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Report

Enhancement of the Antiinflammatory Effect of Ethyl 4-Biphenylyl Acetate in Ointment by β-Cyclodextrin Derivatives: Increased Absorption and Localized Activation of the Prodrug in Rats

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Ethyl 4-biphenylyl acetate (EBA) is a prodrug of the antiinflammatory 4-biphenylyl acetic acid (BPAA). The inclusion complexes of EBA with β-cyclodextrin (β-CyD), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD), and 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) at a molar ratio of 1:2 (EBA:cyclodextrin) were prepared and used to make hydrophilic antiinflammatory ointments. The *in vitro* release of EBA from the ointments was enhanced by complexation in the order of β-CyD < DM-β-CyD ≤ HP-β-CyD. The improvement correlated with the improved solubility and not with the decreased diffusibility observed to occur upon the complexation of EBA. *In vivo* the complexation with cyclodextrin derivatives increased both the release of EBA from the vehicle and its conversion in the underlying tissue to BPAA, but the total of EBA and BPAA in the tissue was decreased. *In vitro* studies confirmed that the effects of cyclodextrin derivatives on the conversion were exerted indirectly. The combination of the enhanced release and of the enhanced prodrug hydrolysis by esterases in the site where the antiinflammatory action is required resulted in increased therapeutic effects. In the model of carrageenan-induced acute edema in rat paw, the complexation improved the therapeutic effects over those of EBA alone in the order of β-CyD < DM-β-CyD < HP-β-CyD. HP-β-CyD may be a particularly useful cyclodextrin derivative since it improves the topical availability and does not irritate tissues.

KEY WORDS: antiinflammatory ointments; 4-biphenylyl acetic acid; ethyl 4-biphenylyl acetate; β-cyclodextrin derivatives; percutaneous absorption; prodrug activation.

INTRODUCTION

The prodrug approach has often been used to optimize the dermal delivery of drugs, especially of those with antiinflammatory and antipsoriatic effects. The prodrugs, however, must be delivered to the focal regions rapidly and converted immediately into the pharmacologically active species, since both the slow entry of prodrug and the escape of drug into systemic circulation decrease the therapeutic value of the ointment. Thus, the steps of prodrug release from vehicle, permeation through the skin, and prodrug conversion to drug are of critical importance (1,2).

Cyclodextrins are cyclic oligomers of glucose which form inclusion complexes (host–guest type) with lipophilic drugs as guests and thus improve their water solubilities (3). Widespread uses of cyclodextrins as hosts for drugs are, however, restricted by their low aqueous solubilities, particularly that of β-cyclodextrin (β-CyD) (4–6). Alkylation or hydroxyalkylation of the hydroxyl groups of β-CyD has been used to solve the problem of low solubility (7,8). Among the various derivatives evaluated, heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD), which is a defined mixture of oligo substituted species, have gained some acceptance in pharmaceutical applications (9,10).

Both DM-β-CyD and HP-β-CyD were found to improve the percutaneous absorption and antiinflammatory efficacy of an ointment containing 4-biphenylyl acetic acid (BPAA) in rats (11). Presently, we have examined the effects of cyclodextrin derivatives on ointments containing a prodrug of BPAA, ethyl 4-biphenylyl acetate (EBA; Fig. 1). Surprisingly, indirect effects of cyclodextrin derivatives on hydrolysis of EBA by esterases were found which improved the therapeutic effects considerably. Since the use of esters as prodrugs for drugs possessing carboxylic groups is quite common, the approach described here may have many other uses.

MATERIALS AND METHODS

Materials

EBA, BPAA, and 4-hydroxy-4-biphenylyl acetic acid

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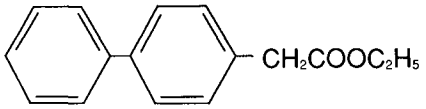

Fig. 1. The chemical structure of ethyl 4-biphenylyl acetate (EBA).

