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Note

Effect of 1-*O*-ethyl-3-butylcyclohexanol on the skin permeation of drugs with different physicochemical characteristics

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Abstract

The effects of 1-*O*-ethyl-3-butylcyclohexanol (OEBC) on the in vitro skin permeation of ten model drugs with different physicochemical properties across excised rat skin were evaluated. The results showed that the addition of OEBC significantly improved the in vitro skin permeation of the model drugs compared with the control (without OEBC). To clarify the promoting mechanism of OEBC, a multiple regression analysis was employed. When the permeation study was performed without OEBC, the permeability coefficient was quantitatively predicted as a linear function of molecular weight (log MW) and their lipophilicity (partition coefficient of drugs between octanol and water ($\log K_{o/w}$)) with a sufficiently high correlation coefficient ($r = 0.842$). It was suggested that skin permeation of drugs without OEBC was explained as a function of diffusion of drugs through the skin and partitioning of drugs to the skin. Although OEBC was administered, the permeability coefficient of drugs cannot be predicted as a linear function of log MW and $\log K_{o/w}$ ($r = 0.572$).

Keywords: 1-*O*-ethyl-3-butylcyclohexanol; Absorption enhancer; Skin permeation; Lipophilicity; Molecular weight; Physicochemical properties; Multiple regression analysis

We previously found that cyclic monoterpenes such as D-limonene and L-menthol greatly enhanced the skin permeation of drugs (Obata et al., 1993). Based on these previous results, we synthesized and evaluated the promoting activity of *O*-alkylmenthol and *O*-acylmenthol derivatives (Negishi et al., 1995). Among them, *O*-ethylmenthol (MET) showed the greatest promoting activity and caused relatively little skin irritation. Then, we synthesized cyclohexanol derivatives that have mono- or di-substitute groups and an *O*-ethyl group to use as a skin permeation enhancer (Obata et al., 2000). Their enhancement activity for percutaneous absorption of ketoprofen and the skin irritancy were evaluated in rats in vivo. Among those compounds, 1-*O*-ethyl-3-butylcyclohexanol (OEBC) showed the greatest efficiency at relatively low concentrations and caused relatively little skin irritation (Obata et al., 2001; Wu et al., 2001). Furthermore, we clarified the promoting mechanism and the site of action of OEBC (Li et al., 2001).

The aim of this study was to evaluate the enhancement effect of OEBC on the skin permeation of various drugs having a wide range of lipophilicity as indicated by the logarithm of the octanol–water

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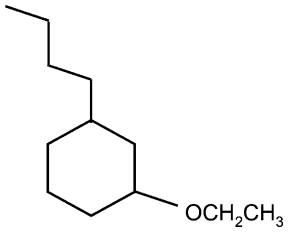

Fig. 1. The chemical structure of 1-*O*-ethyl-3-butylcyclohexanol (OEBC).

partition coefficient ($\log K_{o/w}$, 0.23–3.86) and molecular weight (MW: 138.12–394.44). The physicochemical properties–permeability relations of the model drugs were investigated in detail employing a multiple regression analysis.

The chemical structure of OEBC was shown in Fig. 1. OEBC was synthesized by the method described by Obata et al. (2000), and was characterized by elemental analysis, nuclear magnetic resonance (NMR) spectroscopy (Jeol GSX 270F, Tokyo, Japan) and gas chromatography (GC) (Shimadzu GC-7A, Kyoto, Japan). The purity was greater than 99%. Salicylic acid (SA), antipyrine (ANP), ibuprofen (IP), aminopyrine (AMP), flurbiprofen (FP), pentoxifylline (PT), indomethacin (IND), hydrocortisone (HC), triamcinolone (TA) and carboxyvinyl polymer (HIVIS-WAKO 105) were purchased from Wako (Osaka, Japan). Ketoprofen (KPF) was purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of reagent grade.

