



## Research paper

# Lidocaine self-sacrificially improves the skin permeation of the acidic and poorly water-soluble drug etodolac via its transformation into an ionic liquid

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## ABSTRACT

Poor transdermal penetration of active pharmaceutical ingredients (APIs) impairs both bioavailability and therapeutic benefits and is a major challenge in the development of transdermal drug delivery systems. Here, we transformed a poorly water-soluble drug, etodolac, into an ionic liquid in order to improve its hydrophobicity, hydrophilicity and skin permeability. The ionic liquid was prepared by mixing etodolac with lidocaine (1:1, mol/mol). Both the free drug and the transformed ionic liquid were characterized by differential scanning calorimetry (DSC), infrared spectroscopy (IR), and saturation concentration measurements. In addition, *in vitro* skin-permeation testing was carried out via an ionic liquid-containing patch (Etoreat patch). The lidocaine and etodolac in ionic liquid form led to a relatively lower melting point than either lidocaine or etodolac alone, and this improved the lipophilicity/hydrophilicity of etodolac. *In vitro* skin-permeation testing demonstrated that the Etoreat patch significantly increased the skin permeation of etodolac (9.3-fold) compared with an etodolac alone patch, although an Etoreat patch did not increase the skin permeation of lidocaine, which was consistent with the results when using a lidocaine alone patch. Lidocaine appeared to self-sacrificially improve the skin permeation of etodolac via its transformation into an ionic liquid. The data suggest that ionic liquids composed of approved drugs may substantially expand the formulation preparation method to meet the challenges of drugs which are characterized by poor rates of transdermal absorption.

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## 1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for various inflammatory diseases. The side effects of NSAIDs include the risk of digestive tract disorders, kidney function impairment, and liver function impairment, and these are considered a problem when there is a need for frequent use [1,2]. Accordingly, parenteral administration methods have been developed. Some methods have adopted the form of an external preparation for the transdermal absorption of NSAIDs [3–8]. A patch of Ketoprofen, which is referred to as the Mohrus<sup>®</sup> Patch, is a typical successful example. However, skin permeability of some NSAIDs is extremely poor. Therefore, the therapeutic effects are remarkably decreased in cases of external administration by comparison with oral administration. Therefore, an improvement in the technology of the skin permeability of NSAIDs is required.

Ionic liquids are defined as organic salts, which are characterized by melting points that are below 100 °C [9,10]. Ionic liquids that are liquid at ambient temperatures and pressures are referred to as room-temperature ionic liquids [11]. Ionic liquids are used as solvents in chemistry and in catalysis during synthesis, and their use has been extended to pharmaceutical applications with the ultimate goal of improving the dissolution, solubility and bioavailability of active pharmaceutical ingredients (APIs) [12–14]. Sahbaz et al. [15] demonstrated that transformation of the weakly basic, poorly water-soluble drugs, itraconazole, cinnarizine and halofantrine, into lipophilic ionic liquids facilitates incorporation into lipid-based formulations and improves their bioavailability via lipid absorption pathways after oral administration. In addition, ionic liquids have sparked interest for their use as additives in formulations for topical drug delivery [16–21].

Hough and co-workers [22,23] have used ionic liquid in the preparation of several compounds that combine biologically active cations and anions. The cations of lidocaine, ranitidine, and didecylmethyl ammonium are capable of forming ionic liquids, while

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the anions of docusate and ibuprofen are compounds that have been approved by the FDA for use in pharmaceutical applications. Together, these are used to form three ionic liquids: lidocaine-docusate, ranitidine-docusate, and didecylmethyl ammonium-ibuprofen. Lidocaine-docusate combines the cation lidocaine with the anion docusate (an emollient) to produce an ionic liquid that has shown better efficacy than free lidocaine as a topical anesthetic. Ranitidine-docusate combines the same anion with the cation ranitidine, which is a histamine H<sub>2</sub>-receptor antagonist, and provides an advantage over ranitidine alone that allows the avoidance of problems with polymorphism and impurities. Didecylmethyl ammonium-ibuprofen has shown anti-inflammatory and antibacterial properties that can be associated with the use of anions and cations, respectively. These developments led us to the assumption that NSAIDs themselves might create an ionic liquid with the counter ion of another API and consequently increase the skin permeability and therapeutic efficacy of each.

Etodolac, an anionic ion of NSAID, is known to reduce the levels of hormones that cause inflammation and pain in the body [24]. It is used to treat mild to moderate pain, osteoarthritis, or rheumatoid arthritis [25]. Lidocaine, a cationic ion, is used to numb tissue in a specific area and to treat ventricular tachycardia. It can also be used for nerve blocks [26]. Etodolac and lidocaine might be good candidates for ionic liquid formation. In the present study, therefore, we tried to prepare an ionic liquid by mixing etodolac and lidocaine (1:1, mol/mol). The transformed ionic liquid was characterized by differential scanning calorimetry (DSC), infrared spectroscopy (IR) and saturation concentration measurements. In addition, *in vitro* skin-permeation testing was carried out with ionic liquid-containing patches (Etoreat patch).

## 2. Materials and methods

### 2.1. Materials

Etodolac and lidocaine were purchased from ABIC Inc. (Netanya, Israel) and Delta Synthetic Co., Ltd. (New Taipei City, Taiwan), respectively. Propylene glycol, diethyl sebacate and 1-octanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### 2.2. Etodolac–Lidocaine salt preparation

The Etodolac–Lidocaine ionic liquid was obtained by mixing an equimolar mass of etodolac and lidocaine at 60 °C until each was completely dissolved.

### 2.3. DSC analysis

DSC measurement was performed on an EXSTAR6000 (Seiko Instruments Inc., Chiba, Japan) using a sample pan made of aluminum. The temperature range was –100 to 200 °C, and the heating rate was adjusted at 5 °C/min. All samples were kept at room temperature before the DSC measurement.

