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The effects of ICI 182,780, a pure anti-oestrogen, on the hypothalamic–pituitary–gonadal axis and on endometrial proliferation in pre-menopausal women

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ICI 182,780 has shown pure oestrogen antagonism *in vitro* and *in vivo* in animals. A total of 17 women with normal menstrual cycles were administered ICI 182,780, 12 mg daily for 7 days in the follicular phase prior to hysterectomy; 11 normal women were used as controls. Of the 17 patients, three (18%) experienced a luteinizing hormone (LH) surge in the treatment group compared with five (45%) in the controls ($P = 0.24$), and these patients were only included up to the surge. There were no differences in the daily mean plasma LH and follicle stimulating hormone concentrations between the treatment ($n = 17$) and control ($n = 10$) groups. The mean plasma oestradiol was higher in the treatment group than controls ($P < 0.05$) on days 5, 6 and 7. However, there was no increase in endometrial thickness in the treatment group throughout the study. In the control group, endometrial thickness increased during the study and was significantly higher ($P < 0.05$) on day 7. There was no ultrasonic evidence of ovarian hyperstimulation and no serious adverse events reported. This study shows that treatment for 7 days with ICI 182,780 does not cause ovarian hyperstimulation and has a potent anti-oestrogenic action on the endometrium. We conclude that ICI 182,780 may be a useful compound in the treatment of oestrogen-dependent gynaecological disease.

Key words: anti-oestrogen/endometrium/gonadotrophins/oestrogen

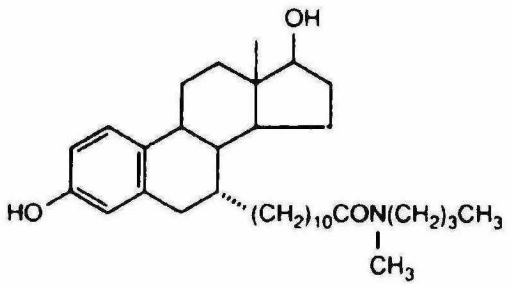
Introduction

ICI 182,780 is 7α-[9-(4,4,5,5,5-pentafluoro-pentylsulphinyl) nonyl]estra-1,3,5(10)-triene-3,17β-diol and competes with endogenous oestrogen for binding to the oestrogen receptor. It is similar to ICI 164,384 (Wakeling and Bowler, 1988) and it has been demonstrated to show specific anti-oestrogenic activity *in vivo* in the immature and mature rat (Wakeling *et al.*, 1991). The chemical structures of both these compounds are shown in Figure 1. It also showed potent anti-tumour activity *in vivo* in xenografts of MCF-7 and Br10 human breast cancers in nude mice and in cultures of MCF-7 cells (Wakeling *et al.*, 1991). It does not demonstrate any oestrogenic activity or effects on gonadotrophin secretion in rats (Wakeling *et al.*, 1991). It shows anti-uterotrophic activity in ovariectomized (Dukes *et al.*, 1992) and intact female macaques (Dukes *et al.*, 1993). This pure anti-oestrogenic activity means that it may have clinical potential in the treatment of benign and malignant disease that is oestrogen dependent in both pre- and post-menopausal women. This paper reports the effects of administration of the compound to pre-menopausal women on the plasma concentrations of reproductive hormones and upon endometrial proliferation.

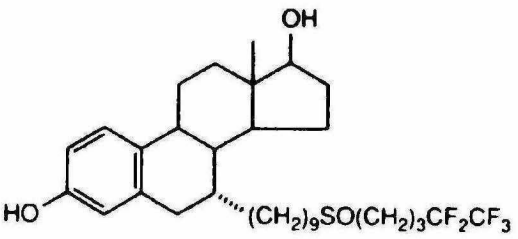
Materials and methods

Study design

This was an open, randomized, controlled study of seven daily i.m. injections of ICI 182,780, 12 mg, compared with no treatment, on the plasma concentrations of luteinizing hormone



ICI 164,384



ICI 182,780

Fig. 1. Structure of ICI 164,384 and ICI 182,780.

