Author's response to reviews

**Title:** Evaluation of the in vitro skin permeation of antiviral drugs from penciclovir 1% cream and acyclovir 5% cream used to treat herpes simplex virus infection

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**Version:** 3  **Date:** 9 January 2009

**Author's response to reviews:** see over
Title: MS: 8370183982091332
Evaluation of the in vitro skin permeation of antiviral drugs from penciclovir 1% cream and acyclovir 5% cream used to treat herpes simplex virus infection

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Version: 3 Date: January 9, 2009

Author’s response to reviews

To: Scott Edmunds PhD
Senior Editor
BMC-series Journals

Dear Dr Edmunds,

Please find below our answers to reviewers, who enabled us to increase the clarity of this manuscript. In addition, Figure 1C was added to depict the results as percentage of the applied dose.

We hope that our changes will meet with you and with reviewer’s expectations.

We greatly appreciate your cooperation and we are looking forward to hearing from you very soon.

With best regards,

Nathalie Hasler-Nguyen, PhD
REFEREE 1  
Version: 2 Date: 22 december 2008  
Reviewer: Charles M Heard

Reviewer's report:  
The revised manuscript has addressed most of my concerns. I am particularly pleased with the western blot analysis of keratin 5 ('keratin 5' needs to be added to Figure legend).  
Answer : Legend of figure 4 was completed.  

I still feel the molecular modelling section adds little to the paper - certainly, the significance of the images needs to be explained better.  
Answer : The molecular modeling enabled to generate and display the molecular surface properties such as electrostatic potential, lipophilicity potential or polar surface revealing parts of the molecule which are involved in hydrophobic or electrostatic interactions and which may cause bioavailability difference. Our findings show two additional hydrophobic moieties in the acyclovir molecule, which are captured for the first time in the present study. Although both molecules are very similar these additional hydrophobic moieties might interact within the lipophilic stratum corneum and might decrease the paracellular passage of acyclovir. This is discussed under discussion.  

I would like to see some discussion of the statistics in-text.  
Answer : levele of significant diffrenece was added under statistical analysis.  
Statistics of Table 1 were discussed under Results.  

REFEREE 2  
Version: 2 Date: 17 December 2008  
Reviewer: John J Docherty

Reviewer's report:  
I have read the authors response to my questions and now have a significant problem with this paper as written. The authors state that their skin samples are not viable. The relevance of their study to the penetration of drug in viable tissue is questionable but not discussed by the authors. What relationship do their results in non-viable tissue have to that of viable tissue?  
Answer: The scope of the paper was to compare the passive diffusion of two hydrophilic molecules from similar formulation through the excised skin which was frozen before use. The freezing step has been reported to result in non viable tissue lacking metabolism and enzymatic activities [31].  

Based on the comment made in the first review by the reviewer asking if the concentration differences that were found between the two drugs at the different skin layers might be simply due to the different intracellular half lives of acyclovir (0.7-1 h.) and penciclovir (10-20 h.), we clarified in the last paragraph under discussion that enzymatic activity does not happen in the excised skin tissue which was frozen before use. Thus, this might exclude the drug to be phosphorylated and retained in
the cells under these experimental conditions. However it would be of interest in future experiments to use fresh excised viable skin infected or not with the HSV-1 to evaluate the contribution of the viral enzymatic activity on the drug bioavailability into the deeper epidermal skin layers, where target cells are present.

For example in the second paragraph of their discussion the authors talk about the classic episode of herpes labialis causing erythema, edema, ulcer, etc. None of this can happen in their model and is misleading to the reader. Theirs is a passive model with none of the inflammatory features or enzymatic activity of viable tissue and it is the authors responsibility to clearly enunciate this in their discussion.

Answer: Paragraph 2 was removed. Extrapolation to in use condition was discussed under conclusion.