International Journal of Pharmaceutics 518 (2017) 59–65

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Cutaneous iontophoresis of μ-conotoxin CnIIIC—A potent $Na_V1.4$ antagonist with analgesic, anaesthetic and myorelaxant properties

Sergio del Río-Sancho[a], Cecile Cros[b], Bethsabée Coutaz[b], Muriel Cuendet[a], Yogeshvar N. Kalia[a,*]

[a] School of Pharmaceutical Sciences, University of Geneva & University of Lausanne, 1 Rue Michel-Servet, 1211 Geneva, Switzerland
[b] Activen SA, 2 Ruchonnet, 1003 Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 25 September 2016
Received in revised form 22 December 2016
Accepted 23 December 2016
Available online 26 December 2016

Keywords:
Biodistribution
Conopeptide
Cutaneous
Iontophoresis
Topical delivery
XEP-018

ABSTRACT

Cutaneous iontophoretic delivery of μ-conotoxin CnIIIC (XEP), a potent peptide antagonist of the $Na_V1.4$ sodium channel, was investigated using porcine ear skin and validated using human abdominal skin. Initial results demonstrated that cutaneous deposition of XEP following iontophoresis was superior to passive delivery and increased with current density. XEP deposition after iontophoresis at 0.1, 0.3 and 0.5 mA/cm^2 for 2 h and 4 h was 22.4 ± 0.4, 34.5 ± 1.4, 57.4 ± 7.6 μg/cm^2 and 30.6 ± 5.4, 53.9 ± 17.2, 90.9 ± 30.8 μg/cm^2, respectively (cf. corresponding passive controls – 9.8 ± 1.1 and 16.9 ± 1.0 μg/cm^2). Moreover, tape-stripping studies showed that XEP was mainly adsorbed on the skin surface when administered passively. Co-iontophoresis of acetaminophen demonstrated that XEP was present in the skin as it significantly reduced convective solvent flow as evidenced by the ~7-fold decrease in acetaminophen permeation. Shorter duration iontophoresis (15, 30 and 60 min) was performed and the effect of current density (0.1, 0.3 and 0.5 mA/cm^2) and concentration (0.1 and 1 mM) investigated. Skin deposition of XEP was already quantifiable after iontophoresis for 15 min at the lower concentration. There was no statistically significant difference between XEP deposition in porcine and human skin. Confocal laser scanning microscopy enabled post-iontophoretic visualization of FITC-labelled XEP in the epidermis.

1. Introduction

The novel μ-conotoxin CnIIIC (XEP), which is commercialized as XEP^{TM}-018, is a highly potent 22-residue conopeptide (Fig. 1), identified in marine snail venom (*Conus consors*; MW = 2375.73 Da), with myorelaxant, anaesthetic and analgesic properties (Favreau et al., 2012; Markgraf et al., 2012). It is a potent antagonist of the voltage-gated $Na_V1.4$ sodium channel with an IC_{50} of 1.3 nM acting at the neuromuscular junction. In addition to its potential pharmaceutical applications, it has already been used in aesthetic dermatology to smooth the appearance of fine facial lines, where it has been applied topically to the skin surface from a cream (Lirikos Marine Botoxin®; Amorepacific). A related conopeptide, ziconotide (Prialt®; Jazz Pharmaceuticals), which is a synthetic version of the naturally occurring ω-conotoxin MVIIA, present in the venom of the piscivorous marine cone snail *Conus magnus* (Duggan and Tuck, 2015; Lewis et al., 2012; Schroeder and Craik, 2012), is a potent $Ca_V2.2$ calcium channel blocker and it is administered intrathecally for the management of severe and chronic pain in patients refractory to systemic analgesics (McDowell and Pope, 2016; Pope and Deer, 2013). Given XEP's physicochemical properties – an isoelectric point (pI) of 8.29, a net positive charge of 3.9 at pH 5.6 and 2.3 at physiological pH (http://www.scripps.edu/~cdputnam/protcalc.html) and its hydrophilic surface (GRAVY (Grand Average of Hydropathy) value of −0.864 (Kyte and Doolittle, 1982), http://www.gravy-calculator.de – it is clear that its passive penetration into and diffusion across the stratum corneum is a challenge. However, its potency means that it is effective at very low concentrations and the presence of nanogramme amounts is sufficient to elicit effective blockade of the $Na_V1.4$ sodium channel and its pharmacological effect is facilitated

