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Variability of topical penetration data: what do we learn from a be-study using dermal open flow microperfusion

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References

[4] Bodenlenz et al., Clin. Pharmacokinet. 2016

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Purpose

Topical permeation studies on human skin are associated with considerable data variability. The sources of this variability and their impact on the outcome of bioavailability-bioequivalence (BA-BE) studies have not yet been fully decoded. The decryption of variability requires an appropriate data set comprising both the BA-BE endpoint data and data on variables that potentially influence topical penetration in the specific study setting. Such a data set has recently been created in a clinical BA-BE study in healthy subjects using dermal open flow microperfusion (dOFM).

We performed an extensive statistical analysis of this comprehensive data set aiming to investigate the sources of variability and their potential impact on BA-BE endpoint variables.

Data Set analysed

The dOFM study delivered 240 intradermal acyclovir profiles from 20 healthy adults (36 hours per profile). The kinetic BE endpoint variables (logAUC, logCmax) enabled the verification of topical BE of a reference product vs. itself and the identification of a test product as non-bioequivalent [4]. In addition it delivered data on the individuals' skin properties and potential methodological factors (Fig. 1).

Results: Where does the variability come from?

Results for the logAUC of reference product (R) are shown throughout. logCmax data and data derived from the test product (T) showed comparable results.

Total variability: The total coefficient of variation (CV) for logAUC was 39 % for R, and 45 % for T. This is in line with results from previous studies using dermal microdialysis.

Inter-subject variability of logAUC for R and T accounted for 82% and 91%, respectively, of the total variability (Fig. 2). Inter-subject variability is most likely due to differences in the subjects' stratum corneum (SC). The skin impedance method developed recently by our group enabled the reflection of SC properties and correlated well with logAUC ($r = -0.71$, $p<0.0001$), while the established TEWL-method seemed to be more prone to outliers and thus showed a low correlation ($r = 0.30$, $p = 0.054$). Consequently, the rather robust impedance method will be developed further and both methods re-evaluated in the subsequent BA-BE studies.

Intra-subject variability of logAUC for R and T was low with 18% and 9%, respectively. ANOVA indicated that sites and probes contribute similarly. However, further analysis showed that the sites in fact contributed little to variability: LogAUCs were rather reproducible between the sites on the same leg and between left and right leg (R: $r=0.91$, $p<0.0001$: Test: $r=0.94$, $p<0.0001$; Fig. 3).

Surprisingly, most intra-subject variability was clearly attributable to probe-to-probe differences. Adjacent probes within the same test sites did not show lower AUC-differences than two arbitrary probes in a subject (Fig. 4). These probe-to-probe differences in AUCs were not explainable by differences in dOFM probe depth, flow rate or relative recovery. Interestingly, such probe-to-probe differences have also been observed in microdialysis studies before, where they have remained unexplained.

We therefore hypothesized that dermal sampling probes detect locally increased penetration, e.g. through skin appendages. IVPT studies with replicates, and studies of follicular penetration using follicle-plug and skin-sandwich techniques seem to support our assumption that such local differences are linked to skin microstructure, the impact of which getting particularly evident for drugs with low SC permeability like acyclovir. This clinical in vivo acyclovir study might be the first with sufficient power and sensitivity to reveal the local variability of topical penetration in vivo in human skin.

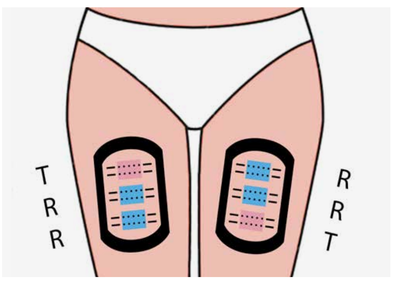

Figure 1: Test setting in 20 volunteers and type of data. *Twelve dOFM probes per subject delivered 36-hour-profiles of intradermal acyclovir released from a topical test (T) and a reference product (R). The study delivered (i) dermal BE endpoint data (AUC0-36h, Cmax), (ii) data describing the individuals skin properties and (iii) data describing dOFM quality parameters*

AUC0-36h, Cmax (240 x in 20 subjects)

- Subject-related data
  - TEWL
  - Skin impedance
  - Skin temperature
- Method-related data
  - dOFM probe depth
  - dOFM flow rate
  - dOFM relative recovery

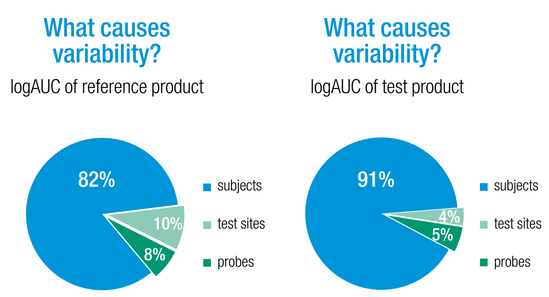

Figure 2: ANOVA results describing the sources of variability for the penetration of R (left) and T (right). *The pie-chart shows the relative contributions of three sources of variability to the total variability. Inter-subject variability is the dominant source of variability.*

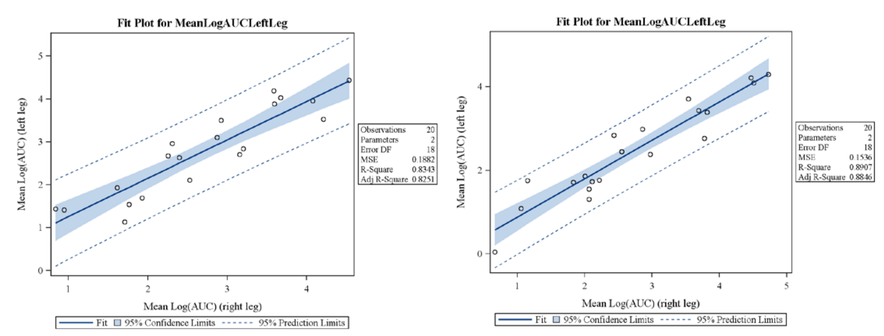

Figure 3: logAUCs left versus right leg. *The reproducibility indicates that the factor test site is not adding significant variability. (a) logAUCs 0-36h for R, r=0.91, p<0.0001 (b) logAUCs 0-36h for T, r=0.94, p<0.0001.*

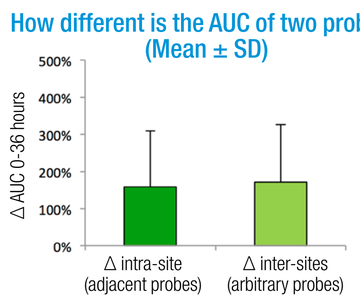

Figure 4: *Deviations between the AUCs of two adjacent probes within the same site (Δ intra-site) and between arbitrary probes (Δ inter-site) within the same individual. The comparison reveals that intra-subject AUCs variation is predominantly caused by probe-to-probe differences, while sites do not add significant variation.*

Conclusions

- **Inter-subject variability accounted for more than 82% of total variability and was clearly attributable to inter-subject differences in skin barrier properties. This highlights the power of kinetic approaches for topical BA-BE assessments, such as dOFM and microdialysis that enable simultaneous head-to-head comparison of products.**
- **Intra-subject variability accounted for less than 18% of total variability. This indicates reasonably good control and reproducibility of the OFM test setting. The intra-subject variability might reflect the local variability of skin permeation caused be the skin microstructure.**
- **Further clinical studies with different topical drugs are required to verify our findings.**