DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(9), 1699-1708 (1994)

THE INFLUENCE OF 2-HYDROXYPROPYL-β-CYCLODEXTRIN ON DIFFUSION RATES AND TRANSDERMAL DELIVERY OF HYDROCORTISONE

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ABSTRACT

The influence of 2-hydroxypropyl-β-cyclodextrin (HPβCD) on the permeability of hydrocortisone through semi-permeable cellophane membrane and hairless mouse skin was investigated. When the hydrocortisone concentration was kept constant and the HPβCD concentration in an aqueous vehicle was gradually increased the flux through the semi-permeable membrane was increased until all hydrocortisone had dissolved, after that the flux decreased with increasing HPβCD concentration. A maximum flux was obtained when just enough HPβCD was used to dissolve all the drug in the aqueous vehicle.

INTRODUCTION

The main barrier to dermal and transdermal drug delivery is the outermost layer of the skin, the stratum corneum. Usually, drugs applied topically must

penetrate this barrier before they can reach their site of action. The clinical effectiveness of many drugs is often limited by their inability to pass this barrier. One way to enhance drug penetration into and through the skin and make topical therapy more efficient and reliable is to add to the drug vehicle a compound, a penetration enhancer, which temporarily alters or damages the skin barrier[1-4]. Thus, penetration enhancers disrupt the protective function of the stratum corneum[5]. An other way to enhance drug penetration is to increase the supply of dissolved drug molecules at the skin surface without affecting the skin barrier.

Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic cavity in the center[6,7]. The three commonly known CDs are made up of six (αCD), seven (βCD) or eight (γCD) glucopyranose units but various derivatives of these three CDs have been made. The number of glucopyranose units determines the size of the central cavity. CDs can form inclusion complexes with a wide variety of hydrophobic drug molecules by taking up a whole drug molecule or some part of it into the cavity. No covalent bonding is involved in the complex formation and in aqueous solutions the drug molecule within the cavity is in rapid equilibrium with free drug molecules out in the solution. The complex formation depends on how well the drug molecule fits into the cavity and the CD which is the most useful for complexation of drugs is βCD[6]. In our present study we used 2-hydroxypropyl-β-cyclodextrin (HPβCD) which is obtained by treating βCD with propylene oxide[8]. The molar substitution (MS), i.e. the average number of propylene oxide molecules that have reacted with one glucopyranose unit, was 0.6 or 0.9. HPβCD has a very good aqueous solubility (over 60% w/v) and forms stable complexes with many drugs[8,9].

In general, the very hydrophilic HPβCD molecules do not penetrate biological membranes. It is thought that in aqueous topical drug formulations HPβCD keeps lipophilic water-insoluble drug molecules in solution and delivers them to the skin surface where they partition into the skin barrier[10,11]. Under normal conditions HPβCD improves dermal and transdermal delivery of drugs without penetrating into the skin itself. However, under occlusive conditions, considerable amounts of HPβCD have been found to penetrate the skin, probably due to action of surfactants involved in the ointment base on the skin barrier[12]. The purpose of this study was to investigate the effect of HPβCD on the release of drugs from topical vehicle systems. We used hydrocortisone as a sample drug.

MATERIALS AND METHODS

Materials

Hydrocortisone was obtained from Sigma Chemical Co. (St. Louis, Missouri), and HPβCD MS 0.6 and 0.9 from Wacker-Chemie (Munich, Germany). All other chemicals used were of pharmaceutical or special analytical grade. Semi-permeable cellophane membrane (Spectrapor® membrane tubing no. 2) was obtained from Spectrum Medical Industries (U.S.A.). The hairless mice (C3H/Tif hr/hr) were obtained from Bommice (Denmark).

Quantitative analysis

The quantitative determination of hydrocortisone was performed on a high-performance liquid chromatographic (HPLC) component system from Milton Roy consisting of a ConstaMetric 3200 solvent delivery system operated at 1.50 ml/min flow rate, a Rheodyne 7125 injector, a Beckman Ultrasphere ODS 5 μm (4.6 x 150 mm) column and a Spectro Monitor 3200 uv/vis variable wavelength detector operated at 254 nm. The mobile phase consisted of acetonitrile, tetrahydrofuran and water (35:1:64) and the retention time was 3.2 min.

Solubility studies

A phase-solubility experiment was conducted by adding excess amount of hydrocortisone to aqueous solutions containing up to 7.25×10^{-2} mol/litre HPβCD MS 0.6. The suspensions formed were sonicated in an ultrasonic bath for 2 h and heated in an autoclave in sealed containers to 120°C for 20 min. After equilibration at room temperature (approximately 23°C) for 3 days the suspensions were filtered through a 0.45 μm membrane filter, diluted with a mixture of methanol and water (7:3) and analysed by HPLC.

