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Effect of 2-Hydroxypropyl-β-cyclodextrin on Percutaneous Absorption of Methyl Paraben

MUNEO TANAKA, YUHEI IWATA, YUICHI KOUZUKI, KAZUYO TANIGUCHI, HAJIME MATSUDA, HIDETOSHI ARIMA* AND SEISHI TSUCHIYA*

*Research Center, Shiseido Ltd, 1050 Nippa-cho, Kohoku-ku, Yokohama 223, and *School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1 Horinouchi, Hachioji, Tokyo 192–03, Japan*

Abstract

A potential use of 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) to solubilize methyl paraben and to suppress its percutaneous absorption was examined, and compared with nonionic surfactant HCO-60.

HP-β-CyD significantly increased the solubility of methyl paraben in water, where the apparent 1 : 1 stability constant of the soluble complex was determined to be 2150 M^{-1}. The in-vitro cutaneous permeability of methyl paraben through an isolated skin of hairless mouse was suppressed by HP-β-CyD, thus promoting the bioconversion of methyl paraben to the less toxic metabolite, *p*-hydroxybenzoic acid (p-HBA) in the epidermis. These effects of HP-β-CyD were greater than those of HCO-60. HP-β-CyD (2% w/v) reduced the in-vivo percutaneous absorption of methyl paraben by 66% 24 h after the topical application of a solution containing [^{14}C]methyl paraben to hairless mouse skin. Additionally, the percutaneous absorption of [^{14}C]HP-β-CyD was confirmed to be extremely low.

These results suggest that HP-β-CyD is useful in liquid preparations of methyl paraben for topical application.

p-Hydroxybenzoic acid esters (parabens) have an antibacterial function and they are widely used as preservatives for pharmaceutical preparations, cosmetics and foods. However, practical use of parabens in liquid preparations has been limited because of their low solubility in water, hence requiring a solubilizing agent such as a surfactant. In addition, from the viewpoint of safety, it is desirable to lower the percutaneous absorption of parabens after application onto skin.

Cyclodextrins (CyDs) can form inclusion complexes with various guest molecules (Saenger 1980). The low aqueous solubility of natural CyDs, especially β-CyD, however, has restricted its range of applications. To improve the solubility, chemical modification including alkylation and hydroxyalkylation of the hydroxyl group of β-CyD has been used (Szejtli 1983; Pitha & Pitha 1985). Of the modified CyDs evaluated, 2-hydroxypropyl-β-CyD (HP-β-CyD) has high solubility (> 50%) in water, and is expected to be useful as a parenteral drug carrier because of its excellent safety (Carpenter et al 1987; Yoshida et al 1988).

Lehner et al (1993, 1994) have reported that the antimicrobial activity of parabens against several microorganisms was reduced by complex formation with HP-β-CyD. The loss in activity of parabens afforded by HP-β-CyD correlated with the complexed fraction of the preservatives.

The present study deals with the effects of HP-β-CyD on the solubility of methyl paraben, a representative compound of parabens, on the in-vitro cutaneous permeability of methyl paraben, and on the bioconversion of methyl paraben to *p*-hydroxybenzoic acid (p-HBA), a less toxic metabolite, by using isolated skin of hairless mouse. These effects were compared with those of the nonionic surfactant, polyoxyethylene hydrogenated castor oil 60EO (HCO-60). In addition, the ^{14}C-radiolabelled HP-β-CyD ([^{14}C]HP-β-CyD) and methyl paraben ([^{14}C]methyl paraben) were employed to investigate the effects of HP-β-CyD on in-vivo percutaneous absorption of methyl paraben after application of [^{14}C]methyl paraben solution onto the dorsal skin area of a hairless mouse.

Correspondence: S. Tsuchiya, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1 Horinouchi, Hachioji, Tokyo 192–03, Japan.

Materials and Methods

Materials

Methyl paraben was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). [^{14}C]Methyl paraben (sp. act. 2·4 MBq mg^{-1}) was obtained by esterification of [^{14}C]p-HBA (New England Nuclear Co., MA, USA) using diazomethane. HP-β-CyD was donated by Nihon Shokuhin Kako Ltd (Shizuoka, Japan); the average substitution degree of 2-hydroxypropyl groups per β-CyD molecule was determined to be 4·8 by ^{1}H NMR (Pitha et al 1986). [^{14}C]HP-β-CyD (sp. act. 257 kBq mg^{-1}) was obtained from Pharmatec Inc. (FL, USA). All other chemicals and solvents were of analytical reagent grade.

