Penetration of Minoxidil from Ethanol/Propylene Glycol Solutions: Effect of Application Volume and Occlusion

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Abstract - We have previously established that the relative concentrations of propylene glycol and ethanol as a binary solvent system have a significant effect on the skin penetration of 2% solutions of minoxidil at 50 uL/cm2. The present work extends these studies and investigates the penetration of minoxidil from the different vehicle combinations as functions of application volume and occlusion. Decreasing the application volume has a variable effect, which depends on vehicle composition. Penetration of minoxidil from 100% ethanol solutions decreased linearly with application volume. Generally, irrespective of the volume applied, the penetration of minoxidil increased with increasing ethanol fraction with a maximum penetration at 90% ethanol. Penetration from all the formulations was enhanced upon occluding the skin, with greatest increase evident in solutions with higher volatile fraction. Penetration of minoxidil in vivo showed trends similar to those seen in vitro.

Introduction

In an earlier study we described the influence of ethanol and propylene glycol cosolvents on skin penetration of minoxidil when 50 uL/cm2 of 2% solutions was applied. The penetration of minoxidil into and through the skin generally increased as the ethanol fraction of the binary solvent vehicle was increased. This effect could be due to one or a combination of the following: (1) an effect wherein ethanol evaporates rapidly and concentrates the drug in the residual formulation that remains on the skin (The increased thermodynamic activity would tend to drive the drug into the stratum corneum.) and/or (2) a penetration enhancement effect wherein ethanol alters the physical integrity of the stratum corneum barrier resulting in an increase in the ability of the drug to penetrate the skin.

We reason that the first of these two mechanisms would be much more dependent on the volume of formulation applied than would the latter. Obviously, light applications of the media evaporate off in a shorter time, increasing the thermodynamic activity of the drug, but limiting the period of deposition and solvent contact. We further reason that slowing or preventing evaporation by occluding the application will increase the solvent contact time and perhaps enhance penetration, while at the same time minimize thermodynamically forced deposition, allowing us to determine which effect predominates. We have therefore studied the effect of incrementally decreasing the application volume of 2% minoxidil solutions in the nonoccluded state and comparing selected volumes under occlusion.

Coldman et al. studied penetration of fluocinolone acetonide and its acetate from different combinations of volatile/ nonvolatile solvent mixtures (2-propanol and isopropyl myristate or propylene glycol) through human cadaver skin in vitro. They found that up to relatively high 2-propanol composition, penetration of drug increased dramatically with an increase in the fraction of the volatile solvent when administered in the open application, but occlusion severely limited its penetration. The maximum drug penetration occurred when the formulation contained 85-95% 2-propanol. Delivery dropped off dramatically when neat 2-propanol was used. Keying in on these past observations, a 90% ethanol formulation was included in our study.

Coldman et al found that the in vitro penetration of fluocinonide through human skin from DMSO solutions increased with a decrease in application volume. They proposed this to be due to influx of water through the skin from the receiver due to the hygroscopic nature of DMSO. This dilution of the application film would cause a decrease in saturation solubility of fluocinonide in the solution with resultant increase in thermodynamic activity. Since the smallest initial volumes would be diluted to the greatest extent by the influx of water during the first few hours, these solutions would develop the highest thermodynamic activities which was believed to enhance penetration of fluocinonide. More recently, Tsai et al. reported on penetration of minoxidil from different volumes of a 2% minoxidil solution in 20% propylene glycol/20% water/20% ethanol. They observed that epidermal, dermal, and receiver concentrations of minoxidil measured at 20 h increased between 17.5 and 31.25 uL/cm2 application volumes, but not between 31.25 and 62.5 uL/cm2. To gain a clearer understanding of the processes behind these application volume effects for minoxidil, we simplified the vehicle by removing water and used solutions with different ethanol/propylene glycol ratios.

Materials and Methods

Materials - Nonradioactive (cold) minoxidil (2,4-diamino-6-piperidinopyrimidine 3-oxide) and [3H]minoxidil with specific activity of 44 mCi/mmol were provided by the Upjohn Co. (Kalamazoo, MI). Propylene glycol (Sigma Chemical Co.) and neat (200 proof) ethanol (Midwest Grain Products, Perkin, IL) were used to prepare the formulations.

