In Vitro Effects of Testosterone, Dihydrotestosterone and Estradiol on Cell Growth of Human Hair Bulb Papilla Cells and Hair Root Sheath Fibroblasts

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The influence of testosterone, dihydrotestosterone (each 10 ng/ml up to 300 ng/ml) and estradiol (0.2 ng/ml up to 10 ng/ml) on the growth behaviour (cell count, [^{3}H]thymidine uptake, cell doubling time) of subcultured human hair bulb papilla cells and hair root sheath fibroblasts was studied. Papilla cells and root sheath fibroblasts were isolated by microdissection from the same anagen hair follicles obtained from biopsies of androgen-sensitive scalp regions in 6 healthy male subjects. Dihydrotestosterone and testosterone concentrations above 30 ng/ml significantly reduced the growth of both cell types; lower doses had no effect. Estradiol had no distinct influence on the growth curves of either cell type up to 10 ng/ml, whereas higher concentrations significantly increased the growth of both cell types as shown by [^{3}H]thymidine uptake. Papilla cells reacted more sensitively than root sheath fibroblast to dihydrotestosterone and testosterone, as shown by the growth curves, [^{3}H]thymidine uptake, and cell doubling time. ***Key words: Cell culture; Growth behaviour.***

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As shown in animal studies, the anagen hair bulb papilla plays a fundamental role for the induction and maintenance of hair growth (1,2). Recently, hair bulb papilla cells (PC) were characterized as a special population of fibroblasts by virtue of certain in vitro properties, e.g. morphology as well as growth pattern, chemotactic response, and collagen type synthesis (3,4,5,6). Moreover, PC as compared with root sheath fibroblasts (RSF) proliferate remarkably more slowly under subcultivation conditions. Additionally, in subcultured human PC, androgen receptors as well as high 5α-reductase activity were found and PC were assumed to be one of the targets for sex hormones influencing the hair growth (7).

The present study was therefore designed to eluci-

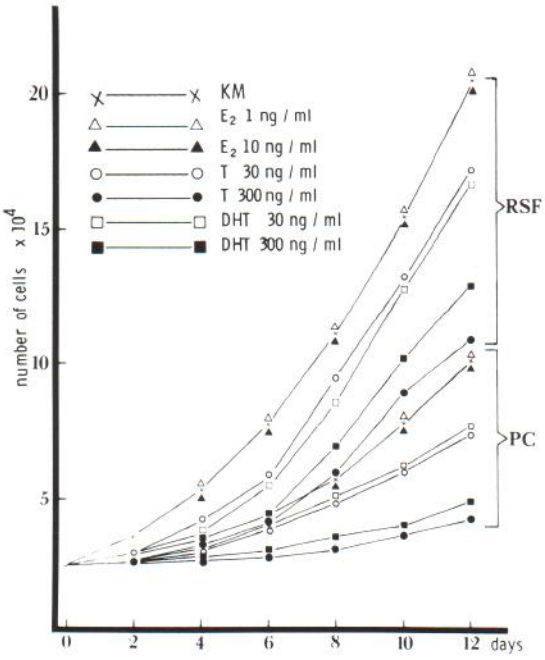

Fig. 1. Growth curves of PC and RSF subcultured with control medium and medium supplemented with testosterone, dihydrotestosterone and estradiol.

date the influences of testosterone (T), dihydrotestosterone (DHT) and estradiol (E2) in different concentrations on the growth behaviour of subcultured PC compared with RSF from identical subjects.

