乳酸-エタノール-ミリスチン酸イソプロピル混合系による種々薬物の皮膚透過促進効果

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The effects of the L-lactic acid-ethanol-isopropyl myristate mixed system on the skin permeation of several drugs

異なる脂溶性を有する種々酸性, 塩基性および中性薬物の *in vitro* 皮膚透過性に及ぼす多成分経皮吸収促進剤である乳酸-エタノール-ミリスチン酸イソプロピル(IPM)混合(LEI)系の効果をヘアレスラット皮膚を用いて測定した. LEI 系に加えて, IPM のみ, エタノール-IPM(EI)系, およびシリコーン液を比較のために基剤として用いた. その結果, (ⅰ)IPM とシリコーン液からの皮膚透過性の比較より, IPM は薬物の荷電や脂溶性にほとんど依存せず一様に薬物の皮膚透過性を促進すること, また, (ⅱ)IPM と EI 系の比較より, エタノールはそれ自身に近い脂溶性を有する(溶解度パラメータが近い)薬物に対して特に高い効果を示すこと, さらに, (ⅲ)EI 系への乳酸の添加(LEI系)は, アミノ基を有する塩基性薬物の皮膚透過速度を増加するが, カルボン酸を有する酸性薬物の透過速度を減少することが明らかとなった.

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Although transdermal delivery systems for a few therapeutic drugs have been clinically used, application to many kinds of drugs is limited due to their low skin permeations. To overcome this shortcoming use of permeation enhancers is one option. Useful permeation enhancers such as oleic acid[1)], isopropyl myristate(IPM)[2,3)], cyclic monoterpene[4)] and ethanol[5,6)] have already been applied to the systems and the related topical formulations. Recently we reported that a lipophilic multicomponent system consisted of L-lactic acid (1%), ethanol (10%) and IPM(89%)(LEI system) markedly enhanced the skin permeation of ketotifen fumarate (KF), a model compound, compared to their single uses.[7)]

Objective of the present study is to search drug candidates suitable for this system and to find a role of each component in this system on its skin penetration enhancing effect. In this experiments, nine acidic, basic or neutral drugs having different lipophilicities were used. IPM alone and 10% ethanol/IPM (EI system) were selected as vehicles in addition to the LEI system, and an inert vehicle, silicone fluid was also used as a control vehicle. *In vitro* permeations of these drugs from the vehicles were measured using excised hairless rat skin, and steady-state flux through the skin was obtained for each combination of a drug and a vehicle. Dependency of the flux ratios from the vehicles with and without an additive was evaluated against solubility parameters of the drugs.

Experimental

(1) Materials

Ibuprofen(IP), aminopyrine(AMP), antipyrine(ANP), ketotifen fumarate(KF), ethylparaben(EP), dichlofenac sodium(DC-Na), indo-

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Table 1 Solubility parameter, molecular weight and solubility in various
vehicles of drugs used in this study

	pKa (acid/base)	SP (cal/cm³)¹ᐟ²	MW	silicone fluid	solubility (mg/ml)		
					IPM	EI system	LEI system
Ibuprofen (1, IP)	5.20 acid	10.22	206.27	2.56	172.59	301.95	274.41
Aminopyrine (2, AMP)	5.00 base	10.86	231.29	2.36	24.03	52.96	136.83
Antipyrine (3, ANP)	1.50 base*	11.27	188.23	0.31	7.35	43.72	44.23
Ketotifen fumarate (4, KF)	6.05 base	11.89	425.50	0.01	0.10	0.86	2.23
Ethylparaben (5, EP)	8.4 acid*	12.82	166.17	0.05	28.70	134.58	137.38
Dichlofenac sodium (6, DC-Na)	4.00 acid	12.93	318.13	0.02	0.06	1.03	0.618
Indomethacin (7, IDM)	4.50 acid	13.03	357.81	0.02	2.47	15.24	17.36
Isosorbide dinitrate (8, ISDN)	—	13.09	236.14	1.31	23.67	26.22	24.17
Methylparaben (9, MP)	8.4 acid*	13.31	152.14	0.04	38.64	103.22	100.86

SP : solubility parameter. MW : molecular weight
*These are very weak base and acids.

methacin(IDM), isosorbide dinitrate(ISDN) and methylparaben(MP) were used as acidic, basic and neutral penetrants having different lipophilicities(**Table 1**). IDM and ISDN were supplied by Toko Pharmaceutical Ind. Co., Ltd. (Tokyo, Japan). IP, DC-Na and KF were by Nissei Chemical Co., Ltd.(Tokyo), Nichiban Co., Ltd.(Tokyo) and Sandoz Pharmaceutical Co., Ltd.(Tokyo), respectively. AMP was obtained from Wako Pure Chemical Industries, Ltd.(Osaka, Japan). ANP, MP and EP were from Tokyo Kasei Kogyo Co., Ltd.(Tokyo). Silicone fluid(Medical fluid 360, 19cP viscosity, Dow Corning Asia Co.) (Kanagawa, Japan) was used as an inert vehicle. Other vehicle additives (ethanol, L-lactic acid and IPM) were of reagent grade and used without further purification.