### 2.4. IR analysis

Fifty mg each of the Etodolac–Lidocaine ionic liquid, the etodolac, and the lidocaine was dissolved in 0.5 mL of chloroform to gain chloroform solutions. In addition, 50 mg each of the Etodolac–Lidocaine ionic liquid, the etodolac, and the lidocaine was dissolved in 1 mL of propylene glycol to gain propylene glycol solutions. Each sample of the chloroform and propylene glycol solutions was held between sodium chloride plates to measure the infrared absorption spectrum. IR spectral measurements were carried out using an Infrared Spectrophotometer (IRPrestige-21; Shimadzu, Kyoto,

Japan). The optical resolution was 2 cm<sup>-1</sup> and the data were accumulated 20 times. Using the spectrum data of each solution, the wave numbers (cm<sup>-1</sup>) of the carbonyl groups in the carboxylic acid in the etodolac, the carbonyl groups of amides in the lidocaine, the carbonyl groups of carboxylate anions (–COO<sup>-</sup>), and the amides in the Etodolac–Lidocaine ionic liquid all were compared, and thereby the presence/absence of salt formulation in the Etodolac–Lidocaine ionic liquid was determined.

### 2.5. HPLC

The concentration of etodolac and lidocaine was determined using an HPLC system equipped with a UV detector (LC-2010C HT; Shimadzu). Chromatographic separation was performed using a YMC-Pack Pro C18 RS (5 μm, 4.6 × 150 mm i.d.; YMC Co., Ltd., Kyoto, Japan) at 40 °C. The mobile phase was 0.18% sodium lauryl sulfate in a 0.1% phosphoric acid solution/acetonitrile/methanol (50/40/10, v/v) at a flow rate of 1.0 mL/min. The wavelength of the UV detector was set at 225 nm. The retention times for the lidocaine and etodolac were approximately 8 and 14 min, respectively.

### 2.6. Solubility determination

The saturation solubility of etodolac and lidocaine in water was determined. An excess of each of the Etodolac–Lidocaine ionic liquid, the etodolac, and the lidocaine was added to 5 mL deionized water in 10 mL glass vials and then sealed. The vials were placed in a shaker water bath and agitated at 60 oscillations/min at 25 °C. The samples were withdrawn after 24 h, then filtered, and assayed for drug concentrations using HPLC, as described above. The saturation solubility of etodolac and lidocaine in propylene glycol, diethyl sebacate or 1-octanol was also determined.

### 2.7. Patch preparation

To prepare the Etoreat patches, Etodolac–Lidocaine ionic liquid, solvents, formulation bases, PSA matrix, and hexane were mixed at 60 °C. A general casting method was used to prepare a PSA tape (150 μm thickness) containing 4.4% Etodolac–Lidocaine ionic liquid. An etodolac-alone patch (E-alone patch) and a lidocaine-alone patch (L-alone patch) were also prepared using the same method.

Each Etoreat patch contained 4.4% Etodolac–Lidocaine ionic liquid. The E-alone patch contained 2.4% etodolac only and the L-alone patch contained 2.0% lidocaine only.

### 2.8. Skin preparation

Frozen Yucatane Micro Pig (YMP) skin sets (female pigs: 5 months old) were obtained from Charles River Japan Inc. (Yokohama, Japan). Each YMP skin set consisted of 16 skin sheets (sheet size: approximately 10 × 10 cm) enclosed in a plastic bag identified by number.

To prepare the intact YMP skin sheet, subcutaneous fat was removed from the purchased YMP skin and the skins were dermatomed to a nominal thickness of approximately 1 mm using an electric dermatome (KED-101; Keisei Medical Industries Co., Ltd., Tokyo, Japan). The prepared YMP skin sheets were stored at –20 °C until use. Before starting the skin-permeation tests, the frozen YMP skin sheets were thawed at room temperature and cut into 25 mm rounds.

To prepare the stripped YMP skin sheet, tape stripping was performed 30 times using Scotch tape (BK-18; 3M Japan Ltd., Tokyo, Japan) on the intact YMP skin sheet 25 mm rounds immediately before the skin-permeation tests.

### 2.9. *In vitro* skin-permeation testing

The skin-permeation tests were performed using Franz-type diffusion cells (effective diffusional area: 1.0 cm<sup>2</sup>, PermeGear Inc., Hellertown, USA) [27]. The intact YMP skin or the stripped YMP skin was used for the permeation tests. The YMP skin was mounted on receptor cells filled with 8.0 mL of 0.01 M phosphate buffered saline (PBS, pH 7.4). The receptor cell was maintained at 32 °C by a constant-temperature circulator water bath. After equilibrating for 10–30 min, transepidermal water loss (TEWL) was measured using a Vapometer (SWL4001JT; Delfin technologies Ltd., Cuopio, Finland) and a Standard Adapter (Delfin technologies Ltd.). After TEWL measurement, the YMP skin was removed from the receptor cell. Each of the Etoreat, E-alone and L-alone patches (1.1 cm<sup>2</sup>, diameter: 12 mm) was applied to the skin surface. The receptor cell medium was maintained at 32 °C and stirred with a Teflon stirrer driven by a constant speed motor at 1000 rev/min throughout the test. The receptor fluid (0.2 mL) was withdrawn at 2, 4, 8, and 24 h for the test with the intact YMP skin or at 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 h for the test with the stripped YMP skin. The same volume of fresh PBS was added to the receptor cell to keep the volume constant. Samples were filtered using a cellulose acetate membrane (0.45 μm pore size, Advantec Toyo Kaisha, Ltd., Tokyo, Japan), and were assayed for drug concentration using HPLC, as described above. The cumulative amount of drug permeation was calculated using the following formula:

$$Q_s(\mu\text{g}/\text{cm}^2) = C_{sn} \times V_s/A_s + \sum_{i=1}^{n-1} C_{si} \times S/A_s$$

$Q_s$ : The cumulative amount of drug permeated per unit area (μg/cm<sup>2</sup>).

