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Evaluation of chemical enhancers in the transdermal delivery of lidocaine

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Abstract

The effect of various classes of chemical enhancers was investigated for the transdermal delivery of the anesthetic lidocaine across pig and human skin in vitro. The lipid disrupting agents (LDA) oleic acid, oleyl alcohol, butenediol, and decanoic acid by themselves or in combination with isopropyl myristate (IPM) showed no significant flux enhancement. However, the binary system of IPM/*n*-methyl pyrrolidone (IPM/NMP) improved drug transport. At 2% lidocaine dose, this synergistic enhancement peaked at 25:75 (v/v) IPM:NMP with a steady state flux of $57.6 \pm 8.4\ \mu g\ cm^{-2}\ h^{-1}$ through human skin. This observed flux corresponds to a four-fold enhancement over a 100% NMP solution and over 25-fold increase over 100% IPM at the same drug concentration ($p < 0.001$). NMP was also found to co-transport through human skin with lidocaine free base and improve enhancement due to LDA. These findings allow a more rational approach for designing oil-based formulations for the transdermal delivery of lidocaine free base and similar drugs.

Keywords: Transdermal drug delivery; Chemical enhancers; *n*-Methyl pyrrolidone; Lidocaine

1. Introduction

Lidocaine is a widely used local anesthetic for a variety of medical procedures including treatment of open skin sores and lesions, surgical procedures such as suturing of wounds, and venipuncture (Smith et al., 1999). Lidocaine is also a first line anti-arrhythmic drug when administered to the heart in larger doses (Sleight, 1990). The most common method of lidocaine delivery is through intravenous or hypodermic injection. When lidocaine is injected as an analgesic agent, the discomfort caused by the application is counterproductive to the pain relieving effect of the drug. For purposes such as preparation for pediatric venipuncture, a painless means to administer lidocaine would be an important procedure. This makes local transdermal delivery of lidocaine a favorable avenue of research. Transdermal lidocaine products such as EMLA® cream (AstraZeneca) and Lidoderm® (Endo Laboratories) are commercially available. However, further improvement in enhancement of transdermal lidocaine delivery is desirable.

The primary barrier to transdermal drug delivery is the outermost layer of the skin, the stratum corneum (SC) (Ranade, 1991). The SC consists of keratinocytes embedded in a continuous lipid phase, forming a tortuous network preventing the infiltration of exogenous agents into the body (Johnson et al., 1997). Two main transport pathways have been proposed through human SC—the polar, aqueous pathway and the lipid pathway (Ranade, 1991). Due to the hydrophobicity of the SC, transport of drug through the aqueous pathway has proven very difficult (Peck et al., 1995). Since lidocaine free base is a lipophilic molecule (log octanol–water partition coefficient = 2.48), we decided to explore lipid based enhancers to improve drug flux. A variety of methods for increasing transdermal drug transport have been

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studied. These include chemical enhancers (Walters, 1989), therapeutic and low frequency ultrasound (Mitragotri et al., 1995), iontophoresis (Burnette, 1989), and electroporation (Prausnitz et al., 1993). In this study, the effects and interactions of chemical enhancers were evaluated.

Chemical enhancers with different proposed mechanisms of action were tested for their effects alone and in combination. The more commonly studied chemical enhancers can be broken down into three broad categories. The first is the class of lipid disrupting agents (LDAs), usually consisting of a long hydrocarbon chain with a *cis*-unsaturated carbon–carbon double bond (Francoeur et al., 1990; Kim and Chien, 1996). These molecules have been shown to increase the fluidity of the SC lipids, thereby increasing drug transport. In this study, oleic acid, oleyl alcohol, decanoic acid, and butene diol were investigated as lipid disrupting agents. A second class of permeation enhancers relies on improving drug solubility and partitioning into the skin (Guy and Hadgraft, 1987). The lipophilic vehicle isopropyl myristate (IPM) (Gorukanti et al., 1999) as well as the organic solvents ethanol (Liu et al., 1991) and *N*-methyl pyrrolidone (NMP) (Yoneto et al., 1995) were studied. The third class of enhancers studied was surfactants. These molecules have affinity to both hydrophilic and hydrophobic groups, which might facilitate in traversing the complex regions of the SC. An anionic surfactant lauryl sulfate (SDS) and a nonionic surfactant polysorbate 80 (Tween 80) (Sarpotdar and Zatz, 1996) were tested for their effect on lidocaine delivery. It has been reported in the literature that combinations of various enhancers result in a synergistic increase in drug flux that is far greater than either chemical by itself (Johnson et al., 1996; Sasaki et al., 1990; Priborsky et al., 1987; Karande et al., 2004). Therefore, various mixtures of enhancer combinations were also tested to identify useful trends in lidocaine free base delivery.

