Influence of combinations of enhancers on skin permeation of meloxicam

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メロキシカムの皮膚透過性に及ぼす吸収促進剤の併用効果

非ステロイド性消炎鎮痛剤 (NSAIDs) の一つであるメロキシカムを用い、皮膚透過性に及ぼす作用機序の異なる吸収促進剤の併用効果について評価した。その結果、薬物の溶解度に寄与する添加剤と皮膚中での拡散または皮膚への分配に寄与する添加剤を併用することで、高い皮膚透過性が得られた。また、製剤 (テープおよびパップ剤) では、薬物の溶解度に寄与する添加剤の性質により皮膚透過性が異なることが明らかとなった。以上より、高い皮膚透過性を得るには、溶解度に寄与する添加剤と拡散または分配に寄与する添加剤の併用や、基剤に適した添加剤の選択が重要であることが明らかとなった。

Permeation of meloxicam (MEL) through excised hairless mouse skin under the influence of various additives was investigated with respect to mechanisms of action. Of a total of 23 additives, such as alcohols, fatty acids, esters, terpenes, amines and surfactants, 12 were found to enhance the skin permeation of MEL, and these could be classified into three groups that are influencing diffusion in the skin, partition to the skin and solubility of the drug. Combination of additives enhancing diffusion and partition resulted in approximately 6-fold increase in permeation as compared to the water control case, whereas diisopropanolamine enhancing solubility combined with l-menthol enhancing diffusion or glycerin monocaprylate enhancing partition caused 500-fold elevation. Therefore, to achieve high enhancement with combinations having differing mechanisms of action, use of an additive enhancing solubility with either of the other two types may have advantages.

With tape type patches containing diisopropanolamine to increase solubility of MEL with l-menthol to increase diffusion in the skin or glycerin monocaprylate to increase partition to the skin, permeation was well enhanced, but this was not the case with poultice type patches. With these latter, improvement was obtained on substitution of polyoxyethylene oleylamine (5E.O.), a hydrophobic solvent, for the hydrophilic diisopropanolamine.

These results suggest that combinations of enhancers and confirmation of the miscibility of additives enhancing solubility with the base is important for achieving high permeation of drugs from transdermal patches.

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Meloxicam (MEL) is a nonsteroidal anti-inflammatory drug (NSAID) which inhibits cyclooxygenase (COX), the enzyme responsible for the first step in synthesis of various prostaglandins (PGs) from arachidonic acid. COX exists as two isoforms, COX-1, which presents in almost all cells and constitutively producing PGs concerned with protection of the gastric mucosa and kidney functions, and COX-2, which is induced by cyto-

kines or hormones in inflammatory regions¹⁾. Although MEL that is unlike conventional NSAIDs selectively inhibits COX-2, some of COX-1 is also inhibited²⁻⁵⁾. Therefore it holds promise in reducing side effects and improving local disorders with transdermal application.

Transdermal administration of drugs has many advantages when compared with oral or injection administration, such as the avoidance of the first pass effect in the liver and side effects on the gastrointestinal tract, achievement of a prolonged steady-state blood concentration of the drug and

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the potential for rapid withdrawal by removal⁶⁾. However, this approach is often precluded because of the stratum corneum barrier. In the development of transdermal patches it is therefore necessary to overcome this barrier and various techniques have been employed to enhance the skin permeation of drugs. One generalized approach is to use additives. The process of skin permeation of drugs seems to follow Fick's theory of diffusion⁷⁾, that is, passive diffusion that derives a driving force from the concentration gradient of drug. In this theory, skin permeability of drugs can be enhanced by additives which have effects on solubility of drugs, partition to the skin, and diffusion in the skin. There have been many reports regarding mechanisms of action of enhancers of skin permeation⁸⁾, and best results are generally achieved with combinations rather than single enhancers alone⁹⁾. However, enhancing effects on permeation differ with the enhancer and drug¹⁰⁾, and making suitable choices is complicated by the range of enhancers that are available. With transdermal patches, the additives solving drugs influence diffusion in the patch and release onto the skin, these varying with the base¹¹⁻¹⁴⁾. According to Higuchi's report¹⁵⁾, such variation in the thermodynamic activity of drugs is very important for skin permeation. Therefore it is important for achieving high permeation of drugs to select suitable additives for high thermodynamic activity of drugs in the patch.

