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Mechanism study on ion-pair complexes controlling skin permeability: Effect of ion-pair dissociation in the viable epidermis on transdermal permeation of bisoprolol

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ABSTRACT

Though ion-pair strategy has been widely used in transdermal drug delivery system, knowledge about the molecular mechanisms involved in the skin permeation processes of ion-pair complexes is still limited. In the present study, a homologous series of fatty acids were chosen to form model ion-pair complexes with bisoprolol (BSP) to rule out the influence of functional groups on polar surface area, stability and other physicochemical properties of ion-pair complexes. The ion-pair complexes were characterized by FTIR, thermal analysis, and ^{1}H NMR. The skin permeability of BSP as well as its ion-pair complexes was investigated by *in vitro* skin permeation experiments then visualized by CLSM. The skin permeability coefficient (k_p) of BSP ion-pair complex was negatively related to its *n*-octanol/water apparent partition coefficient ($P'_{o/w}$) in the hydrophobic vehicle caprylic/capric triglyceride, ($\log k_p = -1.657 - 1.229 \log P'_{o/w}$), suggesting that the instability of ion-pair complexes due to their dissociation in the viable epidermis (VED) played an important role in controlling the skin permeability of BSP, which was further proved by ^{1}H NMR and molecular docking. These findings broadened our understanding about the molecular mechanisms involved in the skin permeation processes of ion-pair complexes.

1. Introduction

Transdermal drug delivery system (TDDS), a successful controlled release technology, has been used to deliver a number of suitable drugs (Liu et al., 2016a,b; Lv et al., 2016; Malik et al., 2012; Weng et al., 2016; Wiedersberg and Guy, 2014; Zhang et al., 2014). Among the strategies used in TDDS, ion-pair, as a simple but effective approach plays an important role in dual-directional regulation of drug permeation profile. On one hand, for drugs whose skin permeability is poor, forming ion-pair complexes can increase their percutaneous absorption, thus enhance the effectiveness of their transdermal patch (Liu et al., 2016b; Xi et al., 2013). On the other hand, for drugs with extremely high skin permeability, their permeation amount can be down regulated to a moderate level by forming ion-pair complexes in order to prepare long-acting transdermal patches (Song et al., 2016, 2012).

Only a small number of studies have been carried out to investigate the mechanism of ion-pair complex on the skin permeability modulation of drugs. It was pointed out in the previous studies that polar surface area (Cui et al., 2015), stability (Xi et al., 2012b) and other physicochemical properties such as molecular weight and apparent partition coefficient (Song et al., 2016, 2012) were important factors influencing the skin permeability of ion-pair complexes. To build general permeability-structure relationships, ion-pair complexes with diverse functional groups were used in these studies. Nevertheless, it is logical to choose homologues as model ion-pair complexes in order to systematically discuss the mechanisms in their skin permeation processes. The homology in structure can rule out the influence of functional groups on polar surface area, stability, and other physicochemical properties, which would be extremely useful for revealing the underlying mechanisms involved in the permeation processes of ion-pair complexes.

Few studies focused on homologous ion-pair complexes. Ogiso mentioned the influence of homologous fatty acids on the skin permeability of propranolol (Ogiso and Shintani, 1990). The variable enhancing effects were roughly related to the difference in lipophilicity and skin affinity of the corresponding ion-pair complexes, without further determining their physicochemical properties. Megwa reported the enhancing effects of homologous tertiary amines on the skin permeation of salicylic acid (Megwa et al., 2000). Though the physicochemical properties of ion-pair complexes were measured, and the

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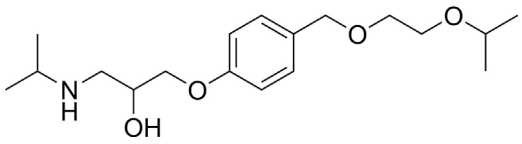

Fig. 1. Chemical structure of BSP.

different enhancing effects were attributed to the diversity in their partition coefficients, mechanisms involved in the permeation processes of ion-pair complexes were still unclarified.

In the present study, bisoprolol (BSP, Fig. 1), a basic drug which has been studied previously (Song et al., 2015, 2012) in our laboratory was used as model drug. A homologous series of saturated fatty acids (SFAs) were chosen to form model ion-pair complexes with BSP. Fourier transform infrared spectroscopy (FTIR), thermal analysis, and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy were used to confirm the formation of ion-pair complexes. The permeability of BSP and its ion-pair complexes through rat skin was investigated by *in vitro* permeation experiments, and was visualized by confocal laser scanning microscopy (CLSM). Mechanisms involved in the skin permeation processes of ion-pair complexes were investigated from three key aspects, including polar surface area, stability and other physicochemical properties of ion-pair complexes. The stability of ion-pair complexes in the VED was explored using ^{1}H NMR and molecular docking. What's more, molecular docking was also conducted to explain the permeability pattern showed in the skin permeation experiments of BSP ion-pair complexes. The aim of present work was to systematically elucidate the underlying molecular mechanisms involved in the skin permeation processes of BSP ion-pair complexes.

