Assessing Bioavailability and Estimating Bioequivalence of Acyclovir Creams by In Vitro Permeation Tests with Excised Human Skin

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Purpose
Dermal pharmacokinetic approaches, including in vivo skin-stripping, in vivo dermal microdialysis and in vitro permeation tests (IVPT) with excised human skin can directly measure or may be predictive of human in vivo bioavailability for topical dermatological drug products. The purpose of the current study was to evaluate the utility of an IVPT method for comparing the bioavailability and estimating the bioequivalence (or lack thereof) of various 5% acyclovir creams.

Methods
IVPT experiments were performed using a flow-through in-line diffusion cell system. The initial screening tests involved U.S. Zovirax® cream as the reference product and U.K. Zovirax®, 1A Pharma® and Aciclostad® creams as test products – all containing 5% acyclovir. Subsequently, a pivotal study was conducted comparing the bioavailability of acyclovir from U.S. Zovirax® and U.K. Zovirax® creams on split-thickness ex vivo human skin obtained from 6 donors with a minimum of 4 replicates per treatment per donor. Following barrier integrity testing, an un-occluded finite dose of 15 mg of formulation per cm² was applied and receptor samples were collected every 4 hours up to 48 hours for analysis by a validated high performance liquid chromatography (HPLC) method.

Results
The 1A Pharma® and Aciclostad® creams exhibited lower flux and lower total permeation of acyclovir across the study duration compared to the U.S. and U.K. Zovirax® creams. For 1A Pharma® and Aciclostad® creams, only a few samples contained detectable peaks for acyclovir despite a highly sensitive HPLC method (LLOQ: 5 ng/mL). By contrast, the arithmetic mean (± SE) maximum fluxes (Jmax) across 6 donors were 0.094 (± 0.02) and 0.051 (± 0.02) µg/cm²/h for U.S. Zovirax® (reference) and U.K. Zovirax® (test) creams, respectively. The arithmetic mean (± SE) cumulative amounts of acyclovir permeated over 48 hours were 2.36 (± 0.83) and 1.45 (± 1.04) µg/cm² for U.S. Zovirax® and U.K. Zovirax®, respectively. The geometric mean ratio for the test/reference was 0.520 for Jmax and 0.548 for the total amount of acyclovir permeated. The 90% confidence interval for the ratio of means was 0.38 – 0.72 for Jmax and 0.43 – 0.69 for the total amount of acyclovir permeated. For both PK-parameters, the within-reference variability was high (exceeding 0.294), supporting the use of a scaling approach for estimating BE. According to this approach, the two products were determined to be bio-inferior based upon both PK-parameters. The same statistical method accurately reached a conclusion of bioequivalence between two sets of triplicate skin sections dosed with the same product, for both the U.S. and the U.K. Zovirax® creams.
Additionally, power simulations showed that under certain conditions (i.e., an equivalence interval of 0.75-1.33), such studies can attain statistical power of at least 80% with a minimum of 12 donors in the case of Jmax, and a minimum of 6 donors in the case of the total amount permeated.

Conclusion
The current study demonstrated that IVPT is a potentially sensitive and discriminating method, able to detect differences in the bioavailability of acyclovir from Zovirax® creams sold in the U.S. and U.K. markets. It is necessary to compare the current in vitro results with the clinical bioavailability or bioequivalence of acyclovir from these acyclovir creams to evaluate whether the current results are predictive of human in vivo bioavailability.

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