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THERAPEUTIC POTENTIAL OF PURE ANTIOESTROGENS IN THE TREATMENT OF BREAST CANCER

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Summary—Novel 7α-analogues of 17β-oestradiol like *ICI 164,384*, differ from all antioestrogens described previously in being entirely free of partial agonist activity. In adult rats, ICI 164,384 blocks completely the stimulatory effects of endogenous or exogenous oestrogens and produces a castration-like involution of the uterus without affecting the hypothalamic-pituitary–ovarian axis. If analogous effects were achieved in patients, peripherally-selective *complete* oestrogen withdrawal would occur, which presents a novel pharmacological option not achieved by any current treatment. Studies with human breast cancer cells showed that ICI 164,384 reduced to a greater extent than did tamoxifen, the mitotic fraction. This difference may reflect a synergistic stimulatory interaction between serum growth factors like insulin, and the partial agonist effect of tamoxifen which is not seen with ICI 164,384. In long-term culture in the presence of ICI 164,384 no resistant cell lines developed, as has been observed previously in studies with tamoxifen. Pure antioestrogens might thus have a further therapeutic advantage over partial agonists like tamoxifen in reducing the probability of treatment failure due to the regrowth of tumours from resistant cells.

RATIONALE FOR SEEKING NOVEL ANTIOESTROGENS

The non-steroidal antioestrogen tamoxifen (Nolvadex*) has proved to be a very effective therapy for the treatment of breast cancer in both the advanced disease and in the adjuvant setting [1, 2]. The impressive clinical efficacy of this compound, which is achieved with minimal side-effects, might seem to render unlikely the possibility that alternative, more effective compounds could be discovered. The reasons why this may, in fact, be incorrect and the properties of some new antioestrogens with which to challenge this conclusion are described here.

Although tamoxifen affects a number of cellular events linked to the proliferative capacity of breast cancer cells [3], the balance of evidence favours the view that the most important of these is the competition of tamoxifen with oestradiol for binding to specific oestrogen receptors (ER) [4, 5]. The tamoxifen–ER complex attenuates, but does not always block completely the growth stimulatory mechanisms controlled by endogenous oestradiol–ER. This is manifest in the complex pharmacology of tamoxifen, and of other similar non-steroidal antioestrogens. In the absence of endogenous oestrogens, the effects of treatment may be equivalent to those of oestradiol (full agonist), similar, but of smaller magnitude (partial agonist) or, when endogenous oestrogens are present, there may be partial or complete blockade of normal stimulatory responses (partial or complete antagonist) [6, 7]. This inherent variation is observed in both experimental animals and man at the gene, cell and organ level. We therefore sought to identify novel, ER-specific ligands which would complete effectively with oestradiol without inducing any oestrogen-like actions. Such compounds would be defined pharmacologically as *pure antioestrogens*.

At the inception of our search for pure antioestrogens two important *potential* advantages over partial agonist/antagonist antioestrogens like tamoxifen were recognized. Firstly, *complete* blockade of all stimulatory actions of oestrogens from whatever source in breast cancer patients may result in a more rapid, complete and longer-lasting tumour remission. It should be noted that no current therapy satisfies this criterion. Secondly, pure antioestrogens *may* have an improved toxicology profile in rodents and, therefore, may be more likely to satisfy drug regulatory requirements

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*Nolvadex is a Trade Mark, property of Imperial Chemical Industries plc.

Table 1. Activity of C_7-derivatives of 17β-oestradiol in the immature rat uterotrophic/antiuterotrophic assay

C_7-substituent $(CH_2)_{10}R$	Dose (mg/kg s.c.)	% Agonism[a]	% Antagonism[b]
1. R = —COOH (mixed isomers)	25	23	30
2. —CH_2OH (mixed isomers)	25	28	33
3. —$CH_2N(C_2H_5)_2$ (mixed isomers)	10	33	62
4. —$CONH(CH_2)_5COOH$ (mixed isomers)	25	19	33
5. —$CONH(CH_2)_3CH_3$ (7:3α/β)	10	−3	92
6. —$CONH(CH_2)_3CH_3$ (7α)	10	−3	100
7. —$CONH(CH_2)_3CH_3$ (7β)	25	0	0
8. —$CON(CH_3)(CH_2)_3CH_3$ (7α)	5	−5	102
Tamoxifen	10	41	59

[a]Percentage agonist = $C - A/B - A \times 100$.

