Similarities and distinctions in the mode of action of different classes of antioestrogens

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Introduction

Since the description of the first antioestrogen, MER 25, more than four decades ago (Lerner et al. 1958), and of tamoxifen ten years later (Harper & Walpole 1967a,b), a large number of antioestrogens with diverse chemical structures have been described in the literature. The high level of interest in the synthesis and testing of new antioestrogens has been sustained by the therapeutic and commercial success of tamoxifen in the treatment of breast cancer. More recently, the first demonstration that tamoxifen can reduce the incidence of breast cancer in healthy women at high risk of developing the disease (Fisher et al. 1998) and that raloxifene can prevent osteoporosis (Bryant & Dere 1998) have provided a new impetus for drug discovery. Of the many analogues of tamoxifen described, only one other, chlorotamoxifen (toremiphene, Kangas 1990), has been marketed for breast cancer whilst several others are in various stages of clinical evaluation (e.g. idoxifene, Coombes et al. 1995; droloxifene, Bruning 1992). These molecules have very similar chemical and pharmacological properties: they are all non-steroidal, triphenylethylene-derived structures and all are partial agonists (mixed agonist/antagonist activities). A number of other non-steroidal structures with antioestrogenic activity have been reported, including substituted tetrahydropyranophanes (e.g. nafinoxifene, Duncan et al. 1963; trioxifene, Jones et al. 1979), indole derivatives (e.g. zindoxifene, Stein et al. 1990; ZK 119010, von Angerer 1990), benzoanthiophenes (e.g. LY117018, Black & Good 1980; LY156758 (keoxifene), Clemens et al. 1983) and benzopyrans (Sharma et al. 1990, Grese et al. 1996). All of these antioestrogens share with tamoxifen the pharmacological characteristic of partial agonist activity; thus, whilst they antagonise the trophic action of oestrogens, this activity is incomplete because each compound has intrinsic oestrogen-like stimulatory (agonist) activity in vivo. The effect of drug treatment is a balance between agonist and antagonist activity. Whilst the term ‘partial agonist’ accurately conveys the idea that all of these antioestrogens have some oestrogenic activity, it does not adequately describe their complex organ, cell and gene-specific actions (Furr & Jordan 1984, Jordan & Murphy 1990, Wakeling 1995, Macgregor & Jordan 1998).

The major impetus for research has remained the desire to reduce or preferably to eliminate partial agonism. Removal of oestrogen-like activity might confer a clinical advantage compared with tamoxifen in the form of more rapid, more complete or longer-lasting remissions in breast cancer (Wakeling 1993). Although this objective was approached incrementally by trioxifene (Jones et al. 1979), LY117018 (Black & Good 1980), LY156758 (keoxifene, Clemens et al. 1983) and ZK 119010 (Nishino et al. 1991), which showed an ordered reduction in oestrogenic (uterotrophic) activity compared with tamoxifen, it was apparent that synthetic chemistry based on non-steroidal structures was unlikely to produce antioestrogens completely free of partial agonism. A new chemical approach was needed and studies on the preparation of an affinity matrix to facilitate purification of the oestrogen receptor (ER) suggested one such possibility (Bucourt et al. 1978). Attachment of an alkylene bridge structure between the ligand and the matrix material at carbon atom 7 of oestradiol provided the optimum site on the steroid nucleus for substituents to retain a high affinity for ER. At ICI (now AstraZeneca, formerly Zeneca) this observation was explored by the synthesis of a series of 7-alkyl analogues of oestradiol (Bowler et al. 1989). The first compound which completely blocked the trophic action of oestradiol on the rat uterus and was devoid of uterotrophic activity, ICI 164384, emerged from this approach (Wakeling & Bowler 1987). ICI 164384 was described as a ‘pure’ antioestrogen to emphasise that it is clearly distinguished pharmacologically from tamoxifen and other partial agonists. The most striking difference between ICI 164384 and the partial agonists is the capacity of the former to block the uterotrophic action of the latter (Wakeling & Bowler 1987). Further chemistry on 7α-substituted oestradiol produced a more potent pure antioestrogen, ICI 182780 (Wakeling et al. 1991). This drug candidate molecule has been formulated in a long-acting solution for monthly intramuscular injection (Faslodex, a trademark property of the AstraZeneca group of companies) and has demonstrated therapeutic efficacy in...
advanced breast cancer resistant to tamoxifen (Howell et al. 1995).

Following the discovery of ICI 164384, a number of other steroid-derived non-agonist antioestrogens have been reported, including further modifications of 7α-substituted oestradiol (Levesque et al. 1991) and 11β-amidoalkoxyphenyl oestradiol derivatives (e.g. RU 58668, Nique et al. 1994). Further synthetic chemistry at Zeneca and elsewhere produced non-steroidal molecules completely free of agonist activity; for example ZM 189154, in which the alkylsulphonyl side-chain of ICI 182780 is substituted on a 2-methyl tetrahydronaphthalene (Dukes et al. 1994), EM-800, a benzopyran derivative (Gauthier et al. 1997), and dichlorotriarylcyclopropanes (Day et al. 1991).

