

***In Vitro* Evaluation of Percutaneous Absorption of an Acyclovir Product using Intact and Tape-stripped Human Skin**

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ABSTRACT Purpose. To evaluate the use of a flow-through diffusion cell system to assess the absorption and penetration characteristics of drug (acyclovir) products. **Methods.** *In vitro* studies were performed to assess the absorption/penetration of acyclovir using a flow-through diffusion cell system with human skin sections obtained from 19 healthy women following mammoplasty. The skin sections, 200-400 μm thick, were prepared using a dermatome. Acyclovir ointment (~10 mg) spiked with ^3H -labelled acyclovir was applied onto the stratum corneum/epidermis side. The skin sections were continually perfused on the dermis side with sterilized culture medium, Buffered Hanks' Balanced Salt Solution, saturated with a CO_2/O_2 (5/95%).

Results. After 24 hours, the percentages of acyclovir-derived radioactivity (based on dose applied) in different components were as follows: stratum corneum (SC), 0.20 ± 0.28 ; viable skin (VS), 0.40 ± 0.38 ; effluent fluid (EF), 0.25 ± 0.53 . A second set of experiments was performed using tape stripped (10X) skin sections. Levels of acyclovir-derived radioactivity were VS, 1.21 ± 1.43 and EF, 2.65 ± 2.61 , which were threefold higher ($p < 0.05$) for VS and elevenfold higher ($p < 0.05$) for EF compared to the

results obtained with the intact skin sections.

Conclusions. The SC is the main barrier layer for the penetration of acyclovir through human skin. The use of the flow-through diffusion cell system provides an appropriate *in vitro* model to assess the absorption/penetration of acyclovir through human skin layers and therefore can potentially be used for dermal formulation characterization and development.

INTRODUCTION

Acyclovir {9-[(2-hydroxyethoxy)methyl]guanine} is a synthetic purine nucleoside analog derived from guanine. It differs structurally from guanine by the presence of an acyclic side chain (1). The activity of acyclovir is directed primarily against the herpes group of DNA viruses (2-3). Susceptible members of the herpes virus family include herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella-zoster, Epstein-Barr virus, and murine cytomegalovirus (4-7). The unique feature of acyclovir is its selectivity as a substrate for virus-specified thymidine kinase (8-10). Since acyclovir is selectively converted to its active form in herpes virus-infected cells, it is not toxic to normal, uninfected cells (11-14). Acyclovir is indicated for the treatment of infections caused by the herpes virus and is considered the safest and most efficacious for this purpose.

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Acyclovir can be administered orally or intravenously for treating herpes virus infections (15-16). Topical application of acyclovir in polyethylene glycol (PEG) is also a common practice, but has been shown to be less effective compared with oral or intravenous routes. Lack of effectiveness of topically applied acyclovir has been attributed to an inadequate delivery of the drug to the skin cells (17-19). Nevertheless, convincing evidence to conclude lack of absorption into the skin layer appears to be lacking.

Absorption of chemicals through skin can be characterized using both *in vivo* and *in vitro* methods. In this respect *in vitro* studies are generally conducted using a diffusion cell system with either static or a flow-through cell. A flow-through diffusion cell method, by virtue of continuous replenishment of perfusion medium, helps in maintaining the viability of the skin tissue thus would mimic better to a physiological environment than a static cell. In addition, by collecting effluent (perfusion medium) fractions it offers a convenient method to develop drug absorption and penetration profiles through skin over time. The use of a flow-through diffusion cell system has been described in the literature for the assessment of pharmacological and toxicological characteristics of chemicals (20).

This report describes an application of the flow-through diffusion system for the evaluation of absorption and penetration characteristics of acyclovir from an ointment product.

MATERIAL AND METHODS

Skin sections were prepared using an Electrodermatome, model B purchased from Padgett Instruments, Missouri, USA. The percutaneous absorption experiments were conducted using a flow-through diffusion cell system (Crown Glass, Inc. PA, USA). A 5% acyclovir ointment, in a polyethylene glycol (Burroughs Wellcome Co., Research Triangle Park, N.C. USA) was obtained from a local market. [³H]-acyclovir (specific activity: 35 mCi/mmol and concentration: 1000 µCi/ml) was obtained from ICN, CA, USA. The Liquid Scintillation Counter, System

1400TM was from Fisher Scientific, USA. All other chemicals and reagents were of analytical grade and were obtained from commercial sources.