The formulae of hydrogels containing the model drugs used in this study are listed in Table 1. Full-thickness abdominal skin was excised from male Wistar rats weighing 180–200 g, whose hair had been previously removed with electric clippers. The excised skin was used as a permeation membrane. A Franz diffusion cell having an available diffusion area of 1.77 cm^2 was employed. The receiver side was filled with 16 ml of phosphate buffer solution (pH 7.2) and the donor side was filled with the test hydrogel (1 g) under occlusive conditions. The Franz cell was thermoregulated at 37 °C and the receiver side was stirred with a magnetic stirrer. At appropriate times, an aliquot of the receiver fluid was withdrawn and the same volume of fresh buffer solution was supplied to the receiver side. Each aliquot was mixed with methanol containing an internal standard. The sample was filtered through a disposable filter unit (Ekikuro-Disc 3CR, Gelman Science Japan, Tokyo, Japan). The concentrations of drugs in the filtrate were analyzed using an HPLC. All experiments were quadricated.

Table 1
Formulae of the model drug hydrogels

Drugs[a] (g)	3.0
Carboxyvinylpolymer (g)	1.5
Triethanolamine (g)	2.0
Ethanol (g)	40.0
OEBC (g)	0.5
Water (ad)	100.0

[a] Salicylic acid, antipyrine, ibuprofen, aminopyrine, flurbiprofen, ketoprofen, pentoxifylline, indomethacin, hydrocortisone, triamcinolone.

Determination of the concentration of the model drugs in the sample solutions was performed by high-performance liquid chromatography (HPLC). The sample solution was injected onto the column using an auto-injector equipped with a system controller (SIL10A, SCL10A; Shimadzu, Japan), a pump (LC10AS; Shimadzu) and a UV detector (SPD6A; Shimadzu). The determination conditions for each drug are summarized in Table 2.

An excess amount of drugs was added to phosphate buffer solution (pH 7.2) containing 40% ethanol, which was then incubated at 37 °C for 24 h. After centrifugation, the supernatant was filtrated using disposable filter unit (Gelman Science Japan, Ltd., Ekikuro-Disk 3CR). The concentration of drugs in the solution was determined using HPLC.

The cumulative amount of drugs permeated per unit skin surface area was plotted against time. The permeation rate (steady-state flux (J_{ss})) was obtained from the slope of the linear portion of the graph. The permeability coefficient (P) was calculated based on Eq. (1):

$$P = \frac{J_{ss}}{S} \quad (1)$$

where S is the solubility of the drug in the vehicle. When the drug was completely dissolved in the vehicle, the intrinsic $J_{ss(int)}$ was estimated based on Eq. (2):

$$J_{ss(int)} = J_{ss} \times \frac{S}{C_d} \quad (2)$$

where C_d is the concentration of drug in the vehicle.

Table 2
HPLC conditions for determination of the concentration of various drugs used in this study

Drugs	Mobile phase	Detection (nm)	Internal standard	Column (nm)
Salicylic acid	Acetonitrile:100 mM pH 7.0 phosphate buffer (17:83)	275	*p*-Methoxybenzoic acid	C_{18} 4.6 × 150
Antipyrine	Acetonitrile:0.057% phosphoric acid (80:20)	245	*p*-Hydroxybenzioc acid *n*-hexyl ester	C_{18} 4.6 × 150
Ibuprofen	Acetonitrile:0.057% phosphoric acid (50:50)	220	*p*-Hydroxybenzioc acid *n*-hexyl ester	C_{18} 4.6 × 150
Aminopyrine	Acetonitrile:0.057% phosphoric acid (80:20)	255	*p*-Hydroxybenzioc acid *n*-hexyl ester	C_{18} 4.6 × 150
Flurbiprofen	Acetonitrile:0.057% phosphoric acid (60:40)	250	*p*-Hydroxybenzioc acid *n*-hexyl ester	C_{18} 4.6 × 150
Ketoprofen	Methanol:0.057% phosphoric acid (65:35)	254	*p*-Hydroxybenzioc acid *n*-butyl ester	C_{18} 4.6 × 150
Pentoxifylline	Acetonitrile:0.057% phosphoric acid (20:80)	270	*p*-Hydroxybenzoate methyl ester	C_{18} 4.6 × 150
Indomethacin	Methanol:0.057% phosphoric acid (65:35)	254	*p*-Hydroxybenzioc acid *n*-hexyl ester	C_8 4.6 × 200
Hydrocortisone	Acetonitrile:water (7:13)	254	*p*-Hydroxybenzoate ethyl ester	C_{18} 4.6 × 250
Triamcinolone	Acetonitrile:water (1:3)	240	Prednisolone	C_{18} 4.6 × 150

For statistical evaluation of results, the one-way analysis of variance (ANOVA) was employed. A *P*-value smaller than 0.05 was considered as significant.

