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EVIDENCE FOR THE METABOLIC ACTIVATION OF NON-STEROIDAL ANTIOESTROGENS: A STUDY OF STRUCTURE-ACTIVITY RELATIONSHIPS

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1 The oestrogenic and antioestrogenic activities of tamoxifen and monohydroxytamoxifen have been compared with those of *para*-methoxy, -methyl, -fluoro, and -chloro tamoxifen in the 3 day immature rat uterine weight test.

2 The oestrogenic activity of mestranol, a steroid with low oestrogen receptor binding affinity which is believed to be demethylated to ethinyl oestradiol before exerting its effects, was less potent than ethinyl oestradiol when assayed in the 3 day immature rat uterine weight test. Similarly, *para*-methoxytamoxifen was less active than monohydroxytamoxifen in oestrogenic and antioestrogenic tests.

3 The introduction of a *para*-methoxy group into tamoxifen did not affect oestrogenic or antioestrogenic activity.

4 All the derivatives of tamoxifen were partial oestrogen agonists when compared with oestradiol benzoate in the 3 d immature rat uterine weight test. All test compounds inhibited the uterotrophic activity of oestradiol benzoate (0.16 μg daily) in a dose-related manner. The order of potency was: monohydroxytamoxifen > tamoxifen ≡ methoxytamoxifen > *p*-fluoro ≡ *p*-chloro ≡ *p*-methyl-tamoxifen.

5 Tamoxifen was approximately equiactive with its *p*-methyl, *p*-fluoro and *p*-chloro derivatives in the ability to inhibit [^{3}H]-oestradiol binding to rat uterine oestrogen receptors *in vitro*.

6 Tamoxifen was approximately equiactive with its *p*-methyl and *p*-fluoro derivatives in the ability to inhibit vaginal cornification of ovariectomized rats upon intravaginal administration with oestradiol (3.2 ng total dose).

7 Since tamoxifen *in vivo* was more active as a partial oestrogen agonist and antagonist than the *para* substituted fluoro, chloro and methyl derivatives that cannot undergo metabolic hydroxylation to monohydroxytamoxifen, whereas the antioestrogenic activity of the compounds upon local application in the vaginal cornification test was equivalent as was their ability to inhibit [^{3}H]-oestradiol-17β binding to the oestrogen receptor *in vitro*, it is suggested that at low doses; i.e. over the range of the partial agonist dose-response curve, the biological activity of tamoxifen is the net result of the activities of the parent compound and its metabolites.

8 The results demonstrate that metabolic activation of non-steroidal antioestrogens is only an advantage and not a requirement for antioestrogenic activity.

Introduction

During the past 20 years considerable interest has focused upon the development of non-steroidal antagonists to oestrogen action. Many compounds have been screened in the laboratory for antioestrogenic and antifertility activity (Lerner, Holthaus & Thompson 1958; Holtkamp, Greslin, Root & Lerner 1960; Harper & Walpole 1967; Lednicer, Lyster & Duncan 1967; Collins, Hobbs & Emmens 1971) but only a few have been used successfully in clinical endocrinology. Perhaps the most important compounds are clomiphene (a mixture of *cis* and *trans* isomers of 1-(4-β-diethylaminoethoxyphenyl-1,2-diphenyl-2-chloro-ethylene); used for the induction of ovulation (Bishop 1970) and tamoxifen (*trans* 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene) used for the treatment of advanced breast cancer (Mouridsen, Palshoff, Patterson & Battersby 1978). With an increasing clinical use of the nonsteroidal antioestrogens it has become important to understand how they exert their effect; however, the precise mechanism of action of

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these compounds at the subcellular level has remained obscure.

As a first step towards an understanding of antioestrogenic mechanisms it is important to identify the biologically active ligand at its target site *in vivo*. Recently Ferguson & Katzenellenbogen (1977) have suggested that non-steroidal antioestrogens are prodrugs; being converted into more active polar metabolites before binding within oestrogen target tissues (Katzenellenbogen, Katzenellenbogen, Ferguson & Krauthammer 1978). In support of this suggestion, tamoxifen is known to be metabolized in laboratory animals to monohydroxytamoxifen (Fromson, Pearson & Bramah 1973) which has greater antioestrogenic activity than the parent compound (Jordan, Collins, Rowsby & Prestwich 1977). However, in support of the concept that the parent compound has inherent biological activity, tamoxifen can control the growth of oestrogen-sensitive human breast cancer cells in long term tissue culture (Lippman, Bolan & Huff 1976) under conditions where no metabolic changes can be detected (Horwitz, Koseki & McGuire 1978).

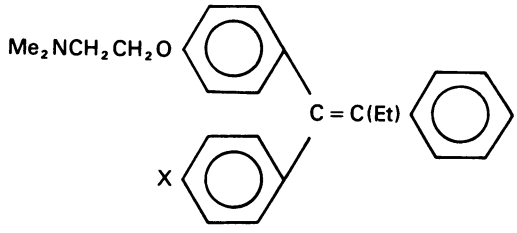
To investigate the contribution of metabolic activation in the actions of antioestrogens *in vivo*, we have evaluated the oestrogenic and antioestrogenic properties of a series of structural derivatives of tamoxifen. For comparison we have determined the oestrogenic activity of mestranol which is believed to be metabolically activated to ethinyl oestradiol before exerting its uterotrophic effects (Hahn, McGuire, Greenslade & Turner, 1971).

