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Efficacy of 5% Minoxidil versus Combined 5% Minoxidil and 0.01% Tretinoin for Male Pattern Hair Loss

A Randomized, Double-Blind, Comparative Clinical Trial

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Abstract

Background: 5% topical minoxidil solution has been widely used to stimulate new hair growth and help stop hair loss in men with androgenetic alopecia (AGA). However, it is not convenient for patients to continue applying the solution twice daily on a regular basis. Tretinoin is known to increase the percutaneous absorption of minoxidil and, therefore, to enhance the response of AGA to minoxidil. For this reason, it was assumed that tretinoin would be helpful in alleviating the inconvenience associated with the recommended twice-daily application of minoxidil.

Objective: To compare the efficacy and safety of therapy using a combined solution of 5% minoxidil and 0.01% tretinoin once daily with those of the conventional 5% topical minoxidil therapy applied twice daily in the treatment of AGA.

Methods: A total of 31 male patients (aged 28–45 years, mean 39.7 ± 4.5) with AGA (Hamilton-Norwood classification type III–V) were randomly assigned into two groups, one in which 5% minoxidil was applied to the scalp twice daily and the other in which the combined agent was applied once daily at night together with a vehicle placebo in the morning. The efficacy parameters were: (i) changes in total hair count, non-vellus hair count, anagen hair ratio, linear hair growth rate, and mean hair diameter assessed by macrophotographic image analysis; and (ii) the patient's and investigator's subjective assessments.

Results: After therapy, increases in the macrophotographic variables of total hair count and non-vellus hair count were shown in both treatment groups. There were no statistically significant differences between the two treatment groups with respect to changes in macrophotographic variables or scores on subjective global assessments by patients and the investigator. The incidence of adverse effects such as pruritus or local irritation was similar in the 5% minoxidil group (4 of 14 subjects) and the combined agent group (5 of 15 subjects).

Conclusion: The efficacy and safety of combined 5% minoxidil and 0.01% tretinoin once-daily therapy appear to be equivalent to those of conventional 5% minoxidil twice-daily therapy for the treatment of AGA.

Androgenetic alopecia (AGA), the most common form of alopecia in men, is a genetically determined, cosmetic disorder usually beginning from about the late twenties and affecting approximately half of all adult males aged >50 years.[1,2] Shortening of the anagen phase and miniaturization of the follicles are prominent in AGA.[3]

Minoxidil, originally developed for the treatment of hypertension, is the most popular topical agent for AGA approved by the US FDA. Minoxidil lengthens the duration of the anagen phase and shortens the latent period of the hair cycle.[4] In addition, topical application of minoxidil increases the size of the hair follicles.[5] However, the exact mechanism of action of the drug has not been fully elucidated.[6]

The effect of minoxidil correlates with the concentration and amount of the applied agent. Olsen et al.[7,8] showed that twice-daily application of minoxidil was superior to once-daily applica-

tion for the treatment of AGA. Therefore, its application twice daily for the treatment of AGA has been generally recommended.[9] Regular application of minoxidil is an important factor in effective treatment.[10] However, from a practical point of view, it is not easy for patients to regularly apply minoxidil daily.

In a previous study,[11] the percutaneous absorption of 2% minoxidil was increased nearly 3-fold by 0.05% tretinoin, which increases the permeability of the stratum corneum. When minoxidil combined with tretinoin was applied only once daily, the urinary excretion of minoxidil was significantly higher than that of minoxidil alone applied twice daily.[11] Moreover, 0.5% minoxidil plus 0.025% tretinoin (95% alcohol plus 5% propylene glycol vehicle) applied twice daily to the affected scalp area was reported to prolong the anagen hair ratio and induce new hair growth.[12] These findings suggest that tretinoin can be helpful in alleviating the inconvenience associated with the recommended twice-daily application of minoxidil. In this study, we compared the efficacy and safety of the combined 5% minoxidil and 0.01% tretinoin topical preparation applied once daily with those of 5% minoxidil applied conventionally twice daily in the treatment of AGA.

Patients and Methods

Patient Selection

Thirty-one male patients ranging from 28 to 45 years of age (mean 39.7 ± 4.5 years of age) with a clinical diagnosis of AGA type III–V (Hamilton-Norwood classification) volunteered to participate in this study. Patients with other medical problems were excluded. No patients had used any products or taken any drugs that might have affected hair growth for ≥6 months prior to this study.

