Spironolactone Directly Inhibits Proliferation of Cultured Human Facial Sebocytes and Acts Antagonistically to Testosterone and 5α-Dihydrotestosterone In Vitro

Hirohiko Akamatsu, Christos C. Zouboulis, and Constantin E. Orfanos
Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Berlin, Germany

Spironolactone produces antiacne effects and has recently been shown to inhibit 5α-dihydrotestosterone (5α-DHT) receptors in human sebaceous glands. We applied spironolactone alone and combined with testosterone and 5α-DHT to investigate its effects on the proliferation of human sebocyte cultures derived from facial skin. Secondary human facial sebocytes in 96-well culture plates were treated for 10 d by a single or combined application of testosterone (10−8−10−5 M), 5α-DHT (10−8−10−5 M), and spironolactone (10−12−10−7 M) in serum-free basal medium. Cell proliferation was assessed in six wells using a fluorometric assay. Testosterone and 5α-DHT significantly stimulated sebocyte proliferation in a dose-dependent manner, the effect being strongest with 5α-DHT. Spironolactone, on the other hand, caused a dose-dependent inhibition (25% and 50% at 10−9 and 10−7 M, respectively). Combined treatment of human facial sebocytes with spironolactone and testosterone or 5α-DHT resulted in a lower proliferation than with androgens alone. The fact that spironolactone directly and dose dependently inhibits the proliferation of cultured human facial sebocytes and acts antagonistically to testosterone and 5α-DHT at the cellular level is indicative of a receptor-mediated effect. J Invest Dermatol 100:660–662, 1993

Spironolactone is an aldosterone antagonist that has been clinically used as an antihypertensive and diuretic drug. The fact that gynecomastia is a common side effect of this compound reflects its antiandrogenic property [1,2]. Spironolactone has also been successfully used for treatment of acne [3,4], hirsutism [5], and androgenic alopecia [6]. The following mechanisms have been thought to explain its action: 1) interference with steroid synthesis by blockage of cytochrome P450 enzymes in the testes and adrenal glands [7,8]; 2) reduction of 5α-reductase activity [9]; and 3) peripheral action by a competitive decrease in 5α-dihydrotestosterone (5α-DHT) activity at the receptor level, as has been demonstrated both in experimental animals and in humans [10,11].

Our aim was to better elucidate the effects of spironolactone on the sebaceous glands at the cellular level by investigating its direct influence on the proliferation of cultured human facial sebocytes in vitro as well as its possible modification of testosterone and 5α-DHT activity in this connection.

MATERIALS AND METHODS

Human Sebocyte Cultures Human sebaceous glands were isolated from facial skin and seeded on monolayer 3T3 cells, as previously described [12]. Primary sebocyte cultures were derived from the periphery of the gland lobules and were maintained to confluence before subcultivation. All experiments were performed using secondary sebocyte cultures, which have been demonstrated to consist of cells undergoing sebocytic differentiation [13,14].

Treatment with Testosterone, 5α-DHT, and Spironolactone

Human sebocytes were seeded in 96-well culture plates (Falcon, Jersey, NJ) at a concentration of 104 cells/well and were left to attach for 2 d at 37°C with 5% CO2 in culture medium consisting of Dulbecco’s modified Eagle’s medium and Ham’s F12 medium (1:1) (Gibco, Berlin, Germany) supplemented with 8% fetal calf serum (Seromed, Berlin, Germany), 2% human serum, 10 ng/ml epidermal growth factor (Sigma, Deisenhofen, Germany), 10−9 M choleratoxin (Calbiochem, Frankfurt, Germany), 3.4 mM l-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all from Seromed). The medium was then aspirated, and serum-free keratinocyte basal medium (KBK) (Clonetics, San Diego, CA) without additives supplemented with testosterone (10−8−10−5 M) (Sigma), 5α-DHT (10−8−10−5 M) (Sigma), or spironolactone (10−12−10−7 M) (Searle Yakuhin, Osaka, Japan) or their combination was added to six wells at each concentration. The compounds were added to the medium as a 0.2% dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) solution. KBM with 0.2% DMSO was concomitantly added to another six wells serving as controls. The plates were incubated at 37°C for 10 d before evaluation. KBM with and without compounds was changed every 2 d.

Cell Proliferation

Cell numbers of untreated human sebocytes in 96-well culture plates were assessed over 9 d by counting single-cell suspensions in Neubauer chambers and were compared with the absolute fluorescence units (AFU) of parallel wells obtained using the 4-methylumbelliferone heptanoate (MUH)–fluorescence assay [15,16]. This assay is based on the hydrolysis of the fluorogenic...
substrate MUH by esterases of proliferating cells. Briefly, a stock solution of 10 mg/ml MUH (Serva, Heidelberg, Germany) was prepared in DMSO and kept frozen at −20°C until use. On the day of assessment, KBM was removed, and the cells were washed twice with phosphate-buffered saline without Ca++ and Mg++ (pH 7.2) (Serva). The MUH stock solution was diluted in phosphate-buffered saline up to 100 µg/ml, and 100 µl of the final solution was added to each well. The plates were then incubated for 30 min at 37°C and read automatically on a Titertek Fluoroscan II (Flow, Meckenheim, Germany). The MUH-fluorescence assay was used to assess the effects of androgens and spironolactone on sebocyte proliferation. The results are given as AFU using 355-nm excitation and 460-nm emission filters.