The physicochemical characteristics of the model drugs such as MW and the log $K_{o/w}$ value are summarized in Table 3. As can be seen in Table 3, antipyrine, aminopyrine and pentoxifylline are hydrophilic drugs (log $K_{o/w}$: 0.23, 0.50 and 0.72, respectively). On the other hand, indomethacin, ibuprofen and flurbiprofen are lipophilic drugs (log $K_{o/w}$: 3.19, 3.51 and 3.86, respectively), followed by ketoprofen (2.94), triamcinolone (2.40), salicylic acid (2.25) and hydrocortisone (1.61). Moreover triamcinolone has the greatest molecular weight (MW: 394.44) among the drugs examined in this study. The solubility of drugs was listed in Table 4.

The permeability coefficients of the model drugs from the hydrogels containing 40% ethanol without OEBC are shown in Fig. 2A. Flurbiprofen and indomethacin showed the greatest permeability coefficient. In contrast, pentoxyphiline showed the smallest permeability coefficient.

Fig. 2B represents the permeability coefficient of the model drugs from hydrogels with 0.5% OEBC as an enhancer. The permeability coefficients of all the permeants were markedly enhanced compared with that of the control (without OEBC). The results suggest that OEBC was a powerful skin penetration enhancer for various drugs having different physicochemical properties.

Table 3
Physicochemical properties of model drugs used in this study

Model drugs	MW	log $K_{o/w}$
Salicylic acid	138.12	2.25[a]
Antipyrine	188.23	0.23[b]
Ibuprofen	206.27	3.51[a]
Aminopyrine	231.29	0.50[c]
Flurbiprofen	244.27	3.86[c]
Ketoprofen	254.29	2.94[b]
Pentoxifylline	278.31	0.72[b]
Indomethacin	357.79	3.19[c]
Hydrocortisone	362.47	1.61[d]
Triamcinolone	394.44	2.40[a]

[a] Phillips and Michiniak (1995).
[b] Lee et al. (1994).
[c] Hatanaka et al. (1990).
[d] Cronin et al. (1999).

Table 4
Solubility of drugs in 40% ethanol

Drugs	Control (mg/ml)	0.5% OEBC (mg/ml)
Salicylic acid	104.42	115.99
Antipyrine	755.24	738.33
Ibuprofen	42.21	30.19
Aminopyrine	379.59	369.29
Flurbiprofen	5.22	13.12
Ketoprofen	99.12	80.16
Pentoxifylline	508.33	540.37
Indomethacin	6.35	6.41
Hydrocortisone	11.32	18.58
Triamcinolone	4.50	4.82

Each value represents the mean of three determinations.

