

Differential Penetration of Skin by Topical Metronidazole Formulations

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ABSTRACT

This in vitro study compared the release and penetration of various metronidazole formulations: 0,75% gel, 0,75% cream, and a new 1% cream. Six skin samples from each of six women (28 to 58 years of age) were used to test the three formulations, for a total of 12 samples per formulation. A 10-mg target dose of each metronidazole formulation (75 µg of 0,75% gel and 0,75% cream, 100 µg of 1% cream) was applied to a skin surface of 1 cm² per cell. The randomized application schedule controlled for variability in skin origin, individual cell thickness, and formulations. Seven fluid samples were collected over a 15-hour period. Metronidazole concentrations were measured in both the skin samples and through the fluid from a dynamic diffusion system with the use of high-performance liquid chromatography method with ultraviolet detection. Cutaneous penetration of metronidazole was significantly greater with the 0,75 % gel and cream formulations than with the 1% cream. These results suggest that the delivery vehicle may be more important than the active drug concentration in cutaneous delivery of metronidazole.

Keywords: metronidazole; cutaneous delivery; cutaneous penetration

INTRODUCTION

Metronidazole has been available in gel (MetroGel* Topical Gel/ Rosex Gel, 0,75 %) and cream (MetroCream+ Topical Cream/ Rosex Cream, 0,75%) formulations for a number of years, and both have been highly effective treatments of inflammatory papules, pustules, and erythema of rosacea /1-3/. Recently, a 1% cream formulation was introduced (Noritate** Cream, 1%) and demonstrated efficacy as therapy for rosacea /4/. Although there are no clinical comparisons between either 0,75% formulation and the 1% formulation, it might be assumed that the higher drug concentration will result in the best cutaneous absorption. Cutaneous penetration of metronidazole is not just a function of drug concentration, however; the delivery system also is critical /5,6/. For example, vehicle hydration, among other vehicle factors, can help counteract the formidable resistance of the stratum corneum, ultimately enhancing topical drug penetration through the skin /5,6/.

This in vitro study assessed the cutaneous penetration of three topical metronidazole formulations: two involving 0,75% drug concentrations and one involving a 1% concentration.

* Registered trademark of Galderma Laboratories, Inc. Fort Worth, Tex, USA

+ Trademark of Galderma laboratories, Inc, Fort Worth, Tex, USA

** Trademark of Dermik Laboratories, Inc., Collegeville, PA, USA

MATERIALS AND METHODS

The in vitro liberation-penetration study followed recommendations of the FDA and AAPS during the workshop on principles and practices of in vitro percutaneous penetration studies, and the European Centre of the Validation of Alternative Methods (ECVAM) during the workshop on methods for assessing percutaneous absorption /7,8/.

Formulation Studied

The following formulations were commercially available: metronidazole topical gel, 0,75% (w/w) and metronidazole topical cream, 0,75% (w/w) were obtained from Galderma Laboratories. Metronidazole topical cream, 1% (w/w) was a new 1% cream commercialized by Rhone-Poulenc Rorer Inc.

Human Skin

Human skin biopsies were obtained from plastic surgery on human breast and abdomen. After removal, the skin was separated from subcutaneous fat and stored at -18°C until used. Six full-thickness skin samples excised from six human female subjects were used in the experiment. The thickness of the skin varied with the donor and the anatomic site. Therefore, in order to avoid bias in the comparison of the test articles, the thickness of the skin was determined. The age of the subjects, anatomic location of sample, and mean thickness per subject are given in Table 1 /3/.

Experimental Conditions

The in vitro liberation-penetration of metronidazole was assessed using “GH” flow-through diffusion cells with a diffusion area of 1 cm² and a receptor volume of approximately 3 mL /9/. A phosphate buffer saline solution with a pH of 7,2 was used as a receptor fluid.

