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Comparison of Metronidazole Dermal Pharmacokinetics Between Mini-pig and Rabbit

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PURPOSE

The most appropriate animal model to study the dermal pharmacokinetics (dPK) of topical dermatological products (TDDP) has typically been mini-pig due to its similarity with human skin. However, the use of in vivo mini-pig is challenging from a technical and expertise perspective. In contrast, the rabbit is less technically demanding, yet allows for the dermal bioavailability characterization of multiple formulations in parallel.

The goal of this meta-analysis is a first attempt to correlate the observed dPK parameters with the anatomical and physiological characteristics of the two animal species from which data are available by using dermal microdialysis (dMD) .

OBJECTIVES

- To compare the dPK from identical TDDPs containing metronidazole (MTZ) in New Zealand Albino rabbit and Yucatan mini-pig utilizing dMD.
- To independently characterize the in vivo dermal disposition parameters for each species using a "dermal infusion" technique that allows the estimation of dermal clearance (dCL), dermal apparent volume of distribution (dVd), and dermal elimination half-life. [1,2]

METHODS

Bioavailability experiments

The MTZ-TDDP evaluated were a generic topical gel (0.75% Tolmar), and a generic topical cream (0.75%, Fougera Pharmaceuticals). The applied dose was 10 mg/cm^2 in both Yucatan mini-pigs and New Zealand albino rabbits. Details of the experiments were previously reported [3, 4]

Disposition experiments

New Zealand Rabbit:

Six (6) probes were inserted on the rabbit's dorsum in one (1) New Zealand albino rabbit and were perfused with a solution containing 1, 5, or 10 µg/mL MTZ in lactated Ringer's, in duplicate, at a flow rate of 0.5 µL/min for 4 hours with a sampling interval of one hour. At the end of the fourth hour, the perfusion solution was switched to plain lactated Ringer's solution containing the probe marker acetaminophen (APAP, 1 µg/mL) and sampling continued for 5 hours. Samples were then analyzed via a validated HPLC-UV method.

Yucatan mini-pigs:

Two probes were inserted in three Yucatan mini-pigs and perfused with MTZ in lactated Ringer's (40 ng/mL) at a flow rate of 0.5 µL/min for 10 hours. The perfusion solution was then switched to plain lactated ringer solution containing deuterated metronidazole (MTZ – D3) as a probe marker. Samples were collected every hour until 48 hrs and then all samples were analyzed via a validated LC-MS/MS method.[2, 5]

RESULTS

Bioavailability Experiments

- Figure 1 reports the dermal concentration profiles resulting from the application of MTZ-TDDPs in the two species.
- The dPK parameters after application of MTZ cream and gel products are reported in Table 1.
- In the rabbit model, dermal exposure is 8-16 times higher than in the mini-pig after application of cream and gel formulations,
- The topical cream application consistently had a higher exposure compared to the gel in both animal species.
- The absorption process, into the dermis, lasted much longer in the mini-pigs with a median Tmax of 16.5 hours for both the cream and gel formulations compared to a median Tmax in rabbits of 2 and 2.5 hours, respectively.
- In both cases, dMD probes were inserted in the dermis, as verified by the ultrasound measurements, however, in rabbits the average depth of the probes was 0.061 ± 0.027 cm versus 0.27 ± 0.08 cm in the mini-pigs. Moreover, Rabbits have also a higher follicular density (400-500 per cm^2) as compared to mini-pigs (11 per cm^2) that might substantially contribute to the percutaneous permeation process.

Disposition Experiments

- Figure 2 shows the dermal concentration profiles resulting from the dermal infusion in the two species.
- The dermal disposition parameters are reported in Table 2.
- In both species, the terminal elimination slope from the dermal infusion experiment is significantly smaller ($p < 0.05$) than that observed from the application of the TDDPs, indicating flip/flop dermal PK; the terminal phase reflects the rate of absorption rather than elimination rate after topical product application.
- dCL (p=0.057) and dVd (p=0.715) are not statistically different yet the dermal elimination half-life differs (P=0.0003) between the two species, while the mini-pig exhibited a longer half-life.