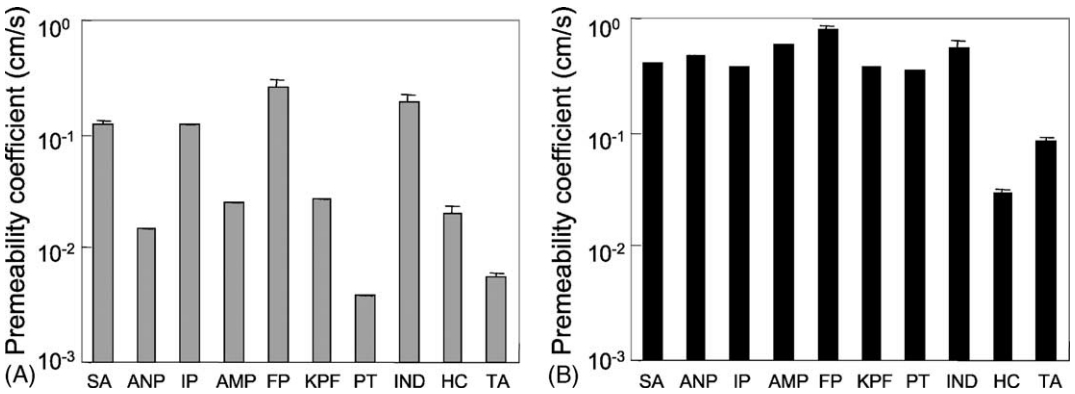

Fig. 2. The permeability coefficient of the model drugs without OEBC (A) or with OEBC (B). The brevity codes of drugs are as follows: SA, salicylic acid; ANP, antipyrine; IP, ibuprofen; AMP, aminopyrine; FP, flurbiprofen; KPF, ketoprofen; PT, pentoxifylline; IND, indomethacin; HC, hdrocortisone; TA, triamcinolone. Each column represents the mean ± S.D. of four determinations.

In the research field of transdermal drug delivery, there are several methods to predict skin permeability of drugs. One of the concepts was that the permeation route of drugs was taken into consideration such as the lipid pathway or polar pathway (Ghanem et al., 1992). It was suggested to be an outstanding method to evaluate the effect of permeation enhancers focused on the particular permeation pathway. On the other hand, there was a proposal for predicting the entire permeation of many drugs (Potts and Guy, 1992; Moss and Cronin, 2002). That concept sought the ordinarity and universality of the skin permeation of drugs. Furthermore, the model that expressed the skin as a two-layer membrane was developed (Okamoto et al., 1989). These methods were excellent to evaluate skin permeation of drugs, however, they sometimes needed many hypothesis or rather complicated steps for evaluation.

In this study, we selected OEBC as a promising compound and evaluated the promoting activity based on Fick's law of diffusion using several model drugs. We tried to evaluate skin permeation of drugs basically and simply.

The permeability coefficient (P) at the steady-state was expressed as

$$P = \frac{D \times K}{h} \tag{3}$$

where D is the diffusion coefficient of the drug, K is the partition coefficient and h is the effective diffusion path length in the skin. The logarithmic expression of both sides of Eq. (3) was given as

$$\log P = \log D + \log K - \log h \tag{4}$$

In general, the diffusion coefficient is inversely proportional to the cube root of the molecular weight of the permeant. Therefore, the diffusion coefficient is expressed as a function of the molecular weight of the permeant with an adequate coefficient. The partition coefficient is substituted by the partition coefficient between octanol and water solution. Moreover, the effective path length in skin was suggested to be constant. Thus, Eq. (4) can be written as

$$\log P = \alpha \log \mathrm{MW} + \beta \log K_{\mathrm{o/w}} - \log h \tag{5}$$

where MW is molecular weight, α, β are coefficients. Using this equation, the relation between the P values obtained in the experiment and physicochemical properties of the model drugs (MW, $K_{\mathrm{o/w}}$) was evaluated employing a multiple regression analysis.

In the case of hydrogels containing the model drugs without OEBC was administered, a regression equation for $\log P$ values was obtained as

$$\begin{aligned} \log P = {} & -1.85(\pm 2.14) \log \mathrm{MW} \\ & + 0.373(\pm 0.232) \log K_{\mathrm{o/w}} + 2.21, \\ & r = 0.842,\ n = 10,\ s = 0.380, \\ & F(2, 7) = 8.51\ (P < 0.05) \end{aligned} \tag{6}$$

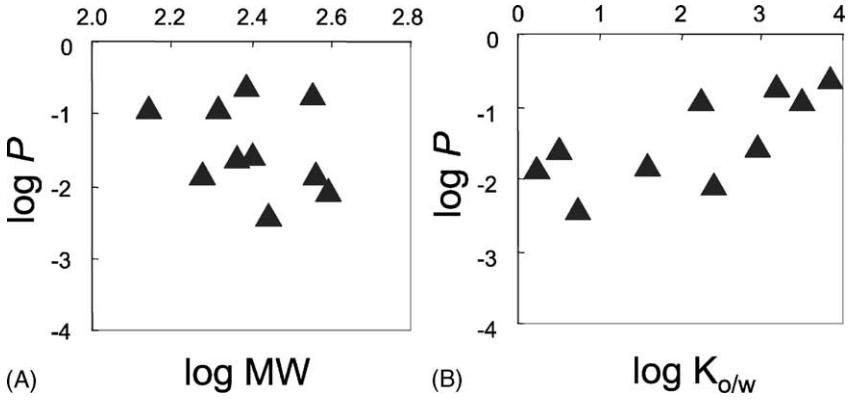

Fig. 3. Relation between log MW (A) or $\log K_{o/w}$ (B) and $\log P$ of the model drugs.

