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Influence of Fatty Acid-Alcohol Esters on Percutaneous Absorption of Hydrocortisone Butyrate Propionate

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Transcutaneous drug absorption is influenced by the physicochemical properties of both the drug and the vehicle. The effect of the vehicle is very important for the maximum drug effect. The effect of the vehicle can be considered from two standpoints, drug release and interaction with the skin, and therefore absorption of the vehicle itself may affect drug absorption. Because of the wide variety of vehicles mixed in ointments, it is difficult to measure the percutaneous absorption of all of them. It is important to study the relationship between the physicochemical properties of vehicles and the enhancement of absorption of the drug.

In this experiment, a series of fatty acid-alcohol esters have been selected since they are frequently used as vehicles for drugs and cosmetics. The effects of the physicochemical properties of these vehicles on the percutaneous absorption of hydrocortisone butyrate propionate (HBP), a topical antiinflammatory corticosteroid, were examined. The partition coefficients ($\log P$) of vehicles were determined by calculation.

The gel vehicle was applied to the abdominal skin of anesthetized rats. The skin was excised after an appropriate interval and drug absorption was calculated from the unabsorbed drug amount. Drug absorption was enhanced by several additives. The physicochemical property values of additives that may result in maximum drug absorptions were calculated. The optimum values were approximately 12 for the partition coefficient and about 400 for molecular weight. Additives that have quite high hydrophobicity were found to be suitable for HBP ($\log P=3.3$). When isopropyl myristate was used as an additive, after about 15 h the absorption rate of HBP was found to have increased. It was suggested that isopropyl myristate has a direct effect on the barrier function. The physicochemical properties of the absorption enhancer affect not only the release of the drug from the vehicle to the skin, but also the interaction between the vehicle and the skin after penetration into the skin.

Keywords—percutaneous absorption; hydrocortisone butyrate propionate; fatty acid ester; partition coefficient; solubility; molecular weight; rat; *in vivo*; skin; corticosteroid

Transcutaneous drug absorption is influenced by the physicochemical properties of both the drug and the vehicle. Vehicle selection is therefore very important in terms of the maximum drug effect. The effect of the vehicle is usually considered from two standpoints, that is, the solvent effect on the drug and the interaction with skin.^{1,2)} A vehicle which produces increased solubility of a sparingly soluble drug can increase transcutaneous drug absorption, even if the vehicle itself can not permeate through the skin.³⁾ Since the skin is a barrier for substances, the diffusion rate in the skin is much lower than that in the vehicle. Therefore, the rate of diffusion in the skin tends to be the rate-limiting step in percutaneous absorption,⁴⁾ and increasing the solubility and releasability alone are inefficient as approaches to increase the permeability of drugs. For such a purpose, the vehicle must alter the barrier function of the skin. Differences in the physicochemical properties of the vehicle affect the

skin penetration of the vehicle itself as well as the penetration of the drug. In this case, the skin penetration rate of the vehicle is correlated with the enhancement of drug absorption. In general, the drug is expected to act on a particular tissue, while it is undesirable for the vehicle to act on the skin. Absorption of drugs by the skin has been widely investigated, but the same cannot be said regarding vehicles. No difference of physicochemical properties can be observed between drugs and vehicles, so it is possible that both substances are transported *via* the same mechanism in the skin. Because of the wide variety of vehicles which are used in ointments, it would be difficult to measure the percutaneous absorption of all vehicles. However, it is important to study the relationship between the physicochemical properties of vehicles and the enhancement of drug absorption, since such knowledge would be widely applicable for the development of absorption enhancers or dosage design for ointments. In this study, a series of fatty acid-alcohol esters were selected since they are frequently used as vehicles for drugs and cosmetics. The effects of the physicochemical properties of these vehicles on the percutaneous absorption of hydrocortisone butyrate propionate, a topical antiinflammatory corticosteroid, were investigated.