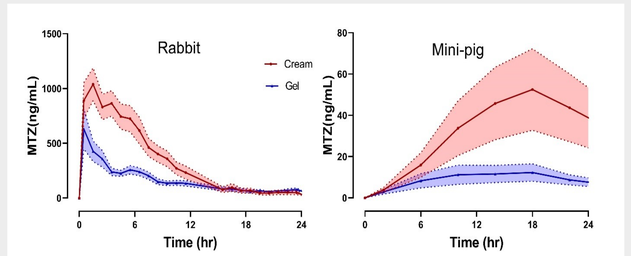

Figure1 - Dermal exposure of MTZ in rabbit and mini-pig measured via dMD following the application of the same product-dose (note the difference in y-axis scale), *GeomMean (GeoSE)* n= 14 probes for each formulations in rabbit and n= 6 probes for each formulations in mini-pig

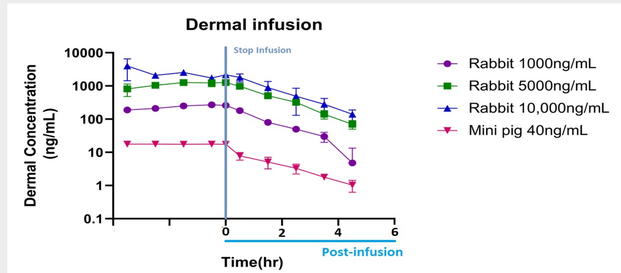

Figure2 - Dermal concentrations resulting from the dermal infusions (GeomMean (GeoSD) n= 2 probes for each dose in New Zealand Rabbit and n=6 for Yucatan mini-pig. Since the duration of the infusion was different in the two species, the time is reported relatively to the end of the infusion for an clearer comparison.

Table 1 – Dermal pharmacokinetic parameters of unbound MTZ after application of topical formulations (cream and gel) in rabbit sampling was (t=24 hrs) vs (t=48) hrs sampling in mini-pig (GeomMean (GeoSD) ;rabbit n=14 for cream n=14 for gel, mini-pig n=6 for cream n=8 for gel)

	Tmax (hr)		Cmax (µg/mL)		AUC0-t (µg/mL/hr)	
Topical Application	**Rabbit**	**Mini-pig**	**Rabbit**	**Mini-pig**	**Rabbit**	**Mini-pig**
Cream	1.50 (0.5-8.5)	21.0 (14.5-46.5)	1.19 (0.14)	0.04 (0.08)	8.09 (1.19)	0.97 (0.08)
Gel	1.50 (0.5-7.5)	13.5 (1.5-37.5)	0.73 (0.19)	0.01 (0.09)	3.93 (0.58)	0.24 (0.10)

Table 2 –Dermal disposition parameters of unbound MTZ estimated by dermal infusion studies (GeomMean (GeoSD),n=6 probes for rabbit and mini-pig.

	dCL (mL/hr)		dVd (mL)		dHL (hr)	
Dermal Disposition (N=6)	0.10 (1.12)	0.05 (1.44)	0.16 (1.15)	0.11 (1.58)	1.14 (1.05)	1.56 (1.17)

CONCLUSIONS

- Physiological and anatomical differences may have a significant effect on the dermal exposure of MTZ. Comparing dPK across different species might help to elucidate the specific permeation pathways contribution toward the overall dermal exposure after TDDP application.
- Indeed, the MTZ dermal absorption in the mini-pig model is much slower than in the rabbit, as it might be explained by the thicker stratum corneum, and the sparse hair follicles in mini-pigs.
- The mini-pig exhibited a longer elimination half-life, which might be explained by lower blood perfusion in mini-pig skin. However, note that two dermal infusion probes were inserted in each of 3 pigs, whereas six (6) probes were inserted in a single rabbit, therefore more experiments are needed to further evaluate the effect of inter-subject variability.
- It is worthy to notice that in both species, the cream formulations exhibited higher exposure than the gel formulations and thus both species may be suitable models to discriminate amongst formulations.
- The dermal disposition parameters estimated via dermal infusion may help to further comprehend the dermal PK of TDDPs.

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