Effect of Several Hydrophilic Polymers on the Permeation of Morphine and Salicylic Acid through Excised Hairless Rat Skin

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Several hydrophilic polymers changed the cumulative amount of morphine (MOR) permeated through excised hairless rat skin from 1% MOR hydrochloride solution containing ethanol and *l*-menthol at concentrations of 40% and 5%, respectively, as permeation enhancers. Anionic polymers (carboxyvinylpolymer and methylvinylether–maleic anhydride copolymer) in the test solutions decreased the skin permeation of MOR, whereas cationic polymers (polyethyleneimine and chitosan) increased it, compared with that without polymers. Little change, however, was observed by the addition of nonionic polymers (hydroxypropylcellulose and polyethyleneoxide). On the other hand, the cationic and anionic polymers in the test solutions decreased and increased, respectively, the skin permeation of salicylic acid (SA) from the same enhancing system containing sodium salicylate. These opposite results were probably caused by the change in escaping tendency of the drugs from the vehicles, which was due to the drug-polymer interaction. (The escaping tendency has a great effect on the drug partition from the polymer solution to the skin barrier). The effect of hydrophilic polymers on the partition was then evaluated by Donnan membrane theory. The partition of MOR was increased and decreased by the presence of polymers having identical and opposite charge to MOR. The low partition of the drugs to skin may also be caused by low diffusion of the drugs in the polymer solutions. The drug release from the hydrophilic polymer solutions was then measured, and the release rate was found to have decreased in the presence of polymers having opposite charge to MOR and SA. It is suggested that these drug–polymer interactions changed the drug partition to skin thus changing the skin permeation of the drug.

Key words skin permeation; morphine; hydrophilic polymer; enhancer; interaction; charge

A transdermal drug delivery system (TDDS) has many benefits of clinical drug therapy: *i.e.* simple application without pain, avoidance of the hepatic first-pass effect, maintaining effective blood level of a drug, and easy termination of drug input by simple removal. Hydrophilic polymers are generally utilized as preparation materials for TDDS. Polymers as a component in a drug reservoir of TDDS sometimes form a polymer–polymer network in a drug reservoir by covalent, coordinate, ionic or hydrogen bonds, so that adequate viscosity of the drug reservoir can be maintained. Drug molecules are dispersed and/or dissolved in a microspace of the polymer network.[1] They passively diffuse across the polymer matrix to release (distribute) and penetrate into the skin. Such polymers could affect the drug diffusion across a vehicle medium.[2,3] They probably also affect the drug partition into skin,[4–6] in a case where skin is not a big barrier compared to vehicle. Thus, one should select a suitable polymer by considering the physicochemical characteristics of a drug in the formulation design of TDDS.

We have reported that the skin permeation of morphine (MOR), a basic compound, was greatly enhanced by a mixed system of *l*-menthol, ethanol and water.[7–11] MOR-TDDS is believed useful for pain control in terminal cancer patients,[12,13] however, few investigations have been done on the formulation design of MOR-TDDS containing a potential penetration-enhancing system. Polyacrylic acid analogs are utilized as a main vehicle material in water soluble preparations. Recently, gel and/or cataplasm which consists of polyacrylic acid containing a nonsteroidal anti-inflammatory drug,[14,15] such as indomethacin, flurbiprofen or ketoprofen, is utilized for clinical therapy. These drugs are acidic compounds having the identical negative charge to the polyacrylic acid analog. On the contrary, since MOR is a basic drug with a positive charge, it may interact with the polyacrylic acid analogs. In the present study, we selected several hydrophilic polymers and their effects on the skin permeation of MOR were evaluated using *in vitro* skin permeation techniques. Polyethyleneimine and chitosan as cationic polymers, carboxyvinylpolymer and methylvinylether–maleic anhydride copolymer as anionic polymers, and hydroxypropylcellulose and polyethyleneoxide as nonionic polymers were utilized in this experiment. The skin permeation experiment was done using excised hairless rat skin, with donor solution containing 1% MOR, 40% ethanol, 5% *l*-menthol as penetration enhancers and 1—3% of each polymer. Salicylic acid (SA) as an acidic compound, was also used as a comparative drug having an opposite charge to MOR.

Experimental

Materials MOR hydrochloride and naloxone hydrochloride were purchased from Takeda Pharmaceutical Industries Co., Ltd. (Osaka, Japan) and Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.), respectively. Sodium salicylate, hydroxypropylcellulose (HPC, HPC-M, M.W. 130000), polyethyleneimine (PEI, 30% polyethyleneimine P-70 solution, M.W. 70000) and chitosan (CHI, chitosan 1000, M.W. 200000) were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Polyethyleneoxide (PEO, Alkox® E45, M.W. 700000), carboxyvinylpolymer (CVP, Carbopol® 934, M.W. 3000000) and methylvinylether–maleic anhydride copolymer (MEM, Gantrez® AN-169, M.W. 67000) were supplied by Meisei Chemical Industries Co., Ltd. (Kyoto, Japan), BF Goodrich Co., Ltd. (Cleveland, OH, U.S.A.) and Gokyo Sangyo Co., Ltd. (Osaka, Japan), respectively. Other reagents were reagent-grade or HPLC-grade products.