The spectrum of actions of partial agonists like tamoxifen in patients, which encompasses some benefits of oestrogen-like effects, for example on bone and lipids (Macgregor & Jordan 1998), as well as less desirable agonist effects on the uterus (Fornander et al. 1993), with therapeutic efficacy in the treatment and prevention of breast cancer (Fisher et al. 1998), has opened-up other applications for antioestrogens. For example, the oestrogenic effects of raloxifene (LY139481.HCl, previously known as keoxifene) on bone and lipids, together with its reduced uterotrophic activity compared with tamoxifen, may provide an alternative to conventional hormone replacement therapy to prevent osteoporosis (Delmas et al. 1997, Bryant & Dere 1998). Raloxifene is described as a selective oestrogen receptor modulator (SERM, Kauffman & Bryant 1995). Despite the fact that raloxifene was less effective than tamoxifen in a rat model of breast cancer (Gottardis & Jordan 1987), it has poor efficacy in advanced breast cancer after tamoxifen treatment (Buzdar et al. 1988), clinical trial data in patients treated for prevention of osteoporosis indicate that raloxifene may reduce the incidence of breast cancer (Cummings et al. 1999). The utility of raloxifene in breast cancer prevention is being assessed in a comparative trial with tamoxifen.

There remains considerable potential to define and discover the ideal SERM to combine the benefits of oestrogen replacement in postmenopausal women on the brain, cardiovascular system and bone whilst removing adverse effects on the uterus and preventing breast cancer (Mitlak & Cohen 1997). Thus, a third generation of SERMs (where tamoxifen and raloxifene represent the first and second generations) is emerging which show gains in potency or selectivity in animal studies; for example, analogues of raloxifene where the carbonyl is replaced by oxygen (Palkowitz et al. 1997) or the side-chain is conformationally restricted (LY 357489, Grese et al. 1998), novel triphenylethylene homologues (e.g GW 5638, Willson et al. 1997) and tetrahydronaphthalenes (e.g. CP-336,156, Ke et al. 1998). As yet no clinical studies on third generation SERMs have been reported.

The pure antioestrogens, ICI 164384 and ICI 182780, are clearly differentiated from tamoxifen and SERMs pharmacologically; the key questions are how are these different actions explained at the molecular level and can this knowledge be translated to facilitate the discovery of new ‘targeted’ oestrogens and antioestrogens for specific therapeutic application?

Mode of action

Classical oestrogen receptor pathway

The cell response to oestrogens is dictated by the presence or absence of ER; oestradiol binds to ER with high affinity and specificity to initiate a sequence of events which include dissociation of heat shock proteins from ER, receptor dimerisation, preferential nuclear localisation and dimer binding to discrete DNA sequences, termed oestrogen response elements (EREs), in the regulatory regions of target genes, and activation of transcription mediated by two activation functions, AF1 and AF2 respectively, of ER (Beato 1989, Tsai & O’Malley 1994). The ligand-bound receptor regulates gene activity through AFs by recruiting other proteins to the general transcription complex. These proteins act as coactivators or corepressors of oestrogen-regulated transcription (Horwitz et al. 1996, White & Parker 1998). The activity of the aminoterminal AF1 is regulated by growth factors acting through the MAP kinase pathway (Kato et al. 1995), whereas the carboxy-terminal AF2 located in the ligand binding region of ER is activated by oestradiol (Kumar et al. 1987); full agonist activity requires both AF1 and AF2 to be active. The activity of AF1 is cell type specific (Tora et al. 1989). These actions are illustrated in Fig. 1.

The molecular effects of tamoxifen (or its higher affinity metabolite 4-hydroxytamoxifen which is often used instead of tamoxifen in in vitro studies), bound to ER (see Fig. 2) are markedly similar to those of oestradiol (McDonnell et al. 1995, Katzenellenbogen et al. 1996). The most significant difference is that AF2 is no longer functional in the tamoxifen-ER complex whereas AF1 remains active (Berry et al. 1990), and the partial agonism attributed to AF1 is dependent both on cellular and promoter context (Berry et al. 1990, Tzukerman et al. 1994). Comparison of the molecular structure of ER bound to oestradiol or raloxifene (Bzozowski et al. 1997) or 4-hydroxytamoxifen (Shiau et al. 1999) has shown that ligand-specific conformational changes in the ER strongly influence protein-protein interactions in the transcriptional complex, since such changes alter the orientation of AF2 relative to the coactivator recognition sequence of ER (Shiau et al. 1999). Ligand-dependent conformational changes in ER have been analysed further by using phage display peptide affinity mapping of different ER complexes to show that there are distinctive differences between...
Figure 1 Mode of action of oestradiol. 1. Oestradiol (E) binds with high affinity to ER and displaces receptor associated proteins (RAPS) e.g. HSP90. 2. E-ER complex homodimerises and localises preferentially in the cell nucleus. 3. E-ER homodimer binds DNA sequence at palindromic ERE in the promotor region of oestrogen-sensitive genes. 4. Activation of transcription by ER involves interaction of the two transcription activation functions AF1 (activity independent of E) and AF2 (activated by E binding to ER) of ER with transcriptional coactivators or corepressors to stimulate or inhibit the activity of RNA polymerase II (RNA POLII).

