Journal of Drug Delivery Science and Technology 41 (2017) 191–196

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Evaluation of peptide drug delivery via skin barrier-impact of permeation enhancers☆

Flavia Laffleur [a, b, *], Andreas Bernkop-Schnürch [a]

[a] Department of Pharmaceutical Technology, Institute of Pharmacy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Austria
[b] Massachusetts Institute of Technology, Koch Institute for Integrative Cancer Research at MIT, Langer Lab, 77 Massachussets Ave, Cambridge, MA, 02139, USA

ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form
2 June 2017
Accepted 9 July 2017
Available online 12 July 2017

Keywords:
Skin delivery
Peptide
Surfactants
Franz diffusion cells

ABSTRACT

Background/purpose: Non-invasive topical delivery of peptides and proteins will emerge the pharmaceutical research by circumventing the disadvantages along with invasive route. Therefore it was aimed to investigate the impact of permeation enhancers ionic as well as non-ionic on peptide permeation through porcine abdominal skin.
Methods: Tripeptide Leu-Gly-Gly was evaluated for is toxicity profile along with Caco-2 cells. Furthermore Leu-Gly-Gly was fluorescence labelled with Fluorescein-isothiocyanate. For permeation studies, porcine abdominal skin was mounted on Franz-diffusion cells and investigated due to the addition of 0.5% (w/w) anionic and non-ionic surfactants in comparison to tripeptide alone.
Results: The safety profile of tripeptide show no toxicity at all according to Resazurin assay. Pre-studies with sodium fluorescein set the parameter for peptide permeation studies. Tripeptide was successfully labelled with FITC determined by TNBS assay. Tween20 revealed the most promising results for permeation enhancing potential with a P_{app} of 2.78×10^{-6} cm/s.
Conclusion: Taking these findings in consideration, ionic surfactants are safe to use and exhibit the most beneficial permeation enhancing effect in peptide delivery via skin.

1. Introduction

Peptides and proteins gain a huge interest in the pharmaceutical technology sector [8]. A variety of therapeutic peptides and proteins such as insulin are needed for different medications and treatments. So far, peptides are peculiarly delivered invasively [2]. Being administered via the parenteral pathway, negative effects of oral delivery such as acidic degradation, first pass effect and denaturation are overwhelmed [9]. However, invasive delivery is associated with challenges first of all the administration which needs to be administered by professionals, secondly it is related to pain and thirdly leading to reduced patients compliance.

Therefore, there is a need for an innovative attempt of alternative administration route when thinking of peptide and protein delivery [11]. Transdermal or through the skin application exhibit plenty of benefits [18]. The skin is the most accessible and the largest organ of the human body. Moreover, this surface convince with its easy access. Topical formulations are comfortable, user-friendly, riskless and safe application procedure. Topical formulation are for example adhesive formulations such as transdermal patches, ointments, creams, liquid and solid formulations such as powder.

Advantages associated with topical application are the circumventing first pass effect, the overcoming acidic and enzymatic degradation as well as the painless application rendering this admiration route a well-accepted and comfortable one. But as there are always two sides of the same coin, the skin reveals remarkably limitations as main barrier, as it is semipermeable e.g. water outwards, but (im) permeable for debris inwards as it acts like a protection wall [5]. This barrier allows only small, potent and moderately lipophilic molecules to deepen layers of the skin [14]. For this reason, it was the aim of this study to utilize surfactants [20,21] being well known as permeation enhancers-for topical administration [15,16]. Surfactants in low concentrations— ionic as

☆ Novelty statement: Peptide delivery via skin is nowadays a key ambition in the pharmaceutical research. Within this study, a tripeptide was investigated for transdermal delivery by the addition of enhancers.

* Corresponding author. Department of Pharmaceutical Technology, Institute of Pharmacy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria.
E-mail address: Flavia.Laffleur@uibk.ac.at (F. Laffleur).

http://dx.doi.org/10.1016/j.jddst.2017.07.007
1773-2247/

well as anionic were investigated according to their permeation enhancing effect to deliver a tripeptide Leu-Gly-Gly.

2. Materials and methods

2.1. Materials

Tripeptide Leu-Gly-Gly (CAS: 1187-50-4), sodium dodecyl sulfate (SDS) (CAS 151-21-3), polyethylene glycol sorbitan monolaureate (Tween 20) (CAS 9005-64-5), polysorbate 80 (Tween 80) (9005-65-6), fluorescein sodium (SF) (CAS 518-47-8), fluorescein isothiocyanate (FITC) (CAS 3326-32-7), Resazurin salt, Minimum Essential Medium (MEM) and Triton®X-100 were obtained from Sigma-Aldrich (Vienna, Austria). All other reagents used were of analytical grade. Cell culture supplements were purchased from Biochrom AG, Germany. Multiwell plates and tissue culture flasks were received from Greiner bio-one, Austria. Caco-2 cells were purchased from the European Collection of Cell Culture (ECACC), England.