In the present study, additives, such as alcohols, fatty acids, esters, terpenes, amines and surfactants having different mechanisms of action were investigated for their effects, alone or in combination on permeation of MEL through excised hairless mouse skin, and then tape and poultice type patches containing additives that showed the enhancement effect were also evaluated.

Materials and methods

1. Materials

Meloxicam was given from Nippon Boehringer Ingelheim Co., Ltd. Diisopropanolamine was pur-

Tab.1 Additive and its concentration in the donor solutions (w/w)

Additive	Concentration (%)
Alcohols	
Lauryl alcohol	3
Stearyl alcohol	5
Hexyldecanol	20
Octyldodecanol	35
Isostearyl alcohol	3
Fatty acids	
Capric acid	0.9
Stearic acid	10
Esters	
Diethyl cebacate	40
Diisopropyl adipate	12
Isopropyl myristate	45
Triacetin	30
Terpenes	
Eucalyptus oil	1
l-menthol	4
Amines	
Diethanolamine	1
Triethanolamine	4
Diisopropanolamine	5
Triisopropanolamine	1
Surfactants	
Polysorbate 80	10
Polyoxyethylene hydrogenated castor oil	3
Glycerin monocaprylate	5
Polyethylene glycol monostearate	1
Polyoxyethylene stearyl ether	1
Polyoxyethylene oleylamine (5E.O.)	1

chased from Nakalai Chemicals, triacetin and isopropyl myristate from Wako Pure Chemical Industry, lauryl alcohol and stearic acid from Tokyo Kasei Kogyo Co., Ltd., and stearyl alcohol, isostearyl alcohol, hexyldecanol and octyldodecanol from Higher Alcohol Industries. Capric acid was obtained from the NOF Corporation and diethyl cebacate, diisopropyl adipate, glycerin monocaprylate, polysorbate 80, polyoxyethylene hydrogenated castor oil, polyoxyethylene glycol monostearate, polyoxyethylene stearyl ether, and polyoxyethylene oleylamine (5E.O.) from Nikko Chemicals. Eucalyptus oil was purchased from Sakae, l-menthol from Takasago International Corporation, triisopropanolamine from MTB and diethanolamine and triethanolamine from Shell. All other additives, pharmacopoeial grade, were purchased from each supplier.

Excised skin from male hairless mice at 7 weeks of age was purchased from Japan SLC Inc.

Tab.2 Formulations of tape type patches used in this study

	Formulation (% w/w)			
	1	2	3	4
Meloxicam	1.25	1.25	1.25	1.25
Diisopropanolamine		2.5	2.5	2.5
l-menthol			4	
Glycerin monocaprylate				5
Acrylic adhesive	98.75	96.25	92.25	91.25

Tab.3 Formulations of poultice type patches used in this study

	Formulation (% w/w)			
	5	6	7	8
Meloxicam	0.1	0.1	0.1	0.1
Diisopropanolamine		1		
Polyoxyethylene oleylamine (5E.O.)			1	1
l-menthol				4
Water-soluble polymers	9	9	9	9
Glycerin	35	35	35	35
pH regulator and other miner additives	q.s.	q.s.	q.s.	q.s.
Add purified water to 100				

2. Preparation of donor solutions

Additives were added to purified water and MEL was suspended in excess in the solution, which was then used as the donor solution. Concentrations of additives are listed in **Tab.1**. Suspensions were all prepared the day before testing and stirred overnight at 32°C.

3. Preparation of test patches

The composition of tape type patches is shown in **Tab.2**. These patches were prepared using a laboratory coating unit. MEL was dissolved in additives and mixed with the acrylic adhesive. The mixture was spread onto plastic release liner at 14.3 mg/cm². The systems were dried at 70°C for 15 min and covered with polyethylene terephthalate film.

