ICI 176,334: A NOVEL NON-STERoidal, PERIPHERALLY SELECTIVE ANTIANDROGEN

B.J.A. Furr, B. Valcaccia, B. Curry, J.R. Woodburn, G. Chesterson* and H. Tucker*

Bioscience I and *Chemistry Departments, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

ABSTRACT

Pure antiandrogens, like flutamide, antagonize androgen action both peripherally and centrally at the hypothalamic-pituitary axis, which leads to an increase in LH and testosterone secretion. A new non-steroidal antiandrogen ICI 176,334 ((2RS)-4'-cyano-3-(4-fluorophenylsulphonyl)-2'-hydroxy-2'-methyl-3'-trifluoromethyl)propionanilide) has now been discovered which causes regression of the accessory sex organs but does not increase serum concentrations of LH and androgens. ICI 176,334 binds to rat prostate androgen receptors with an affinity around fourfold that of hydroxyflutamide. When administered s.c. concurrently with testosterone propionate (200μg/kg) for 7 consecutive castrated rats, ICI 176,334 (10mg/kg) significantly (P<0.001) inhibited growth of the seminal vesicles and ventral prostate gland. Oral administration of ICI 176,334 at doses of 1, 5 and 25mg/kg for 14 days to adult rats caused a dose-related reduction in accessory sex organ weights but had no effect on the testes. None of these doses caused a significant increase in serum LH and testosterone. Flutamide was around fourfold less potent and significantly increased serum LH and testosterone at the higher doses. ICI 176,334 was well tolerated. ICI 176,334 should, therefore, prove useful for the treatment of androgen-responsive benign and malignant diseases.

INTRODUCTION

Three antiandrogens, cyproterone acetate, flutamide and anandron, are currently available for clinical studies in androgen-responsive diseases. Flutamide and anandron are non-steroidal pure antiandrogens and although effective in animals, including man, cause an increase in serum concentrations of luteinizing hormone (LH) and testosterone by antagonism of the action of androgen at the hypothalamic-pituitary axis (Neri & Peets, 1975; Raynaud, Bonne, Bouton, 1979; Neumann & Jacobi, 1982; Mogulwewky, Piet, Tournemine & Raynaud, 1986). The steroidal antiandrogen cyproterone acetate, on the other hand, does not cause increases in serum LH and testosterone but shows other properties, particularly progestational activity (Neumann & Jacobi, 1982), which probably account for the failure of antiandrogenic effects at the hypothalamic-pituitary axis to be manifested. A pure non-steroidal, but peripherally selective, antiandrogen has theoretical advantages over these existing agents and, after extensive chemical syntheses and biological evaluation, such an agent has recently been discovered.

This communication describes the structure and some of the biological properties of ICI 176,334, a peripherally selective pure antiandrogen.

MATERIALS AND METHODS

Flutamide and ICI 176,334 ((2RS)-4'-cyano-3-(4-fluorophenylsulphonyl)-2'-hydroxy-2'-methyl-3'-trifluoromethyl)propionanilide) were synthesized in the Chemistry Department of ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire.

[17α-Methyl-3H]R1881 (87.6 Ci/mole), a synthetic androgen used in binding studies, was obtained from New England Nuclear Research Products, Stevenage, Herts; dextran T-40 from Pharmacia, Uppsala, Sweden; charcoal (activated, acid washed) from BDH Chemicals Limited, Poole, Dorset and unlabelled R1881 from New England Nuclear Research Products, Stevenage, Herts. The radioligand binding assay procedure was essentially similar to that of Wakeling, Furr, Glen & Hughes (1981). The rat prostate tissue supernatant (100μl), [3H]R1881 (5nmol; 86mCi) and a series of concentrations in triplicate containing standards of R-1881 (0.3-88nmol), 5α-dihydrotestosterone (0.3-88nmol), hydroxyflutamide (21nmol-5.5μmol) or ICI 176,334 (1.5nmol-3.7μmol) were mixed and incubated for 2h at 0°C. Non-specific binding was assessed by addition of an approximately 100-fold excess of R-1881. Dextran coated charcoal suspension (500μl; 1% charcoal and 0.1% dextran T-40 in assay buffer) was added and, after mixing, the tubes were centrifuged at 1500g for 5 min at 4°C. The supernatant was removed, added to a proprietary scintillant cocktail (HPO Ready Solv, Beckman R1C Limited, High Wycombe, Bucks) and the radioactivity counted in a liquid scintillation spectrophotometer (Intertechnique SL 4000).

