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Simultaneous effects of tocopheryl polyethylene glycol succinate (TPGS) on local hair growth promotion and systemic absorption of topically applied minoxidil in a mouse model

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Abstract

In this study, topical minoxidil solutions supplemented with TPGS in cosolvent systems of various compositions consisting of water, alcohol, and polyethylene glycol 400 were designed to evaluate the efficacy of promoting hair growth after topical application and the safety in terms of the amount of minoxidil absorbed through the skin into the circulation using C57BL/6J mice as a model. The commercial product of 2% Regaine® was used as the positive control. The role, which sulfotransferase activity plays in hair growth with treatment using minoxidil, was determined as well. The results revealed that the addition of 0.5% TPGS was able to enhance the proliferation of hair, but an increase in the amount of TPGS to 2% led to deterioration in the enhancement of hair growth. At the higher added amount (2.0%) of TPGS, the promotion of hair growth was slightly reduced for both cosolvent formulations F1 (100% water) and F3 (100% PEG 400), whereas it was reduced to a greater extent for the cosolvent formulations F8–F10. In comparison, the influences of cosolvent compositions with TPGS amounts of 0.0 and 2.0% on the promotion of hair growth were similar. On the contrary, variability in the promotion of hair growth by different solvent formulations was minimal when the added amount of TPGS was 0.5%. In general, a relationship between hair growth and sulfotransferase activities after topical application of 2% Regaine® and minoxidil formulations containing various amounts of TPGS was not demonstrated. Plasma concentrations of minoxidil with 2% Regaine® were found to be greater than those of 2% minoxidil in those cosolvent formulations containing various amounts of TPGS, while showing insignificant differences among those 10 cosolvent formulations with a fixed amount of TPGS. A tendency for the plasma concentration of minoxidil to increase after the topical administration of minoxidil formulations containing the higher amount of TPGS (2%) was noted.

Keywords: TPGS; Minoxidil; Topical application; Sulfotransferase; Systemic effect

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1. Introduction

Androgenetic alopecia (AGA) is hereditary and is the progressive, androgen-dependent thinning of scalp hair, which follows a definite pattern. With the aims of increasing hair coverage of the scalp and retarding the progression of hair thinning, current available treatment modalities with proven efficacy, which have been approved by the US Food and Drug Administration are oral finasteride at a dose of 1 mg per day and topical solutions of 2 and 5% minoxidil (Price, 1999). Minoxidil, a pyrimidine derivative (2,4-diamino-6-piperidinopyrimidine-3-oxide), remains the only medical treatment with proven efficacy when used topically and is the only treatment approved for hair loss in women.

The 2% topical product of minoxidil was first marketed for hair regrowth in men in 1986 in the US, and the 5% topical product became available in 1993. Molecular mechanisms of action by minoxidil on hair growth were reviewed by Messenger and Rundegren (2004). Recently, it was suggested by Han et al. (2004) that minoxidil stimulates the growth of human hair by prolonging anagen through proliferative (by activating both ERK and Akt) and antiapoptotic (by increasing the ratio of Bcl-2/Bax) effects on dermal papilla cells (DPCs) of human hair follicles.

Although there are inconsistencies in the results and they are viewed as unproven, both antihypertensive and hair-regrowth effects have been attributed to the action of both minoxidil and its sulfated metabolite (minoxidil sulfate). Minoxidil and minoxidil sulfate act directly on hair follicles of mouse vibrissae and human scalp hair follicles. Some studies have also shown that sulfation is a critical step in the hair-growth effects of minoxidil, and that the sulfation of minoxidil is catalyzed by sulfotransferase (Buhl et al., 1990). Minoxidil sulfotransferase activity has since been demonstrated in human platelets, liver, scalp skin, hair follicles, and epidermal keratinocytes as well as in mouse vibrissa follicles. Human sulfotransferase enzyme expression varies among individuals. Functionally significant genetic polymorphisms for sulfotransferase enzymes in humans have been reported (Anderson et al., 1981). There are interindividual variations in scalp sulfotransferase activity, and these correlate with the level in platelets (Anderson et al., 1998). In a clinical setting, scalp sulfotransferase activity was higher in men who responded to minoxidil compared with those did not respond to it (Buhl et al., 1994). A measure of the level of human blood platelet minoxidil sulfotransferase activity should predict an individual's physiological response to minoxidil.

