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In vivo skin pharmacokinetics of liarozole: percutaneous absorption studies with different formulations of cyclodextrin derivatives in rats

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Summary

The aim of the study was to evaluate whether 2-hydroxypropyl-β-cyclodextrin (HPβCD) can be used as transdermal absorption enhancer for liarozole. Therefore, we used an in vivo model where transdermal drug absorption in rats can be studied under physiological conditions by cannulating the peripheral skin vein draining the area of the skin which is used for drug application, and collecting the blood (Vollmer et al., 1992a). We compared liarozole, applied as a 1% aqueous solution, in different formulations. We varied the pH, the concentration of HPβCD and tested another cyclodextrin derivative (2,6-dimethyl-β-cyclodextrin, DIMEB). In addition, we compared the absorption with a formulation of 40% propylene glycol/10% oleic acid (PG/OA) and after stripping the stratum corneum. HPβCD was a moderate enhancer at a concentration of 20% (4.9% HPβCD was ineffective) and increased the flux of liarozole at pH 4 from 0.138–0.151 (control) to 0.421–0.487 nmol per h (i.e., 3-fold) after a lag time of 84–104 min (95% limits of confidence), whereas the same formulation at pH 7 did not reach steady state during 4.5 h. The absorption of liarozole in 20% aqueous solution of DIMEB was slightly decreased (by a factor of 0.6), in PG/OA it increased by a factor 1.7 and the flux after stripping the stratum corneum reached 91.4–101.4 nmol/h with a lag time of 10–16 min. Therefore, a 20% aqueous solution of HPβCD appears to be a suitable transdermal absorption enhancer for liarozole.

Introduction

One of the functions of the skin is to protect the internal body components against the external environment and thus to control the passage of chemicals into and out of the body. Nevertheless, the skin is an accepted application site for drug delivery, either for local therapy or, especially during the last decade, for systemic availability. However, the transdermal barrier is often too effective to allow sufficient drug flux and this is the reason for the use of penetration enhancers. According to Barry (1983), a penetration enhancer should have some attributes like pharmacological inertness, controllable and reversible

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enhancing action, chemical and physical stability and compatibility with drugs and pharmaceutical adjuvants, solvent character for drugs, no odour, taste or colour, and last but not least no toxic, irritating or allergenic potency. Of course, these attributes are ideal, but many of them turn out to be true for the adjuvant hydroxypropyl-β-cyclodextrin (HPβCD), namely, stability, solvent character for lipophilic drugs (Mesens et al., 1991), no odour and colour as well as safety and tolerability (Coussement et al., 1990; Monbaliu et al., 1990). Thus, the aim of the present investigation was to evaluate HPβCD as a transdermal absorption enhancer. Therefore, we used an in vivo model in rats, as described by Dehn et al. (1988), but in a modified version (Vollmer et al., 1993a). Under physiological conditions 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. An 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 Wauve et al., 1989), as model permeant. We investigated the influence of pH and HPβCD concentration, the effect of pH in the presence of HPβCD, the influence of dimethyl-β-cyclodextrin (DIMEB) and propylene glycol/oleic acid and permeation without stratum corneum.

Materials and Methods

Experimental set-up

Male Sprague-Dawley rats, weighing about 300 g, were anaesthetized with 1.5 g/kg urethane i.p. and kept at a body temperature of 37°C. The iliolumbal skin vein draining the area which was used for drug application was cannulated to collect the blood for determination of the amount of absorbed drug at 10-min intervals for about 300 min. The blood loss was compensated by infusion of heparinized donor blood from the fourth fraction onwards (Vollmer et al., 1993a). The radiolabelled drug was applied after the second fraction (i.e., after 20 min) as solution of 200 μl in a closed teflon chamber (area of 1.13 cm^2) fixed on the shaved skin with rapid 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. Furthermore, the skin of the application area was taken for measurement of radioactive content at the end of the experiment. Liarozole was applied in all experiments as a 1% aqueous solution (29 mmol/l liarozole, about 445 MBq/mmol specific activity, ^{14}C-labelled). The formulation of liarozole in 40% propylene glycol and 10% oleic acid was applied as an emulsion that was ultrasonificated for 5 min prior to application. For the experiments after stripping the stratum corneum, the stratum corneum of the shaved dorsal skin was removed by three consecutive strippings with cyanoacrylate glue spread on glass slides. They were pressed on the skin for 1 min before removal.

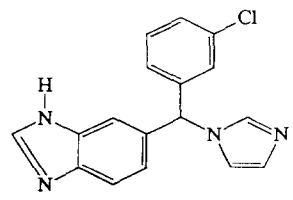

Fig. 1. Chemical structure of liarozole.

