International Journal of Pharmaceutics 529 (2017) 161–167

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

An insight into the molecular mechanism of the temporary enhancement effect of isopulegol decanoate on the skin

Xiaochang Liu[a], Meiying Liu[b], Chao Liu[a], Peng Quan[a], Yongshan Zhao[b,*], Liang Fang[a,*]

[a] Department of Pharmaceutical Science, School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang, Liaoning, 110016, China
[b] School of Life Science and Bio-Pharmaceutics, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang, Liaoning, 110016, China

ARTICLE INFO

Article history:
Received 24 February 2017
Received in revised form 25 May 2017
Accepted 9 June 2017
Available online 10 June 2017

Keywords:
Temporary enhancement effect
Molecular mechanism of skin recovery
Transdermal chemical enhancers
Molecular dynamic simulation
Intercellular lipid bilayer

ABSTRACT

Chemical enhancers are widely used to facilitate drug permeation in transdermal drug delivery system (TDDS) and the effect of chemical enhancers is desired to be temporary. Though temporary enhancement effect of chemical enhancers has been widely discussed, there is still a lack of knowledge about the molecular mechanism of temporary enhancement effect. Using the skin permeation of flurbiprofen as a probe, the temporary enhancement effect of isopulegol decanoate (ISO-10) was evaluated with *in vitro* permeation experiment and confocal laser scanning microscopy (CLSM). In addition, molecular mechanism of skin recovery was explored with skin retention of ISO-10, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), molecular dynamic (MD) simulation and transepidermal water loss (TEWL). Temporary enhancement effect of ISO-10 was observed by the permeation of flurbiprofen after the treatment of 180 min. Furthermore, temporary enhancement effect of ISO-10 on the diffusion of intercellular lipid in the stratum cornuem (SC) was observed by ATR-FTIR, molecular dynamic (MD) simulation. The SC barrier function recovered with the existence of ISO-10 in the lipid bilayer as indicated by the retention study and TEWL. In conclusion, the lipid bilayer accepted the enhancer as a new component to form a new stable arrangement, resulted the recovery of the skin barrier function. This work processed a novel mechanism of the recovery of skin barrier function after the addition of chemical enhancers.

1. Introduction

Transdermal drug delivery system (TDDS) is playing an important role in current controlled drug delivery systems (Rai et al., 2016; Serpe et al., 2016; Strasinger et al., 2016). This non-invasive drug delivery system provides many advantages, such as good patient compliance, stable plasma drug concentration and avoiding first–pass metabolism (Gratieri et al., 2013). However, the most outer layer of the skin, the stratum corneum (SC), is the major barrier of drug permeation. The SC is a thin (about 15–20 μm) layer and it is comprised of keratinized epidermal cells that are embedded within a lipid matrix with a brick and mortar organized structure (Liu et al., 2016; Elias, 1983). Because of formulation flexibility and easy processing, chemical enhancers are most commonly used excipients to facilitate drug permeation in transdermal preparations (Lane, 2013). To date, hundreds of substances are synthesized to enhance drug permeation across the skin (Marwah et al., 2016).

Although lots of substances are reported to show excellent enhancement effect on transdermal drug delivery, the associated safety problems are still barriers to their applications (Mohammed et al., 2014; Welss et al., 2004). An ideal enhancer is also supposed to be reversible, non-toxic and non-irritated (Williams and Barry, 2012). Some solvent enhancers, such as high concentration of DMSO, induce significant irritation and irreversible effect on the skin. The first synthesized enhancer – Azone was reported as an effective chemical enhancer by many studies, but has never been used commercially because of safety issues (Lane, 2013). Therefore, the evaluation of the safety was one of most important parts in the development of chemical enhancers. Whether the chemical enhancers can be used in clinical depend on if their effect on the skin barrier function was temporary (Kang et al., 2007). Irreversible effect may disturb the physiological function of the skin and increase the risk of infection. Thus, the temporary

* Corresponding authors.
E-mail addresses: zhao09081@163.com (Y. Zhao), fangliang2003@yahoo.com (L. Fang).

http://dx.doi.org/10.1016/j.ijpharm.2017.06.023
0378-5173/

enhancement effect is an important property of chemical enhancers and is a preliminary indicator of safety.

Temporary enhancement effect of chemical enhancers has been reported in several studies, but there is nearly no research focused on the molecular mechanisms of the temporary enhancement effect. (Jiang et al., 2014; Novotný and Kovaříková, 2009; Zhao et al., 2001). Theoretically, except for permanent damages which can only be repaired by the SC regeneration, most temporary disorder of the skin caused by chemical enhancers can be repaired through the rapid recovery of the skin barrier function (Scheuplein and Ross, 1970). Zhao et al. (Zhao et al., 2001) discussed that actively metabolizing skin repairs itself within a time period or the skin removes the enhancer through some enzymatic or other neutralizing pathway. However, the hypotheses have not been proved yet, and there is still a lack of evidence of how the skin recovers its barrier function. Therefore, the question of what the molecular foundation of the temporary enhancement effect was arisen.

In the present study, we investigated the temporary enhancement effect of isopulegol decanoate (ISO-10) with *in vitro* permeation study and confocal laser scanning microscopy (CLSM). Based on the temporary enhancement effect of ISO-10, molecular mechanism of how the skin recovered its barrier function was further explored with skin retention study, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), molecular dynamic (MD) simulation and transepidermal water loss (TEWL).

