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Comparative Antiandrogenic Potency of Spironolactone and Cimetidine: Assessment by the Chicken Cockscomb Topical Bioassay[1] (42363)

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Abstract. Spironolactone and cimetidine are effective antiandrogens *in vivo,* although they differ by five orders of magnitude in affinity for androgen receptors *in vitro.* To explore this discrepancy, we directly compared the antiandrogenic potency of these two compounds *in vivo* using the chicken cockscomb topical bioassay. In this assay, the growth of the androgen sensitive cockscomb of immature chicks after stimulation by various doses of androgen (dihydrotestosterone 5, 20, or 100 μg/day sc) is inhibited by antiandrogens in cream vehicle applied topically to the cockscomb itself. At low levels of androgen stimulation (5 μg/day), 0.5% topical cimetidine produced maximal suppression of cockscomb growth, while at high levels of androgen stimulation (100 μg/day), topical cimetidine in concentrations as high as 4% did not suppress cockscomb growth. In contrast, topical spironolactone in concentrations as low as 0.06% produced maximal inhibition of cockscomb growth at all androgen doses. Using an intermediate androgen dose (20 μg/day), the minimally effective antiandrogenic concentration of topical cimetidine was between 0.5 and 1.0%, while that for topical spironolactone was less than 0.001%. We conclude that the chicken cockscomb topical bioassay is a useful method for assessing relative potency of antiandrogens. With this method, spironolactone appears to be at least 500 times as strong an antiandrogen *in vivo* as cimetidine.

In addition to their primary therapeutic actions, the diuretic, spironolactone (1, 2), and the antihistamine, cimetidine (3–6) act as androgen antagonists. In men treated with these drugs, this antiandrogenic action is an unwanted side effect, resulting in gynecomastia and impotence (7, 8). By contrast, in women this antiandrogenic effect has been usefully employed in the treatment of conditions associated with androgen excess, such as hirsutism (9, 10). In fact, cimetidine and spironolactone are currently the only commercially available drugs in the United States which have antiandrogenic actions. Both drugs block the interaction between androgens and the cellular androgen receptor, but spironolactone appears to be about five orders of magnitude more effective than cimetidine in blocking androgen receptors *in vitro* (11). By contrast, animal studies *in vivo* have indicated that both drugs have significant, but submaximal, antiandrogenic effects in rats at similar dose ranges (30–50 mg/kg/day) (2, 6, 12). Such *in vivo* bioassays for antiandrogen are based on the inhibition of growth of androgen responsive organs, usually the prostate or seminal vesicles, by the test compound. Another androgen-sensitive target tissue whose growth reflects androgen action is the chicken cockscomb. Since this organ is external, potential antiandrogens can be applied directly, and the chicken cockscomb topical bioassay has been used in the past to assess potential antiandrogens (13–17). Since previous studies of the *in vivo* antiandrogenic effects of cimetidine and spironolactone have used only a small number of antiandrogen doses and have not directly compared the two drugs, we carried out a detailed comparison of the antiandrogenic potencies of cimetidine and spironolactone using the chicken cockscomb topical bioassay.

[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

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Materials and Methods. Day-old Dekalb XL cockerels (Bowman's Hatchery, Westminster, Md.) were housed in brooders in separate groups of 17 at 95°F and given free access to water and food (Startina; Ralston Purina, St. Louis, Mo.). To provide androgenic stimulus for cockscomb growth, animals were given daily subcutaneous injections of dihydrotestosterone (5, 20, or 100 μg in corn oil) beginning at age 1 day. Also beginning at the same age, 0.1 cc topical antiandrogen (spironolactone or cimetidine) in cream base, or cream base alone, was applied twice daily to the cockscomb with a syringe and spread by finger. Cimetidine (Tagamet for injection; Smith, Kline and French, Philadelphia, Pa.) was combined with cream base in concentrations from 0.5 to 4.0% (weight/weight), while spironolactone powder (obtained from Searle and Co., San Juan) was compounded in the same vehicle in concentrations from 0.001 to 4.0% (weight/weight). At age 15 days, animals were sacrificed by asphyxiation with dry ice and the cockscombs removed using scissors run along the skull. Cockscomb and body weights were measured, and the relative cockscomb weight (cockscomb weight/100 g body wt) was used as an index of androgenic effect. For easy graphic comparison of different groups, the mean relative cockscomb weight in the absence of any antiandrogen was defined as 100% for each androgen/antiandrogen series (raw data are presented in Table I). Statistical analysis was done by ANOVA with Scheffe's test to determine doses producing significant or maximal suppression of cockscomb weight. Maximal suppression was defined by the lowest antiandrogen dose for which the ANOVA indicated no significant difference from all other higher doses. Students t test was used for the two-group comparison involving 0.001% spironolactone. Each combination of androgen and antiandrogen doses was tested on 14–18 animals.

