Evaluation of Metronidazole Bioavailability from Dermal Products Using an In Vitro Permeation Test (IVPT) Across Human Skin

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Purpose

Metronidazole is used to treat moderate to severe rosacea, a chronic, inflammatory skin condition and is commercially available in different products at the same strength. The purpose of the present study was to characterize the rate and extent of metronidazole absorption through excised human abdominal skin from topical drug products using an in vitro permeation test (IVPT). The IVPT studies were conducted to characterize the cutaneous pharmacokinetics (PK) of metronidazole from topical products at the same strength but with different formulations, thereby comparing bioavailability and supporting an evaluation of bioequivalence (BE). The ultimate goal is to support the development of a consistent study protocol for the evaluation of BE for topical products using characterization-based approaches.

Methods

Study Design

Metronidazole topical gel, 0.75% (reference) was used as the reference and an approved generic metronidazole topical gel, 0.75% (generic) and generic metronidazole topical cream, 0.75% (generic), were used as positive and negative controls, respectively, for evaluating BE (Table 1). Drug products were rubbed on 0.95 cm^2 areas of skin pieces with an inverted high-performance liquid chromatography (HPLC) vial after dispensing a pre-determined amount of 13.5 µL gel or 13 µL cream to achieve a target dose of 10 mg/cm^2. The IVPT was conducted with a PermeGear® In-Line flow-through diffusion cell system (PermeGear; Hellertown, PA). Skin surface temperature was maintained at 32.0 ± 1.0°C with a circulating water bath, verified prior to dosing using a traceable® infrared thermometer. Isotonic phosphate buffer (pH 7.4 ± 0.1) was used as the receiver solution; pumped continuously through diffusion cells at a rate of 1 rpm (approximately 1.0 mL/h). The fraction collector was programmed to continuously collect samples every 2 h for 24 h. Each of the three metronidazole topical products was tested on ex vivo human skin from the same five different donors, with four replicate skin sections/donor/product. Data are presented as mean ± SEM, n=5 donors.

Table 1. Inactive ingredients in each metronidazole product

	Metronidazole gel, 0.75% (reference)	Metronidazole gel, 0.75% (generic)	Metronidazole cream, 0.75% (generic)
Product	Topical gel	Topical gel	Topical cream
Inactive ingredients	Carbomer 940	Carbopol 980	emulsifying wax
			isopropyl palmitate
	propylene glycol	propylene glycol	glycerin
	methylparaben	methylparaben	benzyl alcohol
	propylparaben	propylparaben	
	sodium hydroxide	sodium hydroxide	sodium hydroxide /lactic acid
	purified water	purified water	purified water
	edetate disodium	edetate disodium	sorbitol solution

Analytical Method

Metronidazole concentrations of collected receiver solution samples and skin were analyzed using a validated HPLC method.

Bioequivalence Evaluation

Bioequivalence was assessed using a mixed scaled statistical approach similar to the one described within the product-specific guidance for generic drug development of acyclovir topical cream, 5%.

Methods

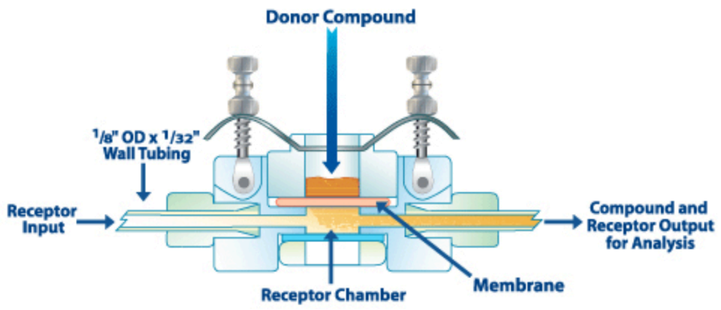

Figure 1. Cross-sectional view of an In-Line cell with HPLC fittings (Source: PermeGear, http://www.permegear.com/inline.htm)

Results

The cutaneous PK of metronidazole was similar between the gels, while the cutaneous PK of the cream was different compared to both gels. Following the early peak in metronidazole flux from the gels, flux from the gels declined after the 4 h time point. In contrast, the flux profile of generic cream showed an increasing flux during the initial 12 h followed by a relatively steady flux during the remaining 24 h study. The maximum flux, J_{max} ±SEM, was at 4 h for both gels; 0.69 ±0.38 µg/cm^2h for the reference gel and 0.85 ±0.44 µg/cm^2h for generic gel. The J_{max} for the generic cream was 0.72 ±0.34 µg/cm^2h and was observed at ≥12 h.

