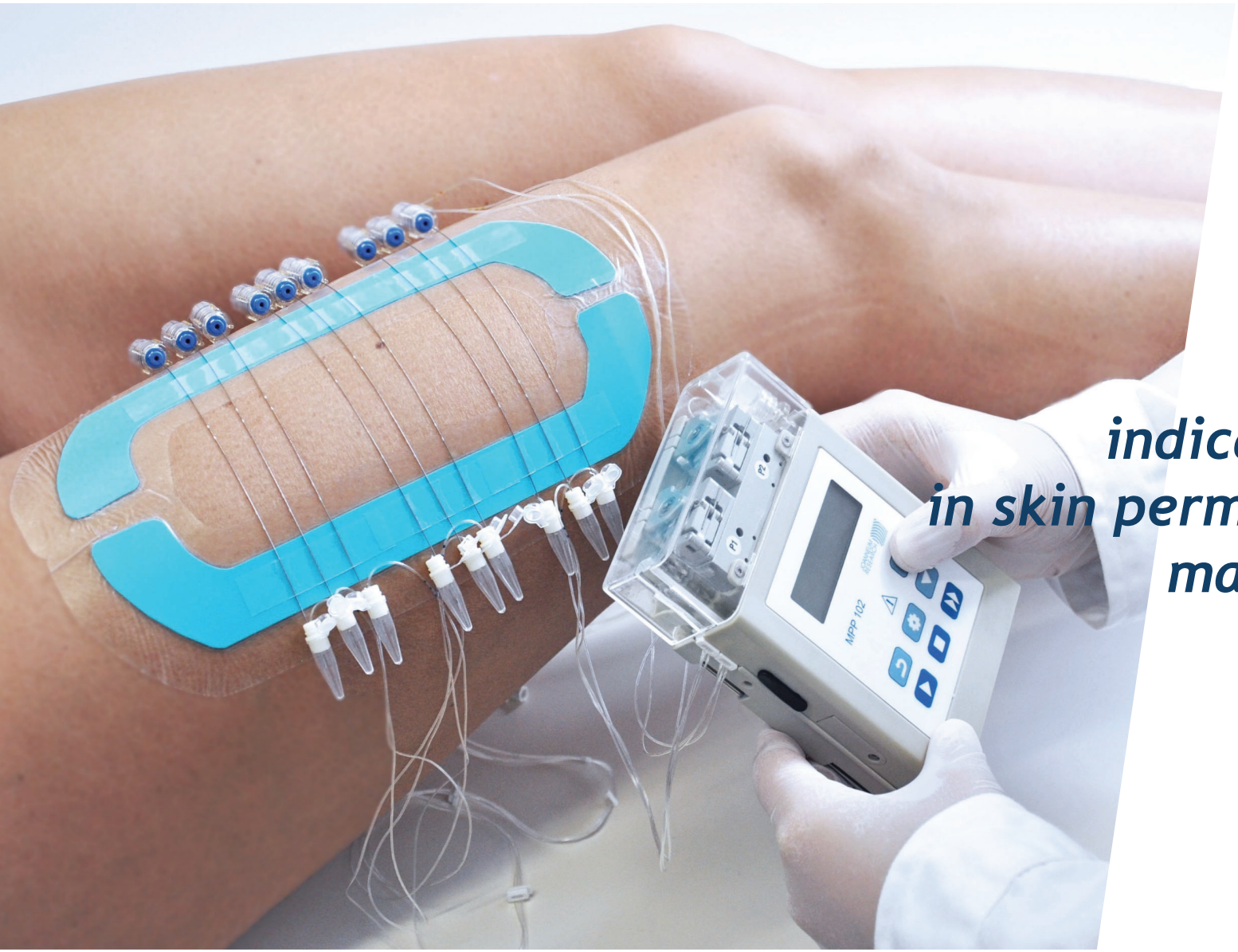

JOANNEUM RESEARCH HEALTH

Poster#6: Dermal OFM indicates differences in skin permeation between males and females

Manfred Bodenlenz

late breaking at GRC 2023

OFM

Poster#6

Dermal OFM indicates differences in acyclovir skin permeation between males and females

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[1] Bodenlenz et al. "Open Flow Microperfusion as Dermal Pharmacokinetic Approach to Evaluate Topical Bioequivalence", Clin. Pharmacokinet. 2015.

[2] Bodenlenz et al. "Variability of skin pharmacokinetic data: Insights from a topical BE study using dermal open flow microperfusion" Pharm. Res. 2020.