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Preparation of Test Solutions Containing Drug and Hydrophilic Polymer One kind of hydrophilic polymer was mixed with 1% MOR hydrochloride or sodium salicylate, 5% *l*-menthol and 40% ethanol in water. The resulting solution was adjusted to pH 6 with adequate amount of acetic acid, L-lactic acid or triethylamine. The concentration of the hydrophilic polymer was 3%, except that of CHI and CVP (1%) (their 3% solutions were not suitable for these tests due to their high viscosity). Table 1 shows the viscosity of the test solutions. These solutions were used for skin permeation and release experiments.

***In Vitro* Skin Permeation Experiment** A vertical type diffusion cell (effective diffusion area, 0.95 cm^2) with a water jacket was used for the *in vitro* skin permeation experiment. The water jacket was connected to a water bath at 37 °C. Hairless rat skin was excised from the abdomen of male HBA/ILA-Ht rats (150—180 g, Ishikawa Laboratory Animals, Saitama, Japan) under i.p. anesthesia of 50 mg/kg of sodium pentobarbital (Somnopentyl,® Pitman Moore, Inc., IL, U.S.A.). The obtained skin piece was immediately mounted on the receiver cell. Test solution (0.2 g/cm^2, 0.22 ml/cm^2) was applied to the donor compartment (stratum corneum side), and the receiver compartment (dermal side) was filled with distilled water (4.4 ml). At predetermined time intervals, samples (0.5 ml) were withdrawn from the receiver compartment for assay and the same volume of fresh distilled water was added to keep the volume constant. A sink condition was always maintained in this compartment.

Equilibrium Experiment using Dialysis Membrane A dialysis membrane (pore size, 24 Å, Sanko Pure Chemical Co., Tokyo) was mounted between the two half cells of a 2-chamber diffusion cell set with a water jacket connected to a water bath at 37 °C, each cell having 0.95 cm^2 effective diffusion area. One cell was filled with 2.8 ml of 10 μg/ml MOR or SA containing 10 μg/ml hydrophilic polymer, and the other cell was filled with the same volume of the drug solution without polymer. Samples (0.5 ml) were withdrawn from both cells after achieving equilibrium condition.

***In Vitro* Release Experiment** A larger vertical type diffusion cell (effective diffusion area, 3.14 cm^2) was used for the release experiment. A cellulose acetate membrane (pore size 0.2 μm, Advantec Toyo Co., Ltd. Tokyo) was mounted between the donor and receiver compartments. Each test solution (0.2 g/cm^2, 0.22 ml/cm^2) was applied to the donor compartment, and the receiver compartment was filled with 20 ml of distilled water. Each sample (0.5 ml) was withdrawn from the receiver compartment at predetermined times.

Assay MOR was assayed by HPLC (LC-Module I, Waters, MA, U.S.A.) with a LiChrospher 100 RP-18(e) 5 μm column (4.0 i.d. × 250 mm, Kanto Chemical Co., Tokyo). Conditions were: mobile phase, 0.1% phosphoric acid–acetonitrile containing 5 mM sodium dodecylsulfate (65 : 35); flow rate, 1.0 ml/min; column temperature, 40 °C; detection, UV 230 nm. Each sample (200 μl) was added to 200 μl of methanol containing naloxone hydrochloride as an internal standard. After the mixture was centrifuged at 15000 rpm for 5 min, the supernatant (20 μl) was injected to HPLC. SA was also determined by HPLC (LC-9A, Shimadzu Seisakusyo Co.), with a 4.6 i.d. × 150 mm MCM column (type C18 DF-5-120A, MC Medical Co., Tokyo). A mixture of 0.2% phosphoric acid–acetonitrile (75 : 25) was used as a mobile phase, and elution was at 1.0 ml/min and 40 °C. Each sample (200 μl) was added to the same volume of acetonitrile containing *p*-ethylhydroxybenzoate. Following centrifugation, the supernatant (20 μl) was injected to HPLC. MOR was calculated as morphine hydrochloride, and SA was calculated as sodium salicylate.