It is necessary to focus on providing a means to limit the efficacy of hypertrichosis to local sites with the percutaneous delivery of minoxidil and to avoid excess minoxidil entering the blood circulation, which can produce side effects, including systemic hypotensive effects. Tocopheryl polyethylene glycol succinate (TPGS) is a water-soluble derivative of a natural source of vitamin E and functions as a surfactant with an HLB value of 13.2. Our previous studies (Sheu et al., 2003) showed that interfacial coverage of TPGS with an increasing TPGS concentration and hindrance of the partitioning of estradiol with an increasing alcohol content might play a role in influencing the permeability of estradiol. It was thought that this effect of TPGS on the permeability of estradiol could be applied to the topical delivery of minoxidil by enhancing the local retention in the skin leading to an improved therapeutic effect but with minimization of the systemic side effects. In this study, topical minoxidil solutions supplemented with TPGS in cosolvent systems of various compositions consisting of water, alcohol, and polyethylene glycol 400 were designed. In order to evaluate the safety and efficacy of these minoxidil solutions for topical application, the amount absorbed through the skin into the circulation and the enhancement of hair growth using C57BL/6J mice were evaluated. These results were compared with the commercial product Regaine®. It was also necessary to determine if individual variations in sulfotransferase activity play a role in hair growth. Sulfotransferase activity in C57BL/6J mice was assayed using the formation of 3′-phosphoadenosine-5′-phosphate (PAP) as well.

2. Experimental

2.1. Materials

Minoxidil was obtained from Fabbrica Lombarda Ammino Acdi (Italy). A 2% Regaine® topical solution, sold by Pharmacia Corporation (Sweden), was purchased from a local market. Polyethylene glycol 400 (PEG 400) was supplied by Fluka (Switzerland).

Table 1
Cosolvent formulations of topical minoxidil solutions designed using Design Expert®

Formulation	Water (%)	Alcohol (%)	PEG 400 (%)
F1	100	0	0
F2	0	100	0
F3	0	0	100
F4	50	50	0
F5	50	0	50
F6	0	50	50
F7	66.67	16.67	16.67
F8	16.67	66.67	16.67
F9	16.67	16.67	66.67
F10	33.33	33.33	33.33

Alcohol was obtained from Taiwan Tobacco and Liquor Corporation (Taiwan). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was supplied by Eastman Chemical Company (USA). Docusate sodium, 3′-phosphoadenosine-5′-phosphosulfate (PAPS), 3′-phosphoadenosine-5′-phosphate (PAP), and sulfotransferase 1A3 were purchased from Sigma–Aldrich (USA).

2.2. Methods

2.2.1. Formulation designs

According to the mixture design, 10 cosolvent formulations (F1–F10) containing three kinds of cosolvents (water, alcohol, and PEG 400) in different ratios were designed using Design Expert (V5.0, Stat-Ease, Minneapolis, MN, USA). One of these was the center point of the mixture design. Details of the formulations are listed in Table 1. Since patients demonstrate the phenomenon of vehicle-dependent allergic contact dermatitis (ACD) to propylene glycol present in Regaine®, it was replaced with PEG 400 in this study to eliminate the ACD syndrome of subjects.

2.2.2. Hair-growth studies

Seven-week-old male C57BL/6J mice were obtained from the Animal Center, National Taiwan University, Taiwan. The hair of these mice was removed by the topical application of calcium thioglycolate. The next day, mice without visible wounds were selected and randomized for the studies. Topical minoxidil solutions (2%), prepared with the addition of various amount of TPGS (0, 0.5 and 2%) in a variety of compositions of cosolvent systems consisting of water, alcohol, and PEG 400, were applied to the dorsal skin of mice and compared to 2% Regaine®. Control animals received the vehicle solution alone. In the in vivo studies, hair-regrowth activities when applying each solution was measured by analyzing photographs taken of each mouse every 5 days. All experiments lasted for 20 days. Fig. 1 shows the scale for evaluating hair growth in this mouse alopecia model.