Data were analyzed by linear regression of the time courses of the mean cumulative amounts of drug in the collected plasma, using the Statistical Consultans Inc. computer package PCNONLIN. The linear regression model was of the form: (1) $y = mx + b$, where x is the time, y denotes the cumulative amount of penetrated drug in the collected blood samples, m is the slope of the regression line, representing the flux and (2) $y = m(x + a)$, where $-a$ is the lag time until absorption reaches steady state. The permeability coefficient was calculated as the ratio between the flux and the product of area and concentration (integrated form of Fick's first law of diffusion) (Scheuplein, 1967; Flynn et al., 1988). The data are expressed with their 95% confidence limits.

TABLE 1

Comparison of the fluxes, lag times, permeability coefficients and enhancement factors of the different formulations

Formulation: 1% liarozole in	Flux (nmol/h)	Lag time (min)	Permeability coefficient (cm/h) ($\times 10^3$)	Enhancement factor
Water/pH 4/solution	0.138–0.151	77– 90	4.8– 5.2	1.0
Water/pH 7/suspension	0.336–0.353	43– 51	11.6– 12.2	2.4
4.9% HPβCD/pH 4	0.149–0.165	70– 85	5.1– 5.7	1.1
20% HPβCD/pH 4	0.421–0.487	84–104	14.5– 16.7	3.1
20% HPβCD/pH 7	–	> 280	–	–
20% DIMEB/pH 7	0.009–0.091	52– 67	3.1– 3.4	0.6
40% PG, 10% OA/pH 77	0.235–0.246	97–102	8.1– 8.5	1.7
Water/pH 4/stripped SC	91.4 –101.4	10– 16	3152 –3510	666

Data are expressed as 95% confidence limits, except for the enhancement factors which are calculated from the mean values. PG, propylene glycol; OA, oleic acid; SC, stratum corneum.

Drugs

The drugs and chemicals used were urethane (25% in 0.9% NaCl solution, Riedel-de-Haen, Seelze, Germany) and liarozole HCl (^{14}C-labelled and unlabelled, Janssen Pharmaceutica, Beerse, Belgium).

2-Hydroxypropyl-β-cyclodextrin (Janssen Biotech, Lammerdries, Belgium), 2,6-di-*O*-methyl-β-cyclodextrin (Cyclolab, Budapest, Hungary), 1,2-propylene glycol (Merck-Schuchard, Hohenbrunn, Germany), oleic acid (Sigma Chemie GmbH, Deisenhofen, Germany) and heparin-Na 5000 IE/ml (Liquemin 25000, Hoffmann-La Roche, Grenzach-Whylen, Germany) were obtained from the indicated sources. All substances used otherwise were of analytical grade (Merck, Darmstadt, Germany).

Results and Discussion

The transdermal absorption of liarozole (1%) in water of pH 4 reached a steady state of 0.138–0.151 nmol per h after a lag time of 77–90 min (values are 95% limits of confidence; Table 1 and Fig. 2). This experiment was devised as a control

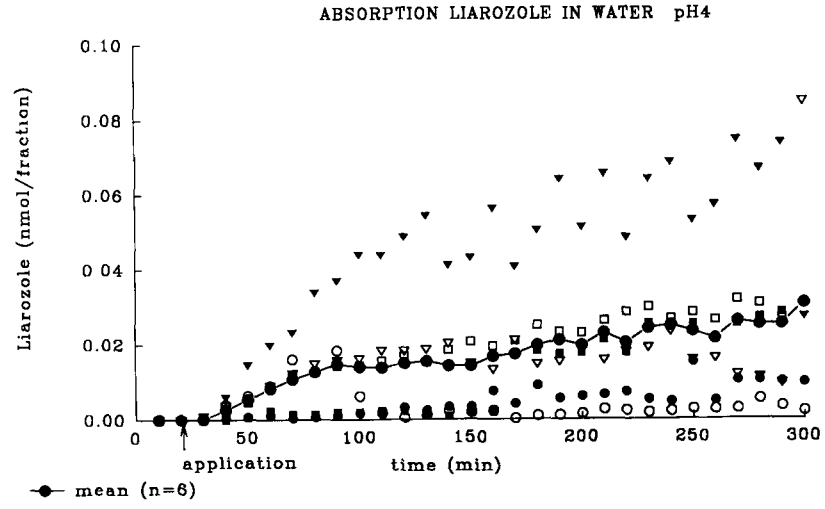

Fig. 2. Time courses of the amount of liarozole absorbed in the blood from an aqueous solution of pH 4. Application followed after 20 min. The closed circles connected by the solid line represent the mean values. The other symbols represent the individual values.

