Estimation Of In Vivo Skin Permeation (Flux) And Cumulative Amount Input of Metronidazole Formulations in Mini-pigs' Dermis

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BACKGROUND

- A dermal microdialysis (dMD) probe placed within the dermis below a topically applied formulation can measure the changes in drug concentrations in the dermis over time; this does not distinguish absorption from distribution and elimination occurring in the dermis.
- For systemic administrations, the absorption process can be clarified by deconvolution of the plasma concentrations with the unit impulse response (UIR), i.e., the plasma concentration resulting from the instantaneous administration of a unit amount of drug directly in the sampling compartment. Plasma UIR is usually estimated from intravenous administration.
- We propose a retrodialysis/microdialysis approach to deliver a drug directly to the dermis to estimate the dermis unit impulse response (dUIR). The dUIR would provide a measure of dermal disposition independent of the absorption process.
- The in vivo flux into the dermis (input-rate) and the cumulative amount absorbed can be calculated via numerical deconvolution of the dermal concentration profiles detected at the topical formulation administration sites with the estimated dUIR.
- The in vivo flux into the dermis and the cumulative amount absorbed can then be compared with the in vitro skin permeation testing data to develop an in vitro in vivo relationship (IVIVR).

PURPOSE

1. Estimate the dermis disposition function (dUIR) for metronidazole (MTZ) by utilizing microdialysis and retrodialysis techniques.
2. Calculate the MTZ flux and cumulative amount permeated in vivo by deconvolution of the concentration profiles in the dermis.
3. Correlate MTZ flux and cumulative amount permeated in vitro with the MTZ flux and cumulative amount permeated in vivo.

METHODS

- Three Yucatan mini-pigs were used to evaluate the effect of formulation application-time with a gel and a cream formulation of MTZ[1]. Formulations were wiped off at predetermined times post dose: 6-hr Dose, 12-hr Dose, and 48-hr Dose (no wipe off) (Figure 1).
- Two probes were placed in the dermis, 3-4 cm away from the topical application sites and perfused with a 40 ng/mL solution of MTZ at a flow rate of 0.5µL/min for 10 hr (retrodialysis phase) and then switched to 20 ng/mL solution of D_3- MTZ for the remaining duration of the experiment (microdialysis phase). dMD samples were collected every hour for 48 hr.

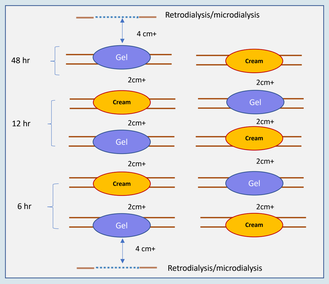

Figure 1: Schematic representation of dMD probe location and wipe off schemes. Twenty probes were placed under the MTZ formulation sites, and two probes were used to estimate the dUIR.

- Dose administered was calculated as:

$$X_{5.5-9.5} = (C_{perfusate} - C_{ss}) \times V_{perfused(5.5-9.5)}$$

Where X is the dose administered between 5.5 and 9.5 hours, $C_{perfusate}$ is the concentration in the perfusate, C_{ss} is the concentration in the steady-state portion of retrodialysis phase, and $V_{perfused}$ is the volume of solution perfused for the 5.5-9.5hr duration.

- Dermis clearance was calculated as:

$$CL_{dermis} = \frac{X_{5.5-9.5}}{AUC_{5.5-9.5}}$$

Where CL is the clearance in the dermis, X is the dose administered for the 5.5-9.5hr, AUC is the area under the curve for the 5.5-9.5hr duration.

- The dermis unit impulse response was calculated as:

$$dUIR = \frac{1}{V} e^{-kt}$$

Where V is the dermis volume of distribution, k is the dermis elimination rate constant, and t is the time.

- The cumulative amount delivered to the dermis and the input-rate (flux) were calculated by deconvolution of the dermis concentration profiles with the estimated dUIR (Phoenix® deconvolution module; Certara®, Princeton, NJ).
- MTZ IVPT data were provided by Dr. N. Murthy, the University of Mississippi. Note that the in vitro experiments were conducted with human cadaver skin.
- Levy's plots to compare in-vivo absorption with in vitro permeation (T_{vitro} versus T_{vivo}) were performed with Phoenix® IVIVC Toolkit.

RESULTS

Dermis Disposition Parameters

- Dermis concentrations declined mono-exponentially following the delivery phase (Figure 2) as the concentrations decrease in a straight line on a semi-log-scale.
- Dermis elimination half-life was 1.47 hr (19.5) (geometric mean (CV%)), while the half-life of MTZ at the formulation sites for the 6-hr, 12-hr and 48-hr doses were 9.01 (30.29), 10.51 (34.06), 10.32 (32.84) hours for the gel and 7.32 (25.95), 10.50 (57.75), 23.86 (86.74) hours for the cream, respectively.