[b]Percentage antagonist = $B - D/B - A \times 100$ where A, B, C and D are mean uterine weight (mg/100 g body weight) in groups of animals ($n = 5$) treated with vehicle alone (arachis oil), 17β-oestradiol (0.5 μg/rat), compound alone or together with oestradiol, respectively.

for therapeutic application in non-malignant oestrogen-responsive conditions like endometriosis and benign breast and uterine disease. It has long been recognized that chronic treatment of rats or mice with oestrogens leads to developmental abnormalities, including carcinomas of the reproductive tract. More recently similar abnormalities have been reported in rodents treated with partial agonist antioestrogens [8, 9]. Elimination of oestrogenic actions on any target organ, consistent with a pure antagonist profile, might therefore allow significant extentions of the therapeutic use of antioestrogens beyond their current confinement to the treatment of established malignancy.

The key problem in instituting a search for novel ER-specific ligands was to identify a suitable chemical lead. It was considered unlikely that pure antagonists could be found amongst non-steroidal variants of triphenylethylene- or benzothiophene-derived molecules since we and others [10, 11] had already explored, at length such medicinal chemistry. Literature review revealed that high affinity for ER is retained uniquely in oestradiol analogues bearing an extended methylene chain at the 7α position of the steroid nucleus [12, 13]. Those studies were designed to facilitate generation of antibodies for oestradiol radioimmunoassay, or affinity matrices for ER purification and no data on the biological activity of such compounds was reported. Synthesis of analogues with a variety of functional groups attached to the 7-position of oestradiol by a decamethylene bridge produced compounds with mixed agonist/antagonist activity ([14] and Table 1) but also, for the first time, molecules devoid of oestrogen-like actions on the uterus which also blocked completely the uterotrophic action of co-administered oestradiol [14, 15]. Thus, such compounds exemplified by ICI 164,384 (compound 8, Table 1) represented the first reported steroidal pure antioestrogens.

In the remainder of this presentation the properties of ICI 164,384 will be reviewed briefly, in particular the data which are relevant to the potential utility of pure antioestrogens in breast cancer therapy.

PHARMACOLOGY OF PURE ANTIOESTROGENS

Initially, in screening novel compounds for activity the classical uterotrophic/antiuterotrophic assay employing a 3-day treatment of immature female rats with escalating doses of compound alone or together with oestradiol was used [16]. The effects of ICI 164,384 in this assay and in analogous assays in immature mice and in ovariectomized adult rats and mice have been reported elsewhere [15] and are uniformly consistent with pure antagonist activity. Structure–activity studies have shown that orientation and composition of the 7-substituent radically affect both potency and profile of activity [14, 15]. Other investigators have confirmed the absence of uterotrophic and mammatrophic activity of ICI 164,384 [17]. A particularly interesting property of ICI 164,384 is its ability, when co-administered with tamoxifen to block the trophic activity of the partial agonist antioestrogen in both the uterus [15] and mammary gland of the rat [17]. These data provide a persuasive case that each class of ligand is acting through common ER-mediated mechanisms.

Chronic treatment of intact rats with ICI 164,384 produced a castration-like involution of the uterus without affecting hypothalamic-pituitary activity [18]. If a similar selectivity of action were achieved in patients it would be

possible to treat breast and uterine pathologies in pre-menopausal women without disturbing the hypothalamic-pituitary–ovarian axis. In the latter connection, and with respect to issues of toxicology raised previously (*vide infra*) it should be noted that ICI 164,384 treatment of neonatal rats, in contrast to oestradiol or tamoxifen, does not cause premature puberty (vaginal opening) or affect subsequent fertility [18, 19].

BIOCHEMISTRY OF PURE ANTIOESTROGENS

ICI 164,384, like tamoxifen, inhibits competitively the binding of oestradiol to cytosolic ER of the rat uterus [15] but its affinity is substantially greater than that of tamoxifen (0.19 vs 0.025 cf. oestradiol = 1). Unlike tamoxifen ICI 164,384 does not bind to "antioestrogen-specific binding sites" [20] which occur commonly in breast tumours and are distinguished from ER by their ability to bind tamoxifen (and other triphenylethlene-derived antioestrogens), but *not* oestradiol.

Direct studies of ligand receptor interaction using tritium-labelled ICI 164,384 and partially purified ER from a human uterus showed that the equilibrium dissociation constant, 0.69 ± 0.10 nM, is similar to that of oestradiol, 0.44 ± 0.20 nM, and that the kinetics of binding, and number of binding sites for oestradiol and ICI 164,384 are similar [21]. The complex formed between ER and ICI 164,384 is deficient in transformation to a conformation which recognizes DNA [21] and this inability to activate the receptor is reflected by the inability of the pure antioestrogen to activate transcription of oestrogen-responsive genes [22–24]. The pure antioestrogen appears to "disable" ER and thereby prevent its biological effects.

