

REVIEW ARTICLE

Minoxidil: mechanisms of action on hair growth

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Summary

We have known for over 30 years that minoxidil stimulates hair growth, yet our understanding of its mechanism of action on the hair follicle is very limited. In animal studies, topical minoxidil shortens telogen, causing premature entry of resting hair follicles into anagen, and it probably has a similar action in humans. Minoxidil may also cause prolongation of anagen and increases hair follicle size. Orally administered minoxidil lowers blood pressure by relaxing vascular smooth muscle through the action of its sulphated metabolite, minoxidil sulphate, as an opener of sarcolemmal K_{ATP} channels. There is some evidence that the stimulatory effect of minoxidil on hair growth is also due to the opening of potassium channels by minoxidil sulphate, but this idea has been difficult to prove and to date there has been no clear demonstration that K_{ATP} channels are expressed in the hair follicle. A number of *in vitro* effects of minoxidil have been described in monocultures of various skin and hair follicle cell types including stimulation of cell proliferation, inhibition of collagen synthesis, and stimulation of vascular endothelial growth factor and prostaglandin synthesis. Some or all of these effects may be relevant to hair growth, but the application of results obtained in cell culture studies to the complex biology of the hair follicle is uncertain. In this article we review the current state of knowledge on the mode of action of minoxidil on hair growth and indicate lines of future research.

Key words: androgenetic alopecia, hair, minoxidil

Minoxidil was introduced in the early 1970s as a treatment for hypertension. Hypertrichosis was a common side-effect in those taking minoxidil tablets^{1,2} and included the regrowth of hair in male balding.³ This led to the development of a topical formulation of minoxidil for the treatment of androgenetic alopecia in men and subsequently in women. The 2% product was first marketed for hair regrowth in men in 1986 in the United States and the 5% product became available in 1993.

Despite much research over 20 years we still have only a limited understanding of how minoxidil

stimulates hair growth. Nevertheless, understanding minoxidil's mechanism of action is important, both from the point of view of developing more effective treatments for hair loss disorders and for the insights it may give into the biology of hair growth. In this article we review what is known about the pharmacology of minoxidil, with particular reference to its action on hair growth, and suggest directions for future research.

Response of the hair follicle to minoxidil

There are a number of ways in which a drug may stimulate hair growth; it may increase the linear growth rate of hair, increase the diameter of the hair fibre, alter the hair cycle, either shortening telogen or prolonging anagen, or act through a combination of these effects. Present evidence suggests that minoxidil acts mainly on the hair cycle; it may also increase hair diameter.

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Conflicts of interest: Dr Messenger is a dermatologist and has been a consultant for Pharmacia and other pharmaceutical companies with an interest in the field of hair growth. Dr Rundegren is an employee of Pharmacia.

Animal studies

Mori and Uno⁴ studied the effect of topical application of minoxidil on spontaneous hair cycles in the rat from birth to 80 days of age. Minoxidil had no effect on the duration of anagen, but telogen was shortened. The telogen phase of the third cycle lasted approximately 20 days in untreated animals, whereas follicles re-entered anagen after only 1–2 days in telogen in minoxidil-treated animals. The same shortening of telogen by minoxidil treatment was also seen in the fourth cycle. The effect of minoxidil on hair growth has been studied extensively in the stump-tailed macaque, a primate that develops postadolescent scalp hair loss closely resembling human androgenetic alopecia. Topical minoxidil prevents the development of scalp hair loss in periadolescent macaques and promotes re-growth of hair in balding animals. Histological studies showed that treatment with minoxidil causes an increase in the proportion of follicles in anagen, a reduction in telogen follicles, and an increase in hair follicle size.⁵

Humans

Little is known of the effect of minoxidil on normal human hair growth and studies have been limited mainly to the response of androgenetic alopecia to topical minoxidil. In male pattern balding (male androgenetic alopecia) there is a gradual reduction in the duration of anagen and a prolongation of the latent period of the hair cycle (the time between shedding of the telogen hair and the onset of the next anagen).⁶ Hair follicles also become miniaturized.⁷ There is some controversy over whether female androgenetic alopecia is the same entity as male balding. Nevertheless, the follicular changes are very similar,^{8,9} if not identical, although prolongation of the latent period has not yet been demonstrated in women. Clinical trials of topical minoxidil in male and female hair loss all show a remarkably rapid increase in hair growth, measured by hair counts or hair weight. The increase is evident within 6–8 weeks of starting treatment and has generally peaked by 12–16 weeks (Fig. 1). It seems improbable that a response of this rapidity can be accounted for by reversal of follicular miniaturization, and a more likely explanation is that minoxidil triggers follicles in the latent part of telogen into anagen. The hypertrichosis that develops in humans taking minoxidil orally, and occasionally following topical use, may affect the forehead as well as other sites such as the

