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Treatments for Androgenetic Alopecia and Alopecia Areata

Current Options and Future Prospects

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Abstract

Androgenetic alopecia and alopecia areata are common disorders of the hair follicle which may heavily influence self esteem and self image. Androgenetic alopecia is caused by the heightened sensitivity of scalp follicles to dihydro-

testosterone whereas alopecia areata is induced by an autoimmune reaction. Current drug treatment approaches include the use of regrowth stimulators such as topical minoxidil and oral finasteride for androgenetic alopecia, as well as topical minoxidil, dithranol (anthralin), corticosteroids, contact sensitisers, and psoralen plus ultraviolet A irradiation (PUVA) therapy for alopecia areata. Combination regimens are also proposed. However, extreme cases of either type of alopecia do not generally respond well to these existing treatments. For this reason, new therapeutic strategies are directed towards both improving the targeting of existing agents, as well as the development of novel hypertrichotic modalities.

Although scalp hair serves no critical physiological function, many people find that the loss of hair can adversely effect their self confidence. The magnitude of this effect can be gauged from the fact that each year in the US, about 60 million people spend approximately $US1.5 billion on various hair regrowth treatments.[1] Historically, hair loss was treated with the application of different herbal remedies, which were frequently of dubious and unproved efficacy. Another approach involved masking baldness by the manipulative use of dyes, cosmetics and hairstyling. Fortunately, recent decades have witnessed the development of clinically effective drug treatments for hair growth stimulation. The objective of this article is to discuss the various drug therapies available today for alleviating both androgenetic alopecia and alopecia areata – the most common forms of baldness. The article reviews the currently available treatments for alopecia and discusses the development of novel research tools for hair regrowth research and the treatment modalities which may well become available in the near future.

1. Background

1.1 The Hair Cycle

The hair follicle and its associated erector pili muscle and sebaceous glands constitute what is generally termed the pilosebaceous unit. The hair follicle consists of a hair bulb and shaft enveloped in an inner root sheath, an outer root sheath and an outermost acellular basement membrane commonly known as the glassy membrane. The sebaceous glands are joined to the follicle by ducts and discharge sebum into the upper third of the follicular canal, creating a lipophilic environment in this region. Hair growth regulation is largely controlled by the follicular papillae and hair matrix cells, located at the base of the hair follicle as well as the mid-follicle bulge area. Scalp hair undergoes a process of continuous turnover throughout life. The formation, growth and shedding of hair shafts result from epithelial-mesenchymal interactions which are regulated by a complex interplay of genetic, immunological and endocrinological factors. During the actively growing stage or anagen, the hair matrix cells divide rapidly and migrate upwards to form the hair shaft. On the scalp, this phase lasts 3 to 8 years but it is invariably followed by catagen, a brief period characterised by dramatic morphological changes such as the cessation of mitosis as well as reabsorption and cell death of the lower follicle segment. The scalp follicle then enters a 3-month resting phase termed telogen, prior to the hair being shed. Anagen then recurs as the hair matrix cells start dividing and the lower follicle redevelops. As a result of hair cycling, in healthy young adults, the amount of scalp hair lost by shedding is approximately matched by the amounts regrown, with typical hair densities ranging between 300 to 350 hairs per cm^2. Despite several decades of hair research, the detailed mechanisms controlling hair growth and cycling have remained elusive.

1.2 Androgenetic Alopecia

This progressive condition is the most common type of hair loss, affecting about 50% of Caucasian males and females beyond age 40 years.[2] In men, where the condition is also known as male pattern

baldness, hair loss can start as early as late adolescence. Androgenetic alopecia can vary in its severity from merely accentuated recession of the frontal hairline to loss of all hair except along the temporal and occipital margins. In females, the condition is usually milder and is associated with diffuse thinning of scalp hair. Clinical onset in both men and women is generally by age 30 years. Although its precise mechanisms are still unclear, cell culture work by Hibberts and co-workers[3] suggests that androgenetic alopecia is caused by the heightened sensitivity of scalp follicles to post-pubertal androgens, particularly dihydrotestosterone. Sawaya and Price[4] showed that androgen receptor protein concentrations, within the outer root sheath and dermal papilla fibroblasts are some 30% greater in the balding frontal follicles than in the nonbalding frontal hair follicles. The resultant increased androgen binding at these receptor sites triggers pronounced effects on follicular physiology. First, the anagen phase becomes progressively shorter over the course of several hair growth cycles. This means there is a decrease in the numbers of actively growing scalp hairs at any given time. The second transformation is progressive follicular miniaturisation which leads to the gradual transformation of thick, long terminal hairs into shorter vellus hairs or vellus-like follicles.[5] The overall effect is to reduce visible scalp density. Importantly, the affected follicles show no abnormalities and they maintain the potential for cyclic growth until a very advanced stage of baldness is reached.

1.3 Alopecia Areata

Alopecia areata is a common, chronic inflammatory disease, which occurs in 0.01 to 0.1% of the Caucasian population.[6] Twin studies indicate that both genetic as well as environmental factors play a role in its induction.[7] Although the aetiology and pathogenesis of this condition are poorly understood, it is known that the hair shedding process is associated with the a CD4+ lymphocytic infiltrate surrounding the follicles.[8] Recently acquired data suggests that alopecia areata is triggered by T lymphocyte recognition of a follicular autoantigen.[9] The clinical course of the condition is extremely variable with some cases showing spontaneous remission and others becoming progressively worse. Onset before puberty is usually indicative of a poor prognosis. Because of its unpredictable severity and recurring frequency, the disease can be psychologically devastating for those affected. This is particularly true for girls and young women who become bald. Fortunately, since the immunological events of alopecia areata do not lethally damage crucial elements of the hair follicle, symptomatic treatment of the condition is possible.