(LH), follicle stimulating hormone (FSH), oestradiol and sex hormone binding globulin (SHBG) and on endometrial thickness as determined by ultrasound, in normal pre-menopausal female patient volunteers. A total of 30 volunteers were recruited and it was planned to randomize with a 2:1 ratio between those receiving active agent and controls. Three patients withdrew prior to starting the study and were substituted randomly. This resulted in 19 patients being randomized to receive ICI 182,780 and 11 to receive no treatment. All patients started the study between day 5 and day 9 of the menstrual cycle and then underwent hysterectomy between days 12 and 18 of the cycle. Plasma was sampled on each day of the study for the estimation of the concentrations of LH, FSH, oestradiol, progesterone and SHBG. Vaginal ultrasound was performed to measure endometrial thickness immediately before the study and on the fourth and seventh days of treatment. Ethical approval was given by the Joint Ethics Committee of Southampton and South-West Hampshire Health Authority and Southampton University, and all volunteers gave written consent.

Volunteers

Women aged <50 years with menstrual cycles of between 21 and 42 days who were scheduled for hysterectomy for either menorrhagia or fibroids were asked to join the study. All patients had had a normal dilatation and curettage, including endometrial histology within the past 2 years, had received no exogenous steroids for 3 months prior to starting the study and had no severe intercurrent illness.

Plasma assays

All samples from a single subject were run in the same assay. LH and FSH were measured using the radioimmunoassay technique described by Ferguson *et al.* (1982). For LH, the intra-assay coefficient of variation (CV) was 5.8% and the inter-assay CV 7.8%. For FSH, the intra-assay CV was 4.2% and the inter-assay CV 6.5%. Plasma oestradiol was measured by radioimmunoassay [DPC Coat-a-Count; Diagnostic Products (UK) Ltd, Abingdon, UK]. Analyses were initially conducted on samples from three treated patients using lipidex chromatography separation prior to assay using a system which separated ICI 182,780 from oestradiol (chloroform:hexane: methanol, 50:50:1). No significant difference was found between the results obtained with or without chromatography and the analyses were therefore conducted without this step. The intra-assay CV was 4.9% and the inter-assay CV 8.8%. Plasma SHBG was assayed with the Farmos immunoradiometric assay method (Organon Teknika, Cambridge, UK). The intra-assay CV was 3.2% and the inter-assay CV 5.3%. Plasma progesterone was measured with the DPC Coat-a-Count radioimmunoassay.

Vaginal ultrasound

Vaginal ultrasonography was performed with an Ultramark 4 ultrasound scanner with a 5 MHz probe (ATL, Stevenage, UK). Endometrial thickness was measured three times on each occasion and the mean value calculated. The measurements were taken from the external edge of each echogenic interface between the endometrium and the myometrium (Santolaya, 1992). Follicular volume was measured using the average of three diameters (Thomas *et al.*, 1986). The ultrasonographer was unaware of the allocation to either treatment or control.

Data analysis

The endocrine data were not assumed to have a normal distribution and have been logarithmically transformed for analysis, as this has been shown to normalize the distribution of the plasma concentrations of oestradiol and gonadotrophins (Kletzky *et al.*, 1975). Comparisons within groups were performed with repeated analysis of variance (ANOVA) and between groups with multivariate analysis. For clarity, the data are presented in Figures 2 and 3 as arithmetic means and standard deviation. Endometrial thickness was assumed to have a normal distribution and ANOVA was used to compare the two groups.

Results

All 30 volunteers completed the study. The mean ± SD age for those randomized to treatment was 41.5 ± 4.2 years, and this was not significantly different from that of 43 ± 3.3 years for those in the control group. There was also no significant difference in the mean ± SD weight in the treatment (66.7 ± 5.4 kg) compared with the control group (64.3 ± 7.1 kg). The median for the day of the cycle on which the study commenced was 7 (range 5–9) in the control group and also day 7 (range 6–8) in the treatment group. Analysis of the ultrasound and endocrine results showed that one volunteer in the treatment group had an unluteinized follicular cyst at the start of the study and another had elevated plasma LH and FSH with a static oestradiol and a low plasma progesterone concentration, suggesting incipient ovarian failure. These two were removed from further analyses. This left 17 volunteers randomized to receive treatment and 11 controls. There were no serious adverse events or significant side-effects reported in the treatment group. Hysterectomy was performed within 4 days of stopping the treatment.