Experimental

Materials—Hydrocortisone 17-butyrate 21-propionate (HBP) was synthesized at our research center.⁵⁾ Isopropyl butyrate, isopropyl palmitate and carboxyvinylpolymer (CVP: HIVISWAKO®104) were purchased from Wako Pure Chemical Ind., Ltd. Isopropyl caproate, isopropyl caprylate, isopropyl caprate, diisopropyl adipate and ethyl myristate were purchased from Tokyo Kasei Kogyo Co., Ltd. Isopropyl myristate (IPM) was purchased from Sigma Chemical Co., Ltd. Hexyl laurate, butyl myristate, butyl stearate, decyl oleate, octyldodecyl myristate and diisopropyl sebacate were supplied by Kokyu Alcohol Ind., Ltd. All other chemicals were of reagent grade.

Preparation of Test Vehicles—The various additives (at 3%) and 0.02% HBP were combined with the 1% CVP gel prepared as follows: CVP, 1%; ethanol, 48.5%; distilled water, 48.5%; 5% ammonia solution, 2%.

Absorption Experiment—The absorption experiments with HBP were carried out in the manner reported previously.⁶⁾ Male rats weighing 200–250 g were anesthetized with urethane. Abdominal hair was removed with electric hair clippers and the skin was cleansed with 70% ethanol.

A circular 2 cm² area was delineated with a 1-mm-wide strip of 5% CVP gel which did not contain drugs; the overflow barrier was functional when the gel had dried. The test vehicle, 50 mg of gel, was applied on the 2 cm² area by the use of a microinfusion pump (Furue Science Co., Ltd., model JP). The animals were sacrificed after appropriate intervals and the skin with overflow barrier was excised carefully cut up to the subcutaneous tissue with scissors.

Absorption rate of HBP through the skin after application was calculated from the following formula:

$$\text{absorption rate (\%)} = \left(1 - \frac{\text{recovered amount at appointed time after application}}{\text{application amount}} \right) \times 100$$

Determination of HBP in the Skin—Acetonitrile (10 ml) was added to the excised skin and the mixture was homogenized with a Hiscotrom homogenizer (type NS-10). After the addition of 5 g of Na₂SO₃ to the homogenate, the mixture was centrifuged and 8 ml of the organic phase was withdrawn. After reextraction with 10 ml of acetonitrile, the combined organic phase was evaporated to dryness. The residue was dissolved in an high performance liquid chromatography (HPLC) mobile phase.

HBP was analyzed by HPLC (Shimadzu LC-3A pump and SPD-2A UV monitor) with a 150 × 4 mm i.d. column containing TSK-Gel LS 410 (5 μm). The flow rate was 1 ml min⁻¹, the ultraviolet (UV) wavelength was 245 nm, and the column temperature was set at 50 °C. As a mobile phase, a mixture of methanol, water and acetic acid (65.5: 35: 0.5) was used.

Solubility Studies—An excess amount of HBP was added to various vehicles and shaken at 25 ± 1 °C. After equilibration (approximately 24 h), an aliquot was filtered through a membrane filter (0.45 μm) and the concentration of HBP was measured by HPLC.

Determination of Partition Coefficient—The partition coefficients (log *P*_{cal}) were calculated by the method of Hansch and Leo.⁷⁾ The only input information required for this method is the structure of the chemical, since the fragment values and factors are known. Fragment values for atoms or groups and factors used were the reported values.^{7,8)}

Results and Discussion

Influence of Additives

The structural formulas of the fifteen fatty acid alcohol esters used as additives are listed in Table I. A large variety of fatty acid alcohol esters can be formed by combining fatty acids and alcohols; the esters used in this study were selected from among the combinations of C_4 — C_{18} fatty acids and C_2 — C_{20} alcohols.

