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COMMUNICATION

Thiomenthol Derivatives as Novel Percutaneous Absorption Enhancers

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ABSTRACT

Thiomenthol derivatives were synthesized and their promoting activity on the percutaneous absorption of ketoprofen from hydrogels was evaluated in rats. The apparent penetration rate (R_p) of ketoprofen absorbed from the hydrogel was estimated by the pharmacokinetic model derived under the assumption of a constant penetration rate through the skin after a lag time. As an index of promoting activity of thiomenthol derivatives, an enhancement factor (E_f) was defined as the ratio of the R_p value with enhancer to the value obtained with the control not containing enhancer. Skin irritancy evoked by these derivatives was investigated microscopically by using a cross-section of the excised skin onto which ketoprofen hydrogel was applied. Total irritation score (TIS) was estimated by summation of each irritation score in several parts of the skin. The physicochemical parameters of thiomenthol derivatives such as a partition coefficient (log P) and a steric energy were calculated and the quantitative relationships between these parameters and the E_f values or TIS values were investigated on the basis of multiple regression analysis. As a result, a parabolic relationship between log P and E_f was noted. A similar relationship was also observed in the case of TIS.

INTRODUCTION

Success of transdermal drug delivery depends on the permeation of drug through the skin at a rate sufficient to achieve a necessary concentration in the systemic circulation, making a desired therapeutic effect. Achieving this usually demands that the absorption enhancer is included in the transdermal formulations. Many com-

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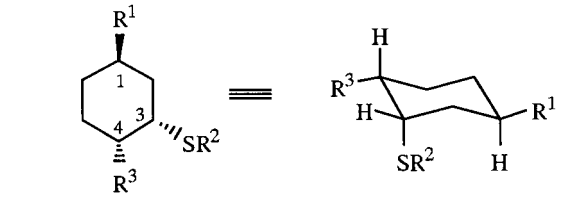
pounds have been challenged to promote drug absorption through the skin. Recently, we synthesized *O*-alkylmenthol and *O*-acylmenthol derivatives and evaluated their promoting activity on the percutaneous absorption of ketoprofen from the hydrogel in rat (1,2). Among these derivatives, *O*-ethyl ether derivative of menthol exhibited the greatest enhancement action with relatively low skin irritancy. In our search for more effective and safer compounds as percutaneous absorption enhancers, we newly synthesized several menthol derivatives containing a sulfur atom in their chemical structures (i.e., thiomenthol derivatives). Their enhancement activity for the percutaneous absorption of ketoprofen from the hydrogel was evaluated in rats in vivo. Pathological study was used to determine the skin damage induced by these derivatives. Quantitative structure–activity and structure–toxicity relationships were investigated by using physicochemical parameters such as a partition coefficient (log *P*) and a steric energy calculated from molecular mechanics potential energies.

MATERIALS AND METHODS

Materials

Chemical structures of the thiomenthol derivatives are shown in Fig. 1. Compounds 1 and 2 were prepared by S-alkylation of the commercially available cyclohexyl mercaptan with methyl or ethyl iodide, respectively, in the presence of sodium methoxide in quantitative yields. Compounds 3, 7, and 8 were synthesized by the method described by Mikolajczyk et al. (3). The reaction sequences for the preparation of compounds 4–6 and 9–13 are outlined in Fig. 2. The cyclohexanone derivatives I, readily available from the alkylation of cyclohexanone or reduction of 2-substituted phenol by the known procedure (4,5), were converted into the equatorial alcohol II by reduction with sodium metal in ethanol (6). The reaction of II with *p*-toluene sulfonyl chloride in pyridine gave the corresponding tosylate III in good yields. Treatment of the tosylate III with potassium thioacetate in dimethyl sulfoxide at 45 C furnished the axial thioacetate IV in 45–57% yield. This reaction was accompanied by the inversion of configuration at the C3 position. The reduction of IV by lithium aluminum hydride in tetrahydrofuran afforded the mercaptan derivatives V. Finally, S-alkylation of compounds V in the same manner as described for the preparation of compound 2 gave the quantitative yield of the desired compounds 4–6 and 9–13. The purity of each compound was characterized by elemental analysis, nuclear magnetic resonance spectroscopy (JEOL PMX 270, Tokyo, Japan), gas chromatography (Shimadzu GC-7A, Kyoto, Japan), and thin-layer chromatography (silica gel 60, with hexane as the solvent system). Thereby, the purity of each compound was over 99%.