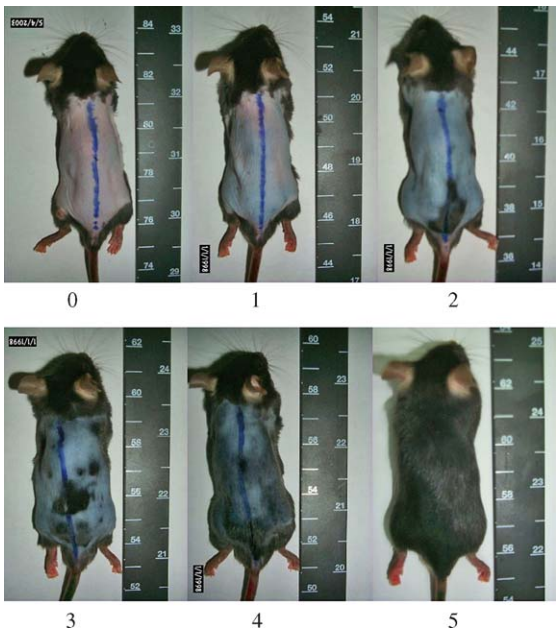

Fig. 1. Scale for evaluating hair growth in a mouse alopecia model. The rating scale was 1, initial state; 2, short visible hair; 3, sparse long hair; 4, dense, long hair; 5, complete hair growth.

2.2.3. Sulfotransferase and protein assays

Blood samples were drawn by venipuncture from the ophthalmic venous plexus of mice and transferred to vacutainer tubes containing 0.05 mL liquid potassium EDTA (0.2 mM). Platelet homogenates were prepared as described by Anderson and Weinshilboum (1980) and stored at −80 °C until being assayed. Sulfotransferase activity was determined by measuring the formation of PAP from PAPS, as described by Duffel, with minor modifications (Duffel et al., 1989). Briefly, the reaction mixture contained 20 μL of platelet homogenate, 10 μL ultrapure PAPS (250 μM), and 70 μL minoxidil (10.57 mM) in 5 mM potassium phosphate buffer containing 2.5 mM dithiothreitol (pH

7.5). The mixture was incubated for 30 min at 37 °C in a shaking water bath. The reaction was terminated by the addition of 30 μL ice-cold methanol and 20 μL supernatant (after centrifugation at 4 °C for 10 min) was measured for PAP using an HPLC analysis. The formation of PAP from PAPS was used as an indicator of sulfotransferase activity with reference to SULT 1A3 (10 units/1.25 μg) as the activity standard. The protein contents of the enzyme preparation were measured by the method of Bradford using the Bio-Rad reagent with bovine serum albumin as the standard.

2.2.4. HPLC analysis of PAP formation

The supernatant from minoxidil incubations was analyzed by a modification of a previous HPLC method (Lewis et al., 2000). An Inertsil ODS-2 column (150 × 4.6 mm) was used with a mobile phase of ammonium chloride (100 mM) and octylamine (2 mM) in potassium phosphate buffer (75 mM, pH 5.45) with 18% (v/v) methanol at a flow rate of 1.2 mL/min and UV detection at 254 nm. PAP standard curves were generated by injecting 20 μL of PAP standard solution (0.1, 0.25, 0.5, 1, 2.5, 5, 7.5 and 10 nmol/mL). The precision and accuracy for intra and inter-day measurements were within an acceptable range of less than 5%.

2.2.5. Plasma concentration of minoxidil

About 0.05 mL of blood was obtained from each mouse at 0, 5, 10, 15 and 20 days. Plasma was immediately separated by centrifugation at 1690 × *g* for 10 min, then transferred to suitably labeled tubes, and stored at −80 °C. Plasma sample preparations and the extraction method are described step by step as follows. The plasma sample (0.05 mL) was mixed with 0.45 mL methanol. After vortex-mixing thoroughly for 5 s, the mixture was centrifuged at 2950 × *g* for 10 min. The supernatant (0.1 mL) was transferred to another clean glass tube and mixed with 0.4 mL methanol. The mixture was centrifuged at 2950 × *g* for another 10 min. Then, 20 μL of the supernatant was automatically injected into the HPLC system for analysis.

