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COMMUNICATIONS

Improved transdermal delivery of prostaglandin E_1 through hairless mouse skin: combined use of carboxymethyl-ethyl-β-cyclodextrin and penetration enhancers

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Abstract—The optimal prescription of transdermal preparations of prostaglandin E_1 (PGE_1) for treatment of peripheral vascular diseases has been investigated. The chemical stability of PGE_1 in fatty alcohol/propylene glycol (FAPG) ointment was markedly improved by carboxymethyl-ethyl-β-cyclodextrin (CME-β-CyD). Application of a PGE_1 ointment containing the penetration enhancer, 1-dodecylazacycloheptane-2-one (Azone) or 1-[2-(decylthio)ethyl]azacyclopentane-2-one (HPE-101), onto the skin of hairless mice showed the increase of blood flow in the skin due to the vasodilating action of PGE_1. In particular, the ointment containing a PGE_1-CME-β-CyD complex supplemented with HPE-101 showed the most prominent increase of the blood flow. Compared with other ointments, this ointment was found to show significantly greater transfer of HPE-101 into in-vitro preparations of the skin of hairless mice. Transfer of PGE_1 into the skin was thought to be facilitated by this increased transfer of HPE-101. These results suggest that a combination of CME-β-CyD and HPE-101 is useful for designing PGE_1 ointments for topical application with good chemical stability and percutaneous permeability.

Since prostaglandin E_1 (PGE_1) has strong vasodilating and platelet anti-aggregating actions, PGE_1 preparations for injection are clinically used primarily for treatment of peripheral vascular diseases (Mizushima et al 1983). To avoid systemic side effects of PGE_1, however, topical application of an external preparation is desirable. PGE_1 is very unstable chemically and shows a poor percutaneous absorption (Yamamura & Yotsuyanagi 1984; Watkinson et al 1990). Thus, a formulation capable of overcoming these problems has to be devised to realize transdermal preparations of PGE_1.

We have previously reported (Adachi et al 1990) that carboxymethyl-ethyl-β-cyclodextrin (CME-β-CyD), a derivative of β-cyclodextrin (β-CyD), in which an ethyl group and carboxymethyl group are introduced to the hydroxyl groups of β-CyD (Uekama et al 1989), markedly improved the chemical stability of PGE_1 in aqueous solution and ointment. In the present study, using hairless mice, we investigated the combination effect of β-CyDs and penetration enhancers on the chemical stability of PGE_1 in fatty alcohol/propylene glycol (FAPG) ointment and on percutaneous absorption of PGE_1. Changes in blood flow in the skin induced by the vasodilating action of PGE_1 after topical application of PGE_1-containing ointment were followed by laser-Doppler velocimetry (Poelman et al 1989). As penetration enhancers, 1-dodecylazacycloheptane-2-one (Azone) and 1-[2-(decylthio)ethyl]azacyclopentane-2-one (HPE-101) were used. HPE-101 is a novel azacycloalkane derivative and known to have a greater solubility in water and less cutaneous irritancy than Azone (Saita et al 1989).

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Materials and methods

PGE_1 was purchased from Fuji Chemical Ind. (Toyama, Japan), [^{3}H]PGE_1 (100 μCi mL^{-1}) was from New England Nuclear (DE, USA), HPE-101 was from Hisamitsu Pharmaceutical Co. (Saga, Japan), and Azone was from Nelson Research (CA, USA). β-CyD and CME-β-CyD were donated by Nihon Shokuhin Kako Co. (Tokyo, Japan) and Wako Pure Chemical Ind. (Osaka, Japan), respectively. The solid complex of PGE_1 and β-CyDs (1:5 in molar ratio) used was prepared by the kneading method (Tsuruoka et al 1981). The FAPG ointment base was employed here based on results of our preliminary studies on the stability and percutaneous absorption of PGE_1 (data not shown). PGE_1 ointments were prepared by mixing PGE_1 or its β-CyD complex (0·01% w/w for PGE_1) and a penetration enhancer (3% w/w) with FAPG base. PGE_1 was completely dissolved in all the systems. The pH of ointments was estimated by measuring the pH of 20% w/v aqueous suspension of the ointments at 25°C.