EFFECTS ON HUMAN BREAST CANCER CELLS

ICI 164,384 inhibits oestradiol-stimulated growth of MCF-7 and ZR-75-1 human breast cancer cells in a competitive and reversible manner: it is approx. 100-fold more potent than tamoxifen and, unlike all of the non-steroidal antioestrogens, is devoid of growth stimulatory activity [15, 18, 25, 26]. In common with observations *in vivo*, ICI 164,384 blocks the proliferative effects of other antioestrogens [26]. Growth inhibition is confined to ER+ cells [25, 26] except that, in common with other antioestrogens high concentrations are cytotoxic [25, 27]. As is the case with other antioestrogens growth inhibition is associated with an accumulation of cells in early G1 [27–29]. However, early studies which indicated that ICI 164,384 is not only more potent than tamoxifen but also more effective in reducing cell growth, i.e. 80% vs 50% decrease in cell number [18] have been confirmed in comparative studies with other partial agonist antioestrogens [25, 26]. This difference between pure and partial agonist antioestrogens is not a simple reflection of differences in affinity for ER [28] but is associated with a marked reduction in the *proportion* of cells which continue to proliferate in the presence of ICI 164,384 compared with tamoxifen [29]. The difference most probably reflects the complete absence of oestrogen-like stimulation in ICI 164,384 treated cells, a particularly important consideration in this model of breast cancer since the weak agonist actions of tamoxifen are synergistically enhanced by the presence of other growth factors like insulin [28, 29]. Extrapolation of these studies to the human disease supports the hypothesis outlined earlier that pure antioestrogens may offer therapeutic advantages over tamoxifen.

Two other features of the action of ICI 164,384 on cultures of breast cancer cells may also prove significant clinically. Firstly, in an assay of the capacity of MCF-7 cells to penetrate basement membrane in which oestradiol enhances invasive capacity, 4-hydroxytamoxifen also stimulated invasiveness. In contrast, ICI 164,384 did not, and was able to block both oestradiol and 4-hydroxytamoxifen stimulation of tumour cell invasiveness [26]. Secondly, no resistant sub-lines of MCF-7 cells could be isolated from cultures exposed continuously to ICI 164,384 under conditions which have been used successfully by others to generate tamoxifen-resistant cells [30]. Extrapolation of these observations to the clinical setting suggests that pure antioestrogens may be more effective than tamoxifen in reducing metastatic spread of breast cancer and that relapse during therapy may occur less frequently.

ANTITUMOUR ACTIVITY

Comparative studies of the effect of tamoxifen and ICI 164,384 on the growth of established DMBA-induced rat mammary carcinoma did not demonstrate any advantage for the pure antioestrogen [31]. Neither treatment was as efficacious as ovariectomy [32]. It should be

noted, however, that these different treatments are not pharmacologically equivalent and are therefore not directly comparable. For example, both tamoxifen and ovariectomy, but not ICI 164,384 peturb neuroendocrine mechanisms, including prolactin secretion and food intake, which affect tumour growth [31, 32]. The significance of such mechanisms in patients is likely to be minimal when compared with direct blockade of ER in the tumour.

In studies of the effects of long-term tamoxifen treatment of athymic mice bearing MCF-7 derived human breast tumours it has been shown that "tamoxifen-resistant" tumours may be isolated [33]. These tumours were not drug resistant in the classical sense but rather appeared to be dependent for growth on the continued administration of tamoxifen. In these tumours growth may still be inhibited by treatment with ICI 164,384 [34] and the pure antioestrogen, unlike tamoxifen did not sustain tumour growth. Similarly, a human endometrial tumour whose growth is athymic mice is stimulated by oestradiol or tamoxifen [35] is also inhibited by pure antioestrogen treatment [36]. Potentially, these observations may have considerable clinical implications since they imply that some tumours recurring after an initial response to tamoxifen will not be cross-resistant to pure antioestrogen treatment.

CONCLUSIONS

The properties of pure antioestrogens highlighted here by reference to ICI 164,384 offer the potential for a novel treatment of human breast cancer. By comparison with existing "antioestrogen" therapy such compounds offer for the first time the opportunity to test the hypothesis that *complete* blockade of endogenous oestrogen action will result in an improved response. The extent and nature of the putative improvement(s) must await clinical trials but several lines of evidence presented here provide powerful presumptive evidence that such improvements will indeed be identified.

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