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Dermal Open Flow Microperfusion as a Pharmacokinetic Approach to Evaluate Bioequivalence for Topical Drug Products

T. Birngruber[1], M. Bodenlenz[1], K. Tiffner[1], R. Raml[1], B. Tschapeller[1], T. Augustin[1], C. Dragatin[1], D. Schimek[1], G. Schwagerle[2], S. Korsatko[2], I. Kanfer[3], F. Sinner[1,2]

CONTACT

1
JOANNEUM RESEARCH Forschungsgesellschaft mbH
HEALTH
Institute for Biomedicine and Health Sciences
Graz, Austria

2
Medical University of Graz
Department of Internal Medicine
Division of Endocrinology and Diabetology
Graz, Austria

3
Faculty of Pharmacy
Rhodes University
Grahamstown, South Africa

Purpose

There is practical utility for exploring methods that may be able to reliably evaluate the bioequivalence (BE) or non-bioequivalence of topical dermatological products based upon a comparative dermal pharmacokinetic measure of bioavailability.

Open-flow microperfusion (OFM) is a technique that provides direct access to target tissues in human volunteers for the continuous in vivo measurement of drug concentrations in the interstitial fluid. Dermal OFM provides continuous in vivo measurement of intradermal drug concentrations up to 48 hours and with no restriction in terms of lipophilicity and size of the drug being investigated (Fig.1). The utility of OFM has been demonstrated by pharmacokinetic-pharmacodynamic studies with a wide range of substances, ranging from small lipophilic drugs to large proteins and antibodies, and these could be monitored in the dermis of both, healthy volunteers and patients.

In this study, we evaluated whether dOFM is an accurate, sensitive, and reproducible in-vivo method to characterize the intradermal bioavailability of acyclovir when using 5% acyclovir creams. Moreover, we characterized sources of variability in a clinical in-vivo BE setting with a focus on factors that could have a negative impact on future BE assessments.

Methods

- 20 healthy volunteers, written informed consent
- 2 test settings per volunteer each involving 3 test sites (Fig. 2)
  - Left leg: R-R-T (Reference-Reference-Test)
  - Right leg: T-R-R (Test-Reference-Reference)
- 2 OFM probes per test site inserted into the dermis and perfused for sampling at 1µL/min (Fig.1)
  - OFM probe 'DEA15003' (0.5 x 15 mm, open mesh, Fig.1)
  - OFM pump 'MPP102' (wearable, operates 3 to 6 probes)
- t=0: Topical dosing of 2 commercial 5% acyclovir creams at 15 mg/cm^2
  - R = Reference = acyclovir cream 5% (Zovirax® cream)
  - T = Test = acyclovir cream 5%
- t=-1h…36h: Continuous OFM sampling in 4h intervals
- Controlled environmental conditions: 22±1°C, 40–60% relative humidity
- eCRF data capture (OpenClinica; validated and 21 CFR Part 11 compliant)
- *Analysis/Assessment:*
  - Acyclovir (UHPLC-MS) and glucose concentration in dOFM samples
  - Skin impedance (in-house tool), TEWL-trans-epidermal water loss (Aquaflux, Biox Ltd), skin temperature, probe depth (ultrasound)
- *Statistics:*
  - BE-evaluation based on dermal acyclovir concentrations (log AUC0-36h, logCMAX) and BE limits of log(0.8) and log(1.25)
  - Sources of variability assessed by Analysis of Variance (ANOVA)
  - Influencing factors identified by regression and correlation analysis

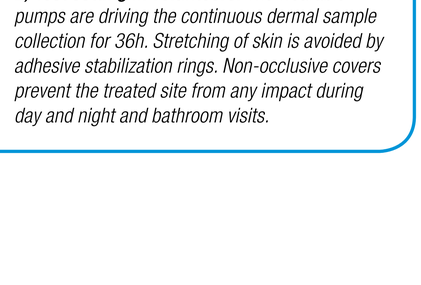

Figure 1: Open Flow Microperfusion (OFM). *dOFM, a universal linear certified probe designed for dermal and subcutaneous use in humans, continuously delivers dermal interstitial fluid for the study of PK and PD in the target tissue. Continuous sample collection is controlled by a wearable pump. All devices are CE-certified for human use and were designed and patented by JOANNEUM RESEARCH, Graz, Austria*

Figure 2: A) Schematic of test setting in volunteers. *Three adjacent topical test sites form one test setting. The setting is implemented twice on each volunteer. Test and reference (lateral) is always compared against the reference in the center, enabling double testing of test vs. reference product, as well as a double testing of the method/setting itself based upon the expectation that the dermal pharmacokinetics of acyclovir from the two sites dosed with the same (reference) product should be the same.*

B) Test setting in volunteers. *The wearable pumps are driving the continuous dermal sample collection for 36h. Stretching of skin is avoided by adhesive stabilization rings. Non-occlusive covers prevent the treated site from any impact during day and night and bathroom visits.*

Acknowledgement

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Study was approved by FDA-RIHSC (FDA Research Involving Human Subject Committee) & local IRB of the Medical University Graz, Austria

Results & Discussion

20 subjects resulted in 240 acyclovir profiles for statistical analysis (each 36 h, in total 8640 h of intradermal data, Fig. 3). No serious adverse events and no dropouts occurred.