Permeation through a semi-permeable membrane

Three groups of vehicle systems (i.e. donor phases) were investigated: 1) Aqueous HPβCD solutions consisting of 1% hydrocortisone in aqueous HPβCD MS 0.6 solutions. 2) A hydrogel consisting of 1% hydrocortisone, 6% HPβCD MS 0.6, and 3.5% sodium carboxymethyl cellulose in water. 3) HPβCD containing oil-in-water (o/w) creams consisting of 0.5 to 3.5% hydrocortisone,

0.25% polysorbate 80, 1.25% cetostearyl alcohol, 1.25% liquid paraffin, 1.5% glyceryl monostearate 40-50, 4% glycerol (85 per cent), 7% sorbitol, and 0 to 10% HPβCD MS 0.9 in purified water. All per cent used to indicate vehicle composition are per cent by weight-in-volume (% w/v) and all vehicle ingredients were of Ph. Eur. grade. In general, hydrocortisone was dissolved or suspended in the HPβCD containing aqueous solution. After addition of other ingredients the solution or suspension was placed in a sealed container and heated in an autoclave to 120°C for 20 min. After heating the hydrocortisone solution or suspension was allowed to equilibrate at room temperature (about 23°C) for at least three days before it was applied to the cellophane membrane or the hairless mouse skin.

The flux of hydrocortisone from the HPβCD containing vehicle systems and Uniderm® 1% creme (an 1% w/w hydrocortisone cream from Schering-Plough, U.S.A.) through a semi-permeable cellophane membrane was investigated. The membrane was placed in a Franz diffusion cell (Vangard International Inc., U.S.A.) containing 10 ml of the receptor phase. The receptor phase consisted of aqueous 5% HPβCD solution and was stirred with a magnetic bar. HPβCD was used to solubilize the drug in the receptor phase and, thus, to help to maintain sink conditions throughout the experiment. The membrane surface area available for diffusion was 3.14 cm^2. At time zero 2 ml of the donor phase was applied to the membrane and donor chamber covered with parafilm. At various time intervals (up to 2.5 h) samples (50 μm) were removed from the receptor phase and analysed immediately by HPLC. The steady-state flux was calculated from the individual cumulative amounts *versus* time plots[13]. Each experiment was repeated three or four times and the results reported are the mean values ± standard error of the mean (SE).

Skin permeation studies

Female hairless mice were killed by cervical dislocation, their full-thickness skins removed and placed in the previously described Franz diffusion cells. The receptor chamber was kept at 37°C by circulating water through an external jacket. The receptor phase consisted of aqueous 5% HPβCD solution containing 0.4% formaldehyde and was stirred with a magnetic bar. At time zero 2 ml of the donor phase, hydrocortisone in aqueous HPβCD solution or hydrogel, or Uniderm® 1% creme, were applied to the stratum corneum. Samples of receptor phase were

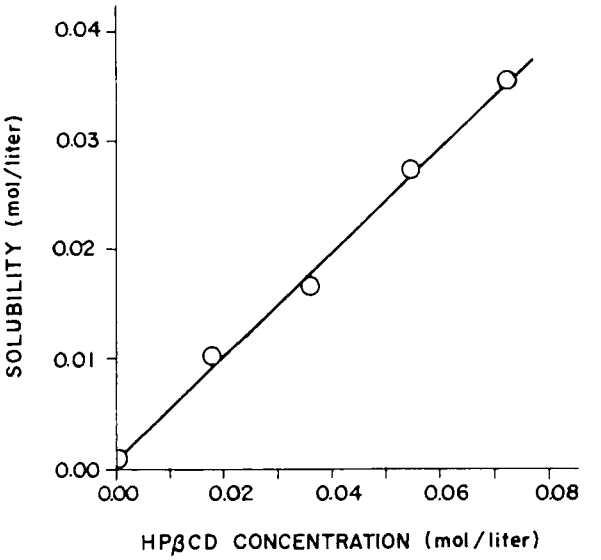

FIGURE 1

A phase-solubility diagram of hydrocortisone in aqueous HPβCD MS 0.6 solution at room temperature.

removed from the cells at every 12 h for 4 days and replaced with fresh buffer solution. The samples were kept frozen until analysed by HPLC. The steady-state flux was calculated as before.