Patients were randomly divided into two groups (the test [n = 16] and control [n = 15] groups) by four-block randomization using a table of random sampling numbers. The random numbers and the allotment table were not made available to investigators and patients until after all study evaluations had been completed.

Written informed consent was obtained from all patients prior to participation in the study. The Institutional Review Board of Seoul National University Hospital approved the conduct of the study.

Products Tested

The 5% minoxidil solution and the combined topical preparation consisting of 5% minoxidil and 0.01% tretinoin were transparent liquids in 95% alcohol plus 5% propylene glycol vehicle prepared by AmorePacific R&D Center (Gyeonggi-do, Korea) and distributed by our clinical research center pharmacy. The two products were indistinguishable in terms of appearance, smell, and viscosity. The patients were instructed to spray 1mL of the agent and massage their scalp softly twice daily. The test group applied the placebo (vehicle, 95% alcohol plus 5% propylene glycol) without minoxidil or tretinoin in the morning and the combined preparation in the evening. Therefore, the test group was treated once a day, in contrast to the control group that applied 5% minoxidil twice daily.

Study Protocol

The patients visited the hospital a total of five times. At the first visit, a baseline global photograph of their scalp was taken, after which the scalp hairs on the transitional zone between the bald region and normal hairy region were shaved to create a round area 1.5cm in diameter. The reference point was tattooed at the center of the shaved round area. Three days later, phototrichogram images were obtained by taking close contact photographs with a digital camera (Coolpix 8400®, [1] Nikon Corporation, Tokyo, Japan) at a magnification of ×30 in the previously shaved region. The camera was mounted with a rigid magnifying lens to ensure that the images were always taken at the same distance from the scalp surface. After application of a drop of water to minimize light scattering, the lens was pressed on to the shaved area so that the newly grown hairs were flattened on to the scalp surface. We recorded the exact time when shaving and taking of the phototrichogram were undertaken in order to calculate the exact duration of new hair growth. Patients were than randomly assigned to one of the two treatment groups.

At the 9-week visit, a global photograph was taken again, and the patient's and investigator's subjective assessments were made.

At the 18-week visit, the final global photograph was taken and final assessments were made by the patient and investigator. The previously tattooed reference area was then shaved again in preparation for the second phototrichogram. Three days later, on the last visit, the second phototrichogram for evaluation of the efficacy of the treatment was taken.

Measurements

Five biologic parameters of hair growth (total hair count, nonvellus hair count, anagen hair ratio, linear hair growth rate, and mean hair diameter) at baseline and post-treatment were measured by macrophotographic image analysis. Total hair count, linear hair length, and hair diameter in the digitized images were measured by

1 The use of trade names is for product identification purposes only and does not imply endorsement.

Table I. Characteristics of study volunteers (n = 29)

No. of subjects	Hamilton-Norwood classification				
	III	IIIa	IIIv	V	Va
Combined minoxidil and tretinoin once-daily group (n = 15)	1	0	9	0	5
Minoxidil twice-daily group (n = 14)	2	1	8	1	2
Total (n = 29)	**3**	**1**	**17**	**1**	**7**

image analysis software (Image J 1.34s, Wayne Rasband, Bethesda, MD, USA). Hairs thicker than 40μm in diameter were counted as non-vellus hair.[13] In addition, hairs with growth rates >200 μm/day were classified as anagen hair, allowing the anagen hair ratio (anagen hair count/total hair count) to be calculated.[14]

At 9 and 18 weeks, patients were asked to rate the improvement in their hair loss on a 10-point scale, where 0 meant no change or worse and 10 indicated complete recovery. They also graded their satisfaction with the result on a 10-point scale, where 0 meant complete disappointment and 10 indicated full satisfaction.

The investigator's assessment of the efficacy of the treatment was conducted by one designated investigator (HSS) and performed by comparing global photographs obtained at the first visit, 9 weeks, and 18 weeks. The results of the evaluation were rated into five grades as follows: 4 = excellent (improved >75%); 3 = good (improved 51–75%); 2 = fair (improved 26–50%); 1 = poor (improved <25%); 0 = no change or worse.

Statistical Methods

A statistical analysis was performed using SPSS 11 software (SPSS, Chicago, IL, USA) with a p-value of <0.05 being considered significant. The non-parametrical Mann-Whitney test and Wilcoxon signed rank test were used to evaluate differences in biologic parameters of hair growth characteristics and the patients' and investigator's assessments between the two groups.

