Relative potencies of Flutamide and Casodex: preclinical studies

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Abstract

After 3 h of incubation at room temperature, hydroxyFlutamide, the active metabolite of Flutamide (Eulexin), and Casodex (bicalutamide), an analog of Flutamide, have comparable potency to displace [3H]R1881 from the human prostate and rat ventral prostate androgen receptors contrary to the previous claims that Casodex was four times more potent using different incubation conditions. Binding data, in fact, must be interpreted with caution, since they are very much dependent upon the duration and temperature of incubation. In the more appropriate intact cell situation, hydroxyFlutamide is approximately three times more potent than Casodex at inhibiting dihydrotestosterone-stimulated proliferation of androgen-sensitive mouse Shionogi carcinoma cells in vitro. Under in vivo conditions, in the orchietomized rat supplemented with androstenedione implants, Flutamide is about three times more potent than Casodex at inhibiting ventral prostate and seminal vesicle weight. When both compounds were administered for 7 days to intact rats, Flutamide and Casodex showed similar apparent potency. However, taking into account the much higher plasma levels of testosterone and dihydrotestosterone in intact animals treated with Flutamide compared with those treated with Casodex, the estimated potency of Flutamide in the intact rat is also at least three times higher than that of Casodex. The present data, contrary to previous claims based upon the inappropriate intact rat model clearly show that Flutamide is at least three times more potent than Casodex in rat and mouse tissues. These data are opposite to those obtained in the inappropriate intact rat model where Casodex was reported to be five to ten times more potent than Flutamide. Using more appropriate models of androgen action, the present data thus indicate a 15- to 30-fold lower estimate of the potency of Casodex. Since the choice of the dose of Casodex to be used for the treatment of prostate cancer patients is based upon such a large overestimate of the true potency of Casodex, the present data should help the choice of a more appropriate dose of this antiandrogen for the treatment of prostate cancer.

Introduction

Prostate cancer is the most frequently diagnosed cancer and the second most common cause of cancer death in men in North America (Parker et al. 1996). In fact, it is predicted by the American Cancer Society that 317 100 new cases of prostate cancer will be diagnosed in the United States in 1996 while 41 400 men will die from the disease during the same period. In the absence of a significant improvement
in the prevention and/or treatment of this cancer, its major medicosocial and economic impact will continue to rise, especially as the population ages.

The best known characteristic of prostate cancer is its high sensitivity to androgen deprivation. In fact, among all hormone-sensitive cancers, prostate cancer is, by far, the one showing the best response to endocrine therapy (Labrie 1995, Labrie et al. 1995). For more than 50 years, following the pioneering report of Huggins & Hodges (1941), the standard treatment of advanced or metastatic prostate cancer has been blockade of testicular androgens by orchiectomy or high doses of estrogens. Since a patient suffering from prostate cancer was first treated with a luteinizing hormone-releasing hormone (LHRH) agonist in 1980 (Labrie et al. 1980), a series of analogous peptides has been used worldwide to achieve medical castration. LHRH agonists have eliminated the psychological limitations of orchiectomy and the life-threatening cardiovascular complications of estrogens. However, although orchiectomy (Huggins & Hodges 1941) or the more recent treatment with LHRH agonists (Labrie et al. 1980, 1986) causes a 90-95% reduction in serum testosterone and dihydrotestosterone (DHT) concentration, a much smaller inhibitory effect is seen on the intraprostatic concentration of DHT. In fact, after elimination of testicular androgens by medical or surgical castration, the intraprostatic concentration of the active androgen DHT remains at 40-50% of the value measured in the prostates of intact control men (Labrie et al. 1985, 1994), thus leaving an important amount of androgens free to continue to stimulate prostate cancer growth after castration.

Contrary to the misconception that the testes are responsible for 90-95% of total androgen production in men as suggested by simple measurement of serum testosterone (Gittes 1991), it is now well demonstrated that prostatic cells efficiently transform the inactive steroid precursors of adrenal origin, namely dehydroepiandrosterone sulfate and dehydroepiandrosterone, into the active androgens testosterone and DHT. In fact, as mentioned above, the amounts of testosterone and DHT synthesized locally in the prostate from adrenal steroid precursors are comparable with the quantity of testosterone and DHT of testicular origin (Labrie 1991, Labrie et al. 1994).