Skin Sample Collection

Nineteen healthy women participated in the study by providing the skin tissue. All subjects gave written informed consent before participating in the trial. The subjects were 19 to 35 years of age (27 ± 5.6) and were judged to be healthy based on medical history and physical evaluation.

The skin specimens were collected from the hospital after plastic breast reduction surgery. The skin tissue was transferred into a container with the sterilised (HEPES buffered Hanks' Balanced Salt Solution, HHBSS) to maintain the tissue viability. To minimize the loss of viability of the skin tissue, experiments were initiated within 4 hours after the surgery.

Set-Up Of Flow-Through Diffusion Cell System

Water, maintained at 35^o, was circulated around the diffusion cell system to maintain a skin temperature of 32^o. The system, including perfusion medium reservoir, flow-through cells and connected tubing, was sterilized using 75% ethanol/water solution. Following the sterilization step, the remaining 75% alcohol was flushed out with sterilized water which was then replaced with HHBSS perfusion media maintaining a flow rate of 2 mL/hour, as described by Bronaugh *et al* (20).

Preparation Of Skin Tissues

The skin tissues for diffusion experiments were prepared by removing any muscle and fat tissues. The tissues were pinned to the surface of a styrofoam block (with stratum corneum side up) and a layer of 200 - 400 µm thickness was removed using a dermatome. The thin layered skin section was then cut into pieces of about 1.8-cm diameter using a cork-borer, which were then mounted onto the cells (with stratum corneum or epidermis exposed to environment). The lower chamber of flow-cell apparatus, which had a volume of approximately 150

μL , was continually replenished with sterilized perfusion medium (HHBSS), saturated with a CO_2/O_2 (5/95%) to perfuse the skin sections and to remove the penetrated drug. For the assessment of drug absorption with tape-stripped sections, prior to mounting the skin sections onto the cells, SC layers were removed by repeated (10X) tape stripping the sections.

Sample Application and Quantitation: Prior to each experiment sufficient quantity of acyclovir ointment was spiked with [^3H]-acyclovir and thoroughly mixed at room temperature. Consistency in mixing of the labelled compound was established by obtaining consistent radioactivity readings of serial samples.

An aliquot of acyclovir ointment (~10 mg) spiked with [^3H]-acyclovir (1.00 $\mu\text{Ci}/\mu\text{L}$), resulting in $\sim 5 \times 10^5$ counts (DPM), were applied on the stratum corneum/epidermis side of the skin sections. The tissues were perfused for 24 hours and the perfusion medium was collected in four fractions of six-hour each.

At the end of the incubation time, skin surface was washed with 1% soap solution followed by distilled water using Q-tip cotton swabs to remove unabsorbed applied ointment. All the Q-tip cotton swabs (three for soap wash and three for water wash) from the individual cells were combined in separate vials, and 10 mL of scintillation liquid was added to enable measurement of the radioactivity.

The skin sections were removed from the cell and were placed on a hard surface with the SC side up. The stratum corneum was removed by repeated stripping with cellophane tape (10X). All the tape pieces were collected in a scintillation vial mixed with scintillation fluid for measuring the radioactivity. For the experiments with the tape-stripped skin sections no further tape stripping was performed.

To solubilize the viable skin sections, i.e. sections without SC, for determining the radioactivity, the tissue was mixed with 3 mL of tissue solubilizing

solution (Soluene 350, Packard, USA) and was kept at room temperature for 24 hr. The mixture was then neutralized by adding 180 μL of acetic acid followed by mixing with 7 mL of LSC-Cocktail, which was used for scintillation counting to determine the amount of acyclovir in the tissue.

To measure the amount of penetrated drug through the skin, one mL of the perfusion fluid was mixed with 9 mL of scintillation liquid and monitored for radioactivities.

Data Analysis

The results were analyzed using the *Student t-test* (SAS software, SAS Institute, Cary, NC). In the description of results, "N" represents the numbers of human volunteers, and "n" represents the numbers of experimental repeats. A difference in results with a *p* value less than 0.05 was considered significant. Data are presented as mean \pm SD.

RESULTS

To minimize the variability in results due to tissue sites, only skin specimens from breast tissues were employed.

At the end of the experiments 60 to 85% of the total applied radioactivity was accounted for, however, only 0.85% of the applied dose was absorbed or penetrated through the skin. The distribution of absorbed acyclovir into different skin layers and the receptor fluid is given in Table 1.