Results

Enrolled Patients

Initially, 31 otherwise healthy patients with AGA were enrolled, but one patient in the control group and another patient in the test group withdrew after missing a follow-up visit. Thus, 15 patients in the test group and 14 patients in the control group completed the study. No significant difference in the age of the patients was observed between the two groups (mean ± SD 39.3 ± 4.2 years for the test group vs 40.2 ± 4.8 years for the control group). The most common AGA subtype was grade IIIv in the Hamilton-Norwood classification (table I).

Biologic Parameters of Hair Growth Characteristics before Treatment

No significant difference in total hair count, non-vellus hair count, anagen hair ratio, linear hair growth rate, and mean hair diameter was observed between the two groups before treatment (table II).

Treatment Efficacy Within Each Group

The total hair count and non-vellus hair count increased significantly after 18 weeks of treatment in both groups ($p < 0.05$). Mean hair diameter was also markedly increased in the control group ($p < 0.05$). In the test group the increase in mean hair diameter was of borderline significance ($p = 0.064$). Neither anagen hair ratio nor

Table II. Biologic parameters of hair growth in the two treatment groups at baseline

Parameter	Combined minoxidil and tretinoin once-daily group[a]	Minoxidil twice-daily group[a]	p-Value
Total hair count (n/cm^2)	124.2 ± 8.5	124.0 ± 7.5	NS
Non-vellus hair count (n/cm^2)	42.7 ± 5.8	33.4 ± 4.3	NS
Anagen hair ratio	0.554 ± 0.020	0.571 ± 0.025	NS
Linear hair growth rate (μm/d)	336.6 ± 10.6	302.2 ± 13.1	NS
Mean hair diameter (μm)	36.2 ± 1.3	34.8 ± 2.1	NS

a Values are mean ± standard error.

NS = not significant.

Table III. Biologic parameters of hair growth at baseline and after 18 weeks of treatment in the two treatment groups

Parameter	Baseline[a]	After treatment[a]	p-Value
Combined minoxidil and tretinoin once-daily group			
Total hair count (n/cm^2)	124.2 ± 8.5	142.4 ± 7.3	<0.05
Non-vellus hair count (n/cm^2)	42.7 ± 5.8	48.8 ± 5.6	<0.05
Anagen hair ratio	0.554 ± 0.020	0.521 ± 0.037	NS
Linear hair growth rate (μm/d)	336.6 ± 10.6	331.1 ± 9.3	NS
Mean hair diameter (μm)	36.2 ± 1.3	38.1 ± 1.4	NS
Minoxidil twice-daily			
Total hair count (n/cm^2)	124.0 ± 7.5	139.9 ± 9.1	<0.05
Non-vellus hair count (n/cm^2)	33.4 ± 4.3	47.4 ± 5.3	<0.05
Anagen hair ratio	0.571 ± 0.025	0.557 ± 0.035	NS
Linear hair growth rate (μm/d)	302.2 ± 13.1	317.7 ± 13.8	NS
Mean hair diameter (μm)	34.8 ± 2.1	37.4 ± 1.8	<0.05

a Values are mean ± standard error.

NS = not significant.

linear hair growth rate showed any significant differences after treatment (table III).

Treatment Efficacy between Groups

No significant differences in percentage changes in biologic parameters of hair growth (total hair count, non-vellus hair count, anagen hair ratio, linear hair growth rate, and mean hair diameter) were observed between the two groups after 18 weeks of treatment. The p-values all exceeded 0.05 (table IV).

Patients' and Investigator's Subjective Assessments

The patient's subjective assessment score and satisfaction score did not show any significant differences between the test group and the control group during the study (table V). Similarly, the investigator's subjective assessment score did not show any significant difference between the test group and the control group during the study (table VI).

Safety Evaluation

Five patients in the test group (n = 15) and four patients in the control group (n = 14) complained of scalp itching or prickling. Among them, two patients in the control group had folliculitis on their scalps. However, symptoms were mild in all cases and the patients were able to continue application of the drugs. All adverse effects were self-limiting and were no longer present after a few days.

Discussion

Among the various drugs used for the treatment of AGA, the most popular topical agent, which is also approved by the FDA, is minoxidil. After application, minoxidil is converted to minoxidil sulfate, an active metabolite of the parent drug, by sulfotransferase enzymes. Minoxidil sulfate opens an adenosine triphosphate-sensitive potassium channel, which functions to relax vascular

Table IV. Comparison of changes in biologic parameters of hair growth between the two treatment groups

Change (%)[a]	Combined minoxidil and tretinoin once-daily group[b]	Minoxidil twice-daily group[b]	p-Value
Total hair count	17.3 ± 3.8	12.9 ± 2.5	NS
Non-vellus hair count	23.4 ± 7.7	55.4 ± 19.6	NS
Anagen hair ratio	−7.0 ± 4.1	−2.9 ± 3.7	NS
Linear hair growth rate	−1.3 ± 1.8	5.6 ± 3.1	NS
Mean hair diameter	5.4 ± 2.5	8.7 ± 3.4	NS

a Change (%) = (parameter at week 18 – parameter at baseline) / (parameter at baseline) × 100.

b Values are mean ± standard error.

