ELSEVIER

International Journal of Pharmaceutics 200 (2000) 127–132

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Influence of cyclodextrin complexation on the in vitro permeation and skin metabolism of dexamethasone

Renata F.V. Lopez [a], John H. Collett [b], M. Vitória L.B. Bentley [a,*]

[a] *Faculty of Pharmaceutical Sciences at Ribeirão Preto, University of Sáo Paulo, Avenida Café s/n, 14040-903, Ribeirao Preto, SP, Brazil*

[b] *Department of Pharmacy, University of Manchester, Oxford Road, Manchester M13 9PL, UK*

Received 27 September 1999; received in revised form 20 January 2000; accepted 7 February 2000

Abstract

The influence of complexation of a model drug, dexamethasone acetate (DMA), with β-cyclodextrin (β-CyD) and hydroxypropyl- β-cyclodextrin (HP-β-CyD) on the in vitro permeation through hairless mouse skin and on skin metabolism have been investigated. Complexation with CyDs increased the amount of DMA permeated in the order of 2.0 and 3.0 times for β-CyD and HP-β-CyD, respectively. The partition coefficient, between stratum corneum and buffer ($K_{SC/buffer}$), for DMA decreased when the drug was an inclusion complex, being greatest for DMA/HP-β-CyD complex. Complexation protected the drug against skin metabolism. The increase of skin permeation and stability of the model drug in the skin suggest that the complexation with β-CyD and HP-β-CyD is a rational way to improve the physical-chemical properties of drugs for use in transdermal delivery systems.

Keywords: Inclusion complexes; Cyclodextrin; In vitro skin permeation; Skin metabolism

1. Introduction

Ideal characteristics of a drug for transportation across the skin include low interaction with the skin and no metabolism by the skin. High lipophilicity and degradation by skin esterases render many drugs unsuitable for transdermal delivery (Potts et al., 1989; Singh and Roberts, 1994; Phillips and Michniak, 1995; Bast, 1997).

One potential method of optimizing the efficacy of drug activity is through the use of rationally designed drug carrier materials such as cyclodextrins (CyDs). They are cyclic oligosaccharides consisting of six, seven or eight glucopyranose units, usually referred to as α, β or γ-CyD, respectively. The CyD cavity exhibits a hydrophobic environment, whereas the exterior of the molecule is hydrophilic. The CDs are capable of forming a variety of complexes in which guest molecules are trapped entirely, or at least partially by the hydrophobic portion. This entrapment leads to changes in the physical–chemical properties of the

* Corresponding author. Fax: + 55-16-6024300.
E-mail address: vbentley@usp.br (M.V.L.B. Bentley)

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00365-3

molecules (Lemesle-Lamache et al., 1996), which can improve their molecular stability and bioavailability. Our previous work, showed that the complexation of a lipophilic drug (DMA) with CyDs can increase boths its solubility and stability in water (Vianna et al., 1998).

In this work we have investigated the influence of the complexation of a lipophilic drug, with CyDs on in vitro permeation and stability to skin metabolism.

2. Material and methods

2.1. Material

β-cyclodextrin (β-CyD) and hydroxypropyl-β-cyclodextrin (HP-β-CyD) were obtained from Roquette. Dexamethasone acetate (DMA), dexamethasone (DM) and desonide were obtained from Sigma Chemical (St Louis, MO). All other reagents were BDH or HPLC reagent grade. The HP-β-CyD used in this work had specified degrees of substitution (number of hydroxypropyl groups per β-CyD unit) ranging from 3.85 to 4.55.

2.2. Solid inclusion complexes

The solid complexes of DMA/β-CyD and DMA/HP-β-CyD were obtained by freeze drying as described previously (Vianna et al., 1998). The stoichiometric ratio was 1:1 for both complexes. The solid complexes were kept in a dessicator over silica-gel prior to use.