(4-hydroxy-BPAA) were obtained from Nippon Lederle Co., Tokyo. β-CyD and HP-β-CyD of the average degree of substitution 5.8 (12) from Nihon Shokuhin Kako Co., Tokyo, DM-β-CyD from Toshin Chemical Co., Tokyo, and the hydrophilic ointment base (Japanese Pharmacopoeia XI) from Iwaki Seiyaku Co., Tokyo. The ointment contained white petrolatum (25%, w/w), stearyl alcohol (20%, w/w), propylene glycol (12%, w/w), polyoxyethylenehydrogenated caster oil (4%, w/w), glycerol monostearate (1%, w/w), methyl 4-hydroxybenzoate (0.1%, w/w), propyl 4-hydroxybenzoate (0.1%, w/w), and purified water (appropriate amount).

Preparation of Solid Complexes

The solid complexes of EBA with cyclodextrin derivatives were prepared by the kneading method (13). The complexes contained 1 molecule of EBA per 2 molecules of respective cyclodextrin derivatives; this stoichiometry was determined on the basis of the phase solubility diagrams for the EBA:cyclodextrin derivative systems as described previously (13). The apparent stability constants (K') given in Table I were calculated from the initial straight-line portion of the solubility diagrams, assuming that a 1:1 complex is initially formed, and were used as a tentative measure of inclusion complexation.

In Vitro Release Studies

The ointments were prepared by mixing, contained EBA or its cyclodextrin complexes (equivalent to 1.0%, w/w, as EBA), and were stable for at least 1 month in the accelerated decomposition condition (40°C, 75% RH). The release of EBA from the ointments (600 mg) into normal saline (9 ml) was determined at 25°C using a horizontal diffusion cell. A silicone membrane (0.85 cm^2, Silastic, Dow Corning Co., Mich.) was used as a barrier for the diffusion of the vehicle. At appropriate intervals, 0.5-ml samples were withdrawn from the release phase. The concentration of EBA was measured by high-performance liquid chromatography (HPLC) (13). The solubility of EBA in the ointment base was determined as described (14). For analysis of the data on the release of EBA or its cyclodextrin complexes

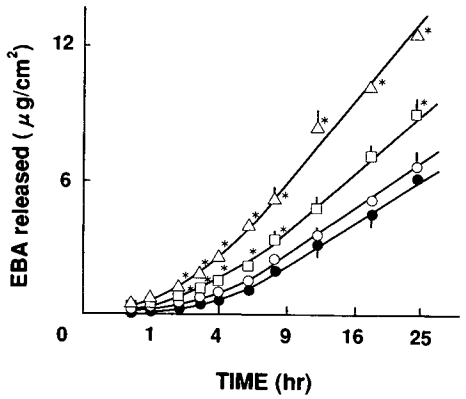

Fig. 2. Release profiles of EBA from hydrophilic ointments containing EBA and its cyclodextrin complexes through a silicone membrane in saline at 25°C. (●) EBA alone; (○) EBA:β-CyD complex; (□) EBA:DM-β-CyD complex; (△) EBA:HP-β-CyD complex. Each point represents the mean ± SE of three experiments. (*) $P < 0.05$, compared to EBA alone.

from the vehicle, Eg. (1) was used when the prodrug was fully dissolved, and Eq. (2) when the prodrug was only partly dissolved in the ointment base (15,16).

$$Q = 2C_o\sqrt{D \cdot t/\pi} \quad (1)$$

$$Q = \sqrt{(2C_o - C_s) \cdot C_s \cdot D \cdot t} \quad (2)$$

Q is the amount of EBA released per unit area at time t; C_o is the initial concentration of EBA, free or complexed; D is the apparent diffusion coefficient; and C_s is the solubility of EBA, or of its cyclodextrin complexes, in the ointment base.