The composition of poultice type patches is shown in **Tab.3**. These patches were prepared using a manual coating unit. MEL was dissolved in additives and mixed with the other additives and water-soluble polymers. The gel was spread onto plastic release liner at 71.4 mg/cm² and covered with non-woven cloth.

All patches were kept at 25°C after preparation.

4. *In vitro* skin permeation tests

Skin permeation tests with the solution system were performed according to the following method. An excised skin sample was held between 2-chamber diffusion cells (effective diffusion area : 0.283 cm²) in which water at 32°C was circulated. To the dermis side (receiver side) was applied 0.9 mL of 0.05 mol/L McIlvaine buffer (pH 7.4), and to the stratum corneum side (donor side) was applied 0.9 mL of each donor solution. At 4,8,12,15 and 24 hours after application, 0.3 mL of receiver fluid was sampled from the receiver side, and the same amount of 0.05 mol/L McIlvaine buffer (pH 7.4) was added to maintain a constant volume of receiver fluid.

Skin permeation tests with patches were performed according to the following method. Using vertical diffusion cells (effective diffusion area : 1.77 cm²), to the dermis side (receiver side) was applied 1.2 mL of 0.05 mol/L McIlvaine buffer (pH 7.4), and to the stratum corneum side (donor side) was applied test patches. Permeation tests were carried out at 32°C. At 4,8,12 and 24 hours after application, 0.6mL of receiver fluid was sampled, and the same amount of 0.05 mol/L McIlvaine buffer was replaced to maintain a constant volume of receiver fluid.

5. Quantitation of meloxicam in the sample and donor solutions

Exactly 150 µL of each sample was added to the same volume of an internal standard (a solution of ethyl anthranilate in methanol, 400 µg/mL). The mixture was thoroughly stirred in a test tube mixer and centrifuged (5°C, 13,000 rpm) for 5 min. After the supernatant was filtered through a syringe filter (pore size 0.2 µm), the concentration of MEL was determined by HPLC on a Agilent HP1100 series with a 4.6 mm × 7.5 cm, d_p = 3 µm, YMC, YMC-Pack Pro C18 column. The mobile phase was phosphate buffer (pH 6)/methanol (3/2 : v/v) and the retention time of MEL was about 4 min.

Tab.4 Parameters of meloxicam permeation through excised hairless mouse skin in the presence of enhancers