Minoxidil concentrations were analyzed using an HPLC method with a reverse-phase ODS-2 column. Measurements were taken with UV detection at a wavelength of 286 nm. The mobile phase consisted of methanol/H_2O/glacial acetic acid (750/250/10 v/v/v, pH 3.0) at a delivery rate of 1 mL/min. This method was validated in the linear concentration range of 0.02–10 μg/mL. The precision and accuracy for intra and inter-day measurements were within acceptable ranges of less than 5%.

3. Results and discussion

In comparison with the application of vehicle only as the normal control and the application of 2% Regaine® as the positive control, 10 cosolvent formulations (Table 1) consisting of water, alcohol, and PEG 400 in different ratios supplemented with different amounts of TPGS (0.0, 0.5 and 2%) were designed to evaluate their efficacy on the promotion of hair regrowth by minoxidil as well as the safety in terms of systemic availability of minoxidil absorbed into the general circulation using C57BL/6J mice as the model. Fig. 2A and B demonstrates hair regrowth at 5-day intervals for the normal control and positive control (2% Regaine®), respectively. In normal control mice, hair regrowth showed only a faint appearance after treatment for 10 days. It became evident that the hair of mice had fully regrown to the original status within approximately 20 days. After the topical application of 2% Regaine®, it took only about 10 days for the hair of mice to fully regrow, indicating the enhancing effect of minoxidil in the proliferative rate of hair growth. Based on this, three levels of normal, medium, and fast were used to classify that it takes 20, 15 and 10 days, respectively, for the hair of mice to fully regrow after treatment.

After topical treatment with 2% minoxidil in those 10 cosolvent formulations containing 0.0, 0.5 and 2.0% TPGS, it was evident that all were able to facilitate hair growth (Fig. 2C shows formulation F5 containing 0.5% TPGS as an example) compared to the normal control. Table 2 lists these results of quantified hair growth after treatment using the scale illustrated in Fig. 1. The results revealed that the increase in TPGS from 0.0 to 0.5% in each corresponding solvent formulation showed a slight increase in the efficacy of promoting hair growth, whereas a further increase in TPGS to 2.0% in the corresponding cosolvent formulation worsened the efficacy of promoting hair growth. At the higher added amount of 2.0% TPGS, the promotion of hair growth was slightly reduced for both cosolvent formulations of F1 (100% water) and F3 (100% PEG 400), whereas it was reduced to a greater extent for

Fig. 2. Hair regrowth in a C57BL/6J mouse alopecia model. After topical application of (A) the normal control, (B) the positive control of 2% Regaine® and (C) the minoxidil formulation (F5) containing 0.5% TPGS for 15 days, photographs of each animal were taken every 5 days (figure in the plots indicates the number of days).

those cosolvent formulations of F8–F10. It was concluded that the addition of 0.5% TPGS was able to enhance the proliferation of hair, and that increasing the amount of TPGS to 2% led to deterioration of this enhancement of hair growth.

In a comparison shown in Table 2, the influence of cosolvent compositions at the same TPGS amount of either 0.0 or 2.0% on the promotion of hair growth was similar. The proliferative rates of hair growth by cosolvent formulations F1–F7 at these two added amounts of TPGS were classified as the level of fast, whereas they were classified as the normal level for F8–F10. It is interesting to note that these three formulations contained all three kinds of cosolvent with a water content of less than 50%. With the added amount of TPGS at 0.5%, the growth rates of hair treated with those 10 cosolvent formulations were all classified as the medium level. Obviously, the variability in the promotion of hair growth by different solvent formulations was minimal when the added amount of TPGS was 0.5%. These results further demonstrated that cosolvent formulations (except F8–F10) containing either amount of TPGS promoted hair regrowth at a rate comparable to 2% Regaine®. It was suspected that the lower efficacy of promoting hair growth by these three formulations all containing less than 50% water

Table 2
Results of quantification of hair growth by test formulations containing different concentrations of TPGS (%) every 5 days using the rating scale [shown in Fig. 1]

