Chemical Agents and Peptides Affect Hair Growth

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During the past decade we have examined both the therapeutic and the prophylactic effects of several agents on the macaque model of androgenetic alopecia. Minoxidil and diazoxide, potent hypotensive agents acting as peripheral vasodilators, are known to have a hypertrichotic side effect. Topical use of both agents induced significant hair regrowth in the bald scalps of macaques. The application of a steroid 5α-reductase inhibitor (4MA) in non-bald preadolescent macaques has prevented baldness, whereas controls developed it during 2 years of treatment. The effects of hair growth were determined by 1) phototrichogram, 2) folliculogram (morphometric analysis), and 3) the rate of DNA synthesis in the follicular cells. These effects were essentially a stimulation of the follicular cell proliferation, resulting in an enlargement of the anagen follicles from vellus to terminal type (therapy) or a maintenance of the prebald terminal follicles (prevention). A copper binding peptide (PC1031) had the effect of follicular enlargement on the back skin of fuzzy rats, covering the vellus follicles; the effect was similar to that of topical minoxidil.

Analyzing the quantitative sequences of follicular size and cyclic phases, we speculate on the effect of agents on follicular growth. We also discuss the triggering mechanism of androgen in the follicular epithelial-mesenchymal (dermal papilla) interaction.

MATERIALS AND METHODS

Stumptailed Macaques The unique development of frontal alopecia in this old world monkey is a species-specific phenomenon and it occurs in nearly 100% of post-adolescent animals in both sexes. The elevation of serum testosterone (T) and dihydrotestosterone (DHT) appears around four years of age when a thinning of hairs begins to show and slowly progresses with age [8, 9]. Our earlier study revealed that baldness was simply due to a progressive miniaturization of the hair follicle per se and it appeared to be androgen (DHT) dependent [10].

Fuzzy Rats A genetic mutant between the hairless and the haired albino rat exhibits progressive thinning of hair in the body after 2 months of age [11]. The back of the adult rat is covered with short vellus hairs and sporadic long kinky hairs are present during young adulthood.

Chemical Agents Minoxidil: A total of 24 adolescent and adult stumptailed macaques were used for a series of studies of hair growth during the past decade. Either 5% or 2% minoxidil in vehicle solution (0.2 ml) was applied topically on the frontal scalp, over an approximately 50-cm² area, once a day, 5 d per week. The total period of the experiment was 10 months for 2% and 2-4 years for 5% minoxidil. Gross photographic recordings were performed once a month. Skin biopsies (4-mm punch) were taken from the frontal scalp of all animals every 3 to 6 months, prepared for serial paraffin sections (10 mi-
of hair. Continuous treatment with either minoxidil or diazoxide showed progressive thickening of hair density in the frontal scalp. However, the effect was reversible and the photographs showed obvious thinning of hairs at 3 months after cessation of treatment [11,14].

The phototrichograms showed clearly the conversion of vellus to terminal hairs after 3 months of minoxidil treatment. In Fig. 1, the terminal hairs, darkly pigmented with large caliber hair shafts, were spread among many less pigmented and fine vellus hairs in the bald scalp at the pretreatment stage. After 3 months of minoxidil (5%) treatment, the most obvious change was an increased number of terminal hairs in the same scalp region. Most terminal hairs from the pretreatment stage showed no change after treatment, but new, additional terminal hairs appeared to be converted from vellus hairs. The average population rate between vellus and terminal hairs was 23 ± 2/77 ± 3 in the bald scalp of four adult macaques at the pretreatment stage. After 3 months the rate of the minoxidil group became 31 ± 1/69 ± 2 and that of the vehicle group accounted for 20 ± 2/80 ± 3. An approximately 10% conversion of vellus hairs to terminal size appeared to be sufficient to show increased hair growth in gross photographs of unclipped scalp hairs.