$C_{sn}$ : Concentration of drug (μg/mL) determined in the receptor fluid at  $n$ th sampling interval.

$V_s$ : Volume of the receptor cell chamber (mL), 8.0 mL.

$\sum_{i=1}^{n-1} C_{si}$ : Cumulative drug concentrations in the receptor fluid from the first sampling to the  $n - 1$ th sampling.

$S$ : Volume of the sampling (mL), 0.2 mL.

$A_s$ : Effective diffusional area (cm<sup>2</sup>), 1.0 cm<sup>2</sup>.

### 2.10. Determination of drug content in the skin

After the *in vitro* skin-permeation testing, the YMP skin was removed from the diffusion cell and then divided into stratum corneum, epidermal and dermis to determine the drug content in each of the skin fractions. To obtain stratum corneum from the YMP skin, tape stripping was performed 10 times using Scotch tape (BK-18; 3M Japan Ltd.). The epidermal was obtained by shaving it from the stripped YMP skin. To obtain dermis from the YMP skin, the shaved YMP skin was cut into an effective diffusion area and then into pieces using scissors. Each of the stratum corneum, the epidermal, and the dermis samples was suspended in 2 mL of methanol, and then sonicated for 5 min to extract the drugs (etodolac and lidocaine). After centrifugation at 1500g for 10 min, the resultant supernatant was filtered with a nitrocellulose membrane (0.45 μm pore size, Merck Millipore, Ltd., Billerica, USA), appropriately diluted with methanol, and assayed for drug content in each of the skin fractions using HPLC, as described above.

### 2.11. Calculation of permeation parameters

The skin-permeation parameters of the drugs were calculated using SKIN-CAD<sup>®</sup> [28] on the basis of the results of *in vitro* intact skin and stripped skin-permeation testing as well as testing for drug release. SKIN-CAD<sup>®</sup> is simulation software for the skin and body pharmacokinetics following transdermal and topical drug

delivery. This software can optimize the design of transdermal drug delivery systems (prediction of clinical performance and setup of administration schedule) as well as evaluation of the effects of various factors on skin permeation and blood concentration profiles. The drug-release tests were performed using a dissolution apparatus (NTR-6100A; Toyama Sangyo Co., Ltd., Osaka, Japan). The paddle rotation speed was set at 50 rpm with 900 mL of 0.025 M phosphate buffer (32 °C, pH 6.8). Each of the patches was cut into 30 mm rounds that were applied to an adhesive on a stainless steel disk (diameter: 41.2 mm; Toyama Sangyo Co., Ltd.). The release liner was peeled off and the disk assembly was carefully placed in the dissolution vessel parallel to the paddle blade with the adhesive side up. Dissolved solutions were withdrawn at 1, 3, 6, and 24 h and assayed for drug concentration using HPLC, as described above.

For the calculations, the skin (thickness of stratum corneum, 20 μm; thickness of whole skin, 1 mm) and patch design parameters (thickness of the matrix, 150 μm; etodolac concentration in the matrix, 2.28 mg/cm<sup>3</sup>; lidocaine concentration in the matrix, 1.87 mg/cm<sup>3</sup>) were also considered. The mean steady-state fluxes and lag times of the drugs through the intact and stripped YMP skin were calculated using SKIN-CAD<sup>®</sup> based on the results of the skin-permeation testing. In addition, the diffusion coefficients for the matrix of each patch, stratum corneum and viable skin sample were calculated using SKIN-CAD<sup>®</sup> based on the results of the skin-permeation and drug-release testing.

### 2.12. Statistics

All data are reported as the mean ± S.D. Statistical analyses of the data were conducted using a Welch *t* test.

