Control of Human Sebocyte Proliferation In Vitro by Testosterone and 5-Alpha-Dihydrotestosterone Is Dependent on the Localization of the Sebaceous Glands

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

Androgens stimulate the activity of sebaceous glands in vivo. In this study the in vitro effect of androgens on the proliferation of cultured human sebocytes derived from facial and non-facial skin were assessed.

Human sebocytes from sebaceous glands isolated from the face and the upper and lower legs of five individuals were cultured in vitro with or without testosterone or 5-alpha-dihydrotestosterone (5α-DHT) at different concentrations ($10^{-11}-10^{-5}$ M). Cell proliferation was assessed in 96-well culture plates using a fluorometric assay.

Testosterone and 5α-DHT stimulated the proliferation of human facial sebocytes in a significant dose-dependent manner. In our system 5α-DHT exhibited the strongest effect; on the contrary, the proliferation of non-facial sebocytes was inhibited by testosterone, whereas 5α-DHT enhanced their growth. The stimulatory effect of 5α-DHT was more prominent on facial than on non-facial sebocytes.

These results provide first evidence that the effect of testosterone and 5α-DHT on the proliferation of cultured human sebocytes may depend on the localization of the sebaceous glands at different skin regions. *J Invest Dermatol 99:509–511, 1992*

The pilosebaceous unit responds to androgens in vivo in a manner corresponding to its localization at different skin regions [1,2]. In vitro studies, however, using dermal papilla cells [3], did not reproduce this androgen effect. The present study provides first evidence that testosterone and 5-alpha-dihydrotestosterone (5α-DHT) exert different influences on human sebocyte proliferation in vitro, depending on the localization of the sebaceous glands.

MATERIALS AND METHODS

Human Sebocyte Cultures Skin specimens of the face and the upper as well as the lower legs of five young patients (three male, ages 20, 22, and 23; two female, ages 22 and 23) undergoing operation were used in this study. Primary sebocyte cultures were obtained as previously described [4]. Briefly, full-thickness skin was incubated for 20 h in 2.4 U/ml dispase (Boehringer, Mannheim, Germany) at 4°C to separate epidermis from dermis. Epidermis was then maintained for 15 min in 0.02% desoxyribonuclease (Sigma, Deisenhofen, Germany) at 37°C. Intact sebaceous glands were isolated by using microsurgical instruments under microscopical observation and their ducts were removed. The isolated gland lobules were cultivated on mitomycin C (Sigma)–inactivated 3T3 cells in sebocytes' medium consisted of Dulbecco's modified Eagle's medium (DMEM) and Ham's F 12 medium (3:1; Gibco, Berlin, Germany), 8% fetal calf serum (Seromed, Berlin, Germany), 2% human serum, 10 ng/ml epidermal growth factor (Sigma), 10^{-9} M cholera toxin (Calbiochem, Frankfurt, Germany), 3.4 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all Seromed) by 37°C with 5% CO_2. Primary sebocyte cultures resulted 2–3 weeks later as outgrowths from the periphery of the gland lobules.

Treatment with Androgens Sebocytes of confluent primary cultures were trypsinized and seeded in 96-well culture plates (Falcon, Jersey, NJ) in sebocyte medium at a concentration of 10^4 cells/well. Cells were left 2 d to attach and then were supplied with serum-free keratinocyte basal medium (KBM, Clonetics, San Diego, CA) without additives. Testosterone and 5α-DHT (both from Sigma) were added at concentrations of $10^{-11}-10^{-5}$ M in dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany; 0.2% final DMSO concentration) in six wells for each androgen concentration. KBM with 0.2% DMSO without androgens was added in six wells serving as controls. The plates were incubated at 37°C and 5% CO_2 for 10 d before evaluation. Medium with and without androgens was changed every 2 d.