[3] Bialik W, Waters RA, Brain KR, Hadgraft J. „Some factors affecting the in vitro penetration of ibuprofen through human skin", Int J Pharm. 1993.

[4] Fahrenkopf et al. "Male versus female skin: What dermatologists and cosmeticians should know". Int J Womens Dermatol. 2019.

Acknowledgement

This project is supported by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services (HHS) as part of grants (U01FD004945, and U01FD005951) totaling $ 3.55 M with 70 percent funded by FDA/HHS. The views are those of the author(s) and do not necessarily represent the official views of, nor an endorsement by, FDA/HHS or the U.S. Government.

We thank Maike Sophie Riedler and Elinor Valdmeier for poster design and graphics.

Purpose

Clinical dermal open flow microperfusion (dOFM) can provide time-resolved dermal concentration profiles that have the potential to support pharmacokinetics- based topical bioequivalence (BE) assessments. A study evaluating acyclovir products in 20 volunteers demonstrated the reproducibility of dOFM data to evaluate the BE of a reference cream product to itself and to discriminate a non-BE product and the reference cream [1]. Initial data analysis characterized the overall sources of inter- and intra-subject variability but did not focus on the factors that may affect the discrimination of products.

This analysis investigated which methodological and biological factors may affect the sensitivity of clinical dOFM studies to discriminate topical acyclovir products.

Methods

Summary of the clinical study with dOFM [1]:

- 20 healthy volunteers (7 females, 13 males)
- Two topical products investigated by dOFM for 36 hours (Fig. 1)
  - Controlled clinical conditions: 22 ± 1°C, 40 – 60% relative humidity
  - R – Reference – acyclovir cream 5% (Zovirax®, USA)
  - T – Test – acyclovir cream 5% (Aciclovir 1A Pharma-Crème, Austria)
  - T and R have previously been shown to exhibit substantial differences in drug release and skin permeation in vitro (e.g., using an in vitro permeation test (IVPT)).
- Analysis of BE, variability, and subpopulations
  - Average bioequivalence (ABE) evaluation of R vs. R and T vs. R based on $\log AUC_{0-36h}$ and $\log C_{max}$ of dermal acyclovir concentrations
  - BE criteria based on the 90% confidence intervals of geometric mean ratios of logAUC and $\log C_{max}$ falling between 0.80 - 1.25
  - Analysis of the sources of variability for T and R by Analysis of Variance (ANOVA); analysis of distribution, regression, correlation and probe-to-probe differences of various methodological and biological parameters
  - Analysis of factors affecting the ratios T vs. R and R vs. R, including separate statistical analysis of N=7 females and N=13 males.

Results

- Joint data analysis of N=20 subjects
  - The data enabled the verification of topical ABE for a reference cream vs. itself and the identification of a test product as being non-bioequivalent [1].
  - Data analysis demonstrated that methodological factors (test site location, probe depths, flow-rate, relative recovery) did not significantly contribute to data variability. ANOVA attributed >- 92% of the variability to subjects. The remaining variability of <-1 9% was attributed to local variability of drug permeation [2].
- Separate analysis of females and males is shown in Figure 3.
  In female subpopulation the negative control produced more discriminating results, compared to the male subpopulation.
  - 7 Females: Profiles rose slowly showing clear differences T vs. R.
    - Negative control (T vs. R) and positive control (R vs. R) were confirmed.
  - 13 Males: Profiles rose faster showing no consistent differences.
    - Negative control (T vs. R) and positive control (R vs. R) not confirmed.
  - Results of ex vivo dOFM in male and female skin confirmed the difference (data not shown). Significant differences between male and female skin penetration had already been reported from IVPT-studies in 1993 by Bialik et al. [3].

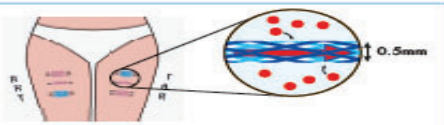


Fig. 1: Schematic of the application sites with dOFM inserted in the dermis during the clinical study in 20 volunteers (7 females, 13 males). The study delivered 240 profiles of intradermal acyclovir for BE evaluation of a reference product itself (R vs R) and a test product vs reference (T vs R).