Lidocaine free base is a commonly studied drug for transdermal delivery (Johnson et al., 1996; Johnson et al., 1995) as its hydrophobicity and molecular size (MW 234.3) characterize it as a typical transdermal drug candidate. By studying the effects of a wide range of chemical enhancers across both full thickness pig skin and heat separated human cadaver skin, some general trends of transdermal permeation enhancement can be hypothesized.

2. Materials and methods

2.1. Preparation of lidocaine solutions

Sample solutions were prepared in 20 ml scintillation glass vials and saturated with drug. In binary systems the sample contained 50% (w/w) of each liquid. All vehicles studied formed miscible, single-phase liquids.

2.2. Determination of saturation concentration

All samples were mixed with a magnetic stir-bar in the presence of lidocaine free base crystals for at least 24 h at room temperature. The saturated solutions were then filtered through a 0.2 μm filter to remove precipitated drug. Concentrations of the filtered solutions were determined by high performance liquid chromatography (HPLC) after dilution to a suitable range.

2.3. Preparation of skin samples

Human cadaver skin from the chest, back, and abdominal regions was obtained from the National Disease Research Interchange (Philadelphia, PA). The skin was stored at −80 °C and thawed at room temperature prior to use. The epidermis-SC was separated from the full thickness tissue after immersion in water (60 °C) for 2 min. Heat separated epidermis was immediately mounted on diffusion cells. Full thickness pig skin was prepared by removing the dermal tissue from a freshly sacrificed pig. Pig skin samples were subsequently frozen and stored at −20 or −80 °C.

2.4. Lidocaine transport experiments

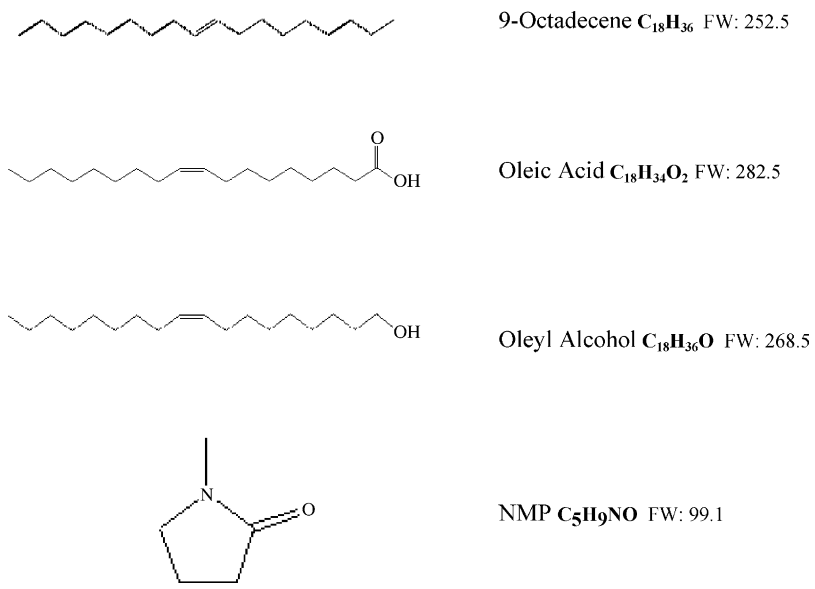
The skin was mounted onto side-by-side glass diffusion cells with an inner diameter of 5 mm. The two halves of the cell were clamped shut and both reservoirs were filled with 2 ml of phosphate buffered saline (PBS, 0.01 M phosphate, 0.137 M NaCl, pH 7.4). The integrity of the skin was verified by measuring skin's electrical impedance at 10 Hz using a mV rms potential (HP 33120A Waveform Generator). Skin samples with an initial impedance of at least 20 kΩ cm^2 were used for the diffusion studies. Prior to introducing the donor solution, the skin sample was thoroughly rinsed with PBS to remove surface contaminants. At $t = 0$, the receiver compartment was filled with 2.0 ml of PBS, while 2.0 ml of sample was added to the donor compartment. Both compartments were continuously stirred to maintain uniform concentrations. At regular time intervals, 1.0 ml of the receiver compartment was transferred to a glass HPLC vial. The remaining solution in the receiver compartment was thoroughly aspirated and discarded. Fresh PBS (2.0 ml) was dispensed into the receiver compartment to maintain sink conditions. After the last time point was collected, the conductance across the skin membrane was checked again in PBS. All flux experiments were conducted in triplicate at room temperature to ensure that the skin was not damaged during the experiment.