In Vivo Absorption Studies

Male Wistar rats weighing 160–200 g were used and a day before the experiment their backs were shaved by electric clippers under pentobarbital anesthesia (30 mg/kg, intraperitoneal). The ointment samples (30 mg) were spread uniformly over the surface of the two sheets of paper (2 × 2 cm^2), and these applied to the shaved areas and covered with a film (Saran Wrap, Asahi Kasei Kogyo Co., Tokyo) which was again fastened by adhesive tape. At intervals, rats were killed, the ointment was recovered from the skin by wiping with an absorbent paper soaked in saline, and the remaining prodrug was extracted from appropriate materials with methanol (5 ml). The content of prodrug and cyclodextrin derivatives in the extract was analyzed by HPLC (13). Then

Table I. Effects of Complexation on the Properties of EBA at 25°C

System	K'^a (M^{-1})	k'^b ($\mu g/cm^2/hr^{1/2}$)	C_s^c ($\times 10^{-2}$ $\mu g/cm^3$)	D^d ($\times 10^8$ cm^2/hr)
EBA alone	—	1.966	4.87	40.069
β-CyD complex	3050	1.964	43.24	5.090
DM-β-CyD complex	12500	2.785	1499.00	6.088
HP-β-CyD complex	4200	3.392	475.00	9.034

[a] The apparent stability constant of the EBA: cyclodextrin complexes in water.
[b] The apparent release rate constant of EBA from the hydrophilic ointment base.
[c] The solubility of EBA in the hydrophilic ointment base.
[d] The diffusion coefficient of EBA in the hydrophilic ointment base.

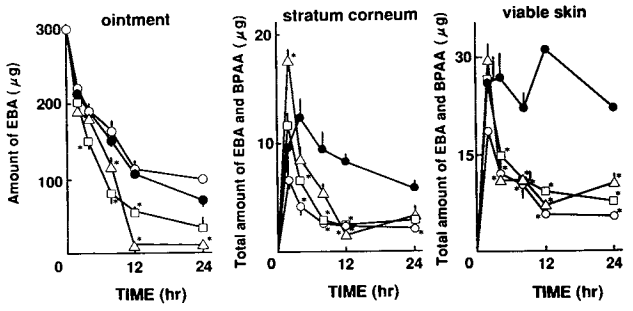

Fig. 3. The time dependence of distribution of EBA and of its hydrolytic product BPAA after the application of hydrophilic ointments containing EBA and its cyclodextrin complexes to the skin on the backs of rats. (●) EBA alone; (○) EBA:β-CyD complex; (□) EBA:DM-β-CyD complex; (△) EBA:HP-β-CyD complex. Each point represents the mean ± SE of four or five rats. (*) $P < 0.05$, compared to EBA alone.

the stratum corneum was removed by stripping with adhesive tape and its EBA and BPAA contents were determined in the same manner.

The underlying skin and subcutaneous tissues were excised, cut finely, and extracted with 7 ml of methanol. The addition of methanol stabilized EBA against enzymatic hydrolysis. For example, the recoveries of EBA and BPAA from the viable skin were determined to be 96 ± 5 and 98 ± 3%, respectively. The delay in processing the tissues before the extraction resulted in a slight loss of EBA. However, the data were not corrected for the enzymatic degradation of EBA because such loss of the prodrug was confirmed to be only less than 5% in all the cases. The extracts were evaporated, and the residues were dissolved in 5 ml of solvent (cyclohexane and ether, 3:1) and analyzed by HPLC. Cyclodextrin derivatives in stratum corneum and in viable skin were analyzed by HPLC (13) with slight modifications. Concentrations of BPAA in serum and of 4-hydroxy-BPAA (17) in urine were measured by HPLC. For analysis of BPAA and 4-hydroxy-BPAA, a HPLC instrument Hitachi L-6000 equipped with a ERC-ODS-1161 column (3 μm, 6 ϕ × 100 mm, Erma Optical Works, Tokyo) was used. Mobile phase was methanol–0.1 *M* acetic acid (7:3%, v/v), flow rate was 1.0 ml/min, and compounds were detected fluorometrically (650-10 LC fluorescence spectrophotometer, Tokyo) using excitation at 285 nm and emission at 320 nm. The area under the serum BPAA concentration–time curve (AUC) and the mean residence time of the drug in serum (MRT) were calculated by moment analysis (18).