**Folliculogram:** Folliculograms of the bald scalp showed a similar pattern in all adult animals that exhibited moderate to advanced degrees of baldness. Over 70% of the hair follicles belonged to the telogen phase; the length ranged from 0.5 to 0.9 mm (mean length 0.65 - 0.9), and less than 20% were of the late anagen phase (Tables I and II). The average length of anagen follicles was approximately 1 mm, and the rest were either mid-anagen or catagen. Compared to the length in non-bald pre-adolescent animals, follicular size in bald scalps was much smaller [11,12]. The patterns of the folliculogram of the bald scalp also suggest that most follicles stay in the telogen phase and have a very short anagen phase. Thus, the length of hairs in the bald scalp do not grow long.

Sequential patterns of folliculograms showed that most telogen follicles in the bald scalp were stimulated to progress in their cyclic growth. After 3 to 4 months of treatment, about half of the telogen follicles converted to early to mid-anagen follicles and the population of late anagen follicles also increased. This cyclic growth was seen in both drug- and vehicle-treated scalps. However, the increased size of both telogen and anagen follicles was seen only in the minoxidil or diazoxide groups, and not in the vehicle group (Fig 2). Continuous treatment with the drugs induced a gradual increase in follicular size and progressive patterns of cyclic growth. An increased anagen population can produce longer hairs. The patterns in the vehicle group were not consistent, but sometimes the population of late anagen follicles was substantially increased; this represents the so-called placebo effect (Table I, vehicle). Quantitative analysis of follicular growth, mean length of telogen and anagen

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**RESULTS**

**Hair and Follicular Growth** Minoxidil (5%) induced obvious thickening of hairs in the bald frontal scalp viewed by unclipped natural photographs compared to pre- and post-treatment stages (after 3 months). Although the degree of this initial effect was slightly weaker, diazoxide (5%) induced an increase in thickening of hair.  

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both drugs were a stimulation and maintenance of the bulbar cell proliferation resulting in a prolongation of the anagen phase. Enlargement of the anagen follicles from the previous vellus follicles to terminal size and a prolongation of the anagen phase results in a growth of thicker and longer hairs in the bald scalp. The rate of follicular growth with 2% minoxidil was smaller than that with 5% minoxidil, but it was sufficient to maintain follicular size and cyclic elongation of follicular size after 2 to 4 months of treatment compared to the previous vellus follicles. After 5 d of minoxidil and 10 d of vehicle treatment many telogen follicles showed growth of the secondary germ and the cells containing a BrdU-positive nucleus were largely found in the secondary germ.

Analysis of the folliculograms was also performed in the fuzzy rat and showed large follicles in all cyclic phases and the majority of the follicles belonged to the anagen phase with both the mid and late phases. This non-bald pattern of folliculogram was maintained by either topical application of antiandrogen (4MA) or minoxidil (5%) in the frontal scalp during the peripubertal age (about 3 to 5 years) [10, 11]. In the same age group the macaques treated with vehicle showed a conversion of the folliculogram from the non-bald to the bald pattern.

Unlike other rodents, the hair follicles in fuzzy rats exhibited non-synchronized cyclic growth after the second postnatal cycle. At the age of two and a half months, the ratio of telogen and anagen follicles was about 50/50 and all follicles were small; the average length was 0.3 mm in telogen follicles and 0.75 mm in anagen follicles. Both minoxidil and PC1031 (5% respectively) induced an 80% increase in the population of anagen follicles and an enlargement to almost double in size.

**DNA Synthesis in Follicular Cells**

The skins of fuzzy rats were taken after 2 h of intraperitoneal injection of BrdU every 5 d after topical application of either minoxidil or vehicle solution. After 5 d of minoxidil and 10 d of vehicle treatment many telogen follicles showed growth of the secondary germ and the cells containing a BrdU-positive nucleus were largely found in the secondary germ.