Each PGE_1 ointment (5 g) was packed in an aluminium tube with the inside coated with phenol resin, and the stability of PGE_1 was tested at 40°C and 75% relative humidity. The intact PGE_1 in the ointment was extracted with 0·01 M KH_2PO_4–acetonitrile (3:2), and assayed by HPLC under the following conditions: pump, Hitachi L-6000 (Hitachi, Tokyo, Japan); detector, Hitachi L-4000 (201 nm, Hitachi, Tokyo, Japan); column, TSK-GEL ODS-120T (5 μm, 4·6 cm × 150 mm, Toyo Soda, Tokyo, Japan) and mobile phase, 0·01 M KH_2PO_4–acetonitrile (3:2).

Female hairless mice, about 9 weeks old, were used. The animals were anaesthetized by intraperitoneal injection of 6 mL kg^{-1} of physiological saline containing 25% urethane, then 10 mg of each ointment was topically applied onto a 10 × 10 mm area of the back skin, and the area was covered with Saran Wrap (Asahi Kasei Kogyo Co., Tokyo, Japan). The blood flow in the skin was measured by a blood perfusion monitor (TSI, Laserflo BPM403A, MN, USA).

For in-vitro experiments to measure percutaneous penetration, a skin sheet isolated from the back of hairless mice was attached to a Loveday-type diffusion cell (Loveday 1961). Ten mg of PGE_1 ointment containing 0·25 μCi [^{3}H]PGE_1 (a tracer) was applied onto the stratum corneum of the isolated skin, and the transfer of PGE_1 to the skin and the permeation to the receptor phase (physiological saline 9 mL, 25°C) were examined. Sink conditions for PGE_1 were maintained throughout this study. Radioactivity due to [^{3}H]PGE_1 in the ointment, the skin, and the receptor phase was counted with a liquid scintillation counter (LSC-3500, Aloka, Tokyo, Japan). Azone and HPE-101 were assayed by HPLC under the following conditions: pump, Hitachi L-6000; detector, Shodex SE-51 (Showa Denko, Tokyo,

Japan); column, μ Bondapak C_{18} (10 μm, 3·9 cm × 300 mm, Waters, Tokyo, Japan), and mobile phase, water–methanol (15:85).

Results and discussion

Effects of β-CyDs on the chemical stability of PGE_1 in FAPG ointment are shown in Fig. 1. The stability of PGE_1 was slightly improved by addition of Azone and HPE-101 to the ointment, and markedly increased by addition of CME-β-CyD. When the PGE_1-CME-β-CyD complex was mixed with the FAPG base, the pH of the ointment dropped to 4·17, probably due to the acidity of the carboxymethyl groups in CME-β-CyD (pK_a about 4 (Uekama et al 1989)). PGE_1 is known to be most stable under the weak acidic condition of about pH 4 (Monkhouse et al 1973). Thus, in addition to stabilization by inclusion of PGE_1 into the hydrophobic cavity of CME-β-CyD molecules, the weak acidic environment produced by CME-β-CyD was thought to contribute to stabilization of PGE_1.

Fig. 2 shows changes with time in blood flow in the skin following topical application of FAPG ointments containing PGE_1, β-CyD complexes or enhancers. Under our experimental conditions, the blood flow was not significantly changed by PGE_1 or β-CyD complexes (containing 1 μg PGE_1) when enhancer-free ointments were used. On the other hand, addition of Azone or HPE-101 to PGE_1 ointment showed marked increase in blood flow in the skin. Since the ointment base or the enhancer itself did not increase the blood flow, it was inferred that the blood flow was increased by enhancer-induced facilitation of transfer of PGE_1 to the skin. Particularly in the presence of HPE-101, the ointment containing a PGE_1-CME-β-CyD complex greatly increased the blood flow, and, 2 h after topical application, the increase of the blood flow was more than 2-fold of that induced by the ointment containing PGE_1 alone or as the β-CyD complex.

