International Journal of Pharmaceutics 474 (2014) 212–222

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Lysine-based surfactants as chemical permeation enhancers for dermal delivery of local anesthetics

Raquel S. Teixeira [a], Tânia F.G.G. Cova [b], Sérgio M.C. Silva [b], Rita Oliveira [c], Maria J. Araújo [d], Eduardo F. Marques [d], Alberto A.C.C. Pais [c], Francisco J.B. Veiga [a,*]

[a] Pharmaceutical Technology Department, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal
[b] Chemistry Department, Faculty of Science and Technology, University of Coimbra, 3004-535 Coimbra, Portugal
[c] Life Sciences Department, Fernando Pessoa University, 4249-004 Porto, Portugal
[d] CIQ-UP, Department of Chemistry and Biochemistry, Faculty of Science, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form 1 August 2014
Accepted 2 August 2014
Available online 7 August 2014

Keywords:
Dermal drug delivery
Chemical permeation enhancers
Cytotoxicity
Tetracaine
Ropivacaine
Molecular dynamic simulation

ABSTRACT

The aim of this study is to investigate the efficacy of new, biocompatible, lysine-based surfactants as chemical permeation enhancers for two different local anesthetics, tetracaine and ropivacaine hydrochloride, topically administered. Results show that this class of surfactants strongly influences permeation, especially in the case of the hydrophilic and ionized drug, ropivacaine hydrochloride, that is not easily administered through the stratum corneum. It is also seen that the selected permeation enhancers do not have significant deleterious effects on the skin structure. A cytotoxicity profile for each compound was established from cytotoxicity studies. Molecular dynamics simulation results provided a rationale for the experimental observations, introducing a mechanistic view of the action of the surfactants molecules upon lipid membranes.

1. Introduction

The stratum corneum (SC) is the outermost layer of the skin. It is a highly organized structure that constitutes the major barrier to the permeation of drugs topically applied. In order to reversibly alter the barrier properties of the SC, and consequently increase the efficacy of the delivery of drugs across the skin, many strategies have been employed (Madison, 2003). Chemical penetration enhancers (CPE) are compounds that are known to have the ability of reducing the barrier properties of the SC and also increasing the partition of the drugs to the skin (Asbill and Michniak, 2000; Bach and Lippold, 1998; Finnin and Morgan, 1999). However, the practical use of CPE requires a careful balance between the toxicity to the skin and permeation enhancement benefits. Surfactants comprise a broad class of amphiphilic compounds, with surface-active properties, consisting of a lipophilic chain linked to a hydrophilic headgroup. These compounds are usually added to formulations to solubilize lipophilic active ingredients and therefore have a great potential to be used as permeation enhancers. Some surfactants are used alone as CPE, or in combination with other active methods (Ashton et al., 1992; Babu et al., 2005; Cappel and Kreuter, 1991; Kitagawa and Kasamaki, 2000; Nokhodchi et al., 2003; Shin et al., 2001; Shokri et al., 2001; Tan et al., 1993; Walters et al., 1993). Surfactants have, in general, a low chronic toxicity (Williams and Barry, 2012), and the major concerns on its use in dermal delivery are associated to the possible occurrence of local irritation, erythema or itching, depending on the amphiphilic structure and concentration employed (Effendy and Maibach, 1995; Naik et al., 2000; Scheindlin, 2004).

Surfactants have often been used either incorporated in a vehicle or dissolved in a solvent system, and their activity has been found to be dependent on its lipophilicity, charge and chain length (Karande et al., 2005). Nonionic surfactants are generally regarded as safe, but reputedly at least as irritating as cationic surfactants, a fact which has not excluded its use in cosmetic formulations. Nonionic surfactants have also been reported as possessing antibacterial properties (Schott, 1985; Shukla and Tyagi, 2006).

Amino acid-based surfactants, in particular, have attracted considerable interest because they are environmentally friendly (Moran et al., 2004) due to their enhanced biodegradability (Brito et al., 2009; Infante et al., 2004) and low ecotoxicity (Perez et al., 2005), and also for displaying a moderate human cytotoxicity, as determined by their alkyl chain length and headgroup properties (Macián et al., 1996; Sanchez et al., 2006; Silva et al., 2013b). It has been found that these class of surfactants

* Corresponding author. Tel.: +351 239488434.
E-mail address: fveiga@ci.uc.pt (F.J.B. Veiga).

http://dx.doi.org/10.1016/j.ijpharm.2014.08.002
0378-5173/

exhibit lower toxicity when compared with conventional surfactants, making them promising alternatives to commercially available anionic and nonionic surfactants in pharmaceutical and cosmetic formulations (Infante et al., 2004, 2010; Sanchez et al., 2004, 2006; Vives et al., 1997, 1999). Additionally, these surfactants seem to be promising candidates to be used in topical pharmaceutical formulations, since they are less irritating and more biodegradable than the commercially available surfactants already tested (e.g., hexadecyltrimethylammonium bromide and sodium dodecyl sulphate) (Doi et al., 2001; Sanchez et al., 2004).

Unlike conventional surfactants that are composed by a single hydrophobic tail connected to a polar headgroup, the lysine-based surfactants employed in this work consist of double-chained amphiphiles with a gemini-like configuration (Brito et al., 2008, 2013). In fact, as can be seen in Fig. 1a, the lysine side chain (a four-methylene group) acts as a spacer group between the two (dissimilar) polar regions, which imparts the surfactants with a dimeric type structure. The molecules used are either anionic sodium alkylcarboxylates with C10, C12, C14 and C16 long alkyl chains, or nonionic ester derivatives with C10 and C16 chains (Fig. 1b).

