JPP Journal of Pharmacy and Pharmacology

JPP 2010, 62: 702–708
© 2010 The Authors
Journal compilation © 2010
Royal Pharmaceutical Society
of Great Britain
Received October 29, 2009
Accepted February 1, 2010
DOI 10.1211/jpp/62.06.0006
ISSN 0022-3573

Research Paper

Effect of lipopeptides and iontophoresis on aciclovir skin delivery

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Abstract

Objectives Lipopeptides are compounds derived from microorganisms that exhibit pronounced surface and emulsifying activity. The ability of lipopeptides to interact with stratum corneum lipids makes them candidates as transdermal penetration enhancers. We have investigated the potential of two lipopeptides, fengycin and surfactin, to act as enhancers for the transdermal penetration and skin accumulation of aciclovir.

Methods To investigate a possible synergistic effect, surfactin and fengycin were associated with anodal iontophoresis. Permeation experiments were performed using vertical diffusion cells and pig ear skin as barrier. Differential scanning calorimetry was used to study the interaction between fengycin and stratum corneum lipids.

Key findings The results obtained indicated that surfactin and fengycin were not suitable to enhance aciclovir flux across the skin, not even when associated with iontophoresis. Aciclovir flux was slightly decreased in passive conditions and unchanged (fengycin) or decreased (surfactin) in anodal iontophoretic conditions. When applied in passive conditions, fengycin and surfactin increased aciclovir concentration in the epidermis by a factor of 2.

Conclusions Surfactin and fengycin did not enhance aciclovir transport across the skin (not even when associated with iontophoresis) although they increased aciclovir concentration in the epidermis by a factor of 2.
Keywords biosurfactant; epidermis; iontophoresis; lipopeptide; transdermal penetration

Introduction

Biosurfactants are compounds derived from microorganisms that exhibit pronounced surface and emulsifying activity. In addition to their detergent properties, some of these compounds exhibit a strong membrane destabilizing action at concentrations below their critical micellar concentration. Very recently they have been studied for their potential application in medicine. The biosurfactant category comprises a wide range of chemical structures, such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids.[1]

Among the several categories of biosurfactants, lipopeptides are particularly interesting because of their high surface activity and antibiotic potential. They partition preferentially at the interface between fluid phases that have different degrees of polarity, such as oil–water or air–water interfaces.[1] Surfactin and fengycin are cyclic lipopeptides, produced by various strains of *Bacillus subtilis*. Both biosurfactants possess a relatively long hydrocarbon chain linked to a peptide cycle. Fengycin is composed of two isoform compounds (isoform A and B with D-Ala and D-Val in position 6 of the peptide moiety, respectively), each of them containing fatty acid side chains varying from 13 to 17 carbon atoms (Figure 1a). Surfactin is composed of several homologues which differ by the length of their fatty acid chain (from 13 to 15 carbon atoms) (Figure 1b). The peptide moiety of fengycin is globally more polar than the one of surfactin.[2] At neutral pH fengycin bears two negative charges (glutamic acid residues) and one positive charge (ornithine residue), while surfactin has only two negative charges (glutamic and aspartic acid residues) (Figure 1). Moreover, compared with surfactin, fengycin exhibits a more disparate distribution of its hydrophobic and hydrophilic amino acid residues.

Experiments conducted on phospholipids, ceramides and mixtures of ceramides, fatty acids and cholesterol have shown the ability of fengycin and surfactin to interact with biomimetic

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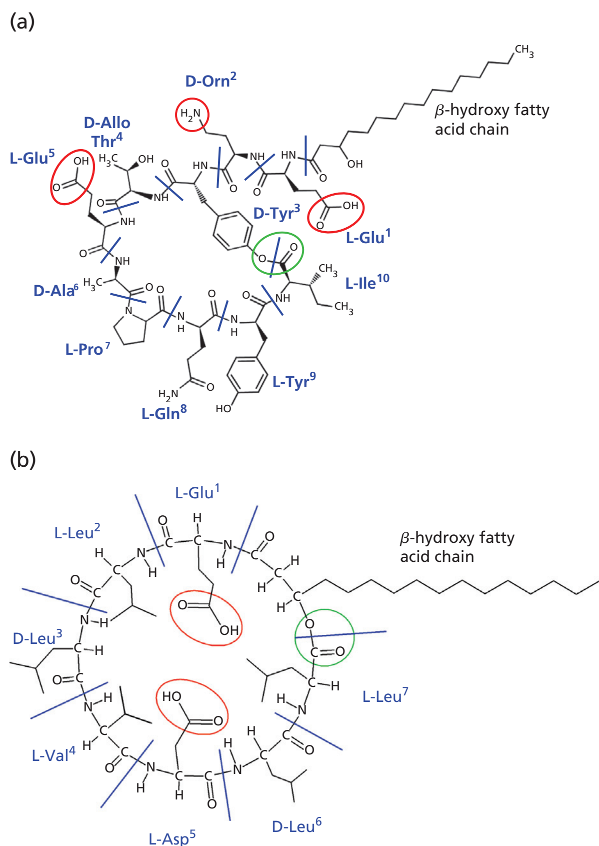

