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Comparative Evaluation of the Permeation of Metronidazole Using Static Diffusion Cells and Flow-Through Diffusion Cells

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PURPOSE

Diffusion cells are extensively used to evaluate the transport of an active ingredient across a membrane (e.g., skin) from topical products during drug development. The most commonly used diffusion cells are the static (vertical) Franz diffusion cells (FDC) and the Bronaugh flow-through cells (FTC). The aim of the study was to evaluate and compare the impact of the differences in the design of these two diffusion cell types on the permeation of an active ingredient across human cadaver skin. Metronidazole topical cream, 0.75% was used as a model drug product for the study.

METHODS

Skin Preparation: Cryo-preserved, dermatomed, human cadaver skin (New York Firefighters Skin Bank) was stored at -70ºC before use. On the day of the study, the skin was thawed and cut into sections large enough to fit the active permeation area of the diffusion cells. Before use, the skin sections were gently rinsed with water for 3 minutes. The barrier integrity of the skin tissue was evaluated using a frequency of 1 kHz and amplitude of 100mV before initiating the permeation experiments by measuring Trans-epithelial electrical resistance (TEER) using a wave form generator and multimeter (Agilent Technologies, Santa Clara, CA). Skin sections that had a resistance value of greater than 10 KΩ per cm^2 were used.

FDC: The static (vertical) Franz diffusion cells (active diffusion area of 1.76 cm^2 and receptor chamber volume of 7.2 ml) were first mounted on a thermostatic mantle and the skin surface temperature was maintained at 32 ± 1ºC. The receptor solution, phosphate-buffered saline (pH 7.4) with 0.01% gentamycin sulfate, was constantly stirred with the help of a magnetic stirrer at approximately 600 rpm. The skin sections were sandwiched between two rubber washers. A dose of 26.5 ± 1 mg of metronidazole topical cream, 0.75% was weighed and applied uniformly on the skin surface (15 mg/cm^2) with the help of a spatula. The receptor solution was completely replaced every 4 hr for 48 hr.
FTC: The skin was cut into 1 cm^2 sections that were positioned in the bottom half of each FTC atop the active diffusional area. Metronidazole topical cream, 0.75% was weighted (15 mg ± 1 mg) and applied on to the skin surface (15 mg/cm^2) with a spatula. The top half of the diffusion cell was then secured in place and the cells were mounted on a thermostatic mantle. The skin surface temperature was maintained at 32 ± 1ºC. The reservoir was filled with the receptor solution, phosphate-buffered saline (pH 7.4) with 0.01% gentamycin sulfate, and a peristaltic pump was used to maintain a constant flow rate of the receptor under the active diffusion area. The air bubbles underneath the skin were removed by setting the peristaltic pump at a maximum flow rate and slightly tilting the cells. Once the absence of air bubbles was confirmed, the cells were positioned in the racks and an automated sample collector was used to collect the receptor solution every 4 h for 48 h. The permeation of metronidazole using the flow-through cells were evaluated at two different flow rates to determine the influence of flow rate on the permeation of metronidazole from the product. For the first set of studies, the flow rate was set at 15 µL/ minute to collect 3.6 mL of sample every 4 hours. For the second set of studies the flow rate was set at 30 µL/ minute to collect 7.2 mL every 4 hours (matching the volume exchanged from the FDC every 4 hours).

Metronidazole sample concentrations were quantified by reverse-phase high-performance liquid chromatography (HPLC). Results are based on 6 replicates from each of 3 skin donors for each study condition. Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using unpaired student's t-test at $p<0.05$.

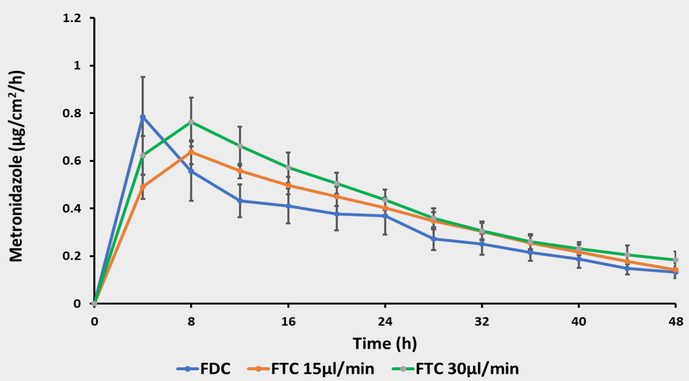
RESULTS

Sink conditions were maintained during all the three study conditions based on the solubility of metronidazole in phosphate-buffered saline (pH 7.4) (9.71 ± 0.17 mg/ml, n=3). The observed cumulative permeation (AUC_{0-t}), maximum flux (J_{max}) and time to reach J_{max} (T_{max}) of metronidazole using both diffusion cells are shown below (n=3 donors, 6 replicates per donor). There was no significant difference in the cumulative permeation (AUC_{0-t}), between the FDC and the FTC (at the 30 µl/min flow rate) (p=0.77). Similar results were observed between the studies conducted using the FTC at different flow rates (p=0.16). Likewise, there was no significant differences in the J_{max} amongst the groups. However, there was an apparent small shift in the T_{max} values between the FTC (using either flow rate) and the FDC.

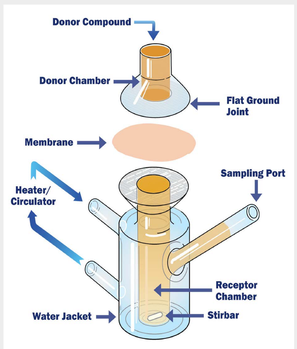
Table 1: Cutaneous pharmacokinetics of metronidazole using different diffusion cells and flow rates (n=6 replicates per donor; 3 donors; mean ±SEM)

Parameter	FDC	FTC	
	Receptor chamber volume: 7.2 ml	Flow rate: 15µl/min	Flow rate: 30µl/min
AUC (µg/cm^2)	16.28 ± 1.84	17.63 ± 1.00	20.00 ± 0.96
J_{max} (µg/cm^2/h)	0.79 ± 0.13	0.64 ± 0.04	0.83 ± 0.06
T_{max} (h)	4.00 ± 0.00	7.73 ± 0.22	6.89 ± 0.22

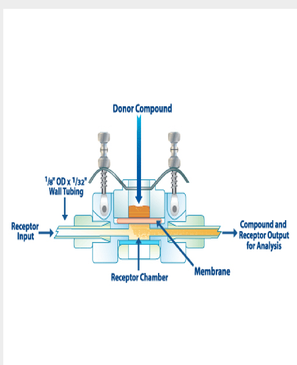
Figure 1: Flux profiles of metronidazole using different diffusion cells and flow rates (n=6 replicates per donor; 3 donors; data presented as mean ±SEM)



FRANZ DIFFUSION CELL



FLOW THROUGH CELL



Images: https://permegear.com/

CONCLUSIONS

The data suggests that the absolute rate and extent of permeation of metronidazole from metronidazole topical cream, 0.75% can be assessed in a comparable manner using both the static Franz diffusion cells and flow-through diffusion cells.

The limited data also suggest that for metronidazole, a relatively hydrophilic molecule, alteration of the flow rate in the FTC did not substantially impact the observed permeation profiles.

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