Methods - Minoxidil solutions were prepared by dissolving 0.04 g of minoxidil in 2 mL of either the neat solvents or binary mixtures of ethanol and propylene glycol in order to achieve 2% solutions in media variously containing 0%, 25%, 50%, 75%, and 100% ethanol. Approximately 1 uCi of [3H]minoxidil was incorporated into the solutions. In a limited initiative toward the end of the study, 0.5% and 1% minoxidil solutions in ethanol were also prepared.

In Vitro Studies – Sixty to ninety day old hairless mice (Skh-hr-1; Charles Rivers Laboratories, Inc., Wilmington, MA) were euthenized, and their skin was excised. Subcutaneous fat was carefully removed, and the skin sections were mounted within Franz diffusion cells. The dermal side of the skin was placed in contact with the receiver medium. The cross-sectional area of the cells was 2 cm2. The receiver medium used in all the studies consisted of isotonic 0.07 M phosphate buffer (pH 7.4) containing 0.02% sodium azide as preservative. Sink conditions (less than or equal to 6% saturation of minoxidil in the receiver solution) were always maintained. A temperature of 32 C was achieved at the skin surface by circulating water at 35 C through the cell jacket. Application volumes of 12.5, 25, 37.5, and 50 uL/cM2 of the 2% solutions were used to investigate the effect of volume. Applications of 25 and 50 uL/cm2 of the 1% minoxidil solution and 50,uL/cm2 of the 0.5% minoxidil solution were also studied in a separate set of experiments. In the occluded experiments, 25 and 50 uL/cm2 of the

2% solutions were applied to the skin and the cells were then sealed with Parafilm, to retard the evaporation of ethanol and propylene glycol. Three to five runs were made at each experimental condition.

At different time intervals during the experiments (1, 2, 3, 4, 5, 6, 8, 12 h), samples of approximately 0.5 mL were withdrawn from the receiver sampling port and replaced with the same volume of fresh buffer. At the conclusion of the experiments, (i.e., at 8 and 24 h), the skin samples were removed and the treated surface was stripped four to six times with Scotch 810 tape (3M, Minneapolis, MN). They were then heat treated at 60 'C for 60 s to facilitate separation of the remaining epidermis from the dermis. Volumes of 2 mL each of the receiver solution were then transferred to scintillation vials. The glass caps which form the donor compartment were rinsed 10 times with water and these rinses were added to scintillation vials. The tape strips and heat-separated epidermis and dermis were analyzed separately as described below.

In Vivo Studies - Glass caps similar to Franz diffusion cell caps were attached to the dorsal skin surface of anesthetized mice with a suitable adhesive (Super glue). Formulation volumes of 12.5,25, and 50 uL/cm2 were added and the samples were left open to the atmosphere. The mice were anesthetized by intraperitoneal injection of 0.25 mL of 1% sodium pentobarbital solution (i.e., ca. 0.1 mg of sodium pentobarbital/g of body weight) and maintained in an anesthetized state throughout the experiments with additional injections of 0.1 mL every hour or as required. A minimum of three animals were used in each individual experiment. At the end of 8 h, the animals were sacrificed with an overdose of pentobarbital. The skin over the treated area was excised and tape stripped and the epidermis separated from the dermis as in the in vitro experiments. The donor caps were rinsed 10 times. All the tissue residues and rinses were added to separate scintillation vials.

Analytical Method - Liquid samples were directly added to scintillation cocktail (Ecolite (+); ICN Biomedicals, Irvine, CA) in scintillation vials. The tape strips and heat-separated epidermis and dermis were also placed directly in Ecolite but were soaked for 48 h and then sonicated for about 10 min before counting. The amounts of 3H were then measured in a liquid scintillation counter (Beckman LS 9000; Beckman Instruments, Fullerton, CA).

