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TAMOXIFEN INHIBITION OF AN *in vitro* OESTRADIOL-INDUCED SURFACE COAT CHANGE ON MOUSE BLASTOCYSTS

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1 Eighty-nine hour post-coitus mouse blastocysts cultured in the presence of oestradiol-17β underwent a histochemically detectable surface coat change.

2 Tamoxifen was found to inhibit this *in vitro* -induced change. The role of Tamoxifen as an inhibitor of implantation is discussed.

Introduction

Tamoxifen (ICI 46,474, *trans* 1(*p*-(2-dimethylamino-ethoxy)-phenyl)-1,2-diphenylbut-1-ene) has been shown previously to prevent implantation of the blastocyst in rats and mice (Harper & Walpole, 1967a, b) and to prevent *in vivo* the oestrogen-dependent surface coat change of the late preimplantation mouse blastocyst (Bloxham, Pugh & Sharma, 1975). These authors pointed out that the latter effect could arise directly, from an inhibition of the uptake of oestradiol by the blastocyst, or indirectly, following interference with the synthesis, release or fate of the post-ovulatory oestrogen surge in the mother.

In order to elucidate further the mode of action of the drug in mice we have studied its effects on the oestrogen-induced surface coat change of the mouse trophoblast *in vitro*.

Methods

Albino random-bred mice were maintained in a chamber with a standardized 10 h dark period centred on 17 h 00 min. In determining the precise time for killing the mice, so as to collect ova of a known age, mating was considered to have taken place at 17 h 00 min on day zero.

The mated mice were randomly divided into three groups. Group I (4 mice) were killed at 89 h post-coitum (h p.c.) and group II (5 mice) were killed at 96 h post-coitum. The blastocysts were collected and fixed for 24 h in 10% neutral buffered formalin, before being air-dried onto slides and stained according to the method of Holmes & Dickson (1973), as used in this laboratory (Bloxham *et al.*, 1975). This technique stains the blastocyst trophoblast red, before, or blue, after, the surface coat change. Group III (26 mice) were killed at 89 h p.c., their blastocysts were collected in Whittingham (1971) culture medium, washed in fresh medium and randomly allocated into ten sub-groups of 25 blastocysts each. The sub-groups were placed in embryological watch glasses containing either 2 ml culture medium (Whittingham, 1971) or medium to which oestradiol-17β (Sigma) or Tamoxifen (ICI), or both, at various concentrations were added (Table 1) as follows. Both the oestradiol and Tamoxifen were made up in absolute ethanol and the amount appropriate to the desired concentration in the culture medium was in each case pipetted into a test tube and dried down; 2 ml of culture medium was added and the tube was vortex mixed for 30 s before tipping the contents into the watch glass. The blastocysts were cultured in an incubator at $37 \pm 0.5°C$, for 16 h, after which time they were removed and washed in phosphate-buffered normal saline before fixation and staining as described above.

Results

All group I (89 h p.c.) mouse blastocysts (38) were red after staining while all the group II (96 h p.c.) blastocysts (53) stained blue, so reconfirming the occurrence of the trophoblast surface coat change between 89 and 96 h p.c. *in vivo*.

The group III 89 h p.c. blastocysts cultured in Whittingham medium (sub-group a) failed to undergo the trophoblastic surface coat change even by 105 h post coitum. The presence of 25 pg/ml or more oestradiol in the culture medium (sub-groups b and c) had enabled the surface coat change to take place by 105 h p.c. in all the blastocysts so treated. Tamoxifen alone, on the other hand, added to give a concentration of 100 pg/ml culture medium did not enable the coat change to occur (sub-group f). This concentration

of Tamoxifen was also able to prevent the anticipated change in the surface coat in the presence of 50 pg/ml oestradiol-17β (sub-group g). Progressive reduction of the amount of Tamoxifen added to successive sub-groups in the presence of 50 pg/ml oestradiol-17β allowed an increasing percentage of the eggs to undergo the surface coat change (sub-groups h, i and j). The composition of the group III sub-groups and the percentage occurrence of the trophoblastic surface coat change in the individual groups of 25 blastocysts are detailed in Table 1.

Discussion

Dickmann, Dey & Sen Gupta (1975) have suggested on the basis of indirect evidence that oestradiol, perhaps originating from the pre-implantation embryo itself, is necessary for the maturation and later implantation of the embryo.

Wilson & Jenkinson (1974) reported that when maintained *in vitro* in simple buffered salt solutions (BSS) containing albumin, development of the embryo ceases at the expanded blastocyst stage and that changes analogous to those of implantation are seen only when otherwise chemically defined culture media are enriched, e.g. with 30% foetal calf serum.

The experiments described in this paper confirm the limitations of culture in BSS + albumin but show that the addition of oestradiol-17β to the fully chemically defined medium allows maturation to progress further in that the pre-implantation surface coat change is enabled to occur. This change is believed to reflect the secretion of acidic glycoproteins by the trophoblastic cells (Pinsker & Mintz, 1973) and our evidence of the occurrence of the coat change *in vitro* would appear to support this possibility, rather than an origin somewhere in the uterus. The necessity for oestradiol-17β for implantation in the mouse is well established (Smith & Biggers, 1968; McLaren, 1971; Holmes & Dickson, 1973). The results of this study raise the possibility that induction of the surface coat change is one mechanism by which oestradiol-17β could act in bringing about implantation. The need for a major change in surface coat properties before the adhesion phase of implantation was postulated by Enders & Schlafke (1974).

Tamoxifen is established as an antifertility agent in the mouse, acting by preventing implantation and has been shown to exert antioestrogenic effects (Harper & Walpole, 1967a). The compound competes with oestradiol-17β for receptors in mouse uterus cytosol (Skidmore, Walpole & Woodburn, 1972) and related drugs decrease the rate of replenishment of cytosol receptors (Clark, Peck & Anderson, 1976). Having already demonstrated that Tamoxifen in preventing implantation in the mouse also prevents the surface coat change *in vivo* (Bloxham *et al.*, 1975), it was of interest to examine its effect on the oestradiol-induced surface coat change under *in vitro* conditions so as to characterize further the anti-implantation action of the drug. In that Tamoxifen was shown to inhibit the *in vitro*, oestradiol-17β-induced, surface coat change in a concentration-dependent manner, it now seems reasonable to suggest that this antioestrogenic action could contribute to the antifertility effect of the drug *in vivo*.

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Table 1 Drug concentrations in the 2 ml of culture medium in which 25 mouse blastocysts were maintained for 16 hours.

Sub-group	*Oestradiol -17β (pg/ml)*	*Tamoxifen (pg/ml)*	*% blastocysts with surface coat change*
(a)	0	0	0
(b)	50	0	100
(c)	25	0	100
(d)	12.5	0	63.7
(e)	6.25	0	0
(f)	0	100	0
(g)	50	100	0
(h)	50	50	16.6
(i)	50	25	88
(j)	50	12.5	100

The percentage occurrence of the surface coat change within each group at this time, as revealed histochemically, is entered in the final column.

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