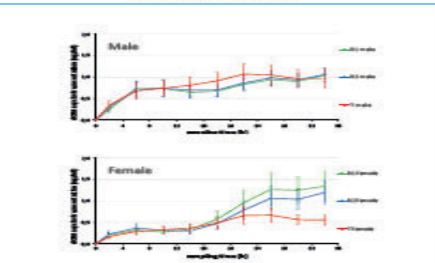


Fig. 2: Acyclovir concentration profiles for R and T. In male concentrations rise fast and do not discriminate T from R. In females dermal concentrations rise slowly and after 20 hours clearly discriminate T from R. Concentrations are plotted as mean ±SE.

Fig. 3: Results of BE evaluation for male (N=13) and female subjects (N=7) for two acyclovir products. In female subpopulation the negative control product shows discriminating results, compared to the male subpopulation.

Conclusions

- Clinical dOFM may reveal sex- and product-related differences in acyclovir skin penetration in a low number of volunteers.
- We hypothesize that the observed differences can be due to differences in the skin microstructure or daily skin care of men and women.
- Further studies may be of value to better understand the underlying biological and pharmacological mechanisms and their impact on clinical BA- BE evaluation.

New data: Do dermal profiles also differ between males and females for topical lidocaine and diclofenac?

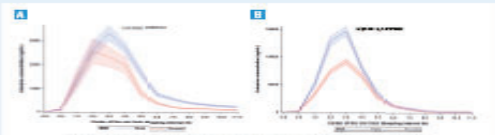


Fig. 4: Lidocaine dOFM concentrations for 15 mg/cm² of the reference cream (Plot A, 14 males + 6 females) and 150 mg/cm² (Plot B, 11 males + 9 females) dosed at t=0 hours, occluded, and removed at t=3 hours (mean ±sem).

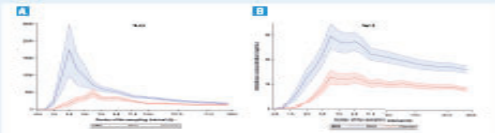


Fig. 5: Diclofenac dOFM concentrations for 20 mg/cm² of test product 1, diclofenac sodium topical solution 2% (Plot A) and drug product 2, diclofenac diethylammonium 1% (Plot B) dosed simultaneously at t=0 hours, both occluded, and both removed at t=3 hours (mean ±sem; 8 males + 8 females).

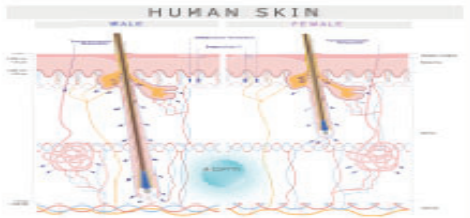


Fig. 6: Morphology of male and female skin with known permeation pathways and a dOFM probe in vivo in the dermis.

Acyclovir, lidocaine, and diclofenac bioavailability appeared to differ in male vs. female dOFM study sub-populations. What makes this difference?

- Skin morphology & appendageal penetration [Fig.6]?
- Hydration, transepidermal water loss, sebum, microcirculation, pH [4]?
- What else is different?

Acknowledgements & Disclaimer

- **Thanks to the HEALTH study team in Graz**
- **Thanks to US-FDA for financial support**
  This project is supported by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services (HHS) as part of grants (U01FD004946 and U01FD005861) totaling $ 3.55M with 70 percent funded by FDA/HHS. The views are those of the author(s) and do not necessarily represent the official views of, nor an endorsement by, FDA/HHS or the U.S. Government.
- **What I say is <u>my personal opinion</u> – this l.b. oral presentation is not cleared by US-FDA!**

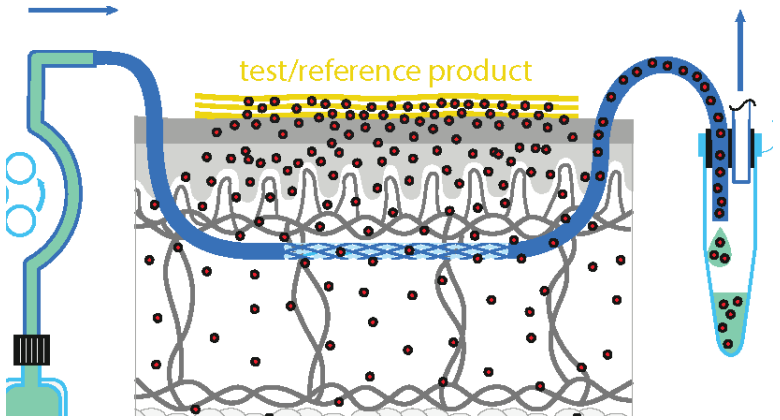
Background: Bioequivalence studies with FDA

