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In Vitro and *in Vivo* Comparisons of Antiandrogenic Potencies of Two Histamine H2-Receptor Antagonists, Cimetidine and Etintidine–HCl (42087)

ROBIN G. FOLDESY, MARY M. VANDERHOOF, AND DO WON HAHN

Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869

Abstract. The antiandrogenic potency of cimetidine was compared to that of a new histamine H2-receptor antagonist, etintidine–HCl (ORF 16753-02). Although both compounds displaced [^{3}H]dihydrotestosterone from androgen receptors *in vitro* and inhibited androgen-stimulated growth of the accessory sex organs of male rats *in vivo*, etintidine–HCl was significantly less antiandrogenic than cimetidine in the analysis of androgen receptor binding and in the inhibition of seminal vesicle weights. Because etintidine–HCl has been shown previously to have more potent gastric antisecretory activity than cimetidine, its lower antiandrogenic activity suggests that etintidine–HCl has a much wider therapeutic ratio and that its use clinically will result in fewer antiandrogenic side effects.

The histamine H2-receptor antagonist, cimetidine, has gained widespread use in the treatment of duodenal ulcers, peptic esophagitis, upper gastrointestinal hemorrhage, and Zollinger-Ellison syndrome. In the latter case particularly, some men treated with doses of 1 g/day or more have developed gynecomastia, impotence, or oligospermia (1–3). It is believed that these symptoms result from the ability of cimetidine to displace androgens from their target cell receptors and thus produce an antiandrogenic effect. Laboratory experiments *in vitro,* in fact, have clearly demonstrated this androgen displacing quality (4–6).

Etintidine–HCl (ORF 16753-02), a new, highly specific histamine H2-receptor antagonist, has been shown to have a greater H2-receptor affinity and a more potent gastric antisecretory activity than cimetidine (7). Because its chemical structure is closely related to that of cimetidine, the possibility that it also may bind to androgen receptors and thus possess antiandrogenic properties was explored both *in vitro* and *in vivo.* The present *in vitro* studies examined the ability of the H2 blockers to competitively displace [^{3}H]dihydrotestosterone ([^{3}H]DHT) from androgen receptors in a cytosolic preparation from rat ventral prostates. The *in vivo* activity was assessed by determining the inhibition of growth of the accessory sex organs in castrate, androgen-supplemented, immature male rats.

The structures of cimetidine and etintidine–HCl are given in Fig. 1.

Materials and Methods. *In vitro activity—androgen receptor assay.* Adult male rats of the Wistar strain, weighing between 300–500 g, were obtained from Marland Breeding Farms Inc., Hewitt, New Jersey. They were maintained on a 12:12-hr light:dark cycle at 22 ± 1°C and given Purina chow and water *ad libitum.* The animals were bilaterally castrated under ether anesthesia through a midline incision in the lower abdomen. Approximately 18–24 hr after surgery, the animals were killed with CO_2 gas and the ventral prostates excised, freed of extraneous tissue, and homogenized in 3 vol of ice-cold buffer with a Polytron homogenizer. The buffer consisted of 0.01 *M* Trizma-7.4 (Sigma Chemical Co., St. Louis, Mo.) 0.0015 *M* EDTA, 0.001 *M* dithiothreitol, 0.01 *M* molybdic acid, and 10% glycerol in distilled, deionized water. The homogenate was centrifuged at 105,000*g* for 60 min at 4°C. The supernatant was decanted and used as the cytosolic receptor preparation.

The affinity of cimetidine and etintidine–HCl for androgen receptors was measured by incubating aliquots of the cytosol solution with [^{3}H]DHT (40–60 Ci/mmole) for 18–24 hr at 4°C in the presence and absence of various concentrations of the H2 blockers. The radioactive ligand was present at a final concentration of approximately 3×10^{-9} *M* and the H2 blockers at final concentrations

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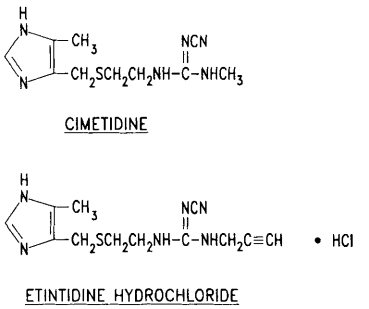

FIG. 1. Molecular structures of cimetidine and etintidine–HCl.

ranging from 10^{-7} to 10^{-3} *M*. The incubation volume was 500 μl. Nonspecific binding was assessed by displacing the radioactive ligand in a separate set of tubes with a 1000-fold excess of radioinert DHT. The nonspecific binding was subtracted from all data points. At the end of the incubation, the amount of radioactivity bound to receptors was separated from the unbound by adding to each tube 500 μl of a charcoal solution containing 1% neutralized, activated charcoal, 0.1% Dextran T-70, and 0.1% bovine γ-globulin in buffer. The tubes were incubated for 10 min after which each received 50 μl of absolute ethanol for an additional 20 min. This latter procedure has been shown by Boesel and Shain (8) to greatly reduce nonspecific binding. The tubes were centrifuged, the supernatants decanted, and the amount of radioactivity in each supernatant was determined by liquid scintillation spectrometry. DHT, cyproterone acetate, and dexamethasone were also tested as a standard, positive control, and negative control, respectively. Each concentration of each compound was assayed in duplicate in four separate trials.