Table 1. Age of Fparticipants Skin-Sample Location and Mean Skin-Sample Thickness

Participant No.	Age Y	Sample Site	Skin-Sample Thickness Mm (+/-SD)
1	38	Abdomen	1,66 +/- 0,05
2	28	Abdomen	0,85 +/- 0,07
3	50	Abdomen	1,18 +/- 0,05
4	41	Breast	1,56 +/- 0,17
5	50	Abdomen	1,17 +/- 0,07
6	58	Abdomen	1,25 +/- 0,21

The diffusion cells were mounted in a dynamic system maintained at 37°C by a circulating Sink conditions, the flow rate was about 3 mL/h with a 3-mL collection/h throughout the experiment. The total collection time was 15 hours, with an hourly collection of the receptor fluid. These hourly fractions were then pooled to finally obtain seven different fractions: 0, 0-1, 1-3, 3-6, 6-9, 9-12 and 12-15 hours /3/.

To compare metronidazole liberation from the three different formulations, each formulation should be run over 12 cells. Each run including six cells was performed with the skin from one single donor by applying each of three formulations to each two skin samples. Thus, six different experiments were performed following an allocation table established so as to control the variability among experiment, donors, and cell positions /3/.

Application of the Formulation

A target finite dose of 10 mg of formulation exactly weighing 75 µg of metronidazole for the 0,75% gel and cream formulations and 100 µg for the 1% cream was applied to a skin surface of 1 cm² per cell. The formulations were applied under nonoccluded conditions.

Bioanalysis

Metronidazole levels in the different skin compartments were measured using a high-performance liquid chromatography with ultraviolet (HPLC-UV) detection. The HPLC-UV method was successfully validated; the lower limit of quantification and detection was determined to be equal to 10 ng/mL of sample. The specificity of the method was good. Precision (for repeatability and reproducibility) and accuracy were always better than 7,6% and 4,7%, respectively.

Data Analysis

For each formulation tested, a metronidazole mass balance was calculated, taking into account the excess and amounts in skin and in receptor fluid. A descriptive comparison of the formulations was performed on the percentage of metronidazole penetrated into the skin and found in the collected fraction as a function of the amount applied and on the amount of the drug in the surface excess, in the skin (epidermis including stratum corneum plus dermis), and in the collected fluid fractions.

Kinetic Analysis

Metronidazole was continuously diffusing into the receptor fluid during the experiment. To determine clearance of metronidazole from the skin sample, the total amount of metronidazole penetrated per cm² versus time was plotted. From this time-course of penetration plot, a descriptive comparison of the formulations was performed on

- the amount of drug in the collected fractions versus time
- the rate of penetration (flux vs time).

Statistical Analysis

The variables analyzed included the amount of metronidazole applied to each skin sample; quantities of metronidazole found in the skin layers (dermis, epidermis including stratum corneum, and dermis plus epidermis); quantities of drug in the collected fractions (0-15 hours); excess drug found on the skin sample; total quantities of drug found in the skin; and collected fluid fractions.

Each variable was logarithmically transformed before being submitted to an analysis of variance controlling for experiment, cell, and formulation. Estimates of ratios between formulations were calculated using geometric means with 90% confidence intervals.

RESULTS

Table 2 shows the comparisons of in vitro release and penetration of 0,75% metronidazole gel, 0,75% metronidazole cream, and 1% metronidazole cream. Total skin absorption with both 0,75 formulations was significantly greater than with the 1% formulation ($P < 0,02$). Similarly, total skin metronidazole concentration was significantly greater with 0,75% cream than with 1% cream ($P = 0,016$).

Over the 15-hour sampling period, the 0,75% gel formulation produced significantly higher metronidazole concentrations in fluid from the dynamic diffusion system than both cream formulations. Drug quantities in collected fluid fractions were significantly greater with 0,75% gel than with 1% cream ($P = 0,006$; Table 2).

The cumulative collected quantity of metronidazole recovered in the fluid receptor from 0 to 15 hours and the individual metronidazole flux per collected interval are seen in Figures 1 and 2, respectively. The cumulative quantity of metronidazole recovered in the fluid

receptor was significantly higher with 0,75% gel than with both cream formulations; no apparent reduction in lag time occurred.