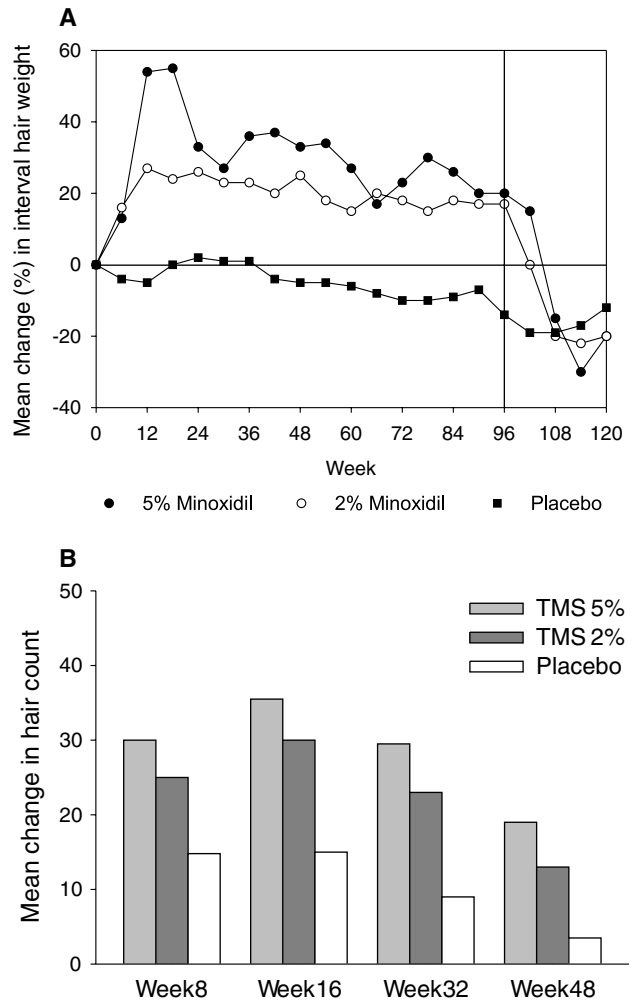


Figure 1. Results of two clinical trials of minoxidil topical solution in the treatment of male androgenetic alopecia using different methods for measuring the response. Both methods show a rapid increase in hair growth which has reached a plateau by 12–16 weeks. (A) Comparison of mean percentage change in interval hair weight per square centimetre for three treatment groups: 5% minoxidil, 2% minoxidil and placebo. Vertical line at 96 weeks indicates cessation of treatment. Adapted from Price *et al.*⁶⁵ (B) Mean change from baseline in nonvellus hair counts (per square centimetre) in men treated with 5% minoxidil solution (TMS), 2% minoxidil and placebo. From Olsen *et al.*⁶⁶

limbs. The increased length of hair at these sites suggests that minoxidil also prolongs the duration of anagen in humans.

The results of histological studies in humans are less conclusive than in the macaque. Abell¹⁰ found a trend towards an increase in anagen/telogen ratios after 12 months of minoxidil treatment in balding men, but the main change was an increase in mean hair diameter. This was most apparent at 4 months and

mean diameter had declined at 12 months. He suggested this might be due to later recruitment of small diameter hairs into anagen. Headington and Novak¹¹ reported that minoxidil treatment caused hypertrophy of follicles but, although there was an increase in mean hair diameter in minoxidil-treated balding men after 12 weeks, a similar increase occurred in control subjects. Care should be taken in interpreting change in mean hair diameters. This does not necessarily imply that individual hair follicles become larger, as an increase in mean diameter may also occur through preferential recruitment of large diameter hairs in a latent phase of the hair cycle.

Minoxidil sulphation

The antihypertensive activity of minoxidil is due to rapid relaxation of vascular smooth muscle by its sulphated metabolite, minoxidil sulphate.^{12,13} The conversion of minoxidil to minoxidil sulphate is catalysed by sulphotransferase enzymes. Minoxidil sulphotransferase activity was initially demonstrated in rat liver¹² and has since been found in human liver,¹⁴ platelets¹⁵ and epidermal keratinocytes,¹⁶ as well as in mouse vibrissae follicles,¹⁷ rat pelage and vibrissae follicles and rat epidermal keratinocytes.^{18,19} In scalp skin of stump-tail macaques, sulphotransferase activity is largely localized in the hair follicle.²⁰ In rat pelage and vibrissae follicles, immunoreactivity for minoxidil sulphotransferase was seen in the outer root sheath.¹⁸

Five human cytosolic sulphotransferase genes have been discovered to date. They encode three classes of enzymes responsible for sulphating phenols and catecholamines, oestrogens and hydroxysteroids.²¹ In human liver extracts, sulphation of minoxidil is catalysed by at least four sulphotransferases. Biochemical evidence for minoxidil sulphation by two phenol sulphotransferases has been found in human scalp skin²² and Dooley²¹ reported finding mRNA expression for four sulphotransferases in human epidermal keratinocytes. There are interindividual variations in scalp sulphotransferase activity and this correlates with the level in platelets.²² In a clinical setting, scalp sulphotransferase activity was higher in men who responded to minoxidil compared with those who did not respond.²³

Minoxidil sulphate is a potassium channel opener

Minoxidil sulphate is one of several chemically unrelated drugs which cause opening of plasma membrane adenosine triphosphate (ATP)-sensitive potassium

channels (K_{ATP} channels), and its relaxant effect on vascular smooth muscle is mediated through this mechanism.^{24,25} K_{ATP} channels are heteromultimers composed of a small subunit that belongs to the inwardly rectifying potassium channel superfamily ($K_{IR6.1}$ or $K_{IR6.2}$), and a large sulphonylurea receptor (SUR1, SUR2A or SUR2B) that binds sulphonylureas and ATP and belongs to the ATP-binding cassette (ABC) superfamily.²⁶ SUR1/ $K_{IR6.2}$ K_{ATP} channels are found in pancreatic and neuronal tissue, whereas SUR2A/ $K_{IR6.2}$ and SUR2B/ $K_{IR6.1}$ (or $K_{IR6.2}$) form the cardiac and vascular smooth muscle K_{ATP} channels, respectively. Potassium channel openers act through binding to the sulphonylurea receptor moiety.²⁶

