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A Study of the Percutaneous Absorption-enhancing Effects of Cyclodextrin Derivatives in Rats

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Abstract—2-Hydroxypropyl-β-cyclodextrin (HP-β-CyD) and 2,6-dimethyl-β-cyclodextrin (D-β-CyD) were studied for transdermal penetration enhancement of the cytochrome P450 inhibitor liarozole by an in-vivo transdermal absorption rat model. The mode of action of penetration enhancement was investigated by differential scanning calorimetry (DSC). In-vivo, HP-β-CyD, as a 20% aqueous solution, increased the absorption of liarozole approximately threefold and a 20% aqueous solution of D-β-CyD decreased the percutaneous absorption of liarozole in blood by a factor of 0·6. However, pretreatment with D-β-CyD (20%, 4 h) enhanced the transdermal absorption 9·4-fold. In the DSC experiments the thermal profile of human stratum corneum was practically unchanged after treatment with HP-β-CyD, but treatment with D-β-CyD revealed an interaction of D-β-CyD with the protein and lipid fraction. Thus the results from DSC and those from the permeability experiments revealed that D-β-CyD acts as a transdermal absorption enhancer by changing the stratum corneum barrier whereas HP-β-CyD influences the partitioning behaviour of the drug in the skin.

The last two decades of considerable effort in dermatological research has resulted only in a few approved transdermal drugs on the market. This reflects the inability to deliver sufficient quantities of therapeutic agents across the skin. The morphology of the stratum corneum, which is commonly accepted to represent the real permeability barrier, has been clarified (Elias et al 1981; Bouwstra et al 1991), the biochemical origin of the barrier function is being uncovered (Wertz 1986) and its relationship to the physicochemical barrier is manifested (Tojo 1987). Together with the insight into the mechanism of stratum corneum transport, understanding of the mode of action of penetration enhancers is increasing. Many of the ideal attributes a penetration enhancer should have (Barry 1983) are to be found in 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD), such as stability, capable of dissolving lipophilic drugs (Mesens et al 1991), odourless, colourless, safety and tolerability (Coussement et al 1990; Monbaliu et al 1990). Thus we have evaluated the penetration enhancing activities of HP-β-CyD and 2,6-dimethyl-β-cyclodextrin (D-β-CyD). For permeability measurement we used an in-vivo rat model. Under physiological conditions the transdermal absorption can be studied by cannulating the peripheral skin vein, draining the area of skin which is used for drug application and collecting the blood. The advantage of this model is the direct measurement of absorption in-vivo in intact skin unaffected by distribution and elimination. We applied liarozole, a cytochrome P450 inhibitor (Fig. 1) (Van Wauwe et al 1989), as a model permeant.

We also investigated the mode of action of these cyclodextrins on human stratum corneum by differential scanning calorimetry (DSC).

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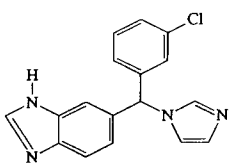

FIG. 1. Structure of liarozole.

Materials and Methods

In-vivo permeability experiments

Male Sprague-Dawley rats, 300 g, were anaesthetized with 1·5 g kg^{-1} urethane, intraperitoneally, and kept at a body temperature of 37°C. The iliolumbal skin vein draining the area to be used for drug application, was cannulated to collect the blood for determination of the amount of absorbed drug continuously in polyethylene vials at 10-min intervals for about 300 min. The blood loss was compensated for by infusion of heparinized donor blood from the fourth fraction onwards. The radiolabelled drug was applied after the second fraction as a 200 μL solution in a closed Teflon chamber (area 1·13 cm^2) fixed on the shaved skin with glue. The amount of drug absorbed was determined by liquid scintillation counting. At the end of each experiment the drained area of the skin was made visible by administration of a methylene blue solution in the dorsal iliolumbal vein and a central blood sample was taken by heart puncture. [^{14}C]Liarozole was applied as a 1% solution (29 mM liarozole, sp. act. 445 MBq $mmol^{-1}$). For pretreatment the animal was tranquilized by light anaesthesia with 80–100 mg kg^{-1} thiopentone, intraperitoneally, to allow for a correct application of the 20% cyclodextrin solution on an occlusive patch for 4 h before the permeability measurement.