Table 2. Metronidazole Levels (mean +/- SD) With 0,75% Metronidazole Gel, 0,75% Metronidazole Cream, and 1% Metronidazole Cream

	Metronidazole Formulation*		
	A (0,75% gel)	B (0,75% cream)	C (1% cream)
Real applied dose, µg	76,3 +/- 1,1	75,1 +/- 0,8	100,1 +/- 1,2
Recovery in surface excess And upper cell washing, µg	42,7 +/- 1,5	47,2 +/- 2,2	61,1 +/- 2,7
% of applied dose	56 +/-2	63 +/-3	61 +/-3
Real applied dose, µg	76,3 +/- 1,1	75,1 +/- 0,8	100,1 +/- 1,2
Epidermis, µg	7,1 +/-1,0	6,2 +/-0,6	4,3 +/-1,2
Dermis, µg	3,9 +/-0,5	3,9 +/-0,7	2,5 +/-0,3
Total skin absorption (1): E+D, µg	10,9 +/-1,4	10,3 +/-0,8**	6,8 +/-1,3
% of applied dose	15 +/-2	14 +/-1	6,8 +/-1,3
Cumulative fractions (0-15 h), µg	7,1 +/-1,3	4,2 +/-0,9	3,9 +/-0,9
Lower cell washing Collected fluid fractions +	ILQ	ILQ	ILQ
Lower cell washing (2): F+L, µg	7,1 +/-1,3	4,2 +/-0,9	3,9 +/-0,9
% of applied dose	9,3 +/-1,7	5,6 +/-1,1	3,9 +/-0,9
Total skin + collected fractions (total cutaneous penetration): 1+2	18,1 +/-1,3	14,4 +/-0,9**	10,8 +/-1,4
% of applied dose	24 +/-2	19 +/-1	10,7 +/-1,3
Mass balance	60,8 +/-1,9	61,6 +/-2,1**	71,9 +/-2,8
% of applied dose	80 +/-2	82 +/-3	72 +/-3

E=epidermis; D=dermis; F=cumulative fraction; L=lower cell washing; ILQ=inferior to the limit of quantification (<10 ng/mL-1).

* Applied over 15 h; n=12 cells

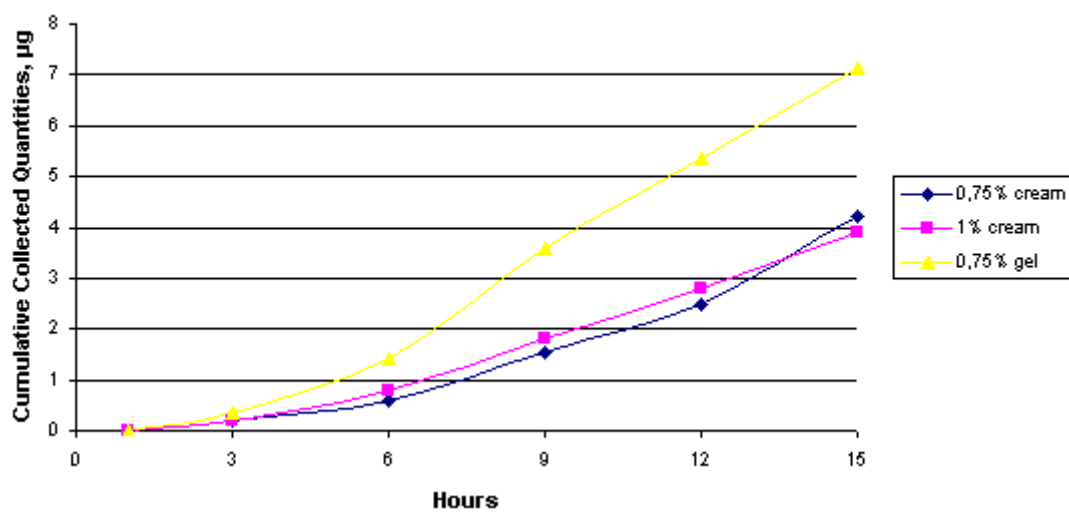
** n=11

Flux results (µg/h/cm²) were compared for each formulation. Steady-state flux was approximately 0,6 µg/h/cm² for the gel formulation and approximately 0,3 µg/h/cm² for both cream formulations (se Fig 2) /3/.

