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In Vivo Dermal Open Flow Microperfusion: Understanding and Controlling Sources of Variability to Evaluate Topical Bioequivalence

M. Bodenlenz[1], T. Birngruber[1], K. Tiffner[1], R. Raml[1], B. Tschapeller[1], T. Augustin[1], G. Schwagerle[4], S. Korsatko[4], I. Kanfer[3], S.G. Raney[2], F. Sinner[1,4]

CONTACT

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

2
Office of Generic Drugs
Center for Drug Evaluation and Research
U.S. FDA
Silver Spring, MD, USA

3
Division of Pharmaceutics
Faculty of Pharmacy
Rhodes University
Grahamstown, South Africa

4
Medical University of Graz
Department of Internal Medicine
Division of Endocrinology and Diabetology
Graz, Austria

Purpose

Open-flow microperfusion (OFM) is a technique that facilitates the direct (in situ) assessment of tissue drug concentrations in human volunteers, enabling the continuous in vivo measurement of drug concentrations in the interstitial fluid. In this study, we evaluated whether dermal OFM (dOFM) could be a suitable in vivo method with which to characterize and compare the intradermal pharmacokinetics (PK) and bioequivalence (BE) of acyclovir from topical acyclovir cream, 5% products based upon an assessment of dermal PK endpoints like maximum concentration (C_{max}) and area under the concentration-time curve (AUC). Moreover, to evaluate how accurate, sensitive, and reproducible dOFM could be as a potential approach to evaluate topical BE, we characterized sources of variability in the clinical in vivo BE study, with a focus on understanding controlled and uncontrollable sources of variability that could impact the precision and power of such BE assessments.

Methods

- 20 healthy volunteers, written informed consent
- Each OFM probe inserted into the dermis was 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)
- Each volunteer had 1 test setting on each leg, with 3 test sites per setting, with 2 probes per test site (Fig. 2)
  - Left leg: R-R-T (Reference-Reference-Test)
  - Right leg: T-R-R (Test-Reference-Reference)
- t = 0 : 2 commercial acyclovir cream, 5% products dosed at 15 mg/cm²
  - R = Reference = acyclovir cream 5% (Zovirax®; USA)
  - T = Test = acyclovir cream 5% (Aciclovir 1A Pharma-Creme; Austria)
- t=-1 h…36 h: Continuous OFM sampling in 4 h intervals
- Controlled environmental conditions: 22 ± 1°C, 40–60% relative humidity
- Data captured in electronic case report forms (OpenClinica; validated and 21 CFR Part 11 compliant)
- *Analysis/Assessment:*
  - Acyclovir (UHPLC-MS) and glucose concentration in dOFM samples
  - Skin impedance (in-house tool); Trans-Epidermal Water Loss (TEWL) (Aquaflux, Biox Ltd); Skin temperature (infrared thermometer); Probe depth (ultrasound)
- *Statistics:*
  - BE evaluation based on dermal acyclovir concentrations (log AUC_{0-36h}, logC_{max}) 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

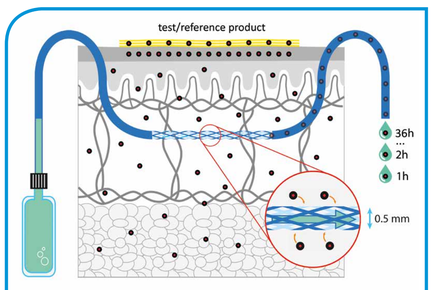

Figure 1: Open Flow Microperfusion (OFM). *dOFM, a linear probe designed for dermal and subcutaneous use in humans, continuously delivers dermal interstitial fluid for the study of PK and PD in the target tissue. Continuous sample collection is controlled by a wearable pump. All devices are CE-certified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