(2) Preparation of skin membrane

Full-thickness skin was excised from the abdomen of male hairless rats (WBN/ILA-Ht, weight about 180 g, Life Science Research Center, Josai University, Saitama, Japan). Excess fat was carefully removed from skin by scissors. The skin sample was used immediately after excision.

(3) Permeation experiments

A side-by-side(2-chamber) diffusion cell (effective area : 0.95 cm², cell volume : 2.5 ml) was utilized for the *in vitro* permeation experiment[8]. Skin membrane was carefully mounted between two half-cells of the diffusion cell. IPM, EI system, LEI system or silicone fluid (2.5 ml) containing one in 9 drugs(**Table 1**) as its suspension (ca. twice solubility) was applied to half-cell facing the stratum corneum, and the dermis side was filled with the same volume of distilled water. The permeation experiments were performed at 37°C. Sample solution (0.2-1.0 ml) was withdrawn periodically from the receiver(dermis) side chamber, and the same volume of distilled water was added after sam-

pling. When (pseudo)steady-state flux was intended to determine, the donor vehicle containing drug was changed periodically to avoid marked decrease in thermodynamic activity of drug and vehicle components in the system throughout the experiment

(4) Determination of drug solubilities in vehicles

The drug solubilities were measured after 24 hour incubation of each drug suspension in vehicles at 37°C. Drug-saturated solution was obtained by filtration of each suspension using a microporous filter (HPLC Sample Plep C02-LG ; Japan Millipore Co. Ltd., Tokyo), and appropriately diluted with methanol or acetonitrile for analysis

(5) Analytical method

All drugs were determined by HPLC. Sample containing each drug(0.2 ml) was added to the same volume of acetonitrile or methanol with or without an internal standard. After centrifugation of the mixed solution, the supernatant was injected to HPLC composed of a pump system(LC-6A, Shimadzu Seisakusho, Kyoto, Japan), a UV detector(SPD-6A, Shimadzu Seisakusho), an integrator(C-R3 A, Shimadzu Seisakusho) and a reverse phase column(Ultron N-C18 ; 150 mm×4.6 mm i. d., Shinwa Kako Co., LTD, Kyoto).

Conditions of HPLC(mobile phase, flow rate, detected wave and internal standard, respectively) was as follows ; IP (0.1% H_3PO_4 : acetonitrile (40 : 60), 0.8 ml/min, 263 nm and heptylparaben), AMP (0.1% H_3PO_4 : acetonitrile (40 : 60) +5 mM sodium dodecyl sulfate, 0.8 ml/min, 254 nm and hexylparaben), ANP (0.1% H_3PO_4 : acetonitrile (70 : 30), 0.8 ml/min, 245 nm and EP), KF(0.1% H_3PO_4 : acetonitrile (70 : 30), 0.8 ml/min, 295 nm and EP), EP (0.1% H_3PO_4 : acetonitrile (70 : 30), 0.8 ml/min, 250 nm and MP), DC-Na (0.1%

H_3PO_4 : methanol (80 : 20), 0.7 ml/min, 286 nm and butylparaben), IDM(0.1% H_3PO_4 : acetonitrile (50 : 50), 1.0 ml/min, 250 nm and hexylparaben), ISDN (0.1% H_3PO_4 : acetonitrile (50 : 50), 0.6 ml/min, 220 nm and EP) and MP (0.1% H_3PO_4 : acetonitrile(70 : 30), 0.8 ml/min, 250 nm and EP).