Figure 1 Chemical structures of a fengycin A isoform and surfactin homologue. (a) Chemical structure of a fengycin A isoform with a β-hydroxy fatty acid chain of 16 carbon atoms. Red circles indicate the chemical functions that can be ionized or protonated depending on the pH. Green circle shows the lactone link between the hydroxyl group of the D-tyrosine and the carboxyl group of the L-isoleucine residue. (b) Chemical structure of a surfactin homologue with a hydrocarbon chain of 15 carbon atoms. Red circles indicate the chemical functions that can be ionized or protonated depending on the pH. Green circle shows the lactone link between the hydroxyl group of the β-hydroxy fatty acid chain and the carboxyl group of the L-leucine residue

model membranes.[3–8] In particular, when lipid mixtures mimicking the lipid composition of the stratum corneum extracellular matrix were used, fengycin has been shown to preferentially partition into fluid-disordered cholesterol-rich phases which surround gel-ordered 2D domains, mainly constituted of ceramide and fatty acid molecules.[8] On the basis of that study, it has been postulated that, by preferentially interacting with cholesterol, fengycin is able to modulate both the surface properties and the molecular organization of stratum corneum lipids.[8]

By definition, penetration enhancers are molecules that are able to interact with the components of the stratum corneum (mainly lipids but also proteins) and are of particular interest for increasing the topical bioavailability of locally applied drugs. The ability of lipopeptides to interact with stratum corneum lipids and in particular with cholesterol makes them potential candidates for enhancing the penetration of topically applied drugs through the skin.

We have investigated the potential of two lipopeptides, fengycin and surfactin, to act as enhancers for the transdermal penetration and skin accumulation of aciclovir, a potent antiviral drug approved for the treatment of *Herpes simplex* infections.[9] The rationale in the choice of aciclovir was its very low oral bioavailability, together with the unsatisfactory efficacy of dermatological formulation, due to insufficient stratum corneum penetration.[10] Additionally, lipopeptides, and in particular surfactin are active against several viruses, and could potentially increase the efficacy of aciclovir-containing formulations.[6]

To investigate a possible synergistic effect, surfactin and fengycin treatments were associated with iontophoresis. Iontophoresis is a physical enhancing technique that consists of the application of an electric current to the skin to increase the transdermal transport of drugs.[11]

Experiments were performed using full-thickness pig ear skin that, owing to its biochemical similarity, is considered as a good model of human skin.[12–14] Additionally, the enhancing activity of chemicals has been reported to be qualitatively similar for pig and human stratum corneum.[15]

Materials and Methods

Materials

The biosurfactants (fengycin and surfactin) used in this study were lipopeptides produced by fermentation of the *B. subtilis* strain S499. The cells were grown in a 20-l fermentor (Biolafitte, Poissy, France) at 30°C for 72 h in an optimized culture media.[16] The stirrer speed was 300 rev/min and the aeration rate (i.e. the volumetric air flow per minute per working volume) was 0.3 v/v/min. The pH was maintained at 7.0. After 72 h of cell growth, the culture medium was centrifuged at 11 000*g* for 25 min to eliminate the cells. The lipopeptides contained in the supernatant (500 mg dry material) were then extracted in a semi-preparative scale by solid-phase extraction on a Bond Elut C18 cartridge (50 g; Varian, Palo Alto, CA, USA).[17] The cartridge, which retained lipopeptides, was rinsed successively with 20 ml water and 4 ml methanol (50% aq.). The lipopeptides were then eluted from the cartridge with 20 ml pure methanol. The eluate was subsequently applied to a silica gel 60 column (30 × 2.5 cm, 45 g, 250–325 mesh; Merck, Darmstadt, Germany) to separate fengycin and surfactin from iturin A, the third type of lipopeptide produced by *B. subtilis,* using the flash chromatography technique and a mixture chloroform/methanol/water/ethanol (7 : 3 : 1.5 : 3.5, v/v) as eluent. The purity of the surfactin and fengycin samples (> 95%) was verified by amino acid analysis, analytical reversed-phase high-performance liquid chromatography (HPLC), and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry.

Aciclovir (MW = 225.2, pK_{a1} = 2.4, pK_{a2} = 9.2) was a gift from Lisapharma (Erba, CO, Italy). For the HPLC analysis, trifluoroacetic acid (HPLC grade) and distilled water were used. All other chemicals were of analytical grade.

Full-thickness skin was excised from the outer part of pig ears, after the animals had been killed at a local slaughter house. The skin was frozen for not more than 30 days.

Before the experiment, the skin was thawed at room temperature for 30 min.