While testicular androgens can be easily and completely eliminated by orchiectomy (Huggins & Hodges 1941) or medical castration with an LHRH agonist (Labrie et al. 1980, 1986), antiandrogens or compounds that block the interaction of testosterone and DHT with the androgen receptor are the best available means of interfering with the action of the androgens made locally in the prostate (Labrie 1991, 1993). The best antiandrogen should be a compound which possesses high specificity and affinity for the androgen receptor while not having any androgenic, estrogenic, progestational, glucocorticoid or any other hormonal or antihormonal activity (Dorfman 1971, Labrie 1993). Such compounds are called pure antiandrogens (Poyet & Labrie 1985, Kennealey & Furr 1991). So far, only Flutamide (Neri et al. 1967) and its analogs Anadron (nilutamide, Moguilewsky et al. 1987) and Casodex (bicalutamide, Furr et al. 1987) have been available for clinical use.

While the non-steroidal pure antiandrogens of the class of Flutamide are well recognized to be clearly superior to steroidal antiandrogens (Neri et al. 1967, 1972, Mowszowicz et al. 1974, Sufrin & Coffey 1976, Poyet & Labrie 1985, Labrie et al. 1987, 1990, Luthy et al. 1988, Plante et al. 1988, Kennealey & Furr 1991, Labrie 1993), it is logical to attempt to develop other pure antiandrogens more potent than Flutamide, the first pure antiandrogen (Neri et al. 1967). The arguments used to support such development are potential improvement in potency, tolerance, and pharmacokinetics (Furr et al. 1987). Considering the rapidly rising interest in antiandrogens, especially following the demonstration that addition of Flutamide to an LHRH agonist prolongs life (Crawford et al. 1989, Denis et al. 1993), we have compared, using the most appropriate in vitro and in vivo assays, the biological characteristics of Flutamide and Casodex, a recently developed analog of Flutamide (Furr et al. 1987). The low 50 mg daily dose of Casodex was chosen for clinical studies (Chodak et al. 1995, Schellhammer et al. 1995), based on the premise that the compound was five to ten times more potent than Flutamide as suggested by the much popularized but inappropriate data obtained in the intact rat model (Furr et al. 1987, Kennealey & Furr 1991, Vershelst et al. 1994, Furr & Tucker 1996). However, the present data using models more representative of the situation in men, show that Casodex is, on the contrary, at best three times less potent than Flutamide, thus making a 15-
to 70-fold difference in the estimation of the clinical dose of Casodex.

**Materials and Methods**

**In vitro assays**

**Androgen receptor assay**

**Chemicals** Both [17α-methyl-3H]Methyltrienolone (R1881) (86 Ci/mmol) and the corresponding unlabeled compound were purchased from New England Nuclear (Lachine, Québec, Canada) while DHT was obtained from Steraloids (Wilton, NH, USA). HydroxyFlutamide was provided by Schering-Plough Research Institute (Kenilworth, NJ, USA) while RU 23908 (nilutamide, Anandron) was provided by Roussel-UCLAF (Paris, France). EM-879 (bicalutamide, Casodex) was synthesized in the medicinal chemistry division of our laboratory. Hydroxyapatite (HAP) (DNA grade Bio-gel) was purchased from BioRad (Mississauga, Ontario, Canada). All other chemicals were of analytical grade.

Stock solutions of the unlabeled steroids were kept at 4 °C in ethanol. The desired steroid solutions were then prepared by appropriate dilution in buffer B (10 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monothioglycerol, pH 7.4) containing 30% ethanol.

**Tissue preparation** Ventral prostates were obtained from adult Sprague-Dawley rats (Charles River Inc., St Constant, Québec, Canada) weighing 200-300 g which had been castrated 24-48 h before being killed in order to eliminate the presence of endogenous steroids which could interfere with the characterization of the steroid-binding component. Human prostatic tissue was obtained through the collaboration of Dr Jean Emond, Hôtel-Dieu Hospital (Lévis, Québec, Canada). Tissues were kept at −80 °C until use.

**Cytosol preparation** All procedures, except when indicated, were performed at 4 °C. Tissues were pulverized frozen in dry ice with a Thermovac pulverizer. The samples were then homogenized in 5 vol (w/v) buffer A (25 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monothioglycerol, 10% glycerol, 10 mM sodium molybdate, pH 7.4) using a Polytron PT-10 homogenizer (Brinkmann Instruments, Canada) at a setting of 5 for two periods of 10 s, with intervals of 10 s for cooling. The homogenate was then centrifuged at 105 000 g for 90 min. The supernatant was used immediately for assay.