To elucidate the mechanism of enhanced pharmacological effect of PGE_1, effects of enhancers and β-CyDs on transfer of PGE_1 to the skin were compared by in-vitro experiments using isolated back skin of hairless mice. Within 2 h of application, the radioactivity due to [^{3}H]PGE_1 was distributed to the ointment and the skin (Fig. 3), while only a small amount was observed in

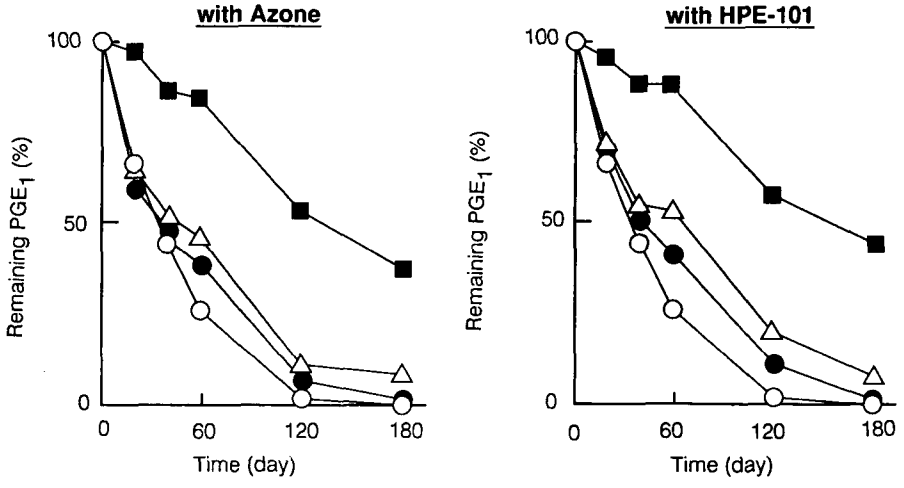

FIG. 1. Chemical stability of PGE_1 in FAPG ointments containing PGE_1, its β-CyD complexes (0·01% w/w for PGE_1), or enhancers (3% w/w). ○ PGE_1 alone, ● PGE_1 with enhancer, △ β-CyD complex with enhancer, ■ CME-β-CyD complex with enhancer. Each value represents the mean of 3 experiments.

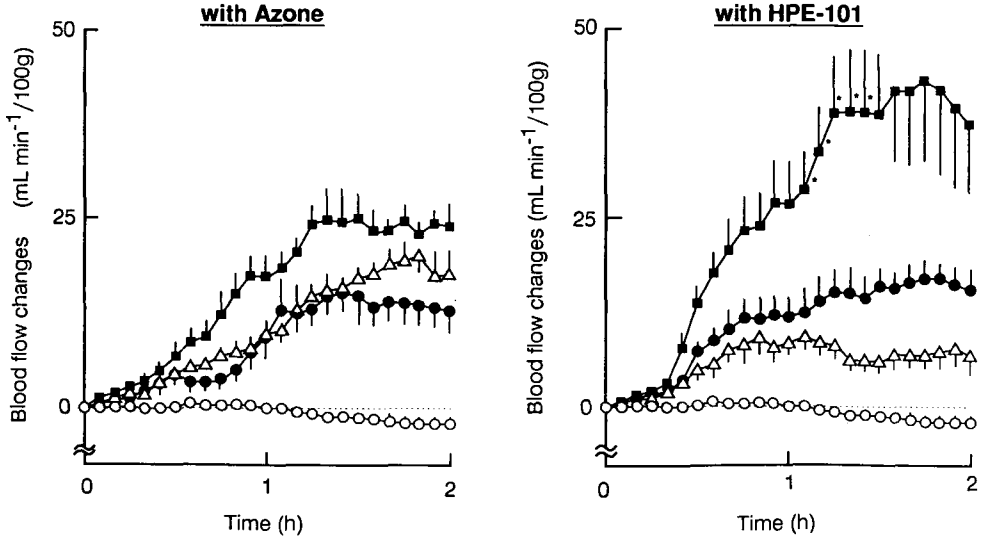

FIG. 2. Changes in blood flow in the skin of hairless mice following topical application of FAPG ointments containing PGE_1, its β-CyD complexes (0·01% w/w for PGE_1), or enhancers (3% w/w). ○ PGE_1 alone, ● PGE_1 with enhancer, △ β-CyD complex with enhancer, ■ CME-β-CyD complex with enhancer. Each value represents the mean ± s.e. of 5 mice. *$P < 0·05$ vs PGE_1 with enhancer.