Data calculation

Data were analysed by linear regression of the time courses of the mean cumulative amounts of drug in the collected

plasma, using the Statistical Consultants Inc. computer package PCNONLIN. The linear regression models were of the forms:

$$y = mx + b$$

where x is the time, y is the cumulative amount of penetrated drug in the collected blood samples, m is the slope of the regression line, representing the flux and

$$y = m(x + a)$$

where a is the lag-time till absorption reaches steady-state. The permeability coefficient was calculated as ratio between flux and the product of area and concentration (integrated form of Fick's first law of diffusion) (Scheuplein & Ross 1974; Flynn & Stewart 1988).

Differential scanning calorimetry (DSC)

For the thermoanalysis, the method with human stratum corneum according to Goodman & Barry (1989) was used. Human skin was obtained after surgical operation and was separated from the epidermis by digestion with 0·1% trypsin in phosphate-buffered saline (PBS) (pH = 7·4) at 37°C for 12 h. The stratum corneum was dried over silica gel and hydrated in a constant humidity chamber (r.h. = 96·1%) to a hydration level of about 0·4 mg mg^{-1}. Approximately 6 mg hydrated stratum corneum (for control) or about 14 mg stratum corneum that had been soaked for 4 h in 5 mL of a 20% cyclodextrin solution, was scanned in hermetically sealed pans with a Perkin-Elmer TAS 7-DSC from 0–140°C using a heating rate of 10°C min^{-1}.

Drugs

The drugs and chemicals used were: urethane (25% in 0·9% NaCl, Riedel-de-Haen, Seelze, Germany), liarozole HCl and [^{14}C]liarozole (Janssen Pharmaceutica, Beerse, Belgium), HP-β-CyD (Janssen Biotech, Lammerdries, Belgium), and D-β-CyD (Cyclolab, Budapest, Hungary). All other drugs used were of analytical grade (Merck, Darmstadt, Germany).

Results

In-vivo permeability experiments

The cumulative penetrated amounts of liarozole are compared in Figs 2, 3 and the 95% confidence intervals of the flux, lag-time, permeability-coefficient and enhancement factor are summarized in Table 1. The effects of the addition of D-β-CyD revealed a decrease in permeability. The penetration enhancement of 20% HP-β-CyD could not be calculated because of lack of linearity of the relationship between the cumulative amount absorbed and the time (Vollmer et al 1993b), but the permeability is increased in comparison with the aqueous solution.

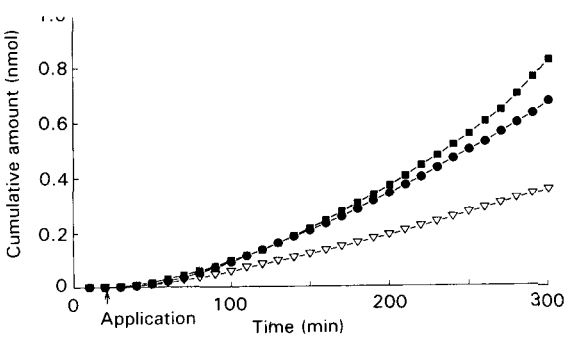

FIG. 2. Comparison of the cumulative absorbed amounts of liarozole in blood in-vivo. ● Water, pH 4; ■ 20% HP-β-CyD, pH 7; ▽ 20% D-β-CyD, pH 7. Values are the mean of six experiments.