**Binding assays**

Incubations were performed at room temperature using 100 μl cytosol, 100 μl [3H]R1881 (5 nM final, which contained 150 nM triamcinolone acetonide in order to saturate progesterone receptors), and 100 μl of the indicated unlabeled compound at concentrations ranging from 0.1 to 10 000 nM. Each assay was performed in triplicate. After 3 h of incubation, unbound steroids were separated by incubation with HAP prepared as follows: 10 g HAP were washed with buffer P (50 mM Tris-HCl, 10 mM KH2PO4, pH 7.4) until the pH of the supernatant reached 7.4, and then, following centrifugation and decantation of the supernatant, 37.5 ml buffer P was added to the pellet. Three hundred microliters of HAP suspension was added to assay tubes for 40 min with agitation every 10 min. The tubes were then centrifuged at 1000 g for 10 min and the pellet was washed four times with 1 ml buffer P. The radioactivity from the HAP pellet was then extracted twice with 1 ml ethanol and counted in 10 ml scintillation liquid at a counting efficiency of 25-30%. Dose-response curves and 50% effective doses (IC50) were calculated using the Marquardt-Levenberg (Marquardt 1963) algorithm to best fit the data points to the four-parameter logistic function.

**Inhibition of Shionogi mammary carcinoma cell proliferation**

An androgen-sensitive cell line (clone SEM-107) derived from Shionogi mouse mammary carcinoma cells (Labrie et al. 1988b) was used at passage 19. Cells were routinely grown as previously described (Labrie & Veilleux 1988, Labrie et al. 1988a). Cells were plated in 24-well plates at a density of 20 600 cells/ml in minimal essential medium supplemented with 2% dextran-coated charcoal-treated fetal calf serum, 1% non-essential amino acids, 10 IU penicillin/ml and 50 μg streptomycin/ml. Steroids and antisteroids were dissolved in ethanol and stock solutions were chosen to yield final ethanol concentrations less than 0.1% in the culture medium. Twenty-four hours after plating, medium was changed and the indicated concentrations of antiandrogens and/or DHT were added to triplicate dishes. Cells were grown for 10 days with medium changes every 2-3 days. Cells were then fixed in methanol, and their number was assessed by measurement of DNA content by a modification of the method of Fiszer-Szafarz et al. (1981).
**In vivo experiments**

**Animals**

Adult male Sprague-Dawley rats (CrL::CD(SD)Br) were purchased from Charles River Canada, Inc. The animals were housed in a light (12 h light/day; lights on at 0715 h) and temperature (22 ± 2 °C)-controlled environment. Purina rat chow and tap water were available *ad libitum*.

**Compounds and androstenedione (4-dione) implants**

Flutamide was kindly supplied by the Schering-Plough Research Institute while Casodex was synthesized in the medicinal chemistry division of the Laboratory of Molecular Endocrinology. The two antiandrogens were dissolved in ethanol and suspended in 8% EtOH-1% gelatin-0.9% NaCl (vehicle). 4-Dione was purchased from Steraloids. Implants of 1.5 cm in length of pure 4-dione were prepared with Silastic tubing (Dow Corning, Midland, MI, USA) measuring 0.062 inch (inner diameter), and 0.125 inch (outer diameter).

**Experimental procedure**

On the morning of the first day of the experiments, rats of the appropriate groups were orchiectomized under isoflurane-induced anesthesia. Rats treated with 4-dione received two implants of 4-dione which were inserted subcutaneously in the dorsal area of each animal at the time of orchiectomy. Treatment with antiandrogens or vehicle was initiated in the afternoon (~1600 h) on the day of orchiectomy. Animals were killed by decapitation 2-4 h after the last administration of the antiandrogen. The ventral prostate and seminal vesicles were then removed, dissected free from fat and connective tissue and weighed.

**Experimental protocols**

In the first experiment, rats weighing 450-500 g were randomly assigned to the following 11 groups, each containing eight animals: (1) intact, (2) castrated, (3) castrated bearing two 1.5 cm long 4-dione implants, or castrated animals bearing two 4-dione implants and treated orally (twice daily) for 7 days with (4) 0.4, (5) 1.2, (6) 4.0 or (7) 12.0 mg Flutamide/kg body weight, or (8) 0.4, (9) 1.2, (10) 4.0 or (11) 12.0 mg Casodex/kg body weight. The rats that were not treated with either Flutamide or Casodex received 0.5 ml vehicle.

In the second experiment, intact rats weighing 215-250 g were randomly divided into the following ten groups, each containing eight animals: (1) intact, (2) intact treated with 0.25, (3) 1.0, (4) 4.0 or (5) 16.0 mg Flutamide/kg body weight, or (6) 0.25, (7) 1.0, (8) 4.0 or (9) 16.0 mg Casodex/kg body weight and control castrated rats receiving the vehicle alone (10).