	Days											
	5			10			15			20		
	0.0	0.5	2.0	0.0	0.5	2.0	0.0	0.5	2.0	0.0	0.5	2.0
Blank	0	X	X	1	X	X	4–	X	X	5–	X	X
2% Regaine®	2	X	X	5	X	X	5	X	X	5	X	X
F1	2+	2	1–	5–	4+	4	5	5	5–	5	5	5
F2	2+	3	3	5–	5	5	5	5	5	5	5	5
F3	2+	2	1	5–	4+	4–	5	5	4+	5	5	5–
F4	2+	1	2–	5–	4+	5–	5	5	5	5	5	5
F5	2	4	2–	4	5	4+	5	5	5	5	5	5
F6	2–	2	2	3+	4+	4+	5	5	5	5	5	5
F7	2	1	2	3+	4	5	5	5	5	5	5	5
F8	1	1	0	3	4	1–	5	5	4–	5	5	5–
F9	1–	1–	0	3	4	1	5	5	4+	5	5	5–
F10	1–	1–	0	3–	4	1+	5	5	4–	5	5	5–

was because the compositions of the solvent formulations were unfavorable for minoxidil partitioning into the skin as a result of the unsaturated condition of the 2% added amount of minoxidil. Fortunately, minoxidil delivered by those cosolvent formulations containing various amounts of TPGS caused no visible abnormal changes (e.g., irritation or allergic contact reactions) in the skin.

Investigations of the sulfotransferases that catalyze minoxidil sulfation can enhance our understanding of the regulation of the response to minoxidil treatment in alopecia. Table 3 lists the changes in sulfotransferase activities in mice after topical application of 2% Regaine® and minoxidil in those cosolvent formulations containing various amounts of TPGS for 15 days. The results showed that sulfotransferase activity was significantly higher in mice for which 2% Regaine® was topically applied compared with those 10 cosolvent formulations containing lower amounts of TPGS (0.0 and 0.5%) for 15 days. When the added amount of TPGS was increased to 2%, significantly higher sulfotransferase activities were noted for the corresponding

Table 3
Sulfotransferase activities (units/μg) after topical application of 2% Regaine® and minoxidil formulations containing various amounts of TPGS (%) for 15 days

	Time (day)											
	0			5			10			15		
	0.0	0.5	2.0	0.0	0.5	2.0	0.0	0.5	2.0	0.0	0.5	2.0
Blank	0.34	X	X	0.86	X	X	1.39	X	X	1.12	X	X
2% Regaine®	2.22	X	X	2.12	X	X	2.05	X	X	1.31	X	X
F1	0.06	0.09	1.91	0.19	0.02	2.41	0.25	0.06	2.33	0.23	0.18	2.76
F2	0.07	0.12	0.75	0.07	0.01	1.47	0.16	0.12	1.72	0.17	0.27	3.45
F3	0.02	0.04	2.22	0.09	0.05	2.24	0.18	0.08	2.59	0.15	0.42	3.80
F4	0.03	0.06	0.75	0.09	0.06	1.72	0.10	0.08	1.72	0.03	0.38	1.68
F5	0.15	0.06	0.73	0.08	0.03	1.38	0.13	0.02	1.29	0.04	0.06	1.23
F6	0.09	0.13	2.61	0.15	0.07	2.85	0.19	0.02	2.76	0.13	0.08	0.67
F7	0.03	0.08	2.20	0.02	0.07	2.85	0.05	0.03	2.16	0.07	0.24	1.49
F8	0.09	0.04	0.34	0.10	0.09	0.86	0.16	0.02	1.35	0.16	0.03	1.51
F9	0.10	0.03	0.69	0.06	0.12	1.03	0.10	0.18	1.60	0.03	0.15	1.43
F10	0.05	0.05	0.78	0.11	0.14	1.55	0.05	0.04	1.42	0.07	0.11	1.62

cosolvent formulations. Data from this study further show inter-animal variations in sulfotransferase activity. In general, a relationship between hair growth and sulfotransferase activities in mice after topical application of 2% Regaine® and minoxidil formulations containing various amounts of TPGS was not demonstrated in the study. The results further intimate that platelet sulfotransferase is not likely an important factor in the biotransformation of minoxidil. It may be that enzymatic mechanisms involved in the sulfation of minoxidil are too complex to show correlations as predicted. Nevertheless, these results are consistent with those reported by Garland's studies (Johnson and Baker, 1987). The significance of platelet sulfotransferase in the pharmacological action of minoxidil seems difficult to assess.