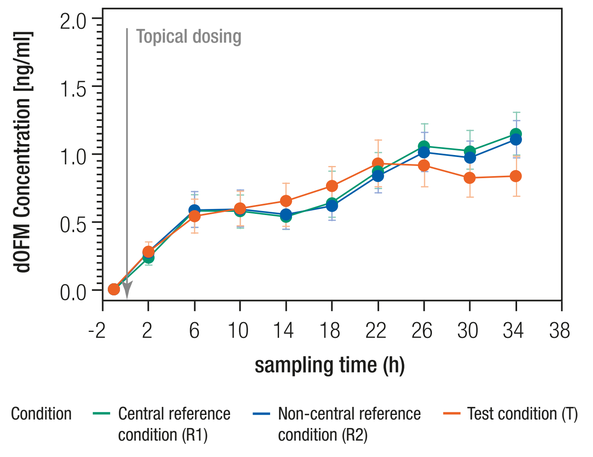

Figure 3: ***dOFM acyclovir concentrations as a function of time.*** *Mean +/- SE. Acyclovir profiles 0-36h for the test and the two reference sites. The AUC0-36h of the adjacent test sites were compared to each other statistically based upon the 90% confidence interval of the mean difference between products (T vs. R_1, R_2 vs. R_1, N=40 test settings in 20 subjects).*

The positive controls (R vs. R) were accurately and reproducibly confirmed to be bioequivalent, while the negative control products (T vs. R) were sensitively discriminated not to be bioequivalent (Table 1).

Table 1: *Test results*

Test condition	Variable	90% confidence interval	Traditional BE-Limits	Mean Difference within 80% –125%
R_2 versus R_1	Log(AUC0-36h)	86.2 – 117.5%	[-0.223; 0.223] or [80–125%]	✔ Passed
R_2 versus R_1	Log(CMAX)	85.7 – 120.9%		✔ Passed
T versus R_1	Log(AUC0-36h)	69.1 – 105.2%		✕ Failed
T versus R_1	Log(CMAX)	60.8 – 102.2%		✕ Failed

Inter-subject variability of logAUC for R (T) accounted for 84% (91%) of the total variability (Fig. 4). This type of variability is most likely due to differences in the subjects' stratum corneum (SC). The in-house skin impedance method was sensitive enough to reflect SC properties and correlated well with logAUC (r=0.68-0.75, p<0.0001), while the established TEWL-method showed a lower correlation (r=0.29-0.37, n.s.)

Intra-subject variability of logAUC for R (T) was low at 16% (9%). Its site-to-site component of 9% (4%) could have been caused by local differences in SC properties and/or local differences in skin temperature (r=0.25, p<0.05). The remaining variability of 7% (5%) is probe-to-probe variability which could have been caused by the user (e.g. variability in probe insertion depths) and variability in the sampling process (e.g. relative recovery). Interestingly, dermal OFM sampling data indicated subclinical effects of skin irritation following the use of Zovirax cream as reference product. A negative effect on BE evaluation of T vs. R was not identified. Statistical analysis of influencing factors is currently ongoing.

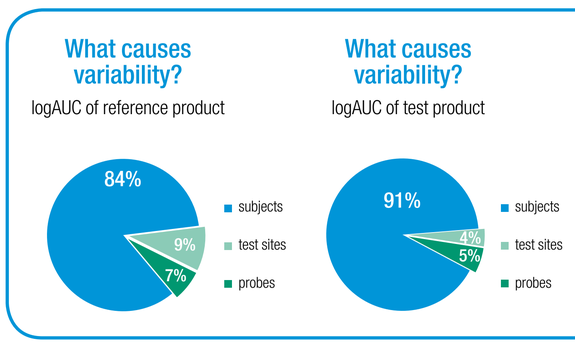

Figure 4: *ANOVA results describing the sources of variability for the penetration of the reference product (left) and the test product (right). The pie-chart shows the relative contributions of three sources of variability to the total coefficient of variation for logAUC, which was 39% for the reference and 46% for the test product. As the charts show, "inter-subject" or "between-subject variability" is the dominant source of variability.*

Conclusions

- **Dermal OFM results showed relatively low variability and high robustness.**
- **Inter-subject variability accounted for more than 84% of total variability in this clinical study setting and is most likely caused by different properties of the stratum corneum in different subjects. Skin impedance was found to be a good predictor of topical penetration.**
- **Intra-subject variability accounted for less than 16% of total variability. This low value indicates reproducibility of the OFM test setting.**
- **Further clinical studies with different topical drugs to investigate dermal OFM as a pharmacokinetic method may be of value.**