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Are all aciclovir cream formulations bioequivalent?

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Abstract

Topical aciclovir cream (ACV, Zovirax Cream) containing 40% propylene glycol (PG), the optimum found for skin penetration, is clinically effective in the treatment of recurrent herpes labialis. One hundred and thirty-nine ACV generic creams were analysed and 80% of these contained less than 20% PG. From this, we hypothesised that these generics might be bioinequivalent to the innovator cream. A pilot in vitro skin permeation study compared the innovator cream with two generics containing about 15% PG. Next, 10 generics containing 0–15% PG were tested in an independent laboratory. Finally, a PG dose-ranging study was conducted in Zovirax cream base. In all studies, human skin was used and ACV analysed by LC-MS-MS. In the pilot study, the innovator cream delivered 7.5-fold more ACV than the two generics. Superiority was confirmed in the second study against all 10 ACV generic creams. By grouping the creams according to PG content, a relationship to ACV skin permeation was suggested. The PG dose effect was confirmed in the third study. These studies suggest that not all marketed ACV creams are bioequivalent to the clinically proven innovator. Given the magnitude of the differences seen, there is concern over therapeutic inequivalence of generic ACV creams to the innovator cream.

Keywords: Bioequivalence; Aciclovir; Propylene glycol; Cream; Topical; Generics

1. Introduction

Aciclovir is a nucleoside analogue indicated for the treatment of HSV-1 (cold sore) and HSV-2 (genital herpes) infections and can be administered topically as well as orally. Topical aciclovir cream, Zovirax® cream (ZOV), has been shown to be effective in the treatment of cold sores (Fiddian and Ivanyi, 1983; van Vloten et al., 1983) including one recent randomised double-blind, vehicle-controlled, multi-centre investigation in over 1300 patients (Spruance et al., 2002).

The bioavailability of the active ingredient in topical dermatological products is particularly sensitive to formulation being dependent upon the degree of satu-

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ration of the drug in the vehicle and the effect of vehicle excipients to increase drug solubility in and/or diffusivity through the stratum corneum, the outer barrier layer of the skin (Lippold and Schneemann, 1984; Williams and Barry, 1991; Megrab et al., 1995). Because of this, 2- to 5-fold or even greater differences in bioavailability of topical formulations containing the same dose of the same active ingredient are not uncommon (Lippold and Schneemann, 1984; Williams and Barry, 1991; Megrab et al., 1995). Importantly, these two to five times differences are not confined to experimental formulations but are also seen within commercially available creams and ointments containing the same dose of the same active ingredient, for example, with topical corticosteroids (Stoughton, 1987, 1992) and topical ibuprofen formulations (Hadgraft et al., 2003). These findings of large differences in bioavailability raise the question of therapeutic equivalence within these formulations.

Early formulation development studies in rat and guinea pig showed the importance of the vehicle on aciclovir topical delivery and on the outcome of herpes infections in such models (Spruance et al., 1984; Mehta et al., 1997). Especially, it was found that there was a dose response to the amount of propylene glycol (PG) in the formulation with between 30 and 40% resulting in the highest skin penetration (Collins and Oliver, 1981; An-eX analytical services, 1999). Since 1993, a large number of generic aciclovir cream formulations have been launched. Following the analysis of 139 aciclovir generic creams, screened for PG content in the period 1993–2004, it was found that the large majority contained less than 20% PG (Breen and Collin, 2005). Based on the early finding of dose response to PG (Collins and Oliver, 1981; An-eX analytical services, 1999), it was hypothesised that a low PG content cream formulation might not be bioequivalent to ZOV containing 40% PG. To test this hypothesis, an in vitro percutaneous flux pilot study was conducted within GlaxoSmithKline comparing ZOV with two leading German generic aciclovir creams. This study was then followed by an external in vitro percutaneous flux study conducted in CTL-Syngenta comparing ZOV with 10 European generic aciclovir creams. Finally, to confirm the role of PG in the difference of permeation observed, a third study was conducted comparing ZOV (40% PG) to a modified ZOV cream containing only 15% PG.