The amounts of minoxidil in the different skin compartments and in the receiver were calculated from the values of 3H obtained from the scintillation counter. These values were normalized to give the final results in the form of ug of minoxidil/cm2 of skin. The pseudosteady-state flux was calculated from the permeation profile as the slope of the first linear portion of the concentration versus time plot that lasts for at least 5 h (with a lag time of about 3 h for most of the solutions). An exception was found in the open application of solutions in 100% ethanol. Here the first part of the curve was linear from 0 to a maximum of 3 h and then the slope decreased with time. Interestingly, in the occluded experiments with 100% ethanol solutions, the penetration curve was similar to that of the other formulations, with a lag time of about 3 h.

Results and Discussion

Table 1 shows the amounts of minoxidil recovered from the receiver compartment at 12 and 24 h. It is clear that an increasing ethanol fraction leads to generally increasing delivery with two notable exceptions. The amount of minoxidil that penetrated the skin from an application of 12.5 uL/cm2 of 2% minoxidil in pure ethanol at 24 h is notably low, lower even than the 50% ethanol/50% propylene glycol solution. Also, penetration of minoxidil peaked at the 90% ethanol solution for all application volumes by the 24 h recovery point. The amounts delivered were roughly double those found when neat ethanol was used as the vehicle. This could be explained in a number of ways, but the most likely reason is that precipitation is delayed and the drug is maintained in solution for a longer duration as proposed earlier by Coldman.

We study the different media to determine if, after evaporation of the ethanol in the formulation, they behave like neat propylene glycol solutions. In other words, the loss of ethanol would concentrate the 75% propylene glycol solution by one third, the 50% propylene glycol solution by a factor of 2, the

Table I - Receiver Concentrations of Minoxidil at 12 and 24 h from Different Volumes of 2% Minoxidil Solutions in Propylene Glycol and Ethanol as Open Applications

Fraction Ethanol	Amount of Minoxidil that Penetrated (Ug/CM2) from 2% Solutions			
	12.5 uL/cm2	25 uL/cm2	37.5 uL/cm2	50 uL/cm2
		12 h		
0.00	3.3 ± 1.6 (1.32)	4.0 ± 0.3 (0.80)	6.4 ± 0.8 (0.85)	7.1 ± 1.5 (0.71)
0.25	4.2 ± 1.8 (1.68)	4.5 ± 1.3 (0.90)	5.2 ± 0.5 (0.69)	12.0 ± 1.6 (1.20)
0.50	5.7 ± 0.3 (2.28)	7.5 ± 1.9 (1.50)	10.0 ± 2.0 (1.33)	13.3 ± 0.8 (1.33)
0.75	8.9 ± 1.9 (3.56)	7.1 ± 1.1 (1.42)	12.4 ± 1.1 (1.65)	23.2 ± 2.6 (2.32)
0.90	18.8 ± 3.5 (7.52)	28.3 ± 4.8 (5.66)	19.6 ± 3.5 (2.61)	31.4 ± 4.3 (3.14)
1.00	9.2 ± 0.2 (3.68)	17.6 ± 0.9 (3.52)	27.1 ± 4.2 (3.61)	32.9 ± 4.1 (3.29)
		24 h		
0.00	9.3 ± 1.6 (3.72)	7.7 ± 0.8 (1.54)	11.8 ± 1.8 (1.57)	16.3 ± 2.0 (1.63)
0.25	13.5 ± 4.0 (5.40)	8.5 ± 0.6 (1.69)	10.5 ± 1.6 (1.40)	21.4 ± 1.5 (2.14)
0.50	23.1 ± 3.1 (9.25)	20.2 ± 3.9 (4.03)	24.6 ± 5.9 (3.28)	26.0 ± 3.1 (2.60)
0.75	28.9 ± 3.6 (11.57)	31.3 ± 5.2 (6.26)	35.0 ± 4.0 (4.67)	57.7 ± 6.2 (5.77)
0.90	33.8 ± 4.1 (13.52)	79.0 ± 6.6 (15.80)	90.6 ± 14.2 (12.08)	157.3 ± 19.7 (15.73)
1.00	15.4 ± 0.3 (6.15)	31.8 ± 1.1 (6.37)	46.7 ± 2.3 (6.23)	63.9 ± 3.3 (6.39)

a Each data point represents mean ± standard error (**n** greater than or equal to **3**). The percentage of the applied dose that penetrated is shown in parentheses.