## 3. Results

### 3.1. Characterizations of the Etodolac–Lidocaine ionic liquid

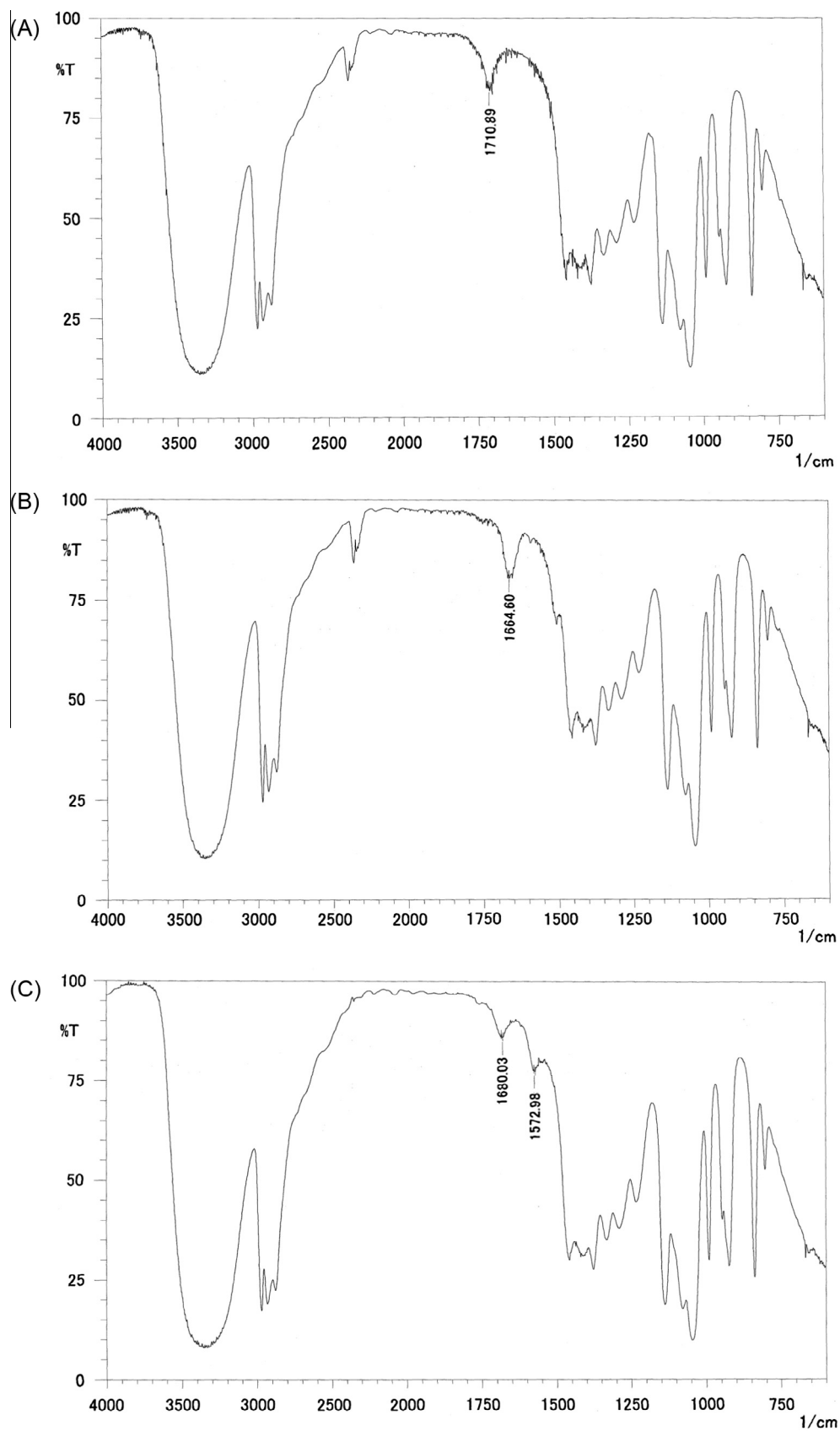
The transformation of the Etodolac–Lidocaine ionic liquid was achieved by mixing equimolar masses of powdered etodolac and powdered lidocaine under the heated conditions described above.

Fig. 1 shows the IR spectra of etodolac powder, lidocaine powder and Etodolac–Lidocaine ionic liquid. In Etodolac–Lidocaine ionic liquid, the absorption bands characteristic to C=O stretching of the carboxyl group of etodolac powder were diminished and a new band appeared at about 1,573 cm<sup>-1</sup> (Table 1). This provided strong evidence of the salt formation in the Etodolac–Lidocaine ionic liquid.

Fig. 2 displays the DSC thermograms of Etodolac–Lidocaine ionic liquid, free etodolac, free lidocaine, and a mixture of etodolac and lidocaine. In the free etodolac and free lidocaine, sharp endothermic peaks were found at the melting points: 148 and 60 °C, respectively. These peaks disappeared, however, though a broad endothermic peak that was due to the eutectic point that persisted in the mixture of etodolac and lidocaine. In addition, no peak was observed in the Etodolac–Lidocaine ionic liquid. This suggests that the Etodolac–Lidocaine ionic liquid, which is a salt of etodolac (148 °C) and lidocaine (60 °C), has no melting point and the Etodolac–Lidocaine ionic liquid is different from a mixture of etodolac and lidocaine.

### 3.2. Saturation solubility

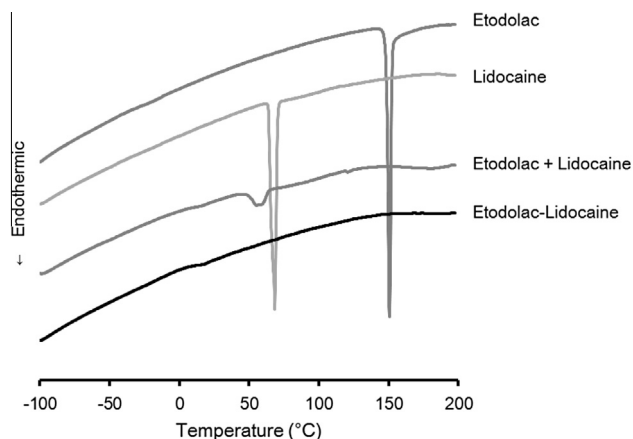
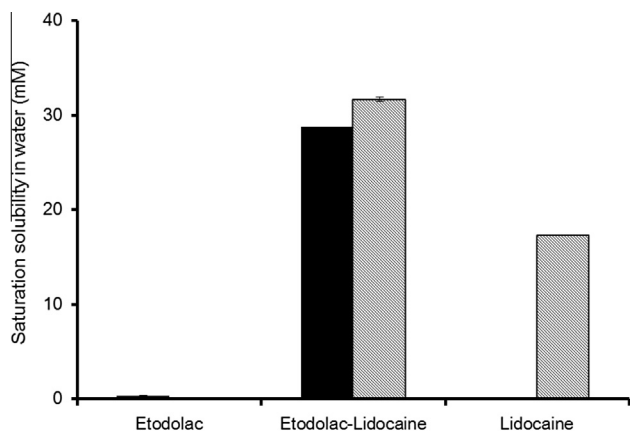
To study the changes in the hydrophilicity/lipophilicity when an ionic liquid is formed, the saturation solubilities of etodolac and lidocaine were determined for several solvents. Etodolac was only scarcely dissolved in water, while lidocaine completely dissolved in water (Fig. 3). The saturated concentration of lidocaine



**Fig. 1.** IR Spectra of Etodolac–Lidocaine ionic liquid in propylene glycol. Each of the samples of the etodolac alone (A), the lidocaine alone (B), and the Etodolac–Lidocaine ionic liquid (C) (50 mg) was dissolved in 1 mL of propylene glycol to gain a propylene glycol solution. Each sample was held between sodium chloride plates. IR spectral measurements were carried out using an Infrared Spectrophotometer (IRPrestige-21). The optical resolution was  $2\text{ cm}^{-1}$  and the data were accumulated 20 times.