2.5. IPM/NMP binary vehicle transport

The two miscible liquids were mixed in the specified (v/v) ratios, with 2% (w/v) lidocaine free base added. Flux cells were set up as described above. At $t = 4$, 21, 23, 25 h, the transport of drug across the skin was measured by HPLC.

2.6. LDA/NMP interaction

Solutions were prepared containing 2% (w/v) lidocaine free base and 1% (w/v) LDA in IPM or NMP. The LDA studied were 9-octadecene, oleyl alcohol, and oleic acid (Scheme 1). Each of these molecules contains C_{18} alkyl chains with a 9-*cis*-double bond, but has different end groups. Although 9-octadecene has not been reported as a LDA, its similar chemical structure allows the comparison of its effect with oleyl alcohol and oleic acid. The



Scheme 1.

flux of lidocaine free base and of NMP through human cadaver skin was determined by HPLC.

2.7. Quantification of lidocaine

Lidocaine was assayed by HPLC based on protocol published in the United States Pharmacopoeia (USP). An HPLC system (Shimadzu model HPLC, SCL-10A Controller, LC-10AD pumps, SPD-M10A Diode Array Detector, SIL-10AP Injector, Class VP v.5.032 Integration Software) with a reverse phase column (Waters μBondapak^{TM} C_{18} 3.9 mm × 150 mm) and ddH_2O (5% acetic acid, pH 4.2)/acetonitrile (35:65, v/v) as the mobile phase under isocratic conditions (1.6 mL min^{-1}) and detection at 237 nm was used. The retention time of lidocaine under these conditions was between 3.4 and 4.3 min. Standard solutions were used to generate calibration curves with linear correlation >0.999 between 100 and 1000 μg ml^{-1} prior to each experiment. NMP was quantified according to previously published methods (Sasaki et al., 1990). A Waters Symmetry® C_{18} 5 μm, 3.9 mm × 150 mm column was used. The mobile phase consisted of ddH_2O:methanol (95:5) at a flow rate of 1.2 ml min^{-1}. Chromatograms were integrated at a peak of 205 nm, with retention time between 3.8 and 4.8 min.

2.8. Calculations

The total mass of drug transported across the skin was determined by HPLC. The flux equation gives:

$$J = \frac{1}{A}\left(\frac{dM}{dt}\right) = k_p \Delta C$$

where, J is flux (μg cm^{-2} h^{-1}), A is cross-sectional area of the skin membrane (cm^2), k_p is the apparent permeability coefficient (cm h^{-1}), and ΔC is the concentration gradient. In this experiment, ΔC was taken as the saturation concentration (given infinite dose and sink conditions), and dM/dt was averaged as the total mass transport over the time course of the experiment. Statistical analyses were performed with the Student's t-test.

