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The effect of polyvinylpyrrolidone on cyclodextrin complexation of hydrocortisone and its diffusion through hairless mouse skin

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Abstract

The effect of nine different cyclodextrin derivatives on the aqueous solubility of hydrocortisone was investigated. Three of these derivatives were selected for further investigation. The effect of methyl-β-cyclodextrin, carboxymethyl-β-cyclodextrin and maltosyl-β-cyclodextrin on the flux of hydrocortisone, from an aqueous suspension or solution, through hairless mouse skin was investigated in vitro. When the hydrocortisone concentration was kept constant and the cyclodextrin concentration was gradually increased, the flux through the skin was increased up to a point when all the hydrocortisone molecules were in solution, then the flux decreased with increasing cyclodextrin concentration. Polyvinylpyrrolidone (PVP) increased the complexation of hydrocortisone which increased solubility of the drug in the aqueous cyclodextrin solutions. Also, the flux of hydrocortisone through hairless mouse skin was increased by addition of small amount of PVP to the cyclodextrin vehicle. The flux of hydrocortisone could be increased even further by addition of oleic acid to the aqueous cyclodextrin PVP vehicle systems.

Keywords: Hydrocortisone; Transdermal delivery; Solubility; Complexation; Cyclodextrin; Polymers; Stability constant; Skin

1. Introduction

The main obstacle to dermal and transdermal delivery of drugs is the thin outermost layer of the skin, the stratum corneum. Usually, a drug molecule must first penetrate this barrier in order to reach its site of action and the clinical usefulness of many drugs is frequently limited by their inability to penetrate. It is well known that various vehicle additives, so called penetration enhancers, can affect the skin barrier and thereby influence the permeability of drugs into or through the skin. Thus, conventional penetration enhancers temporarily alter or damage the skin barrier (Loftsson et al., 1989).

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic cavity in the center. They are able to

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form complexes with various guest molecules by taking up a whole molecule, or some part of it, into the cavity. The cyclodextrin complexation can improve the chemical stability and/or the aqueous solubility of the guest molecules (Duchêne and Wouessidjewe, 1993, Frömming and Szejtli, 1994). The complex formation does not involve the formation or breaking of covalent bonds. The cyclodextrin drug complexes are easily dissociated and free molecules are in fast equilibrium with the molecules bound within the cyclodextrin cavity (Loftsson et al., 1991; Loftsson and Sigurðardóttir, 1994a). The most commonly known cyclodextrins are made up of six (αCD), seven (βCD) and eight (γCD) α-1,4 linked glucopyranose units, but various derivatives of these three parent cyclodextrins have been made. The size of the central cavity is determined by the number of glucopyranose units forming the cyclodextrin (Frömming and Szejtli, 1994). We have previously reported (Loftsson and Sigurðardóttir, 1994a) that the transdermal delivery of hydrocortisone in an aqueous solution of 2-hydroxypropyl-β-cyclodextrin (HPβCD) can be increased by addition of small amount of polyvinylpyrrolidone (PVP) or hydroxypropyl methylcellulose (HPMC). The largest flux was obtained when just enough HPβCD was used to solubilize hydrocortisone in the aqueous vehicle. When an excess amount of HPβCD was added to the aqueous vehicle the flux was significantly decreased. The excess amount of cyclodextrin in the solution leads to a decreased amount of free hydrocortisone molecules in the aqueous solution. The cyclodextrin molecules act as a drug carrier delivering the lipophilic drug molecules to the skin surface where they can penetrate into the skin.