**Table 1**  
IR absorption bands for carbonyl group (C=O stretching,  $\text{cm}^{-1}$ ).

Compound	Assignment	Wave number ( $\text{cm}^{-1}$ )	
		In chloroform	In propylene glycol
Etodolac	—COOH	1707	1711
Lidocaine	—CONR <sub>2</sub>	1675	1665
Etodolac–lidocaine	—COO—	Not clear	1573
	CONR <sub>2</sub>	1676	1680

**Fig. 2.** DSC thermograms of the Etodolac–Lidocaine ionic liquid. DSC measurement was performed on an EXSTAR6000 using a sample pan made of aluminum. The temperature range was  $-100$  to  $200$  °C, and the heating rate was adjusted to  $5$  °C/min. All samples were kept at room temperature before DSC measurement.**Fig. 3.** Solubility of Etodolac–Lidocaine ionic liquid in water. Excess of Etodolac–Lidocaine ionic liquid, etodolac alone and lidocaine alone was added to 5 mL deionized water in 10 mL glass vials, which were then sealed. The vials were placed in a shaker water bath and agitated at 60 oscillation/min at  $25$  °C. The samples were withdrawn at 24 h, then filtered, and assayed for drug concentration using HPLC as described in Section 2. The saturation solubility of etodolac and lidocaine in propylene glycol, diethyl sebacate or 1-octanol was also determined. Data represent the mean  $\pm$  S.D. ( $n = 4$ ).**Table 2**  
Comparisons of the Etodolac–Lidocaine, etodolac and lidocaine.

	Saturation solubility (mM)			
	Etodolac	Etodolac in Etodolac–Lidocaine	Lidocaine	Lidocaine in Etodolac–Lidocaine
In water	$0.3 \pm 0.0$	$28.8 \pm 0.2$	$17.4 \pm 0.2$	$31.7 \pm 0.2$
In propylene glycol	$224.6 \pm 2.2$	$>700$	$>700$	$>700$
In diethyl sebacate	$378.2 \pm 70.9$	$>700$	$>700$	$>700$
In octanol	$298.0 \pm 102.9$	$>700$	$>700$	$>700$

was  $17.4 \pm 0.2$  mM. Interestingly, the formation of ionic liquid increased the saturation solubility of both etodolac and lidocaine in water:  $28.8 \pm 0.2$  mM for etodolac and  $31.7 \pm 0.2$  mM for lidocaine (Table 2). The values for the saturation solubility of lidocaine alone in propylene glycol, diethyl sebacate and octanol were essentially high ( $>700$  mM), while those of etodolac alone were quite low. Surprisingly, the saturation solubility of etodolac in propylene glycol, diethyl sebacate and octanol was markedly improved when it formed an ionic liquid with lidocaine. These results clearly indicate that values for the hydrophilicity and lipophilicity of etodolac and the hydrophilicity of lidocaine were increased via the transformation into an ionic liquid.

### 3.3. Skin permeation

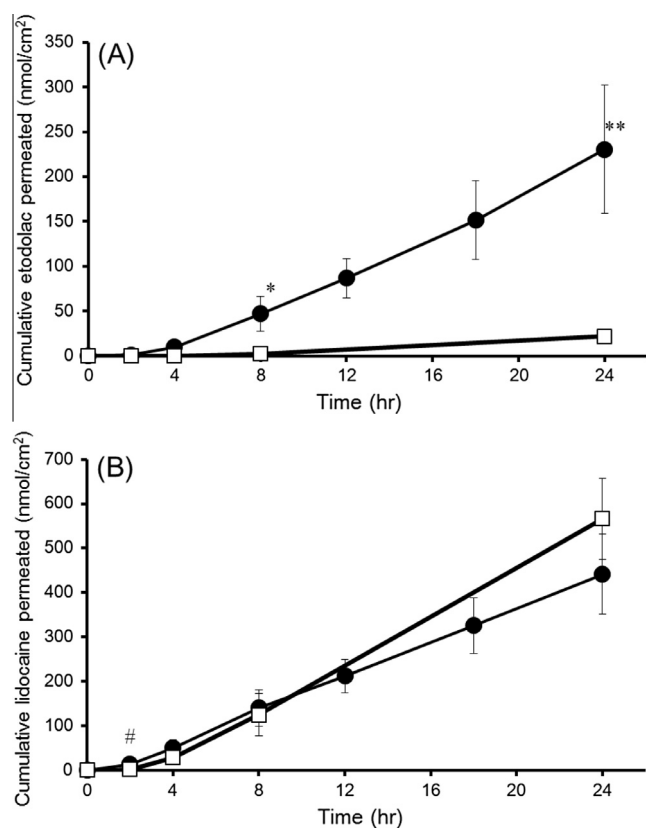
In order to compare the skin permeability of the Etodolac–Lidocaine ionic liquid with that of etodolac alone and lidocaine alone, *in vitro* permeation tests through intact YMP skin or stripped YMP skin with three different patches (Etoeat (a patch containing Etodolac–Lidocaine ionic liquid), E-alone patch and L-alone patch) were carried out. Fig. 4A and B shows the time course profiles of the cumulative permeated amounts of etodolac and lidocaine through the intact YMP skin. The Etoeat patch induced significantly higher etodolac permeation over 24 h after patch application compared with the E-alone patch ( $p < 0.01$ ) (Fig. 4A). On the other hand, the cumulative permeated amounts of lidocaine from the Etoeat patch at 24 h after application ( $441 \pm 91$  nmol/cm<sup>2</sup>) were similar to, or even lower than, those from the L-alone patch ( $566 \pm 91$  nmol/cm<sup>2</sup>).

