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Evaluation of Local Bioavailability of Metronidazole from Topical Formulations Using Dermal Microdialysis: Preliminary Studies in Yucatan Mini-Pig

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BACKGROUND

Microdialysis

- Dermal microdialysis (dMD) can directly monitor the rate and extent to which a topically administered drug becomes available in the dermis, at or near the site of action in the skin.
- Intra- and inter-subject variability may require a large number of subjects to achieve statistically significant differences.[1]
- Using multiple test sites on the same subject, and replicate probes at each test site, permits comparison of the cutaneous pharmacokinetics (PK) of a drug from different products in parallel on the same subject, so that test and reference products can be directly compared in the same subject at the same time under the same conditions, helping to control variability.
- A key study design consideration for such a parallel study design involves characterizing the minimum distance needed between application sites to avoid cross-talk between probes.

Metronidazole (MTZ)

Molecular formula: $C_6H_9N_3O_3$; water solubility > 1 mg/mL; LogP -0.02 ; pKa 2.38

- The MTZ topical gel and cream are indicated for the treatment of rosacea while the mechanism of action is unknown.
- Two different generic formulations were tested, a cream (Fougera 0.75%) and gel (Tolmar 0.75%), of the same strength. These two are not bioequivalent, per clinical endpoint studies, and were expected to demonstrate different PK profiles, also demonstrated in vitro.[2]

PURPOSE

1. Identify the minimum distance required between test sites to prevent cross-talk between probes, i.e., lateral diffusion of drug diffused into the skin from the topical formulation.
2. Evaluate the sensitivity of the dMD method (i) to detect differences in the local concentration of the drug, which is modulated using dosing of 3, 10, and 30 mg/cm^2 and (ii) to discriminate between the cream and gel formulations.
3. Assess the stability of dMD probe sampling over the 48-hour study duration.

METHODS

Twenty-two microdialysis probes were inserted into the dorsum of an anesthetized Yucatan mini-pig using a scheme depicted in Figure 1. Two (duplicate) probes were inserted under each of the (6) test sites at 1 cm distance from each other. Metronidazole (MTZ) cream, 0.75% (Fougera) or a MTZ gel, 0.75% (Tolmar) was applied at different test sites, each at doses of 3, 10, or 30 mg/cm^2 (Figure 1). Eight other dMD probes, designated to assess lateral diffusion (LD), were placed 1, 2, 3, or 4 cm away from the edge of the demarcated dose application area for each test site (Figure 1). An additional two dMD probes were used to evaluate the potential redistribution of MTZ in the skin due to systemic absorption and recirculation. All probes were perfused with 0.5µl/mL of lactated Ringer's solution containing deuterated MTZ (MTZ-D3; 1µg/mL). Trans epidermal water loss and probe depth were measured in triplicate for each test site and probe. Samples were collected every 1 hour for 48 hours and were analyzed for MTZ and MTZ-D3 using a validated LC-MS/MS method (LLOQ: 0.4 ng/mL; ULOQ: 200 ng/mL). An area under the concentration vs. time curve (AUC) plot was used to compare the bioavailability of MTZ between each dose of the cream and gel. This study was replicated in two mini-pigs, and the results were combined for analysis. One AUC outlier value was identified for one probe using the Dean and Dixon Q-test.

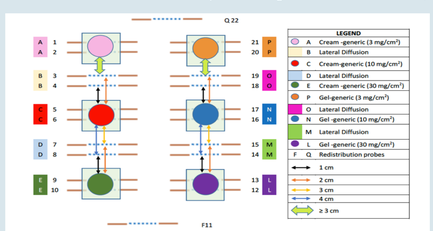
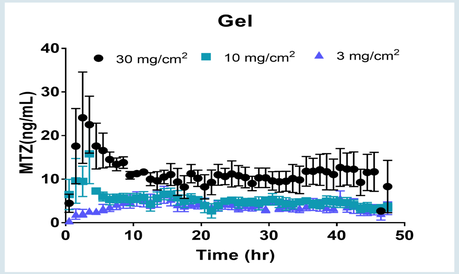


Figure 1: Example scheme for formulation application of lateral diffusion probes

RESULTS



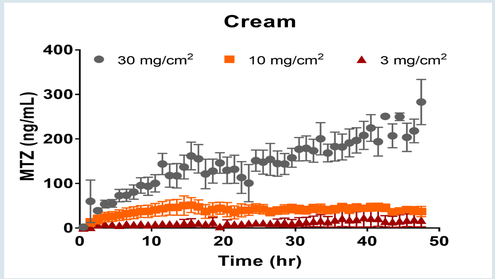


Figure 2: Average Pharmacokinetic profiles for different dosing (n=4, except n=3 for the 3 mg/cm^2 cream treatment group)