Results and Discussion

The influence of hydrophilic polymers on the skin permeation of MOR was investigated. Figure 1a shows the *in vitro* permeation profiles across excised hairless rat skin. No pseudo-steady state flux was obtained and the cumulative amount of MOR permeated over 10 h (Q%-10) without hydrophilic polymers (control) was 73.6 ± 6.0%, suggesting that the system consisting of *l*-menthol, ethanol and water had a high penetration-enhancing effect and that the skin was no longer a large barrier. In the presence of CVP or MEM as an anionic polymer, Q%-10 value was only 24.5 ± 0.9 or 8.4 ± 0.4%, respectively, although a great deal of MOR still remained in the donor solution at 10 h. Further, the maximum permeation rate of MOR in the presence of CVP or MEM was 1/2 or 1/5, respectively, in comparison with that without hydrophilic polymers. On the other hand, when PEI or CHI was used as a cationic polymer, Q%-10 was 97.4 ± 0.8 or 100.7 ± 0.9%, respectively, significantly higher than the control. The maximum permeation rate of MOR with PEI or CHI was 1.5 times higher than the control. Q%-10 of HPC or PEO as a nonionic polymer was similar to the control. The time courses of the MOR permeation were also equivalent.

These results suggested that the skin permeation of MOR was suppressed and increased dramatically by anionic polymers (CVP, MEM) and cationic ones (PEI, CHI), respectively, in comparison with the control. The change in the skin permeation of MOR could be related to electrostatic interaction between carboxyl groups of the polymers and positive charge of MOR in the vehicle. We have already reported that drug molecules having secondary amido groups interacted with acrylic adhesive having carboxylic groups, so that the drug release from the adhesive matrices was influenced by the interaction.[2,3]

In general, an increase in skin permeation of a drug is

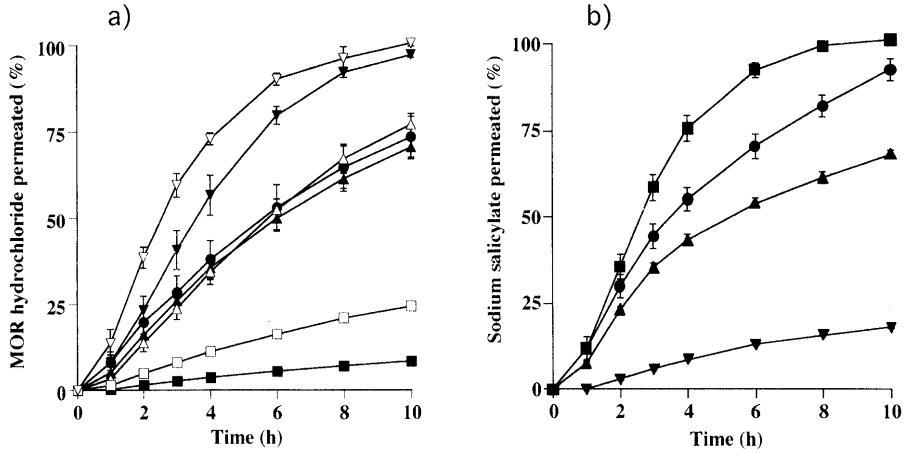

Fig. 1. Effect of Polymers on the Permeation of a) MOR Hydrochloride and b) Sodium Salicylate through Hairless Rat Skin

Each point represents the mean ± S.E. of 3 experiments. ●, without polymer; ▲, 3% PEO; △, 3% HPC; ■, 3% MEM; □, 1% CVP; ▼, 3% PEI; ▽, 1% CHI.

related to its escaping tendency from a vehicle. The escaping tendency has a strong effect on the drug partition to skin. T. Higuchi *et al.* studied the escaping tendency of salicylate from sodium carmellose solution using semi-permeable membrane.[16] Such a membrane equilibrium experiment based on the Donnan membrane theory was carried out to understand the present phenomenon. Skin permeation barriers generally consist of lipid rich domain as a lipid pathway and water rich domain as a pore pathway,[17] and each contribution to the drug permeation is dependent upon the physicochemical properties of a drug and/or the polarity of an application solvent. Since a strong enhancer system of *l*-menthol, ethanol and water was used in this experiment, a new pore domain or a new aqueous domain may be formed by the enhancer.[18] As a result, the theory can be applied also to the enhancer-treated skin. In the treated skin, hydrophilic polymers act as a fixed charge in the drug–donor compartment, whereas low molecular electrolytes easily migrate in both directions across the membrane to maintain electrical equivalence in the system.

The concentration ratio across the dialysis membrane, C2/C1 was calculated to be 1.17 ± 0.02 (mean $\pm$ S.D.) when using PEI, where C1 and C2 are MOR concentration equilibrated in the drug-donor compartment containing hydrophilic polymer and in the drug-receptor compartment, respectively. Thus, MOR moved from the polymer containing side to the polymer free side, suggesting that MOR partition into skin may be increased. On the other hand, C2/C1 was 0.93 ± 0.04 using CVP, and this is the reason why MOR permeation through skin was decreased. These results for SA could not be explained like those for MOR. Dimer complexation of SA may influence this.