K_{ATP} channels are widely distributed in a variety of tissue and cell types, including cells of the heart, pancreas, vascular smooth muscle and the central nervous system, where they couple intracellular metabolic changes to the electrical activity of the plasma membrane.²⁷ These potassium channels sense the metabolic state of the cell—channel opening is inhibited by ATP when energy levels are high and is activated when energy stores are depleted.²⁸ The consequence of K_{ATP} status depends on the cell and tissue type. For example, in pancreatic β cells, K_{ATP} channels are involved in regulating insulin secretion. In vascular smooth muscle cells the vasodilating action of potassium channel openers is due to membrane hyperpolarization and a reduction in Ca^{2+} influx, which reduces the electrical excitability of the cell. It has also been suggested that potassium channel activity is required for early-stage cell proliferation by G_1 progression of the cell cycle.²⁹ Minoxidil was shown to increase DNA synthesis, whereas glibenclamide suppressed DNA synthesis in rat primary hepatocyte cultures.³⁰ Hepatocyte potassium currents were augmented by minoxidil and attenuated by glibenclamide.

Does minoxidil act on hair growth via potassium channels?

Several lines of evidence, from clinical observations, animal studies and *in vitro* experiments, suggest that the promotion of hair growth by minoxidil is related in some way to its action as a potassium channel opener (Table 1).

In vivo studies

In addition to minoxidil, the potassium channel openers diazoxide^{31,32} and pinacidil³³ cause hypertrichosis

Table 1. Does minoxidil act on hair growth via potassium channels?*Evidence for*

1. Chemically unrelated potassium channel openers stimulate hair growth:
 - a. in humans (minoxidil, diazoxide, pinacidil)
 - b. in macaques (minoxidil, cromakalin, P-1075)
2. Chemically unrelated potassium channel openers stimulate thymidine and/or cysteine uptake by mouse vibrissae follicle *in vitro* (minoxidil, pinacidil, cromakalin, nicorandil, P-1075, diazoxide)
3. Stimulation of 3T3 fibroblast proliferation by minoxidil *in vitro* inhibited by potassium channel antagonists (tolbutamide, tetraethylammonium).

Evidence against

1. Stimulation of thymidine/cysteine uptake by minoxidil in cultured mouse vibrissa follicles not blocked by potassium channel antagonists.
2. ^{86}Rb efflux in vibrissae follicle cultures not increased by minoxidil.
3. K_{ATP} channels not demonstrated in cultured hair follicle cells by patch-clamp methods.

in humans. Buhl *et al.*³⁴ tested the effect of topical application of minoxidil and three other potassium channel openers on scalp hair growth in balding macaques. Minoxidil, cromakalin and P-1075 (a pinacidil analogue) all stimulated hair growth over a 20-week treatment period. A fourth potassium channel opener, RP-49,356, was not effective.

Organ culture studies

Buhl *et al.*³⁵ carried out a series of experiments on minoxidil action using cultured mouse vibrissae follicles. In 3-day cultures, 1 mmol L⁻¹ minoxidil preserved follicular morphology, whereas follicles cultured in the absence of minoxidil degenerated rapidly. Follicles cultured in 0.5–5 mmol L⁻¹ minoxidil grew longer than controls and showed higher levels of uptake of radiolabelled cysteine, amino acids and thymidine. This effect appears to be mediated by minoxidil sulphate. The same results were obtained using approximately 100-fold lower concentrations of minoxidil sulphate and the response of cultured follicles to minoxidil, but not minoxidil sulphate, was blocked by diethylcarbamazine and chlorate, agents which interfere with sulphation.¹⁷ The potassium channels openers pinacidil, cromakalin, nicorandil and P-1075 also stimulated uptake of radiolabelled cysteine in cultured vibrissae follicles, although diazoxide did not.³⁶ Harmon *et al.*³⁷ also reported that minoxidil, pinacidil, cromakalin and diazoxide increased uptake of thymidine in a dose-dependent fashion in 4-day cultures of mouse vibrissae follicles. These studies imply that minoxidil stimulates

hair growth in this model by opening potassium channels, but attempts to verify this idea have been unsuccessful. The broad-spectrum ion channel blocker tetraethylammonium chloride and the K_{ATP} channel blockers, glyburide and tolbutamide, failed to inhibit minoxidil stimulation of cultured vibrissae follicles at doses that were not themselves toxic.³⁴ To test whether minoxidil opened ion channels, vibrissae follicles were labelled with $^{86}\text{Rb}^+$, an ion with specificity for potassium channels similar to K^+ . In this model, the potassium channel opener pinacidil increased efflux of $^{86}\text{Rb}^+$ but minoxidil did not.³⁴

Human hair follicle organ culture has been used extensively in hair biology but there is only a single published report describing increased uptake of thymidine by cultured human hair follicles in response to minoxidil.³⁸ Minoxidil causes premature entry of follicles into anagen, and probably prolongs anagen and increases hair follicle size. Of these effects only the prolongation of anagen is possibly modelled by hair follicle organ culture and even here the alteration in follicle survival *in vitro* is measured in days rather than the weeks or months achieved *in vivo*. The rather mixed responses of cultured follicles to minoxidil may therefore be due to insensitivity or inapplicability of the model. However, minoxidil does prolong survival of cultured follicles that would otherwise undergo rapid degeneration *in vitro*, albeit at concentrations which are unlikely to be achieved *in vivo*. This effect appears to be mediated by the sulphated metabolite and there is circumstantial but, as yet, unconfirmed evidence that it involves opening of potassium channels.

