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The molecular assembly of the ionic liquid/aliphatic carboxylic acid/aliphatic amine as effective and safety transdermal permeation enhancers

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ABSTRACT

In spite of numerous advantages, transdermal drug delivery systems are unfeasible for most drugs because of the barrier effect of the stratum corneum. Ionic liquids were recently used to enhance transdermal drug delivery by improving drug solubility. In the present study, safe and effective ionic liquids for transdermal absorption were obtained as salts generated by a neutralization reaction between highly biocompatible aliphatic carboxylic acids (octanoic acid or isostearic acid) and aliphatic amines (diisopropanolamine or triisopropanolamine) (Medrx Co., Ltd., 2009). The mechanism of skin permeability enhancement by ionic liquids was investigated by hydrophilic phenol red and hydrophobic tulobuterol. Further, the skin permeation enhancing effect was remarkably superior in the acid excess state rather than the neutralization state. Infrared absorption spectrum analysis confirmed that ionic liquids/aliphatic carboxylic acid/aliphatic amine are coexisting at all mixing states. In the acid excess state, ionic liquids interact with aliphatic carboxylic acids via hydrogen bonds. Thus, the skin permeation enhancing effect is not caused by the ionic liquid alone. The "liquid salt mixture," referred to as a complex of ingredients coexisting with ionic liquids, forms a molecular assembly incorporating hydrophilic drug. This molecular assembly was considered an effective and safety enhancer of transdermal drug permeation.

1. Introduction

Transdermal drug delivery systems are currently available for various medications, including analgesics, nitroglycerine and nicotine (Barry, 1988, 1991; Benson, 2005; Guy, 1996; Prausnitz and Langer, 2008). This non-invasive drug delivery system can avoid the pain and risks of contamination associated with hypodermic injections and can be self-administered by the patient and released over long periods of time, which improves compliance (Osterberg and Blaschke, 2005; Small and Dubois, 2007). Recently, much attention has been paid to the transdermal delivery of physiologically active substance (i.e., enzyme, hormone, and growth factor) because transdermal drug delivery system can avoid first-pass metabolism (Schmidts et al., 2011, 2012; Tahara et al., 2010; Yang et al., 2012). However, all drugs cannot be applied to transdermal administration by powerful barrier function of the skin. The main barrier to transdermal drug delivery is the stratum corneum, outer layer of the epidermis filled with intercellular lipid matrix containing ceramide and non-living corneocyte cells with a dense keratin network. Further, the stratum corneum cells are connected by the tight junctions closely (Kluger et al., 2013). The stratum corneum barrier prevents not just the entry of foreign material and pathogens, but also moisture evaporation (Barry, 1991; Iwai et al., 2012, 2013; Obata et al., 2010). Therefore, the improvement of the skin permeability must not reduce the barrier function of the skin more than required.

Drug absorption typically involves movement of small molecules or lipid-soluble molecules through the intercellular space and intercellular lipid (Bos and Meinardi, 2000; Kanikkannan et al., 2000). Various enhance method include the physical enhancers have been developed to optimize transdermal drug absorption. 1-dodecylazacycloheptan-2-one (Azone) has reported to increase lipid fluidity and enhances intercellular drug diffusion (Hu et al., 2011; López et al., 2000; Meng-Lund et al., 2014; Michniak et al., 1993; Norlén and Engblom, 2000). The various amphipathic drug carriers have been researched and developed for the improvement of transdermal absorption (Gao et al., 2005; Gupta et al., 2012; Khurana et al., 2013; Manca et al., 2013; Sinico and Fadda, 2009; Yutani et al., 2012, 2014). The microemulsion is reported as suitable form for transdermal drug delivery system (Kitagawa et al., 2010). The solid-in-oil nanosuspension (S/O) of a surfactant was recently developed for the transdermal delivery of anti-inflammatory hydrophilic drugs. These previous studies show the possibility of the transdermal

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enhancing effects by forming the nanoparticles i.e., liposomes, micelles, or other molecular assemblies.