Compound	R^1	R^2	R^3
1	H	CH_3	H
2	H	CH_2CH_3	H
3	CH_3	CH_2CH_3	$CH(CH_3)_2$
4	H	CH_2CH_3	$CH_2CH_2CH_2CH_3$
5	H	CH_2CH_3	$CH(CH_3)_2$
6	H	CH_2CH_3	$CH_2CH_2CH_3$
7	CH_3	CH_3	$CH(CH_3)_2$
8	CH_3	H	$CH(CH_3)_2$
9	H	CH_2CH_3	CH_3
10	CH_3	CH_2CH_3	CH_2CH_3
11	CH_3	CH_2CH_3	H
12	H	CH_2CH_3	CH_2CH_3
13	CH_3	CH_2CH_3	CH_3

Figure 1. Chemical structures of thiomenthol derivatives.

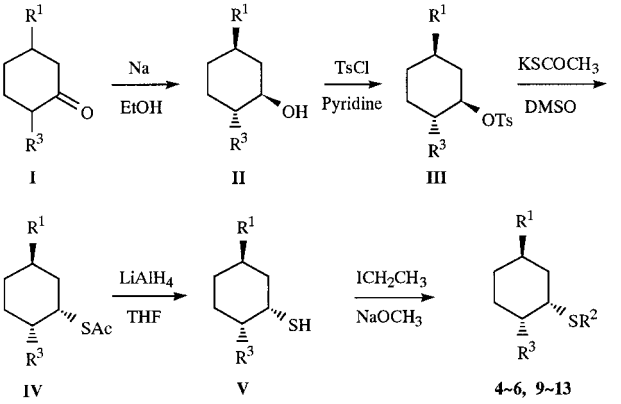


Figure 2. Reaction sequence for the preparation of compounds 4–6 and 9–13.

Table 1

Formulae of Ketoprofen Hydrogels

Ketoprofen	0.30 g
Carboxyvinyl polymer	0.15 g
Triethanolamine	0.20 g
Ethanol	4.00 g
Enhancers	0.05 g
Water	ad. 10.00 g

Ketoprofen was purchased from Sigma Chemical Co. (St. Louis, MO). Carboxyvinyl polymer, marketed as Hiviswako 105, was generously supplied by Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other chemicals used were of reagent grade.

Preparation of Hydrogels

The formulae of ketoprofen hydrogels are listed in Table 1. The gels were prepared as follows. Ketoprofen was dissolved in ethanol with the enhancers. Separately, carboxyvinyl polymer and triethanolamine were dissolved in distilled water. Both components were then well mixed, and the resultant hydrogel was stored before use at room temperature for 24 hr under airtight conditions.

Percutaneous Absorption Study

Male Wistar rats weighing 180–200 g were anesthetized with a carbamic acid ethyl ester solution (25%, 3 ml/kg intraperitoneally) and secured on their backs. Their abdominal hair was gently removed with an electric clipper. A glass cell with a 16-mm inner diameter and 10 mm in height was attached to the shaved abdominal skin with a cyanoacrylate type of adhesive (Aron Alpha A®, Sankyo, Tokyo, Japan) and filled with the test hydrogel (1 ml) under occlusive conditions. Blood samples (300 μl) were taken via the jugular vein 1, 2, 4, 6, and 8 hr after application. Each blood sample was centrifuged (13,000 rpm, 3 min), and the plasma sample (100 μl) was thoroughly mixed with methanol (300 μl) containing an appropriate amount of *p*-hydroxybenzoic acid *n*-butyl ester as an internal standard. The mixture was centrifuged again (13,000 rpm, 3 min) to precipitate the denatured proteins. The sample was filtered through a disposable filter unit (Sample prep® LCR4(T)-LG, Japan Millipore, Yonezawa, Japan). The concentration of ketoprofen in the filtrate was analyzed using an HPLC system (Shimadzu, LC-5A) equipped with a variable wavelength ultraviolet monitor (Shimadzu, SPD-6A). The flow rate was 1 ml/min, and elution was carried out at room temperature. The other analytical conditions were as follows: column, STR ODS-II, 150 × 4.6 mm i.d. (Shimadzu); ultraviolet detection, 254 nm; mobile phase, 0.057% phosphoric acid/methanol (35:65). The absorption study was repeated three times, and the result was expressed as the mean ± SD.

Evaluation of Skin Irritation

Irritation evoked by model formulae on rat skin was microscopically judged at the end of the experiments on percutaneous absorption. The site of application of each formula on the skin was excised from rats. The separated skins were fixed in 10% neutral carbonate-buffered formalin for at least 24 hr before routine processing and then cut vertically against the skin surface at the central region at a width of 4 mm. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were divided into small pieces (about 3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined by light microscopy (Optiphoto, Nikon, Tokyo, Japan). The pathological study was conducted with three animals, and the mean value of irritation scores was used.

