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Optimization of transdermal delivery using magainin pore-forming peptide

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Abstract

The skin's outer layer of *stratum corneum*, which is a thin tissue containing multilamellar lipid bilayers, is the main barrier to drug delivery to the skin. To increase skin permeability, our previous work has shown large enhancement of transdermal permeation using a pore-forming peptide, magainin, which was formulated with N-lauroyl sarcosine (NLS) in 50% ethanol-in-PBS. Mechanistic analysis suggested that magainin and NLS can increase skin permeability by disrupting *stratum corneum* lipid structure. In this study, our goal was to improve conditions that increase skin permeability by magainin by further optimizing the pretreatment time and concentration of magainin exposure. We found that skin permeability increased with increasing pretreatment time. Skin permeability also increased with increasing magainin concentration up to 1 mM, but was reduced at a magainin concentration of 2 mM. Enhancement of skin permeability to fluorescein (323 Da) up to 35-fold was observed. In contrast, this formulation did not enhance skin permeability to larger molecules, such as calcein (623 Da) and dextran (3,000 Da).

Keywords

A. organic compounds; B. electron (confocal) microscopy; D. diffusion

1. Introduction

Multilamellar lipid bilayers comprise the continuum portion around the corneocyte cells of *stratum corneum*, which is the primary barrier in skin [1]. Various physical and chemical methods have been tested to increase the permeability of the *stratum corneum* to drugs, which could enable transdermal delivery of more drugs using a transdermal patch. However, few methods have succeeded to deliver relevant agents at the appropriate flux levels without causing skin irritation or damage [2].

This study addresses the use of a naturally occurring pore-forming peptide, magainin, to increase skin permeability. Magainin is a 23-residue helical peptide isolated from the skin of the African frog, which exhibits a broad spectrum of antimicrobial activity properties. It has a net +4 charge and binds to negatively charged phospholipid membranes with the aid of electrostatic interactions, forming an amphiphilic helix and permeabilizing the bilayers [3,4].

Our previous work shows that the use of magainin disrupts vesicles that are made from lipid bilayer components representative of those found in human *stratum corneum* [5], and that magainin administered in a formulation containing an anionic surfactant, N-lauroyl

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sarcosine (NLS), in 50% ethanol-in-PBS synergistically increased skin permeability. Mechanistic analysis using differential scanning calorimetry, Fourier-transform infrared spectroscopy, and X-ray diffraction suggested that magainin and NLS can increase skin permeability by disrupting *stratum corneum* lipid structure [6].

Building off the results of our previous work, this study sought to further optimize conditions that increase skin permeability. Because the interaction between magainin and the *stratum corneum* is critical to the enhancement mechanism, we varied the pretreatment time and magainin concentration during exposure to skin. We also tested the effect of molecular weight of delivered molecules on skin permeability.

2. Experimental methods

2.1 Skin preparation and permeability measurement

Human epidermis (Emory University) was isolated from dermis using the heat separation method [7]. Before measuring skin permeability, skin was pretreated with magainin and other control formulations. Epidermis was placed in a vertical, glass Franz diffusion cell apparatus (PermeGear) with 0.7 cm² exposed skin surface area. The receiver chamber was filled with PBS and the donor chamber was filled with 0.3 ml of a formulation in PBS containing 50% (v/v) ethanol, 2% (w/v) N-lauroyl sarcosine (Fluka) and, sometimes, 1 mM magainin peptide (Emory University). After a 0-12 h exposure to one of these formulations at 4 °C, the Franz cell was transferred to a heater/stirrer block (PermeGear) maintained at 32 °C and stirred at 455 rpm for 3 h.

After this pretreatment, the receiver chamber was emptied and filled with fresh PBS and the donor chamber was emptied and filled with 0.3 ml of 1 mM fluorescein, calcein (Sigma Aldrich), or fluorescein-tagged dextran (3000 Da, Molecular Probes) in PBS. Every hour for 5 h, the receiver chamber was sampled. Samples were analyzed by calibrated spectrofluorimetry (Photon Technologies International) to determine transdermal flux and permeability.

2.2 Skin imaging by multi-photon microscopy

To image fluorescein and magainin distribution in the skin, skin was pretreated with sulforhodamine-tagged magainin. Fluorescein was then delivered across the skin, as described above, for 1 h. The skin sample was then removed from the Franz cell and placed on a glass cover slip. Skin imaging was carried out using a multi-photon microscope (Zeiss LSM/NLO 510) with an oil-immersion lens of 40× magnification to collect “z-stack” optical slices at a series of depths into the epidermis.