3. Results

3.1. Lidocaine free base solubility

The solubility of lidocaine free base in the various enhancers is given in Table 1. The solubility of lidocaine free base in the hydrophobic enhancers ranged between 300 and 400 mg ml^{-1}. The two solvents that significantly improved the saturation concentration of lidocaine free base were ethanol (618 mg ml^{-1}) and NMP (733 mg ml^{-1}).

3.2. Permeability of lidocaine free base

The in vitro permeability of lidocaine free base across heat separated human epidermis and full thickness pig skin gave an indication of the enhancing effect of each chemical beyond their ability to improve drug saturation concentration. The permeability of lidocaine free base saturated in enhancer solutions is given in Table 1.

3.3. LDAs

The chemical enhancers with proposed lipid-disrupting ability (oleyl alcohol, oleic acid, butene-diol) by themselves did not show significant improvement of lidocaine permeability or flux (Table 1). The flux and permeability of lidocaine free base in the presence of these enhancers was either statistically equivalent or below that of IPM solution.

3.4. Solubility/partition enhancers

Permeability experiments across both human and pig skin indicated that the two chemical enhancers with highest drug

Table 1
Effect of chemical enhancers on lidocaine transport ($N = 3$)

Sample	Saturation concentration ($mg\,ml^{-1}$)	Stripped human cadaver skin		Full thickness pig skin	
		Time averaged permeability ($cm\,h^{-1} \times 10^{-5}$) ± S.D.	Time averaged flux ($\mu g\,cm^{-2}\,h^{-1}$) ± S.D.	Time averaged permeability ($cm\,h^{-1} \times 10^{-5}$) ± S.D.	Time averaged flux ($\mu g\,cm^{-2}\,h^{-1}$) ± S.D.
IPM	246	7.17 ± 1.06	20.4 ± 3.02	0.515 ± 0.117	0.847 ± 0.192
NMP	733	2.92 ± 0.42	26.8 ± 3.86	0.120 ± 0.007	0.706 ± 0.043
Oleyl alcohol	361	3.68 ± 1.59	14.5 ± 6.28	–	–
Oleic acid	428	0.61 ± 0.28	4.04 ± 1.83	–	–
Butene diol	386	1.18 ± 0.47	4.56 ± 1.80	–	–
Ethanol	618	5.14 ± 3.55	31.7 ± 21.9	0.0649 ± 0.0190	0.182 ± 0.053

Table 2
Effect of 1:1 co-solvent systems on lidocaine transport ($N = 3$)

Sample	Saturation concentration ($mg\,ml^{-1}$)	Stripped human cadaver skin		Full thickness pig skin	
		Time averaged permeability ($cm\,h^{-1} \times 10^{-5}$) ± S.D.	Time averaged flux ($\mu g\,cm^{-2} \pm h^{-1}$) ± S.D.	Time averaged permeability ($cm\,h^{-1} \times 10^{-5}$) ± S.D.	Time averaged flux ($\mu g\,cm^{-2} \pm h^{-1}$) ± S.D.
IPM	246	7.17 ± 1.06	20.4 ± 3.02	0.515 ± 0.117	0.847 ± 0.192
IPM/NMP 9:1	365	20.0 ± 6.92	53.4 ± 18.5	1.37 ± 0.69	3.64 ± 1.83
IPM/NMP 1:1	641	18.9 ± 3.10	165 ± 27.1	0.965 ± 0.521	2.78 ± 1.50
NMP	733	2.92 ± 0.42	26.8 ± 3.86	0.120 ± 0.007	0.706 ± 0.043
IPM/oleyl alcohol	345	3.62 ± 1.12	17.7 ± 5.48	–	–
IPM/oleic acid	355	4.93 ± 0.57	32.5 ± 3.73	–	–
IPM/decanoic acid	309	2.47 ± 1.50	7.64 ± 4.65	–	–

solubility were poor permeation enhancers (Table 1). The permeability of lidocaine free base from saturated solutions of ethanol and NMP did not surpass that of IPM. By themselves, these two solvents appeared to be able to increase drug flux only by improving drug solubility.