LH surge

Three patients in the treatment group (18%) and five (45%) in the control group experienced an LH surge during the 7-day period of the study, verified by plasma LH concentrations and raised plasma progesterone concentrations in the subsequent days ($P = 0.24$, Fisher's Exact test). The data for each individual in each group are displayed in Figure 2. In the treatment group, the LH surges occurred on days 2 and 3 of the treatment period, with none appearing after that. In the control group, one surge appeared on day 1, one on day 3, one on day 4 and two on day 5 of the study. Because the LH surge induces such a large transformation in the function of the follicle we have eliminated the data from the beginning of the surge onwards from further analysis so that all comparisons are in the follicular phase of the cycle. This means that, in the treatment group, 17 patients were evaluated on day 1, 15 on day 2 and 14 from then onwards. In the control group, because one patient had started the surge on day 1, this left 10 evaluable patients on days 1 and 2, eight on days 3 and 4 and six from then onwards.

Plasma LH, FSH and oestradiol

Figure 3 compares the geometric mean concentrations of plasma LH, FSH and oestradiol in the treatment group against the

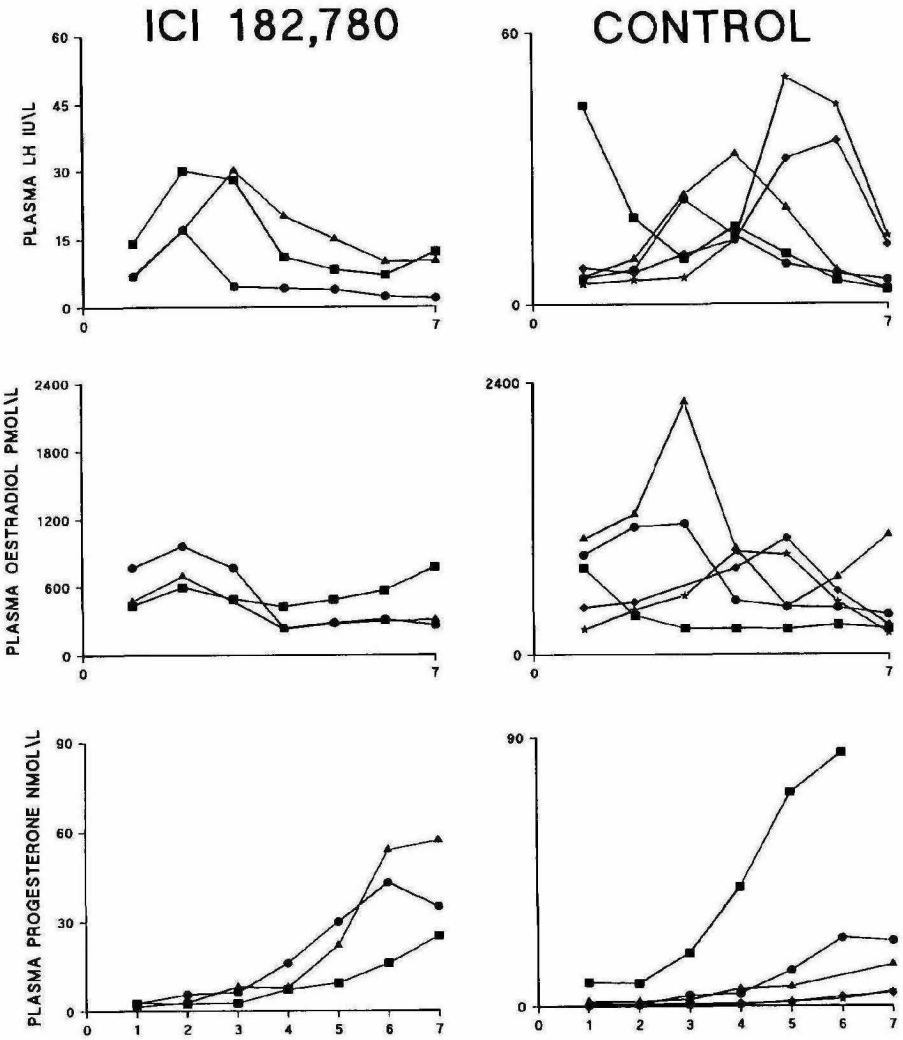


Fig. 2. Comparison of the individual results for the concentration of plasma LH, oestradiol and progesterone in those volunteers who experienced an LH surge in the treatment (n = 3) and control (n = 5) groups.