The rats used in all studies were of the Alderley Park (Wistar-derived) strain and were housed under conditions of 14h light : 10h darkness (lights on at 0600h) and were given free access to rat pellets (Porton Combined Diet, Special Diet Services, Witham, Essex) and water. Some rats were castrated under Fluothane (ICI Pharmaceuticals Division, Macclesfield, Cheshire) anaesthesia.

Serum LH was assayed by a double-antibody radioimmunoassay using well-characterized reagents: a rabbit antiovine LH serum (GN1-15; Professor G. Niswender, University of Colorado, Fort Collins, CO, U.S.A.); purified ovine LH for iodination (LER-1056-C2; Professor L.E. Reichert, Albany State Medical College, New York, NY, U.S.A.) and an ovine standard NIH-LH-521 (NIADDK, Bethesda, Md, U.S.A.). The
sensitivity of the assay was 0.5μg/l and the intra- and inter-assay coefficients of variation were 6.1 and 9.3% respectively.

Serum testosterone was estimated by radioimmunoassay using an antiserum (R45-3) raised in rabbits against testosterone-3-carboxymethyl-oxime-bovine serum albumin. Significant cross-reactions were seen with 5a-dihydrotestosterone (57.1%), 19-nortestosterone (11.4%) and 5a-androstan-3β,17β-diol (10.0%), whilst oestrogens, progestins and corticosteroids showed negligible (<0.1%), cross-reactivity. Serum (50μl) was extracted with 30 volumes diethyl ether; petroleum ether (50:50). After separation of the aqueous and solvent phases by freezing in acetone/solid CO2 (Driko1, ICI Mond Division, Runcorn) the solvent phase was evaporated to dryness by low heat under vacuum (Buchler vortex evaporator). Antiserum at a 1/50,000 dilution and [1,2-3H]testosterone (60 Ci/m mole, Radiochemical Centre, Amersham, Bucks) were added to the dried residue; the solutions were mixed and incubated overnight at 4°C. A suspension of dextran-coated charcoal (see above) was added to separate bound and free hormone. The remainder of the procedure was identical to that described above for receptor binding determinations. The sensitivity of the assay was 0.87nmol/l and the intra- and inter-assay coefficients of variation were 8.4 and 11.8 respectively.

RESULTS

Receptor binding studies (Figure 1) show that ICI 176,334 produces a concentration related reduction in binding of [3H]R1881 to the rat prostate androgen receptor and that the displacement curve produced is parallel to that of R-1881, 5a-dihydrotestosterone and hydroxy-flutamide. The concentrations which cause 50% inhibition of [3H]R1881 binding are 2, 3.8, 190 and 700nmol for R-1881, 5a-dihydrotestosterone,

![Figure 1](image1.png)

Figure 1. Displacement of [3H]R1881 from a rat prostate androgen receptor preparation by increasing concentration of unlabelled R1881 (●), 5α-dihydrotestosterone (○), ICI 176,334 (△) and hydroxy-flutamide (△).

Inhibition of [3H]R1881 binding are 2, 3.8, 190 and 700nmol for R-1881, 5α-dihydrotestosterone, 176,334 and 176,344 Dose (mg/kg)

![Figure 2](image2.png)

Figure 2. Effects of graded daily oral doses of ICI 176,334 and flutamide given for 14 days on seminal vesicle and ventral prostate gland weights and on serum concentrations of LH and testosterone in rats. The % inhibition values for the organ weights refer to an arbitrary scale where placebo-treated controls are given a value of 0 and surgically castrated controls 100%.