Cell culture studies

Sanders *et al.* showed that the stimulatory effect of minoxidil on the growth of 3T3 fibroblasts is inhibited by pharmacological blockade of potassium channels.³⁹ As yet, however, there is no clear evidence that K_{ATP} channels are expressed in cells of hair follicle derivation. Nakaya *et al.* looked for potassium channels in cultured hair follicle outer root sheath and dermal papilla cells using the patch-clamp technique.⁴⁰ They identified large and small conductance calcium-activated potassium channels in cell membranes. These channels were not blocked by ATP or glibenclamide (a specific K_{ATP} channel blocker) and neither minoxidil sulphate nor pinacidil increased efflux of ^{86}Rb , suggesting the absence of K_{ATP} channels. However, the same group has recently reported that human dermal papilla cells express mRNA for the sulphonylurea

receptor SUR2B,⁴¹ the same sulphonylurea receptor expressed in vascular smooth muscle cells.

The cellular response to minoxidil

Whatever the mechanism whereby minoxidil modulates hair growth, there must be a primary effect on cell function (Table 2). The hair follicle is a complex structure comprising epithelial, dermal, pigment and immune cells, and a perifollicular vasculature and neural network. Interactions between these cells are involved in regulating epithelial growth and differentiation and the hair cycle. Several of these cell types have been used in isolation to study minoxidil action, but attempts to localize minoxidil or a minoxidil metabolite binding to a specific cell population within the hair follicle have been unsuccessful.⁴² Uptake studies in mouse vibrissae follicles showed that minoxidil and minoxidil sulphate concentrated in melanocytes and pigmented epithelial cells in the suprapapillary region of the follicle. However, this was probably due to nonspecific binding to melanin as there was no evidence of minoxidil binding in nonpigmented follicles yet pigmented and nonpigmented follicles showed a similar growth response to minoxidil.⁴³

Cell proliferation

Several studies have examined the effect of minoxidil on cell proliferation *in vitro*. A variety of cell types have been used including epidermal keratinocytes, hair follicle keratinocytes and skin fibroblasts from humans, mice and macaques. In some studies, established keratinocyte and fibroblast cell lines have been used. The results have been variable and, to some extent, contradictory.

Table 2. Effects of minoxidil on cell function

Cell growth	Variable effects on growth and survival of cells in culture. In different studies minoxidil has been reported to inhibit or stimulate growth of epithelial and fibroblast cell types. Delays senescence in keratinocyte cultures.
Collagen synthesis	Inhibits lysyl hydroxylase Inhibits collagen production
Prostaglandin synthesis	Stimulates PGE ₂ synthesis Inhibits prostacyclin production
VEGF	Stimulates VEGF synthesis by dermal papilla cells Stimulation of VEGF synthesis mediated by adenosine

VEGF, vascular endothelial growth factor.

Boyera *et al.*⁴⁴ studied the effect of minoxidil on human keratinocytes of epidermal and hair follicle origin using a range of different culture conditions and proliferative markers. They found that micromolar concentrations of minoxidil stimulated proliferation in both cell types and in all culture conditions, whereas millimolar concentrations inhibited cell growth. In cells cultured from the stump-tail macaque, minoxidil stimulated thymidine uptake by follicular keratinocytes but not by epidermal keratinocytes.⁴⁵ O'Keefe and Payne⁴⁶ also failed to show a stimulatory response to minoxidil in cultured human epidermal keratinocytes, although Baden and Kubilus⁴⁷ reported that minoxidil prolonged the time after confluence that keratinocytes could be subcultured.

Studies using fibroblasts have yielded similarly variable results. Murad and Pinnell⁴⁸ reported that high concentrations of minoxidil inhibited growth of human skin fibroblasts. On the other hand, thymidine uptake was increased in macaque follicular fibroblasts cultured in micromolar concentrations of minoxidil, but not in nonfollicular fibroblasts.⁴⁵ Sanders *et al.*³⁹ proposed that the variable results of cell culture experiments may be explained by the potassium channel-blocking activity of aminoglycoside antibiotics, routinely incorporated into cell culture media. Minoxidil stimulated growth of NIH 3T3 fibroblasts cultured in the absence of aminoglycosides but not in their presence, and the proliferative response of 3T3 cells to minoxidil was prevented by the potassium channel blockers tolbutamide and tetraethylammonium. In cultured human keratinocytes, aminoglycoside antibiotics partly suppressed the proliferative response to minoxidil but did not abolish it.⁴⁴

The variations in the cell types and experimental protocols used mean that it is difficult to compare the results from these studies. On balance, they suggest that minoxidil can have a stimulatory effect on cell growth at clinically relevant concentrations, or delay cell senescence, and there is limited evidence that this is mediated by its action as a potassium channel opener.

Collagen synthesis

Two groups have studied the effect of minoxidil on collagen synthesis. Murad and Pinnell⁴⁸ showed that minoxidil suppressed activity of the enzyme lysyl hydroxylase in human skin fibroblast cultures at concentrations down to 25 µmol L⁻¹, leading to production of a collagen deficient in hydroxylysine.⁴⁹ This appeared to be specific for lysyl hydroxylase as the activity of prolyl hydroxylase, which shares the same substrates and cofactors as lysyl hydroxylase, was

unaffected. Minoxidil (0.5 mmol L^{-1}) also suppressed collagen synthesis by rat vibrissae dermal papilla cells, both in monolayer cultures and in cells grown in collagen gels.⁵⁰ The concentrations of minoxidil used in these studies were quite high and the relevance of the results to hair growth is unknown.

