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Research paper

A useful technique using imaging mass spectrometry for detecting the skin distribution of topically applied lidocaine

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ABSTRACT

The skin disposition of topically applied lidocaine hydrochloride was determined in steady-state skin permeation conditions by mass spectrometry imaging (MSI). The distribution of lidocaine in pig ear skin was assessed using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF-MS) with mass images examined using BioMap software. Following the detection of intrinsic signals of lidocaine, the skin concentration–distance curve was obtained using brightness analysis ([M+H+]: m/z 235.18). Although the skin concentration profile obtained by MALDI-TOF-MS did not completely correspond with the calculated distribution obtained from the skin permeation profile, the skin concentration profile could be detected. These results demonstrated that MSI might be a useful method to determine the skin disposition of topically applied non-radiolabeled or non-fluorescent drugs.

1. Introduction

The skin disposition of topically applied drugs is known to be strongly associated with local efficacy and toxicity [8]. Dermato-pharmacokinetics (DPK) [1] is now being utilized to evaluate generic topical formulations in Japan (www.nihs.go.jp/drug/be-guide(e)/Topical_BE-E.pdf). The skin concentrations of topically applied drugs have previously been assessed using several methods, including tape stripping [16,18], heat separation [22], and others [11,23], which generally require drug extraction and tissue homogenization procedures. However, difficulties have been reported in accurately determining the drug distribution or drug concentration–distance profiles in the skin by these methods. The skin permeation profiles of topically applied drugs can be analyzed by Fick's second law of diffusion under the assumption that the skin consists of one or two homogeneous membranes [21]. We reported recently that the skin concentrations of topically applied drugs in a certain logarithmic range of the *n*-octanol/water coefficient, $\log K_{o/w}$ ($1.93 < \log K_{o/w} < 2.81$), could be calculated by a two-layered diffusion model, with a relationship being identified between the calculated and observed steady-state concentrations of drugs. On the other hand, the skin concentrations of hydrophilic drugs ($\log K_{o/w} \leq 0$) could not be accurately determined using the same method. Several studies [14,15,26,27] confirmed that hair follicles and sweat ducts were the primary permeation pathways for hydrophilic drugs and macromolecules. Fluorescence dyes such as calcein, fluorescein, and fluorescein isothiocyanate dextran have been used as model hydrophilic drugs and macromolecules in order to provide insight into their skin disposition following topical application. However, this technique can only be used for fluorescent compounds.

Recently, several studies have used mass spectrometry imaging (MSI) by matrix-assisted laser desorption/ionization (MALDI) to investigate the disposition of a drug after its topical application [4,15]. In addition, D'Alvise et al. reported that the follicular transport of lidocaine in the deeper skin layers and its metabolism in subcutaneous tissue could be detected by desorption electrospray ionization mass spectrometry imaging [5].

The observation of heterogeneous drug distribution in skin, such as localized drug distributions in hair follicles, using a horizontal or

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vertical skin section with a microscope could provide useful information to reveal the skin permeation route of topically applied drugs. Because the pharmacological and toxicological effects of topically applied chemicals can be determined by their concentration at the viable epidermis/dermis, the drug concentration–depth profile in viable epidermis/dermis should be investigated in order to evaluate the safety and effects. Bunch et al. reported that a quantitative skin profile of ketoconazole was acquired with MALDI quadrupole time-of-flight (Q-TOF) mass spectrometry (MS) [2]. However, no comparison of skin concentration profiles obtained by imaging MALDI-TOF MS and a conventional method has been performed to investigate the usefulness of the novel method.

In the present study, an atmospheric-pressure MALDI quadrupole-ion-trap TOF-MS (AP-MALDI-QIT-TOF-MS) instrument was first used with 10-μm spatial resolution to obtain positional information on the concentration of a drug in skin following its topical application. Lidocaine hydrochloride was used as a model drug to observe the drug concentration–distance profile by MALDI-TOF-MS because its skin permeation profile could be successfully calculated using Fick's second law of diffusion from the skin permeation profile.

2. Experimental

2.1. Materials and methods

Lidocaine hydrochloride was obtained as a model drug from Sigma Aldrich (St. Louis, MO, U.S.A.). Other reagents and solvents were of reagent or HPLC grade and used without further purification. Frozen pig ear skin (three-breed cross pigs involving Landrace, Yorkshire and Duroc breeds) was purchased from the National Federation of Agricultural Cooperative Associations (Tokyo, Japan). The skin samples were stored at −30 °C until the skin permeation experiments.

2.2. Preparation of skin membrane

The purchased skin was maintained frozen at −80 °C prior to use. The skin was thawed at 32 °C and excised from the outer surface of a pig ear after being cleaned with distilled water. Stripped skin was obtained by tape stripping of the stratum corneum with adhesive tape (Scotch®; 3M Japan Ltd., Tokyo, Japan) 20 times prior to its excision from the pig ear. Excess fat was carefully trimmed off from the excised skin with a knife.