25% propylene glycol solution by a factor of 4, and the 10% propylene glycol solution by a factor of 10. Upon examining the penetration of minoxidil from 37.5 uL/cm2 applications at 24 h, we see that, relative to pure propylene glycol, 25% ethanol did not lead to an increase in penetration, but the amount that penetrated approximately doubled at 50% ethanol, tripled at 75% ethanol, and increased 8-fold at 90% ethanol (Table 1). Similar ratios were seen across the board with 25 and 50 uL/cm2 applications and up to 75% ethanol composition when 12.5 uL/cm2 was applied. Larger application volumes tended to be associated with increased absorption of minoxidil unlike the results of Coldman. However, at all propylene glycol levels and most notably the 50% level, the efficiency of delivery as determined by the percentage of applied amount that was actually delivered dropped off. Again, it is evident that solutions in pure ethanol behave differently with the efficiency remaining unchanged with volume.

With neat ethanol as the solvent, the entire application is volatile and all such applications were accompanied by complete drying of the vehicle very early into the application period. For this medium, the formation of crystals of minoxidil on the membrane surface was evident within the first halfhour irrespective of the amount of formulation applied up to the maximum of 50 UIJCM2. The processes underlying the evaporation and commensurate deposition of minoxidil into the tissue led to a systematic and virtually linear increase in its penetration over the experimental time frame. The slope of the statistically best line drawn through the data for receiver concentrations at 12 h versus application volumes, forced through the (0,0) point, is 0.67 with a correlation coefficient of 0.995 (Figure 1). The slope of the line through the 100% ethanol data at 24 h, also forced through the origin, is 1.27, which is 1.9 times the slope at 12 h. Hence, there is a near doubling of the amounts penetrated at 24 h relative to

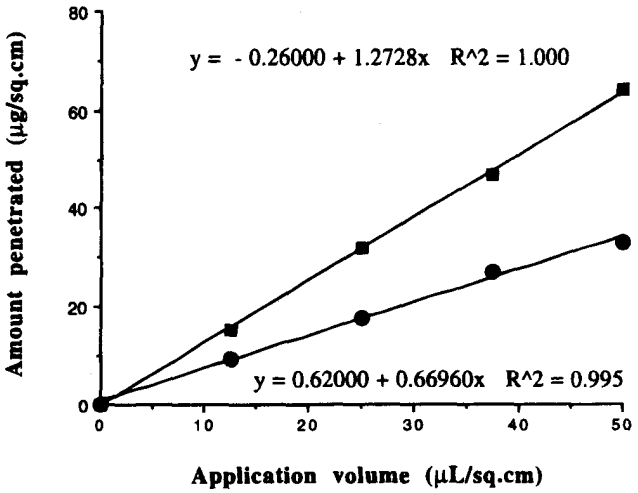

Figure 1 - Amount of minoxidil penetrated per unit area of skin from a 2% solution in ethanol at (CIRCLE) | 12 h and (SQUARE) 24 h as a function of application volume.