Solubility measurements

A constant but excess amount of methyl paraben was added to an aqueous solution containing a given concentration of HP-β-CyD. These were mixed by a magnetic stirrer at 20°C, and after equilibrium was attained (about 12 h) the mixture was centrifuged at 3000 *g* for 5 min and the supernatant was filtered through a pipette with a cotton plug. After diluting the filtrate, methyl paraben was assayed by high-performance liquid chromatography (HPLC), under the

following conditions using a JASCO 860-CO HPLC apparatus (Tokyo, Japan): column, CAPCELL-PAK C18 SG 4·6 × 250 mm (Shiseido, Tokyo, Japan); detection, UV at 256 nm; mobile phase, water : methanol = 1 : 1 (v/v) with 0·1% phosphoric acid; injection, 10 μL; flow rate, 1·0 mL min^{-1}.

Determination of stability constant

The phase solubility diagram was prepared by the method of Higuchi & Connors (1965), and an apparent 1 : 1 stability constant (K_c) for the methyl paraben:HP-β-CyD complex was calculated from the slope and intercept values of the initial straight line portion of the solubility diagram, according to the following equation:

$$K_c = \text{slope}/(\text{intercept}\ (1\text{-slope})) \quad (1)$$

In-vitro cutaneous permeability

The dorsal epidermis of a female hairless mouse (8 weeks old) was attached to a perpendicular diffusion cell (Franz 1975) and the test was performed at 20°C. An aqueous solution of methyl paraben was used on the donor side and physiological saline was used on the receiver side. The concentrations of methyl paraben and HP-β-CyD were 0·2% (w/v) and 2·0% (w/v), respectively. At appropriate intervals, the sample solution (0·8 mL) was withdrawn from the receiver phase. The amounts of methyl paraben and p-HBA were determined by HPLC as described above.

In-vivo percutaneous absorption

One hundred microlitres of 0·2% methyl paraben solution containing [^{14}C]methyl paraben (0·0002% w/v, 480 kBq) in the absence and presence of HP-β-CyD (2·0% w/v) was applied onto the dorsal skin (2 cm^2) of a female hairless mouse (8 weeks old) under sealed conditions using human adhesive test patches (Torii Yakuhin Co., Tokyo, Japan). After the patches had been attached onto the skin for 24 h, the radioactivity in the patches, in the stratum corneum collected by stripping, and in the epidermis and cutis of the skin obtained by peeling off the treated portion, was measured with a liquid scintillation counter (Beckman model LS-1701, Tokyo, Japan) according to the method of Iwata et al (1988). In addition, the amounts of methyl paraben excreted in the urine and faeces and remaining in the body were obtained by driving off the CO_2, collecting and counting, after ashing. Furthermore, the radioactivity excreted by respiration was quantified as [^{14}C]CO_2. To measure the percutaneous absorption of HP-β-CyD in-vivo, 100 μL 2·0% [^{14}C]HP-β-CyD (500 kBq) in the absence and presence of 0·2% methyl paraben was applied in a manner similar to the above experiment. Likewise, the radioactivity excreted in the urine, faeces and respiration and remaining in the body was quantified. Student's *t*-test was used for statistical evaluation of the data, and *P* values of $< 0·05$ were considered to be statistically significant.

Results and Discussion

Solubilization

Fig. 1 shows the phase solubility diagram for methyl paraben and HP-β-CyD in water. The solubility of methyl paraben linearly increased as a function of HP-β-CyD concentration, showing a feature of an A_L type diagram (Higuchi & Connors 1965). The apparent 1 : 1 stability constant (K_c) of the methyl paraben:HP-β-CyD complex was calculated to be 2150 M^{-1}. This solubility behaviour was in sharp contrast to the case of the parent β-CyD, where crystalline complexes precipitated at higher host concentrations (> 0·01 M), showing a typical B_s type solubility curve (Uekama et al 1980).