**Figure 1** Effect of increasing concentrations of unlabeled DHT, hydroxyFlutamide (OH-FLU), Casodex (nilutamide) or Anandron (nilutamide) on $[^3]$HJR1881 binding to (A) human and (B) rat ventral prostate cytosol. The binding assay was performed as described in Materials and Methods.
**Hormone measurements**

Serum testosterone and DHT were measured by RIA after diethyl ether extraction and chromatography on LH-20 columns using antisera developed and characterized in our laboratory (Bélanger et al. 1980, 1989). Serum LH was measured by double-antibody RIAs using rat hormones (LH-1-6 for iodination, LH-RP-2 as standard), and the rabbit anti-rLH-S-8 antisera generously supplied by the National Pituitary Program (Baltimore, MD, USA).

**Statistical analyses**

Statistical significance was assessed by contrast analysis of the main effects and of their combinations such as the type of antiandrogen (Flutamide or Casodex) vs the control and the doses. Levels of significance for each contrast were adjusted to account for an overall type I error of 5% (α=0.05). Multiple a posteriori comparisons between individual experimental groups were performed by t-test when significant effects were demonstrated. The error term was estimated with the pooled within-group variance. Dose-response curves and IC\(_{50}\) values obtained in Shionogi cells in culture were calculated using a weighted iterative non-linear least squares regression (Rodbard 1974). Results are presented as means±S.E.M. of triplicate measurements.

**Results**

**In vitro assays of hydroxyFlutamide and bicalutamide**

After 3 h of incubation at room temperature, DHT, hydroxyFlutamide, Casodex and Anandron displaced \(^{3}\text{H}\)R1881 binding in human prostate cytosol at IC\(_{50}\) values of 28.6±12.8 nM, 2345±395 nM, 2490±690 nM, and 5300±1515 nM (Fig. 1A). In rat prostate cytosol, DHT, hydroxyFlutamide, Casodex, and Anandron displaced \(^{3}\text{H}\)R1881 at IC\(_{50}\) values of 16.7±1.5 nM, 4565±1030 nM, 3595±555 nM, and 18620±3580 nM respectively (Fig. 1B).

Androgen-sensitive mouse Shionogi mammary carcinoma cells have been widely used as a model of human prostate cancer because of their well-characterized responsiveness to androgens and antiandrogens (Labrie & Veilleux 1986, 1988, Labrie et al. 1988a,b, Luthy et al. 1988, Plante et al. 1988). In agreement with their known pure antiandrogenic activity, none of the three antiandrogens exerted any significant effect on basal Shionogi cell proliferation. On the other hand, the stimulatory effect of 0.3 nM DHT was inhibited by hydroxyFlutamide, Casodex and Anandron at IC\(_{50}\) values of 73.3±5.32, 222±25.2 and 375±32.0 nM respectively (Fig. 2).

**In vivo effects of Flutamide and Casodex**

Figures 3 and 4 show that 4-dione implants maintained ventral prostate and seminal vesicle weight at 85% and 80% of the values observed in intact rats respectively. Treatment with increasing doses (0.4, 1.2, 4.0 or 12.0 mg Flutamide/kg body weight) reversed the stimulatory effect of 4-dione on ventral prostate weight by 34%, 50%, 74% and 80% from 133.6±6.78 mg/100 g body weight in control rats to 96.9±7.47 (P<0.001), 79.7±8.46 (P<0.0001), 53.0±6.99 (P<0.0001), and 46.6±5.02 (P<0.0001) mg/100 g body weight respectively (Fig. 3). Treatment with the same doses of Casodex reduced ventral prostate weight by 36%, 36%, 57%, and 64% to
Casodex is known to have potent inhibitory effects on seminal vesicle weight by 46%, 66%, 72%, and 80% from 76.3±2.88 mg/100 g body weight in castrated animals supplemented with 4-dione to 60.8±2.91 (P<0.0001), 54.3±1.10 (P<0.0001), 52.2±2.46 (P<0.0001), and 49.4±2.01 (P<0.0001) mg/100 g body weight. Casodex caused inhibitions of 32%, 40%, 66%, and 68% to 65.6±2.06 (P<0.005), 63.0±2.73 (P<0.005), 54.1±2.11 (P<0.0001), and 53.6±2.65 (P<0.0001) mg/100 g body weight respectively (Fig. 4).