Ionic liquids are defined as salts that are liquid at room temperature. They exhibit high temperature stability, relatively low viscosity, and low vapor pressure. Many ionic liquids are salts of organic acids, combining the properties of organic substances and electrolytes, and some are even amphipathic (Brennecke and Maginn, 2001; Tokuda et al., 2005, 2006). In the pharmaceutical field, ionic liquids are used to enhance the solubility and stability of drugs during their synthesis (Ferraz et al., 2011; Frizzo et al., 2013; Hough et al., 2007; Mizuuchi et al., 2008; Sekhon, 2011; Stoimenovski et al., 2010). Certain ionic liquids show transdermal permeation enhancing effects as drug carriers (Dobler et al., 2013; Moniruzzaman et al., 2010a, 2010b, 2010c). However, some of ionic liquids show bactericidal activity, toxicity, and/or cytotoxicity (Busetti et al., 2010; Dobler et al., 2013; Frade and Afonso, 2010; Obando et al., 2009; Pernak et al., 2003; Vrikkis et al., 2009). Aliphatic carboxylic acids and aliphatic amines possess high biocompatibility. They have been used as stabilizers, reducing agents, pH regulators, emulsifiers, solvents, solubilizing agents, and in pharmaceutical and cosmetic products. The ionic liquids composed of these high biocompatible materials are currently used as transdermal absorption enhancers (Medrx Co., Ltd., 2009, 2014). Similar ammonium salts of carbamic acids are reported as potent enhancers (Holas et al., 2006; Hrabálek et al., 2005; Novotný et al., 2011). However, there is little information available on the mechanism of transdermal absorption enhancement and safety of this category of ionic liquid formulations.

The present study, we tried to investigate the mechanism of transdermal absorption enhancement by amphipathic ionic liquids. Ionic liquid is a salt obtained by a neutralization reaction of an equimolar acid and base. As a new trial, we generated products which were neutralized by various mixed ratio of the aliphatic carboxylic acids and the aliphatic carboxylic amines. In this study, we explained the assembled formulations consisted of these enhancers and drugs.

The phenol red is useful model drug for the transdermal absorption because of the non-absorptive from the epithelial tissue, such as skin and intestinal mucosa. This hydrophilic drug is absorbed by only the intercellular route and excreted without metabolized (Sasaki et al., 1990). Tulobuterol is sympathetic nerve beta 2 receptor operation medicine used for treatment such as asthma, bronchitis, the pulmonary emphysema. This drug used as most successful drug at transdermal absorption (Burioka et al., 2005; Ichikawa and Sugiura, 2013; Kato et al., 2002). The tulobuterol is the most suitable drug to inspect the effectiveness of the ionic liquid enhancement for the existing transdermal administration drug. The skin permeation enhancement mechanism by ionic liquid was inspected by the different drug of these properties of matter.

2. Materials and methods

2.1. Generation of aliphatic carboxylic acid/aliphatic amine/product systems

All reagents were of the first or high grade and purchased from Wako Pure Chemical Industries (Tokyo, Japan). The aliphatic carboxylic acid/aliphatic amine/product system was obtained by the neutralization method. Octanoic acid (OA) or isostearic acid (ISA) was used as the acid, whereas diisopropanolamine (DIPA) or triisopropanolamine (TIPA) was used as the base. Each acid–base pair was mixed in equimolar ratios, incubated overnight (80 °C), stirred using a vortex mixer, and subjected to ultrasonic irradiation (30 min) (Medrx Co., Ltd., 2009). The aliphatic carboxylic acid/aliphatic amine mixing ratio was adjusted to 1/9, 1/2, 1/1, 2/1, and 9/1 (mol/mol).