Methods

Materials

Tamoxifen and monohydroxytamoxifen were obtained from ICI Ltd (Pharmaceuticals Division). Oestradiol benzoate was obtained from British Drug Houses. Ethinyl oestradiol and mestranol were obtained from Sigma Chemicals. [6,7^{3}H-]-oestradiol (sp. act. 58 Ci/mmol) was obtained 98% pure dissolved in benzene/ethanol (9:1 v/v) from the Radio-

Table 1 Table of n.m.r. chemical shifts and microanalyses for new compounds



X	Configuration	Chemical shift (parts/10^6) CH_3(N)	CH_2(N)	CH_2(O)	m.p.°C	Analyses[b] C	H	N
H[c]	*trans*(Z)	2.20	2.54	3.83				
	cis(E)	2.28	2.68	4.02				
CH_3	*trans*(Z)	2.19	2.55	3.84	102–3°	83.8(84.1)	7.95(8.1)	3.95(3.65)
	cis(E)	2.27	2.66	3.99	59–60°			
OMe	*trans*(Z)	2.28	2.65	3.93	84–5°	80.85(80.8)	7.65(7.8)	3.55(3.5)
	cis(E)	2.35	2.75	4.08				
OH[c]	*trans*(Z)	2.27	2.64	3.93				
	cis(E)	2.33	2.75	4.08				
F	*trans*(E)	2.28	2.65	3.93	93–4°	80.25(80.2)	7.15(7.25)	3.55(3.6)
	cis(Z)	2.35	2.75	4.09				
Cl	*trans*(E)	2.20	2.55	3.85	112°	76.75(76.9)	6.8(6.95)	3.3(3.45)

[a] *Trans* indicates a configuration in which the aminoethoxyphenyl ring and the ethyl group are in the *trans* relationship. The unequivocal stereochemical designations using the Z/E nomenclature are given in parentheses.
[b] Microanalyses were determined by the Microanalytical Laboratory, Department of Organic Chemistry, University of Leeds. Calculated values are given in parentheses.
[c] Samples of the *cis* and *trans* isomers of tamoxifen and monohydroxytamoxifen were a gift from I.C.I. Pharmaceuticals Ltd.
[d] n.m.r. spectra were recorded for deuterocholoform or carbon tetrachloride solutions on a Varian 100 MHz spectrometer at the Physicochemical Measurement Unit, Harwell or on a Perkin Elmer 90 MHz spectrometer in the Department of Organic Chemistry, University of Leeds. Tetramethyl silane was used as the reference.

chemical Centre, Amersham and was used without further purification. For experiments *in vitro*, TED buffer (Tris 0.01 mol/l, disodium edetate (EDTA) 0.0015 mol/l and dithiothreitol 0.0005 mol/l pH 7.4) was used. 4-Chloro, 4-methoxy, and 4-methyl-bromobenzenes from which the related *para*-substituted phenyl-magnesium bromides were formed by the standard method were obtained from Koch Light Laboratories Ltd. and 4-fluoro-bromobenzene from the Aldrich Chemical Co.

Synthesis and characterization of test compounds

The compounds used in the study are shown in Table 1.

The chloro, fluoro, methoxy and methyl derivatives of tamoxifen, shown in Table 1, were prepared by reacting the required *para*-substituted phenyl magnesium bromide with 4-(2-*N*,*N*-dimethylaminoethoxy)-2'-phenyl-butyrophenone (Di Paco & Tauro, 1962) and dehydrating the resulting tertiary alcohols by saturating an alcoholic solution with dry hydrogen chloride. Fractional crystallization of the product from petroleum spirit (b.p. 40–60°C) gave, in each case, one isomerically pure product. On the basis of their n.m.r. spectra (Bedford & Richardson, 1966; Collins *et al.*, 1971) these have been assigned the *trans* structures shown. (For convenience, isomers structurally related to tamoxifen have been designated *trans*. The stereochemical assignment using the unequivocal Z or E nomenclature is given in Table 1.) No pure *cis* isomers were isolated, contamination with the *trans* isomers being observed in the n.m.r. spectra of the lower melting point forms of the *p*-fluoro, *p*-methoxy and *p*-methyl compounds. No *cis* form of the *p*-chloro compound was obtained. Assignment of the *trans* structure to the isolated *p*-chloro compound is based on the similarity of chemical shifts of the $CH_3(N)$, $CH_2(N)$ and $CH_2(O)$ protons to the other *trans* compounds (Table 1). In all cases the signals for the *trans* isomers occurred at higher field than for the corresponding *cis* isomers.

Immature rat uterine wet weight test

All subcutaneous injections were made in 0.1 ml arachis oil. Oily solutions of non-steroidal antioestrogens were prepared by taking aliquots from freshly prepared stock ethanolic solutions, adding the required volume of arachis oil and evaporating the ethanol, under a stream of N_2, on a warm (60°C) water bath.

Immature female rats (35 to 50 g) of the Alderley Park strain were randomly divided into groups of at least eight animals. To determine uterotrophic activity, compounds were injected on three consecutive days and on the fourth day animals were killed by stunning and cervical dislocation. To determine antioestrogenic activity, compounds were injected as before except that a standard dose of oestradiol benzoate (0.16 μg daily) was injected at a separate site. In all cases uteri were dissected out and pressed between sheets of blotting paper to remove intraluminal fluid before weighing on a torsion balance.

