Topical Canrenoic Acid
Quantification of the Antiandrogenic Activity in the Hamster Flank Organ

Giuseppe Noto, M.D., Gabriella Pravatá, M.D., Maria R. Bongiorno, M.D., Maria Bosco, M.S., and Mario Aricó, M.D.

Abstract: The topical antiandrogenic activity of potassium canrenoate (CAK), compared with that of spironolactone (SP), was assayed in vivo in female golden Syrian hamsters whose flank organs were stimulated by subcutaneous administration of testosterone propionate. Sebaceous glands and hair were measured by a computerized image analyzer. Pigmented spots, sebaceous gland areas, and the diameter of hairs of the treated flank organs were smaller in the groups that received CAK (1.6 mg/day) and SP (0.4 mg/day). The authors' results showed that CAK may act as a topical antiandrogen on the hamster flank organ when applied in concentrations four times greater than the minimal active dosage of SP. Potassium canrenoate may be a useful weak topical antiandrogen, and it could be used in androgen-related skin disorders involving both sebaceous glands and hair, especially in men. These concentrations could be verified by additional clinical investigations.

Canrenone (CA) is the main circulating metabolite of spironolactone (SP), and SP and potassium canrenoate (CAK) act together as antagonists of aldosterone. Systemic SP caused antiandrogenic side effects in men, and it was used in women as a systemic antiandrogen in cases of acne and hirsutism.[1,2] Actually, SP could be an effective topical antiandrogen, because it has been assayed for local application in the hamster flank organ[3] and used, with good clinical results, in patients with androgen-related disorders.[4]

The flank organ was used to evaluate the antiandrogenic properties of many compounds.[3,5–8] To our knowledge, the activity of topically applied CAK in the hamster has not been quantified, and thus we evaluated the local antiandrogenic activity of CAK in the three structures of the flank organ that can be modified independently by androgens and antiandrogens (ie, the pigmented spot, sebaceous glands, and hair).

Materials and Methods

- Twenty-five female golden Syrian hamsters, which were 13–15 weeks old and weighed 80–110 g, were entered in this study. They had been kept at a constant temperature and humidity and fed with standard commercially available food and water. The costovertebral region was shaved with an electric clipper and the horny layer was partially stripped the day before the study was started, and then every 3 days.

The animals were divided into five groups, according to weight. Every day for 3 weeks (except weekends), all animals, excluding those in group 1, received a subcutaneous dose of testosterone propionate (TP, 250 μg in 25 μl peanut oil) in the dorsal neck fold. On the same days, we applied CAK and SP in water and absolute ethanol (1:4) to the left flank organ, using a micropipette under a continuous air flow to enhance the vehicle evaporation; this was applied as follows: group 1: untreated; group 2: CAK 1.6 mg; group 3: CAK 0.8 mg; group 4: SP 0.4 mg; and group 5: vehicle. It was not applied topically to the right flank organs.

The first day of the fourth week, the two major perpendicular axes of the pigmented spot overlying each flank organ were measured; when these two values were multiplied, we obtained the surface area index (SAI). After the animals were totally anesthetized with diethyl ether, all the flank organs were excised. The organs were divided into two halves along the major axis, immediately fixed in 10% formalin, and paraffin-embedded in parallel. The 5-μm-thick sections were stained with hematoxylin and eosin. Sebaceous glands and hairs were measured in the first three sections of each half of the specimen, with the use of a semiautomatic computerized image analyzer (Vids V), using a video camera (Ikegami ITC-510), which provided direct imaging on a color monitor of samples observed with an optic microscope

From the Department of Dermatology, University of Palermo, Palermo, Italy.

Address correspondence to: Mario Aricó, M.D., Sezione di Dermatologia Sperimentale, Universitá di Palermo, Policlinico "P. Giaccone," via del Vespro 129, I-90127 Palermo, Italy.