controls. Using multivariate analysis, there were no statistically significant differences in the concentrations of LH or FSH between the two groups for any of the days of the study. Using repeated ANOVA there was no difference within the groups in the plasma concentration of LH throughout the study. However, within both groups the decrease in plasma FSH concentration over the study was statistically significant (treatment group: P = 0.01; control group: P = 0.04). Plasma oestradiol concentrations significantly increased in both groups (treatment: $P < 0.0001$; control: $P < 0.05$, using ANOVA) throughout the treatment period. Oestradiol concentrations were significantly higher on days 5, 6 and 7 in the treatment group compared to controls ($P < 0.02$, multivariate ANOVA). The median (range) of plasma oestradiol in the treatment group was 919 (689–1661) pmol/l on day 6 and 1140 (965–1801) pmol/l on day 7. Plasma oestradiol was higher on day 7 than on day 6 in 12 of the treatment group and five of the controls, suggesting that follicular growth was continuing in the majority of volunteers. If all the values for plasma oestradiol are summed to produce an area under the curve, then the distribution is significantly higher in the treatment group ($P < 0.00001$, Wilcoxon) than in the controls.

Endometrial thickness

Figure 3 compares the mean endometrial thickness in millimetres for the treatment group versus controls. The mean ± SD endometrial thickness in the control group was 7.2 ± 1.7 mm on day 1, 8.2 ± 1.3 mm on day 3 and 9.7 ± 1.2 mm on day 7. For the treatment group the values for the same days are 6.2 ± 2, 6.4 ± 1.3 and 6.7 ± 2.1 mm respectively. There is no significant increase in thickness in the treatment group, but there