2.3. HPLC analysis

Analysis of all samples was performed by a Shimadsu Instruments HPLC System, model 5000. UV detector at 254 nm. C_{18} reversed-phase column 125 × 4 mm (5 μm), C_{18} pre-column 4 × 4 mm (5 μm), Intralab 4290 integrator, and 0.01 AUFS. A methanol:water (60:40) mixture was used as the mobile phase, at a flux of 1 ml/min and an injection volume of 20 μl. The extraction was carried out using chloroform. Desonide in methanolic solution (200 ng/ml) was used as internal standard. The retention time for the degraded product dexamethasone (DM), internal standard and DMA were 4.3, 5.4 and 6.9 min, respectively. The assay was linear between concentration of 50–400 ng DMA/ml (r, 0.99) with an injection variability of $< 1\%$ for intra-day variation, and $< 3\%$ for inter-day variation.

2.4. Partition coefficient stratum corneum buffer ($K_{SC/buffer}$)

Stratum corneum (SC) samples were obtained by floating dorsal full thickness hairless mouse for 14 h on a solution of 0.1% w/v trypsin and 0.5% w/v sodium bicarbonate at RT; followed by rinsing of the SC sheets with distilled water. SC samples were spread on filter paper and dried by storage in a desiccator over silica-gel, for at the most 1 week prior to use.

$K_{SC/buffer}$ was determined by agitating gently SC samples in isotonic phosphate buffer (pH 7.2) containing DMA free or complexed at the concentration of 1.0 mg/ml in DMA for periods of time up to 1 h. After allowing to stand, the supernatant was removed and centrifuged for 10 min at 2000 rev·min^{-1}. The aqueous phase was assayed by HPLC at time zero (C_0) and after shaking to ensure partition (C_t). The partition coefficient was $K_{SC/buffer} = (C_0 - C_t)/C_t$. It was found that the values of $K_{SC/buffer}$ were invariant after an agitation period of 5 min and this time period was adopted as a standard equilibration period.

2.5. In vitro permeation

DMA free or complexed was incorporated into hydrophilic gels, hydroxyethyl cellulose (2.0% w/w) in water. Cyclodextrins (β-CyD 1% w/w or HP-β-CyD 10% w/w) were added in order to mantain the solubility of DMA. Full-thickness skin was excised from the abdominal surface of 4–6 weeks old hairless mice HRS/J strain (Jackson Laboratories, Bar Harbor, ME) and mounted in a modified Franz diffusion cell. The available diffusion area of the diffusion cell was 2.54 cm^2. Receptor phase was isotonic phosphate buffer (pH 7.2) with 0.5% w/v of polyoxyethylene 20 cetyl ether and 0.01% w/v of thiomersal. 1.5 g of

the formulations (infinite dose) was placed on the membrane. Samples from the receptor phase were withdrawn at predetermined times over a 24 h period, and the amount of DMA present was analysed by HPLC. After 24 h, the skin was removed, cleaned with cotton soaked in methanol, homogeneized in methanol, filtered, and the amount of DMA assayed.

Permeation profiles were constructed by plotting the total amount of DMA transported across the hairless mouse skin ($\mu g/cm^2$) against time (h). The flux of the drug across the membrane (J) was calculated from the slope of the graph and expressed as $\mu g/cm^2 \cdot h$.

2.6. In vitro skin metabolism

Hairless mouse skin was homogenized with isotonic phosphate buffer (pH 7.2) using a tissue homogenizer to suspend all the tissue. Samples were centrifuged at 3000 rev·min^{-1} at 4°C for 15 min. Protein concentration of the supematant was determined using a Bradford dye-binding assay with γ-globulin as the standard protein (Sapan et al., 1999). The homogenates were adjusted to 2 mg protein/ml for the metabolism studies.