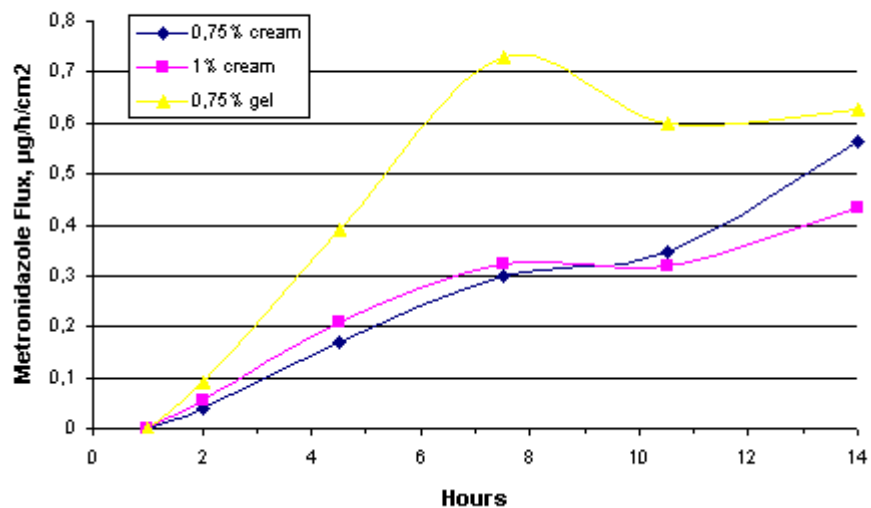
Total cutaneous penetration (total skin + receptor fluid fractions) was significantly greater with 0,75 % gel than with 1% cream (P=,001; Table 2).

The two 0,75% formulations differed significantly only in the quantities of metronidazole recovered in the collected fractions (P=,025; see Table 2, Figs 3 and 4). The mass balance evaluation indicated an average recovery of 80% for 0,75% gel, 82% for 0,75% cream, and 72% for 1% cream.

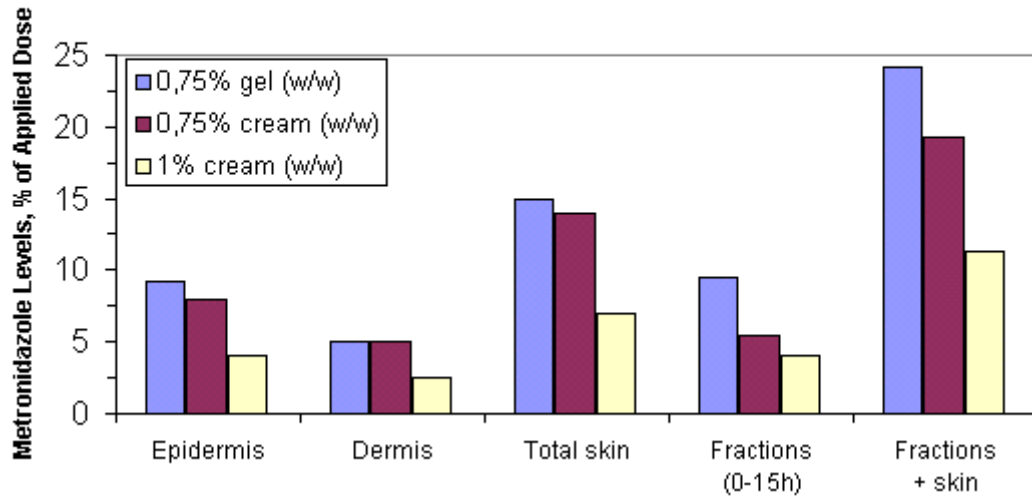
Cumulative metronidazole values (mean) in collected fluid fractions