Aciclovir HPLC analysis

Aciclovir HPLC analyses were performed using a Perkin Elmer instrument (Norwalk, CT, USA) and a Jupiter C18 column (5 μm, 300 A, 250 × 4.6 mm; Phenomenex, Casalecchio di Reno, BO, Italy). The UV detector was set at 254 nm. The mobile phase was distilled water containing trifluoroacetic acid 0.1% v/v, pumped at 1.2 ml/min. In these conditions aciclovir retention time was approximately 9 min. The method was compliant to the system suitability tests according to USP 32.

The absorbance was linear in the interval 0.02–10.33 μg/ml with a relative standard deviation lower than 4.5% and a relative error lower than 7.6%.

Aciclovir solubility

The solubility of aciclovir was determined by adding an excess amount of drug to 1.0 ml of one of the following solutions: pH 7.4 phosphate buffer saline (PBS), 1 mg/ml surfactin in PBS, 1 or 5 mg/ml fengycin in PBS. The dispersions were magnetically stirred for 48 h at 25°C, then filtered through regenerated cellulose filters (pore size 0.45 μm), diluted and analysed by HPLC. Each experiment was replicated six times.

Interaction between lipopeptides and stratum corneum: thermal analysis

Isolation of pig stratum corneum

After elimination of subcutaneous fat, skin samples were immersed in hot water (60°C) for 2 min, to separate epidermis from dermis. The epidermal sheets obtained were soaked at 4°C for 15 h in trypsin 1% solution (w/v) in phosphate buffer pH 7.4. Stratum corneum sheets obtained were individually rinsed with distilled water and then resuspended in fresh trypsin-PBS solution 1% (w/v) for 2 h at 37°C.[18] Tissue pieces were individually washed with distilled water, wiped with filter paper and then oven dried at 37°C for 2 h. Dried stratum corneum was kept in a desiccator until use.

Differential scanning calorimetry measurements

A solution of fengycin (20-μl, concn 110 mg/ml) in water or ethanol : water (66 : 34, w/w) was placed on the separated stratum corneum samples (weight 5–10 mg) and left at ambient temperature and humidity for 30 min. The samples were then transferred to a desiccator for 15 h and then re-equilibrated at ambient humidity until reaching a final water content of 10% of the dry weight. Samples were then placed into sealed aluminium pans and differential thermal analysis was performed by differential scanning calorimetry (DSC) using a 821 STARe Mettler Toledo (Novate Milanese, Italy) with an empty pan as reference, heating from 25 to 150°C at 2°C/min. Samples of stratum corneum were treated with the pure vehicle, namely water or ethanol : water (66 : 34, w/w), and analysed for comparison.

Dynamic light scattering analysis

To verify the possible presence of biosurfactant micelles, dynamic light scattering (DLS) analysis (90Plus apparatus, Brookhaven Instruments Corporation, Holtsville, NY, USA) was performed on 1 mg/ml aciclovir solutions containing either surfactin or fengycin 1 mg/ml in pH 7.4 PBS.

Transdermal permeation and skin accumulation

Permeation experiments were conducted in Franz-type diffusion cells (Disa, Milan, Italy), with an exposed surface area of 0.6 cm². Full-thickness pig ear skin was used as a barrier. The receptor phase was degassed pH 7.4 PBS, thermostated at 37°C and magnetically stirred to prevent any boundary-layer effect. At predetermined time intervals the receptor solution was sampled and analysed by HPLC for the determination of the amount of aciclovir permeated.

The following donor solutions were tested: aciclovir (1 mg/ml) in pH 7.4 PBS; aciclovir (1 mg/ml) and surfactin(1 mg/ml) in pH 7.4 PBS; aciclovir (1 mg/ml) and fengycin (1 mg/ml) in pH 7.4 PBS; and aciclovir (1 mg/ml) and fengycin (5 mg/ml) in pH 7.4 PBS. Each experiment was replicated at least five times.

In the iontophoretic experiments, the current was applied by means of a constant current generator (Iono1, Cosmic, Pesaro, Italy), using silver/silver chloride electrodes. Direct current (0.5 mA/cm², anodal) was applied for the whole duration of the experiment. Each experiment was replicated six times using skin from three different animals.

At the end of the experiment, the donor solution was removed, the cell was dismantled and the skin was carefully washed to remove any residual of donor solution. A disc of tissue was cut, fitting the area covered by donor compartment (0.6 cm²), heated with a hair dryer for approximately 20 s and the epidermis was separated from dermis using forceps. The two skin layers were placed in separate preweighed plastic test tubes, weighed again to determine the amount of tissue and extracted with 0.5 ml distilled water at 60°C for 30 min. Proteins were then precipitated by adding 0.5 ml 1 M perchloric acid. After centrifugation at 11 000 rev/min for 10 min, the solution was filtered through regenerated cellulose filters (0.45 μm; 15-mm diameter, Albet, Barcelona, Spain) and injected into the HPLC.