2. Materials and methods

2.1. Aciclovir cream formulations assayed for propylene glycol content

One hundred and thirty-nine aciclovir creams from 37 different countries, including 21 European countries, were tested. These creams were purchased and tested over the period October 1993 to December 2004. It is possible that some of these creams, since their testing, may have been removed from the market or their formulations changed.

2.2. Propylene glycol content determination

Propylene glycol content in each formulation was analysed by gas chromatography by either of the following methods.

2.2.1. GC method no. 1

The PG content of aciclovir creams was determined by gas chromatography using a CP-Wax-52CB capillary column (25 m × 0.32 mm × 1.2 μm, Varian, UK). An Agilent 6890 gas chromatograph (Agilent, UK) was operated, using helium as carrier gas, under constant flow conditions (2 ml/min) at a temperature of typically or approximately 130 °C. Sample and standard solutions were prepared in water:THF (50:50) containing 2,3-butanediol as internal standard. Typically 0.2 μl of sample and standard are injected under split injection conditions (temperature 250 °C, split ratio 100:1). Flame ionisation detection at 250 °C was used.

2.2.2. GC method no. 2

The PG content of aciclovir creams was determined by gas chromatography–mass spectrometry using a DB5 capillary column (Agilent, UK). The gas chromatograph was operated, using helium as carrier gas, at 2 ml/min. The initial oven temperature was 80 °C held for 2 min, the temperature was raised at 10 °C/min to 150 °C. Sample and standard solutions were prepared in pyridine containing deuterated PG as internal standard. These solutions were derivatised by addition of *N*,*O*-bis(trimethylsilyl)acetamide and heating at 60 °C for 5 min. 0.2 μl of derivatised sample and standard solutions are injected under split injection conditions (split ratio 20:1). Selective ion monitoring of

m/*z* 211 and *m*/*z* 205 was used to detect the trimethylsilyl derivatives of deuterated and non-deuterated PG, respectively.

2.3. Aciclovir cream formulations used for the in vitro skin permeation studies

All aciclovir creams used in the pilot study and external study were commercial cream formulations from different European markets and all contained 5% aciclovir. Zovirax® cream [GlaxoSmithKline] (ZOV/Zovirax control) was used as the reference cream containing 40% PG.

The 10 aciclovir generics creams were: Aciclostad® [Germany, Stada Arzneimittel AG] (cream no. 1), Aciclovir Heumann™ [Germany, Heumann Pharma GmbH] (cream no. 2), Virucalm® [Switzerland, Inpharzam SA] (cream no. 3) Aviral® [Switzerland, Mepha Pharma AG] (cream no. 4), Virasorb™ [UK, Seton Healthcare Group Plc] (cream no. 5), Clearsore [UK, Auden McKenzie] (cream no. 6), Viralief™ [Ireland, Clonmel Healthcare Ltd.] (cream no. 7), Kendix® [France, EG Labo—Laboratoires EuroGenerics] (cream no. 8), Aciclovir Bayvit™ [Spain, Bayvit SA] (cream no. 9), Aciclovir Mabo™ [Spain, Mabo-Farma SA] (cream no. 10).

Zovirax is a registered trademark of the GlaxoSmithKline group of companies, Aciclostad is a registered trademark of Stada Arzneimittel AG, Aciclovir Heumann is a trademark of Heumann Pharma GmbH, Virucalm is a registered trademark of Inpharzam SA, Aviral is a registered trademark of Mepha Pharma AG, Virasorb is a trademark of Seton, Viralief is a trademark of Clonmel Healthcare Ltd, Kendix is a registered trademark of EG Labo—Laboratoires EuroGenerics, Aciclovir Bayvit is a trademark of Bayvit SA, Aciclovir Mabo is a trademark of Mabo-Farma SA.

