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Estimation of In Vivo Percutaneous 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; however, 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 metronidazole (MTZ) 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 (CA) permeated 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 CA permeated can then be compared with results from an in vitro permeation test (IVPT) using excised skin mounted on diffusion cells 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 CA permeated in vivo by deconvolution of the concentration (permeation) profiles in the dermis.
3. Develop an IVIVR to relate IVPT data and dMD data to be able to predict typical pharmacokinetic (PK) parameters (C_{max}, AUC_{0-36}, and AUC_{all}).

METHODS

- Three Yucatan mini-pigs were used to evaluate the effect of MTZ gel and cream dose durations on permeation profiles[1]. The products were wiped off at selected times post dose: labeled 6-hr Dose, 12-hr Dose, and 48-hr Dose (no wipe off) (Figure 1).
- Two (retrodialysis/microdialysis) probes were placed in the dermis, 3-4 cm away from the topical dose 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.

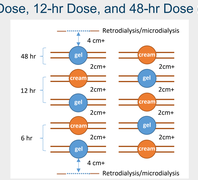

Figure 1: Schematic representation of dMD probe locations and wipe off schemes (dose durations). Twenty probes were placed under the MTZ formulation sites, and two probes were used to estimate the dUIR.

- The 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 hr, $C_{perfusate}$ is the concentration in the perfusate, C_{ss} is the concentration (ng/ml) 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 below, where CL is the clearance in the dermis, X is the dose administered for the 5.5-9.5 hr, AUC is the area under the curve for the 5.5-9.5hr duration:

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

- The dermis unit impulse response was calculated as below, where V is the dermis volume of distribution, k is the dermis elimination rate constant, and t is the time:

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

- The data from 12-hr in vivo study[1] was used to develop the IVIVR and evaluate its internal predictability.

METHODS (CONT'D)

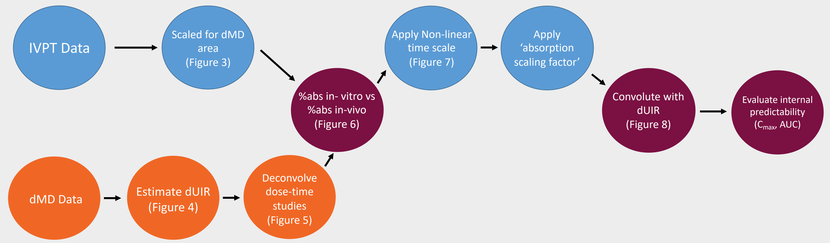

Figure 2: Schematic representation of the development of the IVIVR. The blue circles indicate the in vitro data processing, the orange circles indicate the data from dermal microdialysis (dMD), and the purple circles indicates the intersection of these in vitro and in vivo data sets.

- Figure 2 depicts a schematic of the data processing for the development of the IVIVR.
- The CA permeated 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), where the effective dose was calculated based upon the amount applied on the area immediately above the dialysis window (0.0068 cm^2) of the dMD probe (i.e., based on an area the size of he probe's dialysis window, not the entire (wider) dose application area) as illustrated/explained in Figure 3.
- MTZ IVPT data was provided by Dr. N. Murthy[2], the University of Mississippi. Note that the in vitro experiments were conducted with human cadaver skin.
- Time scaling (TS) on the in vitro profiles was performed using the Inverse Release Function[3] for non-linear time scaling. Then an 'absorption scaling factor' was applied by dividing the in-vitro time-scaled CA permeated by 1.75, which is the ratio between the in-vitro and in vivo data. Finally, the in vitro CA permeated was scaled by the dMD area to derive units of ng (that was needed for convolution with the dUIR).
- The time-scaled, absorption scaled, in vitro permeability profiles were convoluted with the dUIR to predict in vivo dermis concentration profiles that were then compared with the observed in vivo profiles.
- The absolute prediction error was calculated as: $Prediction\ Error = 100 \times \left(\frac{|P_{obs} - P_{pred}|}{P_{obs}}\right)$ for AUC and Cmax.
- Statistical analysis was conducted on the log-transformed PK parameters where significance was determined based upon p<0.05.

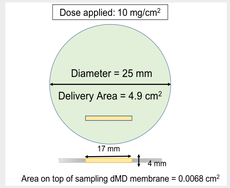

Figure 3: Schematic representation of the dosed area (4.9 cm^2) comparison with the area immediately above the dMD probe window (0.0068 cm^2). We assume that only the MTZ permeating from immediately above the probe's dialysis window is being measured by the dMD probe. The concentration MTZ in the dermis tissues lateral to the probe are assumed to be in equilibrium since MTZ permeates into those areas from the wider (4.9 cm^2) dosed area of the skin surface immediately above those areas. Thus, the effective dose used for deconvolution calculations is 510 ng.

RESULTS

Dermis Disposition Parameters

- Dermis concentrations declined mono-exponentially following the delivery phase as the concentrations decreased in a straight line on a semi-log-scale (Figure 4).
- 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) hr for the gel and 7.32 (25.95), 10.50 (57.75), 23.86 (86.74) hr for the cream, respectively.

Dermis Unit Impulse Response Parameters

- The average dose delivered between 5.5-9.5 hr was 3.5 ng $\pm$ 0.8 (mean $\pm$ 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 calculated as 0.12 $\pm$ 0.06 mL (mean $\pm$ SD)
- The average clearance from the dermis was calculated as 0.057 $\pm$ 0.03 mL/hr (mean $\pm$ SD)

Input Rate

- There was no significant difference in the AUC or J_{max} between the cream and gel formulations for the 6-hr dose application (Figure 5).
- There was a significant difference between the two formulations when comparing AUC and J_{max}, for the 12-hr dose and the 48-hr dose.