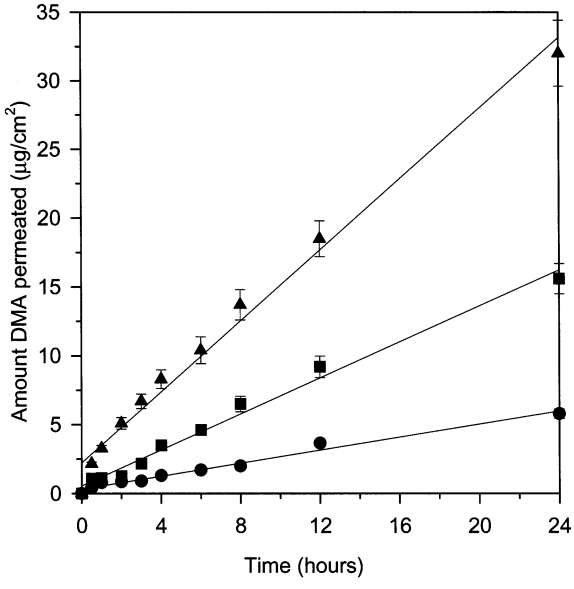

Fig. 1. In vitro permeation profile of DMA and complexes through hairless mouse skin from hydrophilic gels: (●) DMA free; (■) DMA/β-CyD and (▲) DMA/HP-β-CyD.

Homogenate supernatant was incubated at 37°C for 10 min before the start of the study. At time 0 an amount of ethanolic solutions of DMA, DMA/β-CyD or DMA/HP-β-CyD was added in order to obtain a final concentration of 2.3 μM in DMA. Aliquots were removed at appropriate times and the content of DMA remaining and the degradation product (DM) were analysed by HPLC. Controls using phosphate buffer instead of skin homogenate supernatant were also carried out.

2.7. Statistics

The values are expressed as mean ± S.D. of mean. Comparisons were made using the Kruskal–Wallis nonparametric ANOVA test and post-test Dunn's multiple comparisions. Differences were considered significant when P-values were < 0.05.

3. Results and discussion

Fig. 1 shows the in vitro permeation profiles of DMA and DMA/CyD complexes through hairless mouse skin. A linear relationship was obtained when the total amount of DMA/cm^2 in the receptor phase was plotted against time, indicating that the hairless mouse skin is permeable to DMA and that the percutaneous transport can be described by zero order kinetics. Table 1 shows the permeation parameters for free and complexed DMA.

It can be seen that complexation with DMA/β-CyD and DMA/HP-β-CyD increased the amount of DMA transfered across the hairless mouse skin. After 24 h the amount of DMA permeated was 5.8 $\mu g/cm^2$ for the DMA alone. However, when complexed with β-CyD and HP-β-CyD then 15.6 $\mu g/cm^2$ and 32.0 $\mu g/cm^2$ were transfered, respectively. The permeation as measured by the amount transfered was significantly improved when complexed with either CyDs ($P < 0.05$). The flux increased when DMA was in a complexed form. In other words, the percutaneous absorption of DMA was improved when it was complexed with CyDs. It is probable that the CyD increases the DMA flux by increasing drug

Table 1
In vitro percutaneous absorption data of DMA and DMA/CyD complexes through hairless mouse skin after 24 h[a]

Compound	J ($\mu g/cm^2 \cdot h^{-1}$)	DMA permeated ($\mu g/cm^2$)[b]	DMA retained ($\mu g/g$)[c]
DMA free	0.24	5.8 ($\pm$0.34)	10.1 ($\pm$0.6)
DMA/β-CyD	0.65	15.6 ($\pm$1.1)	1.3 ($\pm$0.03)
DMA/HP-β-CyD	1.29	32.0 ($\pm$2.4)	1.4 (0 $\pm$ .09)

[a] Results are expressed as mean $\pm$ S.D. ($n = 5$); abbreviation: J, flux.
[b] DMA free versus DMA/β-CyD and DMA/HP-β-CyD significant ($P < 0.05$ and $P < 0.001$, respectively); DMA/β-CyD versus DMA/HP-β-CyD significant ($P < 0.05$).
[c] DMA free versus DMA/β-CyD and DMA/HP-β-CyD significant ($P < 0.01$ and $P < 0.05$, respectively); DMA/β-CyD versus DMA/HP-β-CyD not significant ($P > 0.05$).