Table 3 summarizes the skin-permeation parameters for etodolac in the E-alone patch and in the Etoeat patch, and it lists the parameters for lidocaine in the L-alone patch and in the Etoeat patch, as calculated on the basis of the data shown in Fig. 4. As for etodolac, the etodolac steady-state flux of the Etoeat patch through intact YMP skin was 9.3-fold larger than that of the E-alone patch. The etodolac lag time for the skin permeation by the Etoeat patch through the intact YMP skin was 1.6-fold shorter than that of the E-alone patch. The etodolac diffusion coefficient in the matrix of patches, stratum corneum and viable skin for the Etoeat patch was approximately 11.0, 5.5 and 0.9-fold larger than that of the E-alone patch. As for lidocaine, the lidocaine steady-state flux of the Etoeat patch through the intact YMP skin was 0.8-fold smaller than that of the L-alone patch. However, the lag time for the skin permeation of lidocaine in the Etoeat patch through the intact YMP skin became 1.5-fold shorter than that of the L-alone patch. The values for the lidocaine diffusion coefficient in the matrix of patches, stratum corneum and viable skin of the Etoeat patch were approximately 0.5-, 4.0- and 1.1-fold larger than those of the L-alone patch.

### 3.4. Drug content in skin fractions

In order to compare the skin distribution values of the Etodolac–Lidocaine ionic liquid, the etodolac alone, and the lidocaine alone, the drug content in the stratum corneum, the epidermal,





**Fig. 4.** Skin-permeation profile for (A) etodolac in Etoreat (●) and E-alone (□), and (B) lidocaine in Etoreat (●) and L-alone (□). The skin-permeation tests were performed using Franz-type diffusion cells. Either intact YMP skin or stripped YMP skin was used. Each of the Etoreat patch, the E-alone patch, and the L-alone patch (1.1 cm<sup>2</sup>, diameter: 12 mm) was applied to the skin surface mounted on receptor cells filled with 8.0 mL of 0.01 M phosphate buffered saline (PBS, pH 7.4), maintained at 32 °C. Receptor fluid (0.2 mL) samples were withdrawn at indicated time points. The samples were assayed for drug concentration using HPLC as described in Section 2. The cumulative amounts of drug permeation were calculated, as described in Section 2. Data represent the mean  $\pm$  S.D. ( $n = 4$ ). \* $p < 0.05$ , # $p < 0.05$  and \*\* $p < 0.01$ .

and the dermis was determined after the *in vitro* skin-permeation testing (Fig. 5 and Table 4). The etodolac content in each of the skin factions was significantly higher in the Etoreat patch than in the E-alone patch at 2, 8 and 24 h (Fig. 5A and B and Table 4). The etodolac content in the stratum corneum in the Etoreat patch at 2, 8 and 24 h was approximately 4.6, 4.2 and 4.0-fold higher, respectively, than that in the E-alone patch. The lidocaine content in each skin faction was lower in the Etoreat patch than in the L-alone patch at 8 and 24 h, although the amounts at 2 h were almost the same (Fig. 5C and D and Table 4). These results are well reflected by the differences in the time course profiles of the cumulative

permeated lidocaine amounts of the Etoreat and L-alone patches shown in Fig. 4B. Interestingly, in the Etoreat patch (Fig. 5B and D and Table 4), the amount of etodolac and lidocaine in each skin faction was similar, although the cumulative amounts that had permeated and the steady-state fluxes were different (Fig. 4 and Table 3). The results described in Figs. 4 and 5 and in Tables 3 and 4 indicate that the lidocaine in the Etoreat patch contributed to an increase in the skin permeation of etodolac as a result of the formation of ionic liquid. The lidocaine in the Etoreat self-sacrificially reduced the limits of etodolac permeation through the matrix and the stratum corneum. However, the skin permeation of the lidocaine in the Etoreat was slightly decreased compared with that of lidocaine alone.