3.5. Permeability in co-solvent systems

To evaluate possible synergistic interactions between the studied enhancers, 1:1 ratios of selected chemicals were mixed to form co-solvent systems (Table 2). IPM was selected as the bulk oil phase to allow mixing with other enhancers since it exhibited the best in vitro permeability of the studied enhancers. Co-solvents of IPM were made with oleyl alcohol, oleic acid, decanoic acid, and NMP. Decanoic acid is chemically similar to oleic acid, and may act in the skin as a lipid-disrupting agent. In these co-solvent systems the LDA had no noticeable enhancing effect on lidocaine free base permeability. The trend appeared similar to that of the neat solvents, suggesting that mixing these LDA with IPM did not result in pronounced enhancement. The only IPM/co-solvent system that had a significant effect on permeability across heat separated human epidermis was the IPM/NMP system ($p < 0.005$). The total transdermal flux across human skin ($165 \pm 27\,\mu g\,cm^{-2}\,h^{-1}$) was roughly eight-fold greater than that of IPM alone, and over six-fold better than saturated NMP ($p < 0.001$). A similar trend was seen in full thickness pig skin (Table 2).

3.6. IPM/NMP co-solvent flux

Based on the high flux observed from the IPM/NMP 1:1 binary system, the effect of varying ratios of the two enhancers on lidocaine free base (2%) flux was studied (Table 3). Between 10 and 75% (v/v) NMP an increase in lidocaine flux was observed that scaled linearly with NMP concentration (Fig. 1). Furthermore, in this range a strong linear correlation was observed between lidocaine flux and NMP flux ($R^2 = 0.97$) (Fig. 2). However, beyond 75% and up to 90% NMP lidocaine flux remained relatively unchanged, although the flux in this range was four times the flux observed from 100% NMP (Fig. 1).

3.7. NMP/LDA flux

Flux of lidocaine free-base through human cadaver skin from IPM phase containing various LDA is given in Table 3. There was no statistical significance ($p > 0.40$) in lidocaine flux from various IPM formulations. When NMP was used as a solvent, an increase in lidocaine free base flux was observed in the following

Table 3
Lidocaine transport from IPM/NMP co-solvent systems through stripped human cadaver skin ($N = 3$)

% NMP (v/v)	Lidocaine $flux_{ss}$ ($\mu g\,cm^{-2}\,h^{-1}$) ± S.D.	NMP $flux_{ss}$ ($mg\,cm^{-2}\,h^{-1}$) ± S.D.
0	1.98 ± 0.57	0
10	4.69 ± 0.84	1.46 ± 0.9
25	16.4 ± 0.5	5.40 ± 0.49
50	32.5 ± 4.0	9.01 ± 0.84
60	40.7 ± 1.2	11.2 ± 1.2
75	56.7 ± 4.9	14.0 ± 0.9
90	57.6 ± 8.4	11.7 ± 0.1
100	15.4 ± 0.6	10.7 ± 0.2

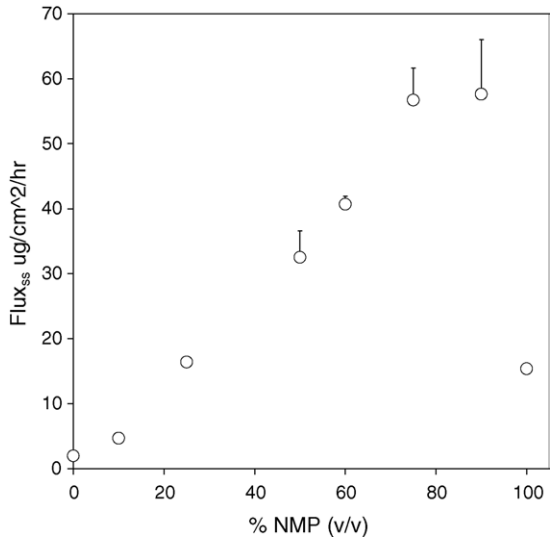


Fig. 1. Lidocaine free base delivery from IPM/NMP binary vehicles through heat separated human cadaver skin. The flux of lidocaine is plotted vs. NMP concentration with S.D. shown for each point $N = 3$.