Results and Discusssion

Table 1 summarizes pKa(acid or base), solubility parameters(SP), molecular weights (MW) and solubilities in each vehicle of nine drugs used in this experiments. Five and three among the nine drugs are acids and bases, respectively. However, acidic or basic properties of ANP, EP and MP are so weak that one can regard them as neutral compounds. The solubility parameters were calculated using the functional method by Fedor[9]. The values were from 10.22 to 13.31 (cal/cm³)$^{1/2}$. Molecular size greatly affects the diffusivity of the drugs in the skin barrier. Reciprocal of square route of molecular weight is generally proportional to permeability coefficient. Among the drugs used in the experiments, however, the square routes for MP(minimum) and KF(maximum) were only 1.7 times different. In contrast, solubilities in vehicles were much different among the drugs. Solubilities of the drugs with low solubility parameters were generally high in all the vehicles although there were several exception. ISDN and two parabens(MP and EP) showed higher solubilities in silicone fluid and IPM than those predicted from their solubility parameters. Among the two vehicles IPM had greater solubilizing effect against all the drugs. Addition of ethanol to IPM(EI system) greatly increased the solubility of the drugs except ISDN. AMP and KF showed higher solubilities in the LEI system than in the EI system. Increase in the solubilities may be due to increase in anion

Table 2   Flux and permeability coefficient of various drugs through hairless rat skin

		Silicone fluid	IPM	EI system	LEI system
1 . IP	J	17.7 ± 0.7	$148.8\pm6.1(8.4)$	$455.1\pm32.3(25.7)$	$208.4\pm23.8(11.8)$
	P	1.92×10^{-6}	2.39×10^{-7}	4.19×10^{-7}	2.11×10^{-7}
2 . AMP	J	30.2 ± 6.7	$199.1\pm26.8(6.6)$	$11312\pm1400(374)$	$21565\pm153(714)$
	P	3.55×10^{-6}	2.30×10^{-6}	5.93×10^{-4}	4.38×10^{-5}
3 . ANP	J	13.9 ± 1.1	$232.4\pm17.3(16.7)$	$11093\pm419(798)$	$11860\pm1138(853)$
	P	1.23×10^{-5}	8.77×10^{-6}	7.05×10^{-5}	7.45×10^{-5}
4 . KF	J	0.3 ± 0.10	$2.5\pm0.2(8.3)$	$2044\pm122(6815)$	$2785\pm254.2(9283)$
	P	9.39×10^{-6}	7.32×10^{-6}	6.60×10^{-4}	3.47×10^{-4}
5 . EP	J	11.8 ± 0.5	$41.3\pm10.3(3.5)$	$259.2\pm13.4(22.0)$	$288.1\pm62.7(24.4)$
	P	6.55×10^{-5}	4.00×10^{-7}	5.36×10^{-7}	5.82×10^{-7}
6 . DC-Na	J	0.8 ± 0.2	$10.0\pm5.3(12.5)$	$4867\pm365(6083)$	$2678\pm167(3348)$
	P	8.57×10^{-6}	4.31×10^{-5}	1.32×10^{-3}	1.20×10^{-3}
7 . IDM	J	0.7 ± 0.2	$3.7\pm0.3(5.2)$	$34.1\pm2.3(48.7)$	$4.0\pm0.3(5.7)$
	P	1.36×10^{-5}	4.15×10^{-7}	6.21×10^{-7}	6.53 ± 10^{-8}
8 . ISDN	J	10.6 ± 0.4	$22.0\pm1.9(2.1)$	$215.9\pm10.2(20.4)$	$267.6\pm16.3(25.2)$
	P	2.24×10^{-6}	2.58×10^{-7}	2.29×10^{-6}	3.08×10^{-6}
9 . MP	J	3.8 ± 0.8	$12.4\pm3.0(3.3)$	$174.4\pm57.7(45.9)$	$129.8\pm26.8(34.2)$
	P	2.63×10^{-5}	8.89×10^{-8}	4.69×10^{-7}	3.57×10^{-7}

J : flux $(\mu g/cm^2/hr)$. P : permeability coefficient (cm/s). mean$\pm$SE, n=3-6.
Values in parentheses indicate the stedy-state flux ratio against silicone fluid.

fraction by addition of L-lactic acid. Since these two drugs contain tertiary amines, interaction of the amine groups and carboxyl group of L-lactic acid may affect their solubilities.

Table 2 shows the pseudo-steady-state flux (J) and permeability coefficient (P) of each drug from silicone fluid, IPM, EI and LEI system. The J value was determined from a slope of linear part after a short lag time in the cumulative amount of the drug permeated through skin-time curve, and P was obtained by dividing the J value by the drug solubility in vehicle. The steady-state flux ratio (each vehicle against silicone fluid) is also shown in **Table 2. Fig. 1a, b** and **c** illustrate the flux ratio from IPM alone/silicone fluid, EI system/IPM alone and LEI system/EI system, respectively, against the solubility parameter of each drug.

