# Effect of Several Enhancers on the Skin Permeation of Water-Soluble Drugs[1)]

Mutsuo OKUMURA, Kenji SUGIBAYASHI and Yasunori MORIMOTO*

*Faculty of Pharmaceutical Sciences, Josai University, 1–1 Keyakidai, Sakado, Saitama 350–02, Japan.* *Received November 25, 1988*

**Since the percutaneous absorption rates of water-soluble drugs are low in general, an enhancing system is needed when using the skin as an administration site for the drugs. We have investigated the effect of various penetration enhancers on the *in vitro* and *in vivo* percutaneous absorptions of catecholamine analogs, *i.e.* levodopa (LD), dopamine hydrochloride (DPH) and isoproterenol hydrochloride (IPH), as model water-soluble drugs. It was found that medium-chain glycerides (Sefsol 318) markedly enhanced the *in vitro* permeation of the drugs through excised hairless rat skin among the enhancers tested in the present experiments; the permeation rates with 5% Sefsol 318 in water were about 65, 34 and 53 times higher than the corresponding control (without enhancer) for LD, DPH and IPH, respectively. In addition, the *in vivo* percutaneous absorption experiments showed that the blood levels of these drugs after application of aqueous gels containing 5% Sefsol 318 on rat skin were higher than those in the absence of enhancer. Drug levels in the liver and kidney were also higher than without Sefsol 318. Percutaneous administration of DPH with Sefsol 318 to hairless rats resulted in lower diastolic blood pressure and a slightly higher heart rate with as compared to administration without the enhancer.**

**These results suggest that Sefsol 318 is a potential candidate to enhance the transdermal absorption of water-soluble drugs.**

**Keywords** percutaneous absorption; water-soluble drug; catecholamine; levodopa; dopamine hydrochloride; isoproterenol hydrochloride; penetration enhancer; medium-chain glyceride; Sefsol 318

## Introduction

In the previous paper,[2)] it was found that the fluxes of several water-soluble drugs through the excised hairless rat skin were relatively high, especially for drugs with low molecular weight and high solubility in water. The high skin permeation rates were a consequence of relatively high permeability coefficients and very high water solubilities of these drugs.

Recently, many investigators have reported that the bioavailabilities of poorly absorbed drugs through rectal mucosa, nasal mucosa or skin are improved by various absorption promoters such as bile salts,[3)] surfactants,[4)] enamine derivatives of amino acids,[5)] glycerin esters,[6)] salicylates[7)] and Azone.[8)] In particular, rectal absorption of a poorly absorbed drug, cefmetazole sodium, was enhanced by medium-chain glyceride.[6)] However, there are few reports on the promoting effect of medium-chain glyceride on skin.

In the present study, the permeation rates of catecholamine analogs (CAA) through the hairless rat skin were measured and the effect of penetration enhancers on the permeation rates of the drugs was investigated *in vitro* by using a 2-chamber diffusion cell.[2)] We also investigated the *in vivo* skin permeation and the pharmacological effects such as blood pressure and heart rate. Levodopa (LD), dopamine hydrochloride (DPH) and isoproterenol hydrochloride (IPH) were selected as CAA.

## Experimental

**Materials** LD, DPH and IPH were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), Katsura Chemical Co. (Tokyo, Japan) and Nikken Chemical Co. (Tokyo), respectively. Radiolabelled compounds, $^{14}$C-LD (11.0 Ci/mol), $^{3}$H-DPH (33.3 Ci/mol) and $^{3}$H-IPH (11.9 Ci/mol) were purchased from Amersham International (Buckingham, UK). Medium-chain fatty acid esters; glyceryl monocaprylate (Sefsol 318, S-318), propylene glycol didecanoate (PDD), propylene glycol dicaprylate (Sefsol-228, S-228), glyceryl tricaprylate (Sefsol 810, S-810) and sorbitan monocaprylate (Sefsol 418, S-418) were kindly supplied by Nikko Chemicals (Tokyo). Sucrose fatty acid ester (S-1670) and laurocapram (Azone) were supplied by Mitsubishi-Kasei Food Co. (Tokyo) and Nelson-Sumisho Co. (Tokyo), respectively. Hydroxypropyl cellulose (150—400 cps) (HPC, Wako Pure Chemical Industries, Osaka, Japan) was used to make aqueous gel. All other reagents were of reagent grade and were used without further purification.

**Animals** Male hairless rats (WBN/kob strain), weighing approximately 150 g each, were purchased from Saitama Laboratory Animals (Saitama, Japan).