where r is the multiple regression coefficient, s is the S.D. of the residual and F is the ratio of the mean square regression to mean square residual (observed F value). The $\log P$ values of the model drugs were estimated using this equation with statistically significant values of each coefficient. The negative coefficient of log MW (−1.85) means that the permeability coefficient of drugs decreases with an increase in their molecular weight (Fig. 3A). On the other hand, the positive coefficient of $\log K_{o/w}$ (+0.373) means that the permeability coefficient of drugs increases with an increase of their lipophilicity (Fig. 3B). Concerning the constant value in Eq. (6), the thickness of skin of several species was previously reported. However, the actual available path length of drugs through skin was not clarified. Consequently, the skin permeation of the drugs were demonstrated by a Fick's law of diffusion.

Then, the permeability coefficient of the drugs obtained by the administration of hydrogel containing OEBC was evaluated in a similar manner:

$$\log P = -1.74 \log \mathrm{MW} + 0.0715 \log K_{o/w} + 3.50, \quad r = 0.572,\ n = 10,\ s = 0.408,\ F(2, 7) = 1.71 \tag{7}$$

In the administration of OEBC, the results of multiple regression analysis showed that there is insignificant relationship between $\log P$ and physicochemical properties of drugs. The accuracy of estimations based on Eq. (7) was rejected. As shown in Fig. 4, the negative relation between $\log P$ values and molecular weight was less clear compared with that without OEBC. In other words, it was considered that there occurred a nonlinear relation between physico-

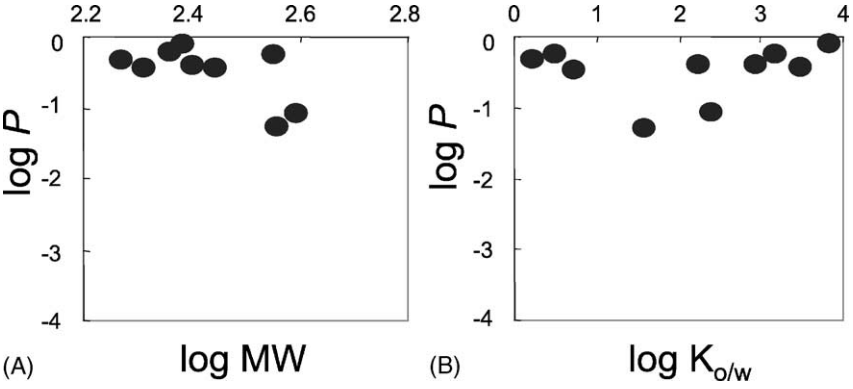

Fig. 4. Relation between log MW (A) or $\log K_{o/w}$ (B) and $\log P$ of the model drugs with OEBC.

chemical characteristics and skin permeation of drugs when OEBC was administered. Moreover, it was suggested that the nonlinear relation between the physicochemical properties and log *P* values might be related to the promoting mechanism of OEBC. From the results of ESR determination, it was clarified that OEBC affected the hydrophobic region of the skin surface (Li et al., 2001). The possible promoting mechanism of OEBC was suggested to relate to the increase in the distribution of drugs to the skin surface and the increase in diffusivity in the stratum corneum (Li et al., 2001). Moreover, OEBC enhanced the fluidity of the most hydrophobic region in lipid bilayer in the stratum corneum compared with L-menthol or *O*-ethylmenthol (data not shown). However, the promoting magnitude of OEBC to each model drug might be uneven. The result was closely related to the actual permeation pathway of the model drugs and the action site of OEBC. In the hydrogel, OEBC and ethanol were coexistent and the possibility of synergistic effects to the skin surface should also be taken into consideration.

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