Dermis Unit Impulse Response Parameters

- The average dose delivered between 5.5-9.5 hr was 3.5 ng ± 0.8 (mean ± SD; n=6) and the corresponding average $AUC_{5.5-9.5}$ was 62.81 ng*hr/mL (4.55) (geometric mean (CV%))
- The average volume of distribution was 0.12 ± 0.06 mL (mean ± SD)
- The average clearance from the dermis was 0.057 ± 0.03 mL/hr (mean ± SD)

Cumulative Amount (Figure 3)

- **CREAM:** The 6-hr dose delivered significantly less MTZ compared to the 12-hr dose (p=0.038) and the 48-hr dose (p=0.046)
- **GEL:** There was no significant difference in the cumulative amount amongst the different dosing times (p>0.667)

Input Rate (Figure 4)

- **CREAM:** The log transform of maximum flux $Ln(J_{max})$ for the 6-hr dose was significantly different from the 12-hr dose (p=0.019) and 48-hr dose (p=0.041). The Ln(AUC) for the 6-hr dose was also significantly different from the 12-hr dose (p=0.018) and 48 hr dose (p=0.013).
- **GEL:** There was no significant difference amongst the different formulation dosing schemes for $Ln(J_{max})$ (p>0.739) and Ln(AUC) (p>0.833)
- **CREAM/GEL:** Comparison between the cream and the gel at the different dosing schemes indicated that both $Ln(J_{max})$ and Ln(AUC) for the 48-hr dosing scheme were significantly different, p=0.010 and p=0.005, respectively; also at the 12-hr dosing scheme the $Ln(J_{max})$ and Ln(AUC) were significantly different between the formulations, p=0.02 and p=0.02, respectively; whereas at the 6-hr dosing scheme there was no difference between the two formulations.

Investigative IVIVR

- Figures 5 shows that both in vitro and in vivo the cream release more MTZ than the gel. Apart from a scaling factor, the profiles look very similar.
- Figure 6 presents Levy plots of in vitro versus in vivo times needed to absorb the same fraction of MTZ as well as the fraction permeated in vitro versus fraction absorbed in vivo for the cream and gel formulations

DISCUSSION AND CONCLUSIONS

- The retrodialysis/microdialysis approach allows the estimation of formulation independent dermal elimination rate, volume of distribution, and clearance.
  - The retrodialysis phase (delivery phase) provides a zero-order drug delivery directly to the dermis, from which the dose administered can be exactly calculated.
  - The microdialysis phase allows the estimation of the actual elimination from dermis.
- Comparison of dermis elimination half-lives at the "formulation-independent" site with the half-lives measured at the topical formulation sites indicates a flip-flop pharmacokinetic scenario where the "apparent" elimination phase reflects the absorption rate, which is the rate-limiting step in the dermis pharmacokinetics of these topical formulations of MTZ.
- Removal of the gel formulation after the drying period[2] did not change the permeation profile; however, the gel permeation slightly increased after the 6-hr dose possibly due to an enhancing effect of the wipe-off procedure that included a small amount of water. Removal of the cream before the drying time reduced the permeation in the case of the 6-hr dose indicating the cream is still delivering MTZ after 6-hr.
- The deconvolution of the pharmacokinetic profile utilizing the dermal disposition of MTZ allowed for the characterization of the absorption process in vivo: in vivo flux and cumulative amount input.
- Comparison of the in vitro in vivo (IVIV) cumulative amount plots clearly shows a consistent higher release of the cream with respect to the gel. IVIV profiles differ at earlier times indicating a slower permeability in vivo than in vitro, possibly due to the differences in skin type and thickness.
- Levy plots show a deviation of the data points from the unity line, indicating that a time scaling factor is necessary to build an acceptable IVIVR model.
- These results offer a promising starting point for further exploration of the microdialysis/retrodialysis approach to study dermis disposition of molecules. It may become the key for the development of quantitative IVIVR for topical dermatological formulations. Additional studies are necessary to further evaluate this hypothesis.

