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The antioxidant action of tamoxifen and its metabolites

Inhibition of lipid peroxidation

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The anti-oestrogen drug tamoxifen is an inhibitor of lipid peroxidation in rat liver microsomes and in phospholipid liposomes. Its *cis* isomer and *N*-desmethyl form are weaker inhibitors, but 4-hydroxytamoxifen is much more powerful. It is possible that the antioxidant property of tamoxifen might contribute to its biological actions.

Tamoxifen; Cancer, breast; Lipid peroxidation; Antioxidant; 4-Hydroxytamoxifen

1. INTRODUCTION

The anti-oestrogen drug tamoxifen (1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1-ene, *trans* isomer), one of a series of synthetic triphenylethylene compounds, is widely used in the chemotherapy of breast cancer (reviewed in [1]) and has been proposed as a prophylactic agent against this disease. The antitumour activity of tamoxifen is believed to be largely due to its binding to oestrogen receptors, but there is also evidence for one or more additional mechanisms of action [1,2] including an ability to inhibit growth [3] and the synthesis of RNA and proteins (H.W., unpublished data) in yeast cells, as well as to inhibit protein synthesis in other eukaryotic cells [4] The *cis* isomer of tamoxifen has much less biological effect. In humans, tamoxifen is extensively metabolized to give 4-hydroxytamoxifen and *N*-desmethyltamoxifen [5]. Their structures are shown in Fig. 1.

Human breast tumour tissue shows differences in the rates of free radical reactions and in antioxidant protection when compared to healthy breast tissue. These include increases in superoxide dismutase activity [6], an ability to produce O_2^- and H_2O_2 from some anti-cancer drugs [7] and probably a decreased ability to undergo lipid peroxidation [8]. Indeed, changes in the rate of lipid peroxidation seem to be a general feature of cancerous cells and may be a prerequisite to cell division [8–13]. The carcinogenicity of the synthetic oestrogen diethylstilboestrol has been suggested to involve DNA damage caused by increased oxygen radical formation [14]. By contrast, oestradiol, oestriol and oestrone have been reported to inhibit lipid peroxidation in vitro [15,16], as do their catechol metabolites [17]. In view of the amount of literature linking oestrogens, free radical reactions and tumour development [6–19], we thought it of interest to examine the action of tamoxifen and its metabolites on lipid peroxidation. The well-established rat liver microsomal system was used as a substrate, and peroxidation was started by adding iron(III) and ascorbic acid [20]. Studies with NADPH-dependent peroxidation [21] are also reported for comparison.

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2. MATERIALS AND METHODS

2.1. *Preparation of microsomes*

Rat liver microsomes were prepared from the livers of adult male rats by standard differential centrifugation techniques as described in [22]. Liposomes were prepared from ox-brain phospholipids [22].

Tamoxifen and 17-β-oestradiol were purchased from Sigma. Purity was at least 99%. 4-Hydroxytamoxifen, *N*-desmethyltamoxifen and *cis*-tamoxifen were kindly supplied by ICI Pharmaceuticals plc.

2.2. *Lipid peroxidation*

Reaction mixtures contained in a final volume of 1.0 ml, microsomal fraction (0.25 mg microsomal protein), 10 mM KH_2PO_4-KOH buffer pH 7.4 and 5 μl of ethanol or test compound dissolved in ethanol. Peroxidation was started by adding, to give the final concentrations stated, $FeCl_3$ and ascorbate (both 100 μM) or $FeCl_3$ (100 μM), ADP (1.7 mM) and NADPH (0.4 mM). The ADP and $FeCl_3$ were premixed before addition to the reaction mixture. Solutions of $FeCl_3$, ADP and ascorbate were made up fresh immediately before use. Incubations were carried out at 37°C for 20 min (unless otherwise stated). The amount of lipid peroxidation was measured by the formation of thiobarbituric acid-reactive substances (TBARS) as described in [22]. HCl (0.5 ml, 2.5% v/v) was added to each reaction mixture, followed by 0.5 ml of thiobarbituric acid solution (1% w/v in 50 mM sodium hydroxide). After heating at 80°C for 30 min the chromogen was extracted with 2 ml of

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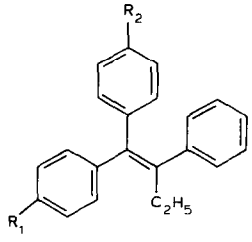

Fig. 1. Structure of tamoxifen and its derivatives.

	R_1	R_2
Tamoxifen	H	$OCH_2CH_2N(CH_3)_2$
4-Hydroxytamoxifen	OH	$OCH_2CH_2N(CH_3)_2$
N-Desmethyltamoxifen	H	$OCH_2CH_2NHCH_3$

butan-1-ol and A_{532} of the upper (organic) layer was measured. Peroxidation of liposomes induced by $FeCl_3$ and ascorbic acid was measured as described above, except that microsomes were replaced by liposomes (1 mg/ml).

