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Glycerol and urea can be used to increase skin permeability in reduced hydration conditions

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ABSTRACT

The natural moisturizing factor (NMF) is a group of hygroscopic molecules that is naturally present in skin and protects from severe drying. Glycerol and urea are two examples of NMF components that are also used in skin care applications. In the present study, we investigate the influence of glycerol and urea on the permeability of a model drug (metronidazole, Mz) across excised pig skin membranes at different hydrating conditions. The degree of skin hydration is regulated by the gradient in water activity across the membrane, which in turn depends on the water activity of the formulation in contact with the skin membrane. Here, we determine the water activity of all formulations employed using an isothermal calorimetric method. Thus, the gradient in water activity is controlled by a novel experimental set-up with well-defined boundary conditions on both sides of the skin membrane. The results demonstrate that glycerol and urea can retain high steady state flux of Mz across skin membranes at dehydrating conditions, which otherwise would decrease the permeability due to dehydration. X-ray diffraction measurements are performed to give insight into the effects of glycerol and urea on SC molecular organization. The novel steady state flux results can be related to the observation that water, glycerol, and urea all affect the structural features of the SC molecular components in a similar manner.

1. Introduction

Transdermal drug delivery is an attractive alternative to oral drug delivery because it avoids first pass metabolic degradation (Prausnitz and Langer, 2008). Optimization of transdermal drug delivery applications include considerations of interactions within the formulation as well as interactions between formulation ingredients and the molecular components of the skin barrier (Barry, 2001). After application of a transdermal or topical formulation onto the skin surface, several new gradients across the skin membrane are established, which may affect the properties of the skin barrier. The understanding of how the skin barrier is affected by changes in physical and chemical gradients is therefore highly relevant for the development of transdermal drug delivery systems. We have previously demonstrated that changes of a gradient in water activity across the skin membrane, which effectively determines the degree of skin hydration, can be used as a switch to regulate the skin permeability to model drugs with different lipophilic characteristics (Björklund et al., 2010). The proposed explanation for these observations is that changes in the water gradient can induce reversible structural alterations in SC lipid or protein components, which can lead to drastic changes in the transport characteristics (Björklund et al., 2010; Björklund et al. 2013a; Sparr and Wennerström, 2001). In the present study we explore the effect of glycerol and urea on the permeability of skin membranes, which are also exposed to a gradient in water activity.

The outermost layer of skin is called the stratum corneum (SC) and constitutes the main barrier towards both inward and outward diffusional transport (Scheuplein and Blank, 1971). The barrier properties of SC are assured by its organization of corneocytes embedded in a multilamellar lipid (Madison et al., 1987; Weerheim and Ponec, 2001). The corneocytes are packed with keratin filaments that are enclosed by the cornified cell envelope (Candi et al., 2005). Despite that SC normally experience low relative humidity (RH), the exposure to very dry environments can lead to defective skin conditions (e.g., winter xerosis). The continuous hydration of SC from the inside of the body is therefore crucial for maintaining healthy skin as water regulates, for example, SC flexibility (Blank, 1953) and enzymatic reactions in SC (Harding et al., 2000). Moisturizers are substances commonly used for

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treatment or prevention of defective dry skin conditions to make the SC more soft and pliable. Humectants comprise a subclass of moisturizers encompassing small polar molecules with hygroscopic properties. Humectants are also naturally present in SC, referred to as the natural moisturizing factor (NMF), which is a mixture of free amino acids and their derivatives, inorganic salts, lactic acid, urea, and glycerol (Choi et al., 2005; Harding et al., 2000). There is a well-regulated interplay between the water gradient in SC and the filaggrin-degradation into NMF components (Harding et al., 2000) and the importance of the NMF molecules is illustrated by the noticeable correlation between the absence of the NMF and conditions of SC abnormality (Marstein et al., 1973; Sybert et al., 1985).

Glycerol and urea are also used in commercial skin care lotions and creams where the beneficial function of these compounds is ascribed to their hygroscopic properties, as the suggested role for NMF. Still, it is clear that the barrier function as well as the mechanical properties of SC do not only depend on its water content, but more important, on the state and molecular organization of non-aqueous SC lipid and protein components. These properties can be affected by hydration (Alonso et al., 1996; Björklund et al., 2010; Björklund et al., 2013a; Blank et al., 1984; Nakazawa et al., 2012; Ohta et al., 2003), and also by the addition of other small polar molecules. For example, the presence of glycerol (10 wt%) in hydrated model skin lipids in a liquid crystalline state impede the transition into a crystalline state at dry conditions (6% RH), as compared to the same lipid mixture in the absence of glycerol (Froebe et al., 1990). In previous studies, we have shown that osmolytes like glycerol and urea can stabilize fluid structures in phospholipid bilayer systems at low RH where the lipids would form solid bilayer structures in the absence of these osmolytes (Costa-Balogh et al., 2006; Nowacka et al., 2012). These observations indicate that glycerol and urea can maintain the physical properties of hydrated lipid systems under dry conditions. It is also possible that a similar mechanism can act on the SC molecular components if these molecules are present inside SC under dehydrating conditions.