The extraction method was validated in blank experiments (specificity) and by applying a known amount of aciclovir to the skin (recovery > 99%) following a previously reported method.[19]

Data elaboration and statistical analysis

From the permeation profiles, fluxes (J, μg/cm²h) were calculated as the slope of the regression line after the achievement of the steady state. From the flux, the permeability coefficient (P, cm/h) was calculated as J/C_D, where C_D (μg/ml) represents the concentration of the donor solution. In the case of nonlinear profiles, the amount permeated after 7 h (μg/cm^2) was used to compare different experimental conditions. Data for skin accumulation were expressed as μg aciclovir/mg tissue.

All results were expressed as the mean ± standard error of the mean (SEM). Statistical differences were determined by Student's t-test and by analysis of variance followed by the Bonferroni test.

Results

Aciclovir solubility

Solubility of aciclovir at 25°C in pH 7.4 PBS was 1.27 ± 0.08 mg/ml and did not vary significantly in the presence of the two lipopeptides: 1.36 ± 0.1, 1.39 ± 0.05 and 1.31 ± 0.02 mg/ml for surfactin 1 mg/ml, fengycin 1 and 5 mg/ml, respectively.

Interaction between lipopeptides and stratum corneum: thermal analysis

The DSC analysis of pig stratum corneum treated with water indicated the presence of a thermal transition at 83°C (Figure 2a). The treatment with fengycin caused a 4-degree reduction of the lipid transition, since the endotherm peak shifted to 79°C. When the stratum corneum was treated with an ethanol : water mixture, dried and rehydrated (Figure 2b), the DSC traces flattened and could not be conclusively interpreted. The effect of ethanol, observed with and without fengycin, was probably due to its solvent properties towards some lipids of the stratum corneum or to the decrease of the strength of H-bonds within the polar headgroups of ceramides.[20,21]

Dynamic light scattering analysis

No micelles or aggregates were found using DLS analysis.

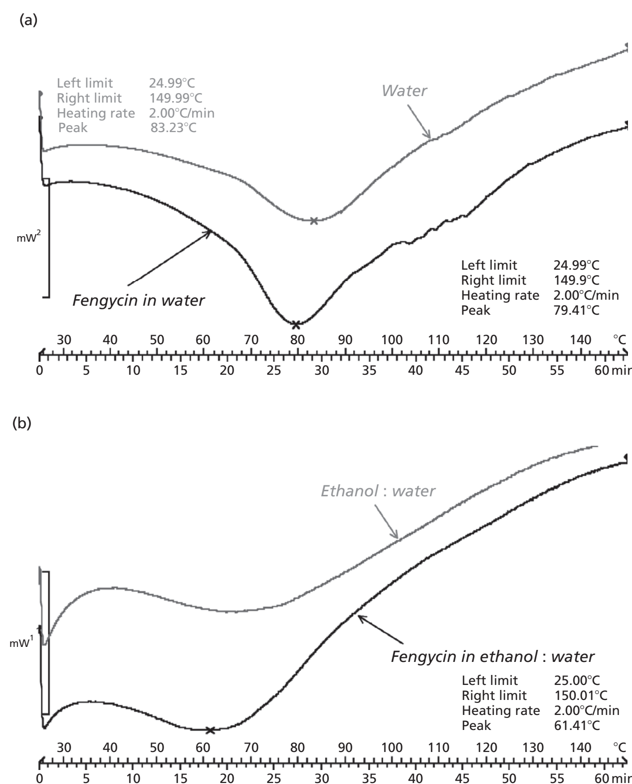

Figure 2 Differential scanning calorimetry traces of pig stratum corneum treated with fengycin. The fengycin was dissolved in water (a) or in ethanol : water (b) in comparison with the pure vehicle treatment

Aciclovir permeation and skin retention: effect of lipopeptides

At first the passive permeation of aciclovir without lipopeptide was determined. The profiles obtained are reported in Figure 3a, while the permeability coefficient values are reported in Table 1. The permeability coefficient was 1.65 (± 0.67) × 10^{-4} cm/h, a value in good agreement with literature data through human skin (3.0 × 10^{-4} cm/h).[22] When the lipopeptides, either fengycin or surfactin, were added to the donor solution, the permeation of aciclovir decreased even if the amounts permeated at 7 h were not statistically significant ($P > 0.05$).