It has been mentioned that the present skin was no longer a large barrier by the strong enhancer system. In such a case, drug diffusion through the vehicle more or less affects the skin permeation. If the diffusion through the vehicle is low, the drug concentration at the vehicle–skin boundary should be lowered to decrease the drug partition into skin. In the present experiment, MOR release was decreased by anionic polymers (Fig. 2a). The decrease in the skin permeation of MOR by anionic polymers (Fig. 1a) is explainable in this manner, probably owing to ion-pair formation between MOR and polymer molecules.

Similar skin permeation and release results for SA are shown in Figs. 1b and 2b. Decrease in the skin permeation of SA by cationic polymers having an opposite charge to SA can be explained as above. Furthermore, the permeability of MOR and SA was not influenced by nonionic polymers (PEO, HPC).

The decrease in the release rate of MOR and SA by hydrophilic polymers in comparison with the control (Fig. 2) was due to high viscosity of the vehicle, drug–polymer interaction and low diffusivity of the drugs through the vehicles. The viscosity of 4% PEO was five times higher than that of 3% PEO and approximately equal to that of 1% CHI (Table 1). The cumulative amount of MOR released over 3 h with 4% PEO ($72.6 \pm 0.7\%$) was lower than that with 3% PEO ($92.6 \pm 0.9\%$). That with 1% CHI ($82.0 \pm 2.2\%$) was higher than that with 4% PEO in spite of a similar viscosity. The MOR release with CHI was thus affected not only by the viscosity of the vehicle but also by electrostatic repulsion. The release behavior of SA with PEI or MEM was also related to the vehicle viscosity. However, in the case of MEM, besides the viscosity and electrostatic interaction, the other factors may be involved. MEM was reported to form ester or

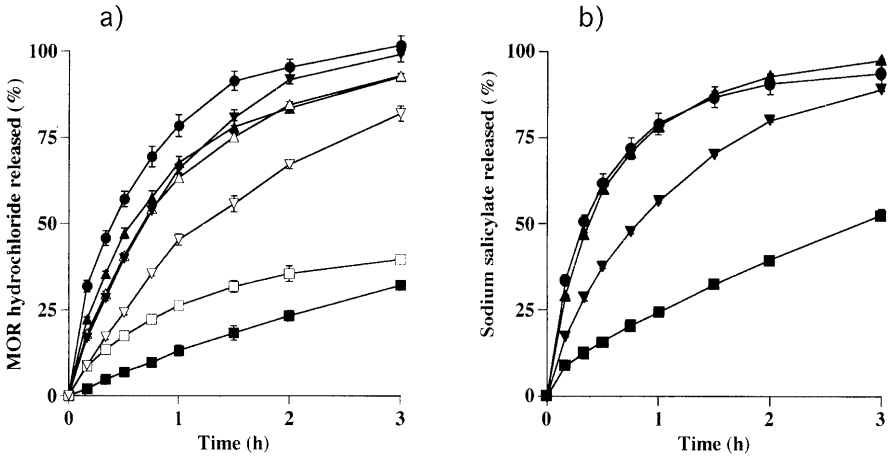

Fig. 2. Effect of Polymers on the Release of a) MOR Hydrochloride and b) Sodium Salicylate

Each point represents the mean $\pm$ S.E. of 3 experiments. ●, without polymer; ▲, 3% PEO; △, 3% HPC; ■, 3% MEM; □, 1% CVP; ▼, 3% PEI; ▽, 1% CHI.

Table 1. Viscosity of the Test Solutions[a]

	Without polymer	Anion		Nonion			Cation	
		3% MEM	1% CVP	3% PEO	4% PEO	3% HPC	3% PEI	1% CHI
Viscosity (cps)	1.8	245.8	58.8	209.4	1118.0	100.0	7.2	1145.0

a) The viscosity of the test solutions was measured using a rotary viscometer system (Rotovisco RV 100 Measuring System CV 100, Haake Inc., Germany).

amide by alcohols, amine and other drugs,[19-23] so that it may react with SA having hydroxyl group. Thus, the release rate of SA was decreased by MEM.

The hydrophilic polymers obviously affected the skin permeation of MOR and SA. This permeation increase in the presence of cationic and anionic polymers, respectively, is related to the escaping tendency from the vehicle and the partition into the skin of the drugs. The kind and concentration of polymer may change the skin permeation of a drug. The present results are very useful for designing TDDS preparations of ionic drugs.

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