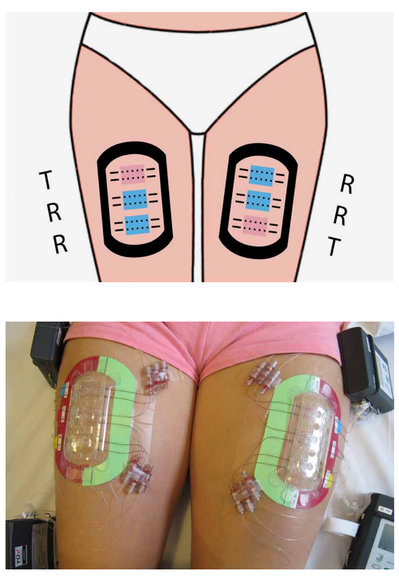

Figure 2: A) Schematic of test setting in volunteers. *Three adjacent topical test sites form one test setting. The setting is implemented twice on each volunteer. Test and reference (lateral) is always compared against the reference in the center, enabling double testing of test vs. reference product, 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 36 h. 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

Twelve probes in each of 20 subjects provided 240 acyclovir dermal PK profiles (each 36 h, in total 8640 h of intradermal data, Fig. 3). No serious adverse events and no dropouts occurred.

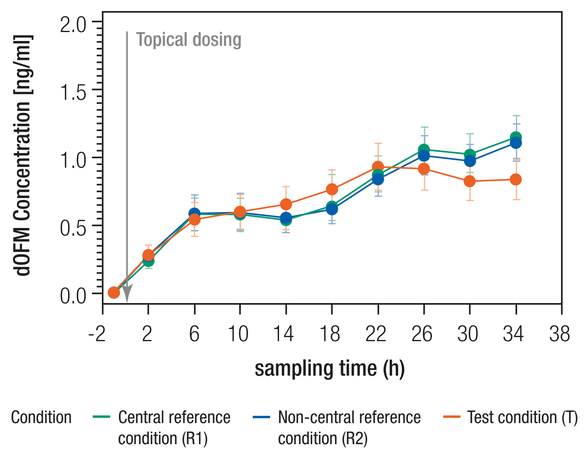

Figure 3: dOFM acyclovir concentrations as a function of time. *Mean +/- SE. Acyclovir profiles 0 - 36h for the test and the two reference sites. AUC_{0-36h} and C_{max} of the adjacent test sites were compared to each other statistically based upon the 90% confidence interval of the mean difference 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(AUC_{0-36h})	86.2 – 117.5%	[-0.223; 0.223] or [80–125%]	✔ Passed
R_2 versus R_1	Log(C_{max})	85.7 – 120.9%		✔ Passed
T versus R_1	Log(AUC_{0-36h})	69.1 – 105.2%		✕ Failed
T versus R_1	Log(C_{max})	60.8 – 102.2%		✕ Failed

Inter-subject variability of logAUC for R and T accounted for 84% and 91%, respectively, 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 with logAUC ($r = 0.69 - 0.75$, $p < 0.0001$), while the established TEWL-method showed a lower correlation ($r = 0.29 - 0.37$, not significant). Similar results were observed for LogC_{max}.

Intra-subject variability of logAUC for R and T was low at 16% and 9%, respectively. The site-to-site variability for R and T (9% and 4%, respectively) 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 for R and T (7% and 5%, respectively) is attributed to probe-to-probe variability which could have been caused by the user (e.g. variability in probe insertion depths) and/or variability in the sampling process (e.g. relative recovery). Similar results were observed for LogC_{max}. A comprehensive statistical analysis of influencing factors is currently ongoing.

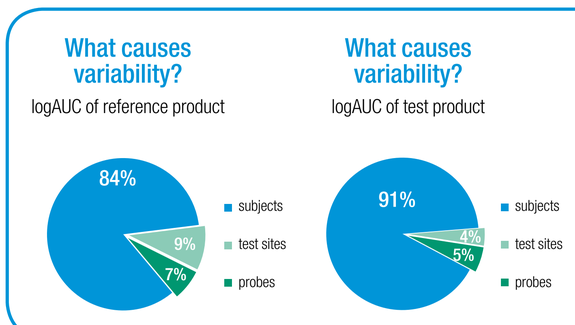

Figure 4: *ANOVA results describing the sources of variability for the penetration of the reference product (left) and the test product (right). The pie-chart shows the relative contributions of three sources of variability to the total coefficient of variation for logAUC, which was 39% for the reference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

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 correlate with topical bioavailability.
- Intra-subject variability accounted for less than 16% of total variability. This indicates reasonably good control and 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.