the amounts seen at 12 h. The linear increase could be explained one of two ways: deposition of minoxidil may be proportional to the time it takes for the ethanol to evaporate completely which increases with increase in volume, evaporation being a zero-order process. This will be true if the deposition of minoxidil comes to an abrupt stop upon exhaustive evaporation of the solvent and a reservoir is set up in the skin which is slowly cleared to the receiver compartment. The other possible explanation is a dependency of penetration on total amount of minoxidil applied to the skin, which also increased linearly with application volume. To determine which one of these two factors was responsible for the unique behavior of solutions in pure ethanol, we conducted a set of experiments in which we applied equal amounts of minoxidil in different volumes of ethanolic solutions by changing the concentration of minoxidil in the solutions. We found that the receiver concentrations of minoxidil at 24 h upon application of 25 uL/cm2 of 1% minoxidil solution and 50 uL/cm2 Of 0.5% minoxidil solution in ethanol were 15.4 ± 2.3 ug/cm2 (flux = 1.7 ± 0.3,ug/h/cm2) and 18.0 ± 0.4,ug/cm2 (flux = 2.2 ± 0.1 /ug/h/cm2), respectively. These values compare well with the value of 15.4 ± 0.3 ug/cm2 obtained at 24 h for 12.5 uL/cm2 Of the 2% minoxidil solution in ethanol. Similarly, the receiver concentration at 24 h from 50 uL/cm2 of 1% minoxidil solution in ethanol was 31.6 ± 3.3 ug/cm2 (flux = 2.9 ± 0.3,ug/cm2) which is close to the value of 31.8 ± 1.1 ug/cm2 observed at 24 h for 25 uL/cm2 of the 2% minoxidil solution. Hence, the net penetration of minoxidil. appears to be dictated by the amount of drug per cm2 of application irrespective of the volume of the solvent. Clearly, the ethanol evaporates rapidly and the 0.5% and 1% solutions in proportionally larger volumes of ethanol pass through a stage very soon after application when they are effectively 12.5 or 25 uL/cm2 of a 2% minoxidil solution. Hence, in the case of pure ethanol solutions, the amount of minoxidil that penetrates the skin depends on the amount of minoxidil present in the application and the duration of the experiment.

The values of the flux for the different volumes of the various formulations are shown in Table 2. These values are consistent with the 12 h accumulations noted in Table 1. They also resolve the ambiguity in the 90% ethanol data by putting them in a more reasonable order (i.e., increase with increasing volume). The fluxes for the 100% ethanol solutions are observed to be greater than those of the 90% ethanol solutions for volumes greater than 12.5 uL/cm2. Thus, the penetration of minoxidil through the skin was faster with neat ethanol, but since this was true only for the first 3 h, the slower flux

Table 2-Flux of minoxidil (ug/h/cm2) through the Skin from Different Application Volumes of 2% Minoxidil Solutions in Propylene Glycol and Ethanol In Vitro

Fraction Ethanol	Flux (ug/h/cm2)			
	12.5 (uL/cm2)	25 (uL/cm2)	37.5 (uL/cm2)	50 (uL/cm2)
0.00	0.28 ± 0.01	0.31 ± 0.04	0.55 ± 0.10	0.73 ± 0.05
0.25	0.25 ± 0.08	0.34 ± 0.03	0.54 ± 0.06	0.89 ± 0.03
0.50	0.50 ±0.03	0.70 ± 0.09	1.00 ± 0.13	0.97 ± 0.07
0.75	0.51 ± 0.05	0.58 ± 0.05	1.13 ± 0.07	2.05 ± 0.30
0.90	1.35 ± 0.24	1.58 ± 0.19	1.96 ± 0.35	2.06 ± 0.19
1.00	1.35 ± 0.11	2.64 ± 0.13	3.70 ± 0.34	4.60 ± 0.06

Each data point represents mean ± standard error (n greater than or equal to 3).

Table 3 - Comparison of Amount of Minoxidil Recovered from the Dermis In Vivo and in Vitro at 8 h from Different Volumes of 2% Minoxidil Solutions in Three Different Formulations

	Amount of Minoxidil (ug/cm2) Recovered in Dermis					
Volume	100% Propylene Glycol		50% Ethanol		100% Ethanol	
(uL/cm2)	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro
12.5	0.15 ± 0.02	0.33 ± 0.08	0.38 ± 0.10	0.47 ± 0.12	0.50 ± 0.04	0.70 ± 0.15
25	0.30 ± 0.00	0.74 ± 0.15	0.55 ± 0.10	1.20 ± 0.30	0.85 ± 0.02	1.55 ± 0.15
50	0.60 ± 0.20	0.90 ± 0.20	1.50 ± 0.10	3.00 ± 0.30	2.30 ± 0.00	3.30 ± 0.70

Each data point represents mean ± standard error (n greater than or equal to 3)

from 90% ethanol solutions over a more extended period (3-8 h) eventually lead to more total minoxidil penetrating through the skin.