Figure 1 shows the absorption rates of HBP from the gel bases containing 3% of the different additives. The absorption rate of HBP from the control gel without additives was about 20% over 24 h. Enhanced absorption was produced by all of the additives except isopropyl butyrate, isopropyl caproate and isopropyl caprylate. The percutaneous absorption of HBP was enhanced approximately 3-fold when IPM and ethyl myristate were added to the gel base.

TABLE I. Structural Formulae of Fatty Acid-Alcohol Esters

Fatty acid ester	Structural formula
1. Isopropyl butyrate	$\text{CH}_3(\text{CH}_2)_2\text{COOCH}(\text{CH}_3)_2$
2. Diisopropyl adipate	$(\text{CH}_3)_2\text{CHOOC}(\text{CH}_2)_4\text{COOCH}(\text{CH}_3)_2$
3. Isopropyl caproate	$\text{CH}_3(\text{CH}_2)_4\text{COOCH}(\text{CH}_3)_2$
4. Isopropyl caprylate	$\text{CH}_3(\text{CH}_2)_6\text{COOCH}(\text{CH}_3)_2$
5. Diisopropyl sebacate	$(\text{CH}_3)_2\text{CHOOC}(\text{CH}_2)_8\text{COOCH}(\text{CH}_3)_2$
6. Isopropyl caprate	$\text{CH}_3(\text{CH}_2)_8\text{COOCH}(\text{CH}_3)_2$
7. Ethyl myristate	$\text{CH}_3(\text{CH}_2)_{12}\text{COOCH}_2\text{CH}_3$
8. Isopropyl myristate (IPM)	$\text{CH}_3(\text{CH}_2)_{12}\text{COOCH}(\text{CH}_3)_2$
9. Hexyl laurate	$\text{CH}_3(\text{CH}_2)_{10}\text{COO}(\text{CH}_2)_5\text{CH}_3$
10. Butyl myristate	$\text{CH}_3(\text{CH}_2)_{12}\text{COO}(\text{CH}_2)_3\text{CH}_3$
11. Isopropyl palmitate	$\text{CH}_3(\text{CH}_2)_{14}\text{COOCH}(\text{CH}_3)_2$
12. Butyl stearate	$\text{CH}_3(\text{CH}_2)_{16}\text{COO}(\text{CH}_2)_3\text{CH}_3$
13. Decyl oleate	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}(\text{CH}_2)_9\text{CH}_3$
14. 2-Octyldodecyl myristate	$\text{CH}_3(\text{CH}_2)_{12}\text{COOCH}_2\text{CH}(\text{CH}_2)_9\text{CH}_3$ $\text{CH}(\text{CH}_2)_6\text{CH}_3$
15. Oleyl oleate	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COO}(\text{CH}_2)_8\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}_3$

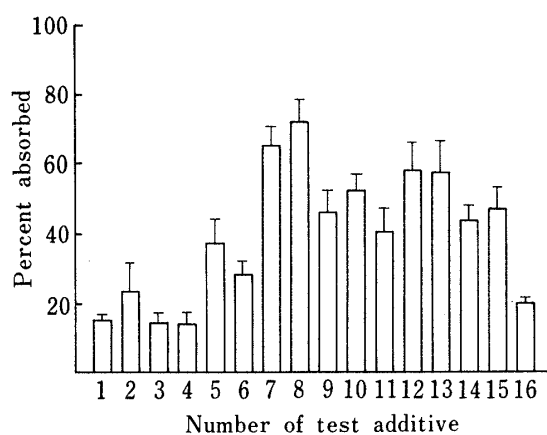


Fig. 1. Influence of Fatty Acid-Alcohol Esters on the Percutaneous Absorption of HBP in Rats

The numbers are the same as in Table I. The number 16 indicates a control experiment (gel base without additives). Values are the means \pm S.D. ($n=4-8$).