2. Current Treatments for Androgenetic Alopecia

Topical minoxidil is currently available for the treatment of androgenetic alopecia in both men and women. The less extensively evaluated oral finaster-

Table I. Compilation of established and proposed treatments for androgenetic alopecia

Drug treatment	Mode of action	References
Approved by US Food and Drug Administration		
Topical minoxidil (2 or 5%, twice daily)	Multiple mechanisms?	2, 9-32
Oral finasteride (1mg, once daily)	Inhibits type II 5α-reductase	33-37
Unvalidated/proposed		
Topical minoxidil + penetration enhancers	Enhances delivery of active agent	38, 39
Topical minoxidil + oral finasteride	Synergism	40, 41
Topical vesicular minoxidil	Targets delivery of active agent to follicles	42
Topical finasteride	Inhibits type II 5α-reductase	43
Antimicrobials	Suppresses follicle inflammation	21, 44
Herbal products	Induces vasodilation/unknown	45

ide represents a novel treatment but its use is limited to males only. Table I summarises treatments for androgenetic alopecia discussed in this section.

2.1 Minoxidil

Minoxidil, a pyrimidine derivative (2,4-diamino-6-piperidinopyrimidine-3-oxide), was the first drug to become available for treating scalp hair loss. Originally synthesised for oral use as an antihypertensive agent, minoxidil was surprisingly found to induce hair growth in patients. This unexpected side effect led to the development of a topical minoxidil-containing lotion for alopecia treatment. The product is currently available as solutions containing 2 or 5% minoxidil, in formulations composed of 60% ethanol, 20% propylene glycol and 20% water.

The mechanism(s) by which minoxidil promotes hair growth is still not fully understood,[10-12] and multiple pathways are thought to be involved. One theory proposes that minoxidil, metabolised to minoxidil sulfate in the hair follicles, acts as a potassium channel agonist to reduce the cytoplasmic free Ca^{2+} concentration. This prevents epidermal growth factor from inhibiting hair formation. Thus, hair growth is promoted.[13] Another possibility is that minoxidil up-regulates the expression of vascular endothelial growth factor and its receptors – an action which subsequently stimulates angiogenesis and anagen.[14] In contrast, Philpot and coworkers[15] recently showed that minoxidil stimulates regrowth in hair follicle cultures where a blood supply is absent. Other researchers have documented that minoxidil is a potent activator of prostaglandin endoperoxide synthase-1 (a cytoprotective enzyme that stimulates hair growth).[11] Immunocytochemistry and autoradiographic analysis of primate scalp has indicated that minoxidil increases the number of DNA synthesising cells in the dermal papilla, bulbar matrix, outer root sheath and perifollicular fibrocytic cells.[16,17] These changes result in the prolongation of anagen and the conversion of vellus hairs to terminal hairs.

Extensive clinical trials on men with androgenetic alopecia have shown that minoxidil treatment is only effective in less than half the cases. For example, the conclusion of a year-long, randomised, double-blind trial involving 56 males, was that cosmetically acceptable hair growth occurred in 32% of individuals.[18] In another 12-month study, minoxidil produced at least moderate cosmetic improvement in only 24% of individuals.[19] Data derived from multiple, double-blind, placebo-controlled trials, involving over 2000 males between the ages of 18 and 50 years, showed that twice daily application of 2% minoxidil solution over one year produced moderate to dense regrowth in approximately 30 to 35% of patients.[2] Fortunately, even when dense regrowth does not occur, minoxidil appears to stop or significantly retard the progression of male pattern baldness.[20] Although significant hair regrowth can occur in patients of all ages, minoxidil treatment is more likely to be successful in individuals under 40 years. Furthermore, a favourable response is more likely when the balding patch is recently developed (<10 years), covers a small area (diameter <10cm) and contains residual, miniaturised hairs, 1cm long or longer rather than no hair at all.[18] Regarding the last parameter, there is a consensus that the greater the density of such miniaturised hairs, the better the cosmetic outcome.[20] Importantly, the agent is more effective on the scalp vertex than on the frontal area where it is generally of little value.[2]

Many researchers have investigated the effect of minoxidil on male scalp as a function of treatment time. De Villez[18] reported that hypertrichosis first became observable some 4 months into the therapy when fine, colorless vellus hairs in the balding area begun to lengthen. By 8 months these hairs were longer and pigmented while other nonvellus hairs had also begun to lengthen and thicken. At this stage, many of the hair follicles were noted to have 2 hairs. Price and Menefee[21] identified the major period of hair growth as occurring within the first 5 months of therapy with either 2 or 5% minoxidil solution. In a recent pilot study, Pierard-Franchimont and co-workers[22] found that 6 months of minoxidil 2% treatment (once a day application) produced an 11% increase in mean hair density and a 7% increase

in median hair shaft diameter in the affected vertex area. Most researchers have concluded that the maximal growth response is attained after 12 months of minoxidil use. Long term treatment is generally characterised by a slow decline in further new regrowth over subsequent years.[23-25] However, even after 4 years of therapy, the number of nonvellus hairs is still greater than at baseline.[25] If minoxidil applications are stopped, accelerated hair loss occurs such that after 4 to 6 months, scalp hair densities return to pre-treatment levels or even below.[2,25]

For male pattern baldness, a minoxidil concentration-response relationship has been demonstrated. A concentration of at least 1% is required in order to obtain a significant effect[26] while a 5% minoxidil solution is slightly more effective than a 2% solution.[21] However, increasing the concentration further does not improve the growth response. This is probably as a result of enhanced minoxidil precipitation from solution, which reduces the thermodynamic activity of the compound.[27,28] Although the influence of application volume has not been clinically assessed, *in vitro* experimentation has indicated that larger initial volumes delay the time to minoxidil precipitation and therefore result in improved drug penetration.[28] It is noteworthy that a twice daily application of minoxidil is more hypertrichotic than a once a day application, but that increasing the administration frequency further does not improve systemic absorption or therapeutic effectiveness.[29] This is because minoxidil accumulates as a reservoir within scalp skin.[30]