- US patients require safe and affordable generic topical products
- US-FDA evaluates PK-based methods for topical bioequivalence (BE)

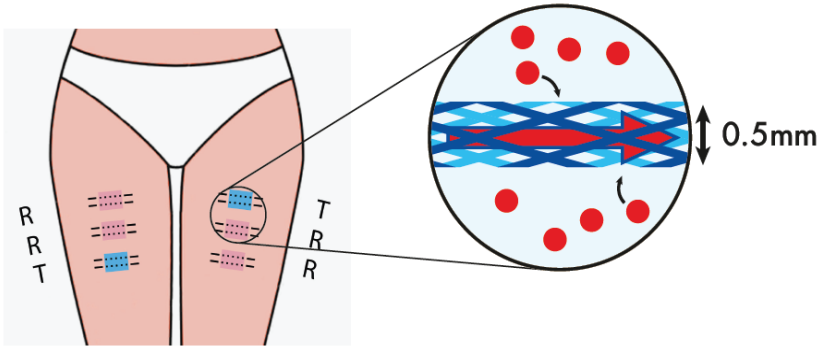
- The method needs to be…
  - **reproducible**,
    to confirm that the generic test product is ~the same as the RLD product (=BE)
  - **sensitive**,
    to show if the generic test product is not BE
  - **usable for different drug products**
    i.e. diff. logP, diff. protein binding, diff. formulations
    ➔ acyclovir, lidocaine/prilocaine, diclofenac products

Method: Dermal Open Flow Microperfusion (dOFM)

- dOFM provides
  - access to molecules in dermal interstitial fluid without discrimination, with time resolution
  - is minimally-invasive, well-tolerable
  - is certified in EU as tool for clinical research

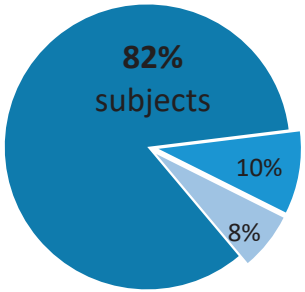


- dOFM is used…
  - in vivo, ex vivo, in humans and animals,
  - **for basic research,**
  - **for drug research (PK, PD)**
    - Pig studies for drug selection & de-risking clinical phases: What is the total & unbound drug concentration in dISF?
    - Human studies for early clinical PoC (PK-PD)
    - Human studies for topical BE?



Study#1 on acyclovir: Variability analysis of data pointed at hair follicles causing noise

- ANOVA of logAUCs for the reference product
  - ≥ **82% inter-subject variability** (cause: subjects)
  - ≤ **18% intra-subject variability** (cause: sites/probes)
  - Similar for test product (91% inter / 9% intra)