**Diffusion Cells** A diffusion cell, consisting of two half-cells with a water jacket connected to a water bath at 37 °C, was used. Each half cell had a volume of 2.0 ml and an effective diffusional area of 1.13 cm$^2$.[2)]

**Skin Membrane Preparation** The abdominal region of the hairless rats was carefully shaved. About 4 cm$^2$ of the left and right abdominal skin was excised and mounted between two half diffusion cells by using a spring clamp.

***In Vitro* Permeation Procedure** The dermis of the skin was in contact with the receiver compartment and the stratum corneum with the donor compartment. The receiver compartment of the cell was filled with 2.0 ml of 0.1 M lactate buffer (pH 4.0) and the donor compartment with 2.0 ml of drug suspension (drug amount; about twice the solubility) in the same buffer with or without penetration enhancer. At appropriate times, a 200 $\mu$l sample was withdrawn from the receiver compartment and the same volume of fresh buffer was added to keep the volume constant.

**Analysis of Drug Concentrations** For the *in vitro* skin permeation studies, the concentration of LD was determined by high performance liquid chromatography (HPLC) (LC-6A, Shimadzu, Kyoto, Japan) under the following conditions: column, 4.6 mm × 250 mm stainless steel column packed with Nucleosil 5C$_{18}$ (Yamamura Chemical Laboratories, Tokyo); mobile phase, 0.05 M phosphate buffer (pH 4.0): acetonitrile (97 : 3); detector (fluorescence HPLC monitor RF 530, Shimadzu), excitation 286 nm and emission 320 nm. The concentrations of DPH and IPH were determined by HPLC under the same conditions as used for LD, except for detection (ultraviolet 280 nm; SPD-6A, Shimadzu).

***In Vivo* Permeation Procedure** For LD or IPH treatment, 3% HPC aqueous gel (2 g) containing 4 mg of LD (8 $\mu$Ci/head) or 20 mg of IPH (10 $\mu$Ci/head) and 400 mg of S-318 with a backing of Cateripad (Nichiban Co., Tokyo) was applied on 22 cm$^2$ (4.0 × 5.5 cm) of the abdominal skin in rats.[8e)] To ensure adequate fixation, an elastic bandage (Elastopore No. 50, Nichiban Co.) was wrapped around the body. For DPH treatment, a 3% HPC aqueous gel (2 g) containing 500 mg of DPH (10 $\mu$Ci/head) and 100 mg of S-318 was applied in a thin layer on 4.91 cm$^2$ of the skin. The application site was then covered with Parafilm (American Can Co., Greenwith, CT, U.S.A.).

At appropriate times, a blood sample (0.2 ml) was withdrawn from the jugular vein into a heparinized syringe to measure each drug concentration. Several tissues (kidney, spleen, lung, liver and heart) were excised at the end of the experiment. Radioactivity in the blood and tissues

was measured by the use of a sample oxidizer (ASC 113, Aloka, Tokyo) and liquid scintillation counter (LSC 700, Aloka).

**Blood Pressure and Heart Rate Measurements** After each hairless rat had been anesthetized with sodium pentobarbital (40 mg/kg, i.p.), heparinized vinyl tubing (1000 unit/ml) was catheterized into the right jugular artery. The operation was performed under sterile conditions. Blood pressure was measured by telemetry with a pressure transducer (Nihon Kohden, Tokyo) connected to the arterial catheter and a transmitter (Nihon Kohden) on the hairless rat. The electrocardiogram was recorded by using electrodes attached to the subcutaneous tissues. The blood pressure and electrocardiogram were simultaneously recorded on a recticorder (Nihon Kohden). Heart rate was determined by counting the pressure pulses for 20 s. Mean blood pressure was determined by adding a third of the difference between the systolic and diastolic pressures to the diastolic pressure.

## Results and Discussion

**Effect of S-318 on the *in Vitro* Skin Permeation of LD** The effects of several enhancers on the permeation of CAA across the excised hairless rat skin were investigated. Five percent was selected as a suitable concentration of each enhancer. These lipid-like enhancers do not affect the solubilities of CAA in water, since CAA are highly water-soluble drugs. Figure 1a and b shows the effect of several skin penetration enhancers on the time course of the cumulative amount of LD that permeated across the skin. No enhancing effect of S-228, S-810 or PDD on the LD permeation was found, and the rate and amount of skin permeation of LD were lower than those of the control (without enhancer). S-418 and S-1670 showed relatively higher skin permeation compared to that without the enhancer. On the other hand, marked enhancing effects of S-318 and Azone on the LD permeation were observed. Simultaneous use of the strong enhancers, S-318 and Azone, gave a greater effect than the use of either singly. The cumulative amounts of LD that permeated over 32 h were 5344, 2930 and 8299 $\mu g/cm^2$ with S-318, with Azone and with S-318 plus Azone, respectively.