Figure 2 Mode of action of tamoxifen. 1. Tamoxifen (T) binds to ER and dissociates RAPS. 2. T-ER complex homodimerises and translocates to the cell nucleus. In T-ER complex, AF1 is exposed but AF2 is not. 3. T-ER dimer binds DNA sequence at palindromic ERE in the promoter region of oestrogen-sensitive genes. 4. Transcription of E-responsive gene(s) is attenuated because AF2 is inactive; partial agonist activity results from AF1 which remains exposed in T-ER complex. RNA POLII, RNA polymerase II.

Figure 3 Mode of action of Faslodex. 1. Faslodex (F) binds to ER and dissociates RAPS. 2. Faslodex binding to ER accelerates receptor degradation i.e. ‘down-regulates’ ER. 3. Rate of dimerisation and nuclear localisation of Faslodex-ER complex is reduced. 4. Reduced binding of Faslodex-ER to ERE. 5. No transcription of E-responsive genes; since AF1 and AF2 are inactive no coactivators are recruited. RNA POLII, RNA polymerase II.
Wakeling: Mode of action of antiestrogens

Oestradiol-ER, tamoxifen-ER and ICI 182780-ER complexes (Norris et al. 1999, Paige et al. 1999).

Schemes more complex than that shown in Fig. 2 are necessary to account for the gene-specific actions of partial agonist antiestrogens (Katzenellenbogen et al. 1996). Such schemes invoke differences in the efficiency of coactivator or corepressor coupling in the transcriptional complex, in addition to ligand affinity and receptor conformation differences. Thus, amongst partial agonists which show varying degrees of agonism depending on which response is measured, agonism is cell, promoter and effector sensitive (McDonnell et al. 1995, Katzenellenbogen et al. 1996). Further complexity is added by the recent description of a third activation domain (AF2a) in ER, which may function when AF1 and AF2 are inactive, and might thus account for how certain ligands act as full agonists in the bone and cardiovascular system whilst being antagonists in the uterus (Norris et al. 1997). Furthermore, ER can be activated in a ligand-independent manner by other signalling molecules, for example dopamine (Smith et al. 1993), epidermal growth factor (Ignar-Trowbridge et al. 1993), cAMP (Aronica & Katzenellenbogen 1993) and insulin-like growth factor-I (IGF-I) (Newton et al. 1994), and so antiestrogen effects, for example on IGF-I synthesis (Huynh & Pollack 1993), could indirectly affect ER activity. Growth factor-induced phosphorylation of ER can amplify the partial agonist effect of tamoxifen (Kato et al. 1995).

In contrast to the partial agonists, studies of the mode of action of the pure antiestrogens, ICI 164384 and ICI 182780, have produced a consensus that the ability of the ER to activate or inhibit transcription in a ligand-dependent or -independent manner in vivo is completely attenuated by ICI 164384 and ICI 182780 (Wakeling 1995). Multiple changes in ER function following pure antiestrogen treatment appear to contribute to this complete abrogation of oestrogen action (see Fig. 3). These include impaired dimerisation (Fawell et al. 1990, Chen et al. 1999), increased receptor degradation (Dauvois et al. 1992, Nicholson et al. 1995, Borras et al. 1996, Pink & Jordan 1996) and disrupted nuclear localisation (Dauvois et al. 1993, Htun et al. 1999). The rapid loss (‘down-regulation’) of ER following ICI 164384- or ICI 182780-treatment of cells in culture (Dauvois et al. 1992, Nicholson et al. 1995, Borras et al. 1996, Pink & Jordan 1996), or from the uterus after in vivo treatment (Gibson et al. 1991), is likely to play a major role in abrogating oestrogen action. ER down-regulation would account for the ability of pure antiestrogens to block the activation of ER by other mediators such as dopamine, cAMP and growth factors (Aronica & Katzenellenbogen 1993, Ignar-Trowbridge et al. 1993, Smith et al. 1993, Newton et al. 1994, El-Tanani & Green 1997). This could have important therapeutic implications in breast cancer, since no other treatment option (tamoxifen, toremiphene, aromatase inhibitors) leads to removal of the ER and consequently to blockade of all mitogens acting through ER. In women with breast cancer treated with ICI 182780 for seven days, the median tumour ER index was reduced from a pre-treatment median of 0.72 to 0.02 (DeFriend et al. 1995), suggesting that this pure antiestrogen also leads to down-regulation of ER in man.

