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

Soo Hyeon Shin[1], Sam G. Raney[2], Elena Rantou[3], Bryan Newman[2], Priyanka Ghosh[2], Hazem E. Hassan[1], Audra L. Stinchcomb[1]

[1]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD
[2]Office of Generic Drugs, CDER, US FDA, Silver Spring, MD [3]Office of Biostatistics, OTC, CDER, US FDA, Silver Spring, MD

Introduction

There are many potential approaches to assess bioavailability (BA) and estimate bioequivalence (BE) of topical dermatological drug products, including *in vivo* vasoconstriction assay, *in vitro/in vivo* skin-stripping, dermal microdialysis and *in vitro* permeation tests (IVPT). These methods can either directly measure or be predictive of BA of topical dermatological drug products *in vivo.* However, except for the vasoconstriction assay which is constrained to glucocorticoids only, no single method and protocol have been agreed upon for evaluating BA and BE for topical dermatological drug products. This lack of established alternative BE methods leads to costly clinical trials being the dominant route for approval of generic topical drug products.

The purpose of the current study was to evaluate the utility of the IVPT for comparing BA and estimating BE (or lack thereof) of various 5% acyclovir creams. The IVPT method with excised human skin sections has been widely used to study percutaneous absorption and there are numerous studies demonstrating the correlation of IVPT data to *in vivo* BA of topically administered drug products. While the method does not directly measure the concentrations of drug within skin layers, the method can be reflective of the rate and extent to which the drug becomes available at or near the site of action, representing a pharmacokinetic approach to evaluate BA and BE of topical drug products. In addition, the IVPT method is generally regarded as more sensitive than the other methods, warranting the need to evaluate IVPT as a useful method in determining BA and BE for topical dermatological drug products.

Methods

In Vitro Permeation Tests

A PermeGear® flow-through In-line diffusion system was used with dermatomed *ex vivo* human abdominal skin with a thickness of 240 ± 60 µm. The receiver solution was isotonic potassium phosphate buffer (pH 7.4) with 0.005% gentamicin. The flow rate was ~0.2 mL/h. The skin barrier integrity was tested by transepidermal water loss (TEWL) measurements prior to dosing. The experiments were performed for 48h with continuous sampling every 4h. A single dose of 15 mg/cm² of formulation was applied at time 0, using a positive displacement pipette (Fig 1). The resulting receiver solution samples were quantified by a validated high performance liquid chromatography (HPLC) method.

Screened and Tested Acyclovir Creams

Four acyclovir creams (Table 1) all containing 5% acyclovir were screened by performing IVPT. Among these four products, two products were chosen to conduct a pivotal study using split-thickness *ex vivo* human skin obtained from 6 donors with 4-7 replicates per donor.

Table 1. Inactive ingredients for the screened acyclovir products

Product	Inactive Ingredients	
Reference	• Cetostearyl alcohol • Mineral oil • Poloxamer 407 • Propylene glycol	• Sodium lauryl sulfate • Water • White petrolatum
Test	• Dimethicone • Propylene glycol • Poloxamer 407 • Cetostearyl alcohol • Sodium lauryl sulfate	• White soft paraffin • Liquid paraffin • Arlacel 165 (glycerol monostearate, macrogol stearate 100) • Purified water
Product A	• Glycerol monostearate • Polyoxyethylene stearate • Dimethicon • Cetyl alcohol	• White Vaseline • Liquid paraffin • Propylene glycol • Purified water
Product B	• Macrogol stearate • Dimethicon • Cetyl alcohol • Liquid paraffin	• White Vaseline • Propylene glycol • Purified water

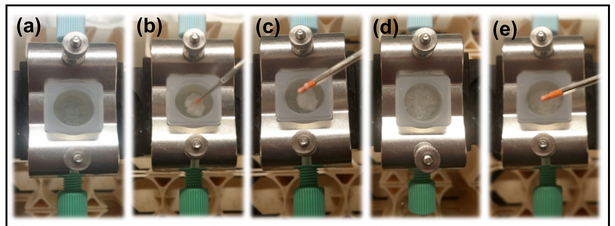

Figure 1. Dose administration using a positive displacement pipette. **(a)** Skin mounted on a diffusion cell, prior to dosing **(b)** Formulation being dispensed **(c)** Exposed Teflon tip for spreading formulation **(d)** Skin surface after spreading formulation **(e)** Minor loss of formulation to the Teflon tip after spreading

Statistical Analysis/ Power Analysis

In BA comparisons, the within-reference standard deviation (S_{WR}) was evaluated. Values of S_{WR} greater than the cutoff point of 0.294 indicated the use of a scaled average bioequivalence (SABE) approach. A determination was made by observing the upper limit of the 90% confidence interval for $(\mu_T - \mu_R)^2 - \theta * \sigma_{WR}^2$, where $\theta = \frac{(\ln \Delta)^2}{\sigma_{WR}^2}$ and Δ is the value of the designated equivalence limit (either 1.25 or 1.33). The statistical power analyses were performed using 500,000 simulations.