#### 4. Discussion

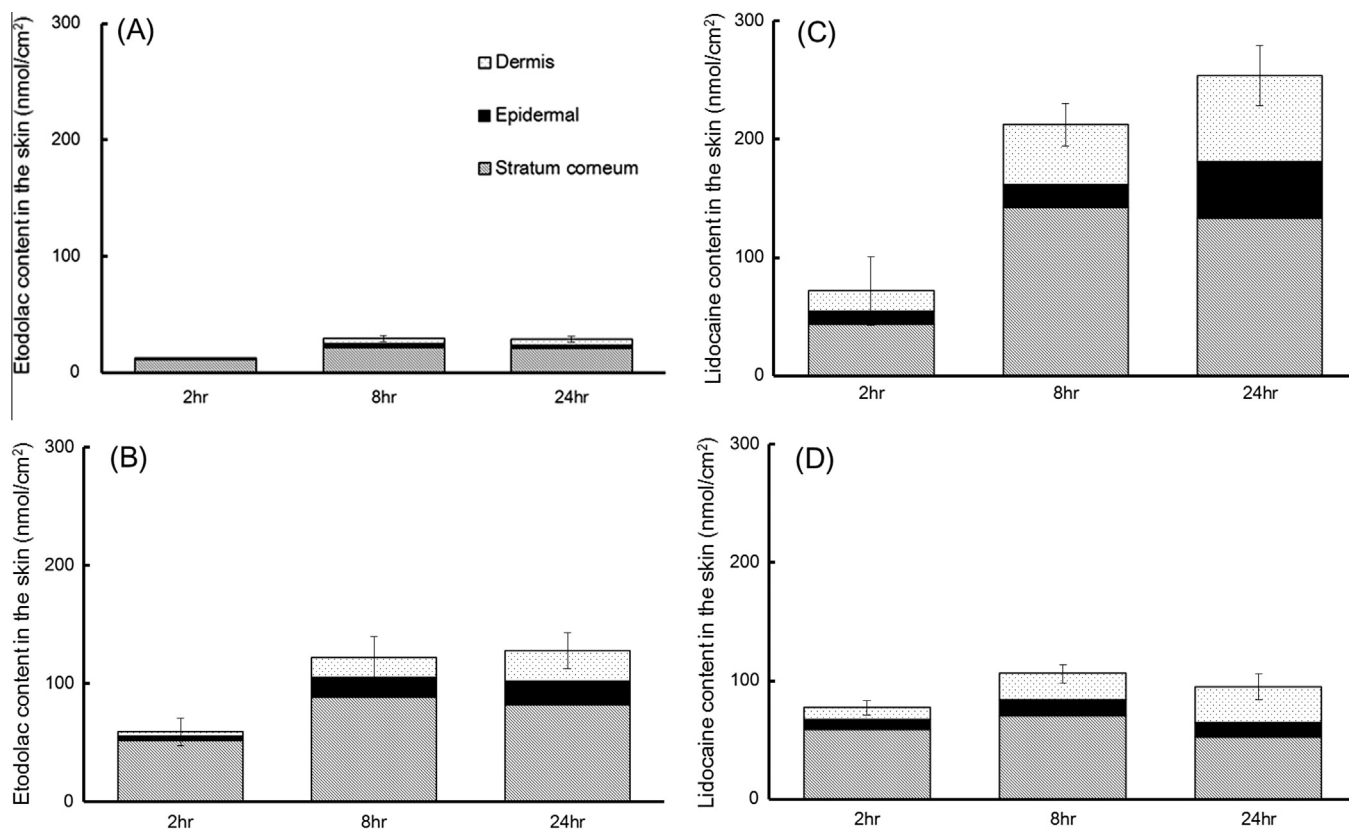
We successfully prepared an ionic liquid by combining an anionic NSAID, etodolac, with a cationic counter ion API, lidocaine. The transformation to an ionic liquid improved the lipophilicity/hydrophilicity of both APIs, etodolac and lidocaine, as we assumed it would. The IR Spectra and DSC thermographs of the Etodolac–Lidocaine ionic liquid indicated that salt formation occurred in the ionic liquid and did not result in API degradations (Figs. 1 and 2). Solubility improvement in ionizable APIs usually is subject to salt screening with sodium, and ionic liquid formulation of APIs has been reported by combining counter ions such as tetrabutylphosphonium (TBP) [29]. Hence, this study represents the first production of an ionic liquid by utilizing the interactions between API molecules with opposite charges.

The saturation level of solubility for etodolac and lidocaine in water was increased to 82.7 and 1.7-fold, respectively, via the formation of ionic liquid (Table 2). The formation of ionic liquids, in principle, reduces the melting point, which increases both the dissociation rate and the solubility [23,29–31]. Our finding is consistent with previous reports [22,23,32,33]. However, it seems that such an improvement cannot necessarily be achieved by the formation of an ionic liquid. Hough et al. [23] demonstrated that the combination of the relatively hydrophobic lidocaine cation with a hydrophobic anion, docusate, forms a hydrophobic ionic liquid salt, lidocaine-docusate, and exhibits reduced water solubility. Balk et al. [29] showed that the respective counter ions control the balance between desired properties (either increased kinetic solubility or a faster dissolution rate in most situations) and undesired properties (either decreased kinetic solubility or a slower dissolution rate in most situations). The creation of an ionic liquid with the desired properties requires either the selection of a suitable counter ion for an API or the combination of respective APIs with opposite charges.

Interestingly, the saturation solubility of etodolac in propylene glycol, diethyl sebacate and octanol was also markedly improved by the formation of an ionic liquid with lidocaine (Table 2). The

**Table 3**  
Comparisons of permeation parameters of Etodolac–Lidocaine, etodolac and lidocaine.

	Etodolac		Lidocaine	
	In E-alone	In Etoreat	In L-alone	In Etoreat
Steady-state flux through intact skin (nmol/cm <sup>2</sup> /h)	1.1	10.5	26.3	19.4
Time lag across intact skin (h)	4.9	3.0	1.8	1.2
Steady-state flux through stripping skin (nmol/cm <sup>2</sup> /h)	5.6	24.3	200.8	64.1
Time lag across intact skin (h)	1.1	1.2	0.5	0.4
Diffusion Coefficient (cm <sup>2</sup> /h $\times 10^{-6}$ )	Matrix	0.9	10.4	40.0
	Stratum corneum	0.4	2.5	1.5
	Viable skin	1494	1390	3568
Matrix/skin partition coefficient	12.5	5.3	2.0	8.3
Stratum corneum/viable skin partition coefficient	17.3	8.8	7.0	5.9



**Fig. 5.** Drug content in the skin fractions: (A) Etodolac in E-alone, (B) Etodolac in Etoreat, (C) Lidocaine in L-alone, and (D) Lidocaine in Etoreat. After the *in vitro* skin-permeation testing, the YMP skin was removed from the diffusion cell and then divided into stratum corneum, epidermal and dermis. Each of the samples, stratum corneum, epidermal and dermis, was suspended in 2 mL of methanol, and sonication was applied for 5 min to extract the drugs (etodolac and lidocaine). After centrifugation followed by filtration, the drug content in each of the skin fractions was determined via HPLC, as described in Section 2. Data represent the mean  $\pm$  S.D. ( $n = 4$ ).