Cyclodextrins are generally poorly absorbed through the skin. On the other hand, the combined action of penetration enhancers and occlusive treatment with ointment bases can result in some cyclodextrin penetration into the skin (Arima et al., 1990; Duchêne and Wouessidjewe, 1991). Pre-treatment of rabbit skin with cyclodextrins did not enhance dermal drug penetration (Uekama et al., 1987). However, cyclodextrins, such as MβCD, have been shown to enhance the penetration of drugs through skin by modifying the skin barrier (Okamoto et al., 1986; Vollmer et al., 1992 and Vollmer et al., 1993). The permeation-enhancing effects of cyclodextrins and conventional penetration enhancers has been shown to be additive, to some extent. Thus, Seo et al. (1992) observed that the combined action of HPβCD and some unsaturated fatty acids improved the transdermal penetration of isosorbide dinitrate through skin more effectively than HPβCD alone.

Solubility experiments (Loftsson et al., 1994b) have shown that the optimal concentration of PVP for increasing the HPβCD complexation of hydrocortisone is 0.25% (w/v). PVP does not have significant effect on the viscosity of the cyclodextrin solutions (Loftsson et al., 1994b). The purpose of the present study was to investigate the effect of PVP on the ability of cyclodextrins to form complexes with hydrocortisone and the hydrocortisone permeability through hairless mouse skin. The effect of combined action of oleic acid and MβCD on the penetration of hydrocortisone was also investigated.

2. Materials and methods

2.1. Materials

Hydrocortisone was obtained from Norsk Medisinaldepot (Norway), 2-hydroxypropyl-β-cyclodextrin of molar substitution 0.6 (HPβCD), methyl-β-cyclodextrin (MβCD) of molar substitution (MS) 0.6, MβCD MS 1.8, carboxymethyl-β-cyclodextrin Na-salt MS 0.6 (CMβCD), methyl-γ-cyclodextrin (MγCD) MS 0.6, MγCD MS 1.8, 2-hydroxy-3-trimethylammoniopropyl-β-CD MS 0.5 (HTMAPβCD), and acetyl-β-CD (AβCD) from Wacker-Chemie (Germany), glucosyl-β-cyclodextrin (glucosylβCD) and maltosyl-β-cyclodextrin (maltosylβCD) from Ensuiko sugar refining Co. Ltd. (Japan), polyvinylpyrrolidone of molecular weight 360,000 (PVP) and oleic acid from Sigma Chemical Co. (USA) and Brij-58 from Aldrich Chemical Company (USA). All other chemicals were commercially available products of special reagent grade. Female hairless mice (3CH/Tif hr/hr) were obtained from Bommice (Denmark).

2.2. Solubility studies

Solubilities were determined by adding excess amount of hydrocortisone to aqueous solutions of various amounts of different kinds of cyclodextrins with or without 0.25% (w/v) PVP. The suspensions formed were heated in an autoclave in sealed containers to 12°C for 20 min, and then allowed to equilibrate for at least 3 days at room temperature (approx. 23°C). After equilibration was attained, an aliquot of the suspension was filtered through a 0.45 μm membrane filter (Millex-HV filter units from Millipore, USA); it was then diluted with 70% (v/v) methanol in water and analysed by HPLC.

The stability constants (K_c) of hydrocortisone-cyclodextrin complexes were calculated from the slope of the phase-solubility diagrams and the solubility of hydrocortisone in water (S_o) (Higuchi and Connors, 1965):