2. Materials and methods

2.1. Materials

Flurbiprofen (FP) was supplied by Shanghai Haiqu Chemical Co., Ltd. (Shanghai, China). PSA DURO-TAK® 87–4098 was obtained from Henkel AG & Co. KGaA (Düsseldorf, Germany). Adhesive tape Micropore™ 1530C-0, release liner ScotchPak™ 9744 and backing membranes ScotchPak™ 9700 were bought from 3 M Co. (St. Paul, USA). ISO-10 was prepared with the methods previously reported by our group (Zhao et al., 2009). Isopropyl myristate (IPM) was purchased from China National Medicines Co., Ltd. (Shanghai, China). Fluorescein was provided by AXROS Organics (New Jersey, USA). Methanol, ethanol and ethyl acetate of HPLC grade were obtained from Concord Technology Co., Ltd. (Tianjin, China). All of other reagents were of reagent grade at least.

Male Wistar rats (180–220 g, 6–8 weeks old) were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. All the procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Shenyang Pharmaceutical University (No.SYPU-IACUC-C2015-0421-202).

2.2. In situ *skin treatment*

The rats were anesthetized with urethane (6 mL/kg, 20% w/v, i.p.). After fastened up with abdomen upward, hair on the abdominal area was trimmed and shaved without damaging the skin. After washed and dried carefully, the skin was assigned into two sections of 0.95 cm^2. One was used as the control and the other one was treated with ISO-10. Pure ISO-10 of 5.0 μL was spread uniformly over the marked shaved area. The skin was disposed for 5, 30, 60, 180, 300, 480, 720, 960, 1200, 1440 min in different groups ($n = 4$). The residual enhancers on the surface were removed carefully and completely (Hoppel et al., 2015) after the treatment. Then, full-thickness skins of rats were excised from the treated sites with scissors after the rat was sacrificed. The adhering subcutaneous tissues were separated carefully with tweezers. All of this work was in a windless house of 22 ± 2 °C.

2.3. The temporary enhancement effect of ISO-10 on the skin permeation

2.3.1. In vitro *permeation experiments*

Modified side-by-side diffusion cells with an effective diffusion area of 0.95 cm^2 were applied to *in vitro* permeation study (Chen et al., 2015). FP patch of 10% drug loading (w/w) was prepared with the solvent evaporation technique. The prepared patch was pasted on the skin (pretreated in Section 2.2) with the adhesive side facing to the SC. After securely clamping the cells together, the receiver compartment was filled with phosphate buffer solution (pH 7.4) of 4.0 mL. At predetermined time points (2, 4, 6, 8, 10 and 12 h), receptor solution of 2.0 mL was withdrawn and the same volume of fresh receptor medium was added to the receiver compartment in order to maintain sink conditions. The samples were analyzed by HPLC and the method was described in our previous works (Chen et al., 2014).

The cumulative penetrated amount per unit area (Q_{12h}, μg cm^{-2}) *versus* time was plotted. Enhancement ratio (*ER*) was used to evaluate the activity of the enhancers.

$$ER = Q_{12h}(\text{with enhancer})/Q_{12h}\ (\text{without enhancer}) \quad (1)$$

The *ER* of control group at t min ($ER_{control,tmin}$) was the ratio of cumulative penetrated amount of the control group at t min ($Q_{control,tmin}$) and the average cumulative penetrated amount of the control group at 5 min ($Q_{control,5min}$).

2.3.2. Confocal laser scanning microscopy (CLSM)

The experimental procedures and the parameter settings were described by our group before (Chen et al., 2014). An excess amount of fluorescein was added into IPM to prepare saturated fluorescein solution. After sonication for 10 min and centrifuged at 5000 rpm for 5 min, the supernatant was filtered with a 0.45-μm filter. The prepared skin (treated with ISO-10 for 5 min, 180 min and 1440 min in Section 2.2) was mounted onto a modified Franz diffusion cells (with a volume of 7.0 mL). 200 μL of fluorescein solutions were added into the donor cells and PBS was added into receptor cells. After a 20 min exposure, the solution was removed carefully. Then, the skin surface was washed gently with diluted water and dried with filter paper. A LSM 710 Laser Scan Microscope (Carl Zeiss, Jena, Germany) was used for the skin imaging. The fluorescein was excited using an argon laser with a wavelength of 488 nm. Z stacks of the samples were taken from the SC to the dermis.

2.4. The changes of the skin barrier function

2.4.1. ATR-FTIR

IR-spectra were collected using a FTIR instrument equipped with an ATR tool and a MCT detector (Bruker, Ettlingrn, Germany). Rat skin samples (pretreated in 2.2) were placed with the SC facing to the zinc selenide (ZnSe) crystal which was set at an incidence angle of 45°. The spectra were collected over the wavenumber region of 4000–700 cm^{-1}. The peak position was analyzed using Peakfit 4.12 software.

2.4.2. Molecular dynamic (MD) simulation

The initial structure of ISO-10 was built using Discovery Studio Viewer and subjected to energy minimization with the software 09 (Gaussian et al., 2009). The atomic charges prepared by PRODRG server (Schüttelkopf and Van Aalten, 2004) were modified by B3LYP quantum mechanical calculation. The initial coordinates of