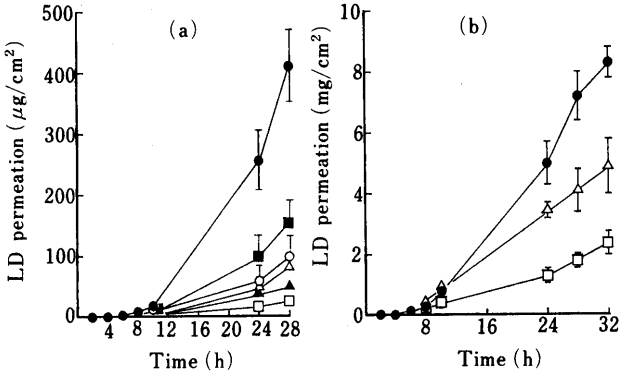


Fig. 1. Effect of Several Enhancers on the Permeation of Levodopa through Excised Hairless Rat Skin

(a) ○, control; △, S-810; ▲, S-228; □, PDD; ■, S-1670; ●, S-418. (b) △, S-318; □, Azone; ●, S-318+Azone. Each value represents the mean±S.E. of 3—4 experiments.

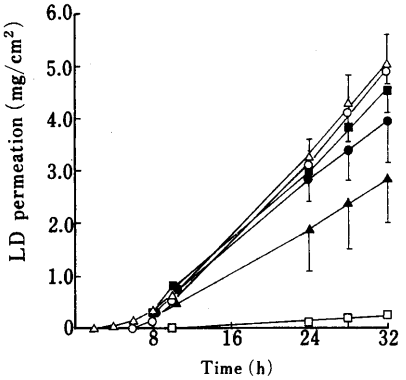


Fig. 2. Effect of Concentration of Sefsol 318 on the Permeation of Levodopa through Excised Hairless Rat Skin

□, 1%; ○, 3%; △, 5%; ■, 20%; ●, 30%; ▲, 50%. Each value represents the mean±S.E. of 3—4 experiments.

**Effect of the Concentration of S-318 on the *in Vitro* Skin Permeation of LD** Figures 2 and 3a, b show the effect of the concentration of S-318 on the skin permeation of LD. S-318 at concentrations from 3 to 30% showed relatively high skin permeation of LD (Fig. 2). Steady-state flux and lag time are plotted in Fig. 3a and b. The highest flux was observed at 3% S-318. With increasing concentration of S-318 from 3 to 50%, however, the flux decreased as shown in Fig. 3a. This may be accounted for by the decrease of effective diffusional area with increasing S-318 fraction in the donor compartment. The lag-time (Fig. 3b) was decreased with the increasing S-318 concentration. Since the permeation flux of drugs is proportional to the partition coefficient of the drugs between the vehicle and skin (skin/vehicle) and diffusivity of drugs in the skin barrier, and lag

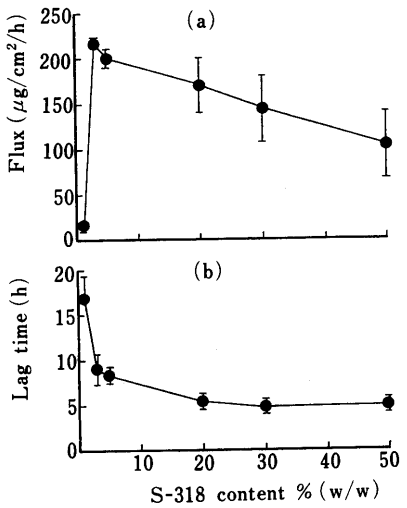


Fig. 3. Relationship between Flux (a), Lag Time (b) and Concentration of Sefsol 318

Each value represents the mean±S.E. of 3—4 experiments.

TABLE I. Effect of Sefsol 318 on the Permeation of Catecholamine Analogs through Excised Hairless Rat Skin

| | | Flux ($\mu g/cm^2/h$) | Lag time (h) | Permeability coefficient[a] (cm/s, $\times 10^6$) |
|---|---|---|---|---|
| LD | Control | 3±1[b] | 17.9±2.2 | 0.17±0.05 |
| | 5% S-318 | 195±10 | 7.2±0.6 | 11.29±0.59 |
| DPH | Control | 458±24[b] | 4.2±0.1 | 0.25±0.01 |
| | 5% S-318 | 15340±2720 | 1.8±0.7 | 8.39±1.49 |
| IPH | Control | 149±17[b] | 2.0±0.2 | 0.12±0.01 |
| | 5% S-318 | 7890±1710 | 3.9±0.7 | 6.54±1.42 |

*a*) Permeability coefficient was calculated from the flux and solubility of CAA in the donor compartment. Since CAA is insoluble in S-318, the solubility of CAA in 5% S-318/buffer is assumed to be the same as that in the buffer solution. *b*) Phosphate buffer (pH 4.0). Each value represents the mean±S.E. of 3 experiments.