Investigative IVIVR

- Figure 6 presents the Levy plots between observed IVPT data and in vivo CA and between non-linear time-scaled (TS) IVPT data and in vivo CA for both cream and gel. The TS plots clearly shows a linear relationship after the time-scaling between in vitro and in vivo data. Thus, the TS in vitro data were used in the following steps of the IVIVR.
- Figure 7 depicts the cumulative permeated MTZ from gel and cream formulations in vitro (from IVPT, which have been time-scaled (TS)) and in vivo. After the TS of the in vitro data, the profiles display a similar permeation profile, and an 'absorption scaling factor' (AS) may correct for the differences in figure 7. The in vitro data were TS and AS time to create the IVIVR.
- Figure 8 presents the observed concentration-time profiles (in the dMD study) and the predicted concentration-time profiles (using IVPT data and IVIVR) for MTZ in both cream and gel formulations. Comparison of the mean profiles yielded the following absolute prediction errors (%): AUC_{all} (3.4) AUC_{0-36} (5.1), and C_{max} (15.1).

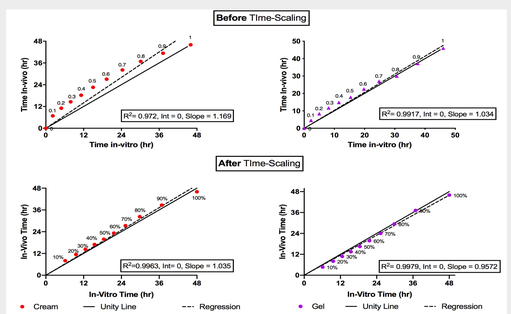

Figure 6: TOP: the relationship between time of delivery in vitro and in vivo at which the same fraction of MTZ is permeated for the cream and the gel. BOTTOM: The Levy plot for the MTZ cream and gel after time-scaling the in vitro data with the inverse release function; The left side of the plots are the cream and the right side are the gel. The slopes are approximately 1 and intercepts are at 0 indicating an IVIVR with good comparison between in vitro and in vivo.

Figure 4: LEFT: 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 is presented per single probe; RIGHT: Comparison of the MTZ elimination from the dermis both after topical administration and after delivery directly into the dermis (n=6).

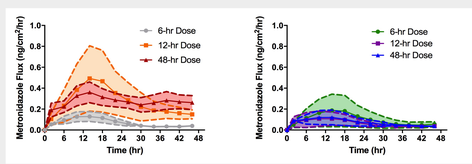

Figure 5: Dermis in vivo flux vs. time profiles. The cream profiles are on the left while the gel profiles are on the right. Data are presented as mean (solid line) $\pm$ SEM (shaded areas); n=3.

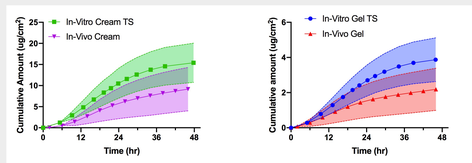

Figure 7: Comparison of in vitro and in vivo cumulative amounts of MTZ delivered to the dermis from the cream product (left) and the gel product (right). The in vitro data are time-scaled (TS) using an Inverse Release Function[3]. Data are presented as mean (solid line) $\pm$ SEM (shaded areas); where in vitro is n=6 and in vivo is n=3.

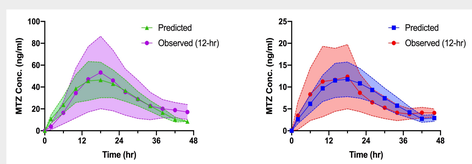

Figure 8: Comparison of the concentration-time profiles for MTZ from cream (left) and gel (right) formulations predicted from in vitro data and the observed in vivo profiles from dMD study. Data are presented as mean (solid line) $\pm$ SEM (shaded areas); where in-vitro is n=6 and in vivo is n=3.

DISCUSSION AND CONCLUSIONS

- The retrodialysis/microdialysis approach allows for the estimation of a formulation independent dermal elimination rate, the volume of distribution, and clearance rate.
  - The retrodialysis phase (delivery phase) provides a zero-order drug delivery directly to the dermis, from which the dose administered can be accurately calculated.
  - The microdialysis phase allows the estimation of the actual drug elimination from the dermis, while the addition of the internal standard allows for a direct measurement of the drug concentration in interstitial fluid.
- Comparison of dermis elimination half-lives at the "formulation-independent" site with the half-lives measured at the topical formulation sites indicate a flip-flop PK scenario; the "apparent" elimination phase reflects the absorption rate, which is the rate-limiting step in the dermal PK of these topical MTZ products.
- Removal of the gel products after the drying period[2] did not change the permeation profile, since the metamorphosis of the formulation had been completed by then; 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 at 6-hr reduced the MTZ flux (compared to 12-hr and 48-hr) because the formulation was not completely dried and the MTZ delivery from the cream would continue beyond 6-hr.
- The deconvolution of the PK profiles utilizing the dermal disposition of MTZ allowed for the characterization of the in vivo permeation parameters of flux and CA permeated.
- Comparison of the in vitro and in vivo CA permeated plots clearly shows a consistently higher MTZ permeation from the cream compared to the gel, and the non-linear time scale helped to account for the differences between the in vitro and in vivo CA permeated.
- The comparison of the observed and predicted in vivo concentration profiles after convolution with the dUIR demonstrates that a reasonable IVIVR was established.
- These results offer a promising starting point for further exploration of the microdialysis/retrodialysis approach to study the disposition of drug molecules in the dermis, which can be useful for the development of a quantitative IVIVR for topical dermatological products. Additional research studies are warranted to further evaluate the utility of this approach, its assumptions, and outcomes.

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