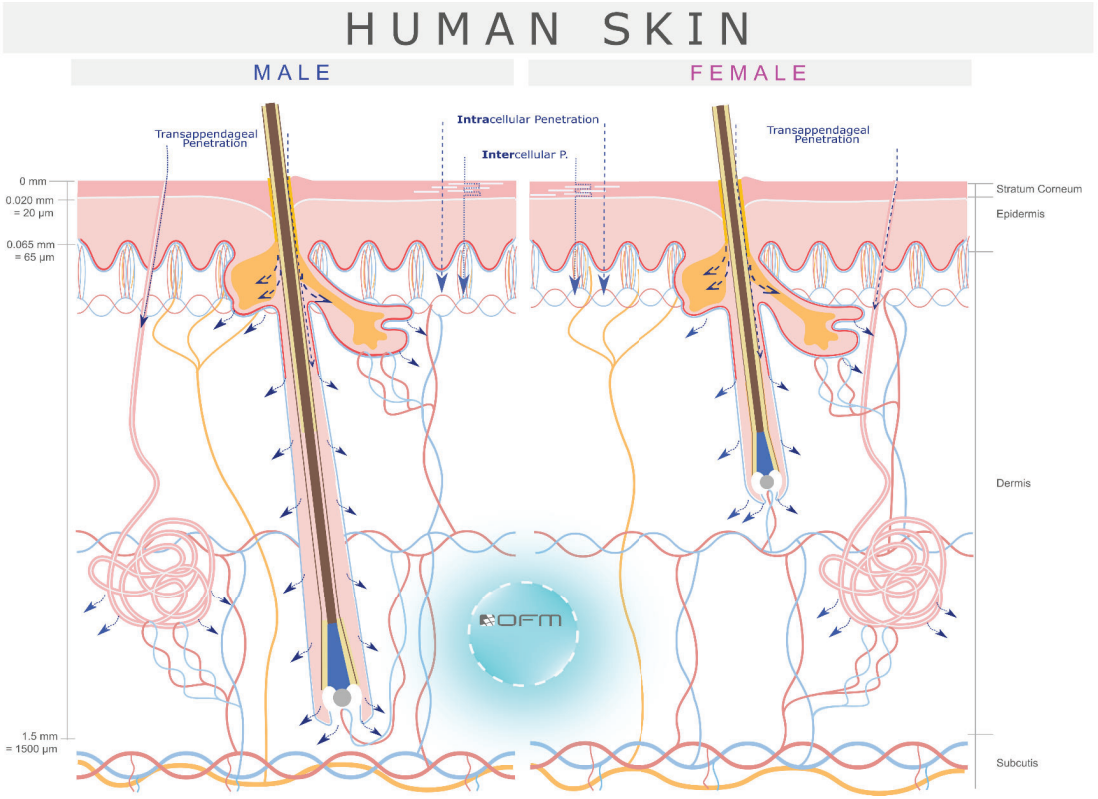


18% intra-subject

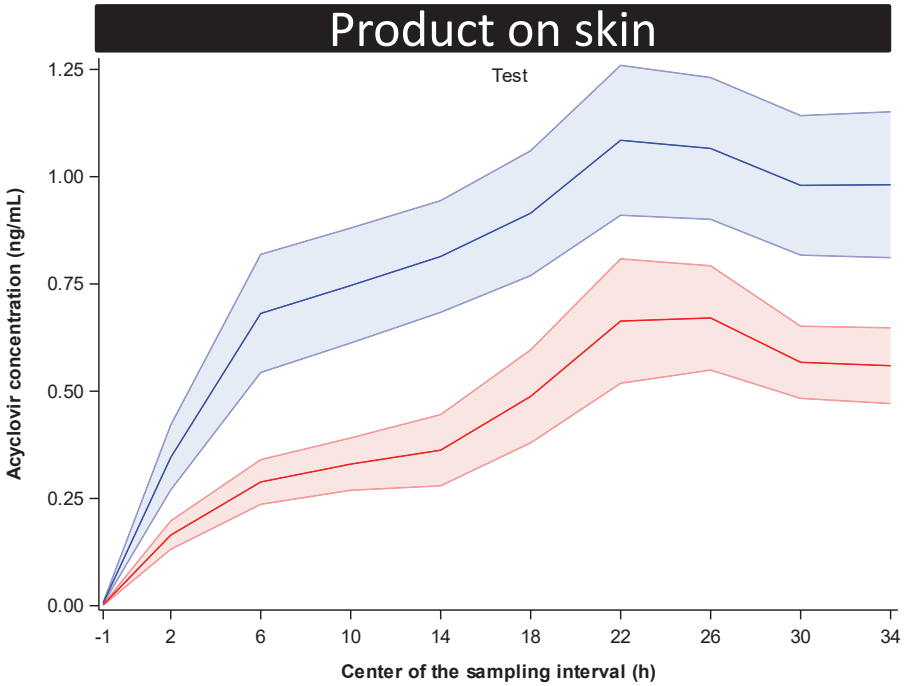
- **Intra-subject PK data distribution was not *normal* - analysed further**
  - Is not caused by sites (excluded by further statistics)
  - Is not caused by probes (excluded all method factors step by step)
  - Literature: Is the local variation of dermal concentration from drug coming through skin appendages!
- Literature study provided evidence
  - IVPT studies with skin (meta-studies) > Log-normal distributed noise
  - IVRT studies with membranes > Gaussian-normal distributed noise
  - Skin sandwich studies **> Skin appendages (hair follicle, seb.gland, sweat duct)**
  - Follicular plug studies **> Follicles!**

Hypothesis

- If this noise is from hair follicles, the dOFM data from males and females may differ/should differ!
  - **Females**: **vellus** hair (thin, ~0.5 mm deep)
  - **Males**: **terminal** hair (thicker, much deeper)



Acyclovir 5% cream products
15 mg/cm², non-occluded, non-removed (13M+7F)