Previous experiments conducted in intact rats have led their authors to conclude that Casodex is more potent than Flutamide (Furr et al. 1987, Kennealey & Furr 1991), a finding which is at odds with the results obtained in the 4-dione model. We therefore compared the effect of increasing doses of the two antiandrogens in intact animals. As illustrated in Fig. 5, ventral prostate weight decreased by 87% 7 days after castration. Twice-daily oral administration of Flutamide or Casodex to intact rats over the same period caused similar inhibitions of 46.5% and 46.9% respectively, at the highest dose used, namely 16 mg/kg body weight. The maximal inhibitory effects of the two antiandrogens on seminal vesicle weight were also comparable with maximal inhibitions of 25.3% and 31.8% for Flutamide and Casodex respectively (Fig. 6).

Since Flutamide and Casodex (Neri et al. 1972, Marchetti & Labrie 1988, Kennealey & Furr 1991) are known to have different effects on the feedback mechanisms controlling LH secretion in the rat, we...
have measured the serum levels of LH, testosterone, and DHT in the same experiment. Serum LH increased from 0.20±0.08 ng/ml in intact animals to 0.46±0.09 ng/ml (127%, not significant (N.S.)), 0.60±0.12 ng/ml (200%, N.S.), 1.20±0.21 ng/ml (500%, P<0.01) and 1.53±0.29 ng/ml (660%, P<0.01) above control at the twice-daily doses of 0.25, 1.0, 4.0, and 16 mg Flutamide/kg body weight, while the same doses of Casodex increased serum LH to 0.39±0.10 ng/ml (95%, N.S.), 0.62±0.11 ng/ml (210%, N.S.), 0.65±0.15 ng/ml (225%, N.S.), and 1.00±0.29 ng/ml (400%, P<0.01) (Fig. 7).

The effects of the 4.0 and 16.0 mg/kg doses of Flutamide and that of the highest dose of Casodex on serum LH would predict that serum testosterone levels would increase correspondingly. In fact, the two highest doses of Flutamide increased serum testosterone by 235% (P<0.01) and 440% (P<0.01) above control respectively, while the same doses of Casodex caused slight but non-significant increases of 81% (N.S.) and 117% (N.S.) over control respectively (Fig. 8). Serum testosterone levels in rats treated with Casodex thus remained significantly lower than those in rats dosed with Flutamide. Similarly, serum DHT increased by 145% (N.S.) and 405% (P<0.01) above control at the 4.0 and 16.0 mg/kg doses of Flutamide while Casodex had no significant effect on this parameter (Fig. 9). Serum DHT levels in rats dosed with 16.0 mg Casodex/kg were significantly lower than in rats receiving the same dose of Flutamide.

**Figure 6** Effect of treatment with increasing doses of Flutamide or Casodex administered orally twice daily for 7 days on seminal vesicle weight in intact rats. Data are expressed as means±S.E.M. **P<0.01 vs intact control animals.

**Figure 7** Effect of treatment with increasing doses of Flutamide or Casodex administered orally twice daily for 7 days on serum LH administered orally twice daily for 7 days on serum LH levels in intact rats. Data are expressed as means±S.E.M. **P<0.01 vs control animals.

**Discussion**

Contrary to previous reports (Furr et al. 1987, Kennealey & Furr 1991), the present data show, using more appropriate models, that Flutamide or hydroxyFlutamide, the active metabolite of Flutamide (Katchen & Buxbaum 1975) is at least three times more potent than Casodex in rat as well as in mouse tissues. *In vitro*, hydroxyFlutamide is three times more potent than Casodex at reversing the stimulatory effect of DHT on the proliferation of mouse Shionogi carcinoma cells (Fig. 2). In contrast to Anandron, which has been shown to possess biological potency and properties comparable with those of Flutamide (Moguilewsky et al. 1987, Raynaud 1988), the present data show that Casodex is at least three times less potent than Flutamide on well-recognized androgen-responsive parameters, namely prostate and seminal vesicle weight (Figs 3 and 4). On the other hand, in a human carcinoma cell line (T47-D), hydroxyFlutamide is seven times more potent than Casodex (J Simard, S M Singh & F Labrie, unpublished data). We believe that these findings have important implications for the choice and dose of antiandrogen to be used in the treatment of prostate cancer. Our interpretation of the results of the study reported here and those reported in the ICI study (Furr et al. 1987, Kennealey & Furr 1991) is
based on a critical analysis of receptor-binding assays, *in vitro* intact cell data and the appropriateness of the animal models used. In addition, such an interpretation appears to be in agreement with the results of clinical studies in men (Chodak et al. 1995).

