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: 2 Date: 13 November 2008

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: 2 Date: November 13, 2008

Author’s response to reviews

To: Scott Edmunds PhD
Senior Editor
BMC-series Journals

Dear Dr Edmunds,

According to the referee’s comments data were subjected to statistical analysis and we evaluated if basal cells were present in tape strips to support evidence that both drugs reach the target cells. As further experiments were performed, M. Bader was added in the author list as well as an acknowledgement part and a Figure 4 complete this new version.

Please find below our answers to reviewers.

We hope that our changes meet with you and the reviewer’s satisfaction.

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: 1 Date: 10 July 2008
Reviewer: Charles M Heard
Reviewer's report:

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)
The authors correctly state that “HSV-1 mainly infects cells in the basal layer..” and that “..antiviral drugs should therefore be able to reach therapeutic concentrations in these cells”. However, the tape stripping procedure involved the taking of only 12 strips, which means that only material from the stratum corneum would have been removed.
Answer: it has been reported that the stratum corneum peels readily from the dermis including all remaining epidermal layers if tape stripping is done 3 h after the product application [see references 21;25]. This is discussed in the discussion.

Therefore, absolutely no evidence is provided to suggest drug has penetrated to the site of action. This must be repeated with further strips that can be used to demonstrate, eg assaying for appropriate keratin markers, the basal cell has been reached.
Answer: A western blot analysis of keratin 5, which is a marker of basal cells was undertaken for all tape strips. Keratin 5 was found in the pooled strips mostly supporting the evidence that the drug could reach the basal epidermal layer. These results were added in Figure 4.

The data must be subjected to the appropriate statistical analysis.
Answer: statistical P value were added in tables 1

The section on molecular modelling proves nothing and should be removed from the paper.
Answer: Molecular modeling gives a new insight on both drugs and should be kept.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
The authors correctly used in-use simulation for applying the test formulations. However, the manner in which this application was made must be stated. The correct procedure would be to use a number of circular massaging actions. Also, they need to state exactly how they successfully and reproducibly administered 5 mg/cm2 to each cell.
Answer: 5 mg/ cm² could be added using a piston syringe, which tip was impregnated with the cream and weighted. Then this system enabled to slightly rub the cream on the surface of the skin. Finally The amount added corresponded to the weight difference of the piston syringe before and after the cream application. The repeatability of the applied dose method showed variation coefficients to be less than 5%.
Must state solubility of drugs in the receptor phase

*Answer:*

Must state the diluent used to prepare calibration curves, ie this must be receptor phase, to avoid solvatochromic artefacts.

*Answer: calibration curve was prepared in phosphate buffer saline. This was added under method.*

**Discretionary Revisions** (which are recommendations for improvement but which the author can choose to ignore)

“It is well established that the outer skin layer.....is the main barrier to skin permeation...”. This is an outdated view and there is increasing evidence that for lipophilic permeants, the viable epidermis is the main barrier.

*Answer: acyclovir and penciclovir are hydrophilic drug, hence the stratum corneum by being lipophilic is the main barrier for these type of drug.*

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**REFEREE 2**

**Version: 1 Date: 16 July 2008**

**Reviewer: John J Docherty**

**Reviewer's report:**

Overview: This paper compares the skin penetrating differences between 5% acyclovir and 1% penciclovir. Using excised human abdominal skin, Franz diffusion cells, adhesive tape stripping of the skin and HPLC the authors conclude that the cream formulation of penciclovir is better at diffusing to deeper layers of the skin than the acyclovir cream preparation.

Reviewers Summary: This paper is an interesting study of the skin penetrating properties of acyclovir and penciclovir and may help explain why penciclovir is more effective at treating cold sores than acyclovir. The studies are appropriately done, well explained and easily understood. The paper is acceptable for publication after appropriate responses to the points below:

**Specific Points:**

1. P. 5 - “Test of Skin Integrity” - please briefly state the results of the study here (not in Results).

*Answer: test of skin integrity was completed under method.*

2. P. 8 - “Discussion” – The authors refer to Figure 4. There was no Figure 4 in the manuscript that I received.

*Answer: Now there is a new figure 4 (Western blot analysis).*

3. P.8 - 10 - “Discussion”-The authors attempt to explain their results by discussing the hydrophobic and hydrophilic differences between acyclovir and penciclovir. The question that this reviewer has is as follows: Could the concentration differences that the authors found between the two drugs at the different skin layers be simply due to the different intracellular half lives of acyclovir (0.7-1 hr.) and penciclovir (10-20 hrs.)? This is especially relevant when considering that the only samples collected were taken 24 hours after the application of the drugs to the excised skin. Taking the samples at 24 hours after
drug application would clearly favor finding penciclovir in the various skin layers because of its longer half life rather than acyclovir. This possibility should be addressed in the “Discussion” of the paper.

Answer: As the skin used is not viable and enzymatic activity allowing to phosphorylated both drugs in order to keep them intracellularly might not be active, it would not be relevant to add this point in the conclusion under these experimental conditions.