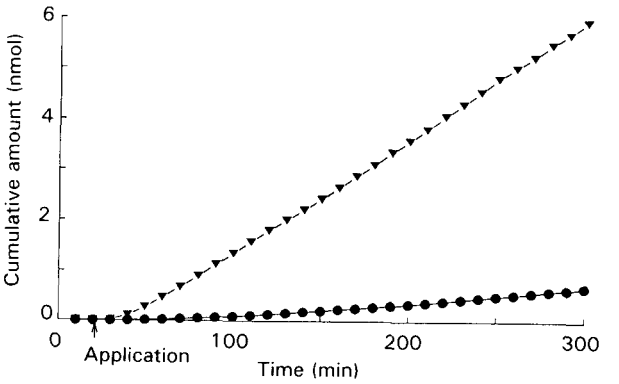

FIG. 3. Comparison of the cumulative absorbed amounts of liarozole in blood in-vivo. ● Water, pH 4; ▼ pretreatment with 20% D-β-CyD. Values are the mean of six experiments.

We extended the in-vivo experiments by the study of the influence of an aqueous solution of 20% D-β-CyD applied 4 h before the application of 1% liarozole at pH 4, because of some promising in-vitro results (data not shown). This pretreatment with D-β-CyD in-vivo led to a 9-fold increase in permeability for liarozole, with a very short lag-time of 21–24 min.

DSC profiles

Fig. 4 shows the DSC transition pattern obtained with stratum corneum hydrated to 40% which has four main endotherm transitions (34, 69, 82 and 100°C). The dotted line represents the profile obtained from stratum treated for 4 h with 20% aqueous HP-β-CyD. This pattern is nearly identical to the control, apart from a different shape at 82°C. Fig. 5 shows the result of stratum corneum treated with a 20% aqueous solution of D-β-CyD. The transition at 100°C was reduced to 79°C, that at 88°C was reduced to 67°C, and the transition at 69°C disappeared. The weak transition at 93°C could not be interpreted.

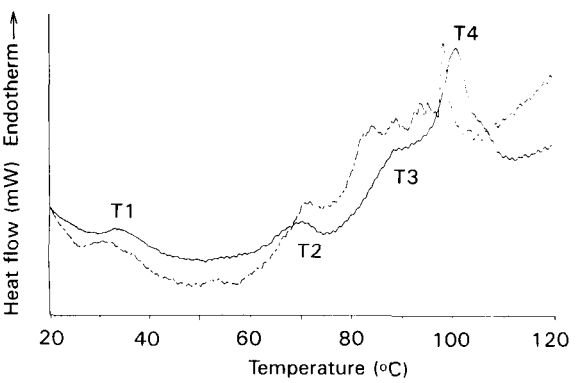

FIG. 4. Comparison of the thermal transitions (T_1, T_2, T_3, T_4) of human stratum corneum hydrated to 40% w/w (——) and stratum corneum pretreated for 4 h with 20% HP-β-CyD (. . . .).

Table 1. Comparison of the fluxes, lag-times, permeability coefficients and enhancement factors of the different formulations in-vivo.

In-vivo formulation: 1% liarozole in	Flux (nmol h^{-1})	Lag-time (min)	Permeability coefficients (cm h^{-1})	Enhancement factor
Water pH 4	0·138–0·151[b]	77–90[b]	$4{\cdot}8–5{\cdot}2 \times 10^{-3}$[b]	1·0[b]
20% HP-β-CyD pH 7[a]	–	>280[b]	–	–
20% D-β-CyD pH 7	0·009–0·091[b]	52–67[b]	$3{\cdot}1–3{\cdot}4 \times 10^{-3}$[b]	0·6[b]
Pretreatment with 20% D-β-CyD	1·258–1·388	21–24	$46{\cdot}8–47{\cdot}9 \times 10^{-3}$	9·4

[a]Steady-state was not reached. [b]From Vollmer et al (1993b).

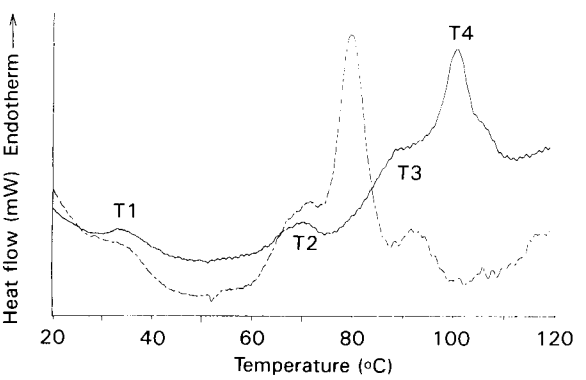

FIG. 5. Comparison of the thermal transitions (T_1, T_2, T_3, T_4) of human stratum corneum hydrated to 40% w/w (——) and stratum corneum pretreated for 4 h with 20% D-β-CyD (–·–·–).