First, it should be considered that the values obtained in a receptor-binding assay vary, among other factors, according to the temperature and duration of incubation. The values obtained in various binding assays should not be taken directly as a reliable indicator of the activity of a compound under conditions of equilibrium in the intact cell.

![Figure 8](image-url)

**Figure 8** Effect of treatment with increasing doses of Flutamide or Casodex administered orally twice daily for 7 days on serum testosterone levels in intact rats. Data are expressed as means± S.E.M. **P<0.01 vs intact control animals; ∼P<0.05, ++P<0.01 Casodex vs Flutamide at the same dose.**

When examining the binding affinity to the androgen receptor after 3 h of incubation at room temperature, the present data show that Casodex and Flutamide have a comparable affinity for the rat prostate androgen receptor while a fourfold higher affinity has been reported previously (Furr et al. 1987, Kennealey & Furr 1991). This difference can probably be explained by the different assay conditions used. In fact, the present results are in agreement with those obtained with the normal human androgen receptor transfected in COS cells where the following order of potency was found in relation to the androgen R1881: hydroxyFlutamide (0.4%), Casodex (0.3%) and Anandron (0.1%) (Furr & Tucker 1996). Moreover, the binding affinities of hydroxyFlutamide and Casodex have been found to be comparable in the dog as well as in the rat ventral prostate (Winneker et al. 1989, Juniewicz et al. 1990).

The fourfold higher binding affinity of Casodex compared with hydroxyFlutamide for the androgen receptor quoted in various clinical publications (Chodak et al. 1995, Schellhammer et al. 1995) as well as in the Casodex product monograph was obtained under special binding assay conditions and it certainly does not translate to the intact cell situation. As further support for the present data and contrary to the claim of Furr et al. (1987), Fuhrmann et al. (1992) have found that Casodex was slightly less potent than hydroxyFlutamide at reversing the induction of chloramphenicol acetyl transferase activity by the androgen R1881 in the cell line 3.9.2 stably transfected with the rat androgen receptor. The IC<sub>50</sub> values of hydroxyFlutamide and Casodex were calculated at 300 nM and 400 nM respectively. In fact, when androgen-sensitive mouse Shionogi carcinoma cells (Fig. 2) or human carcinoma cells (J Simard, S M Singh & F Labrie, unpublished observations) are used, hydroxyFlutamide is three to seven times more potent than Casodex. In T47-D human breast cancer cells, hydroxyFlutamide inhibits DHT-stimulated secretion of gross cystic disease fluid protein-15, a well-characterized androgen-sensitive parameter, with a potency seven times that of Casodex (J Simard, S M Singh & F Labrie, unpublished data). Furr & Tucker (1996) reported that Casodex decreased Shionogi cell growth in culture although no comparison with hydroxyFlutamide was mentioned.

Secondly, Furr (1989) reported that the inhibitory effect of Casodex on ventral prostate and seminal vesicle weight in intact mature rats was approximately five to ten times more potent than that of Flutamide. While the 1 mg dose of Casodex exerted a greater inhibitory effect than the same dose of Flutamide in the ICI as well as in our study, it is not clear from the ICI study if the inhibitory effect of the higher doses of Casodex was significantly greater than that obtained with the same doses of Flutamide. It should be mentioned that the higher doses of the two antiandrogens have more relevance to the clinical situation where maximal androgenic blockade is required. These results, and especially their interpretation, are in obvious contradiction with those reported here since we observed that Flutamide and Casodex were equipotent at doses of 4 and 16 mg/kg.
in intact rats. More importantly, however, the interpretation of Furr (1989) of the relative potencies of Flutamide and Casodex failed to take into account the higher levels of testosterone in the rats dosed with Flutamide. In the ICI study, serum testosterone levels increased significantly in rats receiving 5 and 25 mg Flutamide/kg while Casodex-treated rats showed serum testosterone levels comparable with those found in intact control rats. Thus, the potency of Flutamide in the ICI study was underestimated by at least a factor of two. In fact, we found that Flutamide and Casodex exerted almost superimposable inhibitory effects on ventral prostate weight in intact rats despite the three- to fourfold higher levels of serum testosterone and DHT in the animals receiving Flutamide. Such data clearly indicate that Flutamide is at least three to four times more potent than Casodex in the intact rat since the same dose of Flutamide could antagonize three- to fourfold higher serum levels of androgens.

It is apparent from the results of these two studies that the intact rat model is not an appropriate one in which to conduct comparative potency studies of pure antiandrogens because of the dynamic state of the hypothalamo-pituitary-testicular axis in such animals. The true assessment of the biological activity of antiandrogens in vivo thus requires animal models wherein the circulating levels of androgens are well controlled.