### Table I. The Rate of Follicular Growth

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean length (mm ± SEM)</th>
<th>Population (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Telogen</td>
<td>Anagen (A5)</td>
</tr>
<tr>
<td>Minoxidil 5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>0.65 ± 0.01</td>
<td>0.97 ± 0.07</td>
</tr>
<tr>
<td>4 months</td>
<td>0.70 ± 0.03</td>
<td>1.27 ± 0.04</td>
</tr>
<tr>
<td>1 year 2 months</td>
<td>0.87 ± 0.02</td>
<td>1.41 ± 0.06</td>
</tr>
<tr>
<td>Withdrawal, 1 month</td>
<td>0.80 ± 0.01</td>
<td>1.45 ± 0.15</td>
</tr>
<tr>
<td>Minoxidil 2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>0.90 ± 0.06</td>
<td>1.39 ± 0.11</td>
</tr>
<tr>
<td>4 months</td>
<td>0.74 ± 0.03</td>
<td>1.17 ± 0.09</td>
</tr>
<tr>
<td>7 months</td>
<td>0.78 ± 0.04</td>
<td>1.43 ± 0.10</td>
</tr>
<tr>
<td>Minoxidil 5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>0.78 ± 0.03</td>
<td>1.88 ± 0.07</td>
</tr>
<tr>
<td>10 months</td>
<td>1.16 ± 0.10</td>
<td>1.86 ± 0.12</td>
</tr>
<tr>
<td>2 years</td>
<td>1.17 ± 0.07</td>
<td>1.67 ± 0.13</td>
</tr>
<tr>
<td>3 years</td>
<td>1.14 ± 0.04</td>
<td>1.86 ± 0.06</td>
</tr>
<tr>
<td>4 years</td>
<td>1.05 ± 0.07</td>
<td>1.64 ± 0.11</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>0.71 ± 0.12</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>4 months</td>
<td>0.76 ± 0.07</td>
<td>0.95 ± 0.07</td>
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<tr>
<td>1 year</td>
<td>0.66 ± 0.02</td>
<td>1.31 ± 0.16</td>
</tr>
<tr>
<td>2 years</td>
<td>0.80 ± 0.05</td>
<td>1.46 ± 0.23</td>
</tr>
<tr>
<td>3 years</td>
<td>0.80 ± 0.03</td>
<td>1.15 ± 0.06</td>
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Data based on folliculogram analysis [13].

### Table II. The Rate of Follicular Growth

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<tr>
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<td>Diazoxide</td>
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<tr>
<td>0 time</td>
<td>0.74 ± 0.02</td>
<td>1.28 ± 0.11</td>
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<tr>
<td>4 months</td>
<td>0.91 ± 0.03</td>
<td>1.46 ± 0.08</td>
</tr>
<tr>
<td>1 year</td>
<td>1.06 ± 0.02</td>
<td>1.61 ± 0.09</td>
</tr>
<tr>
<td>Withdrawal, 3 months</td>
<td>0.79 ± 0.03</td>
<td>1.57 ± 0.12</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>0.66 ± 0.02</td>
<td>1.12 ± 0.04</td>
</tr>
<tr>
<td>4 months</td>
<td>0.70 ± 0.07</td>
<td>1.21 ± 0.04</td>
</tr>
<tr>
<td>1 year</td>
<td>0.78 ± 0.02</td>
<td>1.24 ± 0.05</td>
</tr>
<tr>
<td>Withdrawal, 3 months</td>
<td>0.64 ± 0.02</td>
<td>1.06 ± 0.06</td>
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Data based on folliculogram analysis [14].

### Table III. The Rate of Follicular Growth

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* Data based on folliculogram analysis [13].

foliculograms and in non-bald pre-pubertal macaques showed large follicles in all cyclic phases and the majority of the follicles belonged to the anagen phase with both the mid and late phases. This non-bald pattern of folliculogram was maintained by either topical application of antiandrogen (4MA) or minoxidil (5%) in the frontal scalp during the peripubertal age (about 3 to 5 years) [10,11]. In the same age group the macaques treated with vehicle showed a conversion of the folliculogram from the non-bald to the bald pattern.