Every drug flux from IPM was higher with that from silicone fluid (**Table 2** and **Fig. 1a**) (from 2.1 times for ISDN to 16.7 times for ANP), whereas the P values were lower for most drugs (**Table 2**). This is due to much greater solubilities of the drugs in IPM than silicone fluid : High solubility or affinity of a drug in a vehicle sometimes decreases partition of the drug from the vehicle to the skin barrier. IPM is an effective enhancer, because it easily penetrates into the skin barrier to physicochemically or morphologically change the barrier functions, compared to the inert vehicle, silicone fluid. No distinct relation of the flux ratio (IPM alone/silicone fluid), however, was observed against the solubility parameter of the drugs (**Fig. 1a**), although the ratio may have a tendency to decrease with increase in the parameter.

The drug fluxes from the EI system were increased about 20-7,000 folds compared to silicone fluid (**Table 2**). Much higher solubility of the drugs in the vehicle containing ethanol than in silicone fluid is one reason for such high enhancing effects. In contrast, the P values of IP, EP, IDM and MP from the EI system were
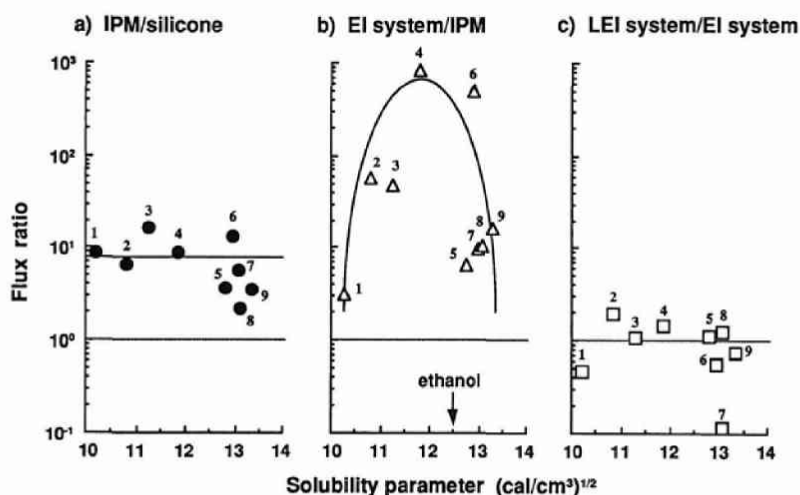
Fig. 1 Flux ratio from IPM alone/silicone fluid(a), EI system/IPM
alone(b) and LEI system/EI system(c) against the solubility
parameter of each drug

lower than those for silicone fluid, as the same phenomena observed for IPM alone. P values from the EI system for AMP, ANP, KF and DC-Na which are soluble in ethanol, however, were much higher compared to the IPM although the values were dependent on the kind of drugs. Thus, addition of ethanol into IPM increased not only the flux but also the P value of ethanol-soluble drugs. It is suggested from these data for fluxes and P values that IPM and ethanol easily penetrate the skin barrier to show marked penetration-enhancing effects for several drugs.

To elucidate the effect of ethanol, the flux ratio of the drugs between IPM vehicles with and without ethanol was calculated, and dependency of the ratio against their solubility parameters was evaluated(**Fig. 1b**). Addition of ethanol into IPM increased the flux ratio of drugs having a similar solubility parameter to ethanol $(12.5(cal/cm^3)^{1/2})$ to represent a bell-shaped curve. Since skin permeation of ethanol from the EI system was very rapid(ca. 60 mg/ cm^2/hr), great amount of ethanol must be contained in the skin barrier. Ethanol flux (i. e.

cotransport or solvent drag) and its solubilizing effect against the drugs in vehicle and skin are thus closely related to its skin penetration-enhancing effect.

Fig. 1c shows the flux ratios of the drugs from the LEI system against the EI system. The flux ratios were more than unity in AMP and KF, which are basic drugs containing tertiary amine groups, whereas they were less than unity for IP, DC-Na and IDM, which are acidic drugs containing a carboxyl group. Since L-lactic acid contains a carboxyl group, the great difference in the enhancing effects by the LEI system especially for the two drug categories may be related to physico-chemical interactions between the drugs and L-lactic acid. Similar ion-ion interactions have been found in the drug diffusions in topical formulations[10].

It is suggested from these results (i) that IPM enhances the skin permeation of a broad characteristics of drugs compared to an inert vehicle, silicone fluid, (ii) that addition of ethanol in IPM is more effective against drugs which are soluble in ethanol, and (iii) that L-lactic acid in the LEI system is probably useful

to increase the skin permeation of basic drugs which electrostatistically interact with the acid to increase their diffusivities. In addition, it was predicted that the LEI system tremendously increased the skin permeations of other basic drugs such as AMP in addition to KF which has already reported in our previous paper[7].

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