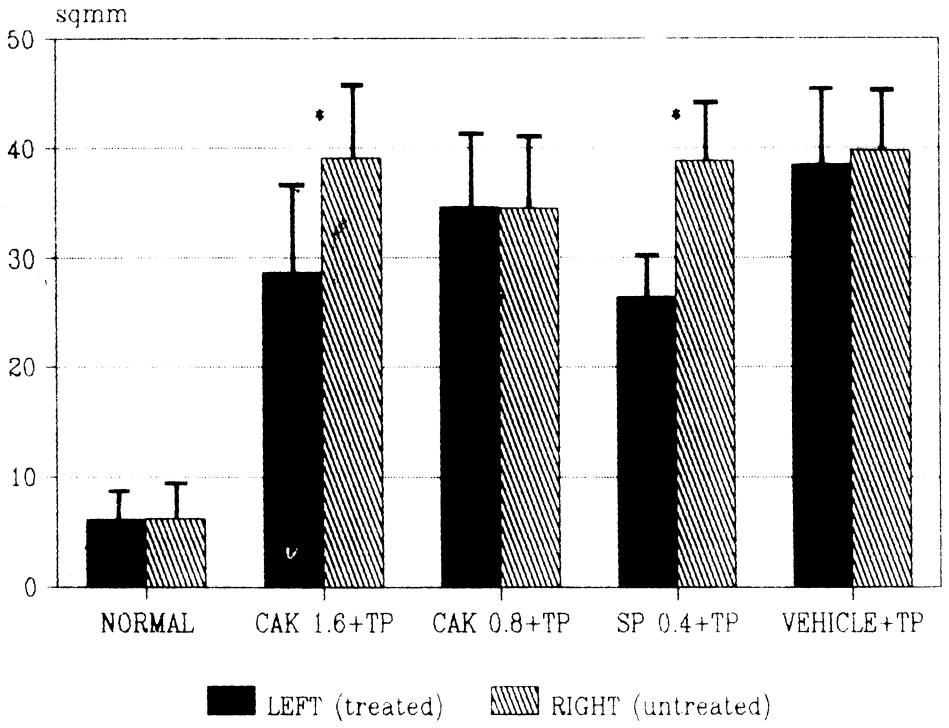

Figure 1. Surface area index of the pigmented spot ($\bar{x} \pm sd$). CAK: Potassium canrenoate (1.6 and 0.8 mg/d); SP: spironolactone (0.4 mg/d); TP: testosterone propionate (250 μg/d). *$p < 0.05$ (left versus right).

(Leitz 20 Dialux); a digitalizing tablet; and an IBM PS/2 personal computer (mod. 50 with 640 KB of base memory, 1 MB of extended memory, and an interface card). The sebaceous gland area in each field was quantified in square millimeters; the field area was 3.1 mm^2. The diameter of the hairs under each flank organ was measured in microns. Statistical analysis was performed with Student's t-test.

Results

Values for the pigmented spot are reported in Figure 1. In group 2, the SAI was decreased in the left (treated) flank organs (CAK 1.6 mg/day), as compared with the contralateral right (untreated) organs ($p < 0.05$). Group 3 (CAK 0.8 mg/day) did not show any difference. Group 4 (SP 0.4 mg/day) demonstrated a significant difference between the left and right flank organs ($p < 0.05$). The values for group 5 (vehicle) did not show any difference, and SAI levels were higher than those of group 1 ($p < 0.01$) in the left and right flank organs.

The average sebaceous gland area (Fig. 2) was decreased in the left flank organs in groups 2 and 4 when compared with that of the contralateral right flank organs ($p < 0.05$). The average values of group 5 were increased ($p < 0.01$) when compared with those of group 1.

Hair diameter (Fig. 3) values were lower in the left flank organs ($p < 0.05$) in groups 2 and 4 and higher in the left flank organs in group 5 when compared with those in group 1 ($p < 0.01$).