Results

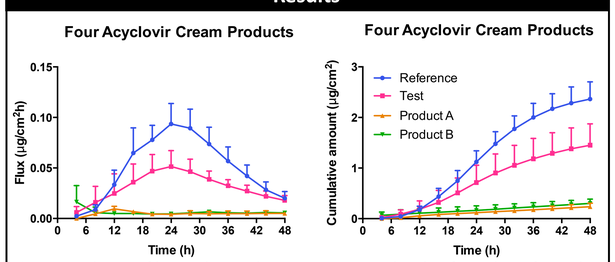

Figure 2. Flux profiles and cumulative permeation levels of acyclovir from the four acyclovir formulations. (Mean ± SE, n= 6 donors with 4-7 replicates per donor for Reference and Test products and n = 2 donors with 3-4 replicates per donor for Products A and B)

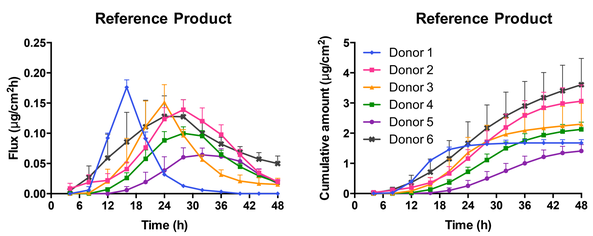

Figure 3. Flux profiles and cumulative permeation levels of acyclovir from Reference product. (Mean ± SE, n= 4-7 replicates per donor)

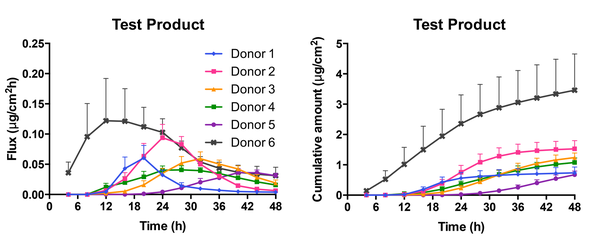

Figure 4. Flux profiles and cumulative permeation levels of acyclovir from Test product. (Mean ± SE, n= 4-7 replicates per donor)

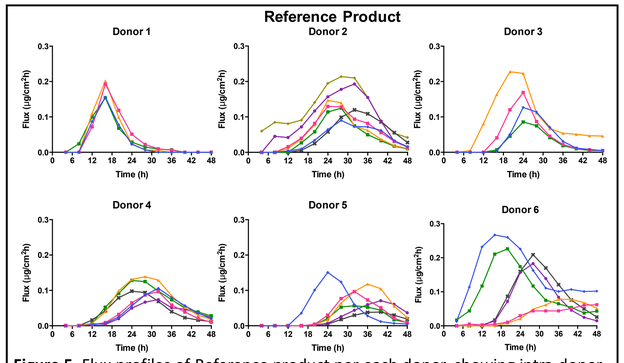

Figure 5. Flux profiles of Reference product per each donor, showing intra-donor variability.

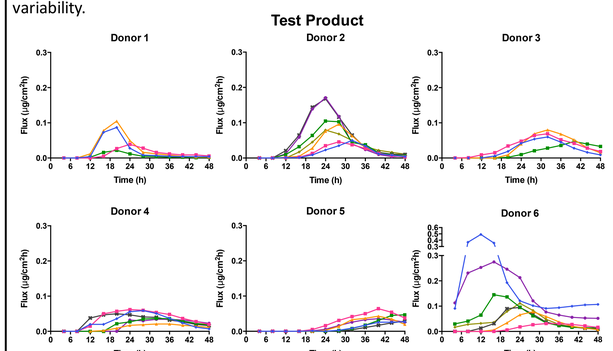

Figure 6. Flux profiles of Test product per each donor, showing intra-donor variability.

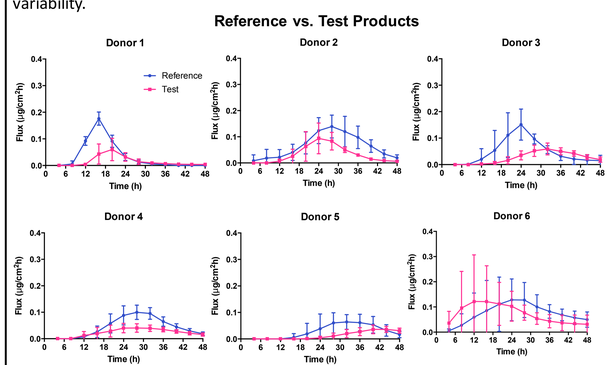

Figure 7. Flux profiles of Reference and Test products per each donor. (Mean ± SD, n= 4-7 replicates per product)