2.4. Percutaneous flux study

For the pilot study, Bronaugh flow through type diffusion cells [Permegear—USA—In line Cells II], having an available diffusion area of 3.14 cm^2 and a receptor volume of 1.2 ml, were employed. These cells sat on a cell holder temperature controlled by circulating water heated at $37 \pm 1\,^{\circ}C$ leading to a measured temperature [measured with a spring loaded surface probe from Hannah Instrument, UK] of $32 \pm 1\,^{\circ}C$ on the skin surface. Dermatomed human skin (stored at $-20\,^{\circ}C$ after collection until used), from the back, from a single donor (Male, 61) was used. Cut skin samples were placed in the diffusion cells sitting on a high flow filter paper (Whatman-541). The receptor phase, phosphate buffer saline [pH 7.4] (Sigma), was pumped at a rate of 1.5 ml per hour. Prior to application of the creams, the diffusion cells were left to equilibrate for 2 h. Three aciclovir creams, ZOV and two leading German generic aciclovir creams [ACV cream nos. 1 and 2] were used in this study. Six or seven replicates were used per formulation. Accurately weighed quantities of creams were applied to the surface of the skin and spread by means of a small bent metal spatula. The dose of cream applied, approximately 20 mg/cm^2 accurately weighed, was selected based on clinical usage of cold sore cream medication. The cells were left unoccluded. Receptor samples were taken at 3, 7, 12, 18, and 24 h time points. In parallel to the tested creams, in order to assess the integrity of the skin sample used, one control solution (5 mg/ml caffeine [Sigma]) was applied on the same skin sample, under infinite conditions and occlusion on separated flow through diffusion cells [Permegear—USA—In line Cells I—0.64 cm2].

For the external CTL-Syngenta study, static diffusion cells having an available diffusion area of 2.5 cm^2 and a receptor volume of 4.5 ml were employed. The receptor chambers were filled with physiological saline (0.85% sodium chloride in water) and the cells placed in a water-bath maintained at $32 \pm 1\,^{\circ}C$. Dermatomed human skin (stored at $-20\,^{\circ}C$ after collection until used), from five different donors was used. The integrity of each skin membrane was assessed by measurement of electrical resistance across the membrane. Membranes with a resistance $<10\,k\Omega$ were regarded as lower than normal and discarded. Prior to application of the creams, the diffusion cells were left to equilibrate overnight. Eleven aciclovir creams, ZOV and 10 leading generic aciclovir creams from different European markets [ACV cream nos. 1–10] were used in this study with 9 or 10 replicates per formulation. Accurately weighed quantities of creams were applied to the surface of the skin at a dose of approximately 20 mg/cm^2. The cells were left unoccluded. Receptor samples were taken at 3, 6, 12, 18, and 24 h time points. As in the pilot study, one control solution

(5 mg/ml caffeine) was tested, under infinite conditions and occlusion in the same type of static diffusion cells.

For the PG dose-ranging study, the same protocol as for the pilot study was used. Two aciclovir creams, ZOV (with 40% PG) and a modified ZOV (with 15% PG) were used in this study. The modified ZOV cream is not available commercially and was made by substituting 25% PG by 25% water while keeping constant all the other excipients. Ten replicates were used per formulation. Receptor samples were taken at 6, 12, 18, and 24 h time points.

2.5. Receptor samples analysis

For the three studies, aciclovir was analysed by LC-MS-MS.

In the pilot study and PG dose ranging study, aciclovir receptor samples were first concentrated via Solid Phase Extraction (SPE). Strata Phenyl cartridges—500 mg/3 ml—[Phenomenex, UK] and a Rapid Trace Work Station [Zymark, UK] were used to perform the SPE. The SPE method was as followed: (1) conditioning of SPE cartridge with methanol followed by water; (2) loading of 3 ml of receptor sample; (3) rinsing with 3 ml of water and 1.5 ml of formic acid; (4) eluting with 1.5 ml methanol which was then evaporated and reconstituted with 0.75 ml of the LC-MS-MS eluent (80/20, 5 mM ammonium formate/methanol). The chromatography was performed on a HTS PAL autosampler [CTC, Switzerland] coupled with an Agilent low pressure pump 1100 series, by injecting 100 μl of the SPE sample onto a 150 mm × 2.0 mm Aqua-3 μ-C18 [Phenomenex, UK] HPLC column. The eluent was run isocratically at 0.3 ml/min into a Quattro LC [Micromass, UK] triple quadrupole mass spectrometer in positive ion electrospray mode. Aciclovir was monitored by multiple reaction monitoring (MRM) of 226 *m*/*z* to 152 *m*/*z*. Limit of quantification was 0.3 ng/ml.