Evaluation of Antiinflammatory Responses

The ointments (100 mg) were uniformly spread on the left hind paws of rats and covered with the film and adhesive tape. After 1 hr the ointment was removed and inflammatory edema induced by a subplantar injection of 1% carrageenan in saline (0.1 ml). The paw swelling was periodically monitored using a water plethysmometer according to the method of Winter *et al.* (19) with slight modifications; the dose of carrageenan and the apparatus to measure the paw swelling were changed.

RESULTS AND DISCUSSION

In Vitro Studies

EBA forms complexes with cyclodextrin derivatives (13); the stabilities of these are in the order β-CyD < HP-β-CyD < DM-β-CyD. The respective apparent stability constants (K') were measured in water (Table I). The complexation increased the solubility of EBA in the hydrophilic ointment base in the order β-CyD < HP-β-CyD < DM-β-CyD (Table I). The ointments investigated contained 1% (w/w) of EBA and thus only when DM-β-CyD or HP-β-CyD was present was EBA fully dissolved in the ointments. The diffusion coefficients of EBA preparations in the hydrophilic ointments decreased in the order of EBA alone ≫ EBA:HP-β-CyD > EBA:DM-β-CyD > EBA:β-CyD (Table I). Amounts of EBA which permeated from hydrophilic ointments through silicone membrane were increased by the complexation of the prodrug with cyclodextrin derivatives in the order EBA alone < EBA:β-CyD < EBA:DM-β-CyD < EBA:HP-β-CyD (Fig. 2). Obviously the solubilization effects of cyclodextrin derivatives were of primary importance, while diffusion effects had only a minor influence. In steady state the amounts of the permeated EBA increased linearly with the square root of time (Fig. 2), indicating that the permeation is diffusion controlled; Table I lists the apparent release rate constants (k') calculated from the data.

In Vivo Studies

The release of EBA from ointments into the skin was assisted by the complexation with DM-β-CyD or HP-β-CyD, whereas β-CyD had no appreciable effect (Fig. 3). These results are similar to the *in vitro* findings. The pretreatment of the skin with the ointments containing only cyclodextrin derivatives without EBA did not assist absorption of EBA applied subsequently (data not shown). Thus, the release of

Table II. The Distribution of Cyclodextrin Derivatives 24 hr After the Application of Hydrophilic Ointments Containing also EBA to the Skin on the Backs of Rats[a]

System	In ointment (mg)	In stratum corneum (μg)	In viable skin (μg)
β-CyD complex	2.50 ± 0.02 (88.1)	37.4 ± 7.5 (1.3)	76.6 ± 6.5 (2.7)
DM-β-CyD complex	1.91 ± 0.07 (57.4)*	76.4 ± 17.0 (2.3)	125.9 ± 15.4 (3.8)*
HP-β-CyD complex	1.72 ± 0.10 (46.8)*	141.9 ± 21.9 (3.9)*	117.0 ± 7.0 (3.2)*

[a] Each value represents the mean ± SE of five rats. Each value in parentheses represents the percentage of the initial amount applied.

* $P < 0.05$, compared to the β-CyD complex.

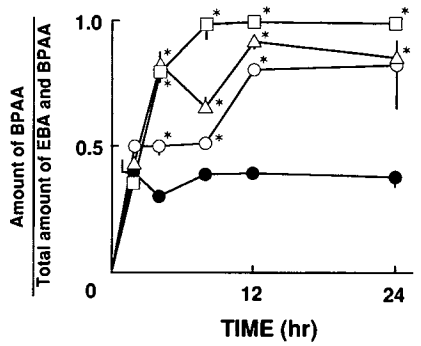

Fig. 4. The bioconversion of EBA to BPAA in the viable skin after the application of hydrophilic ointments containing EBA and its cyclodextrin complexes to the skin on the backs of rats. (●) EBA alone; (○) EBA:β-CyD complex; (□) EBA:DM-β-CyD complex; (△) EBA:HP-β-CyD complex. Each point represents the mean ± SE of four or five rats. (*) $P < 0.05$, compared to EBA alone.