Minoxidil has also been used for treating hair thinning in women.[31,32] Rushton and Fenton[31] used the unit area trichogram technique to measure hair densities in 9 women, both before and after a year's treatment with minoxidil 5%. Topical applications resulted in a 25% increase in the total number of hairs per cm^2 and a 24% increase in the number of nonvellus hairs per cm^2. In a 32-week trial involving 256 individuals, a refined photographic technique was used to evaluate the number of nonvellus hairs regrown.[32] The results showed that 63% of women who applied minoxidil 2% twice daily showed minimal to moderate regrowth as opposed to only 39% of those using a placebo lotion. In fact, in clinical practice, minoxidil appears to be more effective in women than in men. This may be because besides augmenting the number of hairs, the drug also increases the hair shaft diameter, which in a woman's long hair is of particular cosmetic benefit.[33] Women applying minoxidil should be aware that the agent can occasionally cause facial hypertrichosis – an effect produced either systemically or possibly by manual drug transfer. This facial hair growth disappears after cessation of treatment. In conclusion, further clinical trials are required in order to more comprehensively assess minoxidil efficacy for female androgenetic alopecia.

For both men and women, the major disadvantages of minoxidil treatment are the cost and the need for continual, open-ended use. The lotion must be applied twice daily, 7 days a week and good compliance is necessary in order to achieve a positive cosmetic response. Minoxidil may be applied to the scalp either after shampooing (with almost any non-medicated shampoo) or without shampooing. Since the drug is largely absorbed into the scalp some 4 hours after application, individuals can swim or shower after this time.[33] The modality has been associated with virtually no systemic adverse effects although dryness, itching and erythema of the scalp can occur and these are induced by either minoxidil and/or the propylene glycol vehicle. The incidence of local irritation is about 7% with the 2% solution and may be higher with the 5% solution.[12] Although, in another study, no complications were observed after long term treatment with topical minoxidil 3% solution (up to 156 weeks) followed by 5% solution (up to 300 weeks).[34]

2.2 Finasteride

In late 1997, the US Food and Drug Administration (FDA) approved oral finasteride 1 mg/day for the treatment of androgenetic alopecia in men.[1] The agent is not licensed for administration to women because of the potentially teratogenic effects of finasteride on a male fetus, should the woman be

pregnant. Finasteride, a synthetic 4-azasteroid compound, is a specific inhibitor of type II, 5-α reductase. This intracellular enzyme converts testosterone into dihydrotestosterone which affects hair follicle regression. By reducing scalp tissue levels of dihydrotestosterone, finasteride treatment suppresses male pattern hair shedding.[35,36] The efficacy of daily finasteride use for male-pattern hair loss has been recently evaluated in several double blind, placebo-controlled trials. Kaufman and coworkers[37] conducted a randomised study involving 1553 participants exhibiting predominantly mild to moderate vertex hair loss. The researchers employed standardised photographs of the vertex scalp in order to assess the cosmetic response. After 12 months of oral finasteride use, they found that 48% of treated males had improved versus only 7% of the placebo group. Waldstreicher[43] investigated the effect of finasteride in 326 individuals with mostly mild to moderate mid-scalp and anterior baldness. The results showed that finasteride treatment over a 1-year period resulted in a significant hypertrichotic response at the affected regions. Since the drug does not cure the underlying genetic causes of the alopecia, ceasing daily administration results in the shedding of regrown hairs and the resumption of balding. Therefore, continual use of the azasteroid is necessary if the cosmetic benefit is to be sustained.

Finasteride is usually well tolerated. However, there is a small risk of transient impotence as an adverse effect, which is fully reversible upon treatment cessation.[46] Adverse effects could be further reduced if finasteride was applied topically to the affected scalp. This approach was taken by Mazzarella and co-researchers[45] who treated 52 patients with androgenetic alopecia, both male and female, with topical finasteride 0.005% solution in a single-blind, placebo-controlled manner. Treatment efficacy was assessed from both scalp photography and hair loss counts after washing. After 16 months, 73% of the finasteride group have experienced a slight reduction of the balding area compared with none in the placebo group. Monthly hair loss in the finasteride group was approximately 60% of the placebo group ($p < 0.001$). Further trials are required in order to confirm these encouraging early results.

2.3 Herbal Therapies

There are many herbal preparations available that have been used to combat hair loss. For example, cantharidin, extracted from *Cantharis vesicatoria*, has been traditionally incorporated into hydroalcoholic hair lotions. As the agent is highly irritating, it was formulated at strengths of about 0.002%.[47] Another remedy was derived from extracts of jaborandi leaves. The active constituent was pilocarpine which was used in concentrations of up to 0.4%, either alone or in combination with cantharidin or quinine.[47] With nearly all of these preparations, no clinical evidence was supplied to support the claims being made. Greenberg and Katz[48] compared the efficacy of a herbal preparation containing a 7.5% extract of fennel, polygonum, mentha, chamomile, thuja and hibiscus in an aqueous cream base with that of the aqueous cream base alone. A total of 24 balding males were enrolled in the randomised, double-blind study. The volunteers applied either active or placebo cream to their scalps every 24 hours and also washed daily with a supplied shampoo. Hair status was assessed by harvesting, on a bimonthly basis, a selected area of scalp. After 40 weeks of treatment, the mean total hair count increased by 77% in the actively treated group compared with only 3% in the placebo group ($p = 0.003$). Furthermore, the mean terminal hair count for treated men increased by 169% compared with a mere 33% increase for the placebo-treated men. Based on this data, herbal therapy seems to hold great potential as treatment for alopecia and warrants further study.