*P<0.05; **P<0.01. All values are means ± SEM.

ICI 176,334 and hydroxyflutamide respectively. In the first animal experiment immature castrated rats (approximately 80g) were treated s.c. daily with testosterone propionate (200µg/kg) alone or concurrently with ICI 176,334 (10mg/kg) s.c. for 7 days. Comparison of the weights of the seminal vesicles and ventral prostate glands at necropsy on day 8 shows that, at a dose of 10mg/kg, ICI 176,334 completely abolishes the stimulatory effect of the injected androgen. The minimum effective dose in this test system which gave a statistically significant reduction in accessory sex organ weights was ≤0.5mg/kg.

In a second experiment the oral potencies of ICI 176,334 and flutamide were compared in groups of five mature male rats (200-220g) given doses of 1, 5 or 25mg/kg daily for 14 days; two further groups of five animals were treated with placebo, one group being castrated at the start to indicate maximum (100%) inhibition of response and the other remaining intact to provide baseline (0% inhibition) values. The effects of the treatments on inhibition of seminal vesicle and ventral prostate weights and on serum LH and testosterone concentrations are shown in Fig. 2. ICI 176,334 is clearly antiandrogenic and has a potency approximately five times greater than that of flutamide in inducing regression of the accessory sex organs. In contrast, ICI 176,334 has no effect on serum LH and very little effect on serum testosterone concentrations, whereas flutamide causes a dose-related increase in both parameters which is significantly different from control values for testosterone at the 5 (P<0.05) and 25mg/kg (P<0.01) doses and for LH at the 25mg/kg dose (P<0.01).

There was no effect of either drug at these doses on the weight of the testes.

DISCUSSION

Evidence that ICI 176,334 binds to the rat prostate androgen receptor is provided by the radioligand binding studies. Neither of the antiandrogens bound with the affinity of the potent androgens R-1881 and 5α-dihydrotestosterone, but this seems to be a common feature of non-steroidal antiandrogens (Wakeling et al. 1981). In this study the binding affinity of ICI 176,334 appeared to be around fourfold higher than that of hydroxyflutamide, which is consistent with the improved potency of the former compound in vivo.

ICI 176,334 clearly has good oral potency in rats and causes profound reductions in accessory sex organ weights. The surprising finding that it has, in contrast to flutamide, negligible effects on serum LH and testosterone warrants further explanation since, to our knowledge, this is the first description of a peripherally selective antiandrogen. The results are not a consequence of partial androgen agonism, progestational or oestrogenic activity (B.J.A., Furr, B. Valacca & J.R. Woodburn, unpublished results) since ICI 176,334 has no androgenic effect on accessory sex organ weights in castrated immature rats dosed orally at a dose of 25mg/kg. Similarly, it is inactive orally at 10mg/kg in the Claaberg assay for progestational activity and has no oestrogenic effect in a uterotrophic assay in immature rats at a daily oral dose of 25mg/kg. It is possible that the peripheral selectivity of ICI 176,334 is due to its failure to penetrate to the androgen-receptor containing neurones in the hypothalamus.

Alternatively, it may indicate that there are subtle differences in androgen receptors and androgen receptor-mediated actions in different tissues. Differential gene regulation by steroid hormones is, of course, well established for antioestrogens like Nolvadex (tamoxifen citrate) (Wakeling & Slater, 1981) but has yet to be so clearly demonstrated for antiandrogens. One interpretation of the results of these studies with ICI 176,334 is that these provide the first example of selective gene regulation with an antiandrogen. Various possible explanations of these findings are the subject of continuing studies and work on ICI 176,334 and related non-steroidal compounds should improve further our knowledge of androgen action.

Finally, a potent pure antiandrogen with peripheral selectivity should offer considerable clinical advantages over existing agents since it should neither inhibit libido, like cyproterone acetate, nor stimulate a compensatory rise in serum testosterone, like flutamide and anadron. The validity of these assumptions and the clinical merit of ICI 176,334 are now the subject of a clinical trials programme.

REFERENCES