Prostaglandins

The prostaglandin PGH_2 is formed from arachidonate by the action of a cyclooxygenase (COX), also known as prostaglandin endoperoxide synthase (PGHS). PGH_2 is the substrate for subsequent enzymatic modifications leading to the prostaglandins (PGD_2 , PGE_2 , $\text{PGF}_{2\alpha}$), prostacyclin (PGI_2) and thromboxane A_2 . There are two isoforms of PGHS, a widely distributed constitutive form PGHS-1, and an inducible form PGHS-2. The PGHS-1 isoform has been immunolocalized to the dermal papilla of human hair follicles during anagen and catagen.⁵¹ Immunostaining for PGHS-2 was also seen in the dermal papilla but staining was weaker than that for PGHS-1 and was present only in anagen follicles. Minoxidil ($\text{AC}_{50} = 80 \text{ } \mu\text{mol L}^{-1}$) stimulated the activity of purified ovine PGHS-1 *in vitro* and increased production of PGE_2 in cultured human dermal papilla cells and mouse fibroblasts. Lachgar *et al.*⁵⁰ also found that minoxidil ($12 \text{ } \mu\text{mol L}^{-1}$) stimulated PGE_2 production by cultured dermal papilla cells, in this case derived from rat vibrissae, as well as production of leukotriene B_4 . They also found that minoxidil inhibited prostacyclin synthesis by dermal papilla cells (measured as 6-keto-prostaglandin $\text{F}_{1\alpha}$), as had an earlier study using bovine endothelial cells.⁵² Prostanoids have many biological functions in different tissues, acting through specific G protein-coupled receptors⁵³ and, in some cases, via nuclear receptors.⁵⁴ We do not know whether prostanoids have a physiological role in regulating hair growth, although, latanoprost, a topical synthetic $\text{PGF}_{2\alpha}$ analogue used in the treatment of glaucoma, causes hypertrichosis of the eyelashes.⁵⁵ Topical treatment with latanoprost also stimulates hair regrowth on the scalp in balding stump-tail macaques.⁵⁶

Androgen responses

Nuck *et al.*⁵⁷ studied the antiandrogenic potential of minoxidil on androgen-dependent cutaneous structures of the flank organ of female golden Syrian hamsters. Neither 1% nor 5% minoxidil topical solution applied to one flank for 3 weeks prevented the androgen-dependent growth of the pigmented spot, sebaceous glands or

hair follicle diameter induced by subcutaneous capsules filled with testosterone. However, significant inhibition was seen following topical application of 5% progesterone. The effect of minoxidil on human hair growth is not confined to androgen-dependent hair follicles and these findings are consistent with the conclusion that minoxidil does not act through androgen pathways. However, Sato *et al.*⁵⁸ reported that minoxidil stimulates 17β -hydroxysteroid dehydrogenase (17β -HSD) in cultured human dermal papilla cells and also has a small stimulatory effect on 5α -reductase activity. 17β -HSD catalyses the interconversion of testosterone and androstenedione and may therefore increase or reduce androgen responses. A high concentration of minoxidil (0.5 mmol L^{-1}) was used in this study and the relevance of the results to hair growth *in vivo* is unclear.

Vascular effects

The idea that minoxidil stimulates hair growth by increasing cutaneous blood flow has been the subject of two studies giving contradictory results. Wester *et al.*⁵⁹ studied the effect of topical minoxidil (1%, 3%, 5%) on blood flow in balding scalp using laser Doppler velocimetry (LDV) and photopulse plethymography. Both methods showed an increase in skin blood flow following application of minoxidil that was statistically significant with the 5% solution. On the other hand, Bunker and Dowd,⁶⁰ also using LDV, failed to find any change in skin blood flow following application of 3% minoxidil topical solution to the scalp in 10 balding men, whereas all but one showed an increase in blood flow after applying the vasodilator 0.1% hexyl nicotinate. The difference in results may have been due to the higher concentration of minoxidil used in the first study although, as Bunker and Dowd point out, 3% minoxidil topical solution is clinically effective. Sakita *et al.*⁶¹ studied the effect of minoxidil topical solution on the hair follicle vasculature in the rat using transmission electron microscopy. In minoxidil-treated animals there was no difference in the total area of follicular capillaries compared with controls but there was an increase in capillary fenestrations. The authors suggested that the increase in fenestrations may be due to vascular endothelial growth factor (VEGF) (see below), but the functional significance of this observation was not discussed.

Vascular endothelial growth factor

VEGF has a central role in promoting angiogenesis as well as influencing diverse cell functions including cell

survival, proliferation and the generation of nitric oxide and prostacyclin.⁶² The perifollicular capillary network is coupled to the hair cycle, increasing during anagen and then regressing during catagen and telogen. Yano *et al.*⁶³ found that capillary proliferation during anagen was temporally and spatially associated with expression of VEGF in the outer root sheath of murine hair follicles. Transgenic overexpression of VEGF in the outer root sheath increased perifollicular vascularization and led to accelerated hair growth following depilation and the growth of larger hairs. This effect was prevented by systemic administration of a VEGF antibody. Lachgar *et al.*⁶⁴ found that the expression of VEGF mRNA and protein in cultured human dermal papilla cells was stimulated by minoxidil in a dose-dependent fashion. A fivefold increase in VEGF protein occurred in extracts of cells incubated with 12 $\mu\text{mol L}^{-1}$ minoxidil, and there was a similar increase in mRNA expression. A possible mechanism for minoxidil stimulation of VEGF has been proposed by Li *et al.* from experiments on cultured dermal papilla cells.⁴¹ They found that adenosine also increases VEGF release and the VEGF response to minoxidil was prevented by pharmacological blockade of A1 and A2 adenosine receptors. mRNAs for the A1, A2A and A2B adenosine receptors, as well as the sulphonylurea receptor SUR2B, were detected by the reverse transcriptase–polymerase chain reaction. The authors suggested that binding of minoxidil to SUR2B promotes secretion of ATP, which is rapidly converted to adenosine and activates adenosine signalling pathways.