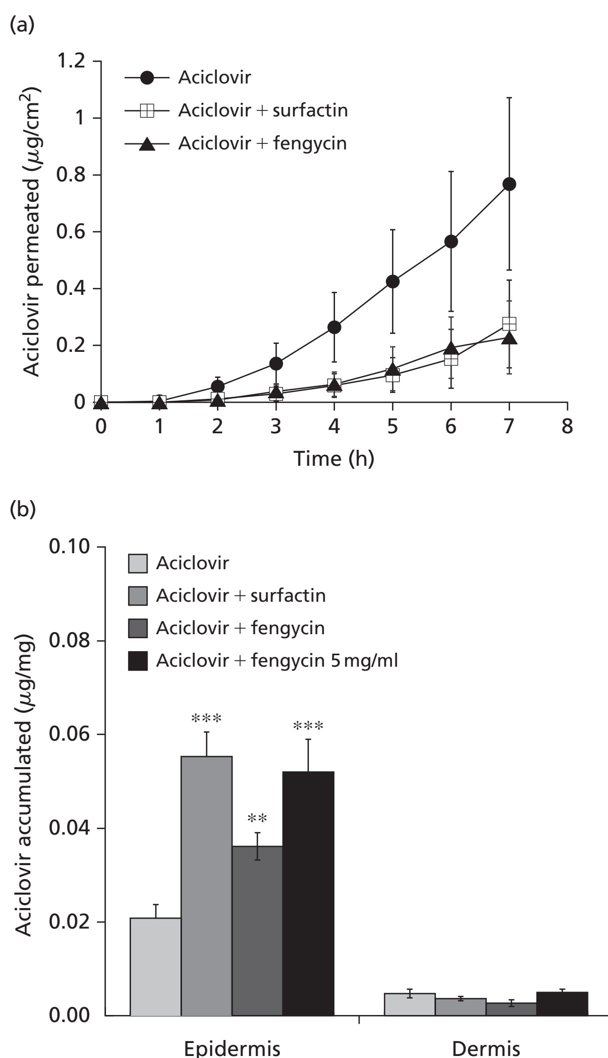

Figure 3 Passive permeation profiles and accumulation levels of aciclovir. (a) Passive permeation profiles of aciclovir (1 mg/ml) through pig skin starting from a pH 7.4 buffer containing aciclovir alone, or aciclovir with surfactin or fengycin. (b) Accumulation levels of aciclovir in epidermis and dermis after passive diffusion experiments starting from a pH 7.4 buffer containing aciclovir alone, or aciclovir with surfactin (1 mg/ml) or fengycin (1 or 5 mg/ml). $^{**}P < 0.01$; $^{***}P < 0.001$ statistical differences with respect to aciclovir alone

Table 1 Permeation and accumulation data of aciclovir under different experimental conditions

	Aciclovir accumulated (μg/mg)		Permeability coefficient (cm/h) × 10^{-4}	Aciclovir permeated after 7 h (μg/cm^2)
	Epidermis	Dermis		
Passive	0.021 ± 0.003	0.005 ± 0.001	1.65 ± 0.67	0.74 ± 0.32
+ surfactin (1 mg/ml)	0.055 ± 0.005***	0.004 ± 0.001	ND	0.17 ± 0.11
+ fengycin (1 mg/ml)	0.036 ± 0.003**	0.003 ± 0.001	ND	0.20 ± 0.11
+ fengycin (5 mg/ml)	0.052 ± 0.007***	0.005 ± 0.001	ND	0.37 ± 0.14
Anodal	0.494 ± 0.038	0.088 ± 0.009	45.51 ± 3.22	25.51 ± 2.24
+ surfactin (1 mg/ml)	0.296 ± 0.021***	0.052 ± 0.003**	16.31 ± 0.713	7.48 ± 0.44
+ fengycin (1 mg/ml)	0.373 ± 0.034***	0.063 ± 0.002*	51.44 ± 4.82	27.77 ± 2.99

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, statistical differences with respect to aciclovir alone in the same condition. ND, not determined.

Beside the transdermal penetration, aciclovir accumulation in epidermis and dermis was also evaluated. In contrast with the permeation data, a significant increase ($P < 0.01$) was found when lipopeptides were present in the donor solution (Table 1 and Figure 3b): the amount of aciclovir accumulated in the epidermis was approximately two- (fengycin) or threefold (surfactin) the reference value. However, dermis accumulation did not change.

When the concentration of fengycin was increased to 5 mg/ml, no difference was found in the permeation profiles (data not shown), but a further increase in the amount of aciclovir accumulated in the epidermis was recorded (Figure 3b).

Aciclovir permeation and skin retention: effect of iontophoresis alone and in combination

Anodal iontophoresis of the simple aciclovir solution (Figure 4a) determined a substantial increase in permeation compared with the corresponding passive condition (Figure 3a), the anodal permeability coefficient being 4.55 (± 0.32) × 10^{-3} cm/h (Table 1). A very similar result (P = 5.14 (± 0.48) × 10^{-3} m/h) was obtained when fengycin was added to the donor compartment, while a much lower ($P < 0.001$) permeability coefficient (P = 1.63 (± 0.07) × 10^{-3} cm/h) was observed in the case of surfactin (Table 1). The accumulation data highlighted a reduction in the amount of aciclovir in the presence of lipopeptides in epidermis and dermis, which was more visible with surfactin (Figure 4b and Table 1).