MATERIAL AND METHODS

Cell culture

Deep scalp biopsies were taken with informed consent from the androgen-sensitive parieto-occipital region in 6 healthy male subjects (age range 20 to 37 years) under local anaesthesia (Mepivacain®). According to techniques previously described (3, 5), anagen hair bulb papillae and root sheath fibroblasts were isolated and primary cultures of both cell types were set up. Once they had attained confluence in primary culture after about 4 weeks, they were trypsinized (trypsin 0.25% and EDTA 0.02%, Gibco, Karlsruhe, FRG) and single cells were subcultivated. All investigations were performed with cells of the third passage, subcultured in 35 mm Petri dishes in control medium (Dulbecco's modified Eagle medium DMEM with 100 U/ml penicillin, 100 ng/ml streptomycin, 0.584 ng/ml L-glutamine and 4% fetal calf serum – all provided by Gibco, Karlsruhe, FRG) supplemented with either T (Sigma, St. Louis, USA, T-1500), DHT (Steraloids, Wilton, USA) or E2 (Sigma, St. Louis, USA, E-8875) in humidified 5% CO_2 atmosphere at 37°C. The hormones tested were dissolved in pure ethanol (100 µl/100 ml medium), which was also added to the control medium as vehicle control.

Cell counts

To establish growth curves, both PC and RSF were separately seeded at a density of 2.5×10^4 cells/35 mm dish in control medium at the onset of experiments. Each concentration of T and DHT (10, 20, 30, 100, 200, 300 ng/ml) as well as E2 (0.2, 0.6, 1, 2, 6, 10 ng/ml) was tested in both cell types separately. In 6 patients the described experiments were done twice each and growth curves for PC and RSF were formed by calculating mean values. The culture media were changed every 3rd day and cell numbers of both cell types were counted every 2nd day over a period of 12 days, using a haemocytometer chamber. The cell doubling time was determined for each hormone concentration by calculation of the linear regression curve.

Table I. *Mean values and standard deviations of cell doubling times (days) obtained from papilla cells (PC) and root sheath fibroblasts (RSF) subcultured in control medium and medium supplemented with testosterone, dihydrotestosterone and estradiol*

	Control	T		DHT		E2
		30	300	30	300	10 (ng/ml)
PC	5.8±0.3	7.2±0.3	15.1±1.2	7.0±0.5	12.0±0.8	5.8±0.4
RSF	3.9±0.2	4.0±0.3	5.2±0.4	4.1±0.3	4.8±0.4	3.9±0.2

$p < 0.001$ for both cell types compared with control values (all hormone concentrations except estradiol (PC and RSF), testosterone (30 ng/ml in RSF) and dihydrotestosterone (30 ng/ml in RSF)).

Relative changes of cell doubling time (compared with control) showed significant differences ($p < 0.005$) between PC and RSF for each hormone, except estradiol.

Table II. *Mean values of stimulation indices obtained from papilla cells (PC) and root sheath fibroblasts (RSF) subcultured for 7 days in control medium and medium supplemented with different concentrations of testosterone, dihydrotestosterone and estradiol*

	Control	T			DHT			E2		
		50	100	500	50	100	500	50	100	500 (ng/ml)
PC	100±3	87±4	78±3	71±4	92±5	83±3	78±4	120±4	131±5	148±7
RSF	100±4	95±4	86±5	81±4	99±4	90±4	80±5	111±5	120±7	131±4

$p < 0.05$ for both cell types compared with control values (all hormone concentrations).
$p < 0.05$ for all nine columns except DHT 500 ng/ml..

[^{3}H]thymidine uptake

In a separate experiment ($n = 6$) PC and RSF were each seeded in a 24-well microtitre plate (Falcon, Becton Dickinson, Heidelberg, FRG) at constant cell densities per well and two wells each patient and each concentration. On the 7th day of subculture with either T, DHT or E2 (each 50, 100, 500 ng/ml) the cells were pulse labelled with [^{3}H]thymidine (1 μCi/ml medium, specific activity 6 Ci/mmol, Amersham, Braunschweig, FRG). After 6 h exposure at 37°C the cell layers were washed twice with phosphate-buffered saline (PBS) without Ca^{++} and Mg^{++} (pH 7.0, 2°C, Gibco, Karlsruhe, FRG). Single-cell suspensions were prepared and cells were collected on Whatman filters (Whatman Inc., Maidstone, England), which were transferred to a scintillation vial containing 7 ml scintillation liquid (analytic quickszint 1, Zinsser, Frankfurt, FRG). Radioactivity of cells was determined with a scintillation counter (1211 Minibeta, Gräfelfing, Munich, FRG) for 180 s. Mean values of the [^{3}H]thymidine labelling indices were denoted as stimulation indices (SI) in comparison with the controls (mean value of controls: 100) and standard deviations were calculated.