Discussion

Topical antiandrogenic action can occur by blocking the enzyme 5-alpha-reductase (metabolic antiandrogens, such as progesterone and 4-androstene-17-beta-carboxylic acid) or by competitive inhibition of the androgen receptor (receptor antiandrogens, such as cyproterone acetate and SP).

Approximately 72% of renal antimineralocorticoid effects of SP can be attributed to CA, which has a half-life of 17 hours; the potency of CAK is approximately 0.68 that of SP, and it has been assumed that the activity of CAK results entirely from CA.[9] The antiandrogenic activity of SP was correlated to its inhibition of cytochrome-P-450–dependent enzymes needed for testosterone biosynthesis.[10] Furthermore, the competitive affinity of SP for the androgen receptor was demonstrated as being one-tenth that of DHT[11]; this action was quantified subsequently as being 20 times less effective than that of DHT, without any inhibition of 5-alpha-reductase.[12]

Canrenone can act competitively with mitochondrial and microsomal cytochrome P-450.[13] In addition, it selectively interacts with the subtypes of cytochrome P-450 that are involved in the steroid hydroxylation reactions, but parenteral administration of CA, unlike SP, does not decrease adrenal content of cytochrome

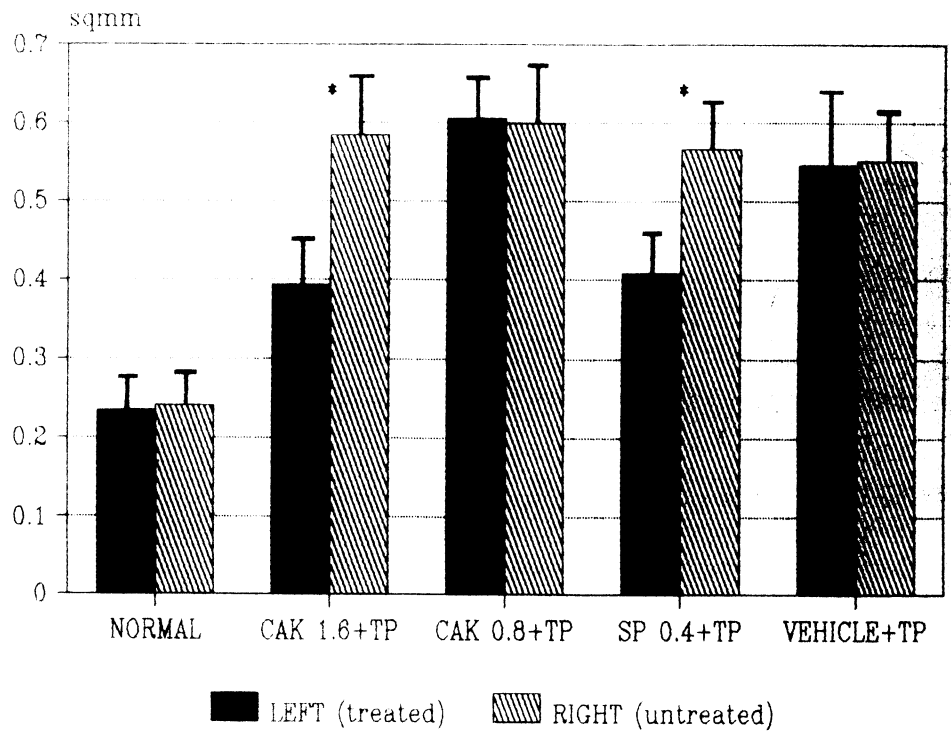

Figure 2. Sebaceous gland area (x ± sd). CAK: Potassium canrenoate (1.6 and 0.8 mg/d); SP: spironolactone (0.4 mg/d); TP: testosterone propionate (250 μg/d). *$p < 0.05$ (left versus right).