Inhibition of [^3H]-oestradiol binding in vitro

Immature rat uteri from oestradiol benzoate-treated rats (0.16 μg daily × 3) were collected and frozen in liquid nitrogen. Pooled uteri (8) were homogenized with an Ultra Turrax homogeniser (2 × 10 s bursts with ice water cooling) in 4 ml TED buffer. The homogenate was centrifuged at 100,000 *g* for 1 h (4°C) in a Sorval OTD2 ultracentrifuge to prepare cytosol. The supernatants were removed, taking care not to disturb the fat layer on the surface, pooled and used immediately. The cytosol was always kept in an ice/water tray. Cytosol (150 μl) was incubated with different concentrations of oestradiol, ethinyl oestradiol, mestranol, monohydroxytamoxifen, tamoxifen and the methoxy, fluoro, chloro and methyl derivatives of tamoxifen in 50 μl TED buffer and [^{3}H]-oestradiol solution (3.1 or 3.5×10^{-8} mol/l; 50 μl) in TED buffer at 30°C for 30 min. Parallel incubation of cytosol (150 μl), [^{3}H]-oestradiol solution (50 μl) and 50 μl TED or 50 μl of a solution of diethylstilboestrol (BDH, 5×10^{-6} mol/l) in TED was used to determine the specific binding of [^{3}H]-oestradiol by subtraction of the counts obtained in the presence of diethylstilboestrol from those obtained for parallel incubates in its absence. All tubes were cooled in ice/water for 15 min and then 200 μl of a suspension of dextran-coated charcoal (250 mg % Norit A, 2.5 mg% dextran) in TED buffer was added and allowed to stand for 20 min in ice/water with occasional shaking. Tubes were centrifuged at 2000 *g* (4°C) for 5 min and 200 μl samples of the supernatant were added to 5 ml tritium scintillator (6 g butyl PBD, 135 ml toluene, 720 ml dioxan, 100 g naphthalene and 45 ml absolute methanol). Samples were counted for 10 min in a Beckman LS3133T liquid scintillation spectrometer. Counting efficiency, as determined by an external standard method, was 40–42%.

Antioestrogenic activity by intravaginal administration

Mature female Sprague Dawley rats (200 to 250 g) were ovariectomized under ether anaesthesia. One week later, animals were primed (s.c.) with 1 μg oestradiol benzoate in 0.1 ml arachis oil. Rats were randomized into groups of 5 and used for experiments 1 week later. Solutions for intravaginal administration were prepared in 2% aqueous Tween 80 as described by Clark & McCracken (1971) except that the small quantity of ethanol (<2%) was not evaporated. The

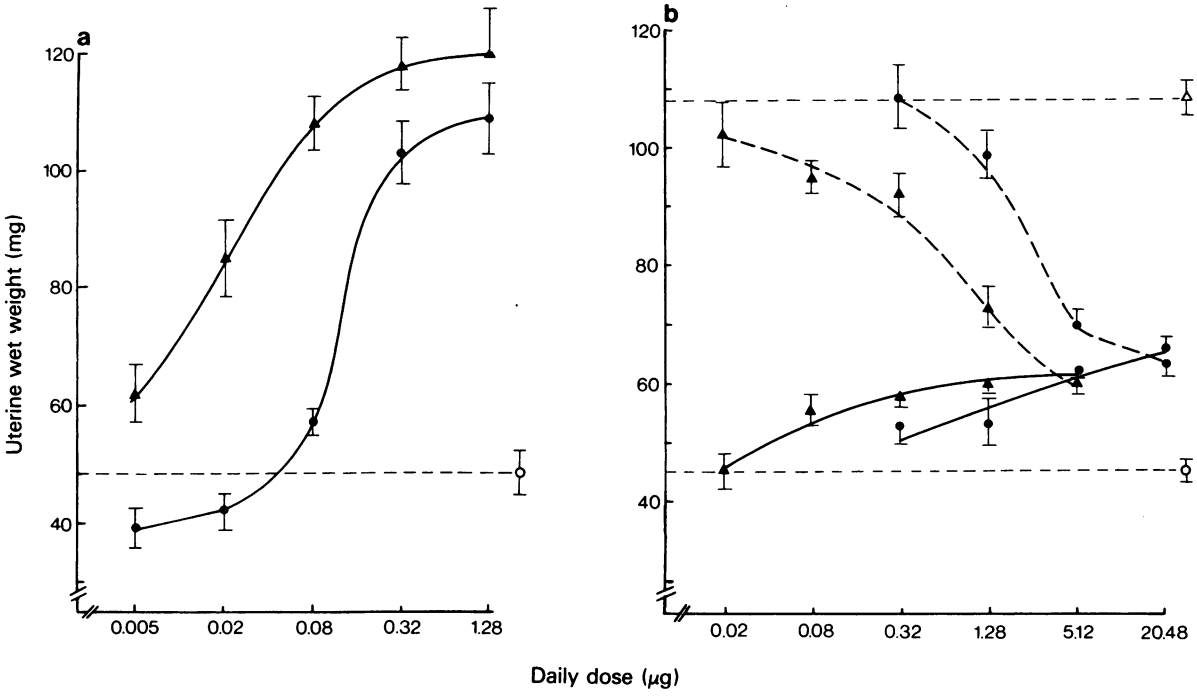


Figure 1 (a) The oestrogenic effects of various daily doses of ethinyl oestradiol (▲) and mestranol (●) and (b) The oestrogenic (solid lines) and antioestrogenic (broken lines) effects of various doses of monohydroxytamoxifen (▲) and methoxytamoxifen (●) in the 3 day immature rat uterine weight test. Antioestrogenic activity was determined by simultaneous administration with oestradiol benzoate (0.16 μg daily) and comparison with results obtained with oestradiol benzoate alone (△). Controls (○) were injected with arachis oil alone. Results represent means with at least 7 rats per group; vertical lines show s.e. mean.