In the external study, 10 μl of aciclovir receptor samples were injected directly into the LC-MS-MS system. The pump and autosampler were of same make and model as in the pilot study. The eluent was run at 1 ml/min with a gradient (95/5 water/methanol to 10/90 water/methanol) through a 100 mm × 4.6 mm-4 μm Synergi Hydro RP [Phenomenex,UK] column. The detection was performed using a Sciex API4000 [Applied Biosystem, UK] triple quadrupole mass spectrometer in negative ion electrospray mode. Aciclovir was monitored by multiple reaction monitoring (MRM) of 224 *m*/*z* to 150 *m*/*z*. Limit of quantification was 0.25 ng/ml.

Caffeine receptor samples were analysed by HPLC-UV and injected without sample preparation. The eluent was 50/50, methanol/water and the UV detection was 280 nm. In the pilot and PG dose-ranging study, the eluent flowed isocratically onto a 250 mm × 4.6 mm-ODS1-5 μm-Spherisorb [Waters, UK] column while in the external study it flowed onto a 150 mm × 4.6 mm-C18-5 μm-Altima [Alltech, UK] column.

2.6. Data analysis: diffusion cells permeability rejection criteria and statistics

One issue with in vitro human skin permeation studies is large variation, which was seen in all three studies. This is partly due to the amount of drug delivered through skin which is very small compared to the applied dose, especially for polar compounds such as ACV, and also because skin permeability is not normally distributed (Williams et al., 1992). For this reason, it is important to carefully define exclusion criteria for outliers.

Three rejection criteria were considered for each cell.

Criteria 1: 3-fold higher permeation than average cell permeation over 24 h: This demonstrates substantially higher permeability of the skin membrane.

Criteria 2: For the most permeable cell, permeation at any single time point is superior to 3-fold the permeation of the second next most permeable cell: This demonstrates substantially inhomogeneous set of permeability for a formulation as well as higher permeability of skin membrane.

Criteria 3: Permeation at first time point is superior to 50% of the permeation over 24 h: This demonstrates unusual profile (burst at first time point).

Data from “outlier cells” (only 0/3 or 1/3 criteria met) were rejected. Data from “equivocal cells” (2/3 criteria met) or where 3/3 criteria were met were included in the analysis.

Student *t*-tests were performed in the three studies to define the *p*-value of each formulation tested against ZOV.

Table 1
Summary of propylene glycol content of all generic aciclovir creams tested by GlaxoSmithKline in the period from October 1993 to December 2004

	Percent (w/w) propylene glycol	
	<20%	≥20%
Number of creams	111	28
Percentage of creams (%)	80	20

3. Results

Of the 139 generic aciclovir creams tested for PG content between October 1993 and December 2004, 80% (111 out of 139) contained less than 20% PG (Table 1). In Table 1, where there are two analytical test results for the same brand of cream obtained in two different years, results for both PG contents measured are given.

Of the 11 aciclovir creams tested in the pilot and external studies, 1 cream had 40% PG (ZOV), 9 generic creams contained approximately 15% PG (creams nos. 1–9) and 1 generic cream had no PG (cream no. 10) (Table 2).

In the pilot study (Fig. 1), aciclovir percutaneous delivery over 24 h was 6.5- and 8.5-fold superior from ZOV compared to the two other aciclovir creams (cream nos. 1 and 2, respectively). There were no equivocal cells (failing only one of the exclusion criteria) and two outlier cells (failing two or three of the exclusion criteria) among the total of 20 diffusion cells used in this study. The average flux over 24 h of the caffeine control cells was 1.4 μg/cm^2/h (Table 3).