2.3. Skin permeation experiments for lidocaine

The skin sample was set in a vertical-type diffusion cell (effective diffusion area: 1.77 cm^2), in which the skin surface temperature was maintained at 32 °C. After 1 h of hydration with phosphate-buffered solution (PB), PB containing 2.5 mg/mL lidocaine (volume: 1.0 mL) was applied to the stratum corneum side as a donor solution for 8 h, and PB (volume: 6.0 mL) was added to the dermis side as a receiver solution. The receiver solution was stirred with a stirrer bar on a magnetic stirrer. An aliquot (500 μL) was withdrawn from the receiver chamber and the same volume of PB was added to the chamber to keep the volume constant. The penetrant concentration in the receiver chamber was determined by high-performance liquid chromatography (HPLC). Three skin permeation experiments were conducted to calculate the skin concentration–distance profile.

2.4. High-performance liquid chromatography conditions

Ethyl paraben was used as an internal standard in the quantitative determination of lidocaine. An aliquot of a sample solution containing lidocaine was mixed with acetonitrile (1:1) and centrifuged (15,000 × rpm, 5 min, 4 °C) to obtain the supernatant. Then, 20 μL of obtained supernatant was injected into the HPLC system. The HPLC system consisted of a pump (LC-20AD), a UV detector (PD-20A), a system controller (SCL-10A^{VP}), an auto-injector (SIL-20A), a degasser (DGU-20A_3), a column oven (CTO-20A), and analysis software (LC solution) (all from Shimadzu, Kyoto, Japan). An Inertsil® ODS-3 5 μm, 4.6 × 150 mm (GL Sciences Inc., Tokyo, Japan) column was kept at 40 °C. The mobile phase was 0.1% phosphoric acid:acetonitrile = 7:3, containing 5.0 mM 1-heptanesulfonate at a flow rate of 1.0 ml/mL. Detection was carried out at UV 230 nm.

2.5. Preparation of skin samples to plot lidocaine calibration curve

The stripped skin was immersed in different concentrations of lidocaine solution (500, 1500, and 2500 μg/mL) for 24 h to obtain skin with homogeneously distributed lidocaine. A calibration curve for the skin concentration of lidocaine was obtained by imaging analysis of the treated skin.

2.6. Skin treatment for MALDI-TOF observation

After the permeation experiment or after immersion in lidocaine solutions to obtain a calibration curve, the skin surface was washed three times using 1 mL of ultrapure water. The skin was then embedded in 2.0% carboxymethyl cellulose and frozen in isopentane at −80 °C. The embedded skin was sliced using a cryostat (CM3050; Leica Microsystems, Wetzler, Germany) to obtain 7-μm-thick vertical sections at −20 °C. Then, each skin section was kept at room temperature for 30 min before subjecting it to MALDI-QIT-TOF-MS. These sections were mounted on an indium tin oxide-coated slide glass (Sigma-Aldrich), dried in a silica gel-containing plastic tube, and then sprayed with 2,5-dihydroxybenzoic acid (50 mg/mL in 70% methanol and 30% water containing trifluoroacetic acid at a concentration of 0.1%) using a 0.2-mm-nozzle caliber airbrush (Procon Boy FWA Platinum; Mr. Hobby, Tokyo, Japan) to conduct MALDI-imaging mass spectrometry in the positive-ion mode. MALDI-QIT-TOF-MS equipped with a 355-nm Nd:YAG laser and microscope allowed mass images to be obtained with a high spatial resolution of 10 μm, in the scan range of m/z 200–900, as described previously for a prototype MALDI-MSI instrument [15] (Mass Microscope; Shimadzu, Kyoto, Japan). Microscopic images were obtained after application of a matrix (300 μL of 2,5-dihydroxybenzoic acid solution) to the skin sections. Mass spectra were subsequently acquired in the positive-ion mode in the designated areas of a specimen.

2.7. Image analysis of skin sections using BioMap®

The distribution of the specific signal intensity of lidocaine ($[M+H^+]$: m/z 235.18) in the skin was assessed using AP-MALDI-TOF-MS with mass images being examined using BioMap® software (Novartis Pharma K.K., Basel, Switzerland). The brightness of the obtained skin section images was analyzed using the image processing software Image J® (National Institutes of Health, Bethesda, MD, U.S.A.). Fig. 1a shows microscopic observation results of a skin section. The measurement area in the skin section was decided by the positional information of the obtained image, and the area, and was then divided into 5 sections (each section: 260 μm long × 500 μm wide) from the surface of stratum corneum