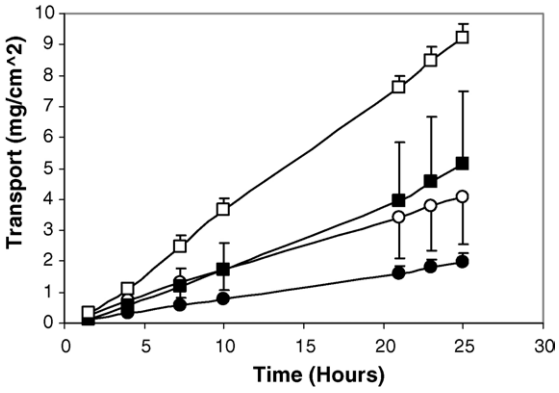


Fig. 2. Transdermal transport of 2% lidocaine through human SC. Drug transport over 25 h in NMP solvent containing no LDA (●), 1% octadecene (◯), 1% oleic acid (■), and 1% oleyl alcohol (□).

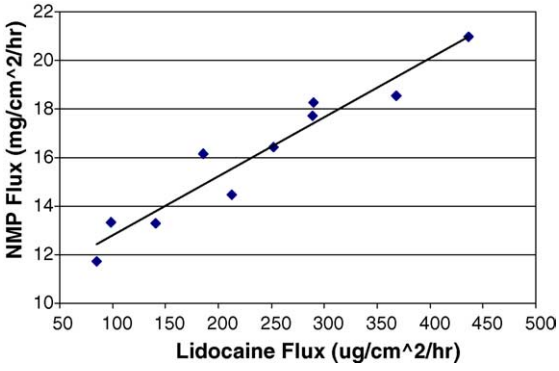


Fig. 3. Correlation of NMP and lidocaine steady state flux in NMP solvent with 1% LDAs. The flux of lidocaine free base (2%, w/v) is plotted against the flux of NMP from the same samples. Although there is a large range of NMP flux, the relative amount of drug flux increases proportionately indicating a relationship between the transport of the two species ($R^2 = 0.93$).

order: pure NMP < 9-octadecene < oleic acid < oleyl alcohol. All three enhancers improved lidocaine free base flux compared to that from pure NMP ($p < 0.05$). The formulation with the greatest flux, NMP/oleyl alcohol (1%, w/v), was statistically better than IPM alone and NMP/9-octadecene (1%, w/v) ($p < 0.05$). We observed that the flux of NMP from these formulations followed that of lidocaine transport, with a R^2 value of 0.93 (Fig. 3).

4. Discussion

Due to the continuous lipid regions in the stratum corneum, it is believed that passive transdermal diffusion occurs predominantly through the lipid phase of the skin (Ranade, 1991; Johnson et al., 1997). For this reason, hydrophobic drugs generally have better transport through skin while water-soluble ionic drugs have very limited permeability (Lee et al., 1994). In this study, lidocaine free base was used as a model drug to study the relative effectiveness of a wide range of lipophilic chemical enhancers. Understanding the effects of combining enhancers can aid the development of improved multi-component transdermal formulations (Lee et al., 2003).

One class of chemical enhancers studied was LDA. These hydrophobic molecules are believed to fluidize the stratum corneum lipids and reduce its barrier properties. In this study, LDA by itself did not improve the permeability of drug across the skin. Saturated solutions of oleyl alcohol, oleic acid, butenediol, and decanoic acid resulted in very poor lidocaine free base transport. This observation is consistent with the generally accepted hypothesis that transport of relatively small molecules such as lidocaine free base is not severely hindered by lipid bilayers, hence altering the bilayer properties would not result in a dramatic increase of flux (Johnson et al., 1996; Mitragotri et al., 1999; Mitragotri, 2001).