13 males

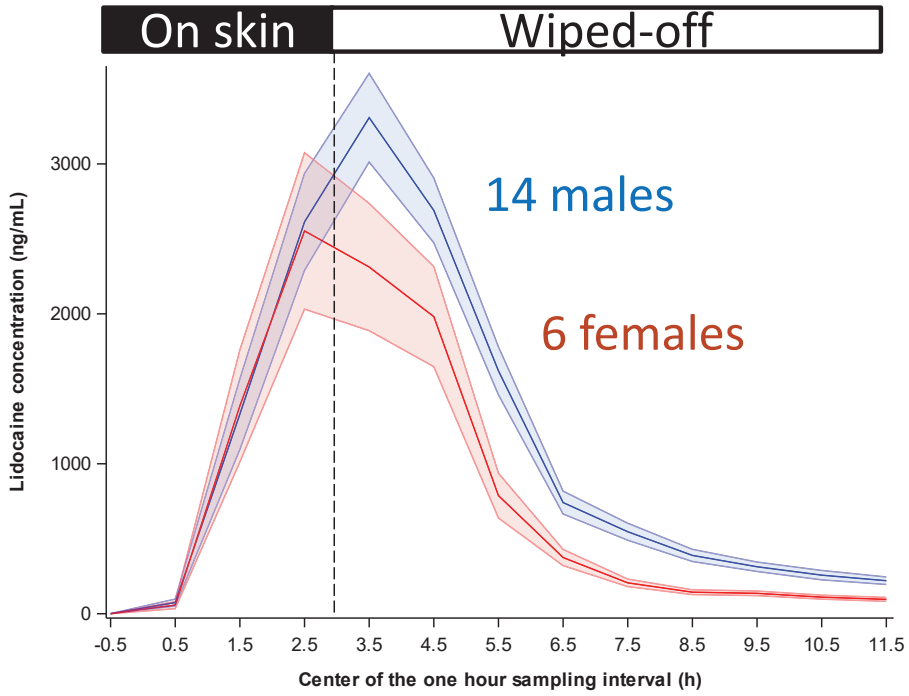
7 females

Mean±SE

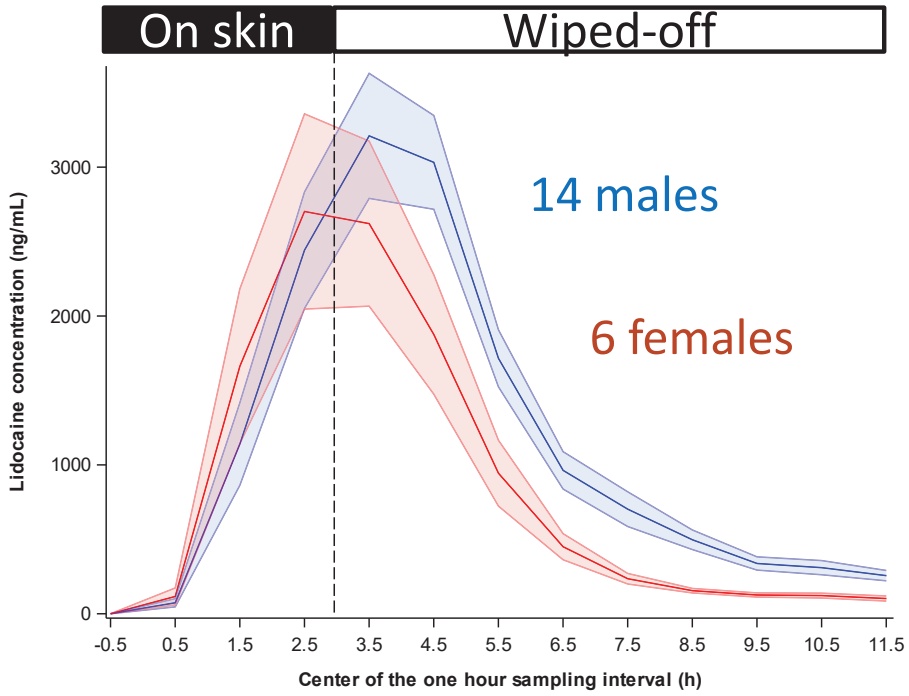
Not a real surprise, Ogiso et al. 2002 demonstrated the follicular pathway for acyclovir.