2. Materials and methods

2.1. Materials

Bisoprolol fumarate (BSP-F) was obtained from Dalian Meilun Biotech Co., Ltd. (Dalian, China). Caproic acid (C_6) was obtained from Shanghai Xinghuo Chemical Factory (Shanghai, China), capric acid (C_{10}) was obtained from Aladdin Industrial Inc. (Shanghai, China), caprylic acid (C_8), lauric acid (C_{12}), myristic acid (C_{14}), palmitic acid (C_{16}) and stearic acid (C_{18}) were purchased from Tianjin Bodi Chemical Holding Co., Ltd. (Tianjin, China). Caprylic/capric triglyceride (ODO) was purchased from Zhengzhou Dahe foods & T Co., Ltd (Zhengzhou, China). Nile red was obtained from Acros Organics (Geel, Belgium). HPLC grade acetonitrile was supplied by Yuwang Pharmaceutical Co., Ltd. (Shandong, China). All other chemicals were of the highest reagent grade available.

2.2. Preparation and characterization of BSP ion-pair complexes

2.2.1. Preparation of BSP ion-pair complexes

BSP free base was prepared from BSP-F according to the method described previously (Song et al., 2012). BSP free base and SFAs at molar ratio of 1:1 were adequately dissolved in acetone. The solution was stirred for 2 h at room temperature and then the solvent acetone was evaporated using a rotary evaporator. Subsequently, the obtained complexes were concentrated under vacuum until constant weight.

2.2.2. Characterization of BSP ion-pair complexes

The formation of BSP ion-pair complexes was characterized by FTIR. The study was carried out at room temperature, using KBr disk coating method in the transmission mode by FTIR spectrometer (Tensor 27, Bruker, Germany) with the help of OPUS 7.2 spectroscopy software. Free BSP, free SFAs, and their ion-pair complexes were separately dissolved in ethyl acetate. The obtained solution was dropped on the KBr disk surface, and ethyl acetate was evaporated. Pure KBr disk was used in the background scanning to eliminate its influence on the spectra. Spectra were obtained in the range of 4000 – 400 cm^{-1}.

2.3. Skin permeation experiments and CLSM study

2.3.1. Skin permeation experiments

Male Wistar rats weighing 180 – 220 g were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All the animal experiment procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals as well as the guidelines for animal use published by Life Science Research Center of Shenyang Pharmaceutical University. Skin preparation was performed according to the process reported previously (Jiang et al., 2014).

Skin permeation experiments were carried out at 32 ± 0.5 °C using horizontal diffusion cells. Thawed skin was placed between the cell halves with the stratum corneum side facing the donor compartment. Suspensions of BSP or its ion-pair complexes in ODO were applied in the donor compartment, and phosphate buffered saline (PBS, pH 7.4, 4.0 mL) was used as receptor medium. Excessive drug was maintained in the donor compartments to keep a constant drug concentration. Each side of the compartment was stirred with a magnetic bar. Sample (2.0 mL) was withdrawn from the receptor phase every 1 h over 8 h and replaced immediately with fresh PBS to maintain sink condition. The collected samples were centrifuged at 1 °C for 7 min, and the supernatants were diluted and analyzed by HPLC (L-2130 pump, L-2200 Auto Sampler, L-2420 UV detector, Hitachi Ltd., Tokyo, Japan) with a Diamonsil C18 reversed-phase column (200 mm * 4.6 mm * 5 μm, Dikma Technologies, Beijing, China). The mobile phase was consisted of acetonitrile and distilled water containing 0.1% triethylamine (23:77, v/v), pH adjusted to 3.0 by phosphoric acid, and detecting wavelength was set at 235 nm.

The slop of the linear portion of the 8 h cumulative permeated amount per unit area (Q_{8h}, μg/cm^2) versus time plot was presented as steady-state flux (J_{ss}, μg/cm^2/h) (Xi et al., 2012b).

The permeability coefficient (k_p, cm/h) was calculated as (Selzer et al., 2013),

$$k_p = J_{ss}/S \quad (1)$$

where S is the drug solubility in donor vehicle ODO.

2.3.2. CLSM study

CLSM study was performed to visualize the skin permeation process. Nile red fluorescent probe (NR) with equimolar C_6, C_{12}, or C_{18} was added into ODO, sonicated for 30 min then centrifuged, and the supernatants were used as donor solutions. NR in ODO without adding acid was used as control group. Full-thickness rat skin was mounted onto the Franz diffusion cells. The receptor compartment was filled with PBS, maintaining 32 ± 0.5 °C. Donor solution of 200 μL was applied to the skin, and was discarded after 30 min, then skin was washed with distilled water and wiped gently with filter paper to remove the excess donor solution on its surface. Skin sample was directly sandwiched between a coverslip and a glass slide. A laser scanning microscope (LSM 710, Carl Zeiss, Jena, Germany) was used in the imaging process. NR was excited at 514 nm wavelength using an argon laser. The detecting wavelength range was 539 – 753 nm. The imaging procedures were carried out according to our previous studies (Che et al., 2014; Cui et al., 2015).

2.4. Determination of physicochemical properties of BSP and its ion-pair complexes

2.4.1. Determination of apparent partition coefficient

The apparent partition coefficient ($P'_{o/w}$) in this paper was defined as the ratio of total equilibrium concentrations of BSP in the *n*-octanol/water system, with the results given in the form of its logarithm to base