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Research Article

Investigation of the Efficacy of Transdermal Penetration Enhancers Through the Use of Human Skin and a Skin Mimic Artificial Membrane

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ABSTRACT

The aim of this study was to investigate the behavior of promising penetration enhancers through the use of 2 different skin test systems. Hydrogel-based transdermal formulations were developed with ibuprofen as a nonsteroidal anti-inflammatory drug. Transcutol and sucrose esters were used as biocompatible penetration enhancers. The permeability measurements were performed with *ex vivo* Franz diffusion cell methods and a newly developed Skin Parallel Artificial Membrane Permeability Assays (PAMPA) model. Franz diffusion measurement is commonly used as a research tool in studies of diffusion through synthetic membranes *in vitro* or penetration through *ex vivo* human skin, whereas Skin PAMPA involves recently published artificial membrane-based technology for the fast prediction of skin penetration. It is a 96-well plate-based model with optimized artificial membrane structure containing free fatty acid, cholesterol, and synthetic ceramide analog compounds to mimic the stratum corneum barrier function. Transdermal preparations containing 2.64% of different sucrose esters and/or Transcutol and a constant (5%) of ibuprofen were investigated to determine the effects of these penetration enhancers. The study demonstrated the good correlation of the permeability data obtained through use of human skin membrane and the *in vitro* Skin PAMPA system. The Skin PAMPA artificial membrane serves as quick and relatively deep tool in the early stages of transdermal delivery systems, through which the enhancing efficacy of excipients can be screened so as to facilitate the choice of effective penetration components.

Introduction

Transdermal drug delivery has received increased attention in recent years thanks to its numerous advantages over the oral and injection routes, such as avoidance of the hepatic “first-pass” metabolism, sustained drug delivery, protection of the gastrointestinal tract from drugs, and good patient compliance. The main penetration barrier for most drugs is the stratum corneum (SC), the outermost 10-15 μm thick zone of the epidermis. The SC consists of several layers of almost nonpermeable, nonviable cornified cells (corneocytes) and intercellular lipid domains. These lipids comprise approximately 50% ceramides, 25% cholesterol, 15% free fatty acids, and some minor components.[1]

The strategies most widely applied with the aim of improving drug penetration involve the use of chemical penetration enhancers and skin hydration.[2] In this respect, we report here on the development of hydrogel formulations containing penetration enhancers for the transdermal delivery of ibuprofen (IBU), a nonsteroidal anti-inflammatory drug frequently used for the treatment of musculoskeletal injuries. The transdermal delivery of IBU is a convenient way to minimize the potential risk of adverse gastrointestinal events (e.g., bleeding, ulceration, and perforation of the stomach).[3] The physicochemical properties of IBU are

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appropriate for its development as a transdermal gel formulation,[4] but the use of a penetration enhancer is unescapable because of the highly structured SC.[1]

Transcutol (TR) and sucrose fatty acid esters (SEs) were chosen as penetration enhancer excipients. TR is a powerful solubilizing agent and enhances the percutaneous penetration of various drugs, especially when it is used in combination with suitable surfactants.[5,6] SEs, a new generation of nonionic surfactants, are likewise favorable penetration enhancers.[7] They have low toxicity, good biocompatibility, and excellent biodegradability, and they cause little dermatologic damage.[8,9] The extent of the ability of the SEs to promote drug release *in vitro* has been reported to depend on the hydrophilic-lipophilic balance (HLB) value and the C atom number of the fatty acid chain in the SEs.[10] The effectiveness of SEs in combination with TR has also been demonstrated in the experiments on porcine ear skin and human skin.[6,11]

The efficacy of a surfactant in enhancing transdermal delivery is dependent on its structure, the physicochemical properties of the drug, and the nature of the vehicle, the main factors defining the surfactant–skin interaction.[12,13] Numerous studies have demonstrated that SEs and TR interact with the skin.[6,11,14,15] However, simple *in vitro*[10] or *ex vivo* animal membrane diffusion measurements[16] are not sufficient to allow an evaluation of the penetration-enhancing effects of different SEs. The SC in animals differs from the human SC in thickness, the number of corneocyte layers, the hair density, the water content, the lipid profile, and the morphology.[17] To select the best formulation for a clinical study, it is important to investigate transdermal formulations with *ex vivo* human skin penetration methods.