availability at the surface of the skin. In addition, the skin retention decreased for DMA/CyD complexes ($P < 0.05$) in contrast to the enhanced percutaneous absorption. It is interesting to consider that the HP-β-CyD might also improve cutaneous drug permeability as a result of the extraction of lipid from the SC (Bentley et al., 1997). Arima et al. (1996) have found that the complexation of a prodrug ethyl-4-biphenylylacetic acid, with CyDs increased its permeation through hairless mice skin. In other work, Arima et al. (1998) reported that pre and post-application of ointment containing HP-β-CyD onto hairless mouse skin did not affect the skin permeation or the conversion of prodrug, but the flux of active drug/HP-β-CyD complex through tape-stripped skin was greater than through full thickness skin, while the conversion of prodrug in skin was slowed by tape stripping. However, Williams et al. (1996), found that the pre-treatment of skin with β-CyD and HP-β-CyD resulted in reduced permeation of toluene through human SC. In the case of pre-treatment with CyDs, the drug can have its cutaneous permeability increased only if does not form an inclusion complex (Legendre et al., 1995). It might be suggested that the method used to assess the effect of CyDs might influence the skin-drug permeation profile (Bentley et al., 1997).

The SC, a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an intercellular lipid-rich matrix, is a known permeation barrier to the transdermal delivery of most drugs (Barry et al., 1983). Because the SC is the first and main barrier for drug permeation through skin, it is interesting to verify the partition of drugs. Table 2 shows that the $K_{SC/buffer}$ was decreased by complexation suggesting that complexation might be decreasing the affinity of DMA for the SC, probably due to the aqueous solubility of the drug being enhanced. This decrease in $K_{sc/buffer}$ is in accordance with Másson et al. (1999), who also predicted decreased partition coefficient with increasing CyD concentration. Since passive diffusion is driven by high drug concentration in the aqueous drug vehicle, the increase in DMA aqueous solubility can explain the improvement of the percutaneous absorption of DMA through the hairless mouse skin by complexation, being greatest when HP-β-CyD was the complexing agent.

Metabolism profiles were developed for DMA free and DMA/CyD complexes in the supernatant of the homogenate of hairless mouse skin in phosphate buffer, at 37°C. Linearity was observed when log concentration remaining was plotted as a function of time, indicating that DMA degradation was first order. Degradation constants (K_{obs})

Table 2
Partition coefficient of DMA between hairless mouse SC and phosphate buffer ($K_{SC/buffer}$) at 25°C[a]

Compound	K_{SC} buffer
DMA free	13.5 ($\pm$1.10)
DMA/β-CyD	1.1 ($\pm$0.06)
DMA/ HP-β-CyD	2.2 ($\pm$0.12)

[a] Results are expressed as mean $\pm$ S.D. ($n = 5$).Statistic-DMA free versus DMA/β-CyD and DMA/HP-β-CyD significant; ($P < 0.001$ and $P < 0.05$, respectively); DMA/β-CyD vs DMA/HP-β-CyD significant ($P < 0.05$).

Table 3
Degradation constants (K_{obs}) and the time for 10% DMA degradation (t_{90}) at 37°C

Compound	$K_{obs} \cdot 10^{-3}$ (min^{-1})[a]	T_{90} at 37°C (min)[b]
DMA free	6.9	15
DMA/β-CyD	4.5	39
DMA/HP-β-CyD	4.4	40

[a] Obtained from degradation curve.
[b] $t_{90} = 2.303/K \log C_0/C_{90}$.

and the time for 10% DMA degradation (t_{90}) were calculated and are presented in Table 3.

After 2 h incubation, about 30 and 65% of the DMA was degraded by skin metabolism for DMA complexed and free, respectively. Complexation with β-CyD and with HP-β-CyD produced the same decrease in degradation. The times to achieve 10% hydrolysis of DMA were 15 and 40 min for DMA free and DMA complexed with CyDs, respectively (Table 3). The possible explanation for the protective effect of these CyDs is that the ester group is at least partially enclosed and hence shielded against the esterases attack. Our previous work verified that the CyD complexation can decrease the hydrolysis of the ester group of DMA molecule in aqueous solution (Vianna et al., 1998). The phosphate buffer solutions of DMA and DMA/CyD complexes did not show degradation of DMA during the period that the experiment were carried out, indicating that the degradation observed with the skin homogenate may be due to the action of esterases. Arima et al. (1998) reported that HP-β-CyD reduced the degradation of ethyl-4-biphenylylacetic acid in skin homogenate.