The efficacy of these lysine-based surfactants as CPE across newborn porcine skin was investigated following a methodology reported elsewhere (Silva et al., 2012). For this purpose, two drugs with distinct physicochemical properties have been selected: tetracaine (non-ionized and hydrophobic) and ropivacaine hydrochloride (ionized and hydrophilic). Tetracaine is a potent amino ester type local anesthetic, indicated for surface anesthesia and spinal block. However, due to its high systemic toxicity, the use in other anesthetic techniques is limited. Tetracaine displays pK_a values of 2.24 and 8.39, and a log *P* value of 2.79 which, compared to other anesthetics, makes this molecule a suitable candidate for skin permeation (Covino and Giddon, 1981). Ropivacaine hydrochloride is a long-acting local anesthetic of the amide type, well tolerated for regional anesthesia, being effective for surgical anesthesia as well as for relief of postoperative and labor pain. Ropivacaine is a white, crystalline powder and is less lipophilic than tetracaine, which diminishes its ability to penetrate the skin (McClellan and Faulds, 2000; McCrae et al., 1995; Zink and Graf, 2004).

The action of lysine-based surfactants as CPE was firstly evaluated for both drugs. The permeation *in vitro* studies were rationalized by molecular dynamics simulation, as a computational approach. Light microscopy and scanning electron microscopy (SEM) techniques were employed to assess morphological changes of the skin in the presence of the CPE. Cytotoxic studies were subsequently performed in cultured human epidermal keratinocytes (HEK), establishing a concentration-toxicity response. Data collected from several complementary techniques thus provide new insight on the use of novel lysine-based surfactants as penetrations enhancers, which is crucial for their prospective use as delivery agents.

2. Experimental

2.1. Materials

Tetracaine base, ammonium acetate, propylene glycol (PG) (Reagent Plus, 99%), sodium bicarbonate, glucose, penicillin G potassium salt and streptomycin were purchased from Sigma–Aldrich (Saint Louis, MO, USA). Hydrochloride ropivacaine was a kind gift from AstraZeneca (London, United Kingdom), and hydroxypropylmethylcellulose (HPMC K15M) a kind gift from Dow Chemical Company (Midland, MI, USA). Hydroxypropyl beta-cyclodextrin (HP-β-CD) with 97% of purity, with an average degree of substitution of 2–6 units of 2-hydroxypropyl (C_3H_7O) per glucose unit, and with an average molecular weight of 1380 g/mol, was purchased from Acros Organics (Geel, Belgium). Methanol (MeOH) and acetonitrile (ACN) HPLC grade were purchased from LiChrosolv Merck (Darmstadt, Germany). Phosphate buffer saline tablets (PBS) were purchased from TIC Gums (Belcamp, MD, USA). Newborn porcine skin tissue was obtained from young pigs (3 weeks, ~5 kg, acquired at a local slaughterhouse). The lysine-based surfactants used in this study were synthesized and purified according to a method described in previous works (Brito et al., 2006; Gomes et al., 2008). The purity of the compounds was determined by NMR, elemental analysis, surface tension and differential scanning calorimetry (Brito et al., 2006, 2008; Gomes et al., 2008). The anionic lysine based surfactants, comprising 10, 12, 14 and 16 carbon atoms are referred in this manuscript as 10Lys10(−), 12Lys12(−), 14Lys14(−) and 16Lys16(−), in an obvious notation, while the nonionic ones are referred as 10Lys10(0), 16Lys16(0). The Tissue-Tek® O.C.T™ compound was purchased from VWR international (Radnor, Pennsylvania, USA). Formaldehyde solution min. 37% was purchased from Merck (White House Station, NJ, USA). The Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and trypsin were purchased from Invitrogen™ (Carlsbad, CA, USA). Human epidermal keratinocytes (HEK) were obtained from DKFZ (Im Neuenheimer Feld, Heidelberg, Germany). Finally, the AlamarBlue® cell viability reagent was obtained from Thermo Scientific (Waltham, MA, USA). All reactants were used as received.

2.2. Porcine skin preparation

Newborn porcine skin tissue used as skin model, was excised and dermatomed using a Padgett® Model B Electric Dermatome (Integra LifeSciences, Plainsboro, NJ). The skin samples with 700 μm thickness were stored at −20 °C for not more than 3 months. Prior to the experiments, skin samples were thawed at room temperature, before immersion in PBS (pH 7.4) for 1 h, for equilibration.

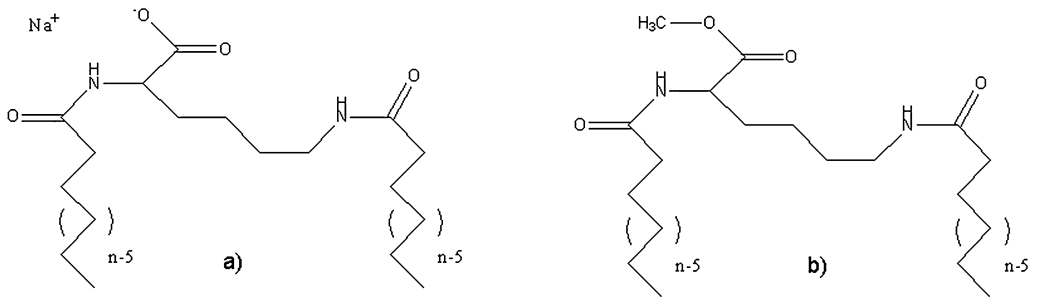

Fig. 1. Structure of anionic lysine-based surfactants (a), *n*Lys*n*(−), where *n* is the alkyl chain length (*n* = 10, 12, 14, 16) and nonionic lysine-based surfactants (b) *n*Lys*n*(0), where *n* is 10 and 16.