Statistical analysis was performed using a *t*-test for paired samples.

RESULTS

The growth curves and cell doubling times are displayed in Fig. 1 and Table I. In the control medium and irrespective of the kind of hormone added, PC grew always slower than RSF. In medium containing T above 30 ng/ml, the growth rates of both cell types decreased (Table I) whereas with lower T-doses the growth rates were not affected (data not shown). With T-concentrations above 30 ng/ml the growth of PC was slightly more depressed, cell doubling times were longer (Table I) and the SI (Table II) showed lower values than those of RSF. Statistically significant differences between PC and RSF could be shown for each T-concentration (Tables I, II).

When applying DHT, similar results were found. DHT concentrations below 30 ng/ml were ineffective, whereas DHT doses above 30 ng/ml reduced the cell growth rate of both cell types, especially that of PC. The effect of DHT on the growth behaviour of both cell types was less pronounced than that of T. Statistically significant differences between PC and RSF were evident for all DHT concentrations except for DHT 500 ng/ml (Tables I, II).

E2 in concentrations up to 10 ng/ml showed no effect on the cell growth of both cell types, whereas E2-doses above 50 ng/ml increased the SI values in both cell types (Table II). Differences between both cell types and also between hormones tested and control experiments were statistically significant (Table II).

DISCUSSION

By the isolation of PC and RSF as well as by special subcultivation techniques it is possible to study the proliferative activity of both cell types in culture (3, 4, 5). As shown recently, addition of different agents, for instance epidermal growth factor, fibroblast growth factor, hydrocortisone, and minoxidil to the culture medium, produced different growth behaviour of PC and RSF (8).

The present study yields first information on the in vitro growth rates of PC under the influence of sex hormones. With a DHT and T concentration of 30 ng/ml, which is about four times the physiological T-plasma concentration in male the growth rates were retarded, especially that of PC. Higher DHT- and T-concentrations revealed statistically significant differences between PC and RSF. Since the DHT- or T-induced decrease in proliferation was greater for PC than for RSF, a higher androgen sensitivity of these specialized fibroblasts can be assumed. The lowered growth rate of PC and the constringent growth pattern in culture – which is

different from that of RSF (5) – may also reflect the androgen target function of PC on hair growth. Interestingly, the growth rates of both cell types were more depressed by T than by DHT. As has been shown by in vitro studies (9, 10, 11, 12) this might be caused by an additional reduction of T to DHT by 5α-reductase or via hydroxysteroid dehydrogenase to androstanediol. Hence, an additional reduction of T via hydroxysteroid dehydrogenase and the resulting metabolites may act as a secondary factor influencing cellular growth under in vitro conditions.

The effects of estrogens on fibroblasts as well as on rodent and human epidermis have been a topic for controversial discussion (13, 14, 15). We found that low E2 concentrations were ineffective, whereas the E2 concentration of 50 ng/ml significantly accelerated the proliferation of PC and RSF. Estrogen sensitivity of PC was greater than that of RSF; this difference was statistically significant.

This effect may be mediated by the enzymatic activity of aromatase or transhydrogenase providing a higher energy supply (16). Further cell kinetic studies on subcultured skin fibroblasts under the influence of E2 are lacking so far.

Our results yield evidence that T, DHT and E2 are able to modify the proliferative activities of hair papilla cells in culture, which can be regarded as major target cells for the effectiveness of these sexual hormones on hair growth.

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