3. Results and discussion

To further optimize conditions that enhance skin permeability by magainin, we studied the effect of the duration and concentration of magainin exposure during pretreatment of the skin and the effect of the molecular weight of delivered molecules on skin permeability.

We first hypothesized that increased magainin pretreatment exposure time should increase skin permeability to fluorescein by enabling more magainin to enter the *stratum corneum*. As shown in Fig. 1-a, the amount of fluorescein delivered across the skin increased when we increased the pretreatment time (ANOVA, $p < 0.01$). The black bars in Fig. 1-a show the increase in skin permeability caused by incubation with the formulation of NLS in 50% ethanol-in-PBS without magainin. This formulation alone increase skin permeability (ANOVA, $p < 0.01$). The white bars show the increase in skin permeability caused by incubation in the same formulation that also contained magainin (ANOVA, $p < 0.01$). The

addition of magainin further increased skin permeability beyond that of the magainin-free formulation after 12 h (ANOVA, $p < 0.05$).

Further examination shows that the permeability increase after 3 h was insignificant (Student's t -test, $p > 0.05$), whereas the permeability increases after 6 h and longer were significant (Student's t -test, $p < 0.01$). This led us to conclude that a minimum pretreatment time of 6 h is required for significant enhancement.

To better understand the mechanism behind these kinetics, we imaged skin after different pretreatment times using red-fluorescence labeled magainin and green-fluorescent fluorescein. The resulting images, shown in Fig. 1-b, indicate that over time more magainin was able to penetrate into the *stratum corneum* (the upper 10 – 15 μm of skin), which corresponded to more fluorescein transport across the *stratum corneum* and into the deeper skin.

We next hypothesized that increased magainin concentration should increase skin permeability to fluorescein. As shown in Fig. 2, increasing magainin concentration up to 1 mM increased skin permeability (ANOVA, $p < 0.05$). However, further increasing magainin concentration to 2 mM decreased the enhancement ratio by more than a factor of two (Student's t -test, $p < 0.01$). This effect might be explained by aggregation of high-concentration magainin in the *stratum corneum* lipids, which may disrupt and occlude the expected pore structures formed by lower-concentration magainin [8]. This explanation requires further study.

Finally, we hypothesized that skin permeability increased by magainin should be more effective for lower molecular weight molecules. Fig. 3 shows skin permeability to molecules of three different sizes: fluorescein (323 Da), calcein (623 Da), fluorescein-tagged dextran (3,000 Da). Skin permeability to fluorescein was significantly increased (Student's t -test, $p < 0.01$), but permeability to the two larger molecules was not significantly affected by magainin (Student's t -test, $p > 0.05$). This can be explained because magainin is believed to form Angstrom-scale pores across lipid bilayers that are known to be large enough for transport only of small molecules [9,10].

4. Conclusion

This study provided three main conclusions. First, we found that increased magainin pretreatment exposure time increased skin permeability to fluorescein by enabling more magainin to enter the *stratum corneum*. Second, increased magainin concentration up to 1 mM was shown to increase skin permeability to fluorescein, but 2 mM fluorescein reduced this effect, perhaps due to magainin aggregation. Finally, skin permeability increased by magainin was effective for low molecular weight fluorescein (323 Da), but not for higher molecular weight calcein (623 Da) or dextran (3,000 Da). Overall, this study shows that magainin-based formulations can be optimized for increased transdermal delivery of low molecular weight compounds.

Acknowledgments

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Figure 1.

(A) Effect of pretreatment time on the enhancement of skin permeability to fluorescein for skin treated with (□) and without (■) magainin. The enhancement ratio is defined as the skin permeability at the condition tested divided by the permeability of untreated skin. (B) Penetration of fluorescein and sulforhodamine-tagged magainin peptide into human epidermis imaged by multi-photon confocal microscopy. Skin was treated with magainin formulation for (1) 1 h, (2) 4 h, and (3) 12 h. Green corresponds to fluorescein, red corresponds to sulforhodamine-tagged magainin, and yellow corresponds to co-localization of fluorescein and magainin. Optical sections taken at 5 μm increments starting at the *stratum corneum* surface on the left and proceeding deeper on the right. Scale bar is 100 μm.

Figure 2.
Skin permeability to fluorescein as a function of magainin concentration during pretreatment..

Figure 3. Skin permeability to molecules of different sizes: fluorescein (323 Da), calcein (623 Da), and fluorescein-tagged dextran (3,000 Da). Pretreatment solutions were with (□) and without (■) magainin.