The molecular mechanism of action of pure antiestrogens other than ICI 164384 and ICI 182780 has been less intensively studied. However, RU 58668 does disrupt nuclear localisation (Devin-Leclerc et al. 1998) and, like ICI 182780, leads to rapid loss of ER in human breast cancer cell lines (Müller et al. 1998). No similar studies have been reported for the nonsteroidal pure antiestrogens but analysis of oestrogen binding capacity of the uterus after long-term treatment of rats or mice with EM-800 indicated loss of ER (Luo et al. 1997, Labrie et al. 1999). This loss of ER may be a consequence of tissue atrophy rather than the acute ‘down-regulation’ of ER observed following treatment with the steroidal pure antiestrogens. In breast cancer cells, EM-652 (SCH 57078), the parent compound of which EM-800 (SCH 57050) is a pro-drug, decreased ER protein levels to a greater extent than raloxifene but to a lesser extent than ICI 182780 (MacGregor & Jordan 1999). The classification of EM-800 as a pure antiestrogen like ICI 182780 is incorrect since it has oestrogenic effects on bone density and serum lipids in rats (Labrie et al. 1999), and is like tamoxifen and raloxifene in this respect. Clearly, if the latter effects are ER-mediated EM-800 cannot ‘down-regulate’ ER in bone cells or in the liver. EM-652 has thus been designated a third generation SERM (Labrie et al. 1999).

SERM-like activity of EM-652 has been observed in cells transfected with ER carrying a naturally occurring point mutation (Asp351Tyr) which changes the pharmacology of raloxifene from antagonist to agonist (Levenson & Jordan 1998). EM-652 similarly acts as an agonist on this ER mutant (MacGregor & Jordan 1999), whereas ICI 182780 remains a complete antagonist (Levenson & Jordan 1998).

As a consequence of the ‘down-regulation’ of ER by ICI 164384 or ICI 182780, the transcription of ER-regulated genes should be completely blocked. This has proved to be the case in cells, in animal models, and in man. In the rat uterus, oestradiol and tamoxifen stimulate the expression of a number of genes, including complement component C3 (Galman et al. 1990), calbindin-D (Blin et al. 1995), IGF-I (Huynh & Pollack 1993), and vascular endothelial cell growth factor and c-fos (Hyder et al. 1997). In each case, ICI 164384 or ICI 182780 showed no induction of transcription and, when administered with oestradiol or tamoxifen, completely blocked oestrogen or tamoxifen induction of these genes. Similarly, these two compounds act as pure anti-estrogens on the transcription of oestrogen inducible genes in human breast cancer cells in vitro (May et al. 1989, Wiseman et al. 1989, Nicholson et al. 1995), in vivo (Osborne et al. 1995), and in patients with breast cancer (DeFriend et al. 1995).
The contrasting actions of tamoxifen and the pure antioestrogens also extend to effects on genes which are down-regulated by oestrogens. For example, expression of the IGF binding protein-3 (IGFBP-3) is suppressed by oestriadiol and tamoxifen whereas ICI 182780 significantly stimulated expression (Huyhn & Pollack 1994). Similarly, blockade by ICI 182780 of oestrogen-suppressed transcription of several other genes, pMGT1 (Manning & Nicholson 1993), IGFBP-5 (Huyhn et al. 1996) and quinone reductase (Montano & Katzenellenbogen 1997), allows these genes to be expressed. ICI 164384 and ICI 182780 also effectively inhibit cell growth and the transcription of oestrogen-regulated genes in human breast cancer cells resistant to growth inhibition by tamoxifen (Coopman et al. 1994, Lykkesfeldt et al. 1994), consistent with the absence of cross-resistance in vivo between the two classes of antioestrogen (Howell et al. 1995, Osborne et al. 1995).

Role of oestrogen receptor β

The discovery of a novel form of ER, designated ERβ (Kuiper et al. 1996, Mosselman et al. 1996, Tremblay et al. 1997) to distinguish it from the classical form of ER (ERα), raised the possibility that cell-specific actions of antioestrogens could be related to differential expression or actions of ERα and ERβ (Kuiper & Gustafsson 1997). Further complexity is added by the discovery that ERα and ERβ can heterodimerise in tissues where both are expressed (Pace et al. 1997, Pettersson et al. 1997, Tremblay et al. 1999) and that a functional variant of ERβ, ERβ₂, is expressed in normal rat tissues (Peterson et al. 1998). It is not known whether ERβ functions in vivo in the control of transcription of specific target genes, but in vitro transfection studies have demonstrated that ERβ can mediate oestrogen-induced reporter gene expression in the absence of ERα, and that ICI 164384, ICI 182780 and EM-652 block this effect (Mosselman et al. 1996, Paech et al. 1997, Tremblay et al. 1998). In reporter gene transcription assays, ERβ did not mediate tamoxifen agonism (Watanabe et al. 1997, McNerney et al. 1998) but the ERα-ERβ heterodimer can do so via ERα AF1 in a promotor- and cell context-dependent manner like the ERα-ERα homodimer (Tremblay et al. 1999).

Studies with transgenic mice lacking ERα or ERβ indicate a dominant role for ERα in reproductive development (Lubahn et al. 1993, Krege et al. 1998), and in ERα knock out (KO) mice tamoxifen has no agonist action on the uterus (Korach 1994), despite the fact that ERα and ERβ are expressed at high levels in the uterus (Kuiper et al. 1997). However, since oestrogens and antioestrogens can have different affinities for ERα and ERβ (Kuiper et al. 1997), induce distinct conformations of ERα and ERβ (Paige et al. 1999), and there are ligands which function differentially as oestrogens or antioestrogens between ERα and ERβ (Sun et al. 1999), there remains the possibility that ERβ-selective ligands could prove to be useful therapeutically in targeting the neuroprotective (Kuiper et al. 1998) and cardioprotective actions of oestrogens (Mäkelä et al. 1999).