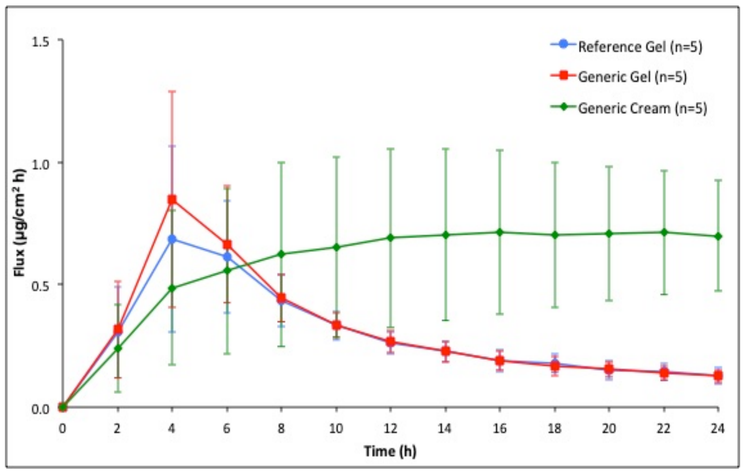

Figure 2. Mean flux (± SEM) for reference gel (blue), generic gel (red), and generic cream (green) [n=5 donors]

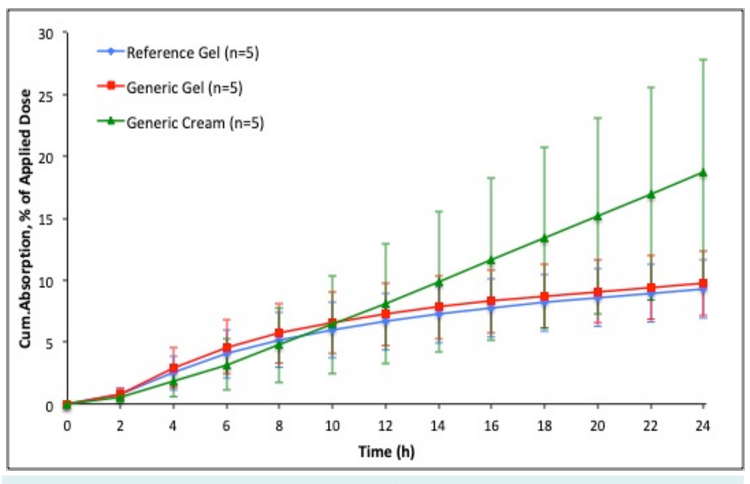

Figure 3. Total cumulative metronidazole amount (± SEM) permeated over 24 h from three metronidazole topical products [n=5 donors]

Results

Highest total cumulative metronidazole amount delivered through the skin per unit area was observed from the generic cream (15.0 ± 7.31 µg) compared to 7.30 ± 1.88 µg and 7.76 ± 2.07 µg for the reference and generic gels, respectively. Total drug absorption at 24 h was similar between the reference gel (9.3%) and generic gel (9.7%) while total absorption from the generic cream (18.7%) was nearly double compared to each gel product. Relative bioavailability (BA) was 1.03 ± 0.04 and 1.62 ± 0.40 for generic gel and generic cream, respectively assuming BA of 1 for the reference gel. Mass balance results indicated a high mean recovery of 88% for reference gel, 90% for generic gel and 90% for generic cream.

Table 2. Bioequivalence analysis

Product comparison	PK-metric	Point Estimate	S_{wr}*	Between-Donor SD	Upper 95% SABE* bound	BE outcome
Generic gel (T) vs.-Reference gel (R)	AUC*	1.020	0.4763	0.0891	-0.1086	✓
	J_{max}	1.065	0.5580	0.1626	-0.1388	
Generic cream (T) vs. Reference gel (R)	AUC	1.4430	0.4763	0.5990	0.7019	✘
	J_{max}	0.9879	0.5580	0.4043	-0.0657	
Generic cream (T) vs. Generic gel (R)	AUC	1.4151	0.5074	0.5129	0.4993	✘
	J_{max}	0.9278	0.4929	0.2907	-0.1967	

***AUC:** Total (cumulative) permeation
S_{wr}: Within-reference standard deviation
SABE: Scaled Average Bioequivalence

Conclusions

The cutaneous PK of three topical drug products with the same metronidazole concentration (strength) was compared using IVPT studies. The in vitro cutaneous PK results for the gels (similar formulation compositions) and a cream (formulation distinct from both gels) were consistent with the expectation that differences in components, composition and physical/structural attributes between topical semisolid drug products can alter the BA of metronidazole. These expectations were also consistent with the statistical assessment of BE using the mixed scaled criterion mentioned earlier. Results also suggested that IVPT studies may help support BE evaluation for topical drug products, since the IVPT results appropriately showed two gels (positive controls for BE relative to each other) had a similar rate and extent of metronidazole delivery and discriminated the cutaneous PK of the cream (negative control for BE relative to reference gel) as being different from both gels (Table 2). The IVPT method employed provided an alternative way to assess BA. A harmonized in vivo study with healthy human volunteers is in progress for the development of in vitro-in vivo correlation.

Acknowledgment

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