3. Current Treatments for Alopecia Areata

Alopecia areata is difficult to treat because of its chronic, inflammatory nature. Research has been hindered by the fact that relatively few double-blind, randomised trials on alopecia areata have been published and the results derived from uncon-

Table II. The established therapies for alopecia areata

Treatment	Proposed mechanism	Typical application	References
Minoxidil	Multiple mechanisms	Patchy/moderate disease	11, 19-37, 43, 45-47
Dithranol (anthralin)	Immunomodulatory	Extensive disease	11, 48, 49
Diphencyprone (DPCP)	Antigen competition	Extensive disease	11, 50
Intralesional triamcinolone	Immunomodulatory	Patchy disease	51, 52
Oral prednisolone	Multiple mechanisms	Extensive disease	53
Photochemotherapy	Immunomodulatory	Patchy disease	54-57

trolled trials are questionable because of the high rate of spontaneous remission. Assessing treatment efficacy can be problematic because of the dynamic nature of the disorder. Apart from spontaneous resolution, it is quite possible to observe both intensified hair growth and accelerated hair loss in different regions of the same scalp. Shapiro and Price[12] have defined a cosmetically acceptable response in terms of 'growth sufficient to cover the scalp although some residual patches of loss may remain'. Despite these problems, several modes of therapy are currently available for alleviating alopecia areata (see table II). Topical treatment with either minoxidil, dithranol (anthralin) or corticosteroids offers limited benefit. Contact sensitisers and intralesional corticosteroids are generally more effective. Combination treatments are also popular.

3.1 Minoxidil

The mechanism by which minoxidil promotes hair growth in alopecia areata is currently unknown but the agent probably stimulates scalp follicles nonspecifically in the same manner as described for androgenetic alopecia.[12] The 2% solution is usually ineffective in alopecia areata and topical minoxidil should be applied twice daily at a 5% concentration. An initial positive growth response is generally observed after 3 months of treatment whereas the maximal effect develops after about 1 year. Clinical trials have demonstrated that the degree of cosmetic improvement obtained is highly dependent upon the severity of the condition. With 25 to 99% scalp baldness, cosmetically acceptable regrowth developed in 20 to 45% of individuals.[20] Higher success rates can be expected when the alopecia is milder. After a successful treatment outcome, minoxidil 5% application must be continued in order to sustain the gain in hair growth. Small regions of hair loss may still periodically develop and then undergo regrowth during this maintenance therapy.[49] Minoxidil is generally ineffective in cases of 100% scalp hair loss.[20]

3.2 Dithranol

The nature of the dithranol effect in alopecia areata is not well understood but it may be immunomodulatory. The treatment is generally used in children or for patients experiencing severe disease. The application protocol involves rubbing dithranol cream, at a 0.5 or 1% concentration, for a short (20 to 60 minute) time interval to the affected scalp. Upon termination of the contact period, the medication is washed off the scalp with a shampoo.[50] In 1 clinical study, it was found that a positive growth response took up to 60 weeks to achieve and was observed in some 30% of individuals with less than 75% hair loss and in 20% of individuals with 75% or greater hair loss. The growth gain was sustained in 75% of patients who continued the therapy.[58] At least 6 months are required before a cosmetically acceptable response is obtained.[12] Adverse effects can include pruritus, erythema, scaling and folliculitis. However, such irritation can be minimised by applying smaller amounts of medication and/or by employing shorter contact periods. It is also necessary to protect the skin against sun exposure and to ensure that dithranol does not get into the eyes.

3.3 Diphencyprone

Diphencyprone (diphenylcyclopropanone; DPCP) is a contact sensitiser which has been shown to be effective in the treatment of severe alopecia areata affecting more than 50% of the scalp. The agent may suppress hair shedding by the process of antigen competition, although research is still being performed to elucidate the exact mechanisms involved. Treatment involves applying diphencyprone solution to one half of the individual's scalp at weekly intervals. The solution is washed off 48 hours after application. Although allergic contact dermatitis is frequently induced, this effect is considered necessary for regrowth to occur. The diphencyprone concentration applied (0.0001 to 2%) is varied according to the intensity of dermatitis provoked by the previous week's application. After successful regrowth develops on one side of the scalp, the other half of the scalp is treated. The effectiveness of diphencyprone therapy, although unpredictable on an individual basis, is dependent upon the initial extent of the alopecia.[59] Shapiro and Price[12] have documented that in severe forms of the disease (50 to 99% baldness), a cosmetically acceptable outcome develops in 40 to 60% of patients. About one third of these, however, may ultimately stop responding to diphencyprone. Pericin and Trueb[59] defined severe disease as those experiencing more than 40% baldness. They reported that following at least 5 months of therapy, the modality induced complete remission in 31% of patients and partial remission in 40% of patients. The main adverse effects of diphencyprone are severe eczema and disseminated contact eczema. Other contact sensitisers include dinitrochlorobenzene and squaric acid dibutylester, although these have not been as extensively evaluated as diphencyprone.