To generate a more detailed dose–response relationship, the *in vitro* receptor assay was repeated with five different concentrations of cimetidine ranging from 10^{-5} to 10^{-3} *M* and of etintidine–HCl ranging from 10^{-4} to 10^{-2} *M*. The concentration of [^{3}H]DHT in the incubation was lowered slightly to maximize the competitive qualities of the H2 blockers. Each concentration of each compound was assayed in duplicate in four separate trials.

In vivo activity—oral administration. The *in vivo* antiandrogenic activity of etintidine–HCl and cimetidine was tested according to the method of Randall and Selitto (9). Briefly, Sprague–Dawley male rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 45–60 g were bilaterally castrated through a scrotal incision under ether anesthesia. Animals were randomized with respect to body weight into groups of eight rats each. Treatments began on the day of surgery and continued for a total of 7 days. All rats received subcutaneously a total dose of 0.4 mg testosterone propionate, dissolved in sesame oil, divided into seven equal daily doses. Androgen-treated rats received orally in 0.5% hydroxymethyl cellulose vehicle total base (active moiety) doses of 50, 100, 200, or 400 mg cimetidine or etintidine–HCl divided into seven equal daily doses. Flutamide was also tested in total doses of 1, 3, and 9 mg as a positive control. A final group was treated only with testosterone propionate to determine the maximal level of androgen stimulation. All animals were autopsied on the day after the last dose at which time the body weights and the weights of the ventral prostates and seminal vesicles were determined.

Statistical analysis. All data are expressed as means ± SEM. The IC_{50} or ED_{50} and relative antiandrogenic potency of the two drugs *in vitro* and *in vivo* were assessed using the technique of parallel line assay (10).

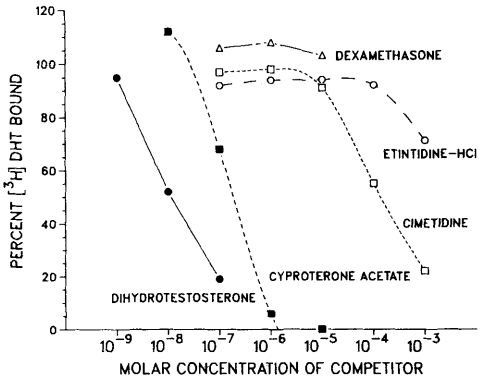

FIG. 2. Ability of various compounds to displace [^{3}H]DHT from rat ventral prostate androgen receptors *in vitro*. [^{3}H]DHT was present at a concentration of approximately 3×10^{-9} *M*. Each data point represents the mean of four trials.

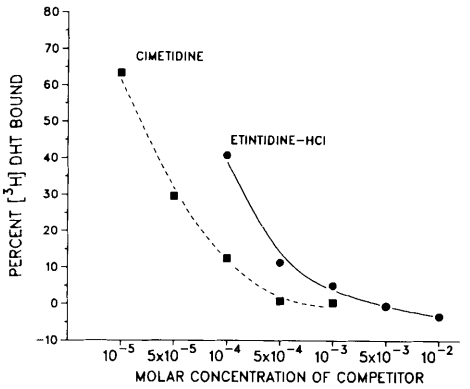

FIG. 3. Ability of cimetidine and etintidine-HCl to displace [^{3}H]DHT from rat ventral prostate receptors *in vitro*. [^{3}H]DHT was present at a concentration of approximately 0.8×10^{-9} *M*. Each data point represents the mean of four trials.

Analysis of variance procedures were used to test the assumptions of the assay. Fieller's theorem was used to construct 95% fiducial limits for the relative potency. Where the responses to the same doses or concentrations of cimetidine and etintidine-HCl were compared, a two-tailed Dunnett's *t* test (11, 12) was used to detect the statistical significance of differences ($P \leq 0.05$).

Results. Radioinert DHT—a potent androgen, and cyproterone acetate—a potent antiandrogen, abolish nearly all [^{3}H]DHT receptor binding at a concentration of 10^{-6} *M*. In comparison, both cimetidine and etintidine-HCl bind to androgen receptors but their ability to displace [^{3}H]DHT is very weak (Fig. 2). Moreover, etintidine-HCl is significantly less potent than cimetidine in this respect. At concentrations of 10^{-4} and 10^{-3} *M*, the percentage of [^{3}H]DHT bound is significantly less with cimetidine than with etintidine-HCl (cimetidine vs etintidine-HCl: 10^{-4} *M*, 55% ± 4 vs 92% ± 3, $P < 0.05$; 10^{-3} *M*, 22% ± 4 vs 71% ± 4, $P < 0.05$; Fig. 2). This is confirmed with a more detailed analysis of the dose-response relationship (Fig. 3). When the potencies of these two compounds are analyzed based upon the latter analysis, cimetidine is 4.4 times more potent than etintidine-HCl in its ability to displace androgen from its cytosol receptor (Table I).