Study 2_Lidocaine 2.5% cream products
15 mg/cm², occluded, removed at 3 hrs (14M+6F)

Reference product



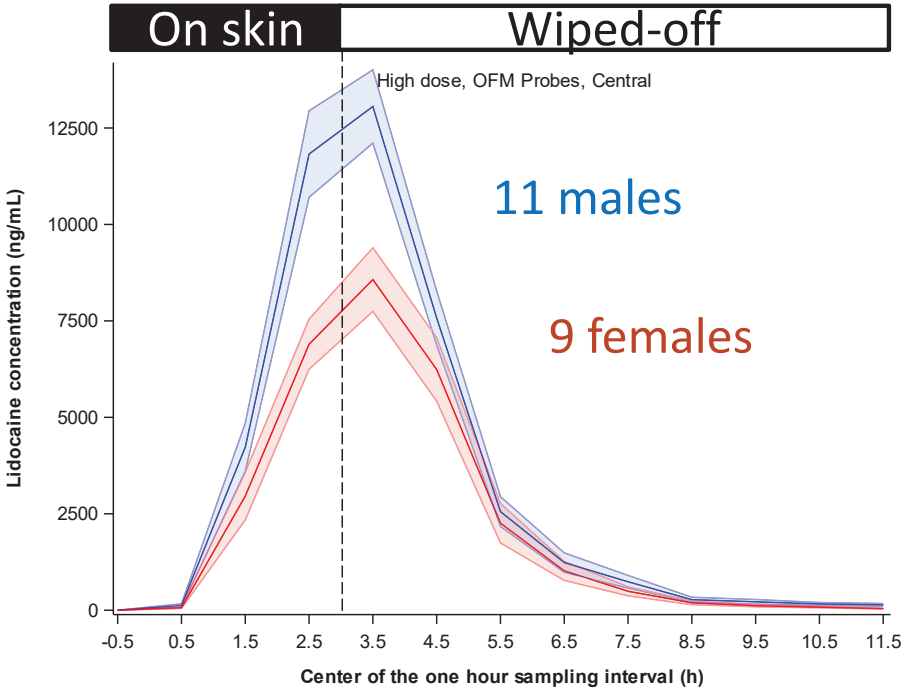
Generic product



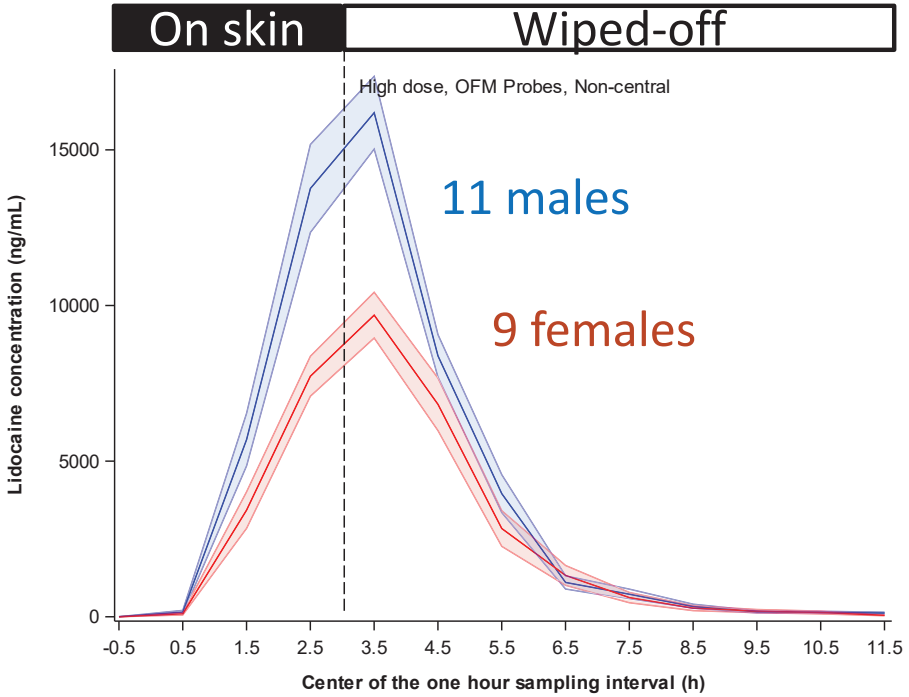
M≠F. Effect of wipe-off at 3h was delayed in males: Different reservoirs?