The difference in efficacy between Flutamide and Casodex reported in intact animals by Furr et al. (1987) could be at least partly explained by the longer duration of treatment in that study (2 weeks versus 1 week in the present study). This is likely to lead to even higher serum levels of androgens in the group of animals treated with Flutamide for longer periods. As observed in the study of Furr et al. (1987), the slight increase in serum testosterone levels in rats receiving Casodex at the twice-daily 4 and 16 mg/kg doses did not reach statistical significance.

As shown by the present data, the in vivo potency of Casodex assessed in an appropriate rat model, such as the 4-dione-treated orchietomized rat, is at least three times lower than that of Flutamide. The conclusions suggested by Kennealey & Furr (1991) with reference to data obtained in the intact rat are thus due to the inadequacy of the model used where Flutamide led to an increase in serum testosterone levels while Casodex had no significant effect on this parameter. As mentioned above, it is expected that a higher dose of Flutamide is needed to counteract such elevated levels of serum androgens in the intact male rat. In fact, Flutamide is well known to increase plasma testosterone levels in the intact rat (Marchetti & Labrie 1988). The potent antiandrogenic activity of Flutamide exerted at the hypothalamo-pituitary level leads to an increased secretion of LH. Consequently, testicular testosterone secretion is stimulated and a progressively higher dose of the antiandrogen is required to neutralize the rising concentrations of serum androgens. On the other hand, in the intact rat, Casodex has a lower stimulatory effect on circulating androgen levels. Nilutamide (Anadron), the first derivative of Flutamide to be used clinically (Labrie et al. 1982, 1983), also produces an important elevation of serum testosterone levels in intact rats (Moguilewsky et al. 1987).

Figure 9 Effect of treatment with increasing doses of Flutamide or Casodex administered orally twice daily for 7 days on serum DHT levels in intact rats. Data are expressed as means ± S.E.M. **P<0.01 vs intact control animals; ++P<0.01 Casodex vs Flutamide at the same dose. N.D., below the limit of detection: 0.468 nM.

Casodex, at the dose of 40 mg/kg has been found to slightly increase serum LH and testosterone concentrations (Chandolia et al. 1991). In another study performed in intact male rats, Casodex and Flutamide increased serum testosterone levels by two- to threefold and seven- to elevenfold respectively (Snyder et al. 1989). In all studies performed in the intact rat, however, Flutamide led to higher levels of serum androgens. In immature castrated
male rats, Flutamide, hydroxyFlutamide and Casodex were found to inhibit testosterone propionate-induced ventral prostate weight with comparable potency while being three- to fourfold more potent than cyproterone acetate, a progestational compound (Snyder et al. 1989). Thus, the purported advantage of Casodex, i.e. peripheral selectivity, has not been demonstrated in laboratory animals other than the rat and, most importantly, it certainly does not translate into the clinical setting.

The data obtained when comparing Flutamide and Casodex in the intact rat (Furr et al. 1987, Kennealey & Furr 1991, Furr & Tucker 1996) have no relevance to the situation in men. The potent stimulation of LH and testosterone secretion in the intact animal by Flutamide is a biological curiosity limited to the rat. It was originally extrapolated, based upon the data obtained in the intact rat model (Furr et al. 1987), that Casodex would exert no stimulatory effect on the hypothalamo-pituitary-testicular axis in men. However, studies in men have rapidly shown that this prediction was incorrect and that Casodex increases serum testosterone levels in men to a degree comparable to and possibly even higher than that observed with Flutamide or Anandron (Kennealey & Furr 1991, Vershelst et al. 1994). In fact, the highest serum concentration of testosterone in men receiving Casodex has been reported at 23.06 ng/ml which corresponds to five times the mean normal value of serum testosterone in men (Kennealey & Furr 1991). It should be mentioned that Flutamide, like Casodex, was known to have no stimulatory effect on serum testosterone in intact male dogs (Furr & Milsted 1988, Lacoste et al. 1988, 1989). The intact rat model is thus well known to be an exception which has no bearing on the use of pure antiandrogens for prostate cancer therapy in men. Surprisingly, despite these well-known facts, the incorrect argument of a higher activity of Casodex is still used to support the use of a low dose of Casodex in the clinic (Schellhammer et al. 1995).