Computer Programs

Physicochemical parameters of thiomenthol derivatives such as log *P* and a steric energy were calculated by using a computer program ''CAChe'' (Oxford Molecular Group Inc., Oxford, England) with a Power Macintosh 8100/80 computer (Apple Japan, Inc., Tokyo, Japan). Multiple regression analysis was performed by a self-made program with an NEC PC-9821 V20 desktop computer (NEC Corp., Tokyo, Japan).

RESULTS AND DISCUSSION

Enhancement Factor of Thiomenthol Derivatives

To evaluate the percutaneous absorption in vivo in rats, the rate of penetration (R_p) of ketoprofen was estimated from a simple pharmacokinetic model based on the assumption that the rate of penetration of ketoprofen absorbed from the hydrogel is constant after a lag time according to the following equation (7):

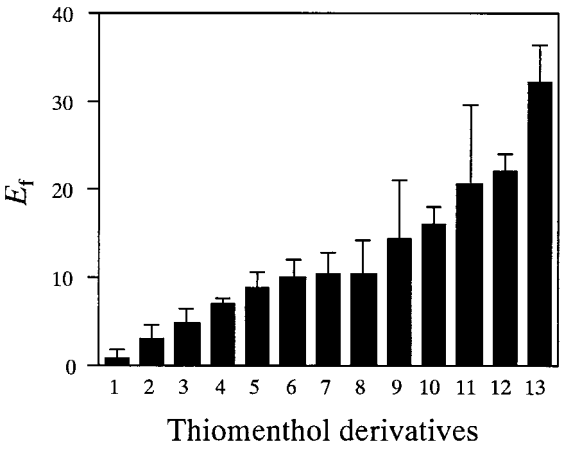


Figure 3. Enhancement factor (E_f) of thiomenthol derivatives. Each column represents the mean ± SD for three determinations.

$$C = \frac{R_p}{V_d k_{10}} \left\{ 1 + \frac{\beta - k_{10}}{\alpha - \beta} e^{-\alpha(t - t_L)} + \frac{k_{10} - \alpha}{\alpha - \beta} e^{-\beta(t - t_L)} \right\} \quad (1)$$

where C is the plasma concentration, R_p is the rate of penetration, t is time, t_L is the lag time, V_d is the distribution volume of the central compartment, k_{10} is the elimination rate constant from the central compartment, and α and β are the hybrid first-order rate constants. The mean values of V_d, k_{10}, α, and β, estimated previously (7), were used in this study to determine R_p and t_L values. As an index for the promoting activity of each enhancer, an enhancement factor (E_f) was defined as follows:

$$E_f = R_p \text{ (with enhancer)} / R_p \text{ (without enhancer)} \quad (2)$$

Figure 3 shows the E_f values of 13 different thiomenthol derivatives (1–13). The strongest promoting activity was observed when the compound 13 was formulated in the hydrogel. From the E_f values observed with compounds 3, 7, and 8, introducing the thioethyl group to the C3 position of menthol was less effective on the drug absorption; however, the combination of thioethyl group at the C3 position and methyl groups at the C1 and C4 positions was thought to be important because it exhibited the strongest activity (compound 13). The methyl group at the C1 position was also effective as can be understood from the fact that the E_f value with compound 13 was significantly greater than that with compound 9. On the other hand, introducing a bulky group at the C4 position brought about disadvantageous results in the promoting activity (compound 4).

Skin Damage Caused by Thiomenthol Derivatives

Skin irritation 8 hr after application of ketoprofen hydrogels was pathologically investigated. The microscopic findings were graded in five levels of irritation, from no change (level 0) to a marked one (level 4), according to epidermis liquefaction and collagen fiber swelling in the dermis and hypodermis, together with edema and inflammatory cell infiltration and degeneration of skin appendages. A total irritation score (TIS) was obtained by summation of each irritation score and used as an index of skin damage caused by the application of ketoprofen hydrogels. Results are given in Table 2. Although the hydrogel not containing enhancer caused no change in any tissues, all other hydrogels containing enhancers induced significant skin damage. The most severe skin damage was evoked by the application of the compound 13. Unfortunately, the TIS values were almost linearly related to the E_f values (Fig. 4), suggesting that the compounds exhibiting strong enhancement action may cause severe damage to the skin.