EBA from the vehicle is probably the rate-limiting step in the cyclodextrin derivative assisted skin permeation of EBA. Recent studies have demonstrated that most of the drug in the skin had to be in free form since the majority of cyclodextrin derivatives remained in the vehicle (20,21). Nevertheless, under the occlusive conditions significant amounts of cyclodextrin derivatives were lost from the vehicle into the skin in the order β-CyD < DM-β-CyD < HP-β-CyD (Table II), a sequence which corresponds to the order of the enhancement of EBA release. A penetration of cyclodextrin derivatives into the skin was also observed from the corresponding prodrug free ointments (data not shown). Only a fraction of the cyclodextrin derivatives was found in the stratum corneum and in the viable skin 24 hr after the application (Table II). These results indicate that considerable amounts of cyclodextrin derivatives passed through the skin, and thus they may exert some biological effects even there (22,23).

The skin is an organ capable of metabolism of topically applied drugs (24,25). Esterase activity was found in both epidermal and dermal layers of human skin, while that in the stratum corneum was minimal (26,27). Since in the skin the hydrophilic product BPAA is the only detectable metabolite of EBA, the time dependence of concentrations of both these compounds in the skin was measured (Fig. 3). The application of EBA in the complexed form resulted in a transient rise and subsequent decrease in the total amount of EBA and BPAA both in the stratum corneum and in the viable skin. Furthermore, the fraction of pharmacologically active BPAA in the viable skin was increased when EBA was applied in the complexed form (Fig. 4), indicating that the complexation assists the bioconversion of EBA to BPAA, and consequently it helped to deliver the active drug to subcutaneous tissues, where its action is most desired (Fig. 5) (28,29). On the other hand, the drugs crossing the skin are efficiently transported away into the capillary. After the application of EBA ointments to the skin, BPAA was found in the blood circulation, and the majority of the metabolites accumulated into urine was 4-hydroxy-BPAA (17). The pharmacokinetic parameters of these compounds are given in Table III. In spite of the enhanced penetration of EBA into the skin seen with cyclodextrin derivatives (Fig. 3), there were no significant differences in these kinetic parameters between all the preparations. Considering the dissociation equilibrium of complexes, the cyclodextrin derivatives no longer affect the pharmacokinetic behavior of the drug and its metabolites after they are entered into the systemic circulation (5). Therefore, the above findings suggest that the cyclodextrin derivatives exert some effects on the eliminating processes of the drug from the skin and, consequently, retain the active drug at the focal regions, where its antiinflammatory action is desired.

The mechanism of the *in vivo* enhanced conversion of EBA to BPAA is intriguing. When hydrolysis of EBA by homogenates of whole skin was investigated, the complexation invariably decreased the conversion (data not shown). The complexation also decelerated chemical hydrolysis of EBA by acids or by bases. All these *in vitro* decelerations were in the order β-CyD < HP-β-CyD < DM-β-CyD, i.e., were proportional to the stability constants of the complexes (Table I). Thus, the complexation must support the *in vivo* hydrolysis in an indirect manner—perhaps by concentrating

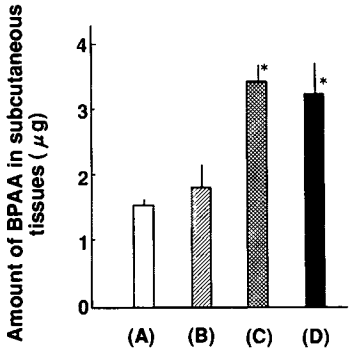

Fig. 5. The amount of BPAA accumulated in subcutaneous tissues 2 hr after the application of hydrophilic ointments containing EBA and its cyclodextrin complexes to the skin on the backs of rats. (A) EBA alone; (B) EBA:β-CyD complex; (C) EBA:DM-β-CyD complex; (D) EBA:HP-β-CyD complex. Each result represents the mean ± SE of four or five rats. (*) $P < 0.05$, compared to EBA alone.