$$K_c = slope\ (S_o(1 - slope))^{-1}$$

2.3. Skin permeation studies

Female hairless mice were sacrificed by cervical dislocation and their full-thickness skins removed. Subcutaneous fat and other debris were carefully removed from the under surface of the skin. The outer surface of the skin was rinsed with 35% (v/v) methanol in water and subsequently with distilled water to remove any contamination. The skin was placed in Franz diffusion cells of type FDC 400 15 FF (Vangard International Inc., USA). The receiver compartment had a volume of 12.3 ml. The surface area of the skin in the diffusion cell was 1.77 cm^2. The receptor phase consisted of phosphate buffer saline pH 7.4 (Ph.Eur., 2nd Ed., VII.1.3.) containing 0.3% (w/v) Brij-58, and 0.4% v/v formaldehyde as a preserving agent. The receptor phase was sonicated under vacuum prior to usage to remove dissolved air. The skin diffusion cells were stirred with a magnetic bar and kept at 37°C by circulating water through an external jacket. The donor phase consisted of suspension or solution of hydrocortisone in aqueous cyclodextrin solution, or aqueous cyclodextrin solution containing 0.25% (w/v) PVP, which had been heated in an autoclave (120°C for 20 min). After equilibration for 3 days at room temperature, 2 ml of the donor phase were applied to the skin surface and the donor chamber covered with parafilm. Samples (150 ml) were withdrawn from the receptor phase every 12 h for 3 days and replaced with fresh buffer solution. The samples were kept frozen until analysed by HPLC. Each experiment was repeated at least three times and the results reported are the mean values ± standard error of the mean (S.E.) in mg hydrocortisone h^{-1} cm^{-2}. Less than 2% of hydrocortisone applied to the skin surface penetrated into the receptor phase during the 3 day period.

2.4. Analytical methods

The quantitative determination of hydrocortisone was performed on a high performance liquid chromatographic (HPLC) component system consisting of ConstaMetric 3000 solvent delivery system operated at 1.50 ml/min, a Merck-Hitachi AS4000A autosampler, a Beckman Ultrasphere ODS 5 mm (4.6 × 150 mm) column, a Spectra-Physic SP8450 UV/VIS variable-wavelength detector operated at 242 nm and a Merck-Hitachi D-2500 Chromato-Integrator. The mobile phase consisted of acetonitrile, tetrahydrofuran, acetic acid and water (35:1:1:63) and the retention time was 2.3 min.

3. Results and discussion

The phase-solubility diagram of hydrocortisone in aqueous solutions of nine different cyclodextrins are shown in Fig. 1 and Fig. 2. At cyclodextrin concentration below 10% (w/v) the solubility diagrams were all of Higuchi's A_L-type, i.e. linear increase was observed with unchanged stoichiometry. At higher concentrations CMβCD, maltosylβCD and glucosylβCD show solubility curves which are of Higuchi"s A_N-type, that is show a negative deviation from linearity (Frömming et al., 1994). Of the somewhat lipophilic methylated cyclodextrins, the ones with a low molar substitution (MS) were generally better sol-

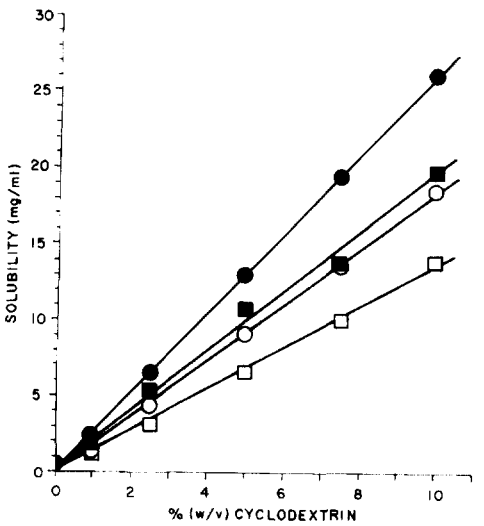

Fig. 1. The phase-solubility diagrams of hydrocortisone in aqueous MβCD MS 0.6 (●), MβCD MS 1.8 (○), MγCD MS 0.6 (■) and MγCD MS 1.8 (□) solution.

ubilizers than cyclodextrins with a high MS (Fig. 1). The hydrophilic cyclodextrins were, as a group, less effective than the methylated cyclodextrins (Fig. 2). The value of the stability constants (K_c) for selected hydrocortisone-cyclodextrin complexes, both when no PVP was present and in the presence of 0.25% (w/v) PVP, are shown in Table 1. Addition of PVP to the cyclodextrin solutions resulted in a 14–186% increase in K_c.