3. RESULTS

When Fe(III) and ascorbic acid are added to rat liver microsomes at pH 7.4, rapid peroxidation occurs that can be measured by the TBA test [20]. Fig. 2 shows that tamoxifen inhibited the peroxidation to approximately the same extent as 17-β-oestradiol. *Cis*-tamoxifen and *N*-desmethyltamoxifen were much less effective inhibitors, whereas 4-hydroxytamoxifen was a much more powerful inhibitor of peroxidation. Table I lists

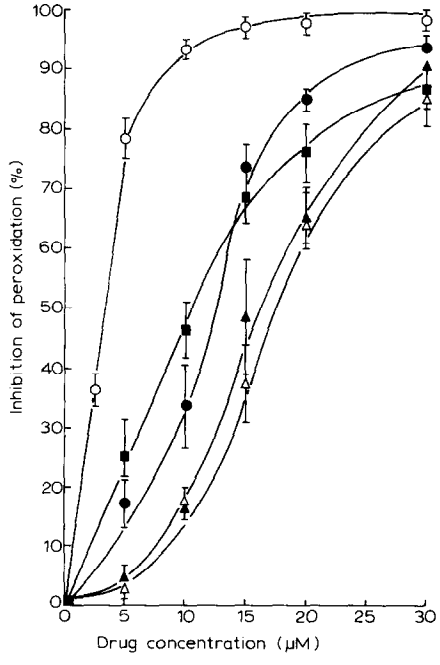

Fig. 2. Concentration-dependent inhibition of microsomal lipid peroxidation induced by $FeCl_3$ and ascorbate. (●) Tamoxifen; (○) 4-hydroxytamoxifen; (▲) *cis*-tamoxifen; (△) *N*-desmethyltamoxifen; (■) 17-β-oestradiol. Results are mean ± SD, n = 3–6.

Table I

IC_{50} values for the inhibition of microsomal lipid peroxidation by tamoxifen, tamoxifen metabolites and 17-β-oestradiol

Compound	IC_{50} (μM)	
	Fe(III)-ascorbate system	Fe(III)-ADP/NADPH system
Tamoxifen	12.5	20
4-Hydroxytamoxifen	3.1	3.5
cis-Tamoxifen	15.6	23.3
N-Desmethyltamoxifen	17.5	25
17-β-Oestradiol	10.6	16

Values are deduced from the graphs shown in Fig. 2, in which each point represents the mean ± SD of 3–6 separate assays

the IC_{50} values for the compounds tested. Tamoxifen and its metabolites inhibited peroxidation by an approximately constant percentage throughout the course of the incubation (Fig. 3): there was no evidence for the 'lag period', followed by an acceleration of peroxidation to the control rate, that is often observed when chain-breaking antioxidants are added to peroxidizing microsomes. None of the compounds tested interfered with the TBA test: no inhibition was seen when the compounds were added to peroxidizing microsomes with the TBA reagents instead of at the beginning of the incubation. The compounds were added dissolved in ethanol: the amount of ethanol present in our reaction mixtures had no significant effect on the rate of peroxidation.

Microsomal peroxidation can also be started by addition of Fe(III)-ADP and NADPH [21]. It was found

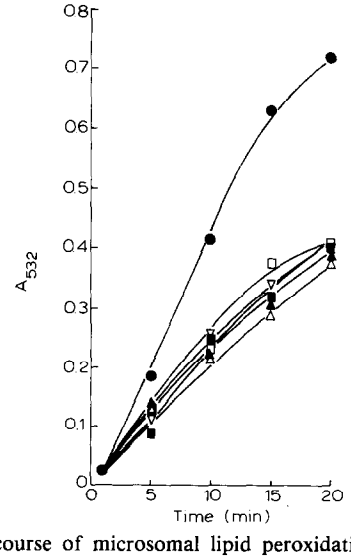

Fig. 3. Time course of microsomal lipid peroxidation induced by $FeCl_3$ and ascorbate: the effect of tamoxifen and its derivatives added at their IC_{50} concentrations. (●) Control (ethanol only added); (□) 12.5 μM tamoxifen; (△) 3.1 μM 4-hydroxytamoxifen; (▲) 17.5 μM *N*-desmethyltamoxifen; (■) 15.6 μM *cis*-tamoxifen; (▽) 10.6 μM 17-β-oestradiol. Concentrations quoted are the final concentrations in the reaction mixtures.

that tamoxifen, *cis*-tamoxifen, *N*-desmethyltamoxifen and 17-β-oestradiol were weaker inhibitors of peroxidation in this enzyme-dependent system than when peroxidation was started with Fe(III) and ascorbate (Table I), whereas 4-hydroxytamoxifen was approximately equally effective. Table I summarizes the IC_{50} values. It has been observed previously (e.g. [23,24]) that several antioxidants are less effective in the microsomal system when NADPH and Fe(III)-ADP are used to induce the peroxidation instead of ascorbic acid and Fe(III).

Tamoxifen and 4-hydroxytamoxifen were also found to inhibit the Fe(III)/ascorbate-induced peroxidation of a completely different lipid substrate, ox-brain phospholipid liposomes. IC_{50} values were 68 and 6.3 μM, respectively.

4. DISCUSSION

17β-Oestradiol has been claimed to be a potent inhibitor of lipid peroxidation [15–17], a result confirmed in the present study. However, we have now shown that tamoxifen exerts a similarly potent effect in the microsomal system.

Examination of the chemical structure of tamoxifen (Fig. 1) shows no apparent reason why it should act as a chain-breaking inhibitor of peroxidation, since there is no group bearing an easily donatable hydrogen atom. In any case, chain-breaking antioxidants usually introduce a lag period into the peroxidation time-course: this was not observed here. The *cis*- and *N*-desmethyl forms of tamoxifen were weaker inhibitors, which perhaps suggests that the conformation of the whole molecule is important and that it acts by modifying the structure of the microsomal and liposomal membranes so as to decrease the propagation rate of lipid peroxidation. The ability of various sterols and steroids to influence membrane structure is well-established (e.g. [25]).

4-Hydroxytamoxifen was a much more powerful inhibitor of lipid peroxidation than tamoxifen. It could be that the phenolic OH group confers chain-breaking antioxidant activity as well as the putative effects on membrane structure discussed above. The fact that tamoxifen and its metabolites will partition into membranes in vivo [26] suggests that their antioxidant activity might have physiological relevance.

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