We reasoned that tissue concentrations of minoxidil would be comparable if in vivo uptake of the drug was mechanistically similar to the in vitro permeation. The in vivo and in vitro dermal minoxidil concentrations at 8 h for different application volumes of three different solutions are compared in Table 3. The in vitro values were 1.2-2.6 times greater than the in vivo depositions. Although the actual amounts penetrated in vitro are greater than those observed in vivo, similar trends are evident. The dermal concentrations increased as the volume fraction of ethanol increased and also as the application volume increased. The higher values in vitro are almost certainly due to underestimation of clearance of drug from the dermis. Due to the presence of functioning epidermal-dermal vasculature in vivo, some of the drug partitions into the blood near the epidermal-dermal junction, limiting the amount which accumulates in the richly vascularized dermis. There is no comparable clearance mechanism in vitro.

The amounts of minoxidil that penetrated the skin from occluded solutions in vitro in 12 and 24 h have been tabulated in Table 4. These values were higher than those for comparable nonoccluded experiments in all cases. The occluded solutions were 1.3-6.1 times more efficient in their delivery. Ratios for the 25 ul/cm2 applications were, in general, greater than their 50 ul/cm2 counterparts. The only reversal was with the 100% ethanol solutions, a composition already shown to be a special case. Minoxidil deposition from the occluded solutions peaked with the 90% ethanol solutions, similar to the open experiments. It should be noted here that the volume of the occluded donor compartment was 4 cm2, providing some space for the solvent molecules to evaporate and even condense on the surface. This may explain the superior efficiency of the 90% ethanol solutions over neat ethanol solutions even in the occluded state. The results in Table 4 reinforce the fact that the duration of solvent residence on the skin is an important determinant, especially for the volatile solvent ethanol. They also suggest a direct penetra-

Table 4-Comparison of the Amount of Minoxidil that Penetrated through Hairless Mouse Skin at 12 and 24 h from 25 and 50 uL/cm2 of Occluded Applications of 2% Minoxidil Solutions in Propylene Glycol and Ethanol

	Amount of Minoxidil Recovered (ug/cm2) from 2% Solutions			
Fraction	12 h		24 h	
Ethanol	25 uL/cm2	50 uL/cm2	25 uL/cm2	50 uL/cm2
0.00	8.0 ±1.6 (2.0)	9.5 ± 1.2 (1.3)	20A ± 3.4 (2.6)	23,9±2.4 (1.5)
0.25	14.2 ±0.5 (3.2)	18.4 ± 0.9 (1.5)	41.9 ± 3.3 (4.9)	52.3 ± 5.2 (2.4)
0.50	20.6 ±1.2 (2.7)	28.1 ±1.8 (2.1)	69.9 ± 7.3 (35)	94.1 ±11.7 (3.6)
0.75	37.7 ± 5.4 (5.3)	50.7 ± 7.4 (2.2)	171.4 ± 8.1 (5.5)	202.7 ± 21.7 (3.5)
0.90	172.2 ± 15.6 (6.1)	170.9 ± 38.4 (54)	222.4 ± 4.6 (2.8)	473.6 ± 20.9 (3.0)
1.00	60.6 ± 13.7 (3.4)	166.4 ± 35.0 (5-1)	99.0 ± 12.0 (3.1)	257.4 ± 52.7 (4.0)

ı Each data point represents mean ± standard error (n greater than or equal to 3). Ratios of the amount penetrated from the occluded applications / open applications are shown in parentheses.

tion-enhancing action for ethanol. The unusual increase in receiver concentration between 12 and 24 h observed in some of the formulations may be due to hydration of the skin, which has been shown to increase penetration of some compounds.

In conclusion, the effect of application volume depends on the vehicle composition, with a greater effect being observed as the fraction of volatile component increased, especially at ethanol concentrations over 50%. The penetration of minoxidil through the hairless mouse skin peaks at 90% ethanol solutions for all the volumes studied, signifying the importance of propylene glycol in the formulations. The in vitro results are in close parallel to the in vivo data as evidenced by dermal accumulations. Penetration-enhancing effects of ethanol play a significant role in minoxidil penetration as the occluded solutions showed increased penetration for all the formulations.