Discussion

For evaluation of the use of cyclodextrins as transdermal penetration enhancers we applied an in-vivo rat model (Vollmer et al 1993a), since this allows direct measurement of absorption into blood without distribution and elimination, in skin under physiological conditions. We observed penetration enhancement by HP-β-CyD but an attenuation of the transdermal absorption by D-β-CyD (Vollmer et al 1993b), which was not expected. The lipophilic drug liarozole has a log P value of 2·88 and needs to be applied at pH 4 to obtain an aqueous solution. At neutral pH, however, the same concentration of liarozole can be obtained by complexation with HP-β-CyD (complex stability constant 8900 M^{-1} as a 1:1 complex (unpublished results)). Obviously liarozole complexed by HP-β-CyD will be absorbed better, whereas complexation by D-β-CyD (complex stability constant 10 152 M^{-1} as a 1:1 complex) decreases penetration. In-vitro results with Franz-typed diffusion cells (data not shown) have demonstrated that pretreatment of the skin with a 20% solution of D-β-CyD, but not HP-β-CyD, before application of liarozole significantly increased the flux. Pretreatment with 20% D-β-CyD in-vivo (Fig. 3) revealed an increase in permeability of 9·4-fold.

To characterize further the mode of action of the cyclodextrins we studied the thermal behaviour of stratum corneum by DSC (Barry 1991). Because of the difficulty in separating stratum corneum from the fully-haired rat skin we used human skin; however, the outermost integument of all mammals has a similar structure as it developed very early in the evolution of life from water to land. According to the findings of Goodman & Barry (1989), the thermal profile at a hydration level of about 40% is reproducible. The precise interpretation of these heat transitions differs in the literature. The third transition is attributed either to protein denaturation (Van Duzee 1975), or to events in the protein-lipid associations at cell membranes (Potts et al 1991) or to the complete disruption of the normal lipid structure of the stratum corneum (Goodman & Barry 1989). The first transition represents melting of sebaceous lipids, the second, a rearrangement of the intercellular lipid structure and the fourth, protein denaturation (a change in the configuration of keratin) (Van Duzee 1975; Goodman & Barry 1989; Potts et al 1991). The thermal profiles of stratum corneum pretreated with HP-β-CyD revealed that this cyclodextrin derivative showed little interaction with the stratum corneum components except for a change in the shape of the third transition, which is difficult to interpret. However, D-β-CyD seems to interact with proteins and the stratum corneum lipid structure as shown by the lowering of the third and fourth transition temperatures and the disappearance of the second transition. The main reason for these changes could be attributed to solvation of the protein structure, thus expanding the structure, and displacement of fatty acids and cholesterol with disordering of the bilayer structure. The extraction of compounds from the skin such as free fatty acids, phospholipids and cholesterols are known from experiments with different cyclodextrins and erythrocytes (Ohtani et al 1989) or liposomes (Miyajima et al 1987). Comparable interactions are described by Sharma & Janis (1991) between surfaces of β-cyclodextrin, but not with its more water-soluble derivatives and lipoproteins. However, the more pronounced interaction of D-β-CyD with the stratum corneum suggests that it will be not as reversible as the more moderate enhancement reached with HP-β-CyD. Although HP-β-CyD may also sequestrate stratum corneum components, the ability of solvation is not as pronounced as that by D-β-CyD and it should be more specific for some lipids that cause a less distinct change in permeability behaviour.

In conclusion, the two cyclodextrin derivatives showed a different mode of action in penetration enhancement as already suggested by Szejtli et al (1986); HP-β-CyD enhances transdermal absorption of liarozole by influencing the distribution and partitioning of the drug in the skin, whereas D-β-CyD enhances liarozole absorption by changing the structure of the permeability barrier.

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