Table 2
Propylene glycol content of each formulation

Cream no.	Percent (w/w) propylene glycol[a]
Zovirax	40
Cream no. 1	14.0 (2003 test)
	14.8 (2004 test)
Cream no. 2	13.7 (2003 test)
	15.0 (2004 test)
Cream no. 3	14.9 (2002 test)
	13.6 (2003 test)
Cream no. 4	15.1 (2002 test)
	15.2 (2004 test)
Cream no. 5	14.3 (2003 test)
Cream no. 6	14.9 (2002 test)
Cream no. 7	14.3 (2003 test)
	14.6 (2004 test)
Cream no. 8	14.8 (2002 test)
	15.0 (2004 test)
Cream no. 9	14.9 (2002 test)
	13.6 (2003 test)
Cream no. 10	None detected (2002 test)

[a] All tested with GC method no. 1.

In the external study (Figs. 2 and 3), aciclovir percutaneous permeation overall was lower than that observed in the pilot study. Skin permeation from ZOV was numerically superior to that from the 10 other creams. Cream nos. 8 and 10 delivered a very small amount of aciclovir compared to the other creams. The variation in the data set was higher than the variation observed in the data set of the pilot study. There were

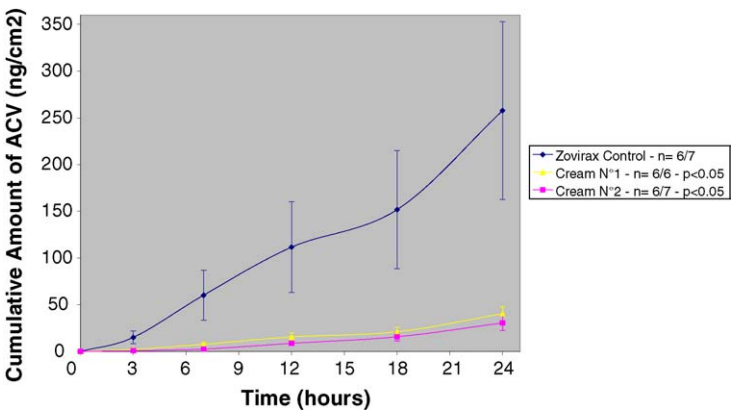

Fig. 1. Percutaneous permeation of aciclovir from three different creams. Pilot study results (standard error).

Table 3
Percutaneous flux of control solutions in pilot, external and PG dose ranging studies

Control solutions infinite dose—occluded	Flux ($\mu g/cm^2/h$) ($\pm$S.E.)
Caffeine (5 mg/ml)—pilot study ($n = 6$)	1.4 ($\pm$0.3)
Caffeine (5 mg/ml)—external study ($n = 12$)	0.48 ($\pm$0.07)
Caffeine (5 mg/ml)—PG dose ranging study ($n = 5$)	0.90 ($\pm$0.36)

6 equivocal cells and 10 outlier cells among the total of 109 diffusion cells used in this second study. The average flux over 24 h of the caffeine control cells was 0.48 $\mu g/cm^2/h$ (Table 3). By grouping the data from the external study on formulations containing approximately 15% PG, a dose response of PG content with aciclovir permeation is suggested. The 40% PG cream delivers 3 (5)-fold more aciclovir than 15% PG creams and 36 (24)-fold more aciclovir than a cream containing no PG (in parentheses: with equivocal cells) (Fig. 4).

In the PG dose-ranging study (Fig. 5), aciclovir percutaneous delivery over 24 h was 10-fold superior for ZOV (40% PG) compared to the modified ZOV cream (15% PG). The average flux over 24 h of the caffeine control cells was 0.9 $\mu g/cm^2/h$ (Table 3). There were 4 outlier cells among the total of 20 diffusion cells used in this study.