REFERENCES

1. Kuzma, B.A., et Al. Effect of formulation wipe-off time on topical bioavailability of metronidazole using dermal microdialysis, AAPS Annual Meeting 2018, Washington D.C., November 2018.
2. Murthy, N. Characterizing the Critical Quality Attributes and In Vitro Bioavailability of Acyclovir and Metronidazole Topical Products FDA Presentation. Oral Presentation at: FDA Workshop on Bioequivalence Testing of Topical Drug Products: October 2018; Silver Springs, Maryland

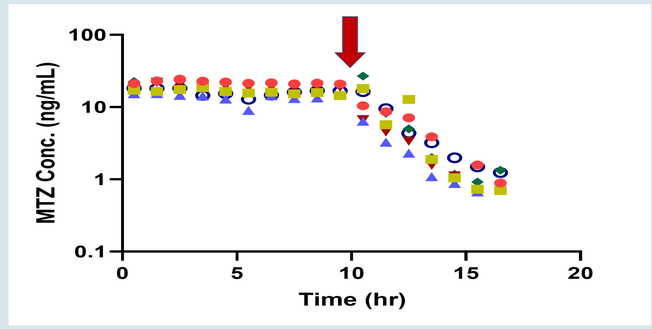

Figure 2: Dermal retrodialysis and microdialysis concentration vs time profiles. The point at which the perfusion solution was switched was 10 hr, indicated by the red arrow. Data are presented per single probe.

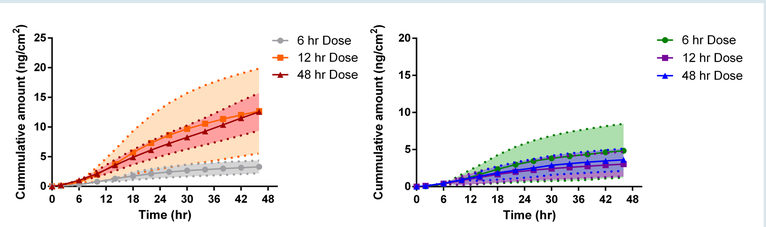

Figure 3: Cumulative MTZ amount present in dermal interstitial fluid vs. time profiles. The cream profiles are on the left while the gel profiles are on the right. Data are presented as mean (solid line) ± SEM (shaded areas); n=6, except for the 48-hr dose where n=5.

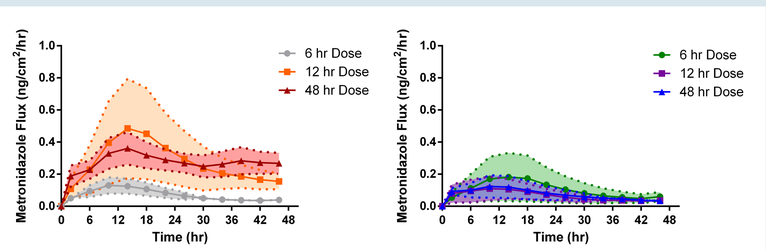

Figure 4: Dermis in vivo flux vs time. The cream profiles are on the left while the gel profiles are on the right. Data are presented as mean (solid line) ± SEM (shaded areas); n=6, except for 48 hours dose where n=5.

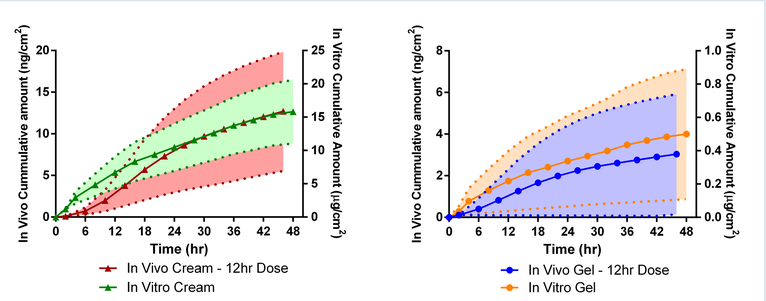

Figure 5: Comparison of in vitro and in vivo cumulative amounts of MTZ delivered to the dermis from the cream product (on the right) and the gel product (on the left) . Data are presented as mean (solid line) ± SEM (shaded areas);

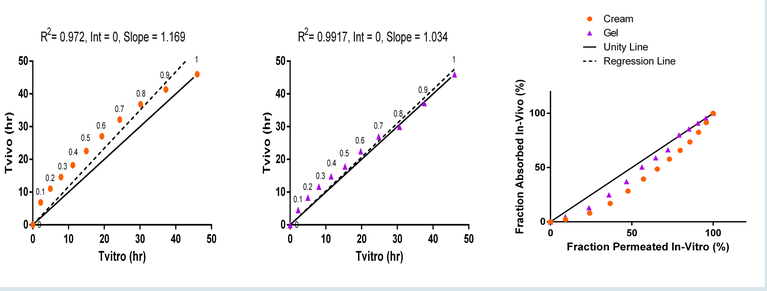

Figure 6: Levy plots – LEFT: in vitro versus in vivo times needed to absorb (permeate) the same fraction of MTZ; RIGHT: plots of fraction permeated in vitro versus fraction absorbed in vivo. The deviation of the data points from the unity line, indicates that a time scaling factor is necessary to build an IVIVR model

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