**Table 4**  
Comparisons of the amount of drugs in skin fractions.

Faction	Time (h)	Drug content in skin fractions (nmol)			
		Etodolac		Lidocaine	
		In E-alone	In Etoreat	In L-alone	In Etoreat
Stratum corneum	2	11.1 $\pm$ 1.5	51.5 $\pm$ 9.9 <sup>**</sup>	43.5 $\pm$ 9.1	58.5 $\pm$ 4.0
	8	21.2 $\pm$ 0.8	88.4 $\pm$ 11.1 <sup>**S</sup>	142.1 $\pm$ 12.4	70.0 $\pm$ 6.2 <sup>##</sup>
	24	20.5 $\pm$ 2.8	82.1 $\pm$ 7.1 <sup>**S</sup>	133.1 $\pm$ 36.5	52.1 $\pm$ 15.8 <sup>#</sup>
Epidermal	2	0.3 $\pm$ 0.5	3.8 $\pm$ 1.4 <sup>SS</sup>	11.1 $\pm$ 4.1	8.5 $\pm$ 1.7
	8	3.1 $\pm$ 0.8	16.7 $\pm$ 2.0 <sup>**S</sup>	19.2 $\pm$ 10.0	13.2 $\pm$ 0.3
	24	2.8 $\pm$ 0.5	19.8 $\pm$ 4.7 <sup>**S</sup>	47.4 $\pm$ 57.6	12.4 $\pm$ 2.4
Dermis	2	0.3 $\pm$ 0.6	3.8 $\pm$ 2.1 <sup>*</sup>	17.1 $\pm$ 20.2	10.2 $\pm$ 4.6
	8	4.9 $\pm$ 2	17.1 $\pm$ 5.5 <sup>*</sup>	50.8 $\pm$ 7.5	22.6 $\pm$ 5.2 <sup>##</sup>
	24	5.2 $\pm$ 1.7	25.8 $\pm$ 10.9 <sup>*</sup>	73.0 $\pm$ 4.9	30.3 $\pm$ 9.1 <sup>##</sup>
Sum of the each fractions	2	11.7 $\pm$ 0.7	59.3 $\pm$ 11.7 <sup>**S</sup>	71.6 $\pm$ 28.9	77.6 $\pm$ 5.9
	8	29.2 $\pm$ 2.4	122.3 $\pm$ 17.6 <sup>**</sup>	212.2 $\pm$ 18.2	105.5 $\pm$ 7.6 <sup>##</sup>
	24	28.6 $\pm$ 2.7	127.5 $\pm$ 15.2 <sup>**S</sup>	253.2 $\pm$ 25.5	95.0 $\pm$ 11.0 <sup>##</sup>

Each value represents the mean  $\pm$  S.D. ( $n = 4$ ).

- \*  $p < 0.05$  significant difference from E-alone for etodolac content using the Welch *t* test.  
 \*\*  $p < 0.01$  significant difference from E-alone for etodolac content using the Welch *t* test.  
 #  $p < 0.05$  significant difference from L-alone for lidocaine content using the Welch *t* test.  
 ##  $p < 0.01$  significant difference from L-alone for lidocaine content using the Welch *t* test.  
 S  $p < 0.05$  significant difference from lidocaine content in Etoreat using the Welch *t* test.  
 SS  $p < 0.01$  significant difference from lidocaine content in Etoreat using the Welch *t* test.

saturation solubility of etodolac alone in those solvents was low (Table 2). Lidocaine has a cationic amino group in its structure, while etodolac has an anionic carboxyl group. The ionic liquid of etodolac and lidocaine was formed through the ionic association between the cationic group of lidocaine and the carboxyl group

of etodolac with a suitable mixing molecular ratio at 1:1. The ionic groups of etodolac and lidocaine were coated with hydrophobic portions of ions, which neutralized the etodolac and lidocaine complex. The neutralization due to the interaction between etodolac and lidocaine might have increased the hydrophobicity of etodolac,

resulting in an increased saturation solubility of etodolac in some organic solvents (Table 2).

The formation of an ionic liquid with etodolac and lidocaine at an equimolar ratio also influenced the skin permeation of both APIs. Interestingly, the ratio of 1:1 created a neutralized ionic liquid complex that increased the skin permeation of etodolac and decreased that of lidocaine (Fig. 5C and D and Table 4). The lidocaine in the Etoreat patch (containing ionic liquid) appeared to self-sacrificially improve the limiting steps, and, thereby, the permeation of etodolac through the matrix and stratum corneum of skin. The underlying mechanism behind the improved skin permeation was not clear, but both APIs might stay together in the skin as ion pairs or clusters rather than as independent solvated ions because the molar ratio of etodolac to lidocaine in each of the skin fractions approximated 1 (Table 4). This theory has been suggested by molecular dynamics simulation studies [34–36] and spectroscopic investigations via NMR techniques [36,37] indicating that ionic liquids do not dissolve as independent ions, but, instead, maintain a nanostructured organization in aqueous media.

The application of ionic liquids has been extended from solvents in chemistry and catalysis for synthesis to pharmaceutical applications with the ultimate goal of improving the dissolution, solubility and bioavailability of APIs [23,30,38–40]. Inactive counter ions are the usual choice when creating an ionic liquid. In the present study, the API lidocaine seemed to demonstrate a dual functionality in improving the skin permeability of etodolac. The transport of etodolac through the skin was improved by its formation of an ionic liquid with lidocaine, which would improve the pharmacological activity. However, the approach explored in this study may be restricted because the skin permeability of lidocaine itself was reduced by the formation of an ionic liquid with etodolac. In order to study the pharmacological activities of etodolac and lidocaine in the Etoreat patch, therefore, some preclinical studies with animal models are now being conducted by our group.

## 5. Conclusions

We successfully prepared an ionic liquid by combining an anionic NSAID, etodolac, with a cationic counter ion, lidocaine. The transformation of the ionic liquid improved the lipophilicity/hydrophilicity of etodolac and increased the skin permeation of etodolac (9.3-fold). Lidocaine appeared to self-sacrificially improve the skin permeation of this acidic and poorly water-soluble drug, etodolac, by transforming it into a 1:1 ionic liquid.

## Conflict of interests

T. Ishida reports receiving a research grant from MEDRx Co., Ltd. Y. Miwa and H. Hamamoto are employees of MEDRx Co., Ltd. No other potential conflict of interests exists.

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