NS = not significant.

Table V. Comparison of patients' subjective assessment scores between the two treatment groups

Subjective score[a]	Combined minoxidil and tretinoin once-daily group[b]	Minoxidil twice-daily group[b]	p-Value
Improvement (at 9wk)	3.1 ± 0.6	2.6 ± 0.6	NS
Improvement (at 18wk)	4.2 ± 0.6	3.7 ± 0.8	NS
Satisfaction (at 9wk)	3.0 ± 0.6	3.1 ± 0.7	NS
Satisfaction (at 18wk)	4.2 ± 0.6	3.9 ± 0.8	NS

a Graded on a 10-point scale, where a score of 0 means no change or complete disappointment and a score of 10 means complete recovery or full satisfaction.

b Values are mean ± standard error.

NS = not significant.

smooth muscle. Therefore, increasing cutaneous blood flow has been regarded as the main mechanism of action of minoxidil.[15,16]

Tretinoin has been shown to alter the stratum corneum barrier and increase the percutaneous absorption of minoxidil which, in turn, enhances the response of AGA to minoxidil.[11] Moreover, retinoic acid promotes the growth of hair follicles and the formation of vessels via a molecular signaling pathway.[17] In addition, we have recently demonstrated that hair growth was significantly enhanced by the combination of minoxidil plus retinol (vitamin A) compared with minoxidil alone via dual mechanisms: (i) activation of extracellular signal-regulated kinase (Erk) and Akt signaling; and (ii) prevention of apoptosis by increasing the B-cell leukemia/lymphoma (Bcl)-2/Bcl-2-associated X (Bax) protein ratio.[18] Therefore, enhancement of transepidermal absorption of minoxidil and a direct stimulatory influence on hair growth have been proposed as the mechanisms of action of tretinoin on hair growth. However, it has not yet been determined which is more predominant in terms of promoting hair growth.

In this study, we found that the application of the combined 5% minoxidil and 0.01% tretinoin solution just once daily showed an equivalent treatment effect to that of 5% minoxidil applied twice daily in terms of changes in hair growth characteristics such as total hair count, non-vellus hair count, anagen hair ratio, linear hair growth rate, and mean hair diameter. The increases in total hair count, non-vellus hair count, and mean hair diameter observed in our study correlate well with those reported in previous studies.[8,19] However, in contrast to previous studies that reported an increase in anagen hair ratio with minoxidil therapy,[4,19] neither the combined agent nor conventional 5% minoxidil were found to improve anagen hair ratio or linear hair growth rate in the current study. Since minoxidil is reported to shorten the latent telogen phase,[4] the lack of change in these parameters with treatment in our study was not expected. It is possible that these results reflected seasonal variations in hair growth, as our study was performed between summer and autumn.[20] Otherwise, some exogen hair might have interfered with anagen hair growth by blocking its path. These factors might at least partially explain why anagen hair ratio and linear hair growth rate were not significantly affected by treatment in this study.

Both groups demonstrated similar improvements in subjective global assessment of therapy by patients and the investigator.

The occurrence of adverse effects such as pruritus or local irritation was similar in both groups. Based on these results, it was inferred that the combined preparation is as safe as conventional minoxidil.

Conclusion

This study was a randomized, double-blind, comparative clinical trial designed to compare the efficacy of a combined solution of 5% minoxidil and 0.01% tretinoin with that of conventional 5% minoxidil in the treatment of AGA. Topical tretinoin has been shown to increase the percutaneous absorption of minoxidil.

Table VI. Comparison of investigator's subjective assessment scores between the two treatment groups

Subjective score[a]	Combined minoxidil and tretinoin once-daily group[b]	Minoxidil twice-daily group[b]	p-Value
At 9wk	1.2 ± 0.2	0.9 ± 0.2	NS
At 18wk	1.6 ± 0.3	1.8 ± 0.4	NS

a Graded on a 4-point scale, where a score of 0 means no change or worse and a score of 4 means >75% improvement.

b Values are mean ± standard error.