Conclusions

The emergence of topical minoxidil for the treatment of androgenetic alopecia in the early 1980s led to the realization that hair loss is potentially treatable and ushered in a new era in hair research. The series of experiments by Buhl and others on cultured vibrissae follicles and on the stump-tail macaque support the view that the hair follicle response to minoxidil is mediated by its sulphated metabolite acting as a potassium channel opener. Nevertheless there are inconsistencies in the results that have yet to be resolved and this idea must be viewed as unproven. A variety of responses to minoxidil have been described in cultured cells. Some have potential relevance to hair growth, such as the effects on cell growth and senescence and the stimulation of VEGF and prostaglandin synthesis. Others, such as the effects on collagen synthesis, are more difficult to explain. Viewed in isolation, the results of cell culture studies must be

interpreted with care. First, the relationship between the complexities of hair growth and the behaviour of a single cell type cultured in a Petri dish is uncertain. Second, the concentrations of minoxidil used have often exceeded those to which the hair follicle is likely to be exposed *in vivo*. Blood levels in subjects taking minoxidil orally are in the upper nanomolar/low micromolar range (20–2000 ng mL^{-1}) and are lower still in those using minoxidil topically ($\approx 2 \text{ ng mL}^{-1}$). Third, the minoxidil target cell population in the hair follicle is unknown. Nevertheless, the stimulation of VEGF and prostaglandin synthesis by minoxidil in dermal papilla cells provides an attractive and logical starting point for future studies and is backed up by evidence from other sources. We need to know more about the signalling mechanisms responsible for these effects—do they involve conventional potassium channel physiology or a novel mechanism as suggested by Li *et al.*?⁴¹ Are K_{ATP} channels operating in the regulation of normal hair growth or the development of androgenetic alopecia and, if so, what is their subtype composition and cellular and subcellular distribution?

Why is minoxidil important? Although the benefits in androgenetic alopecia have been demonstrated in clinical trials, there is perhaps a tendency to dismiss the significance of minoxidil. Yet, it remains the only medical treatment of proven efficacy when used topically and is the only treatment approved for hair loss in women. Minoxidil affects hair cycling, causing premature termination of telogen and probably prolonging anagen. Understanding how minoxidil exerts these effects may lead not only to better treatments for hair loss but also will increase our understanding of the mechanisms responsible for controlling the hair cycle.

References

- 1 Limas CJ, Freis ED. Minoxidil in severe hypertension with renal failure. Effect of its addition to conventional antihypertensive drugs. *Am J Cardiol* 1973; **31**: 355–61.
- 2 Mehta PK, Mamdani B, Shansky RM *et al.* Severe hypertension. Treatment with minoxidil. *JAMA* 1975; **233**: 249–52.
- 3 Zappacosta AR. Reversal of baldness in patient receiving minoxidil for hypertension. *N Engl J Med* 1980; **303**: 1480–1.
- 4 Mori O, Uno H. The effect of topical minoxidil on hair follicular cycles of rats. *J Dermatol* 1990; **17**: 276–81.
- 5 Uno H, Capps A, Brigham P. Action of topical minoxidil in the bald stump-tailed macaque. *J Am Acad Dermatol* 1987; **16**: 657–68.
- 6 Courtois M, Loussouarn G, Hourseau C *et al.* Ageing and hair cycles. *Br J Dermatol* 1995; **132**: 86–93.
- 7 Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. *J Am Acad Dermatol* 1993; **28**: 755–63.