3.4 Corticosteroids

By exerting an immunosuppressive effect, corticosteroids can promote regrowth in alopecia areata. Agents such as betamethasone dipropionate 0.05% [58] and fluocinolone acetonide 0.2%[52] have been documented to produce a positive cosmetic response when applied topically to the scalp. However, the use of topical steroids has, to date, not been adequately assessed.[12] Intralesional triamcinolone (triamcinolone acetonide) represents a much more common therapy which is ideal for stable, patchy hair loss that affects less than half the scalp.[53] This corticosteroid is administered as multiple intradermal injections of 0.1ml per site, about 1cm apart. Anaesthetic cream, applied topically about 1 hour before treatment, decreases the subsequent discomfort of the injections. The protocol is repeated approximately once a month. Any hair regrowth is seen within 3 months but the therapy should be stopped if there is no cosmetic response by 6 months, as such individuals may lack adequate corticosteroid receptors in their scalp tissue.[54] A disadvantage of intralesional triamcinolone is that it may induce slight transient atrophy and occasionally follicular atrophy.

Oral prednisolone treatment can be appropriate for rapidly progressing or extensive alopecia areata affecting more than 50% of the scalp. The mode of action seems to be immunomodulatory but prednisolone may also directly stimulate the hair follicles. The initial daily dose varies between 40 to 60mg, to be reduced by 5mg per week. Winter et al.[55] has documented potential adverse effects including acne, hypertension, cataracts, diabetes mellitus and bodyweight gain. Therefore, blood pressure, ophthalmic function and bodyweight measurements must be monitored before and during treatment. For many patients, continuous administration of systemic prednisolone is inappropriate because the dose required to maintain hair growth is usually high and the associated toxicity is unacceptable.

3.5 Photochemotherapy

Psoralen application used in conjunction with ultraviolet (UV)-A irradiation (PUVA) has been employed to treat alopecia areata.[56,57] This modality appears to act *via* an immunomodulatory mechanism and was suggested to alter T lymphocyte function and perhaps suppress local immunological attack against the hair follicle by depleting Langerhans cells.[60] Photochemotherapy is typi-

cally applied 2 to 3 times per week. Any regrowth is usually detected after 20 to 40 sessions with the maximal effect developing within 1 year. However, the results of PUVA therapy have been less than promising.[61] One study in 70 patients documented that while up to 50% of patients experienced remission with PUVA, rapid hair loss occurred in most when PUVA was stopped such that fewer than 15% of patients experienced a lasting remission.[57] Apart from its mediocre effectiveness, PUVA can induce nausea, possible burning of the scalp, pigmentary alterations, photoaging and squamous cell carcinoma. Consequently, dermatologists no longer prefer this treatment.[61]

3.6 Combination Treatments

The use of drug combinations has now become established practice for treating alopecia areata. For example, topical minoxidil can be combined with topical dithranol in order to restore hair growth. This approach has been assessed in 45 patients with alopecia areata who had not improved on treatment with either agent applied alone or with both agents employed separately. After 6 months of treatment with minoxidil 5% solution and dithranol 0.5%, 5 individuals experienced cosmetically significant hair growth. Adverse effects were almost entirely confined to mild, local scalp irritation.[62] The combination of topical minoxidil 5% plus topical betamethasone dipropionate 0.05% has also been observed to be synergistic for treating severe alopecia.[63] After 4 months of therapy, a fair to good response was found in 56% of individuals receiving minoxidil and the corticosteroid, 27% receiving minoxidil alone, 22% receiving corticosteroids alone and 13% receiving placebo. Synergism may be the result of the differing hypertrichotic mechanisms of the 2 compounds, or alternatively, to prolonged, corticosteroid-induced minoxidil residence time in the dermis.[64] For active disease affecting less than 50% of scalp, the combination of prednisolone 20 mg/day combined with either intralesional triamcinolone every 4 weeks or minoxidil 5% twice daily has been claimed to be more effective than either treatment alone.[65] Although, it should be stated that the prospect of regrowth in individuals who have less than 50% scalp hair loss is quite good even with placebo treatment.

4. New Tools for Hair Growth Research

Until a few years ago, investigations into alopecia treatment were hampered by a lack of suitable research tools. *In vivo* experimentation on the human scalp can only be performed to a limited extent of invasiveness. Despite the fact that some new rodent models have been used, their value is questionable, since the hair growth cycles of humans and rodents are very different.[66] Furthermore, these animals exhibit no readily measurable sexual dimorphism of body hair which is relevant to the important androgen effect found in humans. Therefore, in order to acquire a greater understanding of the molecular mechanisms that control scalp hair growth and in order to test regrowth-promoting drugs, new models such as human scalp histoculture, human scalp explants on mice and the use of the macaque monkey have been developed. A method was proposed for quantitative estimation of hair growth based on changes in hair weight and hair count. Novel research tools include quantitative autoradiography which can be employed to analyse drug localisation within the skin, the use of immunohistochemistry with laser scanning confocal microscopy to study neuropeptide expression in hair follicles and fibre optic confocal imaging (FOCI) which permits the monitoring of dynamic events *in vivo*.

4.1 Hair Follicle Culture Systems

One method for studying hair dynamics involves isolating the complete hair follicle organ and subsequently following its growth in tissue culture. Although many such systems have been documented, the Philpott system has been the most widely used.[15] Developed from human scalp follicles, the technique uses the amputated proximal portion of terminal anagen hair follicles. The follicle portions are then grown for over 1 week in an optimised culture medium. Use of the Philpott sys-

tem has shed light on many aspects of the anagen growth phase.

Another important development has been the histoculturing of whole human scalp tissue.[67] This system consists of scalp skin with abundant hair follicles in various stages of the growth cycle, grown for up to 40 days at the air-liquid interface of a gelatin sponge-supported histoculture. The hair follicles undergo long term growth, shaft elongation and spontaneous regression. Crucially, regression is characterised by the formation of club-shaped hairs, the movement of the hair shaft out of the follicle as well as macrophage association with the follicle. These features indicate the induction of genuine catagen and not simple tissue degeneration. Other physiological changes in the cultured follicles also seemed to parallel those occurring in real scalp tissue. This artificial scalp skin clearly represents a powerful new *in vitro* model for performing hair research. Mechanistic data can be obtained by following drug-histoculture interactions at the cellular level with appropriate techniques such as confocal scanning electron microscopy of fluorescent drugs. The effectiveness of hypertrichotic agents can be evaluated from hair growth measurements.[68] Further improvements in histoculturing can be expected in the near future so the morphology of such membranes should become even closer to that of human skin.