NS = not significant.

Therefore, we presumed that a combined solution of 5% minoxidil and 0.01% tretinoin would be effective for the treatment of AGA, even when it is applied only once daily. There were no significant differences in percentage changes in biologic parameters of hair growth characteristics between the two groups after 18 weeks of treatment. There were also no statistical differences between the two groups in the subjective global assessment of treatment by patients and the investigator. The adverse effects of the combined solution were all mild. In conclusion, although this study had limitations, such as the small number of patients analyzed, the lack of a placebo group, and the fact that no treatment group received 5% minoxidil once daily, our results suggest that the efficacy and safety of combined 5% minoxidil and 0.01% tretinoin administered once daily are equivalent to those of conventional 5% minoxidil administered twice daily for the treatment of AGA. Since there is usually an inverse relationship between administration frequency and compliance,[21,22] use of combined 5% minoxidil and 0.01% tretinoin once daily could be a useful alternative, with a high rate of compliance, for the treatment of AGA.

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References

1. Brodland DG, Muller SA. Androgenetic alopecia (common baldness). Cutis 1991 Mar; 47 (3): 173-6
2. Norwood OT. Male pattern baldness: classification and incidence. South Med J 1975 Nov; 68 (11): 1359-65
3. Bergfeld WF. Androgenetic alopecia: an overview: seventh symposium on alopecia. In: Badin HP, editor. Dermatology capsule and comment. New York: HP Publishing, 1988: 1-10
4. Mori O, Uno H. The effect of topical minoxidil on hair follicular cycles of rats. J Dermatol 1990 May; 17 (5): 276-81
5. Uno H, Cappas A, Brigham P. Action of topical minoxidil in the bald stump-tailed macaque. J Am Acad Dermatol 1987 Mar; 16 (3 Pt 2): 657-68
6. Messenger AG, Rundegren J. Minoxidil: mechanism of action on hair growth. Br J Dermatol 2004 Feb; 150 (2): 186-94
7. Olsen EA, DeLong ER, Weiner MS. Long-term follow-up of men with male pattern baldness treated with topical minoxidil. J Am Acad Dermatol 1987 Mar; 16 (3 Pt 2): 688-95
8. 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 Sep; 47 (3): 377-85
9. Olsen EA, Messenger AG, Shapiro J, et al. Evaluation and treatment of male and female pattern hair loss. J Am Acad Dermatol 2002 Sep; 47 (3): 377-85
10. Buhl AE, Waldron DJ, Kawabe TT, et al. Minoxidil stimulates mouse vibrissae follicles in organ culture. J Invest Dermatol 1989 Mar; 92 (3): 315-20
11. Ferry JJ, Forbes KK, VanderLugt JT, et al. Influence of tretinoin on the percutaneous absorption of minoxidil from an aqueous topical solution. Clin Pharmacol Ther 1990 Apr; 47 (4): 439-46
12. Bazzano GS, Terezakis N, Galen W. Topical tretinoin for hair growth promotion. J Am Acad Dermatol 1986 Oct; 15 (4 Pt 2): 880-3
13. Rushton DH. Chemical and morphological properties of scalp hair in normal and abnormal states. Aberystwyth: University of Wales, 1988
14. Hayashi S, Miyamoto I, Takeda K. Measurement of human hair growth by optical microscopy and image analysis. Br J Dermatol 1991 Aug; 125 (2): 123-9
15. 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 Jun; 245 (3): 751-60
16. 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 Jan; 248 (1): 149-56
17. Madani KA, Bazzano GS, Chou AC. Effects of vitamin A status on cellular retinoic acid-binding protein in rat skin and testes. Eur J Clin Chem Clin Biochem 1991 May; 29 (5): 317-20
18. Yoo HG, Chang IY, Pyo HK, et al. The additive effects of minoxidil and retinol on human hair growth in vitro. Biol Pharm Bull 2007 Jan; 30 (1): 21-6
19. Abell E. Histologic response to topically applied minoxidil in male pattern alopecia. Clin Dermatol 1988 Oct-Dec; 6 (4): 191-4
20. Courtois M, Loussouarn G, Hourseau S, et al. Periodicity in the growth and shedding of hair. Br J Dermatol 1996 Jan; 134 (1): 47-54
21. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med 2005 Aug 4; 353 (5): 487-97
22. Claxton AJ, Cramer J, Pierce C. A systemic review of the associations between dose regimens and medication compliance. Clin Ther 2001 Aug; 23 (8): 1296-310

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