P-450.[13] Potassium canrenoate affinity for the androgen receptor is approximately 100 times lower than that of DHT, without any inhibition of 5-alpha reductase.[12]

Our results showed that CAK may act as a topical antiandrogen on the hamster flank organ when applied in a concentration four times greater than that of SP, which has been used next to the minimal topical dose to obtain an antiandrogenic action in hamsters.[3] On the contrary, CAK did not produce an antiandrogenic effect when applied in a concentration that was two times greater than that of SP. The antiandrogenic ac-

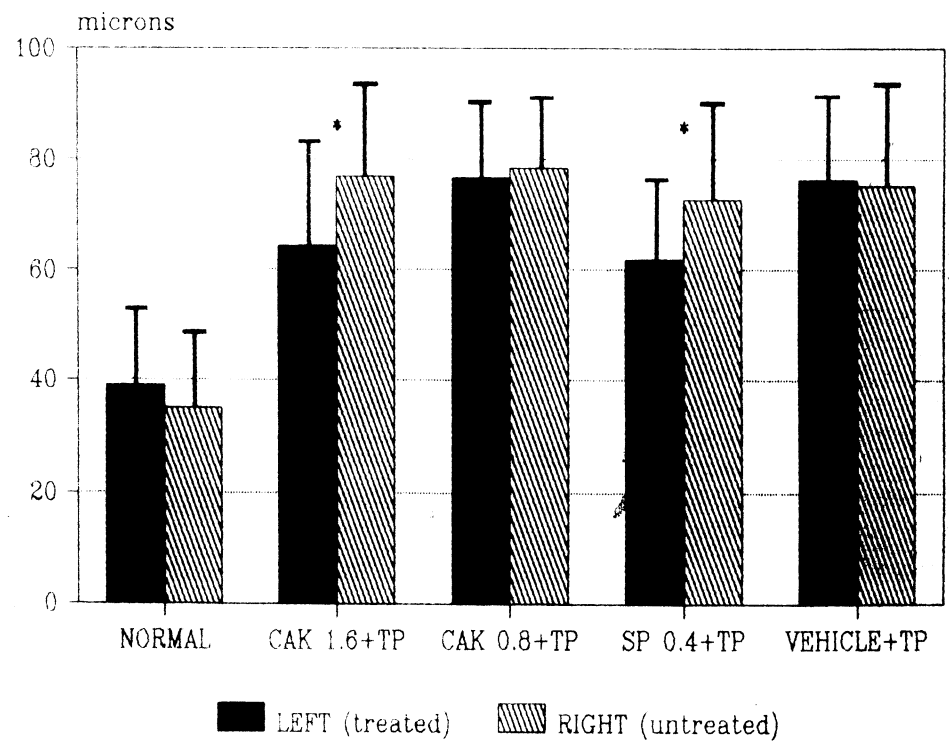

Figure 3. Diameter of the hair under the flank organ (x ± sd). CAK: Potassium canrenoate (1.6 and 0.8 mg/d); SP: spironolactone (0.4 mg/d); TP: testosterone propionate (250 μg/d). *$p < 0.05$ (left versus right).

tion affects both sebaceous glands and hair; the suppression of sebaceous growth, compared with that of hair, appeared to be more evident.

The topical antiandrogenic activity of CAK in humans has been studied in cases of mild hirsutism[14] and acne vulgaris,[15] with discordant results. In our opinion, CAK can be a useful weak topical antiandrogen and it could be used in androgen-related skin disorders, which also may affect sebaceous gland activity, especially in men; however, different concentrations may be used. These indications could be verified by additional clinical investigations.

Acknowledgment

Casimiro Caruso provided technical assistance.