4. Discussion

This work compared the bioavailability of a clinically proven 40% propylene glycol aciclovir formulation, ZOV, with generic aciclovir formulations contain-

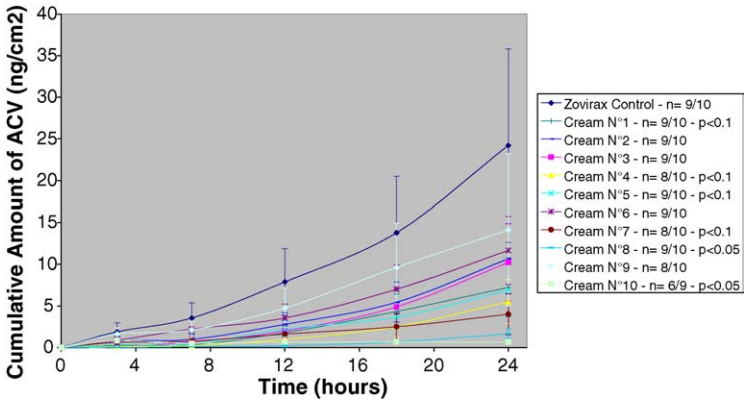

Fig. 2. Percutaneous permeation of aciclovir from 11 different creams. External study results. 3/3 exclusion criteria met (standard error).

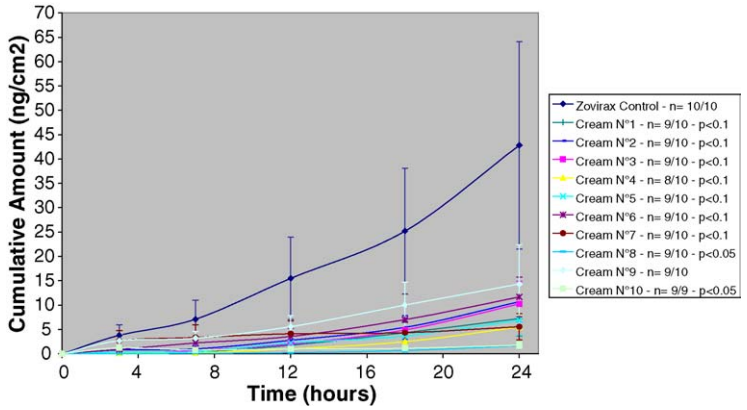

Fig. 3. Percutaneous permeation of aciclovir from 11 different creams. External study results. 2/3 exclusion criteria met (standard error).

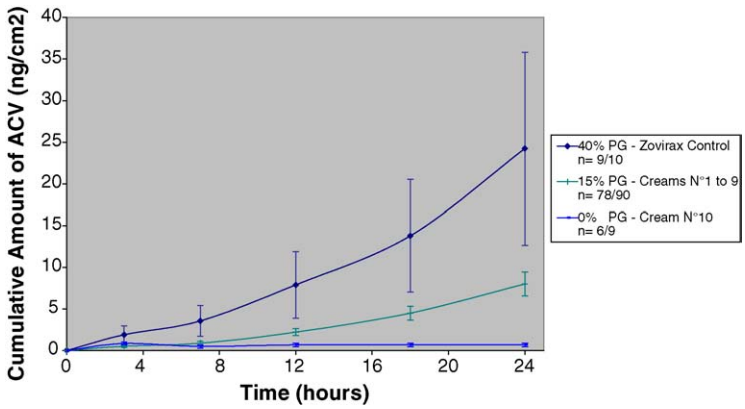

Fig. 4. Effect of propylene glycol content on the percutaneous permeation of aciclovir. 3/3 exclusion criteria met (standard error).

ing 15% PG or less. On the basis of an established dose response to PG it was hypothesised that these generic creams might be bioinequivalent to ZOV in which case, depending upon the magnitude of the differences, the issue of therapeutic inequivalence might also arise.

In the pilot study, ZOV cream containing 40% PG was shown to deliver 6.5 and 8.5 times more aciclovir than two German aciclovir generic creams containing 15% PG. In the external study, ZOV cream containing 40% PG was shown to be numerically superior to all European generic creams containing 15% PG or less. By grouping the creams by PG content, a dose response of PG content to aciclovir skin permeation is suggested. Depending on the method used for data treatment, the 40% PG cream appears to deliver from 3- to 5-fold more aciclovir than the 15% PG creams tested and from 24- to 36-fold more aciclovir than a cream containing no PG. However, it is clear from variation within the 15% PG group that propylene glycol content is not the only formulation variable which influences percutaneous absorption, and that other factors such as differences in other excipients or manufacturing process may also contribute. The last study, however, confirms the important role of PG when all other parameters such as excipients, excipient concentration, aciclovir powder characteristics or manufacturing process are kept constant. In this study, the 40% PG cream delivered 10-fold more aciclovir than the nearly identical formulation containing 15% PG only. A recent presentation has reported very similar findings (Diez-Sales et al., 2003).