solutions of oestradiol or oestradiol and different concentrations of monohydroxytamoxifen, tamoxifen, *para*-methyl and *para*-fluoro tamoxifen were administered intravaginally in two 100 μl aliquots 24 h apart. Controls received vehicle (containing an equivalent quantity of ethanol) alone. Vaginal smears were taken 24 and 31 h after the second injection (Emmens 1950). Squamous cells i.e.: non-nucleated cornified epithelial cells, with no leucocytes present, were taken as a full oestrogenic response.

Results

Immature rat uterine weight tests

Ethinyl oestradiol was a potent oestrogen in the 3 day immature uterine weight test (Figure 1a). Although the 3 methyl ether of ethinyl oestradiol, mestranol, was also an oestrogen, producing a maximum response in the uterine weight test which was not significantly different from that produced by the parent diol ($t = 1.17$, $n = 8$ in each group), it was less potent and the dose-response curves of the two drugs were not parallel (as tested by analysis of variance). Similarly methoxytamoxifen was less potent as a partial oestrogen agonist than monohydroxytamoxifen (Figure 1b). However, the greater activity of monohydroxytamoxifen (Figure 1b) was more apparent when the antioestrogenic dose-response curves were compared. Tamoxifen, monohydroxytamoxifen, *p*-methyl-, *p*-chloro- and *p*-fluoro-tamoxifen were all partial oestrogen agonists when compared with oestradiol-benzoate in the 3 day uterine weight test (Figure 2a). Monohydroxytamoxifen was more potent than tamoxifen and the *p*-methyl, *p*-chloro and *p*-fluoro derivatives were all less active than tamoxifen.

In tests for antioestrogenic activity monohydroxytamoxifen was more active than tamoxifen whereas *p*-fluoro, *p*-chloro and *p*-methyl tamoxifen were all less active. However the fluoro, chloro and methyl-derivatives of tamoxifen all produced a dose-related