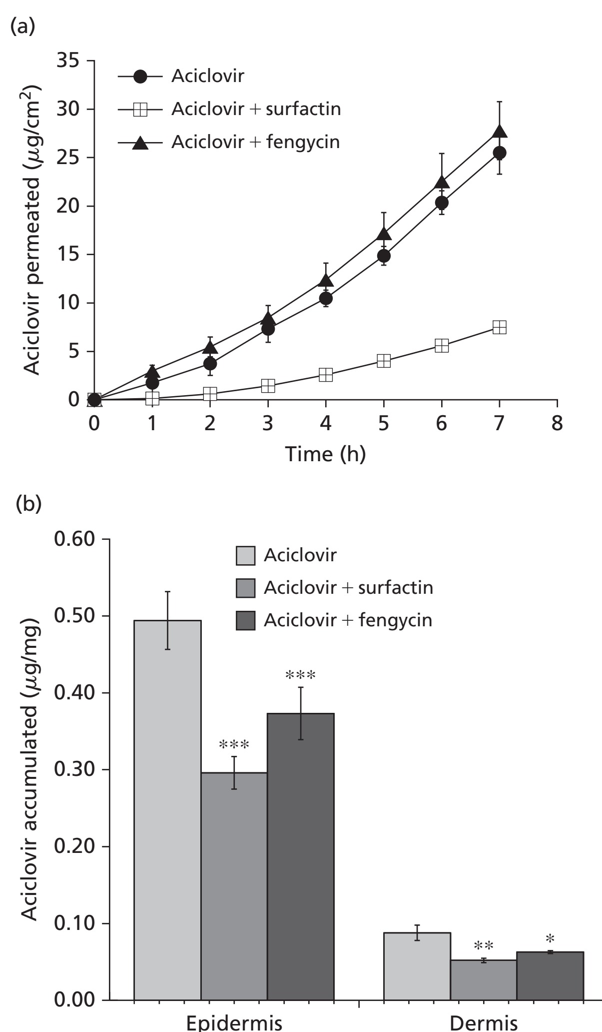

Figure 4 Iontophoretic (anodal) permeation profiles and accumulation levels of aciclovir. (a) Iontophoretic (anodal) permeation profiles of aciclovir (1 mg/ml) through pig skin starting from a pH 7.4 buffer containing aciclovir alone, or aciclovir with surfactin or fengycin. (b) Accumulation levels of aciclovir in epidermis and dermis after iontophoretic (anodal) experiments starting from a pH 7.4 buffer containing aciclovir alone, or aciclovir with surfactin (1 mg/ml) or fengycin (1 mg/ml). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ statistical differences with respect to aciclovir alone

Discussion

It has been postulated that, due to their peculiar structure, lipopeptides have the ability to modify the organization of the stratum corneum extracellular matrix, by intercalating into the stratum corneum lipid lamellae and by specifically interacting with cholesterol.[8] In this work, the ability of fengycin to interact with pig stratum corneum has been confirmed by means of DSC experiments, which highlighted a 4°C reduction of the transition temperature of intercellular lipids, a sign of lipid disorganization.[23] The potential of lipopeptides for disturbing the stratum corneum lipid arrangement could make them excellent penetration enhancers for transdermal delivery of drugs topically applied on the skin surface, as the stratum corneum represents the main barrier to the penetration of drugs, and it is well known that

induced lipid disorder in the uppermost layer of the skin is correlated with increased penetration. Moreover, it is worth noting that penetration enhancement of drugs has been already observed using lipopeptide-related enhancers exhibiting a fatty acid chain linked to an amino acid polar head group (e.g. dimethylamino acid dodecyl esters).[24]

The data presented here, however, highlighted the substantial lack of lipopeptide effect on aciclovir transdermal penetration: even a reduction of the amount permeated through full-thickness skin samples was obtained (Figure 3a). When the donor solution contained lipopeptide, a 2.5-fold increase in the amount of drug accumulated in the epidermis (but not in the dermis) was observed (Figure 3b). In the case of fengycin, there was a concentration-dependent effect, evidence of drug retention but negligible on transdermal penetration.

To explain this behaviour we cannot refer to differences of drug activity in the various donor solutions, since all experiments were performed using the same aciclovir donor concentration and the effect of lipopeptide on aciclovir solubility was found to be negligible. Other explanations are possible. Firstly, due to the amphiphilic nature of lipopeptides and to their tendency to micellization, the effect on aciclovir transport and retention could be attributed to the formation of aciclovir-including lipopeptide micelles.[2] This would lead to a reduction of 'free drug' available for skin permeation and increased partitioning into the stratum corneum, as reported, for instance, for triamcinolone acetonide in the presence of lecithin micelles.[25] Nevertheless, the solubility data and DLS analysis did not support this hypothesis. No micelles were detected under the experimental conditions used, even though critical micellar concentration values of 10 and 11 mg/l have been reported in the literature for surfactin and fengycin, respectively.[2] Probably, differences in terms of ionic strength and/or pH or the presence of aciclovir prevented micelle formation, justifying the discrepancy with the literature data.