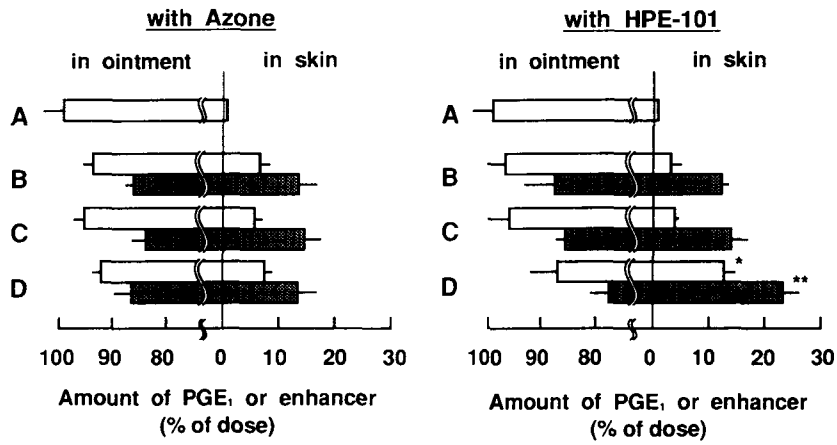

FIG. 3. In-vitro transfer of PGE_1 and enhancers from FAPG ointment base to the skin 2 h after topical application of FAPG ointments containing PGE_1, its β-CyD complexes (0·01% w/w for PGE_1), or enhancers (3% w/w). A PGE_1 alone, B PGE_1 with enhancer, C β-CyD complex with enhancer, D CME-β-CyD complex with enhancer. □ PGE_1, ▨ enhancer. Each value represents the mean ± s.e. of 4 experiments. $*P < 0.05$, $**P < 0.01$ vs PGE_1 with enhancer.

the receptor phase (less than 0·05% of the dose). The transfer of PGE_1 from the ointment base to the skin was greatly increased by addition of Azone or HPE-101. In the ointment containing the PGE_1-CME-β-CyD complex and HPE-101, transfer of PGE_1 to the skin was increased to about 3-fold of that in the ointment containing PGE_1 alone or as the PGE_1-β-CyD complex. On the other hand, no noticeable synergistic effect of Azone with β-CyDs on the transfer of PGE_1 to the skin was observed.

The in-vitro release rate of PGE_1 from the ointment base was decreased by addition of HPE-101 to the base, suggesting the reduced thermodynamic activity of PGE_1 in the ointment. Furthermore, complexation decreased the release rate of PGE_1 from the base containing HPE-101 in the order of PGE_1 alone > PGE_1-β-CyD complex > PGE_1-CME-β-CyD complex. Such negative effects were well compensated for by an increase in partition of PGE_1 to the skin afforded by HPE-101. The rate limiting step in transfer of PGE_1 to the skin was inferred not to be in diffusion processes in the ointment but rather in partition processes to the skin.

HPE-101 is thought to have a similar penetration enhancing action to that of Azone (Saita et al 1989), and the amount of these enhancers transferred to the skin is known to show a good correlation with its penetration enhancing effect. Thus, the transfer of enhancers from ointment bases to the skin was examined. As compared with the ointment containing PGE_1 alone or as its β-CyD complex, the ointment containing the CME-β-CyD complex showed significantly greater transfer of HPE-101 to the skin, while both β-CyDs did not affect the transfer of Azone to the skin (Fig. 3). Many investigators have pointed out that an appropriate ointment base and a solvent must be selected for a penetration enhancer to fully exert its effect (Goodman & Barry 1989; Sasaki et al 1990; Okamoto et al 1990). Propylene glycol has been shown to enhance the ability of Azone to promote cutaneous drug permeation (Wotton et al 1985). The FAPG base contains a large quantity of propylene glycol (70% w/w), which may increase the ability of Azone or HPE-101 to deliver PGE_1 to the skin. Furthermore, the penetration enhancing effect of HPE-101 on PGE_1, was inferred to be exerted through facilitated transfer of HPE-101 induced by CME-β-CyD.