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**DNA Synthesis in Follicular Cells**

The skins of fuzzy rats were taken after 2 h of intraperitoneal injection of BrdU every 5 d after topical application of either minoxidil or vehicle solution. After 5 d of minoxidil and 10 d of vehicle treatment many telogen follicles showed growth of the secondary germ and the cells containing a BrdU-positive nucleus were largely found in the secondary germ.
conversion of short vellus hairs to long terminal hairs (phototrichogram), 2) an enlargement of the follicular size, and a prolongation of

Our observations in the macaque bald scalp obviously showed 1) a

induced a proliferation of germinal cells, the rate of cell prolifera-

in construction of new anagen follicles. Although the vehicles also

aggregated dermal papilla cells in vellus telogen follicles began to

show a gross hair regrowth in the bald scalp by photographs of the

terminal size. This rate of conversion appeared to be enough to

clearly demonstrate a conversion of individual fine vellus hairs to

thick-terminal hairs. Three months of minoxidil (5%) treatment

was remarkably higher in the drug than in the vehicle groups.

enhanced the rate of cell proliferation of follicular germ cells. The

follicular cells strongly suggested that hypertrichotic drugs en-

Figure 2. Hair-follicular growth cycles and folliculograms of vehicle-

(lower row) and minoxidil-treated (lower row) cases represent sequential pat-

terns of follicular transformation from vellus follicles (small sized T, A1, A2

and short length of follicles at each phase) to terminal follicles (large sized T,

A3, A4) with minoxidil treatment. The secondary germs producing primor-

dial anagen follicles (A3 and arrow) appeared much larger and longer in

minoxidil- than those in vehicle-treated cases.

The density of DNA synthesis (S phase) cells and the entire size of the secondary germ were greater and larger in the follicles of the minoxidil- than in the vehicle-treated cases (Fig 3a, d). In the mid-
anagen phase, elongation of the follicular peg and the number of S-phase cells were more pronounced in minoxidil-treated cases (Fig 3b and d). The rate of follicular cell proliferation was continuously increased in the remodeling follicular process and the primordial bulb and follicular sheath of the anagen follicles contained many S

phase cells in minoxidil- compared to those in vehicle-treated cases (Fig 3c and f).

The increased rate of DNA synthesis cells in the follicular ger-

minal cells with minoxidil was also found in the follicles of stump-
tailed macaques in our earlier work [12,13].

In fuzzy rat skin, a longer exposure time to BrdU (time after injection or use of a continual supply with a minipump) increased the number of BrdU-positive cells that belong to either the S or G2 phase. After 14 d exposure to BrdU, BrdU-positive cells were almost saturated in the epidermal basal layer and in the external root sheath of hair follicles in the upper and lower portion of anagen follicles (Fig 4a, b).

DISCUSSION

The trichography, a magnified view of shortly clipped hairs, can clearly demonstrate a conversion of individual fine vellus hairs to thick terminal hairs. Three months of minoxidil (5%) treatment caused approximately a 10% conversion of hairs from vellus to terminal size. This rate of conversion appeared to be enough to show a gross hair regrowth in the bald scalp by photographs of the unclipped natural view.

Studies of sequential data of folliculograms and DNA synthesis in follicular cells strongly suggested that hypertrichotic drugs enhanced the rate of cell proliferation of follicular germ cells. The drugs appeared to have a strong affinity to the follicular cells, but showed no effect on epidermal basal cells [13,15].

Shortly after treatment, the secondary germs associated with ag-

gregated dermal papilla cells in vellus telogen follicles began to

proliferate and their continuous growth and differentiation resulted in construction of new anagen follicles. Although the vehicles also induced a proliferation of germinal cells, the rate of cell prolifera-

Figure 3. The number of DNA synthesis cells (arrow) in early and mid-
anagen follicles showed a marked increase in minoxidil-treated (a,c,f) com-

pared to those in vehicle-treated (a,b,c) cases. a,b,d,e, early anagen. c,f, mid-
anagen. Bar, 0.05 mm.

the anagen phase (folliculogram), and 3) an enhanced rate of cell prolifera-

tion in the follicular germinal cells (DNA synthesis studies) during treatment with minoxidil, diazoxide, and copper-pep-

tide.