A second hypothesis could link flux reduction to lipopeptide interaction with stratum corneum lipids. According to Hadgraft *et al.*[26] some agents could reduce the permeability of the stratum corneum by imparting order to the skin lipids, or by altering the lateral bonding within the stratum corneum lipid lamellae. The increased aciclovir retention in the epidermis (or stratum corneum) could be explained with a higher partitioning of aciclovir into the stratum corneum due to the presence of lipopeptides.

Finally, the decrease of permeation and increase of skin retention could arise from attractive interactions between the hydrophobic molecule of aciclovir and nonpolar moieties located at the surface of the biosurfactant molecules, although the solubility data did not support this hypothesis.

In summary, in the presence of fengycin and surfactin, aciclovir accumulated in the epidermis, but its transdermal flux decreased. The higher aciclovir levels obtained in the epidermis using surfactin compared with fengycin ($P > 0.01$) (Figure 3b) could have been due to the more hydrophobic nature of surfactin compared with fengycin, that favoured its interaction with the stratum corneum extracellular lipid matrix and/or with aciclovir.

It is worth mentioning that the increase in aciclovir accumulation did not involve the dermis, where no difference was observed in the presence or absence of biosurfactants. This was a clear indication that the lipopeptide interaction with the skin was limited to the epidermis and maybe only to the stratum corneum (the experimental set-up used did not allow to distinguish between stratum corneum and viable epidermis).

Iontophoresis is a well known technique used to increase transdermal drug delivery and skin retention.[22,27–29] Even if at pH 7.4 aciclovir existed almost completely in its unionized form, different authors have demonstrated the usefulness of this technique due to the electroosmotic mechanism (at pH 7.4 the electroosmotic flow was in the anode-to-cathode direction.).[30] As expected and in accordance with the literature data, anodal iontophoresis produced a significant increase in aciclovir transport and retention, compared with the corresponding passive conditions (Figures 3 and 4, Table 1).

When iontophoresis was associated with lipopeptides, both a decrease of skin retention and a decrease (or a constant) flux were observed (Figures 3 and 4). Such phenomenon could be explained only by assuming that aciclovir and the lipopeptides interacted in solution, although the solubility data did not support this hypothesis. As both lipopeptides were negatively charged at pH 7.4, the interaction product with aciclovir was attracted towards the positively charged electrode (anode), reducing its epidermis accumulation and flux. It is worth mentioning that in the presence of iontophoresis dermis retention changed in the same way. The existence of potential aciclovir–lipopeptide interactions, as hypothesized in this paper, needs to be further investigated and demonstrated.

Conclusions

Surfactin and fengycin, two lipopeptides produced by *B. subtilis* strains, were not suitable to enhance aciclovir flux across the skin, not even when associated with iontophoresis. In fact aciclovir flux was slightly decreased in passive conditions and unchanged (in the case of fengycin) or decreased (in the case of surfactin) in anodal iontophoretic conditions.

When applied in passive conditions, both lipopeptides increased aciclovir concentration in the epidermis by a factor of 2, although when iontophoresis was simultaneously applied aciclovir epidermal concentration decreased. A possible explanation for this was the ability of the lipopeptides to interact with the stratum corneum lipids (confirmed by DSC analysis), thus increasing the partition of the drug in the uppermost skin layer, although it does not justify the iontophoresis data. Another hypothesis, which has not been demonstrated and needs further investigation, is the interaction between aciclovir and the lipopeptides, which would explain the iontophoretic data. The nature of this interaction is unknown, but it could arise from a molecular attraction between aciclovir, which is hydrophobic, and nonpolar moieties located at the surface of lipopeptide.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The financial support of the Région wallonne (#EPH33103-00R050F/415782) and of the FNRS in Belgium is gratefully acknowledged.

Acknowledgements

Many thanks to Professor P. Thonart and his team (Department of Bioindustries, Gembloux Agro-Bio Tech, Liege University, Belgium) for the production of fengycin and surfactin by the fermentation of *Bacillus subtilis* strain S499. Magali Deleu is a Research Associate of the National Foundation for Scientific Research (F.N.R.S.) in Belgium.