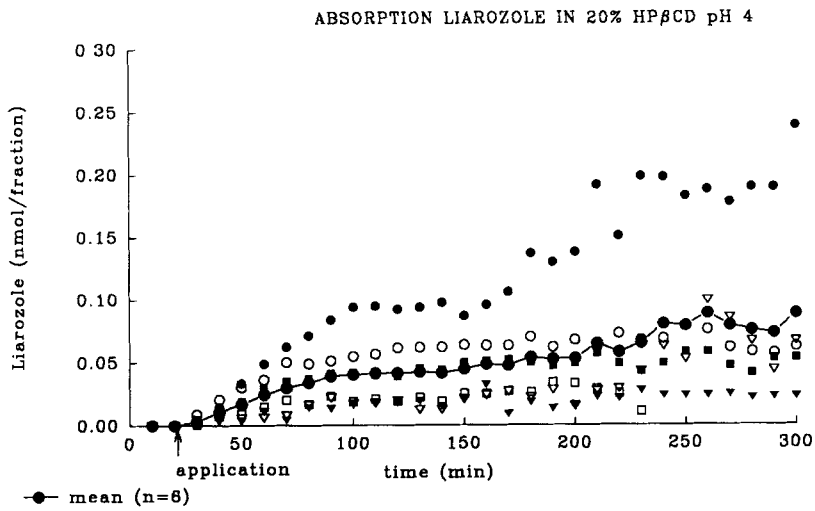

Fig. 3. Time courses of the amount of liarozole absorbed in the blood from a 20% aqueous HPβCD solution of pH 4. Application followed after 20 min. The closed circles connected by the solid line represent the mean values. The other symbols represent the individual values.

experiment to evaluate the differences caused by changes in cyclodextrin concentrations, pH and type of cyclodextrin. The pH of 4 was selected to obtain a solution of the active compound (which is a base with a $pK_{a1} = 4.4$, $pK_{a2} = 6.3$), because only the dissolved drug can be absorbed (Lippold, 1984). In the control experiment as well as in the other experiments, transdermal absorption shows a large interindividual variability. This phenomenon is already known from the high level of barrier variability of human skin (Skelly et al., 1987) and must be accepted as an attribute of biological variability in transdermal absorption studies.

Application of liarozole (1%) in a 4.9% aqueous solution of HPβCD at pH 4 revealed no

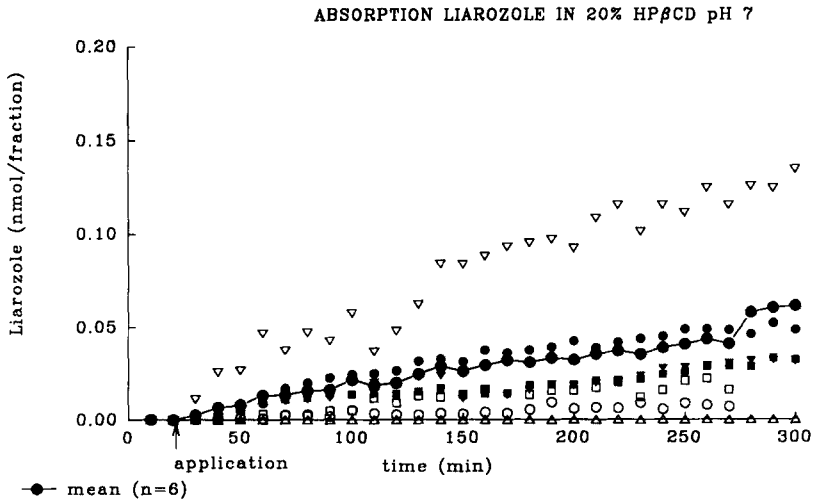

Fig. 4. Time courses of the amount of liarozole absorbed in the blood from a 20% aqueous HPβCD solution pH 7. Application followed after 20 min. The closed circles connected by the solid line represent the mean values. The other symbols represent the individual values.

difference from the control experiments without HPβCD, whereas the 20% aqueous solution of HPβCD under the same conditions enhanced the permeability of liarozole 3-fold to a flux of 0.421–0.487 nmol per h with a nearly unchanged lag time (Table 1 and Fig. 3). This means that the concentration of HPβCD plays an important role in making it act as a penetration enhancer. Although a concentration of 4.9% would be sufficient for solubilizing 1% of liarozole (phase solubility measurements; Peeters, personal communication), this concentration is not efficient in increasing the permeability. Thus, the presence of an excess of HPβCD seems to be necessary.