Alternative mechanisms

An alternative pathway of ER action is suggested by the observation that ER stimulates transcription from promoters containing an AP1 (fos/jun) site (Gaub et al. 1990). This mechanism (see Fig. 4) does not require ER binding to DNA but direct association between ER and AP1 may account for the synergy between growth factors and oestrogens, for example that between oestradiol and insulin on the growth of MCF-7 human breast cancer cells (Wakeling et al. 1989).

Tamoxifen can also activate the ER/AP1 pathway. This was demonstrated by transfecting the human collagenase promoter, which contains a consensus AP1 site linked to a reporter gene, into HeLa cells and co-transfecting with human ER (Webb et al. 1995). This effect was cell specific, being observed in endometrial cell lines but not in breast cells. A similar differential action of tamoxifen has been observed in vivo: in nude mice bearing xenografts of both a human endometrial tumour and a breast tumour, tamoxifen stimulated the growth of the endometrial tumour and inhibited that of the breast tumour (Gottardis et al. 1988). The pure antioestrogens, ICI 164384 and ICI 182780, inhibit tamoxifen- and toremiphene-stimulated growth of endometrial tumours (Gottardis et al. 1990, O’Regan et al. 1998). Similarly, tamoxifen-stimulated growth of MCF-7 breast xenografts, which manifests as tamoxifen resistance after long-term treatment, analogous to the development of tamoxifen resistance in breast cancer patients, is also inhibited by ICI 164384 and ICI 182780 (Gottardis et al. 1989, Osborne et al. 1994). Thus, AP1-mediated effects could account for tamoxifen’s trophic actions on the uterus and endometrial tumours and development of tamoxifen-resistant breast tumours. If this is the case it is clear that the pure antioestrogens effectively inhibit this ER/AP1 pathway in vivo.

ICL 164384 can activate ER/AP1-mediated transcription in transfected HeLa and Ishikawa (human endometrium derived) cells but this effect required expression of supraphysiological levels of ER and did not occur at physiological ER levels (Webb et al. 1995). In untransfected MCF-7 cells the weak growth stimulatory effect of 4-hydroxytamoxifen was amplified in the presence of insulin; under these conditions a weak (non-significant) agonist effect of ICI 164384 was revealed (Wakeling et al. 1989). ICI 164384, unlike tamoxifen, was a pure antagonist in all cell types when anERE-reporter construct was used (Webb et al. 1995). Other studies on the response of Ishikawa cells to 4-hydroxytamoxifen and ICI 164384 showed that ICI 164384 inhibited oestradiol or tamoxifen-induced cell growth, progesterone receptor (PR) synthesis (Jamil et al. 1991) and alkaline phosphatase activity (Albert et al. 1990), while ICI 164384 alone weakly stimulated PR, a modest partial agonist effect.
Figure 4 Oestrogen and growth factor signals interact. Growth factor-mediated phosphorylation (P) cascades activate the fos/jun (AP1) transcriptional complex to promote transcription of AP1-responsive genes. ER binds to the fos/jun complex and enhances transcriptional activation at AP1 sites. Tamoxifen acts as an agonist at AP1 sites in some tissues, e.g. the uterus. ER can interact with AP1 in the absence of oestradiol, possibly by growth factor-mediated phosphorylation of ER. Pure antioestrogens down-regulate ER, block this ‘agonist’ response pathway and can inhibit growth factor signalling in the absence of oestrogens. RNA POLII, RNA polymerase II; RE, response element.

but did not stimulate cell growth (Jamil et al. 1991). A similar dissociation between the effects of ICI 164384 on progesterone receptor synthesis (agonist effect) and cell growth (antagonism) was observed in cells from the fetal guinea pig uterus, whereas in breast cancer cells ICI 164384 acted as a pure antagonist on growth and PR synthesis (Pasqualini et al. 1990). Tamoxifen, raloxifene and ICI 164384 also activate ERβ-mediated AP1 transcription in Ishikawa and MCF-7 human breast cancer cells, whereas oestradiol did not (Paech et al. 1997). However, ICI 182780 exhibited only oestrogen antagonist activity on ERα and ERβ with both ERE- and AP1-reporters in fetal bovine uterine cells (Malayer et al. 1999). These studies raise the possibility that the AP1 pathway could underlie the agonist actions of ICI 164384 reported in some in vitro experiments. These weak agonist effects of ICI 164384 do not compromise the pharmacological definition of this compound as a pure antioestrogen since no such agonism has been demonstrated in vivo. That this has not been possible is consistent with the ‘down-regulation’ of ER by ICI 164384 which will severely limit ER and ER/AP1 signalling but, in in vitro models where ER is overexpressed, sufficient ER may remain to allow ICI 164384 to activate weakly the ER/AP1 pathway. Down-regulation of ER by pure antioestrogens would also account for their capacity to inhibit growth factor signalling through the AP1 pathway in the absence of oestradiol (Wakeling et al. 1989).