NMP and its derivatives are widely used chemical enhancers, which have produced significant results in the transport of various drugs (Sasaki et al., 1990; Phillips and Michniak, 1995). More recently, they have been used in conjunction with more lipophilic molecules to enhance partitioning of more hydrophilic drugs into the skin (Yoneto et al., 1995). Although NMP by itself is an exceptional solvent for drugs, our experiments showed that it did not greatly improve the permeability of lidocaine. However, combining NMP with IPM resulted in substantial flux improvement. In 2% lidocaine systems, the maximum flux occurred between 75 and 90% (v/v) NMP, with a linear relationship below 75% NMP.

The method of transdermal enhancement by NMP was most likely through improving the partitioning of lidocaine free base through the SC barrier. This process may be facilitated by hydrogen bonding between NMP and the drug, as suggested by previous work (Lee et al., 2005). This hypothesis was consistent with the observed correlation between NMP flux and lidocaine flux ($R^2 = 0.93$), suggesting that NMP co-transport was important for drug delivery (Fig. 3). The high permeability of NMP through human SC (1.8×10^{-2} cm h^{-1} in the NMP/IPM systems) might serve as a driving force for lidocaine free base flux. Increasing NMP above 75% quickly diminished permeability of both drug and enhancer. One plausible scenario is that at

Table 4
Lidocaine and NMP flux with LDA ($N = 3$)

LDA (1%, w/v)	IPM solvent	NMP solvent	
	Lidocaine $flux_{ss}$ ($\mu g\,cm^{-2}\,h^{-1} \pm$ S.D.)	Lidocaine $flux_{ss}$ ($\mu g\,cm^{-2}\,h^{-1} \pm$ S.D.)	NMP $flux_{ss}$ ($mg\,cm^{-2}\,h^{-1} \pm$ S.D.)
None	1.95 ± 0.22	92 ± 15	12.6 ± 0.5
9-Octadecene	1.98 ± 0.58	161 ± 52	15.3 ± 1.2
Oleic acid	1.68 ± 0.24	290 ± 103	17.6 ± 2.0
Oleyl alcohol	1.97 ± 0.48	402 ± 52	20.7 ± 1.0

concentrations nearing 100% NMP, molecular level interaction between NMP molecules (via H-bonding) dominant and diminish the thermodynamic activity of NMP. However, upon addition of IPM, disruption of these molecular level interactions due to interaction of IPM, might favor transport of NMP by increasing its thermodynamic activity.

The precise mechanism by which NMP synergizes the effect of LDAs is unclear. It is likely that the synergistic behavior of NMP with LDA appears from the H-bonding capacity of NMP. Based on molecular structures of the LDA studied, oleic acid and oleyl alcohol can be expected to exhibit H-bonding with NMP while 9-octadecene, which lacks a hydroxyl group should not favor such interactions. This could translate into improved penetration of oleyl alcohol and oleic acid into the SC thereby, encouraging disruption of lipid bilayers. This hypothesis is consistent with experimental data (Table 4) and the lack of an enhancing effect of the same LDAs in the absence of NMP (Table 1). In view of NMP's ability to extract epidermal lipids, the role of solubilization of lipids in enhancement of transport cannot be ruled out. It is noteworthy, however that the enhancement at 25:75 IPM/NMP is significantly greater than at 100% NMP, suggesting that the role of lipid extraction in the enhancement might be smaller than expected. This observation is further supported by our findings that the conductance of the skin samples at the end of the experiment was similar to those at the beginning, implying minimal damage if any to the skin (data not shown). Nevertheless, potential permanent damage to skin upon long-term exposure of these formulations need to be studied in rodent models.

5. Conclusion

NMP was found to be an effective enhancer of transdermal lidocaine transport from a hydrophobic formulation. Combinations of NMP with IPM resulted in synergistic enhancements of lidocaine free base delivery. The effect of LDA was also improved in the presence of NMP. Observed trends of NMP and lidocaine co-transport indicate that the IPM/NMP system may have utility in the delivery of other hydrophobic molecules as well. These findings may prove useful in the development of multi-component transdermal enhancer vehicles.

Acknowledgements

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