The results obtained in this work indicate that enhancing effect of the complexation with CyDs on the skin permeation of DMA can be mainly ascribed to an increase in the solubility of the drug. The protective effect of CyDs against skin metabolism leads to the suggestion that CyDs complexation may promote changes in the physico-chemical properties of drugs thereby improving their characteristics for use in transdermal delivery. However, the potential for cyclodextrin to affect degradation directly in the skin must be limited in that most cyclodextrin are not absorbed from the skin due to their hydrophobicity and high molecular weight.

Acknowledgements

The authors would like to thank the Fundação para o Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil for supporting this research.

References

Arima, H., Miyaji, T., Irie, T., Hirayama, F., Uekama, K., 1998. Enhancing effect of hydroxypropyl-beta-cyclodextrin on cutaneous penetration and activation of ethyl-4-biphenyl acetate in hairless mouse skin. Eur. J. Pharm. Sci. 6, 53–59.

Arima, H., Miyaji, T., Irie, T., Hirayama, F., Uekama, K., 1996. Possible enhancing mechanism of the cutaneous permeation of 4-biphenylylacetic acid by beta-cyclodextrin derivatives in hydrophilic ointment. Chem. Pharm. Bull. 44, 582–586.

Barry, B.W., 1983. Dermatological Formulations. Percutaneous Absorption. Drugs And The Pharmaceutical Sciences. Marcel Dekker, New York.

Bast, G.E., 1997. Influence of solubility and permeant size on metabolism of xenobiotics in rabbit skin. Hum. Exp. Toxicol. 16, 435–440.

Bentley, M.V.L.B., Vianna, R.F., Wilson, S., Collett, J.H., 1997. Characterization of the influence of some cyclodextrins on the stratum corneum from the hairless mouse. J. Pharm. Pharmacol. 49, 397–402.

Legendre, J.Y., Rault, I., Luijten, W., et al., 1995. Effects of (-cyclodextrins on skin: implications for the transdermal delivery of piribedil and a novel cognition enhancing-drug. Eur. J. Pharm. Sci. 3, 311–322.

Lemesle-Lamache, V., Wouessidjewe, D., Chéron, M., Duchêne, D., 1996. Study of (-cyclodextrin and ethylated (-cyclodextrin salbutamol complexes, in vitro evaluation of sustained-release behaviour of salbutamol. Int. J. Pharm. 141, 117–124.

Másson, M., Loftsson, T., Másson, G., Stefánsson, E., 1999. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. J. Control. Release 59, 107–118.

Phillips, C.A., Michniak, B.B., 1995. Transdermal delivery of drugs with differing lipophilicities using azone analog as dermal penetration enhancers. J. Pharm. Sci. 84, 1427–1433.

Potts, R.O., McNeill, S.C., Desbonnet, C.R., Wakshull, E., 1989. Transdermal drug transport and metabolism II: the role of competing kinetc events. Pharm. Res. 6, 119–124.

Sapan, C.V., Lundblad, R.L., Price, N.C., 1999. Colorimetric protein assay techniques. Biotechnol. Appl. Biochem. 2, 99–108.

Singh, P., Roberts, M.S., 1994. Skin permeability and local tissue concentrations of nonsteroidal anti- inflamatory drugs after topical application. J. Pharmacol. Exp. Ther. 268, 144–151.

Vianna, R.F.L., Bentley, M.V.L.B., Ribeiro, G., et al., 1998. Formation of cyclodextrin inclusion complexes with corticosteroids: their characterization and stability. Int. J. Pharm. 167, 205–213.

Williams, A.C., Shatri, S.R.S., Barry, B.W., 1996. Transdermal permeation inhibition by cyclodextrins. Pharm. Res. 13, 379.