Cell Proliferation Cell proliferation was assessed by the 4-methylumbelliferyl heptanoate (MUH)–fluorescence assay and measured automatically, as previously described [5,6]. On the day of evaluation, medium was removed and the cells were washed twice with phosphate-buffered saline (PBS) without Ca^{++} and Mg^{++} (pH 7.2, Seromed). One hundred microliters of a 100 μg/ml MUH (Serva, Heidelberg, Germany) solution in PBS were added in each well. Plates were then incubated for 30 min at 37°C and released fluorescence was read on a Titertek Fluoroscan II (Flow, Meckenheim, Germany). The results are given as absolute fluorescence units (AFU) using 355-nm excitation and 460-nm emission filters.

Manuscript received February 19, 1992; accepted for publication May 20, 1992.

Dr. Akamatsu is a postgraduate research fellow from the Department of Dermatology, Kansai Medical University, Osaka, Japan.

Reprint requests to: Dr. Christos C. Zouboulis, Department of Dermatology, University Medical Center Steglitz, The Free University of Berlin, Hindenburgdamm 30, W-1000 Berlin 45, Germany.

Abbreviations:
AFU: absolute fluorescence units
5α-DHT: 5-alpha-dihydrotestosterone
DMEM: Dulbecco's modified Eagle's medium
DMSO: dimethyl sulfoxide
KBM: serum-free keratinocyte basal medium
MUH: 4-methylumbelliferyl heptanoate
PBS: phosphate-buffered saline

0022-202X/92/$05.00

Statistical Evaluation Each value represents the mean of the results obtained from the five different sebocyte cultures ± one standard deviation. The response of each sebocyte culture was determined by the mean value of six wells at each concentration tested. Statistical significance was assessed by the Student t test. Mean differences were considered to be significant when $p < 0.05$.

RESULTS

Testosterone stimulated the proliferation of cultured human sebocytes derived from facial sebaceous glands in a significant dose-dependent manner. A 25%-stimulatory effect was obtained at 10^{-8}–10^{-7} M and a 50%-stimulatory effect at 10^{-6}–10^{-5} M (10^{-8} M, $p < 0.05$; 10^{-7}, 10^{-6}, and 10^{-5} M, $p < 0.01$). On the contrary, the proliferation of cultured human sebocytes derived from sebaceous glands of the upper and lower legs showed a significant dose-dependent inhibition by testosterone. Twenty-five percent inhibition was assessed at 10^{-9}–10^{-8} M and 50% inhibition at 10^{-5} M (10^{-9} M, $p < 0.05$; 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M, $p < 0.01$) (Fig 1*a*).

5α-DHT enhanced the proliferation of facial human sebocytes in a significant dose-dependent manner, whereas its effect was more pronounced than the activity of testosterone. A 25% stimulation was detected at 10^{-9}–10^{-8} M and a 50% stimulation at 10^{-8}–10^{-7} M (10^{-8} M, $p < 0.05$; 10^{-7}, 10^{-6}, and 10^{-5} M, $p < 0.01$). In contrast to testosterone, the proliferation of cultured human sebocytes derived from sebaceous glands of the legs was enhanced by 5α-DHT. This stimulatory effect, however, was markedly weaker than the effect of 5α-DHT on the proliferation of facial sebocytes. Twenty-five percent enhancement of proliferation was obtained at 10^{-8}–10^{-7} M and 50% enhancement at 10^{-5} M (10^{-7} and 10^{-6} M, $p < 0.05$; 10^{-5} M, $p < 0.01$) (Fig 1*b*).

There was no significant difference in the responses to testosterone and 5α-DHT between the sebocyte cultures obtained from male and female individuals examined in this study (not shown).

DISCUSSION

Cultured human sebocytes presented a specialized response to testosterone and 5α-DHT corresponding to the localization of the sebaceous glands at different skin regions. Two patterns of response were detected. The proliferation of facial sebocytes was stimulated by both testosterone and 5α-DHT, but 5α-DHT was the most potent agent. On the contrary, the proliferation of non-facial sebocytes was inhibited by testosterone; 5α-DHT still stimulated cell proliferation, but its effect was weaker than that on facial sebocytes. No differences in the response to androgens between "male" and "female" sebocytes were found; however, the number of specimens examined was very small to evaluate this matter.

