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Transdermal enhancement effect and mechanism of iontophoresis for non-steroidal anti-inflammatory drugs

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ABSTRACT

Iontophoresis is an important approach to improve transdermal drug delivery. However, The transdermal enhancement mechanism of iontophoresis was not well known. The relationship between the physicochemical properties of drugs and the transdermal enhancement effect of iontophoresis was revealed in this study. Non-steroidal anti-inflammatory drugs (NSAIDs) were used as the models, including aspirin, ibuprofen and indomethacin. Their oil-water partition coefficients were measured. The carbomer-based hydrogels of them were prepared. Iontophoresis significantly enhanced *in vitro* transdermal delivery across the rat skins. Strong lipophilicity could lead to high permeation of drugs. However, the dissociation extent (indicated as pK_a) of drugs was the key factor to determine the transdermal enhancement effect of iontophoresis. The more dissociation the drugs were, the higher the transdermal enhancement effect of iontophoresis. The drug-loaded hydrogels combined with iontophoresis improved the treatment of rat raw's inflammatory syndrome. Iontophoresis significantly improved the drugs penetrating into the hypodermis, dermis and epidermis, more deeply than the application of drugs alone according to the experimental result of 5-carboxylfluorescein hydrogels. Iontophoresis led to the unordered arrangement of skin intercellular lipids, the significantly increased flowability and loose stratum corneum structure. Iontophoresis is a promising approach to improve transdermal drug delivery with safety and high efficiency.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one type of most used agents in clinic. Oral NSAIDs may lead to severe gastrointestinal tract side effects. Transdermal delivery of them is sometimes an appropriate alternative approach, such as aspirin (Kaushal et al., 2007), ibuprofen (Steffansen and Herping, 2008), indomethacin (Chauhan et al., 2003). The major barrier for transdermal delivery is stratum corneum (SC). Some methods are used to overcome through the barrier, such as penetration enhancers (Choi et al., 2012), iontophoresis (Yamamoto et al., 2012), sonophoresis (Polat et al., 2010), electroporation (Tanaka et al., 2009).

Iontophoresis was used to improve the transdermal delivery of some drugs, such as ranitidine (Djabri et al., 2012b), phenobarbital (Djabri et al., 2012a), zidovudine (Oh et al., 1998). Commonly, charged drugs were placed on the same charged electrode. A small electrical current ($<0.5\,mA/cm^2$) with a low voltage through the skin is applied so that safety is ensured (Langer, 2004). The major advantages of iontophoretic delivery include safety, high transdermal efficiency, ease application and miniaturized instrument. However, some mechanisms were not well known. Why could iontophoresis improve transdermal efficiency? What's the relationship between iontophoretic delivery efficiency and the physicochemical properties of drugs? Three NSAIDs drugs were selected as the model drugs to explore the relationship, involving aspirin, ibuprofen and indomethacin. They have different pK_a, lipophilicity and molecular weight. The effect of iontophoresis on *in vitro* transdermal efficiency and *in vivo* anti-inflammatory effect

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were evaluated. The relationship between the physicochemical properties of drugs and the transdermal enhancement effect of iontophoresis was discussed. The transdermal enhancement mechanism of iontophoresis was explored by the fluorescence method and pathological analysis.

2. Materials and methods

2.1. Materials

Aspirin was purchased from Hexin Biologic Co., Ltd., (Zhengzhou, China). Ibuprofen was from Dahua Weiye Pharmaceutical Co., Ltd., (Wuhan, China). Indomethacin was from Xinjialing Biochemical Co., Ltd., (Wuhan, China). A type of carbomers, Carbopol 934PNF was from Noveon Inc. (Cleveland, USA). 5-Carboxylfluorescein (5-CF) was from Yunhui Biochemical Technological Co., Ltd., (Zibo, China). Carrageenan was from Spectrum Chemical Co., Ltd., (New Brunswick, CA, USA). Other reagents were of analytical grade.

2.2. Animals

Sprague-Dawley rats (male, body weight, 180 ± 20 g) were from the Laboratory Animal Center of Beijing Institute of Radiation Medicine (BIRM). Principles in good laboratory animal care were followed and animal experimentation was in compliance with the guidelines for the care and use of laboratory animals in BIRM.

2.3. Measurement of LogP

The oil–water partition coefficients (LogP) of NSAIDs was measured using the classical saturated *n*-octylalcohol/water method (Pongcharoenkiat et al., 2002). Sufficient distilled water was mixed with *n*-octylalcohol, stirred for 5 min and then stored for 24 h to obtain water-saturated *n*-octylalcohol. *N*-octylalcohol-saturated water was also prepared using a similar method. Aliquots (10 mg) of aspirin, ibuprofen and indomethacin were separately dissolved in the above *n*-octylalcohol (10 ml) followed by adding the above water (10 ml). The mixture was oscillated at 200 rpm (THZ-D Oscillator, Taicang Experimental Instrument Factory, Jiangsu, China) and 25 °C for 24 h, and then stored statically for 15 min. The oil phase and water phase were separated. Drugs in the two phases were determined using spectrophotometry on an ultraviolet-visible spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co., Ltd., China). LogP was calculated as Eq. (1).

$$\mathrm{LogP} = \mathrm{Log}\left(\frac{C_O}{C_W}\right) \quad (1)$$

where C_O was the concentration of drugs in the water-saturated *n*-octylalcohol, C_W was the concentration of drugs in the *n*-octylalcohol-saturated water.

