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 (5a-DHT) at different concentrations (10^{-11}-10^{-5} M). Cell proliferation was assessed in 96-well culture plates using a fluorometric assay.

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

These results provide first evidence that the effect of testosterone and 5a-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 (5a-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 operations 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% deoxyribonuclease (Sigma, Deisenhofen, Germany) at 37°C. Intact sebaceous glands were isolated by using microsurgical instruments under microscopic 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% CO₂. 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, N[J]) in sebocyte medium at a concentration of 10^6 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 5a-DHT (both from Sigma) were added at concentrations of 10^{-11}-10^{-9} 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% CO₂ 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 Titerfluoroscan II (Flow, Meckenheim, Germany). The results are given as absolute fluorescence units (AFU) using 355-nm excitation and 460-nm emission filters.

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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 1a).

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 1b).

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-
Androgens 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