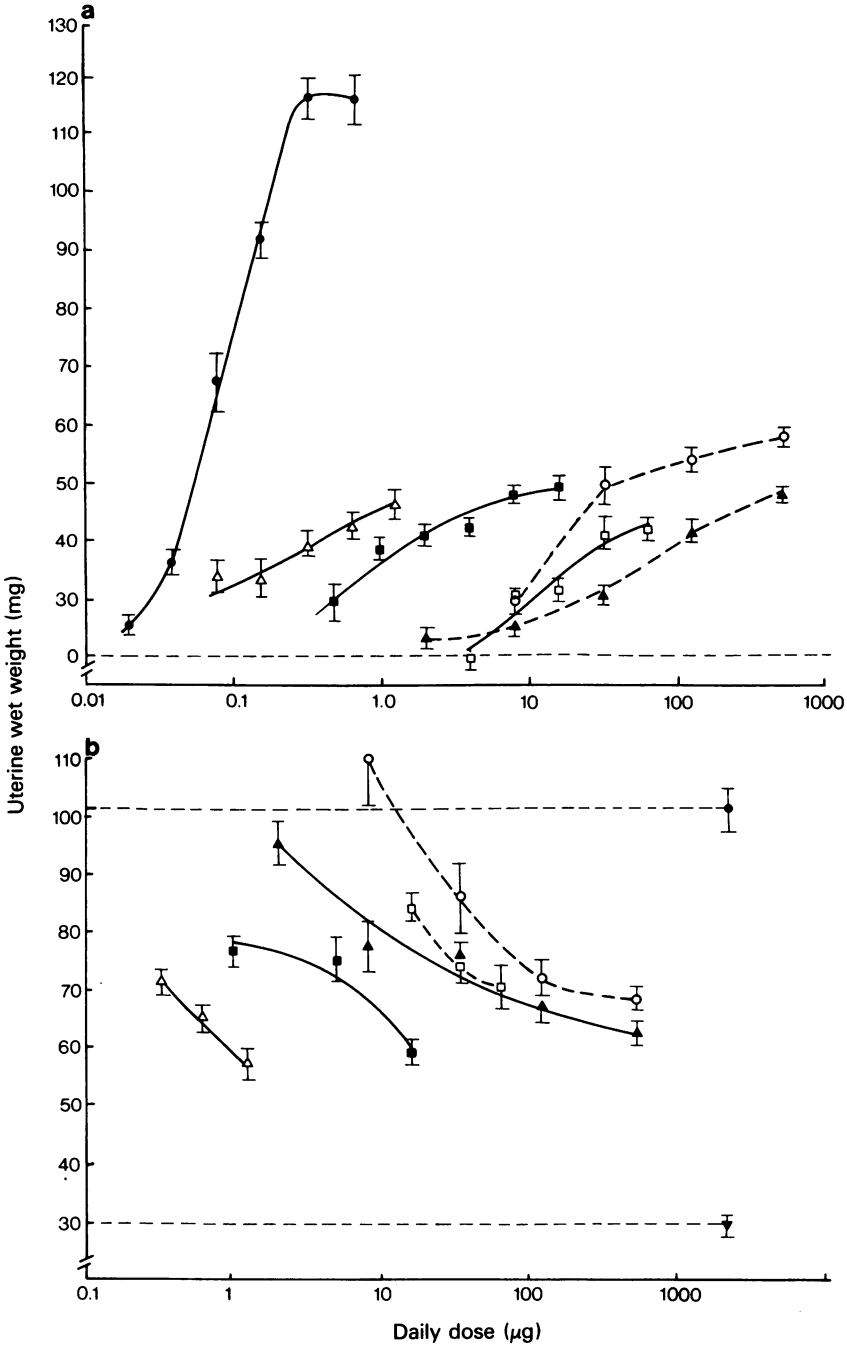


Figure 2 (a) Oestrogenic and (b) antioestrogenic effect of various derivatives of tamoxifen (monohydroxytamoxifen △; tamoxifen ■; fluoro-▲; chloro-□ and methyltamoxifen ○) in the 3 day immature rat uterine weight test. Oestradiol benzoate (●) was used for comparative purposes and in the tests for antioestrogenic activity compounds were administered simultaneously with oestradiol benzoate (0.16 μg daily) and the results compared with oestradiol benzoate alone (---●), Controls (▼) were injected with arachis oil alone. Results represent means with at least 7 rats per group; vertical lines show s.e. mean.

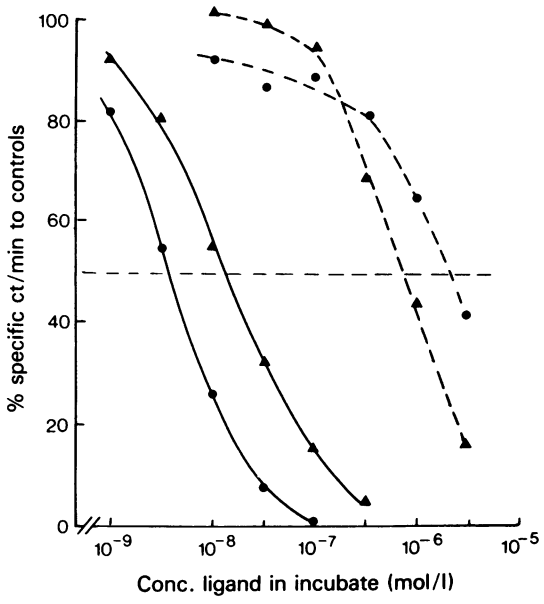


Figure 3 Effect of increasing concentrations of ethinyl oestradiol (●), monohydroxytamoxifen (▲), methoxytamoxifen (▲---▲) and mestranol (●---●) on the binding of [^{3}H]-oestradiol-17β (7×10^{-9} mol/l) to macromolecules in rat uterine cytosol. Incubations were undertaken at 30°C for 30 min. Specifically bound radioactivity (ct/min) is plotted as a percentage of the specifically bound radioactivity in control tubes i.e. those with no competitive ligands.

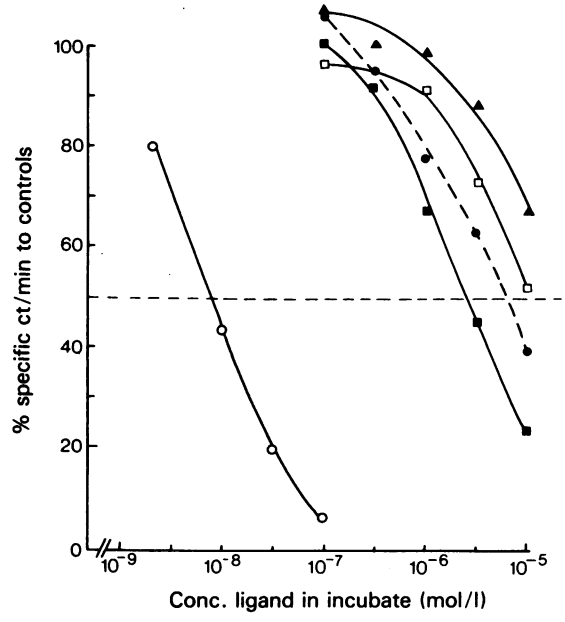


Figure 4 Effect of increasing concentrations of oestradiol-17β (○), methyltamoxifen (■), tamoxifen (●), chlorotamoxifen (□), and fluorotamoxifen (▲) on the binding of [^{3}H]-oestradiol-17β (7×10^{-9} mol/l) to macromolecules in rat uterine cytosol. Incubations and results were plotted as described in Figure 3.

inhibition of the uterotrophic effects of 0.16 μg oestradiol benzoate daily (Figure 2b).

p-Methoxytamoxifen and tamoxifen at both 2 μg and 8 μg dose levels produced uterine weight responses which were not significantly different ($P > 0.7$), the mean responses (± s.e. mean) being 50.0 ± 1.5 and 58.7 ± 2.1 ($n = 10$) for the methoxy compound and 50.7 ± 2.35 and 59.5 ± 1.4 ($n = 7$) for tamoxifen. Their antioestrogenic potencies were also similar, maximum suppression of the uterine weight response to 0.16 μg oestradiol benzoate being obtained with 20.48 and 16 μg respectively (cf. Figures 1b, 2a and 2b).