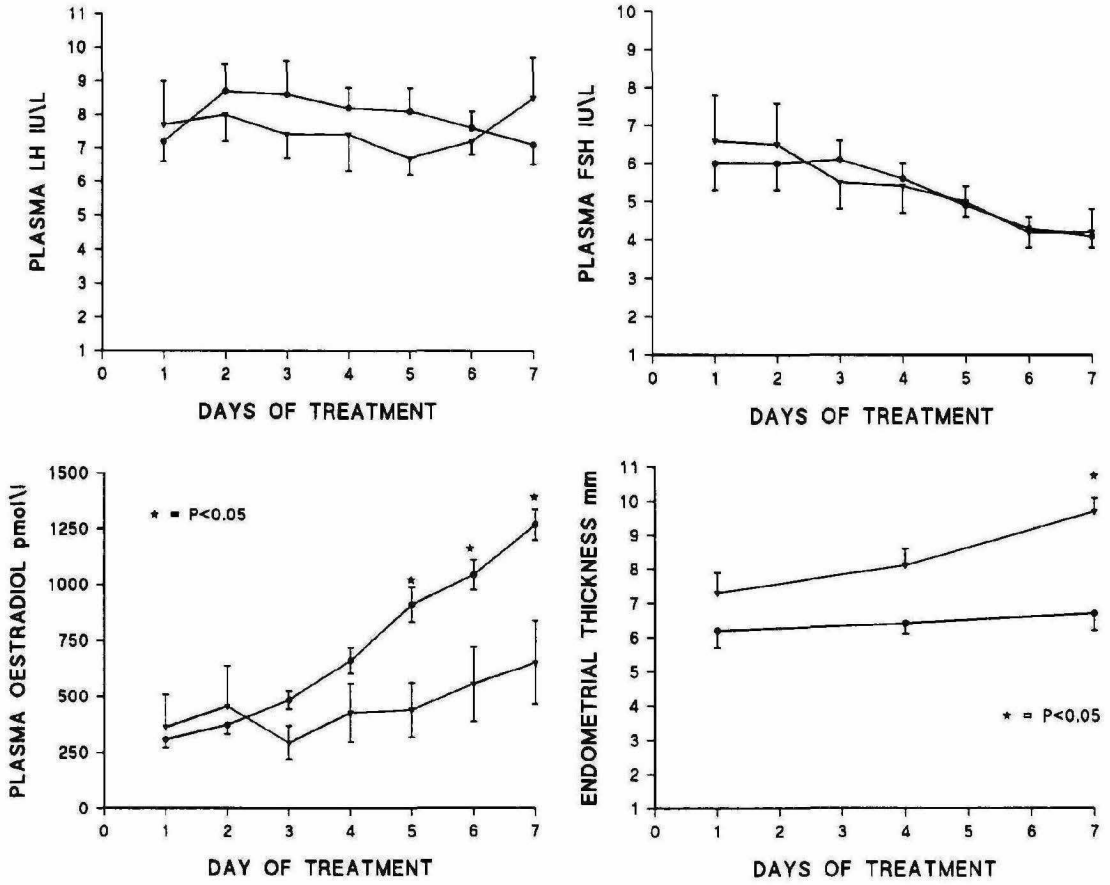


Fig. 3. Comparison of the mean (±SD) plasma concentrations of LH, FSH, oestradiol and the endometrial thickness of the treatment group (●, $n = 17$) and the controls (▼, $n = 10$).

is a significant increase in the control group ($P < 0.03$, ANOVA) throughout the study. The thickness on day 7 is significantly less in the treated volunteers than the controls ($P < 0.05$, multivariate ANOVA) in spite of the plasma oestradiol concentration being higher.

Follicular growth

Only four of the women in the control group had interpretable ultrasonic determination of follicular growth, and therefore a comparison between the two groups was not possible. In the treatment group there was no evidence of multiple folliculogenesis during the administration of the drug. The mean ± SD follicular volume was 0.9 ± 0.2 ml on day 1, 1.2 ± 0.5 ml on day 4 and 1.9 ± 0.5 ml on day 7, and these increases were significant ($P < 0.0001$, ANOVA). In 12 of the 14 women exposed to ICI 182,780 there was an increase in follicular volume on day 7 in comparison to day 4.

SHBG

There were no differences in the mean plasma SHBG concentrations for any day in the treatment group compared with controls.

Discussion

This study reports on the administration of a pure anti-oestrogen to women with regular menstrual cycles. The results showed that after 7 days administration of ICI 182,780 there was no detectable increase of LH and FSH secretion and no ultrasonic evidence of ovarian hyperstimulation. It appears that the LH surge may be suppressed by the anti-oestrogen after 3 days of treatment and that follicular growth continues. In spite of the continued secretion of oestradiol there is no increase in endometrial thickness, demonstrating the powerful oestrogen antagonism of the compound.

The exact mechanism of action of ICI 182,780 is not clear. It binds to the oestrogen receptor with an affinity of 0.89 compared to that of oestradiol. This is greater than the 0.19 affinity of ICI 164,384, which is another example of this series of compounds on which many non-clinical studies have reported, confirming pure oestrogen antagonism (Wakeling *et al.*, 1991). Investigations with ICI 164,384 have shown that it prevents binding of the occupied receptor to DNA cellulose (Wilson *et al.*, 1990). In transient-transfection studies, the addition of the antibody MP16 restored the ability of receptors occupied by ICI 164,384 to bind to DNA. This antibody restores high-affinity binding to DNA to mutant receptors that are defective for

dimerization, and it was concluded that these antagonists of oestradiol prevented receptor dimerization. This hypothesis was given further support by the report that ICI 164,384 causes a rapid loss of oestrogen receptor expression in the mouse uterus (Gibson *et al.*, 1991), and it was also shown to decrease the half-life of the oestrogen receptor from ~5 to <1 h (Dauvois *et al.*, 1992). The authors concluded that this decrease in half-life was probably a result of the inability of the occupied receptors to form homodimers. This inability is likely to prevent binding to DNA and thus it was postulated that it was through this mechanism that the anti-oestrogenic activity was propagated (Fawell *et al.*, 1990).

