ANTI-ANDROGENS AND ESTROGENS:
MODULATORS OF VEGF EXPRESSION IN
CULTURED HAIR DERMAL PAPILLA CELLS
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Introduction
In human androgenetic alopecia, elevated testosterone levels
seem to be responsible for follicular regression (1), with anti-
androgen treatment being able to prevent this (2).

The hair dermal papilla, with its own blood supply, androgen
receptors and types I & II 5α-reductase activity appears to be
a target for many circulating factors such as androgens, estro-
gen and growth factors.

Vascular endothelial growth factor (VEGF) is regarded as the
most important positive regulator of angiogenesis and vascular
permeability (for reviews, see 3, 4, 5).

Previously, we found that cultured dermal papilla cells syn-
thetize a large quantity of this cytokine, which may be respon-
sible for the regulation of hair vascularization (6). The synthesis
of VEGF is also most likely implicated in the regulation of the
hair growth cycle (7).

Thus, in the present study, we chose to investigate the rela-
tionship of hair dermal papilla vascularization and the effect
of certain steroids. This was done using ELISA and RT-PCR
techniques for determining VEGF protein synthesis and gene
expression respectively, in human dermal papilla cells cultured
with or without dihydrotestosterone, or testosterone in associa-
tion with anti-androgens (finasteride). In addition, the effect of 17β-
estra diol was also investigated in parallel.

Material and methods
Dermal papilla cells (DPC) were plated on a 6-well plate at a
density of 1x10⁶ cells/well and incubated for 24h in serum-free
medium containing different agents: testosterone (0.5–10 nM),
dihydrotestosterone (0.5–10 nM), 17β-estradiol (0.01–1.0
nM) or finasteride (0.5–5 nM). Finasteride was also tested on
DPC precultured with testosterone 0.5 nM. VEGF protein
expression was measured in the cell supernatants using the EL-
ISA method. All assays were done in triplicate for each agent.

RT-PCR analysis
Total RNA (1 μg) from cultured DPC, was reverse transcribed
at 37°C for 60 min using cDNA using Access RT-PCR System
(Promega) in a 50 μl volume.

Polymerase reactions were performed using oligonucleotides
complementary to the 5’ and 3’ ends of the coding sequence
(CTG TCT CAC TAC CAG CAC CAC CAC GAC TCA CAC). Amplification was performed for 30
cycles (94°C for 40 s, 57°C for 1 min, 72°C for 1.5 min) in a
DNA thermal cycler heat block (Perkin Elmer, Gene Amp PCR
system 2400). The amplified PCR products were separated by
3% agarose electrophoresis.

Results and discussion
During the 24h treatment period, increasing testosterone or di-
hydrotestosterone concentrations (0.01 to 100 nM) resulted in
a non-significant stimulation of VEGF production in both
supernatants and cell extracts (results not shown) of cultured
dermal papilla cells (Fig. 1).

In the presence of antiandrogen drugs, a significant stimula-
tion of VEGF production is observed (Fig. 1), with maximum
stimulation (1.3-fold) being observed in the presence of 10 nM fin-
asteride. A significant dose-dependent stimulation of VEGF ex-
pression is seen with 17β-estradiol also (Fig. 2).

According to our RT-PCR studies (Fig. 3), 17β VEGF form
is present in cultured DPC. These cells pre-incubated with tes-
tosterone then treated with different finasteride concentrations...
Figure 1. VEGF production by cultured DPC following 24 h treatment with testosterone (T), dihydrotestosterone (DHT) or finasteride (Fina). DPC were also cultured in the presence of testosterone for 2 h, then treated with finasteride for 22 h. VEGF protein was measured in the cell supernatants.

Figure 2. Dose-response curve of VEGF expression for 17β-estradiol incubation. DPC were cultured in the presence of 17β-estradiol for 24 h then VEGF protein was measured in the cell supernatants.

(6.5, 13 & 26 nM) show a stimulation which is dose-dependent on transcriptional levels of the VEGF gene (Fig. 3).

The oestrogen-like stimulation of VEGF production obtained with the 5α-reductase inhibitor (finasteride) points to a possible role of the anti-androgens via the aromatase pathway, on the vascularisation of the follicular papilla and consequently on hair growth.

This hypothesis on the putative new mode of anti-androgen action could be confirmed by associating androgen receptor antagonists with 5α-reductase inhibitors.

These findings reflect the involvement of anti-androgen molecules and oestrogens in hair dermal papilla vascularization and present an important parameter in maintaining the hair follicle in the anagen stage of hair cycle.

References