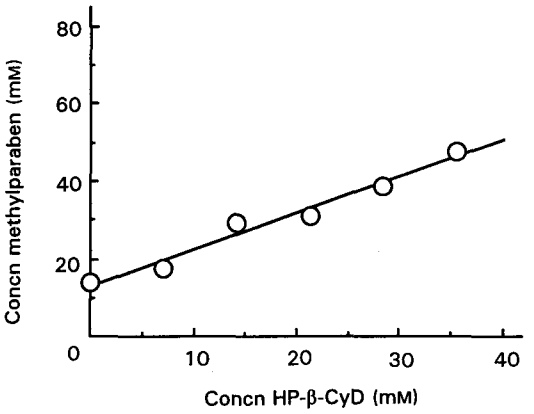

FIG. 1. Phase solubility diagram of methyl paraben:HP-β-CyD system in water at 20°C.

These facts suggest that HP-β-CyD is preferable to β-CyD in liquid preparations because of the formation of highly water-soluble complexes.

In-vitro cutaneous permeability

The effects of HP-β-CyD on the in-vitro permeation of methyl paraben and on the conversion of methyl paraben to p-HBA in hairless mouse skin were examined, in comparison with the nonionic surfactant HCO-60. Fig. 2 shows the time courses of the total amount of methyl paraben and p-HBA in the receiver phase. It is evident that the rate of cutaneous permeation of methyl paraben was markedly decreased by the addition of HP-β-CyD, the suppressive effect of which was greater than that of HCO-60. Under the present conditions, pretreatment of the skin with HP-β-CyD did not suppress the cutaneous permeation of methyl

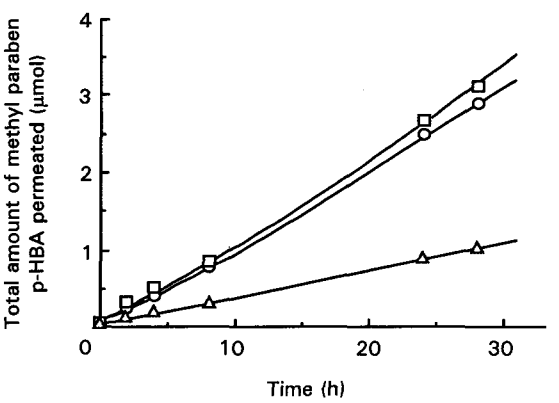

FIG. 2. Effects of HCO-60 and HP-β-CyD on the cutaneous permeation of methyl paraben in water at 20°C. ○ Without HP-β-CyD and HCO-60, □ with HCO-60, △ with HP-β-CyD. Each value represents the mean of two experiments.

Table 1. Effects of HCO-60 and HP-β-CyD on the distribution of methyl paraben (%) 28 h after the application of test solutions onto the isolated skin of hairless mouse at 20°C.

	Donor phase Methyl paraben	Skin Methyl paraben	Receiver phase Methyl paraben	p-HBA	p-HBA/total
Control	93·1	0·7	2·0	4·2	67·7
With HCO-60	93·3	0·6	3·0	3·2	51·6
With HP-β-CyD	97·8	0·2	0·4	1·7	81·0

paraben. This in-vitro result is similar to the in-vivo results reported previously (Loftsson et al 1994; Vollmer et al 1994). Therefore, the reduced cutaneous permeation of methyl paraben by HP-β-CyD could be explained by the complex formation of methyl paraben with HP-β-CyD rather than the indirect effects of HP-β-CyD on the skin. Table 1 shows the effects of HP-β-CyD on the accumulation and bioconversion of methyl paraben in the skin.

Under the present experimental conditions, p-HBA was detected only in solution at the receiver phase, not in the donor phase nor in the skin. The amount of methyl paraben accumulated in the skin decreased in the order of methyl paraben alone > HCO-60 > HP-β-CyD, reflecting the results of the cutaneous permeation of methyl paraben (Fig. 2). The fraction of p-HBA of the total amounts of methyl paraben and p-HBA was increased in the order of methyl paraben alone < HCO-60 < HP-β-CyD, indicating that the complexation may assist the bioconversion. In addition, a negative correlation between the suppressive effects of cutaneous permeation and the bioconversion effects existed. When a relatively low dose of methyl paraben was applied onto the skin, methyl paraben penetrated gradually into the epidermis and may be susceptible to enzymatic conversion in the epidermis itself (Guzek et al 1989). We have recently reported that the conversion rates of methyl paraben in the skin decreased with increasing concentration of methyl paraben in the donor phase (Tanaka et al 1993).

Therefore, in the presence of HP-β-CyD, lower levels of permeation, and consequently, lower concentrations of the permeate in the skin facilitated the metabolic process that is active in the skin. The above results suggest that HP-β-CyD clearly suppresses the in-vitro cutaneous permeation of methyl paraben, and promotes the conversion of methyl paraben to p-HBA in the skin.