In the treatment group there were no LH surges after the first 3 days. This was different from the control group in which there were surges on days 4 and 5. Temporally, it would be expected that LH surges should occur with increasing frequency as the follicular phase develops, and this suggests that the use of ICI 182,780 may suppress the LH surge if it has not already been initiated by the beginning of treatment. However, this study only extended to day 16 of the menstrual cycle, and it is possible that a number of LH surges could have occurred spontaneously after that time. Therefore, the possibility that ICI 182,780 suppresses the LH surge requires more detailed study. There was no increase in LH or FSH secretion as a result of administration of ICI 182,780, and the pattern of decreasing FSH secretion was as expected for a normal follicular phase of the menstrual cycle for both groups (Thomas *et al.*, 1986). This was unexpected, as we had postulated that as the compound was a potent anti-oestrogen, there would be an increase in LH and FSH secretion similar to that seen post-menopausally.

There are no other studies reporting the result of the administration of pure anti-oestrogens to women with which to compare these results. In the human, tamoxifen does not necessarily stimulate LH and FSH secretion, the response appearing to be variable (Tajima, 1984; Tajima and Fukushima, 1983). Clomiphene citrate does appear to increase LH and FSH secretion in the human (Martikainen *et al.*, 1991), although these responses can be variable (Randall and Templeton, 1991). The variability for tamoxifen and clomiphene could be explained by them being both oestrogenic and anti-oestrogenic. However, ICI 182,780 is a pure anti-oestrogen and we have no obvious explanation why it did not cause an increase in gonadotrophin secretion. It is possible that it takes longer than 7 days before this effect becomes apparent. Another explanation is that ICI 182,780 does not act centrally, at least in this treatment schedule, although this would contradict our hypothesis that it may suppress the LH surge. It is possible, however, that the compound may act by controlling the stimulus for the surge of LH at the pituitary rather than the hypothalamic level. This possibility would not then conflict with the lack of central action on the tonic release of LH and FSH. More detailed studies are needed and ICI 182,780 provides a useful experimental tool for the investigation of the role of oestradiol in the control of LH and FSH secretion.

Follicular growth continued in spite of exposure to ICI 182,780. There was no luteinization of these follicles as determined by plasma progesterone concentrations, but this may also have been because no patient was studied beyond day 16 of the cycle. The plasma oestradiol concentration was higher in the treatment group and this did not appear to be mediated by increased gonadotrophin stimulation. An explanation for this may have been that there was change in the ratio of bioactive to immunoactive FSH as a result of ICI 182,780. It also is possible that the anti-oestrogenic effect of the compound alters granulosa and thecal cell function, leading to an increased secretion of oestradiol. Oestradiol has been reported as increasing FSH-stimulated steroidogenesis in cultured marmoset granulosa cells (Shaw and Hodges, 1992). However, this observation, while verifying that oestradiol may have paracrine actions, does not help to explain how ICI 182,780 may alter oestradiol secretion by granulosa cells. It may also be that there were some smaller follicles that were also contributing to the secretion of oestradiol that were not consistently identified using ultrasound. Because this study showed that follicular growth was continuing in 12 of the 14 volunteers given ICI 182,780, longer studies are needed to observe whether the follicles continue to grow or whether they become atretic. Investigation of the fate of the follicles in the treatment group over 4–5 weeks will be vital for determining the long-term use of the compound. Continued follicular growth or the initiation of a follicular cyst will limit its therapeutic value. On the other hand, atresia of the follicle with no further stimulation of folliculogenesis will provide a suitable environment for long-term use.

The data showed that, in spite of continued oestradiol stimulation, there was no significant endometrial growth in the ICI 182,780 group. The rate of growth in the control group was similar to that reported in normal women in the follicular phase (Bakos *et al.*, 1993). This verifies a potent anti-oestrogenic activity *in vivo* and agrees with the data of Dukes *et al.* (1992, 1993), who showed no growth of the endometrium in both the intact and the ovariectomized monkey. This observation provides support for the use of ICI 182,780 to treat disorders of endometrial proliferation, such as endometrial carcinoma, endometriosis and dysfunctional uterine bleeding. Its use in the treatment of these disorders will depend on the side-effects encountered in the long term, specifically, the occurrence of hot flushes and bone demineralization, as well as acceptable effects on folliculogenesis.

In conclusion, this study demonstrated that ICI 182,780 is well tolerated during short-term use. It did not cause an increase in LH or FSH secretion and may suppress the LH surge. There was no evidence of ovarian hyperstimulation although follicular growth continued. There appeared to be a potent anti-oestrogenic effect on the endometrium *in vivo*.

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