4.2 Human Scalp Explants on Severe Combined Immunodeficient Mice

Another experimental model has been described by Kyoizumi and co-workers[69] who engrafted tissue samples, derived from fetal scalp skin, on to the backs of severe combined immunodeficient (SCID) mice. Although the hair in the engrafted tissues fell out several months after implantation, new black hairs, indicative of human origin, reappeared soon after and grew continuously for more than 1 year. Six months after implantation, histological analysis indicated that the engrafted skin exhibited the same tissue morphology as that of human skin. The sweat glands, sebaceous glands and erector pili muscles were identical to that of normal human scalp skin and approximately 10% of follicles were in the catagen or telogen phases. The research team used this explanted scalp skin model to investigate the effect of X-radiation on epilation rates. Moreover, the model can be employed to analyse the efficacy of regrowth-promoters *in vivo*. Gilhar's group[9] recently adapted this method in order to study the pathogenesis of alopecia areata. The researchers transplanted samples of involved scalp tissue from patients with alopecia areata into the subcutaneous tissue of SCID mice. The graft tissue was then injected with autologous T lymphocytes which had been isolated from affected scalp. The workers were able to demonstrate that T lymphocyte recognition of a follicular autoantigen mediates a role in the autoimmune hair shedding process.

4.3 The Macaque Monkey Model

A quantitative *in vivo* model for studying scalp hair growth has been developed by Uno and co-workers[16,17,70] who have used stumptailed macaque monkeys extensively. At puberty, macaques exhibit a species-specific frontal scalp baldness in both sexes. Crucially, the interplay of genetic and hormonal factors which regulate follicular regression in the species are extremely similar to those active in human androgenetic alopecia. Both topical minoxidil[17] and oral finasteride[71] have been found to reduce baldness in these animals, thus validating the suitability of this *in vivo* model for investigating hair growth stimulating drugs.

4.4 Quantitative Autoradiography by Computerised Image Analysis

In order to improve the delivery of topical hypertrichotic drugs, it is necessary to determine whether active molecules reach their target site in the lower follicle by directly penetrating through the follicular opening, by diffusing *via* the bulk strata or by a combination of both routes. To this end, the technique of quantitative autoradiography by computerised image may be particularly beneficial.[72-74] The method involves interfacing microcomputer-based image analysis with histoautoradiography. This approach has already been employed

to quantify and visualise the follicular deposition of tetrahydrocannabinol and oleic acid within hairless rat skin.[72] 24 hours after drug application, the greatest concentration of tetrahydrocannabinol was observed in the epidermis, followed by the appendages and finally the dermis. In contrast, the concentration of oleic acid, localised in the epidermis, was not significantly different from the concentration in the appendages. The technique has also been used to show that caffeine deposition from liposomes was quite pronounced within the appendages of hairless rat skin.[75] Quantitative autoradiography can be used to elucidate the localisation behaviour and absorption kinetics of existing as well as novel topical hair growth promoters.

4.5 Laser Scanning Confocal Microscopy and Immunohistochemistry

Finding new treatments for alopecia, and for associated nervous symptoms such as itching or pain, requires a clearer understanding of the disease at the molecular level. Hordinsky and Ericson[38] have developed a method which combines laser scanning confocal microscopy with immunological labeling to map the expression of neuropeptides and immune proteins which may be involved in pathological processes. This method has been used to look at the distribution of compounds such as substance P, calcitonin-gene-related peptide, protein-gene-related-peptide and interferon-γ.[38,39,76,77] In addition, perifollicular innervation in tissue sections can be observed, giving us a more detailed picture of the cutaneous sensory nervous system and the innervation of hair follicles. A better understanding of the patterns of neuropeptide expression and modulation of inflammatory processes by the nervous system should help us better understand the aetiology of alopecia areata, thereby paving the way for the development of new treatments.

4.6 Fibre Optic Confocal Imaging

Although fluorescence laser scanning confocal microscopes (FLSCMs) have been employed for several years to visualise stained structures *in vitro*, conventional FLSCMs are limited in application as they are bulky, immobile and require accurate alignment of large components. Consequently, the potential for *in vivo* use of this instrumentation is rather limited. Over the last few years, Delaney and coworkers[78] have devised a prototype, miniaturised fibre optic FLSCM or FOCI system which achieves equivalent resolution in conjunction with greater flexibility. Crucially, FOCI has enabled surface and subsurface fluorescence microscopy of hairless mouse skin *in vivo*.[79] The pre-application of certain fluorescent dyes such as acridine orange and 4-di-2-ASP to the anaesthetised animal resulted in the staining of cellular and subcellular structures of particular interest to the investigators. One noteworthy aspect of the technique is that the imaging plane is virtually parallel to the skin surface – a view not well documented in the literature. For hair growth research, the judicious selection of specific dyes that target particular follicular cells would be highly promising. Implicit in this new technology is the potential to monitor the effects of hypertrichotic drugs on hair growth *in vivo*.