Since a decrease to pH 4 for solubilisation of the active compound is not necessary in the presence of HPβCD, the same formulation, liarozole in 20% HPβCD, but at pH 7, was used. This revealed a different absorption profile that did not reach a steady state during the 5 h of measurement (Table 1 and Fig. 4) (The plot of the cumulative amount of liarozole vs time in 20% HPβCD pH 7, penetrating the skin, shows no linearity which can be calculated by means of linear regression (graph not depicted). Therefore, the values of 95% limits of confidence cannot be determined.). At the end of the experiments a flux of approx. 0.3 nmol per h was assessed. In this formulation the drug will be complexed in the uncharged state by HPβCD (complex stability constant is 8900 l/mol for a 1 : 1 guest-host complex; Peeters, personal communication. This condition is not comparable with that at pH 4, where HPβCD complexes more liarozole per molecule, thus exhibiting a complexation behaviour that varies with the pH.). Permeability experiments with ^{14}C-labelled HPβCD (Vollmer et al., 1993b) showed that the cyclodextrin derivative itself will be absorbed very quickly after a lag time of 20–28 min and with a flux of 0.994–1.037 nmol per h. Thus, the complex of HPβCD with liarozole shows either a less permeable character or the active compound will be displaced from the cavity by components of the stratum corneum. Such a phenomenon is very probable because the complex stability constant of, e.g., free cholesterol, is much higher than that of liarozole, about 19 000 l/mol (Frijlink et al., 1991) and the content of free cholesterol in the real transdermal barrier, the stratum corneum lipids (Landmann, 1984), is about 29% (Melnik et al., 1986). Furthermore, there are also phospholipids, free fatty acids and proteins in the skin that can be complexed by HPβCD as shown in experiments with different cyclodextrins and erythrocytes (Ohtani et al., 1989) or liposomes (Miyajima et al., 1987).

The aqueous application form of liarozole at pH 7, which is a suspension, showed a very short lag time of 43–51 min and a 2.4-fold increased permeability coefficient in comparison with the same formulation at pH 4 (Table 1) (the permeability coefficient is defined according to Fick's first law as the ratio of the flux and the applied drug concentration and thus is independent of the degree of solubility of the drug in the applied vehicle). The increased absorption of the suspension emphasizes the preferred permeation of compounds through lipoid barriers when they are uncharged (Wiechers, 1989). Moreover, the suspension has a very high thermodynamic activity compared to the solution. Both explain the increased permeability as already mentioned by Barry (1991).

In the experiments with liarozole in a 20% solution of DIMEB at pH 7 the flux was decreased to 0.009–0,091 nmol per h (Table 1). The complex stability constant of DIMEB is slightly higher (10 152 l/mol) than for HPβCD and DIMEB has a greater lipophilicity than HPβCD and thus it is suspected that liarozole is retained in the layers of the stratum corneum instead of appearing in blood.

In the experiments with liarozole in a formulation of 40% propylene glycol and 10% oleic acid (emulsion of pH 7), absorption during the steady state of 0.235–0.246 nmol per h proceeded later than in the formulation with 20% HPβCD at pH 4 (Table 1). It is known that propylene glycol is not in all cases a penetration enhancer, but that its enhancing capacity depends very strongly on the compound used (Aungst et al., 1990). Nevertheless, it is very commonly applied as a solubilizer and penetration enhancer in transdermal drug application (Walters, 1989). In this example, however, it is possible that the quite lipophilic liarozole (log $P = 2.88$) will accumulate in the oil

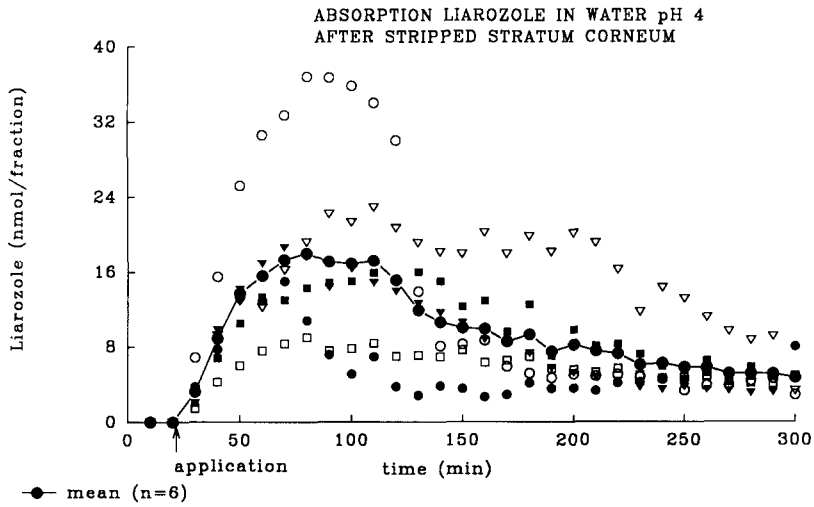

Fig. 5. Time courses of the amount of liarozole absorbed in the blood from an aqueous solution pH 4 after stripped stratum corneum. Application followed after 20 min. The closed circles connected by the solid line represent the mean values. The other symbols represent the individual values.