Inhibition of [^{3}*H*]*-oestradiol binding* in vitro

Ethinyl oestradiol, mestranol, monohydroxytamoxifen and methoxytamoxifen all inhibited the binding of [^{3}H]-oestradiol to the rat uterine oestrogen receptor as their incubation concentrations increased (Figure 3). Ethinyl oestradiol and monohydroxytamoxifen with free phenol groups were both more potent inhibitors of [^{3}H]-oestradiol binding than their related methyl ethers. The overall order of potency was ethinyl oestradiol > monohydroxytamoxifen > methoxytamoxifen > mestranol with the concentrations that inhibited the specific binding of [^{3}H]-oestradiol (6.25×10^{-9} mol/l) by 50% being 4×10^{-9} mol/l, 1.2×10^{-8} mol/l, 8.5×10^{-7} mol/l, and 2.5×10^{-6} mol/l respectively.

The methyl, chloro and fluoro derivatives of tamoxifen all inhibited the binding of [^{3}H]-oestradiol to the rat uterine oestrogen receptor over a similar concentration range to tamoxifen. Only methyl tamoxifen and tamoxifen achieved 50% inhibition of [^{3}H]-oestradiol (7×10^{-9} mol/l) binding at concentrations of 2.5×10^{-6} mol/l, and 6×10^{-6} mol/l respectively (Figure 4). Oestradiol achieved a 50% inhibition of [^{3}H]-oestradiol binding at 7.5×10^{-9} mol/l.

Antioestrogenic activity by intravaginal administration

The administration of increasing doses of monohydroxytamoxifen (3.2, 12.8, 51.2 ng) with a standard dose of oestradiol (3.2 ng) decreased the number of positive smears (Table 2). Tamoxifen, and the methyl and fluoro derivatives of tamoxifen were all much less active than monohydroxytamoxifen but again increas-

ing doses of the compounds (0.32 to 20.48 μg) all inhibited vaginal cornification produced by oestradiol (3.2 ng).

Discussion

The aim of the study was to determine the contribution of metabolic activation to the mechanism of action of tamoxifen by comparing the biological properties of mestranol and its demethylated derivative, ethinyloestradiol, with those of tamoxifen and a series of monosubstituted tamoxifens including its principle metabolite monohydroxytamoxifen.

Aromatic chloro- and fluoro- groups are not normally hydrolysed *in vivo* to hydroxyl, the usual metabolic process in higher animals being the formation of the *p*-substituted mercapturic acid (Williams, 1959). The presence of *p*-chloro- and *p*-fluoro substituents in tamoxifen would therefore be expected to prevent the formation of monohydroxytamoxifen. A methyl substituent would also be expected to prevent the introduction of hydroxyl though in this case oxidation to a carboxyl group is a possibility. In contrast demethylation of aryl methyl ethers is a well established route of metabolic activation and is believed to occur with mestranol. Thus the finding that mestranol is about half as active as ethinyl oestradiol when administered orally in the 3 day immature mouse uterine weight test (Dorfman & Kincl, 1966) and about one fifth as potent as an inhibitor of gonadotrophin release (Kincl, Birch & Dorfman, 1964), but has only 2.5% and 1% the ability of ethinyl oestradiol to inhibit the binding of [^{3}H]-oestradiol to oestrogen receptors derived from rabbit (Korenman, 1969) and rat (Eisenfeld, 1974) sources respectively (cf. Figure 3) lends weight to the suggestion that demethylation of mestranol occurs *in vivo* to yield the biologically active agent, ethinyl oestradiol. Certainly ethinyl oestradiol has been identified in urine after the administration of radio-labelled mestranol to women (Williams, 1969), but perhaps the most compelling evidence for the metabolic activation of mestranol has come from studies in laboratory animals. Following the administration of [^{3}H]-mestranol to rats, Hahn *et al.* (1971) identified both ethinyl oestradiol and mestranol in the uterus. However, the higher oestrogen receptor binding properties of ethinyl oestradiol compared with mestranol indicated that whereas ethinyl oestradiol was probably bound to receptor protein in the target tissue, mestranol was probably free or bound to non-receptor proteins. Consistent with these earlier findings, ethinyl oestradiol was found to be more potent as an oestrogen than its 3-methyl ether mestranol (Figure 1a) and to be approximately 500 times more active at inhibiting the binding of [^{3}H]-oestradiol to rat uterine cytosol (Figure 3). The lack of parallelism exhibited by these compounds in the uterine weight test (Figure 1a) may be a consequence of both mestranol and the metabolically derived ethinyl oestradiol contributing to the observed responses.