In this study, we explore the influence of glycerol and urea on the *in vitro* permeability of excised skin membranes and the molecular structure of SC at varying hydrating conditions. We use an experimental set-up of flow-through diffusion cells, where we have control of the boundary conditions and steady state conditions, to study the situation of opposite gradients in water and humectant across the skin membrane. In the absence of glycerol or urea, a decrease in water activity on the upper side of the skin membrane would lead to dehydration, a reduction of the fraction of fluid SC components (Björklund et al., 2013a), and ultimately a decrease in the skin permeability (Björklund et al., 2010). However, the addition of humectant to the same side of the membrane may prevent the transition from fluid to solid structures and thus retain the permeability of a hydrated skin membrane. To investigate this hypothesis, we study diffusional transport of a model drug (metronidazole, Mz) through pig skin membranes *in vitro* where we control both the gradient in water activity and the gradient in either glycerol or urea. Further, we correlate the effect of glycerol and urea on the skin permeability with their influence on the molecular organization of the SC lipid lamellar structures and the soft keratin proteins by performing small- and wide-angle X-ray diffraction measurements.

2. Materials and methods

2.1. Chemicals

Metronidazole (Mz) was purchased from Mediolast (Milan, Italy). Poly(ethylene glycol) 1500 Da (ultragrade) (PEG), glycerol, urea, trypsin, and methanol were obtained from Sigma–Aldrich. NaCl, $Na_2HPO_4{\cdot}2H_2O$, KH_2PO_4 were obtained from Merck.

2.2. Preparation of skin and silicone membranes

Pig ears were obtained fresh from a local abattoir (Dalsjöfors slakteri, Sweden) and frozen at −80 °C until use. Split-thickness skin membranes (approx. 500 μm thick) were prepared from tissue of the inside of the outer ear by using a dermatome (TCM 3000 BL, Nouvag). Circular membranes (16 mm in diameter) were cut out to fit the diffusion cells (9 mm in diameter). Circular silicone membranes (Speciality Manufacturing, Michigan, USA) were used for reference purposes to confirm that all donor formulations had the same release rate of Mz.

2.3. Preparation of SC

Strips of dermatomed pig ear were placed, dermal side down, on filter paper soaked in 0.2% trypsin in PBS solution for 12 h at 4 °C. Next, the SC was removed with forceps and washed in PBS solution. The SC was rubbed with cotton tipped applicators to remove tissue not belonging to SC and further washed in PBS solution. The SC was dried in vacuum and stored in refrigerator until use.

2.4. Model drug formulations

The model drug used in this work was Mz, which is an antibiotic drug used in commercial formulations for e.g. treatment of the skin disease rosacea. It has low molecular weight (171 g mol^{-1}), is non-charged in the present experimental conditions, and partition approx. equally in octanol and water (log $P_{o/w} = 0$ (Kasprzyk-Hordern et al., 2007)). All Mz formulations were prepared in phosphate buffered saline, PBS (130.9 mM NaCl, 5.1 mM $Na_2HPO_4{\cdot}2H_2O$, 1.5 mM KH_2PO_4, pH 7.4) and varying concentrations of glycerol or urea with or without PEG. The molecular weight of the polymer used in this work is $MW_{PEG} \sim 1500$ Da, which corresponds to roughly $n = 34$ where n is the number of ethylene oxide units according to $H(OCH_2CH_2)_nOH$. The reason for using this particular size is that it is small enough to allow for a considerable decrease in water activity, while at the same time being sufficiently large to assure that the polymer does not penetrate into the skin membrane (Albèr et al., Unpublished results; Tsai et al., 2001, 2003). Thus, the addition of this polymer only act as a dehydrating agent, in analogy with osmotic stress technique measurements (LeNeveu et al., 1976).

2.5. Uniform release rate of the model drug from all formulations

The release rate of Mz from the formulation depends on the chemical potential (activity) of the model drug in the formulation, which is strongly related to the formulation composition. We aim at an experimental set-up where the chemical potential of Mz is the same in all formulations. As we cannot get direct experimental data on the chemical potential of Mz, we use an approximate condition by adjusting the concentration in relation to the total solubility in each formulation. The solubility of Mz was determined for all formulations in three replicates following the procedures in (Björklund et al., 2010). The solubility data are summarized in Table 1. The drug concentration in each formulation was then adjusted by multiplying the total Mz solubility with an arbitrary factor so that the concentration in neat PBS solution was 0.75 wt% (7.5 mg ml^{-1}), which is the concentration used in several commercial topical formulations containing Mz (e.g. Rosex cream and Rosex gel, Galderma Nordic AB). This procedure, i.e. to adjust the Mz concentration to achieve similar chemical potential of Mz, is