* Corresponding author at: School of Pharmaceutical Sciences, University of Geneva, CMU, 1 Rue Michel Servet, 1211 Geneva 4, Switzerland.
E-mail address: yogi.kalia@unige.ch (Y.N. Kalia).

http://dx.doi.org/10.1016/j.ijpharm.2016.12.054
0378-5173/

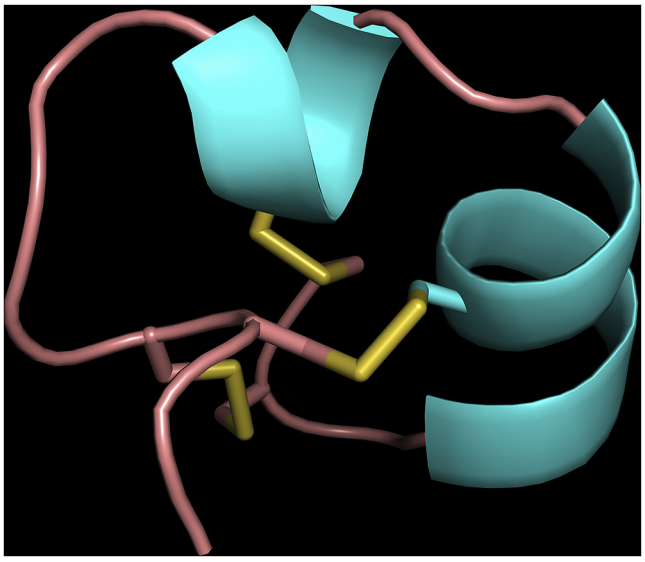

Fig. 1. 3D structure of XEP (2yen.pdb; (Favreau et al., 2012)).

by the relatively lower thickness and reduced barrier function of skin in the periocular region (Ha et al., 2005).

These same properties that make it a "difficult" candidate for passive delivery – i.e. good aqueous solubility and cationic character under physiological conditions – make it an interesting candidate for iontophoresis, a technique that employs a small electric potential to facilitate the electrotransport of water-soluble ionized species. There are two underlying electrotransport mechanisms: electromigration and electroosmosis. The former involves the ordered "diffusion" of the ions under the influence of the electric field whereas the latter is the result of the convective solvent flow across the negatively charged skin (pI of 4–4.5) (Gratieri and Kalia, 2013; Kalia et al., 2004; Marro et al., 2001; Pikal, 1990).

There has been considerable interest in the use of iontophoresis as a non-invasive alternative to the parenteral administration of therapeutic peptides (Gratieri et al., 2011). Previous reports from our laboratory have demonstrated that the technique can be used for the transdermal delivery of therapeutic amounts of triptorelin (Schuetz et al., 2005a) and vapreotide (Schuetz et al., 2005b) and even biologically active proteins (Cazares-Delgadillo et al., 2007; Dubey and Kalia, 2010, 2011, 2014; Dubey et al., 2011). These studies also demonstrated that in addition to transdermal permeation across the skin, there was considerable cutaneous deposition of the peptides and proteins. Indeed, in the lysozyme study, this skin retention was extremely pronounced and although the physicochemical properties of lysozyme suggested that it would be an excellent candidate for iontophoretic delivery, the experimental results showed that its permeation was much lower than anticipated (Dubey and Kalia, 2014). The accumulation of a peptide or protein in the skin might be beneficial in the sense that it may result in the formation of a cutaneous depot that facilitates sustained release for local action – e.g. in aesthetic or clinical dermatology; however, it might hinder transdermal delivery and so prevent systemic drug action.

