The Pure Antiestrogen ICI 182,780 Inhibits Progestin-induced Transcription

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ABSTRACT

The pure antiestrogen ICI 182,780 binds to the estrogen receptor with high affinity and is currently in clinical trials for the treatment of human breast cancer. We now show for the first time that ICI 182,780 also exhibits potent antiprogestin activity at doses frequently used in laboratory investigations. The antiprogestin activity of ICI 182,780 was detected in HeLa, HepG2, and CV1 cells transiently transfected with either the A or B forms of the human progesterone receptor and a luciferase construct driven by a progesterone-response element. ICI 182,780 inhibited progestrone-induced gene transcription in a dose-dependent fashion with maximum inhibition obtained at 10^-6 M and an IC50 of approximately 2 x 10^-7 M. The ICI compound produced the same degree of inhibition as that obtained with the antiprogestin RU-486. The antiestrogen tamoxifen did not display antiprogestin activity in this test system, and ICI 182,780 did not inhibit the activity of transfected androgen or glucocorticoid receptors. These results clearly establish that ICI 182,780 has significant antiprogestin activity in addition to its well-documented antiestrogenic activity and raises the possibility that both antihormonal properties of this compound are exhibited in laboratory studies and in the course of treatment of human breast tumors.

INTRODUCTION

Endocrine therapy has been an important approach to the treatment of breast cancer for many years (1). A number of endocrine therapies have been considered and evaluated for treatment of the disease including surgical ablation of the ovaries and adrenals and the use of pharmacological agents such as antiestrogens, high-dose estrogens, progestins, and antiprogestins (2, 3). At present, the mainstay of endocrine therapy is tamoxifen, which is the agent of choice for all stages of breast cancer (4). Tamoxifen is a mixed estrogen agonist/antagonist, and its therapeutic effect is thought to be due primarily to its action as an estrogen antagonist in the breast (5). Despite its proven efficacy in the treatment of breast cancer, tamoxifen is not a cure for the disease because resistance to the drug commonly develops with time (6). This resistance may be caused by (a) the loss of ER (7); (b) the selection of ER-positive disease that is actually tamoxifen-stimulated for growth (8); or (c) mutations of the ER resulting in estrogen independent growth (9). In addition, tamoxifen displays agonist activity in some tissues, e.g., the endometrium, and this is thought to contribute to untoward side effects of the drug (10–12). A major goal has, therefore, been to develop pure antiestrogens that could be used as second-line therapy after tamoxifen failure.

These efforts have led to the development of “pure” antiestrogens such as ICI 182,780 (Faslodex), which is receiving considerable attention because preliminary clinical studies have shown it to be effective after tamoxifen failure (13, 14). ICI 182,780 binds to the hormone binding site of the ER, and this interaction undoubtedly accounts for many of its actions (15). However, the possibility that steroid hormones and antihormones may produce some effects via nonclassical receptor pathways has been recognized for many years (16), and it is also clear that some antihormones interact with multiple classes of steroid receptors, e.g., the antiprogestin RU-486 displays both antiglucocorticoid and antiestrogenic activity (17, 18). We thus investigated the possibility that ICI 182,780 has activities independent of the classical ER by using HeLa cells, which are devoid of endogenous ER. We found that this pure antiestrogen antagonizes progestrone-induced transcription after cotransfection with a PR expression plasmid and a reporter containing a PRE. This surprising discovery indicates that ICI 182,780 can block PR-mediated transcription and raises the possibility that the therapeutic effects of the drug may include antiprogestin effects in addition to its well-established antiestrogenic action.

MATERIALS AND METHODS

Materials. All of our studies were performed with cells obtained from American Type Culture Collection (Rockville, MD) using Superfect transfection reagent (Qiagen). Fetal bovine serum was obtained from Life Technologies, Inc. (Grand Island, NY). Hormones were obtained from Steraloids (New Port, RI), and tamoxifen was obtained from Sigma Chemicals (St. Louis, MO). The antiprogestin RU-486 (mifepristone) was a gift of Roussel Uclaf (Paris, France) and the AR agonist methyltrienolone (R1881) was obtained from New England Nuclear (Boston, MA). DNA purification kits for the preparation of supercoiled plasmids used in transfections were from Qiagen.