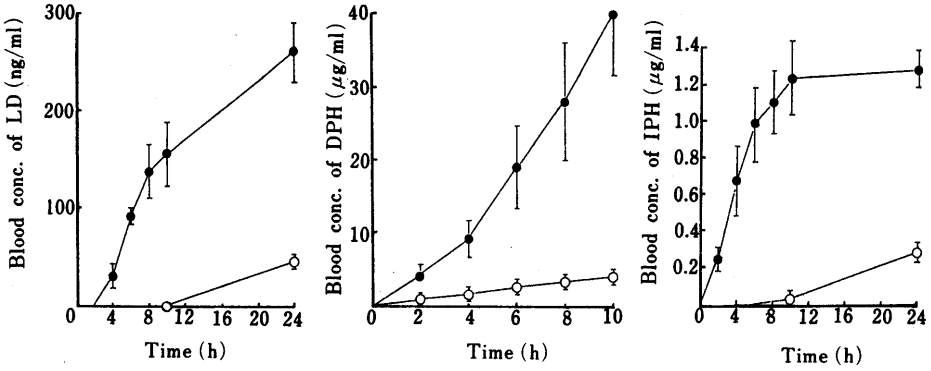


Fig. 4. Blood Levels of Radioactivity after Application of HPC Aqueous Gel Containing $^{14}$C-LD, $^{3}$H-DPH or $^{3}$H-IPH to the Abdominal Skin of Hairless Rats

●, with S-318; ○, without S-318. Each value represents the mean ± S.E. of 3—4 experiments. *a*) Each value was obtained from the radioactivity (dpm/ml) in the blood and specific activity of each drug. Although each value may include radioactivities due to unchanged drug and its metabolites, all values were converted to unchanged drug.

TABLE II. Tissue Levels of Radioactivity after Application of Radiolabelled Catecholamine Analogs on Hairless Rat Skin

| Tissue | | LD[a)] | DPH[b)] | IPH[a)] |
|---|---|---|---|---|
| Kidney | Cont. | 0.61±0.04 | 15.31± 2.10 | 0.86±0.02 |
| | S-318 | 2.23±0.61 | 343.89±91.83 | 9.79±1.12 |
| Spleen | Cont. | 0.04±0.02 | 3.41± 0.39 | 0.46±0.12 |
| | S-318 | 0.09±0.01 | 77.61±34.80 | 1.85±0.30 |
| Lung | Cont. | 0.11±0.02 | 4.59± 0.41 | 0.27±0.05 |
| | S-318 | 0.35±0.08 | 130.86±46.97 | 0.98±0.06 |
| Liver | Cont. | 0.73±0.02 | 3.27± 0.91 | 0.74±0.09 |
| | S-318 | 2.14±0.27 | 130.04±51.76 | 9.18±0.86 |
| Heart | Cont. | 0.07±0.01 | 3.53± 0.67 | 1.10±0.27 |
| | S-318 | 0.24±0.04 | 115.46± 0.67 | 0.51±0.05 |

Each radioactivity was converted to the concentration of unchanged drug (μg/g tissue) (see the legend to Fig. 4) and is given as the mean ± S.E. of 3—4 experiments. *a*) Dermal patch was applied for 24 h. *b*) Dermal patch was applied for 10 h.

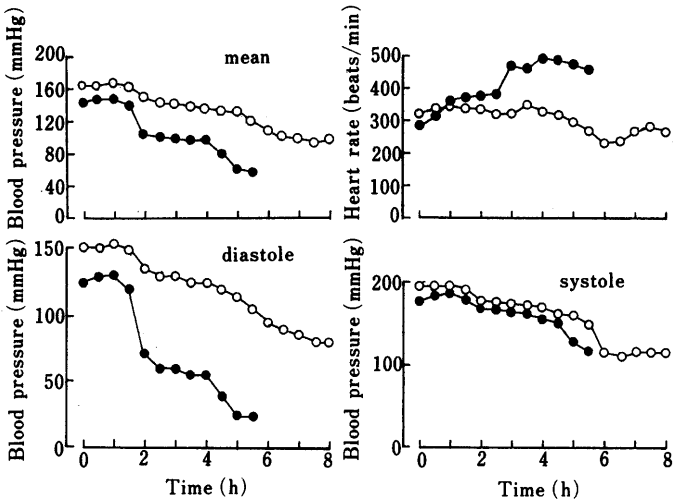


Fig. 5. Pharmacological Effects of Dopamine Hydrochloride after Application of HPC Gel to the Abdominal Skin of Hairless Rat Skin