Formulation	Flux ^a ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Lag time ^b (hr)	Saturation solubility ($\mu\text{g}/\text{mL}$)	Diffusion coefficient ($\text{D}, \text{cm}^2/\text{hr}, \times 10^{-4}$)	Partition coefficient (K)	Permeation coefficient (P, cm/hr)
Purified water (Control)	3.7 ± 0.0	13.6 ± 0.1	52.1	0.221 ± 0.008	135.80 ± 1.50	0.071 ± 0.001
Alcohols						
Lauryl alcohol	3.2 ± 1.0	10.9 ± 0.4	83.3	0.432 ± 0.066	50.52 ± 19.58	0.038 ± 0.011
Stearyl alcohol	11.8 ± 4.2	14.0 ± 0.4	72.0	0.271 ± 0.056	273.69 ± 75.75	0.163 ± 0.059
Hexyldecanol	1.8 ± 0.8	9.3 ± 2.0	186.3	0.309 ± 0.046	15.13 ± 7.91	0.010 ± 0.004
Octyldodecanol	1.6 ± 0.7	10.9 ± 1.1	110.6	0.374 ± 0.053	19.61 ± 8.88	0.014 ± 0.006
Isostearyl alcohol	2.5 ± 1.2	12.1 ± 1.3	70.3	0.293 ± 0.031	62.44 ± 32.90	0.036 ± 0.017
Fatty acids						
Capric acid	22.3 ± 5.4	10.6 ± 1.1	12.0	0.276 ± 0.047	$3,003.56 \pm 962.48$	1.858 ± 0.452
Stearic acid	3.3 ± 0.2	12.4 ± 0.2	8.0	0.215 ± 0.075	795.52 ± 100.08	0.413 ± 0.025
Esters						
Diethyl cebacate	2.0 ± 0.8	10.2 ± 1.7	24.2	0.208 ± 0.052	7.81 ± 4.62	0.004 ± 0.001
Diisopropyl adipate	3.0 ± 0.7	10.6 ± 1.0	39.0	0.420 ± 0.098	10.35 ± 3.89	0.008 ± 0.002
Isopropyl myristate	1.1 ± 0.3	9.0 ± 1.3	15.2	0.237 ± 0.035	14.03 ± 5.89	0.008 ± 0.002
Triacetin	0.8 ± 0.2	7.4 ± 1.4	13.0	0.358 ± 0.058	5.75 ± 2.10	0.005 ± 0.002
Terpenes						
Eucalyptus oil	4.8 ± 1.9	7.7 ± 1.3	64.8	0.674 ± 0.161	73.68 ± 38.94	0.074 ± 0.030
l-menthol	39.7 ± 3.1	2.9 ± 0.8	176.0	1.260 ± 0.641	111.58 ± 40.54	0.226 ± 0.018
Amines						
Diethanolamine	24.6 ± 8.0	11.4 ± 1.4	11,562.2	0.382 ± 0.039	2.82 ± 0.92	0.002 ± 0.001
Triethanolamine	30.2 ± 0.5	14.4 ± 0.2	4,798.0	0.239 ± 0.041	12.28 ± 1.41	0.006 ± 0.000
Diisopropanolamine	110.5 ± 38.8	13.5 ± 0.7	93,511.0	0.184 ± 0.016	2.57 ± 1.03	0.001 ± 0.000
Triisopropanolamine	39.9 ± 29.7	13.6 ± 0.7	7,587.3	0.325 ± 0.116	11.86 ± 9.60	0.005 ± 0.004
Surfactants						
Polysorbate 80	0.7 ± 0.2	12.6 ± 0.5	228.6	0.526 ± 0.171	4.22 ± 1.59	0.003 ± 0.001
Polyoxyethylene hydrogenated castor oil	1.4 ± 0.5	11.3 ± 0.3	71.5	0.382 ± 0.067	29.68 ± 14.22	0.020 ± 0.008
Glycerin monocaprylate	9.7 ± 2.2	12.1 ± 0.6	69.3	0.219 ± 0.030	265.96 ± 72.03	0.140 ± 0.032
Polyethylene glycol monostearate	9.3 ± 5.7	11.5 ± 2.0	86.5	0.345 ± 0.132	204.85 ± 122.07	0.107 ± 0.065
Polyoxyethylene stearyl ether	9.8 ± 1.5	14.4 ± 0.2	62.5	0.204 ± 0.028	326.80 ± 53.57	0.156 ± 0.024
Polyoxyethylene oleylamine (5E.O.)	15.3 ± 5.5	12.2 ± 0.3	411.4	0.373 ± 0.085	50.79 ± 12.32	0.037 ± 0.013

^a The value were calculated from the straight lines of the permeated amount of meloxicam.

^b The value were calculated from the time intercept of the straight-line portion of the cumulative permeation-time curve.

6. Data analysis

Skin permeation parameters were calculated according to the following method. Using equations 1 and 2, the partition coefficient (K), diffusion coefficient (D) and permeation coefficient (P) were calculated from the speed of permeation at steady state (flux), calculated from the slope of the straight-line portion of the cumulative permeation-time curve and the lag time that is defined by the time intercept of the straight-line portion of the curve. Separately, the drug concentration (C) in the sample solution (donor solution) and thickness of the skin (L) were measured at the start of the test.

$$\text{Flux} = K \cdot C \cdot D / L = P \cdot C \text{ (equation 1)}$$

$$\text{Lag time} = L^2 / 6D \text{ (equation 2)}$$

Results

1. Effect of enhancers on meloxicam penetration through hairless mice skin

Skin permeation parameters of MEL that were determined in the test with the solution system are shown in **Tab.4**. Stearyl alcohol enhanced flux of MEL and increased the partition coefficient as compared to those of control. Other alcohols were without enhancing effects. Of the fatty acids, capric acid enhanced, increasing the partition coefficient. None of the esters tested in this study were found to exert any enhancing effects. Of the terpenes, eucalyptus oil and l-menthol enhanced and increased the diffusion coefficient. All amines enhanced and increased the solubility of MEL, this being most marked with diisopropanolamine. Of