4.7 Quantitative Estimation of Changes in Hair Weight and Hair Count

Finding a compound that fosters hair growth necessitates a means for accurately determining changes in hair growth. To this end, Price and Menefee[21] proposed a straightforward and useful means of assessing the effect of treatment on both hair growth promotion and on retardation of hair loss, using total hair weights and counts. In this method, a site is selected on thinning frontal scalp and the hair clipped to about 1mm length using a magnifying glass. Hair is then clipped at 6-week intervals thereafter until the end of the study. In 1 study, individuals treated with minoxidil 2 or 5% showed substantially greater hair mass and change in mean weight, than the placebo groups. In terms of mean weight, the 5% group showed a greater increase than the 2% group.

5. Future Approaches for Hair Regrowth

In the years ahead, the efficacy of existing alopecia treatments may be improved by the use of novel penetration enhancers, combination treatments and the application of targeting technology. An additional strategy is the synthesis or discovery of novel hair growth-stimulating molecules. Exciting breakthroughs in gene therapy may also constitute a radically new approach in the near future.

5.1 Enhanced Delivery and Targeting to the Pilosebaceous Units

A decade ago, Dervault and coworkers[42] reported that applications of 2-n-nonyl-1,3-dioxolane (dioxolane) resulted in accelerated minoxidil transport through both sections of both hairless rat skin and human skin. In other investigations which utilised the *in vivo* stumptailed macaque model, formulations containing either minoxidil, minoxidil and the enhancer, or a placebo vehicle were applied to the animals' balding scalps at fixed intervals over a 16-week period.[80] Hair growth was assessed by weighing shaved hairs at 4-week periods. It was found that the dioxolane treatments enhanced minoxidil absorption, producing earlier and greater stimulation of scalp hair growth. The authors speculated that the effect could occur by either direct penetration enhancement or alternatively by improved minoxidil targeting into the pilosebaceous structures, facilitated by the sebum-miscible dioxolane derivative.

One promising avenue of research involves the use of vesicular systems that can selectively target encapsulated hypertrichotic molecules to their site of action in the hair follicle. However, it must be considered that liposome penetration into the follicle may be physically restricted by the glassy membrane surrounding the entire follicle as well as the keratinous layers of the inner and outer root sheaths. The outer root sheath is of particular importance for drug delivery since this layer is continuous with the epidermis and indistinguishable from it. The upward migration of sebum within the upper third of the follicular canal, may well impede liposome penetration.[81] It has been proposed that by matching formulation polarity to sebum polarity, follicular delivery of a wide range of compounds may be achieved.[82] Effective follicular penetration would probably be facilitated by optimising formulation factors such as pH, ionic strength and lipid composition. Vesicular size is probably also important. Schaefer's group[40] observed that preferential follicular deposition occurred for fluorescent polystyrene beads ranging between 5 to 7 microns in diameter, whereas beads outside this size range tended to accumulate on the skin surface.

Pilosebaceous targeting using liposome-encapsulated drugs was also studied by Li and Hoffman,[41] who compared the *in vivo* deposition of liposome-encapsulated calcein with that of free calcein on mouse skin. Calcein from the vesicular formulation penetrated deeply into the hair shafts and hair follicle cells, in contrast to the free calcein which remained on the skin surface. Crucially, the vesicular system did not deliver any calcein into the systemic circulation over 24 hours, thus indicating that targeted follicular delivery is possible. The penetration of liposome-encapsulated calcein through histocultured human scalp tissue has also been investigated in a recent pilot study.[67] Liposomal applications resulted in significant calcein delivery into the hair follicles without detectable deposition at nonfollicular sites.

Some research groups have employed especially-designed nonconventional vesicular systems. Weiner's team[44] used nonionic liposomes to enhance the *in vivo* delivery of both cyclosporin and interferon-α through hamster ear skin (a model skin containing human-like pilosebaceous units). The results indicated that protein penetration was probably mediated *via* the follicular pathway. Our group recently encapsulated minoxidil within ethosomes (novel vesicular carriers) and applied the formulation to hairless mouse skin *in vitro*.[83,84] Analysis by quantitative autoradiography indicated that minoxidil preferentially accumulated in the hair follicles and sebaceous glands. Importantly, both skin permeation and skin depot formation could be modulated by altering the system composition.

This approach may improve the hypertrichotic effect of minoxidil and further investigations are warranted.

There is mounting evidence that vesicular systems may also facilitate gene targeting to the appendages. Li and coworkers[85] used phosphatidylcholine liposomes to deliver entrapped [^{35}S-labeled]DNA to a mouse skin histoculture complete with hair follicles. After a 44-hour incubation period, large amounts of radiolabel had become incorporated into the cell membrane, cytoplasm and nucleus of the hair follicle cells. In contrast, only minor radiolabel uptake occurred when free [^{35}S-labeled]DNA was incubated with the histoculture. Another report documented the *in vivo* follicular delivery of a liposomally-entrapped bacterial *lac-Z* gene into mouse skin.[86] After a 3-day contact period and appropriate histological processing, it was observed that the *lac-Z* gene was selectively expressed in the hair matrix cells of the hair follicle bulbs and in the follicular bulge area. Topical application of the free *lac-Z* gene did not result in gene transfer. Such liposomal gene targeting to the hair-forming matrix cells suggests that it may be possible to deliver genes that will selectively restore hair growth. Clearly, liposome technology requires further research so that the host of physicochemical parameters which control vesicular targeting to the follicles can be identified and optimised.

5.2 Novel Combination Treatments

Early work provided evidence that the combination of the synthetic retinoid, tretinoin and minoxidil was more hypertrichotic than minoxidil alone.[87] In further studies performed by Ferry and co-workers,[30] 19 men with androgenetic alopecia were treated for 20 days with a minoxidil 2% solution either alone or together with daily applications of tretinoin cream or placebo cream. Tretinoin increased systemic minoxidil absorption by 3-fold. The retinoid also increased transepidermal water loss but did not affect the stratum corneum or epidermal thickness. These results demonstrated that the cornified layer was permeabilised to minoxidil penetration. Unfortunately, the currently available proprietary forms of minoxidil and tretinoin are mutually incompatible within the same solution.[12] Dual treatment would require twice daily minoxidil application as well as a separate tretinoin application, presenting an impractical protocol for most men.