The mechanisms by which SERMs act specifically as agonists on bone, whilst having low or no agonist action on the reproductive tract and the breast, remain unclear but may involve mechanisms other than direct activation of oestrogen-responsive genes (McDonnell & Norris 1997, Willson et al. 1997). One such mechanism may involve oestrogen suppression of the action of osteotrophic factors like parathyroid hormone and interleukin-1 (IL-1). These factors promote interleukin-6 (IL-6)-mediated bone resorption by activating the transcription factors NF-κB and C/EBPβ which bind to and activate the IL-6 promotor (Stein & Yang 1995, Kurebayashi et al. 1997). ER binds to NF-κB and C/EBPβ reducing their transcriptional activity and therefore reducing IL-6 production and IL-6-mediated bone resorption (Kassem et al. 1996). Oestradiol has no effect on the constitutive production of IL-6 but inhibits IL-1-stimulated IL-6 production (Kassem et al. 1996). ICI 182780 blocks this effect of oestradiol, but 4-hydroxytamoxifen had no effect on IL-6 promotor activity (Kurebayashi et al. 1997). The effect of other SERMs on IL-6 production in osteoblasts has not been reported. Other cytokines which are responsive to oestrogens, for example tumour necrosis factor (Kimble et al. 1997), transforming growth factor β (TGFβ) (Yang et al. 1997) and bone morphometric protein-6 (BMP-6) (Rickard et al. 1998) may play a more important role than IL-6 in mediating SERMs’ effects on bone. Both oestradiol and raloxifene increased TGFβ production in oestrogen-deficient rats (Yang et al. 1996), whereas ICI 182780 blocked oestrogen-induced TGFβ production by osteoblasts (Robinson et al. 1996), thus the differential response of TGFβ appears to distinguish between SERMs and the pure antioestrogen.

Another mechanism potentially involved in tamoxifen agonism is the activation of the human retinoic acid receptor
α-1 (hRARα-1) promoter by ERβ (Zou et al. 1999). The activation of hRARα-1 promoter by tamoxifen did not require direct DNA binding of ERβ. Other antioestrogens including raloxifene, ICI 164384 and ICI 182780 also acted as agonists through ERβ, so this mechanism does not distinguish between SERMs and pure antioestrogens.

Conclusions and future directions

The near-term (5–10 years) future for therapeutic use of the antioestrogens will be clarified by the outcome of clinical trials currently in progress. For example, both Faslodex and EM-800 are in Phase III studies in patients with advanced breast cancer who have relapsed on prior endocrine treatment. Most of these patients have been treated previously with tamoxifen, and animal model data and Phase II clinical trials data predict a successful outcome (Gottardis et al. 1989, Osborne et al. 1994, 1995, Howell et al. 1995, Labrie et al. 1999). The efficacy of Faslodex in the treatment of breast cancer at first relapse following therapy for primary disease is also being compared directly with that of tamoxifen. These studies will answer the important question – to what extent, if any, does the partial agonism of tamoxifen limit the quality or duration of response to antioestrogen treatment? Associated benefits, like the elimination of uterotrophic effects during pure antioestrogen treatment, will need to be balanced against the potential adverse effects of oestrogen blockade on bone metabolism, cardiovascular and neuroendocrine parameters. Such adverse effects may assume greater importance if pure antioestrogens are used in pre-menopausal patients or if improved efficacy compared with tamoxifen supports their use in adjuvant treatment or ultimately for breast cancer prevention.

It should not be assumed that the outcome of the clinical trials with different antioestrogens will be identical. It is clear that ICI 182780 and EM-800 have different mechanisms of action, and different outcomes must be expected based on this difference, particularly with respect to effects on tissues other than the breast tumour. For example, it is facile to believe that since EM-800 is a third generation SERM with oestrogen-like effects on the bone it might be preferred to Faslodex to treat both pre- and post-menopausal patients. However, it is clear that the effects of these agents on bone metabolism are not fully understood (Sibonga et al. 1998); complex mechanisms other than those directed by ERα and ERβ-mediated transcription, involving multiple bone morphometric factors and differential effects on different bone parameters remain to be fully elucidated. The lack of a complete understanding of how oestriadiol, SERMs and pure antioestrogens act on bone may prove a significant obstacle in the search for compounds with better activity than raloxifene to prevent osteoporosis.

The ‘third generation’ SERMs, which are targeted at the prevention of breast cancer, osteoporosis and cardiovascular and neurodegenerative disease in the aging female rather than to breast cancer treatment, offer gains in potency in model studies, but it is not yet clear whether this will translate into efficacy or tolerance advantages in patients over more conventional forms of hormone replacement therapy. Moreover, the assessment of oestrogenic activity of SERMs in peripheral tissues may not predict their effects in the brain (Shughrue et al. 1997) and, as with bone, the mechanisms by which oestrogens affect neurones are poorly understood (Paganini-Hill 1997, Wickelgren 1997). The central effects of ICI 182780 and EM-800 also differ significantly and this difference could have profound implications for tolerance and for potential utility in younger women. At doses which provide efficacy on peripheral tissues, ICI 182780 does not enter the brain (Wade et al. 1993, Ordog et al. 1998) and thus would not be expected to exacerbate or precipitate hot flushes or to affect adversely neurone function (mood control, neurodegenerative conditions etc), or to disturb the hypothalamic-pituitary-ovarian axis. The limited clinical data available to date support this assumption (Thomas et al. 1994, Howell et al. 1995). In contrast, EM-800 enters the brain and acts as an antagonist in the hypothalamus (Luo et al. 1998). Similar to EM-800, raloxifene acts like an antagonist in the brain and consequent disturbances of the hypothalamic-pituitary-ovarian axis preclude use of these SERMs to treat benign proliferative diseases of pre-menopausal women (e.g. endometriosis and fibroids) (Baker et al. 1998).