These results are in agreement with the reported limited absorption characteristics of acyclovir both in human and animal studies (10, 19). Compared to intact skin section taped stripped sections resulted in higher ($p < 0.05$) drug levels in viable skin tissue (threefold) and into perfusion medium (elevenfold).

Table 1: Percent distribution of acyclovir, based on radioactivity levels, in different components of skin tissue and the perfusion medium

	Intact Skin (N=9, n = 32)	Tape-Stripped Skin (N=10, n=19)
Stratum Corneum	0.20 ± 0.28	N/A
Viable skin	0.40 ± 0.38	1.21 ± 1.43*
Perfusion Medium (Effluent)	0.25 ± 0.53	2.65 ± 2.61*

Values are the mean ± SD and are expressed as percentage of total applied acyclovir. All comparisons are based on *t*-test. "N" and "n" represents number of volunteers and repeats using tissue from the same volunteer, respectively.

*These values are significantly different at $p < 0.05$ when compared between groups (intact skin vs tape-stripped skin)

DISCUSSION

The topical application of acyclovir for the treatment of HSV skin infections has a long history. However, the topical application of acyclovir has proven clinically disappointing in the therapy of HSV skin infections compared with oral or intravenous administration. Many authors have speculated that the failure of topical acyclovir therapy is due to the inadequate drug delivery to the target site, that is, basal epidermis (18-19). Adequate amounts of drug delivery to skin basal epidermis are necessary for the treatment of HSV skin infections because major virus-induced epidermal pathology occurs in the basal epidermis (21). Previous studies have also demonstrated that a positive correlation exists between drug dose and antiviral efficacy of acyclovir in HSV diseases (21-22). The results of our study support such a hypothesis, since only minimal amounts of acyclovir were absorbed and penetrated through the skin layers following topical application of the drug to intact skin (10, 19). Therefore, it is reasonable to conclude that the limited efficiency of topical acyclovir therapy is due, at least in part, to the inability of acyclovir to penetrate through the stratum corneum barrier layer of the skin and a lack of its reach at the target site the, basal epidermis.

Forty-five years ago Blank (23) demonstrated that progressive removal of stratum corneum by tape-stripping the human skin increases *in vitro* permeability of water. Subsequently, several investigators have shown that the tape-stripping results in an increased permeability of drugs or chemicals through human and animal skin, thus confirming the barrier characteristics of stratum corneum in the absorption and penetration of chemicals through skin (24-25). The data obtained from this study also shows that after removal of stratum corneum the amount of acyclovir is significantly and substantially increased in viable skin layer (1.21% vs 0.20%) and in the perfusion medium (2.65% vs 0.25%). Thus providing a direct evidence of barrier characteristics of stratum corneum to the absorption of acyclovir.

Absorption of drugs/chemicals through skin can be enhanced using chemical enhancers such as dimethyl sulfoxide (DMSO). The exact mechanism of these enhancers are unknown; however, it has been shown that the mode of action involves a combination of elution of DMSO-soluble components from stratum corneum, delamination of the horny layer, and denaturation of its proteins (26). This indirectly supports the conclusion that the stratum corneum is the barrier layer to the absorption of drugs such as acyclovir. The results obtained in this study provide more direct evidence of the barrier characteristic of skin in the absorption of acyclovir. Thus providing an improved methodology to access the barrier characteristics of the skin for absorption of chemical compounds.

In order to determine the mechanism of transdermal drug delivery and develop the optimal drug formulation, an adequate methodology for evaluating the percutaneous absorption of chemicals/drugs is needed. A wide variety of *in vivo* and *in vitro* methods for measuring percutaneous absorption are available. *In vitro* results from this study, with respect to the absorption and penetration of acyclovir, appear to be in good agreement with those *in vivo* and *in vitro* methods reported previously in the literature (18, 19, 27). Thus, the flow-through diffusion cell system provides an appropriate

alternative approach for the direct measurement of skin absorption under controlled conditions and is relatively simple to study the extent of absorption and penetration of drugs, in particular acyclovir, at a relatively low cost.

ACKNOWLEDGEMENT

We thank Michael S.G. Bell, M.D., Ms. Linda Charbonneau, R.N. and other nursing staff working at the Day Surgery, Ottawa Civic Hospital, for collecting and supplying samples. This project was partly funded by the University of Saskatchewan, Saskatoon, Canada.

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