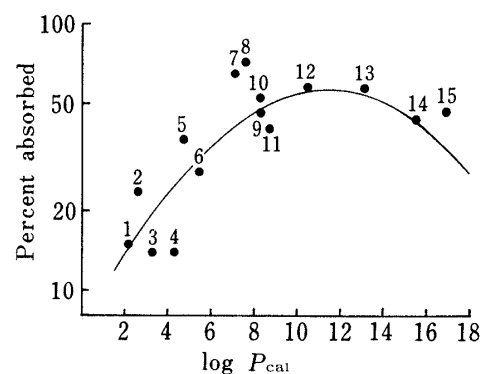


Fig. 2. A Plot of the Percutaneous Absorption of HBP in Rats versus the Calculated Partition Coefficient ($\log P_{cal}$) of Fatty Acid-Alcohol Esters

The numbers are the same as in Table I.

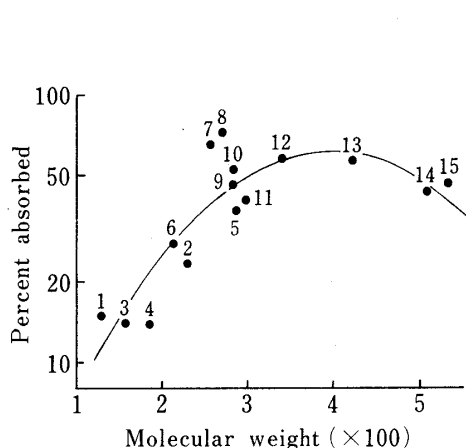


Fig. 3. A Plot of the Percutaneous Absorption of HBP in Rats *versus* the Molecular Weight of Fatty Acid-Alcohol Esters

The numbers are the same as in Table I.

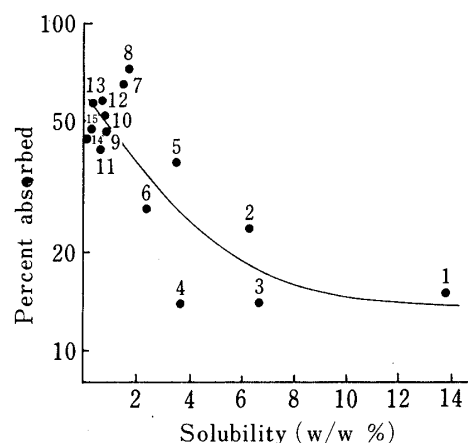


Fig. 4. A Plot of the Percutaneous Absorption of HBP in Rats *versus* the Solubility of HBP to Fatty Acid-Alcohol Esters

The numbers are the same as in Table I.

Physicochemical Properties of the Additives

Partition coefficient ($\log P_{\text{cal}}$), molecular weight (M) and solubility (S) of HBP in the additives were selected as an indicators of the physicochemical properties of the additives, and their affects on the absorption enhancing effect were studied. Plots of the percutaneous absorption of HBP *versus* the physicochemical properties of the additives are shown in Figs. 2, 3 and 4.

The octanol/water partition coefficient (P) is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase.

$$P = \frac{\text{concentration in octanol phase}}{\text{concentration in aqueous phase}}$$

A number of reports have been presented concerning the relationship between the skin permeability of drugs and the partition coefficient of drugs in organic solvent/water or stratum corneum/base. Reports concerning the effect of the partition coefficient of the additives on the skin permeation of drugs are relatively few. Difficulty in measuring the partition coefficient of additives is one reason for the lack of reports. Most drugs have a partition coefficient of less than $\log P=4$, while on the other hand, the partition coefficients of additives can be as high as $\log P=17$ (Fig. 2). Measurement of a partition coefficient with a value of more than $\log P=6$ is technically difficult.⁹⁾ In recent years, several methods have been presented for estimating the octanol/water partition coefficient of organic chemicals.⁸⁾ The partition coefficients ($\log P_{\text{cal}}$) in this report were calculated by the method of Hansch and Leo,⁷⁾ a method which only requires knowledge of the chemical structure. From Figs. 2—4, the regression equations, correlation coefficients and standard deviations were determined to be as follows.