Another area of uncertainty about the mode of action of oestrogens and antioestrogens is how they influence cardiovascular function (Clarkson & Anthony 1997). The cardioprotective effect of oestrogens is due to altered plasma cholesterol levels mediated by direct effects on the liver lipoprotein synthesis, together with vascular protective actions through regulation of vascular smooth muscle proliferation and sensitivity to mediators of contractility like nitrous oxide (Farhat et al. 1996). The relative importance of these different mechanisms in the cardioprotective effect of oestrogens is unknown. Much attention has been devoted to lipoproteins because oestrogens decrease low density lipoprotein (LDL) and increase high density lipoprotein (HDL) levels in humans, and the oestrogen-mediated decrease in LDL cholesterol, but not the increase in HDL, can be modelled in rats (Lunede et al. 1997). Both tamoxifen and raloxifene lower cholesterol in ovariectomised rats but were less effective than ethynyl oestradiol; ICI 182780 alone had no effect on plasma cholesterol (Lunede et al. 1997). Furthermore, the predicted adverse effects of Faslodex on lipid metabolism have not so far been observed in patients (Howell et al. 1996). The rat studies with raloxifene (Lunede et al. 1997) are reflected by recent clinical data which show that raloxifene was less effective than conventional hormone replacement therapy in reducing cardiovascular risk factors (Walsh et al. 1998). As yet, little is known about the direct effects of SERMs on vascular tissues, so it remains uncertain whether the third
generation SERMs will demonstrate any improvement in
cardioprotective activity compared with tamoxifen and raloxifene.

The frontier of drug discovery in ER modulators is
defined by the increasingly detailed understanding of the
molecular consequences of the association of ER with different
classes of ligands (Shiau et al. 1999, Norris et al. 1999)
and the potential utility of this knowledge in defining tissue,
cell and even gene-specific actions of targeted ligands. There
remain, however, huge practical difficulties in translating this
new knowledge into useful therapeutics since the mechanistic
basis for oestrogen actions on two of the most interesting
targets, the prevention of neurodegenerative conditions like
Alzheimer’s disease and the prevention of premature mortality
through vascular diseases, is not understood.

References

Albert JL, Sundstrom SA & Lyttle CR 1990 Estrogen regulation of
placental alkaline phosphatase gene expression in a human
endometrial adenocarcinoma cell line. Cancer Research 50
3306–3310.

von Angerer E, Knebel N, Kager M & Ganss B 1990
1-Aminoalkyl-2-phenylindoles as novel pure estrogen

Aronica SM & Katzenellenbogen BS 1993 Stimulation of estrogen
receptor-mediated transcription and alteration in the
phosphorylation state of the rat uterine estrogen receptor by estrogen,
cyclic adenosine monophosphate, and insulin-like growth
factor-I. Molecular Endocrinology 7 743–752.

Baker VL, Draper M, Paul S, Allerheiligen S, Glant M, Shifren
J & Jaffe RB 1998 Reproductive endocrine and endometrial
effects of raloxifene hydrochloride, a selective estrogen receptor
modulator, in women with regular menstrual cycles. Journal of
Clinical Endocrinology and Metabolism 83 6–13.

344.

Berry M, Metzger D & Chamber P 1990 Role of the two
activating domains of the oestrogen receptor in cell-type and
promotor-context dependent agonistic activity of the anti-
ogen 4-hydroxytamoxifen. EMBO Journal 9 2811–2818.

Black LJ & Good RL 1980 Uterine bioassay of tamoxifen, tri-
oxifen and a new estrogen antagonist (LY117018) in rats and
mice. Life Sciences 26 1453–1458.

Blin C, L’Horset F, Leclerc T, Lambert M, Colnot S, Thomasset
M & Perron C 1995 Contrastings effects of tamoxifen and ICI
182780 on estrogen-induced calbindin-D 9k gene expression in
the uterus and in primary culture of myometrial cells. Journal of
Steroid Biochemistry 55 1–7.

Borras M, Lluis I, El Khissi A, Seo H-S, Lempereur F, Legros
N & Leclercq G 1996 Estrogenic and antiestrogenic regulation
of the half-life of covalently labeled estrogen receptor in MCF-7
breast cancer cells. Journal of Steroid Biochemistry and
Molecular Biology 57 203–213.