In the case of XEP, given that one of the applications of interest is the relaxation of facial lines and the smoothing of the appearance of skin wrinkles, topical cutaneous delivery with retention in the membrane and the absence of any permeation would be highly desirable. Therefore, the aims of the present study were (i) to evaluate whether iontophoresis was able to enhance the transport of XEP in order to increase its cutaneous deposition, (ii) to investigate whether the effects could also be observed using short duration current application, (iii) to confirm the validity of the results obtained with porcine ear skin in experiments using human abdominal skin and (iv) to use confocal laser scanning microscopy to visualize the distribution of FITC-labelled XEP in the skin.

2. Materials and methods

2.1. Chemicals

XEP was provided by Atheris Laboratories (Geneva, Switzerland) and FITC-labelled XEP (FITC-XEP) was sourced from Peptides International, Inc. (Louisville, KY, USA). Acetaminophen (ACE), sodium chloride, and the silver wire and silver chloride used for the fabrication of electrodes, were purchased from Sigma–Aldrich (Buchs, Switzerland). Sodium chloride, potassium hydroxide (KOH), disodium hydrogen phosphate, 2-methylbutane (isopentane), acetic acid and Nile Red were also purchased from Sigma–Aldrich. Bisbenzimide H33258 (Hoechst Blue) was obtained from Applichem GmbH (Darmstadt, Germany) and OCT medium was bought from VWR International, Ptg Ltd. (Leuren, Belgium). Formic acid, MeCN and MeOH were obtained from Biosolve Chimie (Dieuze, France). Potassium dihydrogenphosphate and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) were purchased from Acros Organics (Chemie Brunschwig; Basel, Switzerland). PVC tubing (3 mm ID, 5 mm OD, 1 mm wall) used to prepare salt bridge assemblies was obtained from Fisher Bioblock Scientific S.A. (Illkirch, France). 13 mm Nylon and PTFE syringe filters (0.22 um) were purchased from BGB Analytik AG (Boeckten, Switzerland). Formaldehyde was purchased from Reactolab SA (Servion, Switzerland). All solutions were prepared using deionized water (resistivity >18 M Ω cm). All other chemicals were at least of analytical grade.

2.2. Skin preparation

Porcine ears were obtained from a local abattoir shortly after slaughter (CARRE; Rolle, Switzerland). After cleaning under cold running water, the skin from the outer region of ears was removed carefully from the underlying cartilage and trimmed with clippers to remove any excess hair. Skin was then either dermatomed (thickness 0.75 mm) with an air-dermatome (Zimmer; Etupes, France) or horizontally sliced with a Thomas Stadie-Riggs slicer (Thomas Scientific; Swedesboro, NJ, USA) to a thickness of 1.33 ± 0.53 mm.

Human skin samples were collected immediately after surgery from (i) the Department of Plastic, Aesthetic and Reconstructive Surgery, Geneva University Hospital (Geneva, Switzerland), (ii) Clinique Vert-Pré (Geneva, Switzerland) and (iii) Hôpital de la Tour (Geneva, Switzerland). The fatty tissue was removed and skin was excised with a surgical blade to a thickness of 1.65 ± 0.33 mm. In both cases, the skin was wrapped in Parafilm™ and stored in polyethylene bags at −20 °C for a maximum period of 2 months. The experiments with human skin samples were approved by (i) the Central Committee for Ethics in Research (CER: 08-150 (NAC 08-051); Geneva University Hospital) and (ii) the Commission d'Ethique pour la Recherche Clinique en Ambulatoire (Protocol 10-25; Association des Médecins du Canton de Genève et Société Médicale).

2.3. Iontophoresis experiments

2.3.1. Effect of current density on XEP transport

The experimental set-up used was similar to that described in earlier studies (del Río-Sancho et al., 2012; Kalaria et al., 2014). The skin samples were clamped in vertical two-compartment diffusion