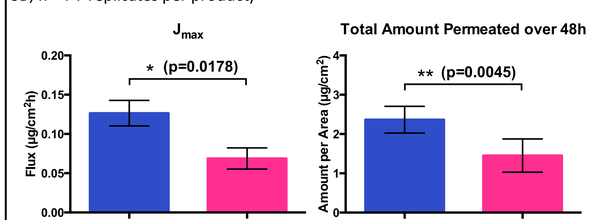

Figure 8. Comparisons of J_{max} and the total amount of acyclovir permeated over 48h between Reference and Test products (Mean ± SE, n= 6 donors with 4-7 replicates per donor)

Table 2. BA/BE comparisons of Test (T) and Reference (R) products

Product Comparison	IVPT PK Parameter	Point Estimate (GMR)	Sigma (WR)	SABE* [0.80,1.25]	SABE* [0.75,1.33]
(T_1) - (R_1)	Total AUC	0.5314	0.4457	**0.5957**	**0.4993**
	J_{max}	0.4926	0.4238	**0.9859**	**0.8950**
(R_2) - (R_1)	Total AUC	0.9439	0.5032	**-0.0864**	**-0.1629**
	J_{max}	0.8339	0.7618	**-0.0326**	**-0.2459**
(T_2) - (T_1)	Total AUC	0.9766	0.7132	**-0.1894**	**-0.3409**
	J_{max}	0.9966	0.7902	**-0.2244**	**-0.3768**

* Values > 0 fail the SABE test for comparable BA within the indicated margins, e.g. [0.80, 1.25]

In all three cases of BA comparisons, the within-reference standard deviation is >0.294, indicating the use of a scaling approach for comparing BA. According to the SABE approach, the T – R product comparison falls outside [0.8, 1.25] limits for both AUC and J_{max}. The GMR is outside the bounds [0.75, 1.33] and the upper bound of the confidence interval is greater than zero. According to the same statistical test, both T and R products are accurately found to exhibit comparable BA to themselves. This holds true even with the stricter limits of [0.80, 1.25].

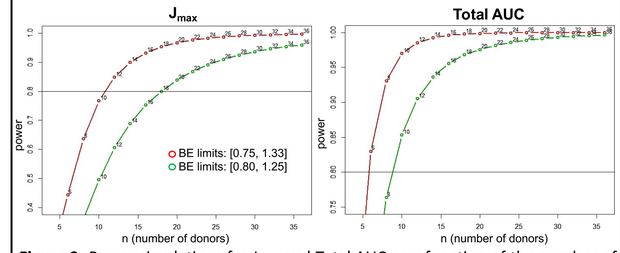

Figure 9. Power simulations for J_{max} and Total AUC as a function of the number of donors (n). When using the wider limit of [0.75, 1.33], statistical power of 80% can be achieved with a sample size of 12 donors for J_{max} and 6 donors for Total AUC. For the tighter limit of [0.80, 1.25], a sample size of 18 donors for J_{max} and 10 donors for Total AUC would be required to achieve the same level of power.

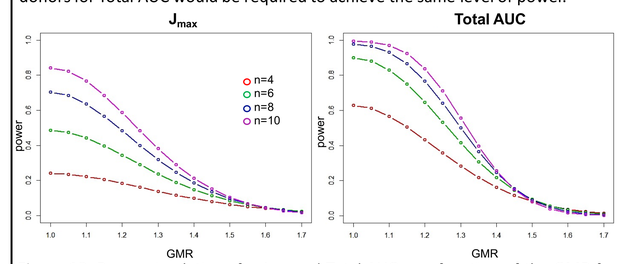

Figure 10. Power simulations for J_{max} and Total AUC as a function of the GMR for different sample sizes (numbers of donors). For values of the GMR inside the limit of [0.75, 1.33], a power of at least 80% can be achieved with a sample size of 10 or more donors for J_{max} and 6 or more donors for Total AUC.

Conclusions

The current study demonstrates that IVPT is a potentially sensitive and discriminating method for determining BA and estimating BE of acyclovir (5%) creams containing different inactive ingredients. Despite the relatively high intra- and inter-donor variability, the IVPT method was able to discriminate the Reference and Test acyclovir products, based on J_{max} and the total amount of acyclovir permeated over 48h. Additionally, power simulations indicated that under certain conditions (e.g. a margin of 0.75-1.33), IVPT studies with a minimum of 12 donors can attain a statistical power of at least 80% for both dermal PK parameters of J_{max} and total AUC. A comparison of these *in vitro* results with the clinical BA or BE of acyclovir from the same creams would help to evaluate whether these results are predictive of human *in vivo* bioavailability.

Acknowledgment

Funding for this project was made possible, in part, by the Food and Drug Administration through grant 1U01FD004947. The views expressed in this poster do not reflect the official policies of the U.S. Food and Drug Administration or the U.S. Department of Health and Human Services; nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.