$$\log A^* = -0.007(\log P_{\text{cal}})^2 + 0.161(\log P_{\text{cal}}) + 0.836 \quad (1)$$

$$r = 0.855, \quad s = 0.136, \quad n = 15$$

$$\log A = -0.102(M/100)^2 + 0.815(M/100) + 0.172 \quad (2)$$

$$r = 0.872, \quad s = 0.128, \quad n = 15$$

$$\log A = 0.00496(S)^2 - 0.113(S) + 1.781 \quad (3)$$

$$r = 0.825, \quad s = 0.148, \quad n = 15$$

* A represents the percent absorption of HBP.

With respect to the partition coefficient and molecular weight, introduction of the squared terms appear to have improved the correlation coefficient. The value that results in the maximum drug absorption was calculated by setting the partial derivative of the equation with respect to that parameter to zero and then solving. The optimum value was about 12 in $\log P_{cal}^{10)}$ and about 400 in M . An enhancer with rather high hydrophobicity was found to be suitable for HBP ($\log P=3.3$), although the effect of the enhancer was also dependent on the hydrophobicity of the drugs.¹¹⁾

Enhancement Factor in Absorption

As suggested from Figs. 2, 3 and 4, $\log P_{cal}$ increased as M increased, while the solubility of HBP was inversely proportional to M . Rosvold *et al.*⁴⁾ showed that the rate-limiting step in the absorption of fluocinolone acetonide from the gel is the diffusion of the steroid through the skin. It is thought that additives function in 2 main ways on the drug-skin interaction: 1) additives that penetrate the skin act as a carrier, and 2) additives that penetrate the skin alter the barrier function. Saket *et al.*¹²⁾ reported that cortisone, hydrocortisone and hydrocortisone acetate exist as monomers in IPM, since the solute-solvent interaction is relatively strong. Drug partition to the skin decreases with an increase in the $\log P$ value of additives, but when additives penetrate the skin and act as a carrier for drugs, skin permeation increases at a relatively high $\log P$ value. Potency reaches a maximum from which it gradually decreases since a lack of drug solubility more than counterbalances increasing $\log P$. Diesters such as diisopropyl adipate and diisopropyl sebacate were not as effective as other additives, since the high solubility of HBP (Fig. 4) and the low $\log P$ (Figs. 2 and 3) counterbalance one another. As can be seen from Fig. 4, the solubility of HBP in decyl oleate, 2-octyldodecyl myristate and oleyl oleate was less than 0.67%, and therefore HBP may crystallize on the skin after ethanol, which is a component of the gel base, had evaporated.¹³⁾ These three additives were further studied by adding a two-fold concentration (6%) of the additive. The concentration of HBP was observed to decrease to only 0.005% for oleyl oleate, a substance which has poor solubility. Figure 5 shows that the poor absorption observed for these three additives was not due to drug crystallization on the skin, although the absorption of HBP tended to increase somewhat. As shown in Fig. 6, the absorption rate of HBP increased in parallel with increasing IPM concentration, although the degree of increase for concentrations above 3% was reduced to some extent. Absorption enhancement was observed at IPM concentrations of not less than 1%, *i.e.* those that can achieve complete solubilization of HBP. This suggests

HBP conc. (%)	additive	conc. (%)	HBP percent absorbed
			20 40 60
0.02	decyl oleate	3	~55
0.02	decyl oleate	6	~65
0.02	2-octyldodecyl myristate	3	~45
0.02	2-octyldodecyl myristate	6	~65
0.005	oleyl oleate	3	~45
0.005	oleyl oleate	6	~45

Fig. 5. The Relationship between HBP or Fatty Acid-Alcohol Ester Concentration and the Percutaneous Absorption of HBP in Rats

Values are the means \pm S.D. ($n=4-6$).