Androgens are the best-known stimulators of the sebaceous glands, enhancing both mitosis and lipogenesis of human sebocytes in vivo [1]. It is generally accepted that testosterone, the major circulating androgen, is not directly active and is converted intracellularly by 5α-reductase to 5α-DHT, which is the most potent androgen in tissue [7,8]. The activity of 5α-reductase is higher in sebaceous glands of scalp and head skin than in other skin areas [8]. This is a possible explanation for the different effects of testosterone on facial and non-facial cultured sebocytes in our study.

5α-DHT binds to androgen receptors [9], which were recently shown to be present in human sebaceous cells [10,11], and exerts its activity after this binding. 5α-DHT, although enhancing sebocyte proliferation in all experiments, was found inactive at concentrations ranging from 10^{-10} to 10^{-9} M, which have been measured extracellularly in vivo [12]. However, possible differences between "in vitro" and "in vivo" have to be taken under consideration when these two conditions are directly compared.

The different responses of facial and non-facial sebocytes to testosterone and 5α-DHT are regulated by the levels of tissue-found 5α-reductase [8], the presence of specific androgen-binding proteins, and also by varying densities of nuclear androgen receptors [10,11].

On the other hand, the inability of androgens to control the proliferation of human dermal hair papilla cells in vitro at any concentration [3], despite the existence of considerable numbers of an-