Table 2. Distribution of radioactivity (% dose) corresponding to [^{14}C]methyl paraben and its metabolites 24 h after topical application of patches containing methyl paraben (2 mg/animal) in the absence and presence of HP-β-CyD (20 mg/animal) onto skin of hairless mouse[a].

	Without HP-β-CyD	With HP-β-CyD
Patch	18·375 ± 4·846	44·483 ± 7·520*
Stratum corneum	0·148 ± 0·083	0·260 ± 0·190
Viable skin	0·094 ± 0·017	0·170 ± 0·023*
Whole body[b]	3·372 ± 0·292	3·751 ± 0·402
Urine	66·393 ± 4·019	42·527 ± 8·266*
Faeces	7·798 ± 1·831	5·451 ± 1·024
Respiration	0·004 ± 0·001	0·004 ± 0·003
Total recovery	96·185 ± 4·019	96·646 ± 0·794

[a]Means ± s.d., n = 5. [b]Except for stratum corneum and viable skin. * $P < 0·05$ compared with experiment without HP-β-CyD.

Percutaneous absorption of methyl paraben and HP-β-CyD

[^{14}C]HP-β-CyD was employed to clarify the suppression effect of HP-β-CyD on the percutaneous absorption of methyl paraben in-vivo. Table 2 shows the percentage of radioactivity in each portion of the dorsal skin of hairless mice, 24 h after the application of test patches containing methyl paraben solution. In the presence of HP-β-CyD (2% w/v), the residual amount of methyl paraben in the patches increased significantly from 18·4 to 44·5%. Moreover, the amounts of methyl paraben excreted in the urine, faeces and respiration, as well as the total in the body fluids—i.e. the amount of methyl paraben absorbed through the skin—decreased by 66%. This indicates that the percutaneous absorption of methyl paraben is significantly lowered by HP-β-CyD, reflecting the results of the in-vitro experiments. Furthermore, HP-β-CyD did not affect the systemic disposition of [^{14}C]methyl paraben after percutaneous absorption when the percentage of methyl paraben in the urine and faeces was compared with the amount in the entire body. On the other hand, as shown in Table 3, the percutaneous absorption of [^{14}C]HP-β-CyD in the presence of methyl paraben was extremely low at about 0·02% of the amount applied onto the skin, and this was almost the same level as that in the absence of methyl paraben. In addition, the absorption rate of [^{14}C]HP-β-CyD in skin from which the stratum corneum had been removed by stripping, was about 24%, suggesting that the stratum corneum may act as a barrier to the percutaneous absorption of HP-β-CyD. These results clearly demonstrate that HP-β-CyD has low permeability through intact hairless mouse skin. Consequently, it was confirmed that only the free fraction of methyl paraben, which is in equilibrium with the complexed fraction, was percutaneously absorbed as pointed out by Gerloczy et al (1988) and Okamoto et al (1986).

The above results suggest that HP-β-CyD greatly suppressed the cutaneous permeability of methyl paraben, and promoted the bioconversion of methyl paraben to the safer p-HBA, while HP-β-CyD permeated only very slightly into

Table 3. Percutaneous absorption of radioactivity corresponding to [^{14}C]HP-β-CyD, 24 h after topical application of patches containing [^{14}C]HP-β-CyD solution (2 mg/animal) in the absence and presence of methyl paraben (0·2 mg/animal) onto skin of hairless mouse[a].

Skin	Test solution	[^{14}C]HP-β-CyD absorbed (%)
Intact	With methyl paraben	0·023 ± 0·007
Intact	Without methyl paraben	0·018 ± 0·003
Stripped	Without methyl paraben	23·987 ± 7·364*

[a] Means ± s.d., n = 3–5. * $P < 0·05$ compared with intact skin.

the skin. Therefore, HP-β-CyD has a significant advantage over surface-active agents with respect to providing high aqueous solubility while maintaining a lack of toxicity (Brewster et al 1990; Frijlink et al 1990) in topical liquid preparations of methyl paraben used in medicines and cosmetics.