Follicular enlargement apparently occurred during the cyclic re-

modeling process in which the germinal cells in telogen follicles prolif-

erate and construct fully grown anagen follicles; the rate of ger-

minal cell proliferation determines the size of new anagen follicles.

The hair follicles in the scalp of both macaque and human andro-

genic alopecia mostly belonged to vellus telogen follicles. Thus, hypertrichotic agents readily stimulate these telogen buds and transfor-

m them to larger anagen follicles than those in previous cycles. This initial conversion from vellus telogen to terminal anagen follicles took about 3 months at which time a dramatic hair regrowth was grossly observed. The rate was less and speed of the follicular enlargement slowed after the initial effect, but they slowly progressed as long as the treatment continued.

Our recent studies using synchronizing hair cycles in rodents revealed that long-term labeling of a DNA precursor BrdU showed a saturation of labeled cells (mostly S- or G2-phase) in all epidermal basal cells as well as throughout the entire external root sheath of anagen follicles. These potential mitotic cells distributed in the follicular sheath can add new sheath and the diameter of the follicle enlarges. Together with the proliferation of the bulbar cells, all of the anagen follicles appear to enlarge by themselves. Either a gradu-

tal thickening or thinning of women's scalp hairs during growth or old age may be the result of this direct (non-cyclic) transformation of the anagen follicle per se. Indeed, female alopecia usually lacks
short vellus hairs, which represent the cyclic conversion of hair from old to new.

Androgens are undoubtedly the triggering hormone for inducing this epigenetic phenomenon called androgenetic alopecia. Furthermore, dihydrotestosterone (DHT) converted from circulating testosterone appears to be a potent androgen for hair follicular transformation in particular body regions such as the beard, chest, and pubic regions and the scalp follicles of bald trait men. The epigenetic expression of hair follicles in both men and women by elevated testosterone exhibit dichotomous effects in such androgen-sensitive follicles, growth versus regression. Growth represents secondary sexual hair growth or hirsutism and regression is androgenetic alopecia.

Thus, the most essential treatment for androgenetic alopecia is a blocking of androgen action on the hair follicles. Hypertrichotic agents induced successful regrowth of the transformed vellus follicles to terminal size. However, androgen is continuously exerting its regressive effect on the regrown follicles. This fact explains that despite the significant initial effect of hair regrowth the growth progression during the following period is much slower and the increased rate of hair length and follicular size shows a certain limit. The regrown hairs maintain their length and thickness, but a complete regrowth to the hairs at the non-bald stage has not been observed. Recent work with a combination of 5α-reductase inhibitor (finasteride) and minoxidil produced a relatively higher effect on hair growth than minoxidil alone [16]. Recent work using the co-culture techniques of dermal papilla cells and follicular epithelial cells opened a new insight into the mechanism of androgenic action on hair follicles [17]. Dermal papilla cells derived from the human beard induced significant proliferation of the follicular epithelial cells with the addition of testosterone in the medium and this effect was blocked by cyproterone acetate. The dermal papilla cells derived from androgen non-sensitive follicles such as in the occipital scalp showed no such androgenic effect. These facts strongly suggest that the dermal papilla cells of the beard have genetic machinery to produce specific factor for 0ICU-1. Indeed, the block of androgenic action by the use of 5α-reductase inhibitor has successfully prevented the development of baldness during the peripubertal age in stump-tailed macaques [10]. Drugs blocking the androgen receptor may have the same effect, but they likely have more universal side effects on masculinization.

The most effective and safest treatments for androgenetic alopecia will be blocking the production of either the suppression factor described above or dihydrotestosterone in the dermal papilla cells. Furthermore, using the ubiquitous action of hypertrichotic agents for an initial regrowth of hair follicles then blocking androgenic actions by the above factor or 5α-reductase inhibitor will be a rational approach for treatment of alopecia.

REFERENCES