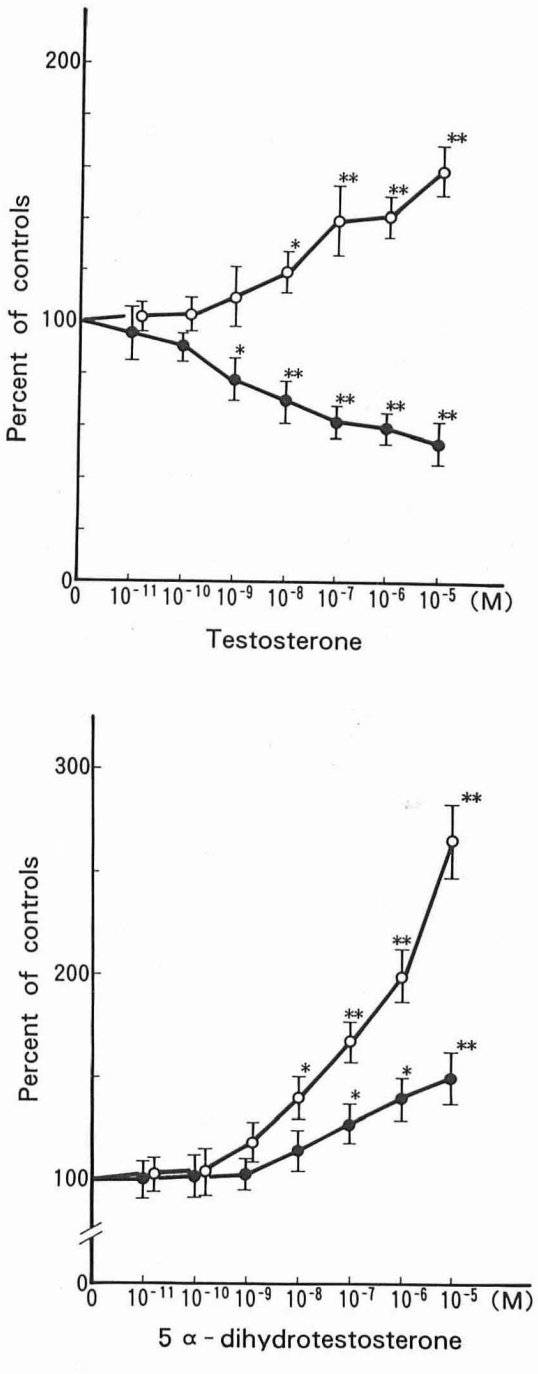

Figure 1. Effects of testosterone *(a)* and 5α-DHT *(b)* on the proliferation of cultured human sebocytes derived from facial (○) and non-facial sebaceous glands (●). The assessment was performed by the MUH-fluorescence assay. The values are means ± one standard deviation of the results obtained from five different sebocyte cultures (sixplicate wells in each culture and concentration) and are presented as percent of controls. *$p < 0.05$; **$p < 0.01$, in comparison to controls.

drogen receptors in human dermal papilla cells in culture and in tissue [13,14], disputes the role of human dermal hair papilla cells as a direct target for androgens [3]. The increased androgen-binding capacity in sebaceous glands located on the scalp of individuals with male-pattern baldness [10] may indicate a formerly postulated, still unknown, but possibly important role of sebocytic activity in this condition.

REFERENCES

1. Thody AJ, Shuster S: Control and function of sebaceous glands. Physiol Rev 69:383–416, 1989
2. Hamilton JB: Age, sex and genetic factors in the regulation of hair growth in man: a comparison of Caucasian and Japanese populations. In: Montagna W, Ellis R (eds.). The Biology of Hair Growth. Academic Press, New York, 1958, pp 399–433
3. Thornton MJ, Messenger AG, Elliott K, Randall VA: Effects of androgens on the growth of cultured human dermal papilla cells derived from beard and scalp hair follicles. J Invest Dermatol 97:345–348, 1991
4. Xia L, Zouboulis ChC, Detmar M, Mayer-da-Silva A, Stadler R, Orfanos CE: Isolation of human sebaceous glands and cultivation of sebaceous gland-derived cells as an in vitro model. J Invest Dermatol 93:315–321, 1989
5. Stadler R, Detmar M, Stephanek K, Bangemann C, Orfanos CE: A rapid fluorometric assay for the determination of keratinocyte proliferation in vitro. J Invest Dermatol 93:532–534, 1989
6. Zouboulis ChC, Garbe C, Krasagakis K, Krüger S, Orfanos CE: A fluorometric rapid microassay to identify anti-proliferative compounds for human melanoma cells in vitro. Melanoma Res 1:91–95, 1991
7. Wilson JD, Walker JD: The conversion of testosterone to 5α-androstan-17β-ol-3-one (dihydrotestosterone) by skin slices of man. J Clin Invest 48:371–379, 1969
8. Takayasu S, Wakimoto H, Itami S, Sano S: Activity of testosterone 5α-reductase in various tissues of human skin. J Invest Dermatol 74:187–191, 1980
9. Jänne OA, Bardin CW: Androgen and antiandrogen receptor binding. Annu Rev Physiol 46:107–118, 1984
10. Sawaya ME, Honig LS, Hsia SL: Increased androgen binding capacity in sebaceous glands in scalp of male-pattern baldness. J Invest Dermatol 92:91–95, 1988
11. Bläuer M, Vaalasti A, Pauli S-L, Ylikomi T, Joensuu T, Tuohimaa P: Location of androgen receptor in human skin. J Invest Dermatol 97:264–268, 1991
12. Lookingbill DP, Demers LM, Tigelaar RE, Shalita AR: Effect of isotretinoin on serum levels of precursor and peripherally derived androgens in patients with acne. Arch Dermatol 124:540–543, 1988
13. Randall VA, Thornton MJ, Elliott K, Messenger AG: Androgen receptors in cultured dermal papilla cells and dermal fibroblasts from scalp, beard and sexual skin (abstr). J Invest Dermatol 92:503, 1989
14. Choudhry R, Hodgins MB: Androgen receptors and regulation of hair growth in organ culture of human hair follicles (abstr). J Invest Dermatol 98:522, 1992