phase of oleic acid and thus will not be available for cosolvency by propylene glycol.

Absorption of liarozole in the same formulation as in the control experiments, but after stripping the stratum corneum, revealed a considerable flux of 91.4–101.8 nmol per h with nearly no lag time (10–16 min) (Fig. 5). This was expected for penetration without a barrier and demonstrates at the same time the effectiveness of this barrier. Furthermore, this experiment demonstrated that the absorption enhancement with cyclodextrin derivatives is by no means the ultimate enhancement which can be reached. The concentration-time curve shows only a steady state of about eight fractions, the penetration decreasing thereafter since the concentration of the applied solution at the end of the experiment was reduced to 30–60% of the originally applied concentration thus reflecting elimination-like kinetics.

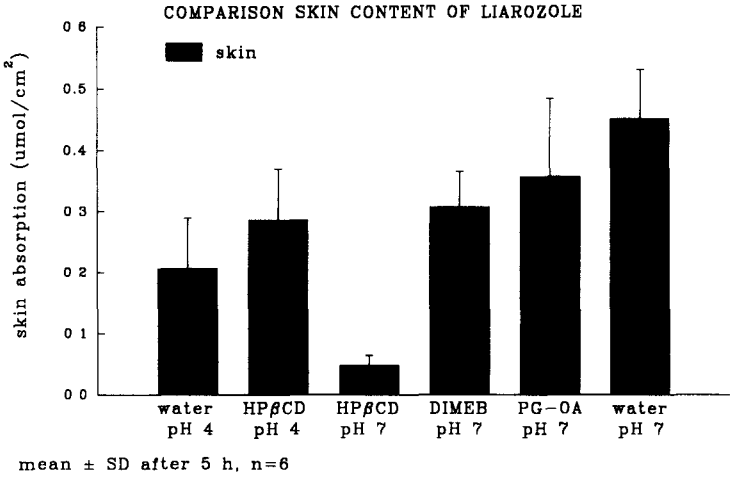

Fig. 6. Comparison of the amount of liarozole in the application area of the skin as obtained after 5 h. Values are the mean of six experiments.

The amounts of liarozole in the skin in the application area (1 cm^2) after 5 h of permeability measurements are shown in Fig. 6. In comparison with the amount of absorbed liarozole in blood during 5 h they show less interindividual variability. In general, the amount measured in the skin at the end of the experiment is about three-tenths of a power higher than the amount absorbed in the blood at the same time. Remarkably, the amount of liarozole in the skin is very low in the case of the formulation of liarozole in 20% HPβCD at pH 7, although the absorption in blood was enhanced (Fig. 4), whereas the amount in the skin on application of liarozole in DIMEB and propylene glycol/oleic acid is rather high although the blood levels were less enhanced if at all (Table 1). The suspension (liarozole in water at pH 7) had the highest skin content of all experiments. The phenomenon of high skin content after suspension application was described by Scheuplein and Ross (1974), who postulated that the solid drug dissolves preferentially in the stratum corneum instead of in the vehicle. Thus, the partitioning of the drug is influenced by the formulation in a manner such that HPβCD as a 20% (pH 4) aqueous solution will target liarozole into the blood, which means it will pass the deeper layers of skin like the basal membrane responsible for development of the epidermis, and become available for systemic efficiency. On the other hand, liarozole applied in DIMEB, propylene glycol/oleic acid mixture or the suspension will result in high skin concentrations, available for local efficiency and acting as a reservoir. Furthermore these data support the finding of the permeability experiments, that the two different cyclodextrin derivatives applied may have a different way of interacting with the skin as pointed out by Szejitli et al. (1986).

In conclusion, this in vivo model is useful to evaluate the influence of different drug formulations such as pH changes, concentration differences or addition of various enhancers on the transdermal absorption behaviour of a drug. In the case of liarozole, a 20% aqueous solution of HPβCD seems to be suitable to enhance moderately the transdermal absorption into blood.

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