Results. Initially, the antiandrogenic potency of topical spironolactone and cimetidine in concentrations of 0.5–4.0% was examined (Fig. 1). For all three doses of dihydrotestosterone examined, 0.5% spironolactone produced significant and maximal reduction in cockscomb weight ($P < 0.05$ by ANOVA). By contrast, the effect of topically applied cime-

TABLE I. EFFECT OF ANTIANDROGENS ON COCKSCOMB WEIGHT

Concentration (%)	DHT dose (μg/day)		
	5	20	100
Cimetidine			
0 (cream alone, no DHT)	130 ± 11	124 ± 11	106 ± 10
0 (with DHT)	182 ± 15	306 ± 23	564 ± 28
0.5	108 ± 10	346 ± 28	606 ± 36
1.0	100 ± 5	206 ± 15	615 ± 37
2.0	127 ± 9	214 ± 15	660 ± 43
4.0	133 ± 11	257 ± 22	637 ± 39
Spironolactone (Study 1—see Fig. 1)			
0 (cream alone, no DHT)	65 ± 6	100 ± 8	96 ± 7
0 (with DHT)	309 ± 19	264 ± 14	664 ± 44
0.5	184 ± 19	160 ± 12	380 ± 29
1.0	161 ± 16	122 ± 8	385 ± 41
2.0	199 ± 10	145 ± 7	359 ± 24
4.0	153 ± 16	156 ± 11	274 ± 16
Spironolactone (Study 2—see Fig. 2)			
0 (cream alone, no DHT)	78 ± 5	158 ± 18	78 ± 6
0 (with DHT)	213 ± 12	339 ± 23	712 ± 52
0.06	96 ± 6	173 ± 9	516 ± 30
0.12	102 ± 4	146 ± 8	389 ± 33
0.25	100 ± 5	195 ± 13	391 ± 24
0.50	132 ± 10	128 ± 8	410 ± 32

Note. All values are mg/100 g body weight (mean ± SEM; n = 14–18).

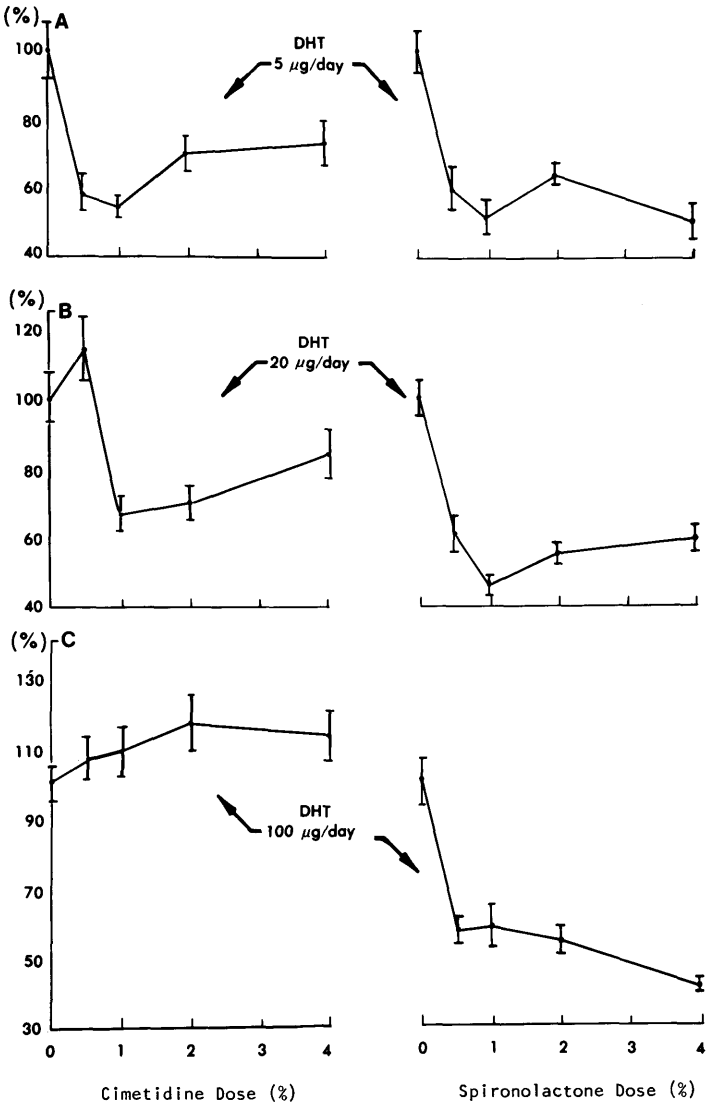

FIG. 1. Effect of different concentrations of topically applied cimetidine (left) and spironolactone (right) on chicken cockscomb weight stimulated by three different doses of dihydrotestosterone (A, 5 μg/day; B, 20 μg/day; C, 100 μg/day). Error bars indicate means ± SEM. Cockscomb weights are corrected for body weight, and for each androgen dose the mean cockscomb weight in the absence of antiandrogen is defined as 100%.

tidine in this dose range depended on the strength of the androgenic stimulus (Fig. 1). At the lowest dose of androgen (5 μg/day dihydrotestosterone), the pattern of the dose-response curve for cimetidine was similar to that of spironolactone; namely, 0.5% concentration produced significant and maximal reduction in cockscomb weight. By contrast, at the highest dose of androgen (100 μg/day dihydrotestosterone) no concentration of cimetidine, up to 4%, was able to reduce cockscomb weight. At the intermediate dose of androgen (20 μg/day dihydrotestosterone), the lowest concentration of cimetidine (0.5%) did not reduce cockscomb weight, but 1.0% cimetidine did, thus defining the range for the minimally effective antiandrogen dose at this level of androgen stimulation.