The transepidermal water loss (TEWL) is an indicator of the integrity of the skin barrier, and measurement of the TEWL is an easy and rapid noninvasive means of examining the effects of new transdermal systems.[18,19] For penetration investigations, measurements were made across excised human epidermis with a vertical Franz diffusion cell.[20] Because the use of human skin in research encounters ethical, health, and supply problems,[17] our transdermal gels were additionally tested in newly developed Skin Parallel Artificial Membrane Permeability Assays (Skin PAMPA),[21] developed as a rapid, reproducible, and cost-efficient system for the prediction of skin penetration. The Skin PAMPA was designed to be biomimetic, that is, the same or similar components have been applied as present in the most important barrier of human skin. As the main barrier of the human skin, it is known to be the outermost layer of epidermis, the SC, Skin PAMPA has been designed to mimic the features of this layer. SC composes of corneocytes embedded into a multilamellar lipid layer. The lipid layer consists of a mixture of ceramides, cholesterol, and free fatty acids as major components and provides the main route of penetration, the paracellular pathway. The Skin PAMPA membrane was created by using free fatty acid, cholesterol, and a synthetic ceramide analog compound (long-chain tartaric acid diamide derivative named certramide) to mimic the features of ceramide in the lipid matrix.[22,23] The optimized artificial membrane mimics molecular transport through the SC, and the commercially already available Skin PAMPA™ system can serve as a good alternative tool for penetration studies of transdermal formulations.[24]

The aims of the present study were to compare different types of SEs (alone or in combination with TR) from the aspect of their ability to enhance the delivery of IBU through human skin and to prove the usefulness of the Skin PAMPA model as a valuable tool in research on transdermal systems. The Skin Pampa model has never been used earlier to investigate transdermal penetration enhancers but the use of it could offer such advantages as a simple and cost-effective early phase screening system of penetration enhancers.

Materials and Methods

Sample Preparation

A penetration-enhancing free hydrogel (control gel) was prepared by the following procedure. A 5 wt% IBU (Sigma-Aldrich, St Louis, MO) was dissolved in polyethylene glycol 400 (20 wt%; Hungaropharma Ltd., Budapest, Hungary) and this solution was added to 3 wt% Carbopol 971 (Lubrizol Corporation/Azelis, Budapest, Hungary) hydrogel prepared with distilled water. The pH was adjusted to 7.0 by adding trolamine (7 wt%; Hungaropharma Ltd.). Sucrose esters, sucrose laurate D-1216 (SL), and sucrose myristate C-1416 (SM) were purchased from Mitsubishi-Kagaku Foods Corporation, Tokyo, Japan. To compare the 2 SEs, 2.64 wt% was incorporated in Carbopol 971-based hydrogel (SL gel and SM gel). A 2.64 wt% was chosen because this gave the maximum SE concentration-containing hydrogel which did not require a centrifugation process to remove the bubbles. Similar compositions were prepared by using 10 wt% TR (Gattefossé and Lubrizol Corporation/Azelis, Budapest, Hungary) (TR gel); 2.64 wt% SE + 10% TR-containing gels too were prepared (SL + TR and SM + TR gel).

TEWL Measurements

Measurements were performed under standardized conditions (room temperature of 21°C-23°C and 40%-50% relative humidity). Seven healthy female volunteers aged between 23 and 60 years with no history of dermatologic diseases or allergy participated in the experiment. Informed consent was obtained from all these volunteers, and the study was approved by the local ethics committee (the Regional and Institutional Human Medical Biological Research Ethics Committee at the University of Szeged). The volunteers were asked not to apply any moisturizer or cosmetic product for at least 24 h before the process. They were given 30 min to adapt to the measurement room conditions. During the experiment, samples were applied to the dorsal region of the hand of all the subjects. TEWL values were determined on the same area of the skin before and after 30 and 150 min of the sample application. The measured values were compared with the values detected on the nontreated skin; the changes in TEWL were expressed in percentage.[25-27] Measurements were performed with a Tewameter® TM 300 instrument (Courage and Khazaka Electronic GmbH, Cologne, Germany).

Drug Diffusion and Penetration Investigations

Preparation of Heat-Separated Epidermis

Excised human skin was obtained during abdominal plastic surgery on a Caucasian female patient (aged between 30 and 40 years). This was approved in advance by the Ethical Committee of the Human Investigation Review Board at Albert Szent-Györgyi Clinical Centre, University of Szeged. The subcutaneous fat tissue was separated from the outer skin layers after the excision, and the skin was stored at −20°C overnight. Then, after thorough defrosting, membrane separation was achieved with a previously reported method.[28] Individual portions were immersed in water at 60°C for 90 s. After removal of the skin from the water, it was placed SC side up on a filter paper, and the epidermis (comprising the SC and the viable epidermis) was gently removed from the underlying dermis with the use of forceps. The latter was discarded, and the epidermal membrane was floated onto the surface of phosphate buffer solution (pH = 7.4) for at least 20 min and then placed on a supporting Porafil membrane (cellulose acetate, pore diameter 0.45 μm).[29,30]