We originally purchased the pure antiestrogen ICI 182,780 from Tocris Cookson, Inc. (Ballwin, MO) and initially observed the inhibition of progestrone-induced transcription with this commercially obtained compound. However, because this antiprogestin activity was so unexpected, we considered the possibility that it might be due to a contaminant in the commercial preparation. We thus tested a second preparation, which was obtained as a gift kindly provided by Dr. Alan Wakeling (Zeneca Pharmaceuticals, Macclesfield, England), and we obtained similar results with the compound obtained from both sources. Because ICI 182,780 used clinically (Faslodex) is provided by Zeneca, the results described in this initial report were obtained with the compound generously provided by Dr. Wakeling.

Plasmids. The PRE-luciferase reporter used in this work was obtained by excising the PsvI–Smal fragment of pPRE/GRE.E1b.CAT (19) and inserting it into the Smal site of pGCL3Basic obtained from Promega (Madison, WI), which provides the luciferase sequence. The fragment of the original pPRE/GRE.E1b provides two copies of the consensus PRE linked to the TATA element from E1b. We used expression plasmids for the human PR that code for the full length PR isoform B or isoform A, and these constructs have been described previously (20). The full-length AR clone (21) was excised from pCMV by digesting with BglII–Xhol and then religating into the BamHII–XhoI site in expression vector PCR3.1 (Invitrogen, Carlsbad, CA). The GR expression plasmid used in these studies was constructed by cloning full-length coding sequence into the BamHI site of pCR3.1 (Invitrogen). The reporter used for the AR and GR is the same as that used for PR inasmuch as all of these receptors bind to the same consensus DNA response element (22).

Transfections. Cells were maintained in DMEM supplemented with 10% fetal bovine serum. Twenty-four h before transfections, cells were plated at 3 x 10^5/well in Falcon six-well dishes in 5% dextran-coated charcoal-stripped serum. Cells were transfected with the indicated plasmids using Superfect reagent (Qiagen) according to the manufacturer’s guidelines. Cells were
progesterone, has the ring structure. The antiprogestin RU-486 is a 19-nor steroid and, like endogenous estrogen, it has a C 18 steroid nucleus with a phenolic A-ring (25). In contrast, progesterone has a C 19 nucleus with a phenolic A-ring structure. The progesterone has a C 19 nucleus with a phenolic A-ring structure. The antiprogestin RU-486 (mifepristone) is a 19-nor steroid and, like endogenous estrogen, it has a C 18 steroid nucleus with a phenolic A-ring (25).

It was, therefore, surprising to observe that ICI 182,780 blocks hormonal ligands with this latter type of A-ring structure in an inverted 1β2α-conformation, whereas the ER favors ligands with a phenolic A-ring (25).

RESULTS

Fig. 1 illustrates the structures of the endogenous ovarian hormones progesterone and estradiol, the antiestrogens ICI 182,780 and tamoxifen, and the antiprogestin RU-486 (mifepristone). Tamoxifen and other early antiestrogens are triphenylethylene derivatives and differ in structure from ICI 182,780 and related steroidal antiestrogens. The ICI compound is a 7 α-alkyl analogue of estradiol, and like the endogenous estrogen, it has a C 18 steroid nucleus with a phenolic A-ring. In contrast, progesterone has a C 19 nucleus with a Δ 4-3-one A-ring structure. The antiprogestin RU-486 is a 19-nor steroid and, like progesterone, has the Δ 4-3-one A ring structure. The PR seems to favor hormonal ligands with this latter type of A-ring structure in an inverted 1β2α-conformation, whereas the ER favors ligands with a phenolic A-ring (25).

Fig. 2. The inhibition of PR-mediated gene transcription by ICI 182,780. HeLa cells were transfected with a PRE reporter construct alone (B) or with the reporter plus an expression plasmid for the human PRs (A). Cells were incubated for 24 h with progesterone (10−8 M) alone; the hormone plus either RU-486, ICI 182,780, or tamoxifen (10−6 M) each; or the antagonist alone (10−6 M). After the incubations, cells were lysed, and luciferase activity and protein content were measured as described in “Materials and Methods.” P, progesterone; RU, RU-486; ICI, ICI 182,780; TAM, tamoxifen. Each value represents the mean of three separate determinations with the indicated SE. *, significant inhibition (P < 0.05) versus the progesterone stimulated group; **, not significant inhibition (P > 0.05) versus the progesterone stimulated group.

Fig. 3 illustrates the dose-response curve for ICI 182,780 inhibition of reporter activity stimulated by 10 nm progesterone. A significant inhibition (P < 0.05 versus the progesterone alone group) is first observed at a concentration of 10−7 M, and hormone-induced activation is virtually abolished at 10−6 M. There seems to be a slight increase in reporter activity at lower doses of the ICI compound, but this effect is not statistically significant and has not been observed in other experiments; we have not investigated it further at this time because our primary interest in this initial report is on the nearly complete inhibition of the progesterone effect caused by the ICI 182,780. The IC50 for ICI 182,780 estimated from the data in Fig. 3 is approximately 2 × 10−7 M.