To define this minimally effective dose for spironolactone, studies were carried out using lower concentration ranges of topical spironolactone (0.06–0.50%; (Fig. 2). Here, too, the lowest dose of spironolactone (0.06%) produced reduction in cockscomb weight that was significant and maximal ($P < 0.05$ by ANOVA) at all three androgen doses used (Fig. 2), indicating that the minimal effective antiandrogenic dose was below this range. In conjunction with a 20 μg/day dihydrotestosterone dose, even a concentration of topical spironolactone as low as 0.001% (tested in a separate study) produced significant reduction in cockscomb weight (170 ± 13 vs 213 ± 13 mg/100 g body wt; $P < 0.05$). Doses lower than 0.001% could not be explored due to the difficulty in compounding the ointment.

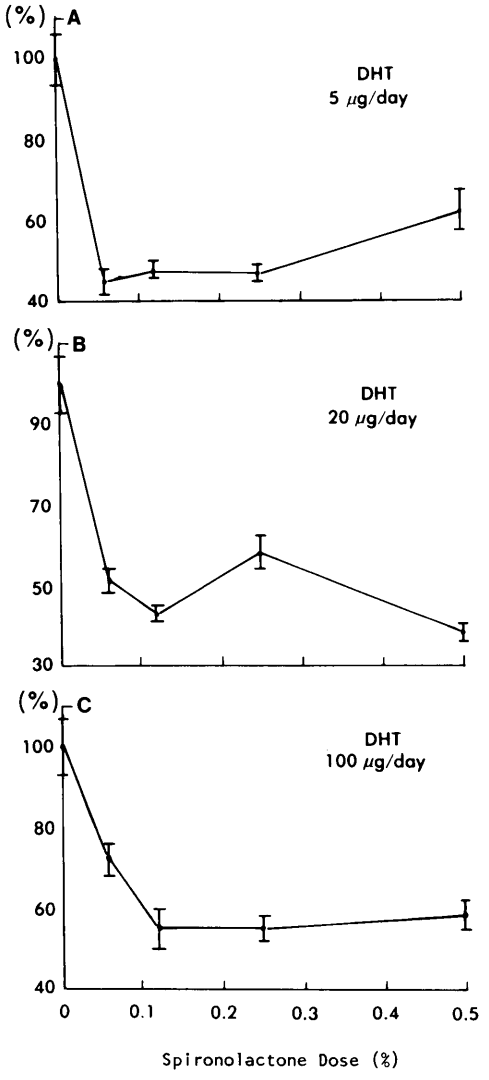

FIG. 2. Effect of low concentrations of topically applied spironolactone on chicken cockscomb weight. See legend to Fig. 1.

Discussion. Our study, using the chicken cockscomb topical bioassay, confirms that spironolactone appears to be a much more potent antiandrogen than cimetidine. Using the same androgenic stimulus (dihydrotestosterone 20 μg per day), the minimally effective dose of topical cimetidine (i.e., the lowest concentration producing significant reduction in cockscomb weight) was between 0.5 and 1.0%, while the minimally effective dose of spironolactone appears to be less than 0.001%. Difficulties in compounding the topical preparation with lower concentrations of spironolactone made more detailed descriptions impractical. By using various dose combinations of antiandrogen and androgen one can get an excellent picture of the antiandrogenic action of a given drug.

There are other potential advantages of the chicken cockscomb topical bioassay for testing androgen antagonists. The assay is very simple, requiring very minimal technical skill, and the animals are very inexpensive, enabling study of large numbers of animals in various groups. Husbandry apparatus for chicks are widely available, as are the animals themselves. An additional theoretical advantage relates to the local absorption and action of the topically applied compounds. Previous studies have suggested that antiandrogens applied to the cockscomb are more effective than those given systemically (14), suggesting that topical application results in local action rather than absorption into the bloodstream prior to action. Therefore, the chicken cockscomb topical bioassay offers the opportunity to assess antiandrogenic activity *in vivo* while minimizing the effects of systemic metabolism of the administered drugs. The extensive *in vivo* metabolism of spironolactone administered systemically (18) may account for the difference, compared to cimetidine, between its potency in inhibiting androgen receptors *in vitro* and its antiandrogenic activity *in vivo*. Thus, the chicken cockscomb topical bioassay complements the standard *in vitro* and *in vivo* assays for antiandrogenic effect.

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