2.2. Structural analysis of aliphatic carboxylic acid/aliphatic amine/product systems

After each neutralization reaction, the structural properties of the reactants and products were analyzed by infrared absorption spectrum analysis. The absorption spectra were recorded on a FT/IR-6100 type A FTIR spectrometer (JASCO, Tokyo, Japan) using thin films on KBr plates (5 × 5 × 1 mm). The KBr plate was measured as blank matrix. The data were collected over a 1000–4000 cm^{-1} infrared region with a 4 cm^{-1} resolution at a 2 mm/s scanning speed by 10 times integration.

2.3. Preparation of ionic liquid formulations

The phenol red/product mixture or tulobuterol/product mixture were prepared by adding 1 mg phenol red or 10 mg tulobuterol (Wako Pure Chemical Industries) to 200 mg of pre-liquefied (65 °C) aliphatic carboxylic acid/aliphatic amine reaction products, measured using a disposable syringe, into a glass sample bottle. The mixtures were incubated (65 °C) overnight. Isopropyl myristate (IPM; 800 μL) was added to these mixtures. The mixtures were then subjected to ultrasonic irradiation (30 min) to obtain the uniform solutions. These uniform solutions were provided as the model formulations. The controls without carboxylic acid/aliphatic amine reaction products (1 mg phenol red/1 mL IPM or 10 mg tulobuterol/1 ml IPM) were prepared using the same process but without the aliphatic carboxylic acid/aliphatic amine/product system. The control for skin permeation by the sample contained model drug (1 mg phenol red or 10 mg tulobuterol) and 10 mg cell biology grade sodium dodecyl sulfate (SDS) purchased from SIGMA-Aldrich (St. Lois. MO, USA) dissolved in 1 mL IPM, and these solutions were subjected to ultrasonic irradiation (30 min). 1-dodecylazacycloheptan-2-one (Azone) provided from KOEI Chemical (Osaka, Japan) was used as the classical hydrophobic enhancer to compare with ionic liquids. Control samples with 3% Azone were prepared by adding 1 mg phenol red or 10 mg tulobuterol to 30 μL of Azone and added 970 μL IPM (Hosoya et al., 1987; Meng-Lund et al., 2014).

2.4. Impact of ionic liquid formulations on the skin permeation amount of phenol red or tulobuterol

The Franz diffusion cell system (vertical reservoir, 8 mL; permeation area, 1 cm^2) (PermGear, Hellertown, PA, USA) was filled with 10 mM pH 7.4 phosphate buffered saline without Mg^{2+} and Ca^{2+} (PBS(−)) as reservoir solution at 34 °C (Mikulásik et al., 2010). Abdominal skin from 5-weeks-old male Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan; certificated by Japanese Society for Laboratory Animal Resources) was used as the transmission skin sample. A 100 μL formulation or control sample (100 μg of phenol red or 1 mg of tulobuterol) was applied to the external surface of the skin, and all the reservoir solution was collected after 6 h. The skin permeation profile analysis of cumulative skin permeation was performed by applying 100 μL formulation containing 100 μg phenol red onto the rat skin. 1 mL of reservoir solution was collected 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h later, and the amount of phenol red permeated was measured. Then, 1 mL of reservoir solution was collected at a measurement time and supplemented with 1 mL PBS(−). Subsequently, 10% (*v*/*v*) 5 mol/L NaOH was added to the collected samples and stirred using a vortex mixer to measure phenol red concentration in the reservoir solution. The absorbance of alkali-treated phenol red was read at 558 nm using a UV-2550 UV–visible spectrophotometer (Shimadzu, Kyoto, Japan) and quantified using a calibration curve and UV Probe 2.31 software (Shimadzu). Since the measured concentration of the phenol red is the concentration diluted by the supplemented reservoir solution, it was corrected by the calculation based on the replenishing amount. The concentration of tulobuterol was measured by HPLC in LC-20AB system (Shimadzu) with YMC-Pack Pro C18 150 × 4.6 mm I.D. column (YMC, Kyoto,