Plasma concentrations of minoxidil from the topical application of 2% Regaine® and these 10 cosolvent formulations containing various amounts of TPGS were simultaneously evaluated in C57BL/6J mice, and the results are illustrated in Fig. 3. Plasma concentrations of minoxidil from 2% Regaine® were obviously greater than those of 2% minoxidil in those cosolvent formulations containing various amounts of TPGS. Plasma concentrations of minoxidil from those 10 cosolvent formulations in which the same amount of TPGS was added showed insignificant differences. A tendency for an increase in plasma concentration of minoxidil in mice after the topical administration of minoxidil formulations containing higher amounts of TPGS (2%) was noted. These phenomena coincided with the high levels of sulfotransferase activity in mice after the topical administration of minoxidil formulations containing the higher amount of TPGS (2%). A possible contribution of TPGS to the increases in the plasma concentration of minoxidil and sulfotransferase activity in mice must be considered. However, there were no positive correlations between plasma concentrations of minoxidil and sulfotransferase, and hair regrowth in mice after topical administration of 2% Regaine® and minoxidil in those 10 cosolvent formulations containing various amounts of TPGS.

In an attempt to enhance the hair growth effects while reducing the systemic effects of minoxidil when delivered percutaneously, the addition of TPGS in cosolvent formulations containing water, alcohol, and PEG 400 seemed to be beneficial. The hair growth

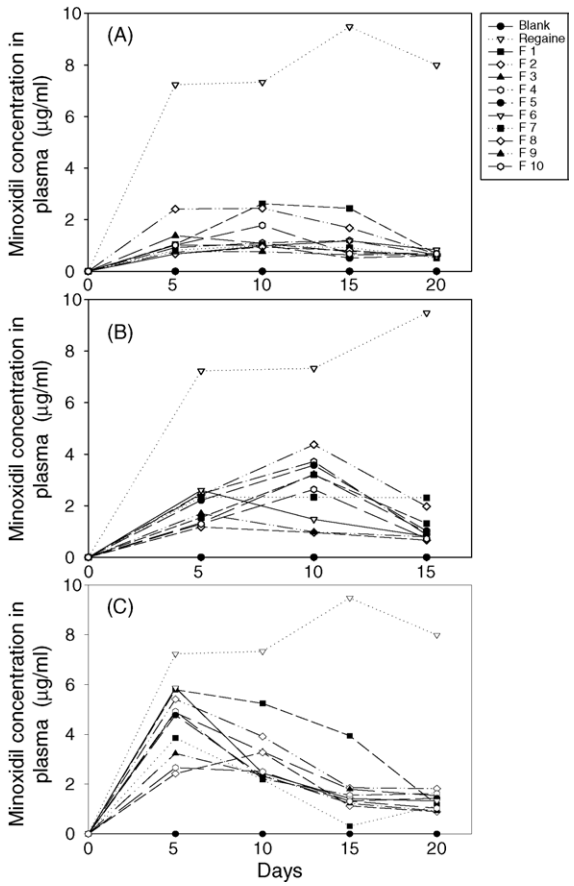

Fig. 3. Plasma concentrations of minoxidil in C57BL/6J mice after application of 2% Regaine® and minoxidil formulations containing various amounts of TPGS: (A) 0% TPGS, (B) 0.5% TPGS and (C) 2% TPGS.

effect was similar to that for Regaine® and the systemic effect was reduced using cosolvent formulations F1–F6 to deliver minoxidil percutaneously.

4. Conclusions

It was concluded that the efficacy in the promotion of the hair growth by minoxidil in these 10 cosolvent formulations was comparable to that by 2% Regaine®, but a lower amount of minoxidil was absorbed into the general circulation by the former than by the latter causing the least-serious systemic effect. The addition of TPGS at 0.5% in these 10 cosolvent formulations

was beneficial in terms of enhancing hair growth. Among them, 2% minoxidil in those formulations with the added water content of ≥50% with 0.5% TPGS seemed to be favorable choices for delivering minoxidil percutaneously with comparable efficacy to 2% Regaine® but with fewer systemic hypotensive effects.

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