The effect of cyclodextrin complexation on the

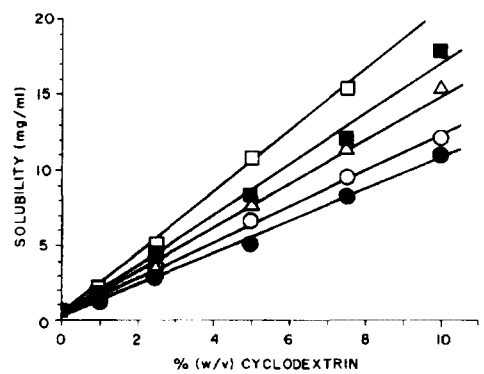

Fig. 2. The phase-solubility diagrams of hydrocortisone in aqueous HPβCD (○), HTMAPβCD (●), CMβCD (□), glucosylβCD (■) and maltosylβCD (△) solution.

hydrocortisone permeability through hairless mouse skin was also investigated. Three different cyclodextrin derivatives were selected for this study, i.e. MβCD MS 1.8, maltosylβCD and CMβCD, with or without PVP. The vehicle consisted of dissolved or suspended hydrocortisone in aqueous cyclodextrin solution. The initial hydrocortisone concentration in the MβCD, maltosylβCD and CMβCD aqueous vehicle systems was 2.3%, 1.6% and 1.3% (w/v), respectively. The hydrocortisone flux from the aqueous vehicles containing the hydrocortisone-CMβCD, hydrocortisone-MβCD MS 1.8 or hydrocortisone-maltosylβCD complex (Fig. 3, Fig. 4 and Fig. 5) was in a good agreement with what we observed previously for the hydrocortisone-HPβCD complex (Loftsson and Sigurðardóttir, 1994a). When hydrocortisone was in suspension, the flux of hydrocortisone increased with increasing cyclodextrin concentration. At higher cyclodextrin concentrations, when all the hydrocortisone was in solution, the flux decreased with increasing cyclodextrin concentration. In a suspension of hydrocortisone, an increasing cyclodextrin concentration resulted in increased amount of dissolved hydrocortisone and, since the release rate of hydrocortisone from the hydrocortisone-cyclodextrin complex was much faster than the rate of hydrocortisone dissolution, this led to a larger flux through the skin. When all hydrocortisone was in solution, increasing the cyclodextrin concentration led to increased cyclodextrin-hydrocortisone complexation. This increased complexation lead to decreased amount of free hydrocortisone molecules in the aqueous solution and consequently a decreased flux through the skin. When hydrocortisone was in suspension, PVP increased the cyclodextrin complexation of hydrocortisone and thereby increased the flux of hydrocortisone through the skin. When hydrocortisone was in solution, the flux drooped rapidly as the amount of cyclodextrin was increased. Complexation of MβCD MS 1.8 with hydrocortisone does have a considerable effect on the flux of hydrocortisone through the skin. Addition of PVP to the aqueous MβCD MS 1.8 solutions had even greater effect on the flux of hydrocortisone through the skin (Fig. 4). The MβCD MS 1.8 complexation of

Table 1
Stability constant (K_c) of hydrocortisone complexes for different types of cyclodextrins and 0.25% (w/v) PVP

Type of cyclodextrin	K_c (M^{-1})		The ratio of K_c
	Aqueous CD solution	Aqueous CD solution containing PVP	
HPβCD	890	1070	1.20
MβCD MS 1.8	1710	2040	1.19
CMβCD	3150	9000	2.86
MaltosylβCD	2330	2660	1.14

hydrocortisone (i.e. the value of K_c) was increased by a factor of 1.19 when PVP was added (Table 1). The increased flux of hydrocortisone in a suspension of MβCD MS 1.8 can partly be explained by the action of MβCD MS 1.8 on the skin itself and partly by the increased amount of dissolved hydrocortisone in the suspension. On the other hand, CMβCD complexation of hydrocortisone was increased by a factor of 2.86 in the presence of PVP. This resulted in an increased amount of dissolved hydrocortisone and consequently a larger flux. The flux increase from a hydrocortisone suspension in aqueous maltosylβCD was not as large in the presence of PVP as for MβCD MS 1.8 and CMβCD. The complexation of hydrocortisone and maltosylβCD was increased by a factor of 1.14 upon addition of PVP.

