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Letter to the Editor

Binding of [^{3}H] Monohydroxytamoxifen in Human Breast Carcinoma Cytosols*

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The clinical use of tamoxifen in the treatment of breast cancer has stimulated attempts to elucidate the mechanism of antioestrogen action. Unfortunately, studies with [^{3}H] tamoxifen *in vitro* are unreliable because of a low affinity for the oestrogen receptor [1] and also tamoxifen is converted to more potent antioestrogens *in vivo* [2, 3].

Monohydroxytamoxifen, a metabolite of tamoxifen in the rat, is a potent antioestrogen [4] with antitumour properties [5]. Its advantage as a research tool is strengthened by an affinity for the oestrogen receptor similar to that of oestradiol [4]. We now report the first studies with a preparation of [^{3}H] monohydroxytamoxifen (50:50 *cis/trans* mixture) with high specific activity (42 Ci/mmole >98% purity; ICI Ltd., Pharmaceuticals Div., Macclesfield, Cheshire, U.K.) (Fig. 1).

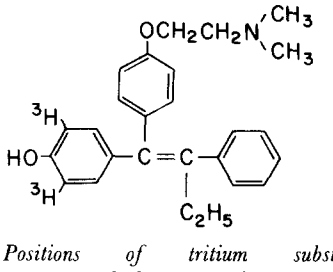

Fig. 1. Positions of tritium substitution in monohydroxytamoxifen.

[^{3}H] Monohydroxytamoxifen and [2, 4, 6, 7-^{3}H] oestradiol-17β (104 Ci/mmole >98% purity; Radiochemical Centre, Amersham, U.K.) both bound to the 8S oestrogen receptor present in some human breast carcinomata (Fig. 2). This binding was inhibited by oestradiol-17β (Sigma Chemicals, St. Louis, MI). Similarly [^{3}H] monohydroxytamoxifen binds specifically to the 8S oestrogen receptor derived from immature rat uteri and

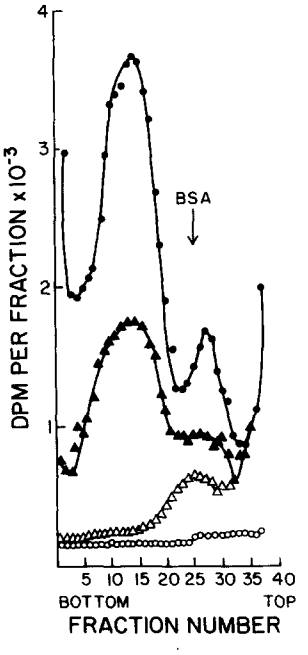

Fig. 2. Sucrose density gradient analysis (10–30%) of a human breast carcinoma cytosol. Cytosol was prepared as in Table 1. Aliquots (480 µl) were incubated (0–4°C) for 2 hr with 10 µl ethanol containing [^{3}H] oestradiol-17β (●) or [^{3}H] monohydroxytamoxifen (▲) to give a final concentration of 4 and 8 nmole/l respectively. Competing tubes for [^{3}H] oestradiol (○) or [^{3}H] monohydroxytamoxifen (△) contained 4 µmole/l oestradiol-17β added in 10 µl ethanol. Non-competed tubes contained 10 µl ethanol alone. Unbound ligands were adsorbed onto charcoal pellets (25 min at 0–4°C). Samples (200 µl) were layered onto gradients and centrifuged for 2 hr (4°C) at 400,000 g using a VTR 865 rotor in a Sorvall OTD 65 centrifuge. Bovine serum albumin (^{14}C-BSA) was used as an internal sedimentation standard (4·6S).

N-nitrosomethyl urea-induced rat mammary tumours. In all these experiments the molar concentration of [^{3}H] monohydroxytamoxifen used was twice that of [^{3}H] oestradiol-17β. This was because the *trans* isomer of monohydroxytamoxifen has a similar binding affinity for the oestrogen receptor as oestradiol, whereas the *cis* isomer has approximately 0.1% this affinity (unpublished findings). Therefore, the *trans* isomer is selectively bound to the

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oestrogen receptor under the present experimental conditions.

The binding of [^{3}H] oestradiol-17β and [^{3}H] monohydroxytamoxifen was examined in breast carcinoma cytosols using either oestradiol-17β or monohydroxytamoxifen as a competitor. The data in Table 1 suggest that a component in some cytosols binds [^{3}H] monohydroxytamoxifen and is competed for by oestradiol. However there seems to be a greater competition by monohydroxytamoxifen. It has been reported [7] that [^{3}H] tamoxifen binds to an 'antioestrogen' receptor in human carcinoma cytosols. Our findings support this observation but we have not been able to demonstrate the 'antioestrogen' receptor using sucrose density gradient analysis. The significance, if any, of the antioestrogen binding component remains to be determined.

In summary, we have demonstrated that the characteristics of [^{3}H] monohydroxytamoxifen make it a potent new tool with which to examine antioestrogen action *in vitro*. Preliminary studies indicate that [^{3}H] monohydroxytamoxifen can be used with confidence to study antioestrogen action *in vivo*.

Table 1. *Binding of* [^{3}H] *oestradiol* (4 nmole/l) *or* [^{3}H] *monohydroxytamoxifen* (8 nmole/l) *in human mammary tumour cytosols*

Tumour	[^{3}H] oestradiol		[^{3}H] monohydroxytamoxifen	
	OE_2	MOH	OE_2	MOH
1	398.3	403.0	361.8	505.7
2	32.9	35.2	25.0	70.4
3	22.1	18.9	0	20.4
4	5.3	11.9	0	0
5	0	0	10.6	83.9
6	0	0	54.6	49.5
7	0	0	0	0

Tumours were homogenised (2 × 10 sec bursts with a Polytron tissue homogeniser with ice/water cooling) in 5 vols. TED buffer (Tris 0.01 mole/l; EDTA 0.0015 mole/l and dithiothreitol 0.005 mole/l, pH 7.4) and centrifuged at 125,000 *g* for 50 min to prepare cytosols. Cytosols (480 μl) were incubated with tritiated ligands (added in 20 μl ethanol) for 18 hr (4°C). Either oestradiol-17β (OE_2) or monohydroxytamoxifen (MOH) (4 μmole/l) was used as a competitor with both radioactive ligands. Unbound ligands were adsorbed by the addition of 1 ml of a 0.25% dextran coated charcoal suspension for 25 min (0–4°C). Cytosol protein was determined by the method of Lowry [6]. Data as fmole/mg cytosol protein.

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