Tamoxifen is metabolised primarily via $N$-demethylation, 4-hydroxylation, $\alpha$-hydroxylation and $N$-oxidation pathways. $\alpha$-Hydroxytamoxifen is a minor metabolite in rat liver but is responsible for the formation of major DNA adducts in this organ. Following sulphotransferase-mediated sulphonation (Glatt et al. 1998; Shibutani et al. 1998), adducts are formed with DNA via highly reactive carbocations (Fig. 1; Osborne et al. 1996). The major adduct formed, $\alpha$-(deoxyguanosine-$N^2$-yl)tamoxifen, exists as four diastereoisomers (Dasaradhi and Shibutani 1997). $N$-Desmethyltamoxifen, a major tamoxifen metabolite in rat liver, is thought to undergo $\alpha$-hydroxylation and sulphonation to form a second major adduct in liver of tamoxifen-treated rats, $\alpha$-(deoxyguanosine-$N^2$-yl-$N$-desmethyltamoxifen (Fig. 1; Brown et al. 1999; Phillips et al. 1999; Rajaniemi et al. 1999; Da Costa et al. 2000). 4-Hydroxytamoxifen is responsible for minor adducts in livers of tamoxifen-treated rats, which probably arise via a quinone methide (Fig. 1; Marques and Beland 1997; Martin et al. 1998). Tamoxifen adducts cause a high incidence of mutations in livers of $\beta$/$\beta$ transgenic rats.

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with an increased proportion of G:C to T:A transversions compared with controls (Davies et al. 1997). Studies in vitro have also shown that tamoxifen adducts have mutagenic potential (Shibutani and Dasaradhi 1997; Lowes et al. 1999).

In tamoxifen-treated women there is an increase in the incidence of uterine endometrial tumours (Fornander et al. 1993; Kedar et al. 1994; van Leeuwen et al. 1994). Low levels of DNA adducts have been detected in the endometrial tissue of tamoxifen-treated women, and these have been shown to co-elute with the major adduct peaks found in the livers of tamoxifen-treated rats (Hemminki et al. 1996; Shibutani et al. 1999). However, there is still some controversy as to whether adducts are actually formed in humans (Carmichael et al. 1999) and if they are their role in the carcinogenic pathway still has to be elucidated.

Because the rat is extremely sensitive to the hepatocarcinogenic effects of tamoxifen, that species has been used in this present study to investigate the dose-response relationship between the formation of DNA adducts and the subsequent development of liver tumours. It has been possible to examine whether a practical threshold exists for the presence of tamoxifen DNA adducts and the development of liver cancer over the 36-month period employed in this study. Phenobarbital treatment was also included to determine whether the DNA adduct initiation could be promoted in a classical two-stage carcinogenicity study.

**Fig. 1** Proposed pathways of activation and detoxication of tamoxifen. Tamoxifen undergoes phase I metabolism including α-hydroxylation, N-demethylation and 4-hydroxylation. 4-Hydroxytamoxifen can be further oxidised to give a quinone methide that reacts with DNA to give adducts α-(deoxyguanosine-N²-yl)-4-hydroxytamoxifen (a). Phase II activation at the α position by hydroxysteroid sulphotransferases yields reactive carboxations which also react with DNA to give adducts α-(deoxyguanosine-N²-yl)tamoxifen (b), α-(deoxyguanosine-N²-yl)-N-desmethyltamoxifen (c) and α-(deoxyguanosine-N²-yl)-N,N-didesmethyltamoxifen (d). The major routes of DNA adduct formation are via α-hydroxytamoxifen and N-desmethyltamoxifen. The major detoxification pathway is by glucuronidation.

**Materials and methods**

**Chemicals**

Tamoxifen was kindly donated by Zeneca pharmaceuticals (Macclesfield, UK). Proteinase K, RNase A and T₁ were from Sigma Chemical Co. (Poole, Dorset, U.K.). Chemical reagents and solvents of analytical grade were purchased from Aldrich Chemical Co. (Poole, Dorset, UK) or Fisher Scientific Ltd. (Loughborough, Leicestershire, U.K.) unless otherwise stated.

**Animals and treatments**

Female Wistar (Han) rats (6–8 weeks of age), 300 in total allocated to 5 groups of 60 animals, were fed 420 ppm tamoxifen in the diet.