The effectiveness of a combined finasteride-minoxidil approach has been investigated by Diani and co-researchers[88] who conducted a 20-week controlled study on balding, stumptailed macaques. The derived data indicated that oral finasteride and topical minoxidil were equally as effective in significantly increasing scalp hair densities in the animal. The combination of topical minoxidil 2% and oral finasteride 0.5mg daily produced an additive hypertrichotic effect. Minoxidil with oral finasteride has so far been evaluated in one case study where it was found to be effective.[89] However, since minoxidil was formulated in a retinoid-containing vehicle, it is difficult to ascertain the growth gain contribution of each of the 3 drugs. Clearly, further clinical work is required on this particular topic.

5.3 Novel Hair Growth Promotors

Some investigators have proposed that a microbial-driven inflammatory reaction abutting the pilosebaceous units might exacerbate the progression of androgenetic alopecia.[23,90] In order to test this hypothesis, Pierard and co-workers[90] treated 20 men, exhibiting male-pattern baldness, with a lotion containing both the antifungal agent, piroctone olamine (0.25%) and the antibacterial agent triclosan (0.3%). The treatment lasted 18 months during which hair densities were evaluated by macrophotography and trichograms taken at 3-month intervals. Although the trial was uncontrolled, the lotion applications produced progressive cosmetic benefit. More recently, ketoconazole, which is active against scalp microflora and exhibits intrinsic anti-inflammatory activity, has also been investigated.[22] 39 males, showing medium-severity vertex androgenetic alopecia, applied either ketoconazole 2% shampoo once daily or a placebo shampoo once daily over a 21-month period. Biop-

sies indicated that there were significant increases in both the proportion of follicles in anagen and in the median hair shaft diameter. Ketoconazole may therefore have considerable potential as a topical treatment for androgenetic alopecia. Nevertheless, this therapy awaits further clinical investigation in a larger group of individuals.

There has also been some progress in the identification of new modalities for treating alopecia areata. McElwee and co-workers[91] have experimented with the macrolide tacrolimus (FK-506), a specific immune cell inhibitor. The topically applied drug was assessed for hair regrowth potential in the Dundee experimental bald rat, a species that frequently exhibits an alopecia areata-type hair loss from the age of 4 months onwards. Hair at the site of drug application regrew within 2 to 3 weeks in tacrolimus-treated rats. At the clinical level, there has been a preliminary report describing the use of pulse corticosteroid therapy for treating children with severe, rapidly evolving alopecia areata.[92] Intravenous methylprednisolone infused twice a day for 3 days resulted in complete regrowth in a large fraction of the patients after one year. There has also been considerable interest in the immunomodulatory potential of novel phototherapies. These would involve the application of non-UV light sources such as lasers and light-emitting diode arrays.[12]

Another approach would be to identify compounds that inhibit the binding of endogenous steroids to the androgen receptor. One such compound, RU-58841, was found to inhibit 70% of dihydrotestosterone binding to androgen receptors purified from human scalp hair follicles, with a calculated Ki of 0.4 nmol/L.[93]

5.4 Gene Therapy

At present, it is unclear how many genes are important to hair follicle morphogenesis, differentiation and growth. Ahmad and coworkers[94] made an important contribution to this field when they identified the gene responsible for papular atrichia, the human analogue of the gene inducing hairlessness in the hairless mouse. The topical delivery of transgenes to the hair follicles represents an exciting possibility. Recently, it was shown that liposome-DNA complexes (lipoplexes) could be used to efficiently transfect the follicles in a human skin xenograft model.[95] Despite the fact that androgenetic alopecia and alopecia areata are both probably polygenic disorders, it is likely that in the future, more hair growth-regulating genes will be identified making gene therapy a potential treatment of the future. Treatment for androgenetic alopecia could involve suppression of the synthesis of 5α reductase or the androgen receptor protein. Follicular stem cell gene therapy may also be developed in the future and would facilitate changes in DNA transcription and RNA translation of enzymes and receptors involved in follicle inactivation.[12] For alopecia areata treatment, gene replacement therapy could eventually allow for permanent correction of defective gene expression.[12]

6. Conclusions

To date, only topical minoxidil and oral finasteride are available as drug treatments for inducing hair regrowth in androgenetic alopecia (see table I). In terms of compliance, finasteride with a once daily tablet administration is easier than the twice a day lotion application of minoxidil. However, this benefit should be weighed against the lesser invasiveness and lower probability of adverse effects that are associated with topical minoxidil treatment. The major limitation in both cases lies in the fact that they exhibit only partial effectiveness. However, data derived from primate studies indicate that a combination of the 2 drugs results in an additive hypertrichotic effect. Trials should be performed in order to determine whether this is the case in humans. There is evidence that the minoxidil effect in androgenetic alopecia can be improved by the incorporation of enhancers in the formulation and further research is warranted in this area. A topical finasteride lotion may offer greater effectiveness than the oral formulation and such formulations should be developed and evaluated. There are indications that topical antimicrobial and antifungal agents may also be beneficial. In the long

term, gene therapy, probably facilitated by liposomal targeting, may constitute an efficacious treatment for androgenetic alopecia.

There are currently several different modalities available for treating alopecia areata and therapeutic efficacy often depends upon individual disease severity (see table II). New treatments are constantly being researched. Vesicular targeting of immunocompetent cells or replacement genes to the follicles may have profound therapeutic implications for influencing hair growth in this disease.

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