References

1. Rodrigues L *et al*. Biosurfactants: potential applications in medicine. *J Antimicrob Chemother* 2006; 57: 609–618.
2. Deleu M *et al*. Interfacial and emulsifying properties of lipopeptides from Bacillus subtilis. *Colloid Surface Physicochem Eng Aspect* 1999; 152: 3–10.
3. Deleu M *et al*. Fengycin interaction with lipid monolayers at the air-aqueous interface: implications for the effect of fengycin on biological membranes. *J Colloid Interface Sci* 2005; 283: 358–365.
4. Deleu M *et al*. Effect of fengycin, a lipopeptide produced by *Bacillus subtilis*, on model biomembranes. *Biophys J* 2008; 94: 2667–2679.
5. Eeman M *et al*. Penetration of surfactin into phospholipid monolayers: nanoscale interfacial organization. *Langmuir* 2006; 22: 11337–11345.
6. Seydlová G, Svobodová J. Review of surfactin chemical properties and the potential biomedical applications. *Cent Eur J Med* 2008; 3: 123–133.
7. Eeman M *et al*. Nanoscale properties of mixed fengycin/ceramide monolayers explored using atomic force microscopy. *Langmuir* 2005; 21: 2505–2511.
8. Eeman M *et al*. Effect of cholesterol and fatty acids on the molecular interactions of fengycin with stratum corneum mimicking lipid monolayers. *Langmuir* 2009; 25: 3029–3039.
9. Lin P *et al*. Changing paradigms in dermatology: antivirals in dermatology. *Clin Dermatol* 2003; 21: 426–446.
10. O'Brien JJ, Campoli-Richards DM. Acyclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 1989; 37: 233–309.
11. Guy RH *et al*. Iontophoresis: electrorepulsion and electroosmosis. *J Control Release* 2000; 64: 129–132.
12. Dick IP, Scott RC. Pig ear skin as an in-vitro model for human skin permeability. *J Pharm Pharmacol* 1992; 44: 640–645.
13. Sekkat N *et al*. Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo. *J Pharm Sci* 2002; 91: 2376–2381.
14. Simon GA, Maibach HI. The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations–an overview. *Skin Pharmacol Appl Skin Physiol* 2000; 13: 229–234.
15. Andega S *et al*. Comparison of the effect of fatty alcohols on the permeation of melatonin between porcine and human skin. *J Control Release* 2001; 77: 17–25.
16. Jacques P *et al*. Optimization of biosurfactant lipopeptide production from *Bacillus subtilis* S499 by Plackett-Burman design. *Appl Biochem Biotechnol* 1999; 77–79: 223–233.
17. Razafindralambo H *et al*. Purification of antifungal lipopeptides by reversed-phase high-performance liquid chromatography. *J Chromatogr* 1993; 639: 81–85.
18. Law S *et al*. Regional variation in content, composition and organization of porcine epithelial barrier lipids revealed by thin-layer chromatography and transmission electron microscopy. *Arch Oral Biol* 1995; 40: 1085–1091.
19. Volpato NM *et al*. Assay of acyclovir in human skin layers by high-performance liquid chromatography. *J Pharm Biomed Anal* 1997; 16: 515–520.
20. Vaddi HK *et al*. Terpenes in ethanol: haloperidol permeation and partition through human skin and stratum corneum changes. *J Control Release* 2002; 81: 121–133.
21. Guillard EC *et al*. Molecular interactions of penetration enhancers within ceramides organization: a FTIR approach. *Eur J Pharm Sci* 2009; 36: 192–199.
22. Volpato NM *et al*. In vitro acyclovir distribution in human skin layers after transdermal iontophoresis. *J Control Release* 1998; 50: 291–296.
23. Ibrahim SA, Li SK. Chemical enhancer solubility in human stratum corneum lipids and enhancer mechanism of action on stratum corneum lipid domain. *Int J Pharm* 2010; 383: 89–98.
24. Novotny J *et al*. Dimethylamino acid esters as biodegradable and reversible transdermal permeation enhancers: effects of linking chain length, chirality and polyfluorination. *Pharm Res* 2009; 26: 811–821.
25. Bentley MV *et al*. Influence of lecithin on some physical chemical properties of poloxamer gels: rheological, microscopic and in vitro permeation studies. *Int J Pharm* 1999; 193: 49–55.
26. Hadgraft J *et al*. Mechanisms of action of skin penetration enhancers/retarders: Azone and analogues. *Int J Pharmaceutics* 1996; 141: 17–25.
27. Padula C *et al*. The influence of iontophoresis on acyclovir transport and accumulation in rabbit ear skin. *Pharm Res* 2005; 22: 1519–1524.
28. Shukla C *et al*. In vivo quantification of acyclovir exposure in the dermis following iontophoresis of semisolid formulations. *J Pharm Sci* 2009; 98: 917–925.
29. Xu Q *et al*. Ion-exchange membrane assisted transdermal iontophoretic delivery of salicylate and acyclovir. *Int J Pharm* 2009; 369: 105–113.
30. Volpato NM *et al*. Iontophoresis enhances the transport of acyclovir through nude mouse skin by electrorepulsion and electroosmosis. *Pharm Res* 1995; 12: 1623–1627.