References

1. Messina M, Manieri C, Bilfignandi P, et al. Antiandrogenic properties of spironolactone: Clinical trial in the management of female hirsutism. J Endocrinol Invest. 1983;6:23–27.
2. Goodfellow A, Alaghband-Zadeh J, Carter G, et al. Oral spironolactone improves acne vulgaris and reduces sebum excretion. Br J Dermatol. 1984;111:209–214.
3. Weissman A, Bowden J, Frank BL, et al. Antiandrogenic effects of topically applied spironolactone on the hamster flank organ. Arch Dermatol. 1985;121:57–62.
4. Messina M, Manieri C, Rizzi G, et al. A new therapeutical approach to acne: An antiandrogen percutaneous treatment with spironolactone. Curr Ther Res. 1983;24:319–322.
5. Gomez EC, Frost P. Hamster flank organ: Relevance of studies with topically applied antiandrogens. In: Maibach H, ed. Animal Models in Dermatology. Edinburgh: Churchill Livingstone, 1975:190.
6. Kaszinsky E. The stimulation of hair growth in the flank organs of female hamsters by subcutaneous testosterone propionate and its inhibition by topical cyproterone acetate: Dose-response studies. Br J Dermatol. 1983;109:565–569.
7. Orentreich N, Matias JR, Malloy V. The local antiandrogenic effect of the intracutaneous injection of progesterone in the flank organ of sexually mature male Syrian Golden hamsters. Arch Dermatol Res. 1984;276:401–405.
8. Nuck BA, Lucky AW. Epitestosterone: A potential new antiandrogen. J Invest Dermatol. 1987;89:209–211.
9. Ramsay L, Asbury M, Shelton J, et al. Spironolactone and canrenoate-K: Relative potency at steady state. Clin Pharmacol Ther. 1977;21:602–609.
10. Menard RH, Martin HF, Stripp B, et al. Spironolactone and cytochrome P-450: Impairment of steroid hydroxylation in the adrenal cortex. Life Sci. 1975;15:1639–1648.
11. Pita JC, Lippman ME, Thompson EB, et al. Interaction of spironolactone and digitalis with the 5-alpha-dihydrotestosterone (DHT) receptor of rat ventral prostate. Endocrinology. 1975;97:1521–1527.
12. Corvol P, Michaud A, Menard J, et al. Antiandrogenic effects of spironolactones: Mechanism of action. Endocrinology. 1975;97:52–58.
13. Greiner JW, Rumbaugh RC, Kramer RE, et al. Relation of canrenone to the actions of spironolactone on adrenal cytochrome P-450-dependent enzymes. Endocrinology. 1978; 103:1313–1320.
14. Gamborg-Nielsen P. Treatment of moderate idiopathic hirsutism with a cream containing canrenone (an antiandrogen). Dermatologica. 1982;165:636–639.
15. Shernaz W, Cunliffe WJ, Lookingbill D, et al. Lack of effect of topical spironolactone on sebum excretion. Br J Dermatol. 1986;114:261–269.

• • • • • • • • • • •

Mammary and Extramammary Paget's Disease (Study of 140 Cases)

106 cases of mammary Paget's disease (MPD) and 34 cases of extramammary Paget's disease (EMPD) corresponding to almost 30 years of personal experience are presented.

MPD appears in women aged between 50 and 60. The lesions are usually unilateral and appear as an eczema-like plaques located on the nipple and areola. The borders are sharply limited.

EMPD is an uncommon condition of middle-aged and old people, appearing on the anogenital area. The lesions are erythematous eczema-like plaques with margins less defined than in MPD. They may frequently begin as multiple foci (multicentric origin). It is habitual to distinguish a "primary" form, which is not associated with any other neoplasm, and a "secondary" one, which is the result of extension of a sweat gland, urogenital or digestive cancer to the skin.

The histopathology shows the so-called "Paget cells", large cells with clear and abundant cytoplasm. Histochemical stains show different results in MPD and EMPD. Carcinoembryonic antigen, GCDFP-15 and monoclonal antibodies to cytokeratins are good markers of neoplastic cells of the disease.—*Mascaro JM, Zemba MC. Mammary and Extramammary Paget's disease. (Study of 140 cases) Med Cut I I A 1990;18:301–313 (Spanish). Submitted by Yehudi M. Feiman, M.D., Brooklyn, N.Y.*