Statistical Evaluation Each value represents the mean of six wells ± 1 SD. Statistical significance was assessed by Student t test. Mean differences were considered significant at p < 0.05.

RESULTS

Sebocyte proliferation was highly correlated with the results obtained using the MUH-fluorescence assay (Fig 1). Spironolactone inhibited the proliferation of cultured human sebocytes derived from sebaceous glands of the facial skin in a significant and dose-dependent manner. The inhibitory effect was 25% at 10⁻⁹ M and 50% at 10⁻⁷ M (10⁻⁹, 10⁻⁸, and 10⁻⁷ M; p < 0.01) (Fig 2).

Testosterone stimulated the proliferation of cultured human facial sebocytes, the effect being 25% at 10⁻⁷ to 10⁻⁶ M and 50% at 10⁻⁵ M (10⁻⁸ and 10⁻⁷ M; p < 0.01) (Fig 2a). 5α-DHT markedly enhanced the proliferation of cultured human facial sebocytes in a dose-dependent manner: 25% at 10⁻⁹ M and 50% at 10⁻⁷ M (for all concentrations tested p < 0.01) (Fig 3b). Spironolactone significantly inhibited the stimulatory effect of testosterone on the proliferation of cultured human facial sebocytes in vitro. Inhibitory effects were 3–5% at 10⁻³ M, 14–22% at 10⁻⁸ M, and 22–36% at 10⁻⁷ M of spironolactone when added together with different testosterone concentrations to human facial sebocyte cultures (spironolactone 10⁻⁸ M plus testosterone 10⁻⁸ M, p < 0.05; spironolactone 10⁻⁷ M plus testosterone 10⁻⁷, 10⁻⁶, and 10⁻⁵ M, p < 0.05; spironolactone 10⁻⁷ M plus testosterone 10⁻⁸ M, p < 0.01) (Fig 3a).

In a similar way, the stimulatory effect of 5α-DHT on the proliferation of cultured human facial sebocytes was dose dependently reduced by spironolactone. Inhibitory effects were 14–20% at 10⁻⁹ M, 19–30% at 10⁻⁸ M, and 22–45% at 10⁻⁷ M of spironolactone when added together with different 5α-DHT concentrations to the proliferating cells (spironolactone 10⁻⁸ M plus 5α-DHT 10⁻⁸ M, p < 0.05; spironolactone 10⁻⁸ M plus all 5α-DHT concentrations tested, p < 0.05; spironolactone 10⁻⁸ M plus 5α-DHT 10⁻⁸ M, p < 0.05; spironolactone 10⁻⁷ M plus 5α-DHT 10⁻⁷, 10⁻⁶, and 10⁻⁵ M, p < 0.01) (Fig 3b).

DISCUSSION

Androgens cause hyperactivity of sebaceous glands, with increased sebum secretion [17]. The resulting seborrhea promotes the formation of acne lesions [18]. The major circulating androgen, testosterone, is intracellularly converted in the skin to 5α-DHT by the enzyme 5α-reductase [19,20]. The androgenic effect of 5α-DHT is probably mediated by its binding to androgen receptors. Androgen receptors have been demonstrated in human sebaceous glands [21,22]. Therefore, hypersecretion of androgens and increased testosterone metabolism to 5α-DHT are closely correlated to the pathogenesis of acne [23–27].

In this study, the proliferation of cultured human facial sebocytes was shown to be significantly stimulated by testosterone and 5α-DHT but markedly inhibited in a dose-dependent manner by spironolactone and testosterone or 5α-DHT simultaneously administered. These observations indicate that spironolactone antagonizes testosterone and 5α-DHT activity, inhibiting their stimulatory influence on the proliferation of human facial sebocytes in vitro. Evidence suggests that such an effect may be due to inhibition of 5α-reductase reduction of testosterone to 5α-DHT [9] and partial or total blockade of 5α-DHT binding to its receptor [10,11]. In addition, increasing doses of spironolactone produced a corresponding proliferation inhibition indicative of a receptor-monitored effect. Thus, spironolactone may antagonize 5α-DHT by binding to its receptor or by modifying the structure of its receptor. It seems likely that the ligand–receptor interaction is disturbed.

An inhibitory effect of spironolactone on 5α-DHT–stimulated proliferation of human sebocytes derived from femoral skin in vitro was previously observed by our study group [28]; however, sebocytes may respond differently to androgens, depending on the location of the sebaceous glands [29]. The results of our study also confirm the inhibitory effect of spironolactone on testosterone- and 5α-DHT–stimulated proliferation of sebocytes derived from facial skin.

In conclusion, spironolactone may produce its anticanic effect by
directly inhibiting the proliferation of human sebocytes and by acting antagonistically on the stimulation of the sebaceous gland by testosterone and, above all, by 5α-DHT at the cellular level. Both effects seem to be receptor mediated.

![Figure 3](image-url)

**Figure 3.** Effect of spironolactone on the stimulatory influence of (a) testosterone and (b) 5α-DHT on the proliferation of cultured human sebocytes derived from sebaceous glands of facial skin, as assessed by the MUH-fluorescence assay. Values are mean ± SD of six wells and are presented as percent of controls. *p < 0.05, **p < 0.01, compared with testosterone- and 5α-DHT–treated controls, respectively.

### REFERENCES