●, with S-318; ○, without S-318. Each value represents the mean of 2 experiments.

time is inversely proportional to the diffusivity, the results shown in Fig. 3a and b suggested that not only drug partition from the vehicle to the skin but also drug diffusivity in the skin would increase with increasing concentration of S-318 in the donor compartment. However, the decreasing ratio of the lag time was very small at higher S-318 concentrations than 3—5%. Therefore, the most effective enhancing concentration of S-318 was estimated to be between 3 and 5%. For the following experiments, 5% S-318 was used.

**Effect of S-318 on the *in Vitro* Skin Permeation of CAA** The effect of S-318 (5%) on the skin permeation of CAA through the excised skin is summarized in Table I. The enhancing effects of S-318 on the skin permeation of CA were remarkable and the differences from the corresponding control (without enhancer) were about 65, 34 and 53 times for LD, DPH and IPH, respectively. The permeabilities of these drugs with S-318, however, were similar to each other. These results suggest that there is a maximum flux which is dependent upon the permeability of the stripped skin.[8c,d] The mechanism of the skin penetration enhancing effect of S-318 is still unclear. Experiments to establish the mechanism are under way in our laboratory.[1] One of the most important mechanisms may be the fluidizing effect on the stratum corneum lipids.

**Effect of S-318 on the *in Vivo* Skin Permeation** The effect of S-318 on the *in vivo* skin permeation of CAA was investigated by measuring radioactivity in the blood and several tissues after application of an aqueous gel, containing radiolabelled CAA (Fig. 4, Table II). The radioactivity could be assumed to be the sum of radioactivities of the unchanged form of CAA and its metabolites. A remarkable enhancing effect of S-318 was observed in terms of the blood concentration. The blood concentrations (converted to concentration of unchanged drug) at 10 h were 0.16, 40.13 and 1.26 μg/ml with S-318 and 0.01, 4.10 and 0.05 μg/ml without the enhancer, for LD, DPH and IPH, respectively. $AUCs_{0-10h}$ of DPH calculated by the trapezoidal method were 161.1 and 20.7 μg/ml/h with and without S-318. $AUCs_{0-24h}$ of LD and IPH were 3.61 and 25.1 μg/ml/h with S-318 and 0.32 and 3.01 μg/ml/h without S-318. These differences of *AUC*s (area under the blood concentration–time curve) were about 10-fold between with and without S-318.

Radioactivities in several tissues at the end of the experiments (24 h for LD and IPH, 10 h for DPH) are shown in Table II. The radioactivities in the kidney, spleen, lung, liver and heart of the CAA and its metabolites after administration with S-318 were markedly higher than in the case without S-318.

**Effect of S-318 on the Pharmacological Action of DPH**

As shown in Fig. 5, the mean blood pressure began to decrease at 2 h after dermal application of HPC aqueous gel (2 g) containing 500 mg of DPH and 100 mg of S-318. The mean blood pressure was decreased from about 150 to 50 mmHg 5 h after administration. The heart rate increased by about 180 beats/min (290 at 0 h to 470 beats/min at 4 h) during 3 to 5 h after administration of DPH with S-318. These pharmacological effects were greater than those in the absence of S-318. The diastolic pressure decreased from about 130 to 25 mmHg from 1.5 h to 5 h with S-318. These phenomena are characteristic of DPH action. The systolic pressure with and without S-318 showed no difference.

## Conclusion

S-318 was emulsified in water. This emulsion was relatively stable at concentrations of S-318 less than 10%. It is evident from Figs. 2 and 3 that a 3—5% concentration of the enhancer was enough to achieve improved skin delivery of LD. S-318 has already been used as a formulation additive and has been proved to be safe. The results obtained in the present study suggest that S-318 is a potential candidate to enhance the transdermal absorption of water-soluble drugs. HPC gel containing S-318, which was used for the *in vivo* experiments, retained the high penetration enhancing effect of S-318 emulsion. The gel may be a prototype of a formulation containing lipid-soluble enhancers such as S-318. LD, DPH and IPH are unstable to high temperature, light and high pH (>8). Nevertheless, adequate pharmacological action of DPH after application as HPC gel (Fig. 5) was obtained, although the possibility that some degradation of DPH occurred can not be ruled out.

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