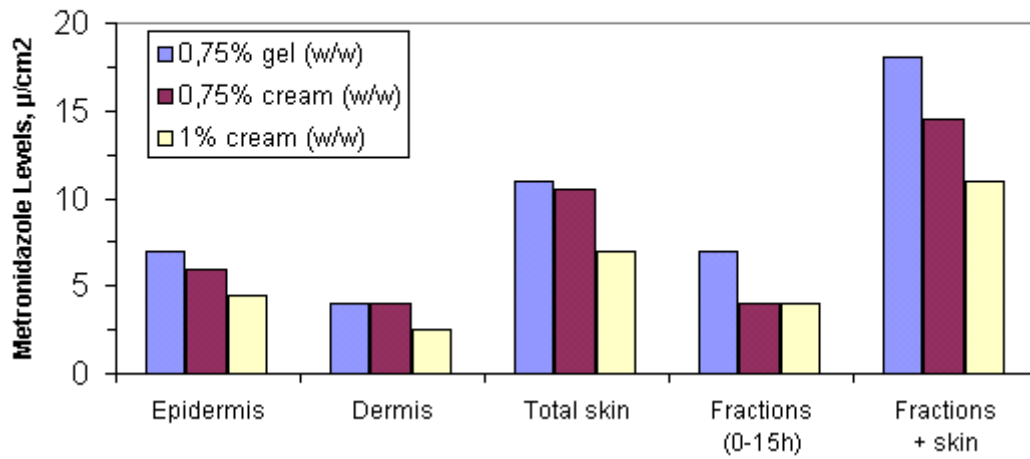
Flux values (mean) of metronidazole 0,75% gel, 0,75% cream and 1% cream



Percentage of applied dose of three metronidazole formulations in skin samples and collected fluid fractions (mean)



Metronidazole quantities in skin samples and collected fluid fractions



DISCUSSION

Metronidazole has relatively high water solubility /10/. The 0,75% cream and gel formulations contain the active ingredient in solution; therefore, as a hydrophilic molecule, more metronidazole will be available for cutaneous penetration. In other forms such as suspensions, metronidazole can precipitate out in crystalline form and be filtered out by the stratum corneum. The formulation's water content also can influence penetration; hydration of the vehicle or use of moisturizers that increase hydration of the stratum corneum can enhance topical drug absorption /6/. Both 0,75% metronidazole formulations contain a high percentage of water and demonstrate a comparably higher level of absorption than the 1% formulation /3/.

Other examples of the vehicle's influence on topical bioavailability and drug potency abound. For example, different formulations of 0,05% diflorasone diacetate have demonstrated different topical bioavailability /11/. The potency of this drug in affecting the hypothalamic-pituitary-adrenal axis can also be a function of the vehicle. The ointment formulation of 0,05% diflorasone diacetate is more potent than the same drug concentration prepared as a cream /12/.

Another vehicle factor explaining the superior penetration of the 0,75% metronidazole gel is the presence of the hydrophilic solvent, propylene glycol. Previous studies of vehicle influence on topical metronidazole delivery found that propylene glycol was important for maximal drug penetration through the stratum corneum /13/.

These favorable vehicle factors, which are present in the 0,75% metronidazole formulations, appear to be more important than the drug concentration itself in affecting cutaneous penetration. Indeed, increasing drug concentrations in topical formulations can influence cutaneous absorption only to a point. Stoughton /5/ has shown that as the concentration of a topical hydrocortisone formulation increases from 1% to 4%, a definite decrease occurs in the percentage of the applied agent that will penetrate into the skin.

These in vitro study results support the conclusion that the 0,75% metronidazole concentration may be optimum for penetration; exceeding this active drug concentration does not increase the amount of drug that is cutaneously absorbed. The gel formulation, with its higher water content, was originally designed to minimize the irritation and dryness that occurred with a previously developed 1% cream /14/. Now another benefit has been demonstrated for both the gel and cream 0,75% formulations: with their high water content, these formulations enhance penetration through the skin.

CONCLUSION

No vehicle is appropriate for all topical drugs. Rather, each topical system must be designed in deference to the active drug for optimal penetration and therefore therapeutic effects /15/. This in vitro study demonstrated that the vehicle composition is critical for topical drug delivery. Both 0,75% products have been formulated to provide optimal release and penetration of metronidazole through the stratum corneum barrier.

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