Tab.5 Additives enhancing the skin permeation of meloxicam and mechanisms of action

Mechanism	Enhancer
Increased drug solubility in vehicle	Diethanolamine
	Triethanolamine
	Diisopropanolamine
	Triisopropanolamine
	Polyoxyethylene oleylamine (5E.O.)
Increased diffusion coefficient	l-menthol
	Eucalyptus oil
Increased partition coefficient	Stearyl alcohol
	Capric acid
	Polyoxyethylene stearyl ether
	Polyethylene glycol monostearate
	Glycerin monocaprylate

the surfactants, glycerin monocaprylate, polyethylene glycol monostearate and polyoxyethylene stearyl ether enhanced and increased the partition coefficient. Polyoxyethylene oleylamine (5E.O.) increased the flux in comparison with the control, affecting the solubility of MEL.

Skin permeation enhancers classified as to whether they act on the partition, diffusion or solubility of MEL are listed in **Tab. 5**.

2. Effect of enhancers in combination on skin permeation of meloxicam

Data for effects of enhancers in combination on skin permeation of MEL are summarized in **Tab.6**. Additives, such as diisopropanolamine, l-menthol and capric acid, that markedly increased each parameter were combined with either of the other two types. Combination of additives enhancing diffusion and partition did not enhance flux. However, combinations of additives enhancing solubility and either of the other two types increased flux to a greater extent than with single agents used alone. Furthermore, it was found that diisopropanolamine in combination with l-menthol or glycerin monocaprylate provided the greatest effects.

3. Effect of enhancers in combination with differing mechanisms of action on skin permeation of meloxicam from patches

Data for skin permeation of MEL from tape and poultice type patches containing enhancers that

markedly enhanced skin permeation of MEL in the skin permeation test with the solution system are shown in **Fig.1** and **2**. Cumulative amounts of MEL permeating from tape type patches were in the order : diisopropanolamine and l-menthol = diisopropanolamine and glycerin monocaprylate > diisopropanolamine > only base without enhancer. This result was in line with solution system findings (**Tab.3**). However, diisopropanolamine did not exert any positive effects with poultice type patches. With these, permeation was enhanced by substitution of polyoxyethylene oleylamine (5E.O.), a hydrophobic solvent, for the hydrophilic diisopropanolamine. Addition of l-menthol further enhanced the skin permeation.

Discussion

The main finding of the present study is that combinations of additives enhancing diffusion or partition are ineffective without a additive enhancing solubility, in line with the obvious necessity for interaction with the stratum corneum (SC)⁸⁾. The lipid protein partitioning theory was introduced by Barry to describe the mechanisms of action of permeation enhancers¹⁶⁾, whether by ① disruption of the highly ordered structure of SC lipids, ② interactions with intracellular protein, or ③ improvement in partitioning of the drug, coenhancer, or cosolvent into the SC.

In this study, we found that diffusion of MEL through the skin was increased by terpenes, eucalyptus oil and l-menthol. It is reported that terpenes enhance diffusion of drugs by disrupting the structure of lipid in the intercellular space of the SC¹⁷⁻¹⁹⁾. Additionally, we found that partition of MEL to the skin was increased by the fatty acid, capric acid, and the surfactants, polyoxyethylene stearyl ether, polyethylene glycol monostearate and glycerin monocaprylate. It is known that fatty acids act on the SC²⁰⁾ and enhance the skin permeation of drugs by increasing partition to the skin²¹⁾. Surfactants change lipid chain fluidity of the SC and improve drug partitioning²²⁾. Thus the mechanism of increasing partition to the skin

Tab.6 Flux through excised hairless mouse skin with pairs of additives

Mechanism	Additive as second component	Single component flux ^a ($\mu\text{g}/\text{cm}^2/\text{hr}$)	Additive as first component		
			Diffusion l-menthol	Partition Capric acid	Solubility Diisopropanolamine
Diffusion	Eucalyptus oil	4.8		7.9	224.4
	l-Menthol	39.7	-	20.9	1,875.8
	Stearyl alcohol	11.8	2.4	1.0	182.1
	Capric acid	22.3	20.9	-	167.9
Partition	Polyoxyethylene stearyl ether	9.8	1.3		223.2
	Polyethylene glycol monostearate	9.3	2.6		214.7
	Glycerin monocaprylate	9.7	22.8	11.3	1,703.0
Solubility	Diisopropanolamine	110.5	1,875.8	167.9	-

^a The value were calculated from the straight lines of the permeated amount of meloxicam.