2.4. Preparation of hydrogels

Carbopol 934PNF (1.2 g) was slowly added to water (75 ml) and continually stirred until dissolving. Aspirin (2 g), ibuprofen (5 g) and indomethacin (1 g) were separately dissolved in the ethanol solutions (25 ml) containing triethanolamin (4 g). The solutions were slowly added to the Carbopol solutions and continually stirred to obtain the ropy and transparent gels. The gels were diluted with water to 100 g to get a 2% (w/w) aspirin hydrogel, a 5% (w/w) ibuprofen hydrogel and a 1% (w/w) indomethacin hydrogel, separately.

The pH values of hydrogels were measured using a pH meter (H2221, HANNA, Woonsocket, USA). The hydrogels were diluted with 5 folds volume of water before measurement. The hydrogel pH values of blank matrix, aspirin, ibuprofen and indomethacin were 8.69, 7.28, 8.45 and 8.28, respectively.

The viscosity of hydrogels was measured using a rotational viscometer (DV-III ULTRA, Brookfield Engineering Laboratories Inc., MA, USA). All measurements were performed in triplicates. The hydrogel viscosity of blank matrix, aspirin, ibuprofen and indomethacin was 23,707 ± 1012, 7540 ± 92, 30,773 ± 46 and 19,173 ± 303 cP, respectively.

2.5. Measurement of drugs in the receptors

The drugs in the receptors were determined after withdrawing the solutions in the receptors in the *in vitro* transdermal experiments. Aspirin and indomethacin were determined using a Shimadzu 10 A high performance liquid chromatographic (HPLC) system (Japan) consisting of LC-10Avp pump, SPD-10Avp UV detector, SCL-10Avp controller and Shimadzu CLASS-VP 6.02 chromatographic workstation software. The Diamonsil C18-ODS HPLC column (5 μm, 250 mm × 4.6 mm, Dikma, China), a manual injection valve and a 20-μl loop (7725i, Rheodyne, USA) were used. The mobile phase for aspirin determination was acetonitrile/water/tetrahydrofuran/acetic acid (20:70:5:5, v/v) and the flow rate was 1.0 ml/min. The detection wavelength was 263 nm. The retention time was about 10 min. The linear range was 0.16–1016 μg/ml. The mobile phase for indomethacin determination was methanol/water/acetic acid (75:25:0.1, v/v) with the flow rate of 1.0 ml/min. The detection wavelength was 265 nm. The retention time was about 8.8 min. The linear range was 1–20 μg/ml. Ibuprofen was measured using spectrophotometry at 243 nm. The linear range was 0.5–30 μg/ml.

2.6. Transdermal experiments

2.6.1. Skin preparation

The back skins of sacrificed Sprague-Dawley rats were cut using a surgical blade and washed with distilled water and physiological saline. The skins were split into the pieces of 2.5 cm^2 area and stored at −80 °C. The pieces were used within 3 months. The skins were defrosted at room temperature and then soaked in phosphate buffer saline (PBS, pH 7.4) for 1 h before transdermal experiments.

2.6.2. Passive diffusion study

In vitro transdermal experiments were done on a transdermal permeation instrument (Tianjin Xinzhou Technical Co., Ltd., Tianjin, China) with the vertical Franz-type diffusion cells of diffusion area of 1.96 cm^2 (Fig. 1). Aliquots (17 ml) of PBS solutions (pH 7.4) were poured into the receptor compartments and stirred at 200 rpm. The skins were sandwiched between the donor and receptor compartments with the epidermal side upward. Aliquots (3 ml) of the drug hydrogels were added to the donor compartments. Diffusion was continued for 8 h at 32 °C (Hu et al., 2011). The samples (3 ml) were withdrawn from the receptor compartments for HPLC or UV analysis and then quickly filled with the same amount of PBS at 32 °C at 1, 2, 3, 4, 6, 8 h. The donor compartments were covered with Parafilm (Bemis Company, Inc. Oshkosh, USA) to prevent water evaporation. Each experiment was repeated for three times.

2.6.3. Iontophoresis study

A small iontophoresis system (Zhengzhou Huihao Co., Ltd., Zhengzhou, China) was used to provide a weak current of 0.06 mA (Fig. 1). Both of anodes and cathodes were submerged in the hydrogels in the donor compartments with 2 mm distance above the skins, and they were connected to the positive and negative terminators of the small iontophoresis system. The other processes were the same as the above passive diffusion experiment.