Bowler J, Litley TJ, Pittard JD & Wakeling AE 1989 Novel

Bruning PF 1992 Droloxifene, a new anti-oestrogen in
postmenopausal breast cancer: preliminary results of a
double-blind dose-finding Phase II trial. European Journal of
Cancer 28A 1404–1407.

Bryant H & Dere WH 1998 Selective estrogen receptor
modulators: an alternative to hormone replacement therapy.
Proceedings of the Society for Experimental Biology and
Medicine 217 45–52.

Bucourt R, Vignau M, Torelli V, Richard-Foy H, Geynet C,
Secco-Millet C, Redeuilh G & Baulieu EE 1978 New biopspecific
adsorbents for the purification of estradiol receptor. Journal of
Biological Chemistry 253 8221–8228.

Buzdar AU, Marcus C, Holmes F, Hug V & Hortobagyi G 1988
Phase II evaluation of Ly 156758 in metastatic breast cancer.
Oncology 45 344–345.

Bzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T,
Engstrom O, Ohman L, Greene GL, Gustafsson J-A & Carlquist
M 1997 Molecular basis of agonism and antagonism in the

Chen D, Pace PE, Coombs RC & Ali S 1999 Phosphorylation of
human estrogen receptor α by protein kinase A regulates

Clarkson TB & Anthony MS 1997 Effects on the cardiovascular
system: basic aspects. In Estrogens and Antiestrogens: Basic and
Clinical Aspects, pp 89–118. Eds R Lindsay, DW Dempster &

Clemens JA, Bennett DR, Black LJ & Jones CD 1983 Effects of a
new antiestrogen, keoxifene (LY156758), on growth
carcinogen-induced mammary tumors and on LH and prolactin
levels. Life Sciences 32 2869–2875.

Coombes RC, Haynes BP, Dowsett M, Quigley M, English J,
Judson IR, Griggs LJJ, Potter GA, McCague R & Jarman M
1995 Idoxifene: report of a Phase I study in patients with

Coopman P, Garcia M, Brunner N, Deroo D, Clarke R &
Rocheft H 1994 Anti-proliferative and anti-estrogenic effects of
ICI 164,384 and ICI 182,780 in 4-OH-tamoxifen resistant
human breast cancer cells. International Journal of Cancer 56
295–300.

Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ,
Cauley JA, Norton L, Nickelson T, Bjarnason NH, Morrow M,
Lippman ME, Black D, Glusman JE, Costa A & Jordan VC
1999 The effect of raloxifene on risk of breast cancer in
postmenopausal women: results from the MORE randomized
trial. Journal of the American Medical Association 281
2189–2197.

Dauvois S, Danielian PS, White R & Parker MG 1992
Antiestrogen ICI 164,384 reduces cellular estrogen receptor
content by increasing its turnover. Proceedings of the National
Academy of Sciences of the USA 89 4037–4041.

Dauvois S, White R & Parker MG 1993 The antiestrogen ICI
182780 disrupts estrogen receptor nucleocytoplasmic shuttling.

Day BW, Magarian RA, Jain PT, Pento JT, Mousissian GK &
Meyer KL 1991 Synthesis and biological evaluation of a series of
1,1-dichloro-2,2,3-triarylcyclopropanes as pure antiestrogens.
Journal of Medicinal Chemistry 34 842–851.

Defriend DJ, Howell A, Nicholson RI, Anderson E, Dowsett M,
Mansel RE, Walton P, Sutcliffe F & Wakeling AE 1999 Idoxifene:
a new pure antiestrogen (ICI 182,780) in women with


El-Tanani MK & Green CD 1997 Two independent mechanisms for ligand-independent activation of the estrogen receptor. Molecular Endocrinology 11 928–937.


Wakeling: Mode of action of antioestrogens

receptor-dependent transcriptional activation of an estrogen-responsive element. Molecular Endocrinology 7 992–998.


Katzenellenbogen JA, O’Malley BW & Katzenellenbogen BS 1996 Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. Molecular Endocrinology 10 119–131.


Macgregor JI & Jordan VC 1999 Classification of the antiestrogen EM-800 using a novel assay system. Proceedings of the

McInerney EM, Weis KE, Sun J, Mosselman S & Katzenellenbogen BS 1998 Transcription activation by the human estrogen receptor subtype β (ERβ) studied with ERβ and ERα receptor chimeras. Endocrinology 139 4513–4522.


Ridner DJ, Hofbauer LC, Bonde SK, Gori F, Speilsberg TC & Riggs BL 1998 Bone morphometric protein-6 production in...
Wakeling: Mode of action of antioestrogens

...human osteoblastic cell lines. Journal of Clinical Investigation 101 413–422.


Stein B & Yang MX 1995 Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-xB and C/EBPβ. Molecular and Cellular Biology 15 4971–4979.


Sun J, Meyers MJ, Fink BE, Rajendran R, Katzenellenbogen JA & Katzenellenbogen BS 1999 Novel ligands that function as selective estrogens or antioestrogens for estrogen receptor-α or estrogen receptor-β. Endocrinology 140 800–804.


White R & Parker MG 1998 Molecular mechanisms of steroid hormone action. Endocrine-Related Cancer 5 1–14.