Table III. Pharmacokinetic Parameters of BPAA in Serum and Cumulative Amounts of 4-Hydroxy BPAA Excreted into Urine After the Application of Hydrophilic Ointments Containing EBA and Its Cyclodextrin Complexes to the Skin on the Backs of Rats[a]

System	BPAA in serum AUC[b] (hr · μg/ml)	BPAA in serum MRT[c] (hr)	4-Hydroxy-BPAA excreted in urine[d] (μg)
EBA alone	17.2 ± 3.2	9.1 ± 0.1	17.6 ± 3.1
β-CyD complex	13.5 ± 3.1	9.5 ± 0.3	17.9 ± 4.4
DM-β-CyD complex	17.3 ± 2.7	9.9 ± 0.3	22.1 ± 4.2
HP-β-CyD complex	16.6 ± 1.9	8.5 ± 0.3	21.5 ± 2.1

[a] Each point represents the mean ± SE of five rats.
[b] The area under the serum BPAA level–time curve up to 24 hr postapplication.
[c] The mean residence time of BPAA in serum calculated from the serum data up to 24 hr postapplication.
[d] The cumulative amount of 4-hydroxy-BPAA excreted into urine up to 24 hr postapplication.

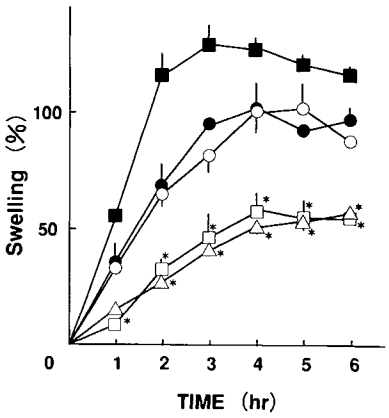

Fig. 6. Effects of EBA and its cyclodextrin complexes in hydrophilic ointment on the carrageenan-induced acute edema in rat paw. (■) Control; (●) EBA alone; (○) EBA:β-CyD complex; (□) EBA:DM-β-CyD complex; (△) EBA:HP-β-CyD complex. Each point represents the mean ± SE of eight to ten rats. (*) $P < 0.05$, compared to EBA alone.

all the important components to a single site. Furthermore, the contribution of shunt pathways in the skin such as hair follicles and sebaceous glands to the percutaneous absorption of the prodrug should be considered for the semisolid preparations used here. It seems likely that the particles of undissolved prodrug migrate well into the skin through the shunt pathways, which would lead to a higher fraction of unconverted prodrug in the skin. Therefore, the increase in dissolved fraction of prodrug in the vehicle by complexation (Table I) may contribute in part to the accelerated bioconversion of EBA seen with the cyclodextrin derivatives.

Antiinflammatory Effect

The antiinflammatory effects of ointments were tested using the model of carageenan-induced acute edema in rat paw (19). Results in Fig. 6 show that a dramatic improvement may be obtained when EBA is administered in complexed form; the efficacy increased in the order EBA alone < EBA:β-CyD < EBA:DM-β-CyD < EBA:HP-β-CyD complex; the inhibition of swelling was 26.1, 36.5, 64.3, and 68.2%, respectively.

In conclusion, the complexation with cyclodextrin derivatives may dramatically improve the antiinflammatory effects of ointment preparations through improved release of prodrug into the tissue and through the indirect activation of the prodrug to the drug at a location where its therapeutic effects are more effective. It should also be noted that the very effective complexation agent (HP-β-CyD) has a low toxicity (10,30).