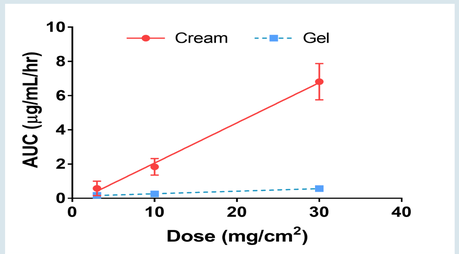


Figure 3: Sensitivity and Dose-Response Relationship of Average AUC vs. Dose Amount of Product (n=4, except n=3 for the 3 mg/cm^2 cream treatment group; data are shown as mean ± standard deviation)

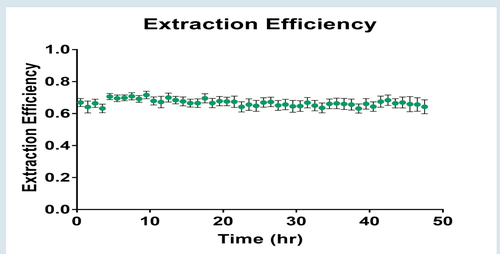


Figure 4: Probe stability as indicated by extraction efficiency. Data are presented at mean ± SEM from the two studies.

Lateral Diffusion

- MTZ concentrations were slightly above the LLOQ (0.40 ng/mL) in 168 of the 765 samples collected by the LD probes, and 8 samples contained MTZ above the LLOQ at the redistribution sites, while the average concentration above the LLOQ was 0.47 ± 0.12 ng/ml.
- There was no trend in MTZ concentrations relative to probe distance from formulation application edge. However, the number of samples with a quantifiable concentration was significantly higher in the second 24 hours compared to the first 24 hours (p = 0.002).

Dose sensitivity

- Figure 2 shows the profiles for the different formulations and doses. The probe depth was relatively consistent across all probes (0.226 ± 0.077 cm; n=132) except for one probe (cream, 3mg/cm^2) whose depth was 0.069 ± 0.015 cm (n=3). This probe was located much closer to the surface of the skin, and the AUC value was identified as an outlier relative to the other probes with the same treatment
- There is a linear relationship (R^2>0.99) between the dose applied and the AUC of the dose (Figure 3) for both the gel and cream products.

Discrimination between formulations

- At the 10mg/cm^2 (p=0.017) and 30mg/cm^2 (p=0.001) the AUC of the cream formulation was significantly higher than the gel formulation (Figure 3), whereas at the 3mg/cm^2 dosing there was not significant difference between the AUCs of the two formulations.

Probe Stability

- MTZ-D_3 extraction efficiency in the first 24 hours compared to the second 24 hours did show a significant difference (p=0.025), upon further probing only the third block of 12 hours (24hr-36hr) for probe recovery was significantly different from the first 12hr block (0-12hr) and the last 12hr block (36-48hr). The first 12 hours and the last 12 hours were not statistically different. These data do not indicate probe instability, but rather that there are random fluctuations within the recovery of the MTZ – D_3. The use of the correction factor accounts for these fluctuations (Figure 4).

DISCUSSION AND CONCLUSIONS

- The negligible lateral diffusion and undetectable systemic redistribution observed suggest that the MTZ concentrations measured by the dMD probes can be specifically associated with the local bioavailability from the topical dose of the cream or gel just on top of the probe.
- Analysis of D_3-MTZ standards had shown that D_3-MTZ contains approximately 0.1 % of MTZ that corresponds to 1 ng/mL in the perfusate. This would be an explanation for the concentration of metronidazole detected in the lateral diffusion probes, regardless of the distance from the application site.
- dMD was sensitive to capture the differences in bioavailability between the cream and the gel.
- The maximum concentration (C_{max}) of MTZ, time to maximum concentration (T_{max}), and half-life of MTZ were difficult to estimate because the complete PK profile was not adequately characterized during the 48-hour study duration.
- These results suggest the necessity to shorten the study duration by wipe-off the formulation at earlier times to better characterize the complete PK profile and compare the rate and extent to which topically administered MTZ becomes available in the dermis from different products.

The results of this study indicate that:

- Formulations can be safely placed at a 2 cm distance one from the other without cross-talk (limitation of formulation application template).
- This type of microdialysis probe is stable for 48 hours.
- The addition of an internal standard to the perfusate help to correct for relative probe-recovery fluctuations.

REFERENCES

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2. Roberts, M. *Correlation of physicochemical characteristics and in vitro permeation test (IVPT) results for acyclovir and metronidazole topical products*. Oral Presentation at: FDA Workshop on Bioequivalence Testing of Topical Drug Products: October, 2018; Silver Springs, Maryland

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