Study 3_Lidocaine 2.5% cream products
150 mg/cm², occluded, removed at 3 hrs (11M+9F)

Reference product – central site



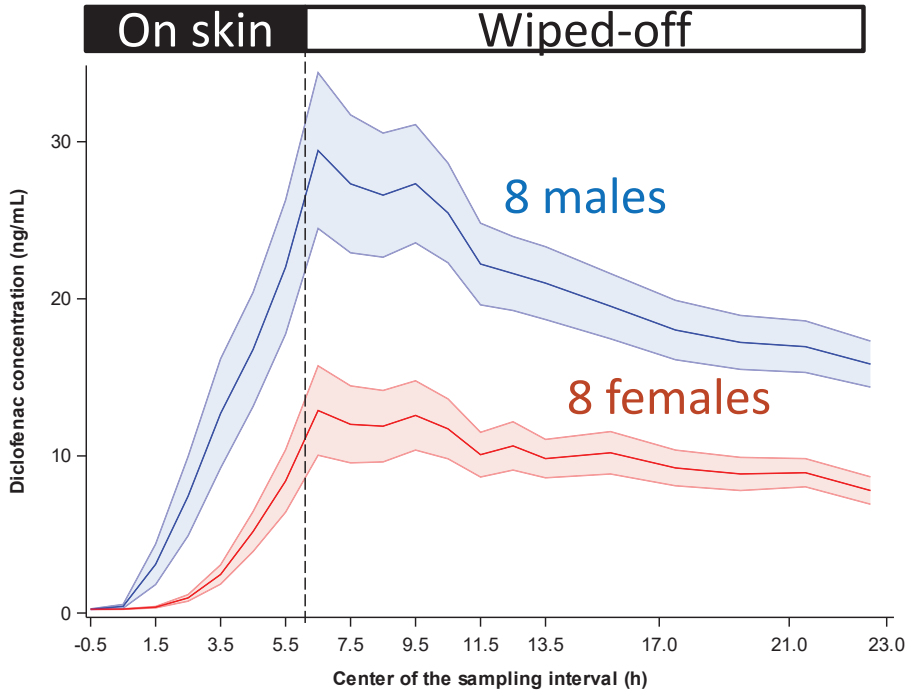
Reference product – lateral site



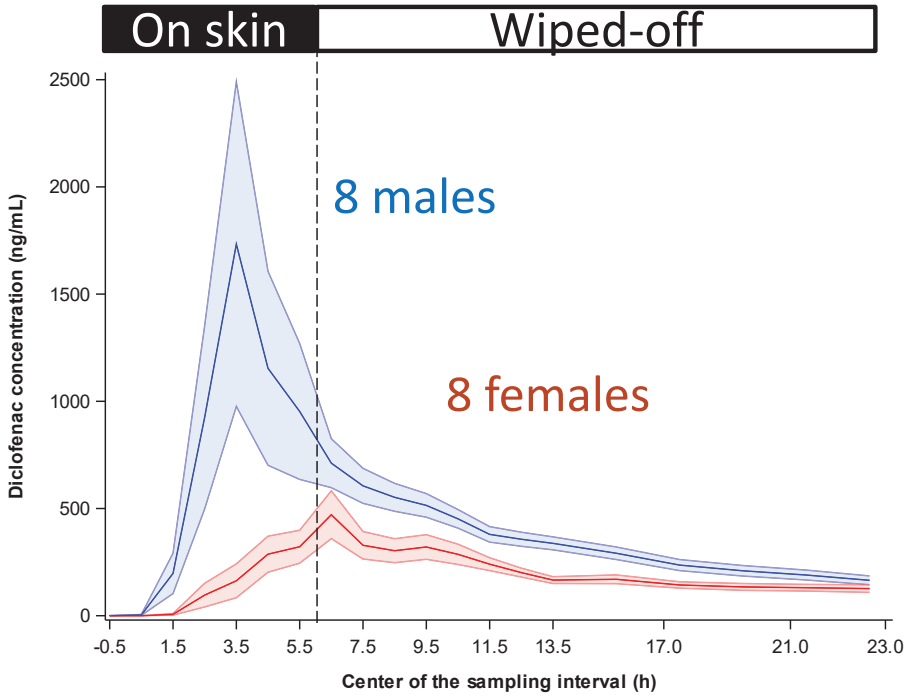
M≠F. Difference in the delivery phase. Effect of wipe-off similar.

Study 4_Diclofenac sodium 1% gel and 2% solution
20 mg/cm², non-occluded, removed at 6 hrs (8M+8F)

Generic 1% gel



Aggressive 2% solution incl. DMSO



M≠F. Differences appear to reflect differences in formulation & penetration routes.

For discussion …

- What is different between male and female skin?
  - Skin morphology & appendageal penetration?
  - Hydration, transepidermal water loss, sebum, microcirculation, pH?
    [e.g. Review by Rahrovan et al., Int J Womens Dermatol. 2018:
    "Male versus female skin: What dermatologists and cosmeticians should know"]
  - What else is different?
- Which of those can lead to the differences seen by dOFM?
- Can this sensitive tool be used to learn more about skin & formulations?