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REFERENCES

1. S. Y. Chan and A. L. W. Po. *Int. J. Pharm.* 55:1–16 (1989).
2. D. A. W. Bucks. *Pharm. Res.* 1:148–153 (1984).
3. J. Pitha. *J. Incl. Phenom.* 2:477–485 (1984).
4. J. Szejtli. In V. F. Smolen and L. A. Ball (eds.), *Controlled Drug Bioavailability*, John Wiley & Sons, New York, 1985, pp. 365–420.
5. K. Uekama and M. Otagiri. *CRC Crit. Rev. Ther. Drug Carrier Syst.* 3:1–40 (1987).
6. W. Saenger. In J. L. Atwood, J. E. D. Davies, and D. D. MacNicol (eds.), *Inclusion Compounds, Vol. 2*, Academic Press, London, 1984, pp. 231–259.
7. J. Szejtli. *J. Incl. Phenom.* 1:135–150 (1983).
8. J. Pitha, T. Irie, P. B. Sklar, and J. S. Nye. *Life Sci.* 43:493–502 (1988).
9. K. Uekama and T. Irie. In D. Duchêne (ed.), *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris, 1987, pp. 393–439.
10. A. Yoshida, H., Arima, K. Uekema, and J. Pitha. *Int. J. Pharm.* 46:217–222 (1988).
11. H. Arima, H. Adachi, T. Irie, and K. Uekama. *Drug Invest.* (in press) (1990).
12. J. Pitha, J. Milecki, H. Fales, L. Pannell, and K. Uekama. *Int. J. Pharm.* 29:73–82 (1986).
13. H. Arima,, T. Irie, and K. Uekama. *Int. J. Pharm.* 57:107–115 (1989).
14. K. Uekama, K. Masaki, K. Arimori, T. Irie, and F. Hirayama. *Yakugaku Zasshi* 107:449–456 (1987).
15. T. Higuchi. *J. Pharm. Sci.* 50:874–875 (1961).
16. W. I. Higuchi. *J. Pharm. Sci.* 51:802–804 (1962).
17. F. S. Chiccarelli, H. J. Eisner, and G. E. V. Lear. *Arzneim.-Forsch./Drug Res.* 30:707–715 (1980).
18. K. Yamaoka, T. Nakagawa, and T. Uno. *J. Pharmacok. Biopharm.* 6:547–558 (1978).
19. C. A. Winter, E. A. Risley, and G. W. Nuss. *Proc. Soc. Exp. Biol. Med.* 111:544–547 (1962).
20. H. Okamoto, H. Komatsu, M. Hashida, and H. Sezaki. *Int. J. Pharm.* 30:35–45 (1986).
21. A. Gerlóczy, S. Antal, and J. Szejtli. In O. Huber and J. Szejtli (eds.), *Proceedings of the 4th International Symposium on Cyclodextrins*, Kluwer Academic, Dordrecht, 1988, pp. 415–420.
22. T. Hoshino, K. Ishida, T. Irie, K. Uekama, and T. Ono. *Arch. Dermatol. Res.* 281:60–65 (1989).
23. K. Ishida, T. Hoshino, T. Irie, and K. Uekama. *Xenobio. Metabol. Dispos.* 3:377–386 (1988).
24. R. J. Martin, S. P. Denyer, and J. Hadgraft. *Int. J. Pharm.* 39:23–32 (1987).
25. J. Hadgraft. In H. Bundgaard (ed.), *Design of Prodrugs*, Elsevier, Amsterdam, 1985, pp. 271–289.
26. D. B. Guzek, A. H. Kennedy, S. C. McNeill, E. Wakshull, and R. O. Potts. *Pharm. Res.* 6:33–39 (1989).
27. R. O. Potts, S. C. McNeill, C. R. Desbonnet, and E. Wakshull. *Pharm. Res.* 6:119–124 (1989).
28. Y. Ozawa, I. Koyama, S. Murayama, and T. Nadai. *Chem. Pharm. Bull.* 33:5113–5118 (1985).
29. Y. Mizushima. *Drug Deliv. Syst.* 2:3–12 (1987).
30. M. E. Brewster, J. W. Simpkins, M. S. Hora, W. C. Stern, and N. Bodor. *J. Parent. Sci. Tech.* 43, 231–240 (1989).