Table 2. Table of the total doses of compounds administered intravaginally in 2% aqueous Tween 80 with a total dose of 32×10^{-4} μg oestradiol-17β to determine antioestrogenic activity in ovariectomized rats using the method described by Emmens (1950)

Compound	*Total intravaginal dose* (μg)	*Number of full vaginal cornifications*
—	—	8
Monohydroxytamoxifen	0.0032	8
	0.0128	7
	0.0512	2
Tamoxifen	0.32	7
	1.28	2
	5.12	0
	20.48	0
p-Methyltamoxifen	0.32	6
	1.28	2
	5.12	3
	20.48	0
p-Fluorotamoxifen	1.28	1
	5.12	1
	20.48	1

Six rats were used per group. Control animals received vehicle alone and had no vaginal cornifications.

The decrease in oestrogenic activity of ethinyl oestradiol by methylation of the 3 phenolic hydroxyl was mirrored by the decreased anti-oestrogenic activity of methoxytamoxifen when compared with monohydroxytamoxifen (Figure 1b). The ability of monohydroxytamoxifen to inhibit the binding of [^{3}H]-oestradiol to the oestrogen receptor was also decreased on methylation (Figure 3). Although monohydroxytamoxifen is more potent than methoxytamoxifen in the 3 day immature rat uterine weight test it has been found that methoxy derivatives of triphenylethylene antioestrogens have a longer biological effect than their free phenolic derivatives (Ferguson & Katzenellenbogen 1977). This is probably because the phenolic compounds are more water soluble than their methyl ethers and as such are more liable to conjugation and subsequent excretion.

It is interesting to note that the introduction of a methoxy group into tamoxifen did not signifiantly alter the partial oestrogenic and antioestrogenic properties. If methoxy derivatives of non-steroidal antioestrogens are demethylated prior to exerting their biological effects, then hydroxylation of tamoxifen *in vivo* to form monohydroxytamoxifen may make

a significant contribution to its antioestrogenic effect over the lower doses of the partial agonist dose-response curve (cf. Jordan *et al.*, 1977). Support for this concept is provided by a comparison of the anti-oestrogenic properties of tamoxifen and the methyl derivative of tamoxifen. Tamoxifen and its methyl derivative have a similar ability to inhibit the binding of [^{3}H]-oestradiol to the rat uterine oestrogen receptor (Figure 4) and to inhibit oestradiol-stimulated vaginal cornification when applied locally (Table 2). However, in the immature rat uterine weight test, the dose of tamoxifen that completely inhibited 0.16 μg oestradiol benzoate daily from increasing the uterine weight above that produced by tamoxifen alone was 16 μg daily, whereas the dose of methyl tamoxifen that produced a similar effect was 512 μg daily. This result indicates that the antioestrogenic effect of tamoxifen in the 3 day uterine weight test probably involves metabolic *p*-hydroxylation.

Nevertheless, the finding that compounds that cannot undergo metabolic *p*-hydroxylation are also actively antioestrogenic *in vivo* demonstrates that these substituted triphenylethylenes have inherent antioestrogenic properties. This supports the view of Horwitz *et al.* (1978) that tamoxifen does not undergo metabolic transformations during long term culture with human breast cancer cells *in vitro* prior to producing an antitumour affect.

The implications of our findings for an understanding of the role of metabolism in the mechanism of action of tamoxifen as an antitumour agent *in vivo* depends upon two major factors: the capacity of the liver to metabolize significant quantities of the drug to active metabolites and the relative importance of metabolic pathways in the species under study. Clearly a consideration of the blood levels of tamoxifen will be important because at very high concentrations the parent compound will be the dominant oestrogen receptor binding ligand whereas at low concentrations metabolism may provide a significantly greater proportion of active metabolites for receptor binding. However, the prime factor for consideration will be the relative importance of the major metabolic pathways. In the rat, a primary route for tamoxifen metabolism is via mono- and dihydroxylation (Fromson *et al.*, 1973), therefore earlier studies on the mechanism of action of tamoxifen in the DMBA-induced rat mammary carcinoma model (Nicholson & Golder, 1974; Jordan, 1976) must now consider that the observed antitumour actions are probably the net result of the individual actions of tamoxifen and its major non-conjugated metabolite monohydroxytamoxifen. We have recently demonstrated that monohydroxytamoxifen has antitumour activity in the DMBA-induced mammary carcinoma model (Jordan & Allen, 1979). In man it has been suggested that the primary metabolic route of tamoxifen is via *N*-demethylation to produce desmethyltamoxifen (Adam, Douglas & Kemp, 1979) although monohydroxytamoxifen has been detected in breast cancer patients during tamoxifen therapy (R. I. Nicholson personal communication). Clearly further study of the metabolism of tamoxifen is necessary to confirm these findings before the contribution of metabolic activation of tamoxifen to its mechanism of action in man can be evaluated.

In conclusion, it appears from the study of the structure-activity relationships of tamoxifen that the ability of an antioestrogen to undergo metabolic activation to a free phenolic compound is an advantage, but not a requirement, for antioestrogenic activity.

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References

ADAM, H. K., DOUGLAS, E. J. & KEMP, J. V. (1979). The metabolism of tamoxifen in humans. *Biochem. Pharmac.*, **27**, 145–147.

BEDFORD, G. R. & RICHARDSON, D. N. (1966) Preparation and identification of *cis* and *trans* isomers of substituted triarylethylene. *Nature*, **212**, 733–734.

BISHOP, P. M. F. (1970). Clomiphene. *Br. med. Bull.*, **26**, 22–25.

