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# Evaluating the Bioequivalence of Topical Dermatological Drug Products Containing Metronidazole Using Dermal Microdialysis: Preliminary Studies in Rabbits

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## PURPOSE

- The ability to evaluate the rate and extent of drug bioavailability (BA) at or near the site of action in the skin may facilitate the development of high quality generic topical dermatological drug products, which can enhance patient access to these medicines.
- Comparative clinical endpoint bioequivalence (BE) studies were historically used for topical drug products. These studies are time-consuming, costly, and considered to be the least sensitive method for evaluating differences in BA between a generic product and the corresponding reference product.
- The assessment of BE by pharmacokinetics (PK)-based endpoints, using in vivo results derived from drug concentrations in dermis, may represent a more efficient and sensitive approach by which to evaluate BE for topical dermatological drug products.
- Dermal microdialysis (dMD) continuously samples the dermal interstitial fluid (IF) and permits the characterization of the PK of topically administered drugs. Hence, dMD may represent a potential alternative to comparative clinical endpoint BE studies for assessment of topical dermatological products.
- Metronidazole (MTZ) is a synthetic nitroimidazole derivative with antiprotozoal and antibacterial activities used in dermatology to treat rosacea.
- MTZ physicochemical parameters: Water solubility > 1 mg/mL, LogP -0.02, pKa 2.38, Plasma protein binding ~ 11%.

## OBJECTIVES

This is a proof of concept study performed in rabbits to:

- Assess the consistency of the microdialysis-probe performance during the 24-hour sampling duration of the experiment.
- Evaluate redistribution of the drug from the systemic circulation to the skin.
- Calculate the maximum dermal concentration ($C_{max}$ ) and area under the dermal concentration curve (AUC) to compare the rate and extent to which MTZ permeates into the dermis from the topical products evaluated.
- Evaluate PK endpoints and BE among the MTZ topical products evaluated.

## METHODS

- dMD probes: Each house-made dMD probe consisted of a 1.7 cm semi-permeable membrane of polyacrylonitrile (MWCO 50kDa, AN69 HF Hospal-Gambro, Inc.; Meyzieu, France) and polyimide tubing arms.
- dMD Probe Location - Nine microdialysis probes were inserted into the dorsum of seven tranquilized rabbits using a predetermined scheme (Figure 1). Two (duplicate) probes were inserted under each of the four test sites at a 1 cm distance from each other. An additional dMD probe was inserted at a distal site, far from the product application sites, to evaluate the potential redistribution of MTZ into the skin due to systemic absorption and recirculation.
- Metronidazole products evaluated:
  - Gels (Reference: MetroGel® topical gel, 0.75% from Prasco Laboratories "brand gel" and Test: metronidazole topical gel, 0.75% from Tolmar "generic gel").
  - Creams (Reference: MetroCream® topical cream, 0.75% from Galderma Laboratories "brand cream" and Test: metronidazole topical cream, 0.75% from Fougera Pharmaceuticals "generic cream").
- Applied dose: 10 mg/cm2.

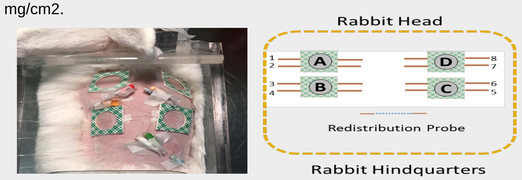


**Figure 1** - Diagram and photographic image of product application sites and locations of dMD probes. In each experiment, gel and cream sites were assigned according to a cross-over design (inasmuch as the test and reference treatments were compared in the same rabbit).

## METHODS (CONT'D)

- dMD conditions: All probes were perfused with 0.5 µL/mL of lactated Ringer's solution containing acetaminophen (APAP) 1 µg/ml as an internal standard. Samples were collected at 1-hour intervals for 12 hours continuously, then the animal was given a break for 3 hours and sampling resumed for another 9 hours. Samples were analyzed for MTZ and APAP using a validated HPLC-UV method (LLOQ: 0.02 µg/mL; ULOQ: 10 µg/mL) for MTZ and (LLOQ: 0.10 µg/mL; ULOQ: 10 µg/mL) for APAP.
- Data Analysis – MTZ dialysate concentrations were corrected by APAP recovery in that sample and plotted versus the mid-point of the dialysate collection interval.
- PK Analysis - The 90% confidence interval (CI) of the ratios of log-transformed parameters (AUC and Cmax) were calculated to assess BE amongst the various formulations. The BE module in Phoenix® (Certara) was used to calculated BE using a cross-over study design.
- Transepidermal water loss and probe depth were measured in triplicate for each test site and probe.