Structure–Activity and Structure–Toxicity Relationships

For a better understanding of the quantitative structure–activity and structure–toxicity relationships, physicochemical parameters of these compounds such as log P and a steric energy were estimated by using a computer program, ''CAChe.'' The log P, the octanol-water partition coefficient, was calculated using the atom typing scheme (8). The steric energy (kcal/mol) of molecules is the sum of the molecular mechanics potential energies calculated for the bonds, bond angles, dihedral angles, nonbonded atoms, and so forth (9). Multiple regression analysis was applied to predict the E_f and TIS values as a function of log P and steric energy. The coefficient of determination, which was doubly adjusted with degrees of freedom, was used as an index for selection of the optimum combination of parameters (10). An optimum regression equation for the E_f values was obtained as follows:

$$\begin{aligned} \log E_f = {} & 8.00\ (\pm\ 1.34) \log P \\ & - 1.17\ (\pm\ 0.20)\ (\log P)^2 \\ & - 0.199\ (\pm\ 0.105)\ \mathrm{SE} \\ & - 9.78\ (\pm\ 1.82) \end{aligned} \quad (3)$$

Table 2

Histopathological Findings of Hairless Rat Skin 8 hr After Application of Ketoprofen Hydrogels Containing 0.5% Thiomenthol Derivatives and 40% Ethanol

		Derivatives												
Pathological Findings	Control	1	2	3	4	5	6	7	8	9	10	11	12	13
Epidermis liquefaction	0	0	0	1	1	1	1	2	2	2	3	3	3	4
Subepidermis edema	0	0	1	0	0	1	1	2	3	2	2	3	3	4
Dermis														
Collagen fiber swelling	0	0	0	0	0	0	0	2	3	2	2	3	3	4
Inflammatory cell infiltration	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hypodermis														
Collagen fiber swelling	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Inflammatory cell infiltration	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin appendages degeneration	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TIS	0	0	1	1	1	2	2	6	8	6	7	9	9	12

0, no change; 1, very slight; 2, slight; 3, moderate; 4, marked.

$r = 0.978$, $s = 0.100$, $F_o = 65.8$, where r is the multiple correlation coefficient, s is the standard deviation of residual, F_o is the ratio of mean square regression to mean square residual (observed F value), and SE is the steric energy. A convex relation between log P and log E_f may suggest that the optimal lipophilicity of the enhancers exists for their promoting activities (Fig. 5). The steric energy values negatively affected the promoting activity of enhancers, that is, the more stable and smaller the steric structure is, the stronger the enhancement action is achieved. An equation for the TIS values was also obtained as a function of log P and steric energy:

$$\log \text{TIS} = 11.2\ (\pm\ 3.6)\ \log P - 1.69\ (\pm\ 0.53)\ (\log P)^2 - 0.214\ (\pm\ 0.182)\ \text{SE} - 14.9\ (\pm\ 5.2) \quad (4)$$

$r = 0.941$, $s = 0.167$, $F_o = 20.5$. Similar to the result obtained with E_f, a convex curvature between log P and log TIS was seen (Fig. 5). In a series of thiomenthol derivatives, the compound exhibiting strong enhancement action led to severe skin damage. Thus, further study to find more effective and safer enhancers is required to

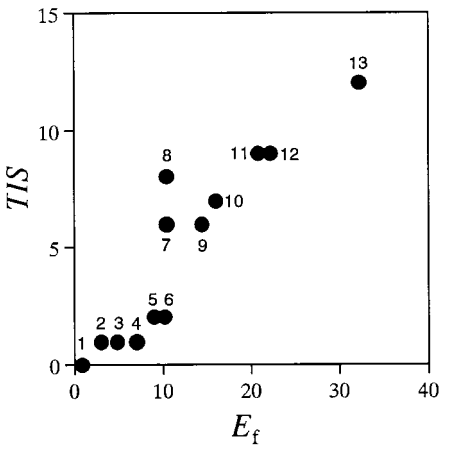


Figure 4. Relationship between enhancement factor (E_f) and total irritation score (TIS).

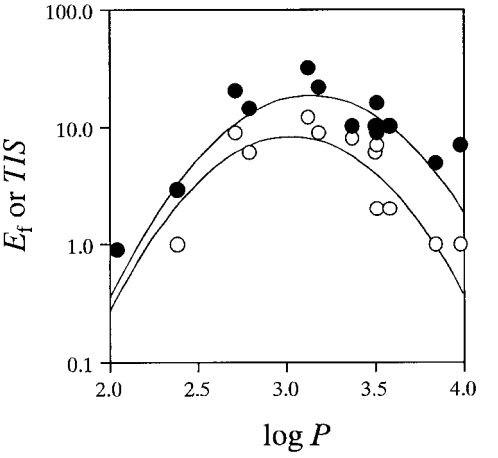


Figure 5. Relationship between log P and (●) enhancement factor (E_f) or (○) total irritation score (TIS).

apply transdermal delivery systems to less permeable drugs through the skin.

ACKNOWLEDGMENTS

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