We next sought to determine whether ICI 182,780 would inhibit the transcriptional activity of other steroid receptors in addition to the PR. Because the hormone response element of the luciferase reporter containing two copies of the consensus PRE linked to a minimal promoter obtained from the E1b gene (24).

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Fig. 3. Dose-response curve for ICI inhibition of PR-mediated transcription. HeLa cells were cotransfected with human PR, and a PRE-luciferase reporter as described in “Materials and Methods.” After transfection, cells were incubated in the presence of 10^{-8} M progesterone alone (−) or with the indicated concentrations of ICI 182,780. Luciferase activity was measured as described in “Materials and Methods.” Each value represents the mean of three determinations with the indicated SE. *P < 0.05 versus progesterone-alone group.

Fig. 4. ICI 182,780 does not block AR- or GR-mediated transcription. HeLa cells were cotransfected with the luciferase reporter plasmid and the AR, GR, or PR. The effects of the AR agonist R-1881 (left panel), the AR agonist R-1881 (middle panel), or the synthetic glucocorticoid dexamethasone (right panel)—alone (at 10^{-8} M) or in the presence of ICI 182,780 (10^{-8} M)—on luciferase activity were then measured as described in “Materials and Methods.” The results in each panel are expressed as the % of maximum luciferase activity respectively produced by the agonist alone in each case. Each value represents the mean of five separate determinations with the indicated SE.

DISCUSSION

We have shown for the first time that ICI 182,780, which is generally considered to be a pure antiestrogen, also blocks transcriptional activation by progesterone. Quantitatively, both ICI 182,780 and the prototype antiprogesterin RU-486 essentially abolish the stimulatory effect of progesterone in the HeLa transfection system, although we have not compared the potencies of the two compounds in this work. This result was unexpected because of the structural differences between ICI 182,780 and ligands known to bind well to the PR. Thus, to investigate the possibility that the observed inhibition was due to a contaminant rather than to the ICI compound itself, we tested two different batches of the drug: one, initially purchased from a commercial supplier (Tocris Cookson, Inc.) and a second, generously provided by Zeneca (formerly ICI) Pharmaceuticals, which had originally developed the drug as an antiprogestin. In separate experiments, both preparations inhibited progesterone-induced transcription, which suggests that the effect is due to ICI 182,780 itself.

The steroidal antiestrogens such as ICI 182,780 and its less potent predecessor ICI 164,384 have numerous activities related to ER binding: (a) they compete with endogenous estrogens for ER binding sites (15); (b) they decrease cellular levels of ER, presumably because drug binding enhances receptor degradation (7); (c) they interfere with nucleocytoplasmic shuttling of the ER (26); and (d) they antagonize the effects of peptide growth factors and other agents that act via kinase signaling cascades (presumably by phosphorylation of the ER; Ref. 7). However, our results clearly establish that the inhibition of PR-dependent transcription by ICI 182,780 does not involve the classical ER pathway inasmuch as: (a) HeLa cells do not contain endogenous ER; and (b) the minimal PRE-luciferase reporter construct that we used does not contain an estrogen response element. Furthermore, tamoxifen did not display a significant inhibition in our studies, which indicates that the effect of the ICI compound is not shared by other mixed estrogen agonist/antagonists.

A qualitatively similar inhibition of PR-induced transcriptional activity is seen in HepG2 and CV1 as well as HeLa cell backgrounds, although there are quantitative differences in the magnitude of inhibition observed in the different systems. Although these studies were not meant to be an exhaustive survey of cell lines, they do establish that the inhibition that we initially observed in HeLa cells is not limited to this cell line. This finding is significant because it indicates that the inhibitory action of ICI 182,780 on PR-mediated transcription does not require a factor(s) unique to HeLa cells but is a more general phenomenon.