Fig.1 Permeation profiles of meloxicam from tape type patches across excised hairless mouse skin with enhancers

◇ : Only base. □ : diisopropanolamine
 △ : diisopropanolamine + l-menthol
 ○ : diisopropanolamine + glycerin monocaprylate
 Each point represents the mean $\pm$ SE (n=3).

Fig.2 Permeation profiles of meloxicam from poultice type patches across excised hairless mouse skin with enhancers

◇ : Only base. □ : diisopropanolamine
 ■ : polyoxyethylene oleylamine (5E.O.)
 ▲ : polyoxyethylene oleylamine (5E.O.) + l-menthol
 Each point represents the mean $\pm$ SE (n=3).

is the same as for increasing diffusion so that combination of additives enhancing diffusion and partition would not be expected to exert synergistic enhancing effects on skin permeation of MEL. On the other hand, the mechanism of additive enhancing solubility increases passive diffusion of drugs, and so this mechanism differs from the mechanism of increasing partition or diffusion. Therefore combinations of additive enhancing solubility with either of the other two types may have advantages. Moreover, diisopropanolamine particularly enhanced flux of MEL without combination of additive. It is reported that dodecylamine

enhance the skin permeation of diclofenac sodium by forming an ion-pair²³⁾. Using Fourier transform infrared spectroscopy (FT-IR), we found that diisopropanolamine-MEL mixture formed a salt. For these results, diisopropanolamine enhanced flux because of not only increasing solubility but also forming an ion-pair with MEL.

Generally speaking, the enhancement of skin permeation of drugs varies with the base and it can not be predicted with certainty that enhancers selected in skin permeation tests with a simple solution system will actually increase skin permeation of drugs from pharmaceutical patches.

Fig.3 Comparison of flux of meloxicam and ketoprofen from patches through excised skin of wistar rat
Each value represents the mean $\pm$ SE (n=3).

In the present study, tape type patches containing diisopropanolamine that increased the solubility of MEL in combination with l-menthol that increased the diffusion in the skin or glycerin monocaprylate that increased the partition to the skin enhanced the skin permeation of MEL, but with poultice type patches diisopropanolamine was ineffective in comparison with polyoxyethylene oleylamine (5E.O.). Skin permeation of a substance in the base depends on the thermodynamic activity^{24,25}. In the tape type patch, it seems that thermodynamic activity of MEL in the base is increased by using diisopropanolamine as a hydrophilic additive, because the base is hydrophobic. On the contrary, in the case of poultice type patches, the thermodynamic activity of MEL in the base might be reduced by diisopropanolamine, but increased by polyoxyethylene oleylamine (5E.O.), which is not miscible with water. Consequently, these results suggest that it is important to select a hydrophilic solvent for a lipophilic base and a hydrophobic solvent for a hydrophilic base.

Comparison of skin permeation of MEL with tape type patch and ketoprofen (KP) with a commercial patch, MILTAX (Daiichi Pharmaceutical Co., Ltd.), through excised wistar rat is shown in **Fig.3**. Although flux of MEL was approximately half that of KP, the IC_{50} for MEL against COX-2 is about a seventh part of that for KP²⁶. Thus, MEL would provide more effective patch for localized

disorders than KP.

In conclusion, combinations of additives increasing solubility of drugs and acting on the lipid in the SC may be effective for enhancing skin permeation of drugs from transdermal patches. Furthermore, it is important to select the solvent most appropriate for the base material of the patch. We found MEL to be a potential candidate for the topical patch.

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