The flux of hydrocortisone from a hydrocortisone suspension in 8% (w/v) MβCD MS 1.8 in the absence of or in a combination with oleic acid and/or PVP was investigated. The flux of hydrocortisone from a hydrocortisone suspension in aqueous MβCD MS 1.8 vehicle was increased by a factor of 2.5 when 1% (w/v) oleic acid was added to the vehicle and by a factor of 10 when the oleic acid concentration was increased to 5% (w/v). Addition of PVP to the above mentioned aqueous suspensions increases the flux even further. The enhanced permeability of hydrocortisone is due to increasing amount of free hydrocortisone in the solution and the effect of oleic acid on the skin barrier. The solubility of hydrocortisone was not markedly affected by the presence of oleic acid.

The results show that cyclodextrins can be used as skin penetration enhancers. The penetration

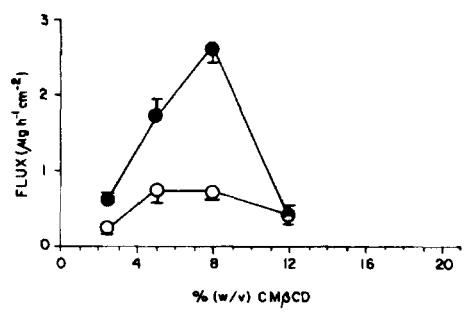

Fig. 3. The effect of CMβCD concentration on the flux of hydrocortisone through hairless mouse skin in vitro. Aqueous CMβCD solution (○); aqueous CMβCD solution containing 0.25% (w/v) PVP (●).

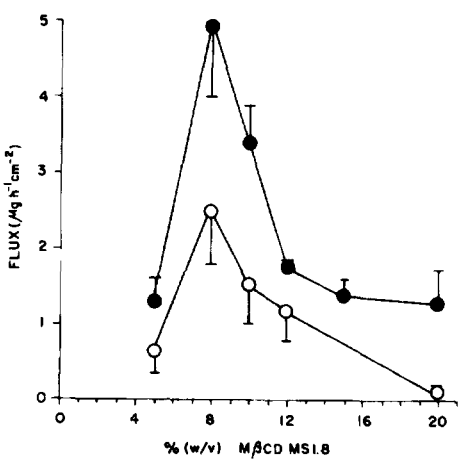

Fig. 4. The effect of MβCD MS 1.8 concentration on the flux of hydrocortisone through hairless mouse skin in vitro. Aqueous MβCD MS 1.8 solution (○); aqueous MβCD MS 1.8 solution containing 0.25% (w/v) PVP (●).

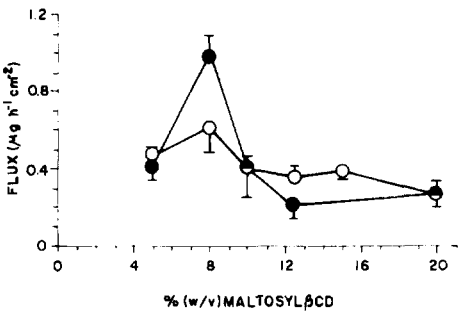

Fig. 5. The effect of maltosylβCD concentration on the flux of hydrocortisone through hairless mouse skin in vitro. Aqueous maltosylβCD solution (○); aqueous maltosylβCD solution containing 0.25% (w/v) PVP (●).

enhancement of cyclodextrins seems to depend on the type of cyclodextrin used. It is critical to use the right concentration of cyclodextrin to dissolve the drug in an aqueous vehicle. Addition of too much cyclodextrin, more than is needed to dissolve the drug, will result in a decreased flux of the drug. PVP both increased the hydrocortisone cyclodextrin complexation and acted as a co-enhancer for the cyclodextrin enhanced transdermal delivery of hydrocortisone.

Acknowledgements

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