CLARK, E. R. & MCCRACKEN, A. M. (1971). Investigation of aqueous Tween 80 as a vehicle for intravaginal administration. *J. Endocr.*, **51**, 797–798.

COLLINS, D. J., HOBBS, J. J. & EMMENS, C. W. (1971). Antiestrogenic and antifertility compounds. 4. 1,1,2-triarylalkan-l-ol and 1,1,2-triaryl-alk-l-enes containing basic ether groups. *J. med. Chem.*, **14**, 952–957.

DI PACO, G. & TAURO, C. S. (1962). *Brit. Patent 9005,903* Sept. 12, 1962.

DORFMAN, R. I. & KINCL, F. A. (1966). Uterotrophic activity of various phenolic steroids. *Acta. endocr. Copenh.*, **52**, 619–626.

EISENFELD, A. (1974). Oral contraceptives: ethinyl estradiol binds with higher affinity than mestranol to macromolecules from the sites of antifertility action. *Endocrinology*, **94**, 803–807.

EMMENS, C. W. (1950). The intravaginal assay of naturally occurring oestrogens. *J. Endocr.*, **6**, 302–307.

FERGUSON, E. R. & KATZENELLENBOGEN, B. S. (1977). A comparative study of antioestrogen action: temporal patterns of antagonism of estrogen stimulated uterine growth and effects on estrogen receptor levels. *Endocrinology*, **100**, 1242–1251.

FROMSON, J. M., PEARSON, S. & BRAMAH, S. (1973). The metabolism of tamoxifen (I.C.I. 46, 474) 1. In laboratory animals. *Xenobiotica*, **3**, 693–709.

HAHN, D. W., MCGUIRE, J. L., GREENSLADE, F. C. & TURNER, G. D. (1971). Molecular parameters involved in the estrogenicity of mestranol and ethinyl estradiol. *Proc. Soc. exp. Biol. Med.*, **137**, 1180–1185.

HARPER, M. J. K. & WALPOLE, A. L. (1967). A new derivative of triphenylethylene and mode of action in rats. *J. Reprod. Fert.*, **13**, 101–119.

HOLTKAMP, D. E., GRESLIN, J. G., ROOT, C. A. & LERNER, L. J. (1960). Gonadotrophin inhibiting and anti-fecundity effects of chloramiphene. *Proc. Soc. exp. Biol. Med.*, **105**, 197–201.

HORWITZ, K. B., KOSEKI, Y. & MCGUIRE, W. L. (1978). Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogen. *Endocrinology*, **103**, 1742–1751.

JORDAN, V. C. (1976). Antiestrogenic and antitumor properties of tamoxifen in laboratory animals. *Cancer Treat. Rep.*, **60**, 1409–1419.

JORDAN, V. C. & ALLEN, K. E. (1980). Evaluation of the antitumour activity of the non-steroidal antioestrogen monohydroxytamoxifen in the DMBA-induced rat mammary carcinoma model. *Eurp. J. Cancer*, **16**, 239–251.

JORDAN, V. C., COLLINS, M. M., ROWSBY, L. & PRESTWICH, G. (1977). A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. *J. Endocr.*, **75**, 305–316.

KATZENELLENBOGEN, B. S., KATZENELLENBOGEN, J. A., FERGUSON, E. R. & KRAUTHAMMER, N. (1978). Antiestrogen interaction with uterine estrogen receptors. *J. biol. Chem.*, **253**, 697–707.

KINCL, F. A., BIRCH, A. J. & DORFMAN, R. I. (1964). Pituitary gonadotrophin inhibitory activity of various steroids in ovariectomized-intact female rats in parabiosis. *Proc. Soc. exp. Biol. Med.*, **117**, 549–552.

KORENMAN, S. G. (1969). Comparative binding affinity of estrogen and its relation to estrogenic potency. *Steroids*, **13**, 163–177.

LEDNICER, D., LYSTER, S. C. & DUNCAN, G. W. (1967). Mammalian antifertility agents. IV. basic 3,4-dihydronaphthalenes and 1,2,3,4-tetrahydro-1-naphthols. *J. med. Chem.*, **10**, 78–85.

LERNER, L. J., HOLTHAUS, J. F. & THOMPSON, C. R. (1958). A non-steroidal estrogen antagonist 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2-p-methoxyphenyl ethanol. *Endocrinology*, **63**, 295–318.

LIPPMAN, M., BOLAN, G. & HUFF, K. (1976). Interaction of antioestrogens with human breast cancer in long-term tissue culture. *Cancer Treat. Rep.*, **60**, 1421–1429.

MOURIDSON, H. T., PALSHOF, T., PATTERSON, J. & BATTERSBY, L. (1978). Tamoxifen in advanced breast cancer. *Cancer Treat. Rev.* **5**, 131–141.

NICHOLSON, R. I. & GOLDER, M. P. (1975). The effect of synthetic anti-oestrogens on the growth and biochemistry of rat mammary tumours. *Eur. J. Cancer*, **11**, 571–579.

WILLIAMS, K. I. H. (1969). The metabolism of radioactive 17α-ethinyl-estradiol 3-methyl ether (mestranol) by women. *Steroids*, **13**, 539–544.

WILLIAMS, R. T. (1959). The metabolism of halogenated aromatic hydrocarbons. In *Detoxication Mechanisms*. 2nd ed. pp. 237–277 London: Chapman & Hall.

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