2.2. Methods

2.2.1. Preparation of the porcine skin

In order to perform diffusion studies through the skin, skin samples were prepared. Porcine abdominal skin was received from the slaughter house. Briefly, abdominal skin was shaved and washed with warm water. The submucosal tissue was removed and the skin section was prepared with a dermatome (GB 228R, Aesculap) set at 1.2 mm. Lastly, skin was cut in 2 × 2 cm^2 pieces while storing in the freezer at −24 °C until further use. One hour prior to experiments skin samples were thawed [17].

2.2.2. Sample preparation by labelling with FITC

Tripeptide as shown in Fig. 1 was dissolved in 0.1 M Na_2CO_3 at pH 9 in a concentration of 2 mg/mL. FITC was dissolved in DMSO (1 mg/mL). The labelling procedure followed a previously published by The et al. [19]. Aliquots of 500 μL of the FITC solution was added dropwise to the tripeptide solution. The solution was protected from light and stirred overnight. Afterwards Nh4Cl solution in a final concentration of 50 mM was added and stirred for 30 min. Labelled peptide was stored in the fridge until further use. Succeeded labelling was determined via TNBS assay.

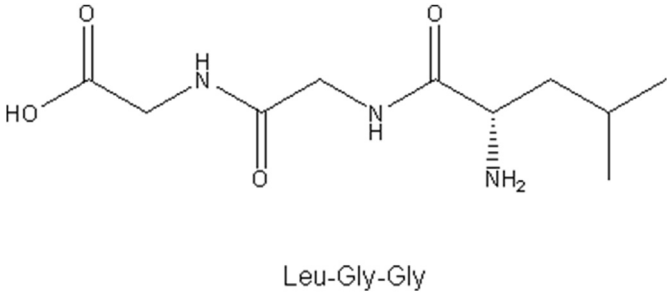

Fig. 1. Chemical structure of tripeptide- Leu-Gly-Gly.

2.2.3. TNBS assay

Primary amino content before and after labelling was determined by using a colorimetric assay with 2,4,6-trinitronenzenesuplphonis acid (TNBS) [3] as shown in Fig. 2.

2.2.4. Pre-study with sodium fluorescein

In order to set up the diffusion experiment with the Franz type cells 0.1% of sodium fluorescein solution was evaluated with respect to its permeation properties [12]. Acceptor chamber was filled with water and kept at 32 °C. Donor chamber was filled with 0.1% (w/w) of SF solution being separated by skin membrane from the receptor part. At predetermined time points 500 μL samples were taken and analyzed via fluorescence measurement (exc. 495 nm/em. 525 nm) with the TECAN infinite 200, Grödig, Austria.

2.2.5. Permeation using Franz −type diffusion cells

Diffusion studies were performed using Franz diffusion cells [6,12]. The permeation area is 1 cm^2. These cells comprise two chambers one acceptor and a donor chamber being separated by the porcine abdominal skin membrane. 1 mL of tripeptide solution with different surfactants in a concentration of 0.5% SDS, Span®20, and Span® 80, respectively, were applied to the donor chamber. These surfactants were investigated on their impact of permeation enhancement. Receptor chambers were mounted with 0.012 M phosphate buffer with pH 7.4. The Franz type diffusion cells were kept at 32 °C mimicking the physiological conditions while aliquots of 500 μL were taken at predetermined time points (0, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180 min) and replaced with fresh permeation buffer. The apparent permeability coefficients (P_{app}) for permeation were calculated according following equation:

$$Papp = \frac{Q}{A*c*t}$$

where Q represents the total amount permeated throughout the incubation time (μg), A expresses the diffusion area of the chambers (0.64 cm^2), c is the initial concentration in the donor chamber (μg/cm^3) and t is the time of permeation study (s).

2.2.6. Safety profile investigations

For safety profile Caco-2 cells of passages 25–50 were investigated [10]. Cells were kept in a humidified chamber with 5% CO_2. Growing cells were fed with Minimum Essential Medium Earle's comprising glutamine and 2.2 g/L $NaHCO_3$ supplemented with 100 μg/mL streptomycin, 100 μg/mL penicillin and 10% fetal bovine

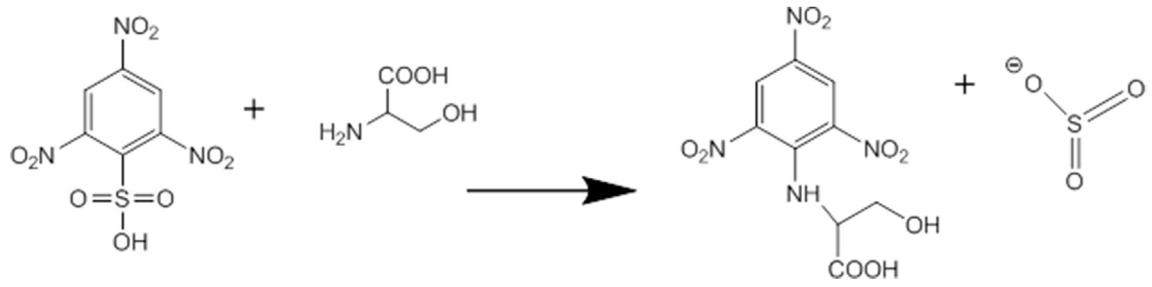

Fig. 2. Schematic overview of TNBS assay.