## RESULTS

- Figure 2 shows the average dermis concentration detected at each formulation site.
- APAP microdialysis recovery was consistent during the 24-hour sampling duration demonstrating that the probe performance was consistent during the entire experiment, as indicated by the lack of a significant difference between the first 12hr and second 12hr extraction ratios (p=0.40) (Figure 3).
- MTZ was not detectable in the dialysate from the probes that were far away from the formulations (i.e., the distal redistribution probes), demonstrating that the MTZ concentrations measured by the dMD probes under the product treatment sites can be specifically associated with the local bioavailability from the topical dose of the cream or gel on the skin atop of the probe.
- Bioavailability parameters (AUC and $C_{max}$) are reported in (Table 1).
- The dMD technique was sufficiently sensitive to discriminate differences in bioavailability between the cream and the gel, since the calculated 90% CIs for this comparison lay entirely outside the 80-125% limits for both AUC and $C_{max}$ (Figure 4).
- By contrast, the comparison of the natural log transformed AUC and $C_{max}$ mean test/reference ratios for cream and gel products were inconclusive. Although the point estimates of the geometric test/reference mean ratio for gel vs. gel or cream vs. cream lay within the 80-125% limits, the 90% CIs extended outside the 80-125% limits for both AUC and $C_{max}$, (Figure 4).

| Formulation | N | AUC (µg/mL/hr) | $C_{max}$ (µg/mL) |
|---|---|---|---|
| R Cream | 14 | 5.85 (1.76) | 0.93 (1.69) |
| T Cream | 13 | 6.85 (1.83) | 1.03 (1.80) |
| R Gel | 14 | 3.03 (2.09) | 0.42 (2.10) |
| T Gel | 14 | 3.36 (2.00) | 0.50 (1.92) |

**Table 1** – PK Parameters: AUC and Cmax are reported as geometric mean with geometric standard deviation in parentheses

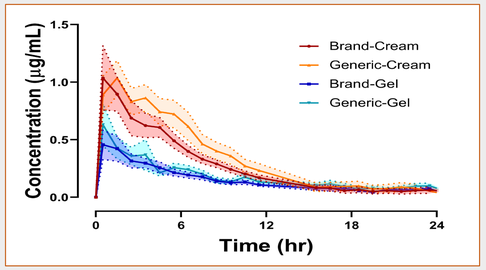


**Figure 2** – Average dermal concentration profiles (mean ± SEM, n=7) sorted by formulation

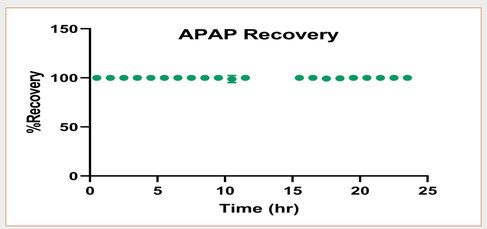


**Figure 3** – Probe stability as indicated by extraction efficiency. Data are presented as mean ± SEM from the two studies. Samples were not collected between 12 and 15 hours to allow rabbits an opportunity to take a respite.

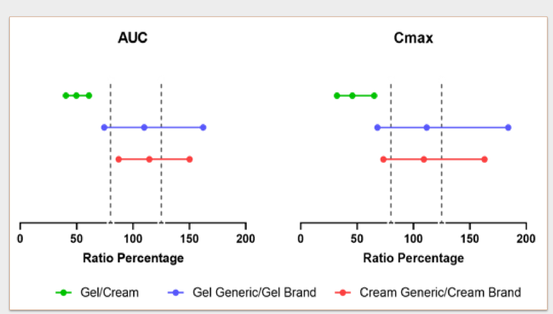


**Figure 4** – 90% confidence intervals of the ratio of the Ln-transformed PK parameters. The dashed lines indicate the BE CI limits of 80-125%

## CONCLUSIONS

- The 24-hour sampling duration appeared to be sufficient to characterize the complete dermal PK profile for MTZ in rabbit skin.
- Probe performance was stable (consistent) for the entire duration of the experiment.
- Redistribution of MTZ from the systemic circulation back into the skin was negligible, below the LLOQ.
- Reference gel vs. cream products were accurately and sensitively discriminated as not being bioequivalent.
- Reference vs. test cream and gel products could not be discriminated. The point estimate of the geometric test/reference mean ratios was close to 1 for cream as well as gel products, however, the 90% CIs lay outside the 80-125% limits, suggesting insufficient power with the relatively small number of rabbits in the study.
- Increasing the number of rabbits in the study may narrow the CIs for test vs. reference products and demonstrate that dermal microdialysis can be used to assess BE of topical dermatological formulation.

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