- 8 Rushton DH, Ramsay ID, James KC *et al*. Biochemical and trichological characterization of diffuse alopecia in women. *Br J Dermatol* 1990; **123**: 187–97.
- 9 Whiting DA. Chronic telogen effluvium: increased scalp hair shedding in middle-aged women. *J Am Acad Dermatol* 1996; **35**: 899–906.
- 10 Abell E. Histologic response to topically applied minoxidil in male-pattern alopecia. *Clin Dermatol* 1988; **6**: 191–4.
- 11 Headington JT, Novak E. Clinical and histologic studies of male pattern baldness treated with topical minoxidil. *Curr Ther Res Clin Exp* 1984; **36**: 1098–106.
- 12 Johnson GA, Barsuhn KJ, McCall JM. Sulfation of minoxidil by liver sulfotransferase. *Biochem Pharmacol* 1982; **31**: 2949–54.
- 13 Meisheri KD, Johnson GA, Puddington L. Enzymatic and non-enzymatic sulfation mechanisms in the biological actions of minoxidil. *Biochem Pharmacol* 1993; **45**: 271–9.
- 14 Falany CN, Kerl EA. Sulfation of minoxidil by human liver phenol sulfotransferase. *Biochem Pharmacol* 1990; **40**: 1027–32.
- 15 Johnson GA, Baker CA. Sulfation of minoxidil by human platelet sulfotransferase. *Clin Chim Acta* 1987; **169**: 217–27.
- 16 Johnson GA, Baker CA, Knight KA. Minoxidil sulfotransferase, a marker of human keratinocyte differentiation. *J Invest Dermatol* 1992; **98**: 730–3.
- 17 Buhl AE, Waldon DJ, Baker CA *et al*. Minoxidil sulfate is the active metabolite that stimulates hair follicles. *J Invest Dermatol* 1990; **95**: 553–7.
- 18 Dooley TP, Walker CJ, Hirshey SJ *et al*. Localization of minoxidil sulfotransferase in rat liver and the outer root sheath of anagen pelage and vibrissa follicles. *J Invest Dermatol* 1991; **96**: 65–70.
- 19 Hamamoto T, Mori Y. Sulfation of minoxidil in keratinocytes and hair follicles. *Res Commun Chem Pathol Pharmacol* 1989; **66**: 33–44.
- 20 Baker CA, Uno H, Johnson GA. Minoxidil sulfation in the hair follicle. *Skin Pharmacol* 1994; **7**: 335–9.
- 21 Dooley TP. Molecular biology of the human cytosolic sulfotransferase gene superfamily implicated in the bioactivation of minoxidil and cholesterol in skin. *Exp Dermatol* 1999; **8**: 328–9.
- 22 Anderson RJ, Kudlacek PE, Clemens DL. Sulfation of minoxidil by multiple human cytosolic sulfotransferases. *Chem Biol Interact* 1998; **109**: 53–67.
- 23 Buhl AE, Baker CA, Dietz AJ *et al*. Minoxidil sulfotransferase activity influences the efficacy of Rogaine topical solution (TS): enzyme studies using scalp and platelets. *J Invest Dermatol* 1994; **102**: 534.
- 24 Meisheri KD, Cipkus LA, Taylor CJ. Mechanism of action of minoxidil sulfate-induced vasodilation: a role for increased K⁺ permeability. *J Pharmacol Exp Ther* 1988; **245**: 751–60.
- 25 Winquist RJ, Heaney LA, Wallace AA *et al*. Glyburide blocks the relaxation response to BRL 34915 (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. *J Pharmacol Exp Ther* 1989; **248**: 149–56.
- 26 Schwanstecher M, Sieverding C, Dorschner H *et al*. Potassium channel openers require ATP to bind to and act through sulfonylurea receptors. *EMBO J* 1998; **17**: 5529–35.
- 27 Yost CS. Potassium channels. Basic aspects, functional roles and medical significance. *Anesthesiol* 1999; **90**: 1186–203.
- 28 Yokoshiki H, Sunagawa M, Seki T *et al*. ATP-sensitive K⁺ channels in pancreatic, cardiac and vascular smooth muscle cells. *Am J Physiol* 1998; **274**: C25–C37.
- 29 Xu D, Wang L, Dai W *et al*. A requirement for K⁺-channel activity in growth factor-mediated extracellular signal-regulated kinase activation in human myeloblastic leukemia ML-1 cells. *Blood* 1999; **94**: 139–45.
- 30 Malhi H, Irani AN, Rajvanshi P *et al*. KATP channels regulate mitogenically induced proliferation in primary rat hepatocytes and human liver cell lines. Implications for liver growth control and potential therapeutic targeting. *J Biol Chem* 2000; **275**: 26050–7.
- 31 Koblenzer PJ, Baker L. Hypertrichosis lanuginosa associated with diazoxide therapy in prepubertal children: a clinicopathologic study. *Ann N Y Acad Sci* 1968; **150**: 373–82.
- 32 Burton JL, Schutt WH, Caldwell IW. Hypertrichosis due to diazoxide. *Br J Dermatol* 1975; **93**: 707–11.
- 33 Goldberg MR. Clinical pharmacology of pinacidil, a prototype for drugs that affect potassium channels. *J Cardiovasc Pharmacol* 1988; **12** (Suppl. 2): S41–7.
- 34 Buhl AE, Conrad SJ, Waldon DJ *et al*. Potassium channel conductance as a control mechanism in hair follicles. *J Invest Dermatol* 1993; **101**: 148S–52S.
- 35 Buhl AE, Waldon DJ, Kawabe TT *et al*. Minoxidil stimulates mouse vibrissae follicles in organ culture. *J Invest Dermatol* 1989; **92**: 315–20.
- 36 Buhl AE, Waldon DJ, Conrad SJ *et al*. Potassium channel conductance: a mechanism affecting hair growth both *in vitro* and *in vivo*. *J Invest Dermatol* 1992; **98**: 315–19.
- 37 Harmon CS, Lutz D, Ducote J. Potassium channel openers stimulate DNA synthesis in mouse epidermal keratinocyte and whole hair follicle cultures. *Skin Pharmacol* 1993; **6**: 170–8.
- 38 Imai R, Jindo T, Miura Y *et al*. Organ culture of human hair follicles in serum-free medium. *Arch Dermatol Res* 1993; **284**: 466–71.
- 39 Sanders DA, Fiddes I, Thompson DM *et al*. In the absence of streptomycin, minoxidil potentiates the mitogenic effects of fetal calf serum, insulin-like growth factor 1, and platelet-derived growth factor on NIH 3T3 fibroblasts in a K⁺ channel-dependent fashion. *J Invest Dermatol* 1996; **107**: 229–34.
- 40 Nakaya Y, Hamaoka H, Kato S *et al*. Effect of minoxidil sulfate and pinacidil on single potassium channel current in cultured human outer root sheath cells and dermal papilla cells. *J Dermatol Sci* 1994; **7** (Suppl.): S104–S8.
- 41 Li M, Marubayashi A, Nakaya Y *et al*. Minoxidil-induced hair growth is mediated by adenosine in cultured dermal papilla cells: possible involvement of sulfonylurea receptor 2B as a target of minoxidil. *J Invest Dermatol* 2001; **117**: 1594–600.
- 42 Buhl AE. Minoxidil's action in hair follicles. *J Invest Dermatol* 1991; **96**: 73S–4S.
- 43 Buhl AE, Kawabe TT, MacCallum DK *et al*. Interaction of minoxidil with pigment in cells of the hair follicle: an example of binding without apparent biological effects. *Skin Pharmacol* 1992; **5**: 114–23.
- 44 Boyera N, Galey I, Bernard BA. Biphasic effects of minoxidil on the proliferation and differentiation of normal human keratinocytes. *Skin Pharmacol* 1997; **10**: 206–20.
- 45 Kurata S, Uno H, Allen-Hoffmann BL. Effects of hypertrichotic agents on follicular and nonfollicular cells *in vitro*. *Skin Pharmacol* 1996; **9**: 3–8.
- 46 O'Keefe E, Payne RE Jr. Minoxidil: inhibition of proliferation of keratinocytes *in vitro*. *J Invest Dermatol* 1991; **97**: 534–6.
- 47 Baden HP, Kubilus J. Effect of minoxidil on cultured keratinocytes. *J Invest Dermatol* 1983; **81**: 558–60.
- 48 Murad S, Pinnell SR. Suppression of fibroblast proliferation and lysyl hydroxylase activity by minoxidil. *J Biol Chem* 1987; **262**: 11973–8.