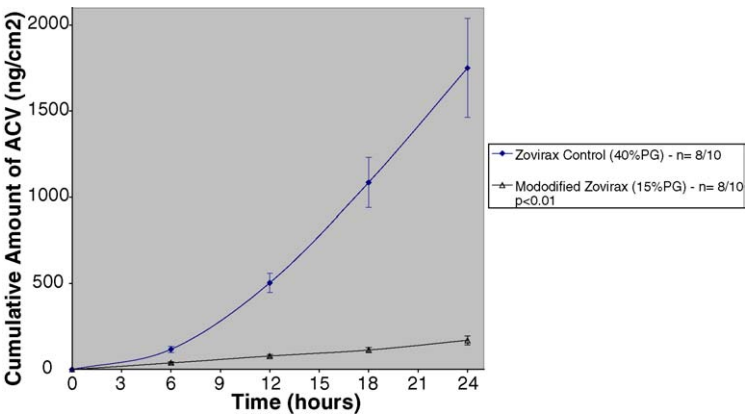

Fig. 5. Propylene glycol dose effect on the percutaneous permeation of aciclovir. 3/3 exclusion criteria met (standard error).

It is noteworthy that compared with limits for bioequivalence for oral dosage forms of ±20%, the differences in bioavailability seen in these three studies are large.

Despite much effort from scientists and regulators (Skelly et al., 1987; Neubert and Wohlrab, 1990; Howes et al., 1996; Surber and Davis, 2002), methods, protocols and standards to determine the bioequivalence of topical dermatological products have yet to be agreed internationally. However, two major reviews from the US and Europe have supported the use of in vitro human skin permeation studies to compare bioequivalence of topical dermatologicals and protocols have been developed which are the basis for that used in these present studies (Skelly et al., 1987; Howes et al., 1996).

These percutaneous studies were performed using an analytical cold assay: LC-MS-MS. This is an important improvement as in vitro skin permeation studies are often conducted using radiolabelled drug "spiked" into the formulation (Brain et al., 2002). Especially where formulation details are not known, this may lead to inhomogeneity between labelled and non-radiolabelled drug and an overestimate of skin permeation as well as inappropriate comparison between formulations.

In the generic registration process, Therapeutic equivalence is assumed on the basis of Pharmaceutical equivalence plus Bioequivalence in which case comparative clinical trials in patients to prove clinical benefit are not required. In this work, all the aciclovir creams compared contained 5% aciclovir in a cream base and thus were Pharmaceutically equivalent. However, given the magnitude of the differences in bioavailability observed, there must be concern over therapeutic inequivalence of these generic ACV creams to the innovator ZOV cream.

These findings with European formulations of the nucleoside analogue aciclovir are similar to the findings of bioinequivalence between innovator and generic topical corticosteroids in the US market (Stoughton, 1987, 1992) and topical ibuprofen on the European market (Hadgraft et al., 2003). FDA pioneered research that has since enabled a validated vasoconstrictor test to be available for bioequivalence testing of topical corticosteroids. However, for other drug classes there are as yet no mutually recognised bioequivalence methods and protocols. As a result we are currently left with two extreme options for generic registration. The first option is to conduct clinical trials which are prohibitively time consuming and expensive. The second approach focuses on pharmaceutical quality, e.g. drug content, release studies, which are relatively easy studies to conduct. However, the literature reports a lack of correlation between release studies and percutaneous permeation/pharmacodynamic response (Malzfeldt et al., 1989; Montenegro et al., 1996; Schwarb et al., 1997; Arellano et al., 1998; Hadgraft and Wolf, 1998; Arellano et al., 1999; Dayal et al., 2002). Neither approach is satisfactory. Further research work on topical bioequivalence methods and protocols is required to ensure that therapeutic standards are maintained within the economic realities of modern healthcare services.

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