Since Flutamide and its analogs Anandron and Casodex have a comparable and small stimulatory effect on serum testosterone levels in men, the intact rat model is clearly not appropriate and the castrated animal should be the exclusive rat model used. This is precisely the model used in the present study. In addition, since pure antiandrogens are used in combination with orchietomy or medical castration with an LHRH agonist in men, the antiandrogen-induced changes in serum testosterone levels observed in the intact rat are irrelevant to the situation in medically or surgically castrated prostate cancer patients where the serum testosterone concentration remains at 5% to 10% of the pretreatment control value and is not affected by the antiandrogen (Labrie et al. 1985, Bélanger et al. 1988).

Another finding of potentially major clinical significance is the observation that the oral administration of doses of 1.0, 3.0 or 10 mg Flutamide once daily or the same doses divided into three equal doses administered every 8 h exert a similar inhibition of 4-dione-stimulated ventral prostate, dorsal prostate, and seminal vesicle weight in the rat (Luo et al. 1996). Similarly, when a total daily dose of 7.5 mg Flutamide was administered subcutaneously every 8 h, every 12 h, once daily or once every second day, a comparable and near-maximal inhibition of DHT-stimulated ventral and dorsal prostate as well as seminal vesicle weight was observed. Comparable results were also observed in mice that received the same total daily dose of Flutamide divided into three doses per day, two doses per day, a once-daily dose or when the compound was administered every second day. In fact, the same maximal inhibitory effects on 4-dione-stimulated prostate and seminal vesicle weight were observed and no effect related to the above-indicated schedules of administration of the antiandrogen could be detected (Luo et al. 1996).

Such data have major implications for the choice of the dosage schedule for therapy of prostate cancer in men. In fact, the above-indicated data show that once-a-day dosing of Flutamide is equivalent to administration of the drug three times a day, an observation which could potentially facilitate compliance by permitting the administration of Flutamide once a day instead of three times a day. It was previously believed that circulating antiandrogen levels had to remain constant in order to exert maximal efficacy. Such a hypothesis originally led to the decision to administer Flutamide as well as Anadron in three equal daily doses (Labrie et al. 1982, 1983). The above-mentioned data strongly suggest that Flutamide could be administered once daily instead of once every 8 h with no change in its therapeutically benefits.

The present data suggest that the true potency of Casodex is thus, at best, one-third that of Flutamide. Since Casodex has been known for some time to have a potency inferior to or at best equivalent to that of
Flutamide (Furhmann et al. 1992), it is somewhat surprising that the low 50 mg daily dose of Casodex has been chosen with the following arguments: 'on the basis of evidence from animal pharmacokinetic studies, the 50 mg daily dose of Casodex is likely to be as effective as the 750 mg daily dose of Flutamide' (Schellhammer et al. 1995). The objectives initially put forth for the development of another analog of Flutamide, namely an improvement in the efficacy, safety, and pharmacokinetics, have not been fulfilled (Furr et al. 1987, Kennealey & Furr 1991). The present data clearly indicate that Casodex is not more potent but, on the contrary, at least three times less potent than Flutamide. The present data are thus opposite to the estimate of a five to ten times higher potency of Casodex (Furr 1990, Kennealey & Furr 1991). There is thus a 15- to 30-fold difference between the estimate of Furr et al. (1987) and Furr (1989) and the present data obtained using more appropriate models of antiandrogen action.

It should be mentioned that the pharmacokinetics of Flutamide could be more favorable than those of Casodex, especially when one considers the much more rapid elimination of Flutamide in the case of serious side-effects. In fact, because of its relatively short half-life (5.2 h) (Katchen & Buxbaum 1975), it was previously felt, as mentioned above, that Flutamide had to be administered three times a day in order to maintain constant serum levels of the antiandrogen (Labrie et al. 1983). Anadron, on the other hand, has a half-life of approximately 2 days in man (Ojasso 1987) while the half-life of Casodex in men has been estimated at 6 to 8 days (Kennealey & Furr 1991). Consequently, the 6- to 8-day long half-life of Casodex could possibly be a disadvantage. Since the two antiandrogens appear to have a comparable incidence of liver function abnormalities (Schellhammer et al. 1995), and it is known that the concentration of the serum liver enzymes returns to normal upon discontinuation of Flutamide (Gomez et al. 1992), it is likely that the 3-4 weeks required to decrease the serum concentration of Casodex below 1 μg/ml could be problematic, since a similar decrease in the blood levels of hydroxyFlutamide could be achieved within 1 day after discontinuation of the drug, thus removing the cause of liver toxicity 20 to 30 times faster with Flutamide than with the slowly metabolized Casodex.

References
Luo et al.: Relative potencies of Flutamide and Casodex


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