The inhibitory effect of ICI 182,780 that we have observed in this...
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Fig. 5. ICI 182,780 inhibits the transcriptional activity of both the A and B forms of the PR in multiple cell types. The inhibitory effect of ICI (10^-8 M) on progesterone (10^-8 M)-induced luciferase activity in HeLa, HepG2, and CV1 cells cotransfected with either the B (top panel) or A (bottom panel) forms of the PR. The results in each panel are expressed as the % of the maximum luciferase activity obtained in the indicated cell/receptor subtype system. Each bar represents the mean of three separate determinations, and the SE values were less than 10% of the mean in all of the cases. C, control (no addition); P (label on x-axis), progesterone alone; P+ICI, progesterone + ICI 182,780. P values are in small boxes for each of the P (progesterone) versus P+ICI groups.

study also displays receptor specificity, inasmuch as the antiestrogen does not block transcriptional activation caused by the ARs or GRs. This rules out: (a) any effects on the general transcriptional machinery; (b) any artifactual effects on the reporter system we have used; and (c) any type of generalized toxicity of the drug toward HeLa cells. Inhibition is observed with both the A and B forms of the PR, which indicates that the inhibitory action of the ICI compound does not require the NH2-terminal sequences present in the longer B form of the PR. However, the quantitative pattern of inhibition seen in three different cell lines (Fig. 5) suggests that these sequences may affect the degree of inhibition. Additional studies in other cell lines are needed to define these differences more completely.

One potential explanation for our results is that ICI 182,780 either decreases PR levels or competes with progesterone for hormone binding sites on the receptor. These possibilities seem very unlikely for several reasons: (a) there are no reports that ICI 182,780 decreases PR levels, and there is even one report that it does not decrease PR levels in the rat hypothalamus (27); (b) the related 7 α-alkyl substituted estrogen analogue ICI 164,384 does not decrease PR levels in vivo in the rat uterus (28), in MCF-7 cells (29), in Ishikawa cells (30), or in a uterine myocyte cell line (31). However, there is one report in the literature that ICI 164,364 causes a modest reduction in PR levels in T47D human breast cancer cells (29), and we, therefore, sought to determine whether ICI 182,780 would have a similar effect. We did not observe a decrease in PR levels measured by Western blots or ligand binding in this cell line (measured by competition of ICI 182,780 for tritiated R5020 binding) in either whole cells or cytosol prepared from T47D cells. Thus, it seems that the effect of ICI 182,780 that we have observed is not caused by the competitive blockade of progesterin-binding sites on the PR nor by altered levels of the receptor itself.

It seems more likely to us that the effects that we have observed are caused either by the binding of ICI 182,780 to a site on the PR distinct from the progesterone binding site or by the binding to a second factor(s) present in transcription complexes (accessory proteins) assembled at PR-responsive promoters. Such a putative factor would have to display some specificity, however, because ICI 182,780 did not block transcriptional activation by the AR or the GRs. Alternatively, the ICI compound could decrease cellular levels of such a factor(s) by affecting its synthesis, degradation, or activity inasmuch as our studies involved an overnight incubation of cells with the compound in the culture media. With regard to the possibility that ICI 182,780 may act via nonreceptor proteins involved in PR-mediated events, it is noteworthy that Parisot et al. (32) have observed in MCF-7 cells the binding of tritiated ICI 182,780 to high-affinity sites that do not represent either the ER or the unique antiestrogen binding sites reported previously by several labs (33). In any case, the simplicity of our assay system argues for some type of inhibitory effect mediated by an allosteric site on the PR itself or by an interaction with a factor selectively involved with the PR-mediated transcription events. Regardless of the exact molecular mechanism(s) involved, the PR sequences that mediate the basic inhibitory effect of the ICI compound seem to be present in both the A and B forms of the receptor.

At present, the molecular mechanism of the effect that we have observed remains unknown. We cannot exclude the possibility that the inhibition of progesterone-induced transcription we have observed is caused by a metabolite of ICI 182,780 formed by HeLa cells rather than the parent ICI compound itself. In addition, it is also important to note that we do not know whether a similar inhibitory effect will be observed for PR-mediated activation of endogenous gene expression. This is a particularly relevant question inasmuch as Weaver et al. (34) showed that a related steroidal antiestrogen, ICI 164,384, has disparate actions on expression of the endogenous pS2 gene and on transfected reporters containing the pS2 estrogen response element. Despite these unanswered questions, our results clearly indicate that ICI 182,780 blocks progesterone-induced transcription in a very simple experimental system that requires PR but does not contain ER. This unexpected discovery may be important for several reasons. ICI 182,780 is often used at a level of 10^-8 M in experimental studies with cultured cells, and the effects observed in this range are generally attributed to the drug’s antiestrogenic activity. However, our results clearly demonstrate that this dose of the drug almost completely abolishes the effects of physiological levels of progesterone. Clearly this effect may also be important for understanding the clinical pharmacology of the drug and its possible therapeutic mechanisms in the treatment of breast cancer.

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REFERENCES

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