- 49 Murad S, Walker LC, Tajima S *et al.* Minimum structural requirements for minoxidil inhibition of lysyl hydroxylase in cultured fibroblasts. *Arch Biochem Biophys* 1994; **308**: 42–7.
- 50 Lachgar S, Charveron M, Bouhaddioui N *et al.* Inhibitory effects of bFGF, VEGF and minoxidil on collagen synthesis by cultured hair dermal papilla cells. *Arch Dermatol Res* 1996; **288**: 469–73.
- 51 Michelet JF, Commo S, Billoni N *et al.* Activation of cytoprotective prostaglandin synthase-1 by minoxidil as a possible explanation for its hair growth-stimulating effect. *J Invest Dermatol* 1997; **108**: 205–9.
- 52 Kvedar JC, Baden HP, Levine L. Selective inhibition by minoxidil of prostacyclin production by cells in culture. *Biochem Pharmacol* 1988; **37**: 867–74.
- 53 Tsuboi K, Sugimoto Y, Ichikawa A. Prostanoid receptor subtypes. *Prostaglandins Other Lipid Mediat* 2002; **68–9**: 535–56.
- 54 Gilmour RS, Mitchell MD. Nuclear lipid signaling: novel role of eicosanoids. *Exp Biol Med (Maywood)* 2001; **226**: 1–4.
- 55 Johnstone MA. Hypertrichosis and increased pigmentation of eyelashes and adjacent hair in the region of the ipsilateral eyelids of patients treated with unilateral topical latanoprost. *Am J Ophthalmol* 1997; **124**: 544–7.
- 56 Uno H, Zimbric ML, Albert DM *et al.* Effect of latanoprost on hair growth in the bald scalp of the stump-tailed macaque: a pilot study. *Acta Derm Venereol* 2002; **82**: 7–12.
- 57 Nuck BA, Fogelson SL, Lucky AW. Topical minoxidil does not act as an antiandrogen in the flank organ of the golden Syrian hamster. *Arch Dermatol* 1987; **123**: 59–61.
- 58 Sato T, Tadokoro T, Sonoda T *et al.* Minoxidil increases 17 beta-hydroxysteroid dehydrogenase and 5 alpha-reductase activity of cultured human dermal papilla cells from balding scalp. *J Dermatol Sci* 1999; **19**: 123–5.
- 59 Wester RC, Maibach HI, Guy RH *et al.* Minoxidil stimulates cutaneous blood flow in human balding scalps: pharmacodynamics measured by laser Doppler velocimetry and photopulse plethysmography. *J Invest Dermatol* 1984; **82**: 515–17.
- 60 Bunker CB, Dowd PM. Alterations in scalp blood flow after the epicutaneous application of 3% minoxidil and 0.1% hexyl nicotinate in alopecia. *Br J Dermatol* 1987; **117**: 668–9.
- 61 Sakita S, Kagoura M, Toyoda M *et al.* The induction by topical minoxidil of increased fenestration in the perifollicular capillary wall. *Br J Dermatol* 1999; **140**: 294–6.
- 62 Zachary I, Glikli G. Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family. *Cardiovasc Res* 2001; **49**: 568–81.
- 63 Yano K, Brown LF, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. *J Clin Invest* 2001; **107**: 409–17.
- 64 Lachgar S, Charveron M, Gall Y *et al.* Minoxidil upregulates the expression of vascular endothelial growth factor in human hair dermal papilla cells. *Br J Dermatol* 1998; **138**: 407–11.
- 65 Price VH, Menefee E, Strauss PC. Changes in hair weight and hair count in men with androgenetic alopecia, after application of 5% and 2% topical minoxidil, placebo, or no treatment. *J Am Acad Dermatol* 1999; **41**: 717–21.
- 66 Olsen EA, Dunlap FE, Funicella T *et al.* A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. *J Am Acad Dermatol* 2002; **47**: 377–85.