Oral administration of high doses of cimetidine or etintidine-HCl to immature rats for 7 days causes some inhibition of androgen-stimulated growth of the ventral prostate and seminal vesicles. This inhibition is weak, however, compared to that caused by a potent antiandrogen, flutamide (Fig. 4). Furthermore, the inhibition in the growth of the seminal vesicles is greater with cimetidine treatment than with etintidine-HCl treatment

TABLE I. ANTIANDROGENIC POTENCY OF CIMETIDINE COMPARED TO ETINTIDINE-HCl BY *IN VITRO* AND *IN VIVO* ANALYSIS

Assay	IC_{50} (*M*) or ED_{50} (mg) (95% fiducial limits)	Relative potency (95% fiducial limits)
Androgen receptor, *in vitro*		
Etintidine-HCl	9.1×10^{-5} (4.9×10^{-5}–1.3×10^{-4})	1.0
Cimetidine	2.6×10^{-5} (2.1×10^{-5}–3.1×10^{-5})[a]	4.4 (3.2–5.8)[a]
Seminal vesicle *in vivo*		
Etintidine-HCL	308 (217–585)	1.0
Cimetidine	154 (117–211)[a]	1.8 (1.2–2.9)[a]
Ventral prostate *in vivo*		
Etintidine-HCl	369	1.0
Cimetidine	352[b]	1.4[b]

[a] Significantly different from etintidine-HCl.
[b] Estimated, significant nonlinearity in the dose-response detected.

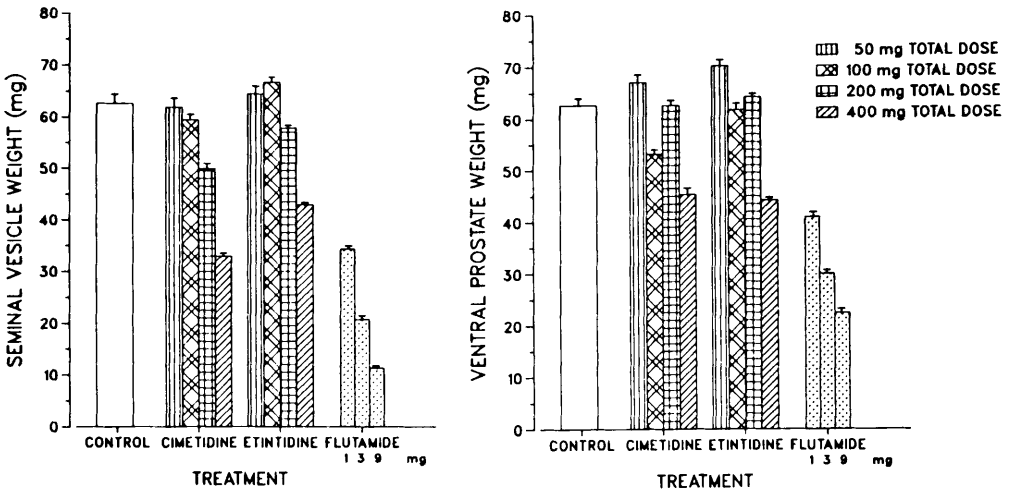

FIG. 4. Inhibition of androgen-stimulated growth of the seminal vesicles and ventral prostates in immature, castrate rats following the administration of cimetidine, etintidine–HCl or flutamide for 7 days. The dose of etintidine–HCl is that of the active moiety.

(Fig. 4). Cimetidine is 1.8 times more potent than etintidine–HCl as an antiandrogen when the dose–responses of the seminal vesicles are compared ($P < 0.05$). When the dose–responses of the ventral prostates are compared, cimetidine is estimated to be 1.4 times more potent although significant nonlinearity in the dose–response was detected (Table I).

Discussion. The present study demonstrates clearly in two different experimental protocols that etintidine–HCl is significantly less antiandrogenic than cimetidine. The results obtained when the compounds were compared by *in vitro* androgen receptor analysis were very similar to those obtained following the oral administration of the drugs to rats. These assays suggest that it would require almost two to four times the dose of etintidine–HCl than of cimetidine to achieve clinically the same degree of antiandrogenic side effects.

Evidence for the antiandrogenic activity of cimetidine has been reported previously both clinically (1–3) and experimentally (4–6). Although cimetidine also may cause increases in prolactin levels (13–15), the feminizing effects observed in men are believed to be due primarily to the ability of cimetidine to displace endogenous androgen from target cell receptors (5, 16). Because of the weak affinity of cimetidine for androgen receptors, however, the antiandrogenic effects are not ubiquitous and require high doses for their presence (2, 16, 17). Etintidine–HCl has been shown in laboratory experiments to be more potent than cimetidine in blocking histamine H2 receptors and in antagonizing gastric acid secretion in rats and dogs (7). The less potent antiandrogenic effects of etintidine–HCl indicate that the two activities, i.e., H2-receptor and androgen receptor affinities, are separate and unrelated. Thus, the greater H2-receptor potency of etintidine–HCl, together with the lower antiandrogenic effects, provide the compound with a wider therapeutic ratio suggesting that its use clinically will result in fewer antiandrogenic side effects.

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