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References

Brewster, M. E., Estes, K. S., Bodor, N. (1990) An intravenous toxicity study of 2-hydroxypropyl-β-cyclodextrin, a useful drug solubilizer, in rats and monkeys. Int. J. Pharm. 59: 231–243

Carpenter, O. T., Pettifor, J. M., Russell, R. M., Pitha, J., Mobarham, S., Ossip, M. S., Wainer, S., Anast, C. S. (1987) Severe hypervitaminosis A in siblings: evidence of variable tolerance to retinol intake. J. Pediatr. 111: 507–512

Franz, T. J. (1975) On the reference of in vitro data in percutaneous absorption. J. Invest. Dermatol. 64: 190–195

Frijlink, H. W., Visser, J., Hefting, N. R., Oosting, R., Meijer, D. K. F., Lerk, C. F. (1990) The pharmacokinetics of β-cyclodextrin and hydroxypropyl-β-cyclodextrin in the rat. Pharm. Res. 7: 1248–1252

Gerlóczy, A., Antal, S., Szejtli, J. (1988) Percutaneous absorption of heptakis(2,6-di-*O*-^{14}C-methyl)-β-cyclodextrin in rats. In: Huber, O., Szejtli, J. (eds) Proceedings of the 4th International Symposium on Cyclodextrins. Kluwer Academic Pub., Dordrecht, pp 415–420

Guzek, D. B., Kennedy, A. H., McNeil, S. C., Wakshull, E., Potts, R. O. (1989) Transdermal drug transport and metabolism. I. Comparison of in vitro and in vivo results. Pharm. Res. 6: 33–39

Higuchi, T., Connors, K. A. (1965) Phase solubility techniques. Adv. Anal. Chem. Instr. 4: 117–212

Iwata, Y., Satoh, I., Akiu, S., Kobayashi, T., Fujiyama, Y. (1988) Studies on the percutaneous absorption and the skin metabolism of arbutin. Proc. Jpn. Soc. Invest. Dermatol. 12: 176–177

Lehner, S. J., Müller, B. W., Seydel, J. K. (1993) Interactions between *p*-hydroxybenzoic acid esters and hydroxypropyl-β-cyclodextrin and their antimicrobial effect against *Candida albicans*. Int. J. Pharm. 93: 201–208

Lehner, S. J., Müller, B. W., Seydel, J. K. (1994) Effect of hydroxypropyl-β-cyclodextrin on the antimicrobial action of preservatives. J. Pharm. Pharmcol. 46: 186–191

Loftsson, T., Friðksdóttir, H., Ingvarsdóttir, G., Jónsdóttir, B., Sigurðardóttir, A. M. (1994) The influence of 2-hydroxypropyl-β-cyclodextrin on diffusion rates and transdermal delivery of hydrocortisone. Drug Dev. Ind. Pharm. 20: 1699–1708

Okamoto, H., Komatsu, H., Hashida, M., Sezaki, H. (1986) Effects of β-cyclodextrin and di-*O*-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. Int. J. Pharm. 30: 35–45

Pitha, J., Pitha. J. (1985) Amorphous water-soluble derivatives of cyclodextrins, nontoxic dissolution enhancing excipients. J. Pharm. Sci. 74: 987–990

Pitha, J., Milecki, J., Fales, H., Pannell, L., Uekama, K. (1986) Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs. Int. J. Pharm. 29: 73–82

Saenger, W. (1980) Cyclodextrin inclusion compounds in research and industry (Review). Angew. Chem. Int. Ed. Engl. 19: 344–362

Szejtli, J. (1983) Dimethyl-β-cyclodextrin as parenteral drug carrier. J. Incl. Phenom. 1: 135–150

Tanaka, M., Nakamura, T., Ito, K., Taniguchi, K., Matsuda, H., Uekama, K. (1993) Movement of skin permeation behavior of parabens by 2-hydroxypropyl-β-cyclodextrin. Nihon Koshouhin Kagakukai Zasshi 17: 185–190

Uekama, K., Ikeda, Y., Hirayama, F., Otagiri, M., Ikeda, M. (1980) Inclusion complexation of *p*-hydroxybenzoic acid esters with α- and β-cyclodextrins; dissolution behaviors and antimicrobial activities. Yakugaku Zasshi 100: 994–1003

Vollmer, U., Müller, B. W., Peeters, J., Mesens, J., Wilffert, B., Peters, T. (1994) A study of the percutaneous absorption-enhancing effects of cyclodextrin derivatives in rats. J. Pharm. Pharmacol. 46: 19–22

Yoshida, A., Arima, H., Uekama, K., Pitha, J. (1988) Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrins. Int. J. Pharm. 46: 217–222