CME-β-CyD improved the stability of PGE_1 in FAPG ointment base, and also facilitated the percutaneous absorption of PGE_1 when it was combined with HPE-101. These results suggest the usefulness of CME-β-CyD in the design of PGE_1 preparations for external application.

References

Adachi, H., Fujimaru, Y., Arima, H., Irie, T., Hirayama, F., Uekama, K., Yano, T., Saita, M., Noda, K. (1990) Possible use of carboxymethyl-ethyl-β-cyclodextrin in the transdermal delivery of prostaglandin E_1. Ther. Res. 11: 649–650

Goodman, M., Barry, B. W. (1989) Lipid-protein-partitioning (LPP) theory of skin enhancer activity: finite dose technique. Int. J. Pharm. 57: 29–40

Loveday, D. E. (1961) An in vitro method for studying percutaneous absorption. J. Soc. Cosm. Chem. 12: 224–239

Mizushima, Y., Yanagawa, A., Hoshi, K. (1983) Prostaglandin E_1 is more effective, when incorporated in lipid microspheres, for treatment of peripheral vascular diseases in man. J. Pharm. Pharmacol. 35: 666–667

Monkhouse, D. C., Van Campen, L., Aguiar, A. J. (1973) Kinetics of dehydration and isomerization of prostaglandins E_1 and E_2. J. Pharm. Sci. 62: 576–580

Okamoto, H., Muta, K., Hashida, M., Sezaki, H. (1990) Percutaneous penetration of acyclovir through excised hairless mouse and rat skin: effect of vehicle and percutaneous penetration enhancer. Pharm. Res. 7: 64–68

Poelman, M. C., Piot, B., Guyon, F., Deroni, M., Leveque, J. L. (1989) Assessment of topical non-steroidal anti-inflammatory drugs. J. Pharm. Pharmacol. 41: 720–722

Saita, M., Shimozono, Y., Sakai, M., Okayama, A., Furuta, K., Tsuji, M. (1989) Evaluation of a new penetration enhancer HPE-101 (3): skin permeation and irritation. Ther. Res. 10: 971

Sasaki, H., Kojima, M., Nakamura, J., Shibasaki, J. (1990) Enhancing effect of combining two pyrrolidone vehicles on transdermal drug delivery. J. Pharm. Pharmacol. 42: 196–199

Tsuruoka, M., Hashimoto, T., Seo, H., Ichimasa, S., Ueno, O., Fujinaga, T., Otagiri, M., Uekama, K. (1981) Enhanced bioavailability of phenytoin by β-cyclodextrin complexation. Yakugaku Zasshi 101: 360–367

Uekama, K., Horiuchi, Y., Irie, T., Hirayama, F. (1989) *O*-Carboxymethyl-*O*-ethylcyclomaltoheptaose as a delayed-release-type drug carrier: improvement of the oral bioavailability of diltiazem in the dog. Carbohydr. Res. 192: 323–330

Watkinson, A. C., Hadgraft, J., Bye, A. (1990) Enhanced penetration of prostaglandin E_2 through human skin in-vitro. J. Pharm. Pharmcol. 42 (Suppl.): 86P

Wotton, P. K., Møllgaard, B., Hadgraft, J., Hoelgaard, A. (1985) Vehicle effect on topical drug delivery. III. Effect of Azone on the cutaneous permeation of metronidazole and propylene glycol. Int. J. Pharm. 24: 19–26

Yamamura, K., Yotsuyanagi, T. (1984) High-performance liquid chromatographic assay for prostaglandin E_1 in various ointment vehicles: separation and stability testing. J. Chromatogr. 303: 165–172