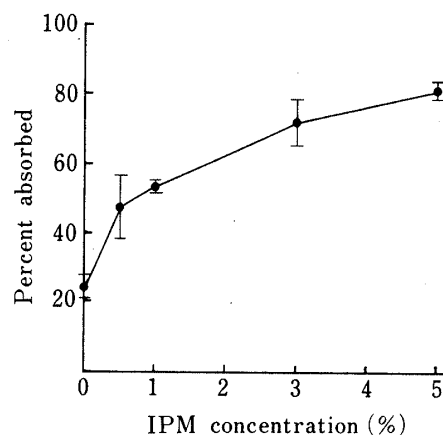


Fig. 6. A Plot of the Percutaneous Absorption of HBP in Rats versus the Concentration of IPM

Values are the means \pm S.D. ($n=4-8$).

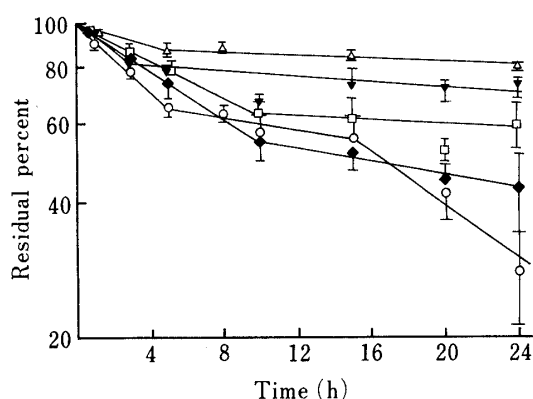


Fig. 7. Time Dependent Profiles of the Influence of Fatty Acid-Alcohol Esters on the Percutaneous Absorption of HBP in Rats

△, no additives; ▼, isopropyl caprate; □, isopropyl palmitate; ◆, decyl oleate; ○, IPM. Values are the means \pm S.D. ($n=4-8$).

that the effect of IPM is derived not only from its solubilizing action, but also the interaction between the skin and IPM.

Effect of IPM

As shown in Figs. 2 and 3, for IPM and ethyl myristate absorption increased and its position shifted from the convex curve of the other additives. It has been reported¹⁴⁾ that the percutaneous absorption of flufenamic acid was markedly enhanced by IPM. *N*-Nitrosodiethanolamine was applied to skin in vehicles having different solubility properties by Bronaugh *et al.*¹⁵⁾ The permeability constant for water was small. In IPM, the permeability constant increased by approximately 250-fold. They thought that this increased permeability was probably due to more favorable partitioning into the membrane. The absorption of antiandrogens, cyproterone acetate and propylmesterolone, after topical application of the substances either in ethanol or in ethanol/IPM (95:5 v/v), was determined in rabbit ear by Ekerdt *et al.*¹⁶⁾ Addition of IPM to the solvent had a profound influence on the percutaneous absorption of both antiandrogens. The percutaneous absorption of ¹⁴C-labeled IPM was studied by Suzuki *et al.*¹⁷⁾ In whole body autoradiography using hairless mice, there was no visible penetration into the skin or organs, whereas microautoradiography with guinea pigs showed local penetration. They suggested that the skin irritation may be correlated with the absorbability, although IPM does not produce erythema in human skin. The time-course of the percutaneous absorption of HBP is shown in Fig. 7. When IPM was used as an additive, approximately 15 h later the absorption rate of HBP had increased. The reason for this enhancement may be that IPM and ethyl myristate have a direct effect on the barrier function, besides the drug carrier action. Mirejovsky and Takruri¹⁸⁾ noted that the enhancing effect of several amides becomes greater as the penetration rate of the amide itself is increased. The physicochemical properties of the absorption enhancer effect not only the release of the drug from the vehicle to the skin, but also the interaction between the vehicle and the skin after penetration into the skin. In this study, it was found that additives which have very high hydrophobicity are effective as absorption enhancers, although measurement of a partition coefficient greater than $\log P=6$ is difficult with the present technique.

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