RESULTS AND DSCUSSION

The phase-solubility diagram of hydrocortisone in aqueous HPβCD MS 0.6 solution is shown in Fig 1. The phase-solubility diagram is of Higuchi's A_L-type and formation of 1:1 complex was assumed[12]. From the slope of the diagram and the solubility of hydrocortisone in water at room temperature (9.8×10^{-4} mol/litre) the stability constant of the hydrocortisone-HPβCD complex was estimated to be 900 M^{-1}.

The diffusion of hydrocortisone from aqueous HPβCD solution containing 1% hydrocortisone in solution or suspension through semi-permeable cellophane

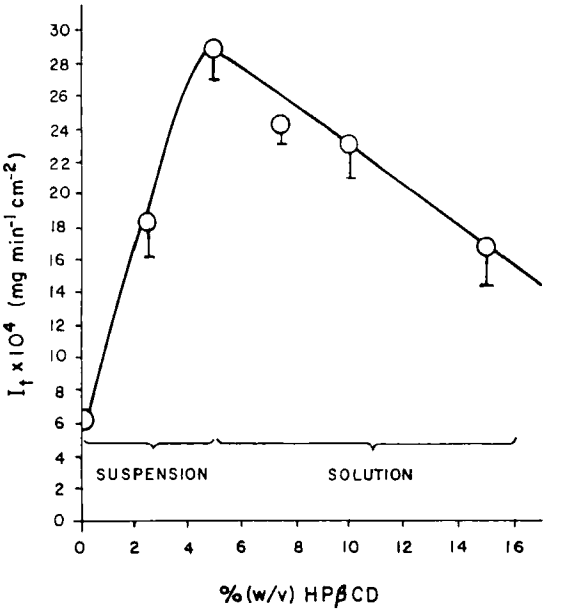

FIGURE 2

The relationship between the HPβCD concentration and the total flux (I_t) of hydrocortisone from aqueous HPβCD MS 0.6 solution containing 1% (w/v) hydrocortisone in a suspension or a solution through a semi-permeable cellophane membrane. Each experiment was repeated three times and the error bars represent the standard error of the mean.

membrane was investigated. At low HPβCD concentration, when hydrocortisone was in suspension, the flux of hydrocortisone through the membrane increased with increasing HPβCD concentration, but when all hydrocortisone was in solution, at HPβCD concentration above 5%, the flux decreased with increasing HPβCD concentration (Figure 2). When hydrocortisone is in suspension increasing the HPβCD concentration increases the amount of dissolved hydrocortisone and, since the rate of hydrocortisone release from the hydrocortisone-HPβCD complex is much faster than the rate of hydrocortisone dissolution, this consequently leads to larger flux through the membrane. On the other hand, when all hydrocortisone is in solution increasing amount of HPβCD results in decreasing amount of free hydrocortisone molecules in the solution. The

TABLE 1

The effect of the HPβCD MS 0.9 concentration and the hydrocortisone concentration in an oil-in-water cream on the flux of hydrocortisone through a semi-permeable cellophane membrane at room temperature.

Hydrocortisone concentration (% w/v)	HPβCD MS 0.9 concentration (% w/v)	Flux x 10^3 [a] (mg min^{-1} cm^{-2})
0.5	0.0	0.612
	2.5	0.946
	5.0	1.01
	7.5	0.874
	10.0	0.690
1.0	0.0	1.18
	2.5	1.66
	5.0	2.38
	7.5	2.05
	10.0	2.11
2.5	0.0	1.39
	2.5	1.60
	5.0	2.21
	7.5	2.43
	10.0	3.06
3.5	0.0	1.17
	2.5	1.93
	5.0	2.27
	7.5	2.85
	10.0	2.81

[a] Average of two experiments.

total flux of hydrocortisone through the membrane (I_t) is the sum of the flux of the free drug (I_D) and the flux of the drug-cyclodextrin complex ($I_{D\cdot CD}$)[11]:

$$I_t = I_D f_D + I_{D\cdot CD}(1-f_D)$$

where f_D is the fraction of free drug and $(1-f_D)$ is the fraction of the drug in the complex. The value of f_D can be calculated from the stability constant of the hydrocortisone-HPβCD complex. Since the hydrocortisone-HPβCD complex

TABLE 2

The effect of vehicle composition on the transdermal flux of hydrocortisone through hairless mouse skin *in vitro* at 37°C. The initial hydrocortisone concentration was 1.0% (w/v). Each experiment was repeated four times and the results shown are the mean flux (F) ± standard deviation (SD).

Vehicle composition	F ± SD ($\mu g\ h^{-1}\ cm^{-2}$)
6% (w/v) HPβCD MS 0.6 aqueous solution	0.139 ± 0.054
8% (w/v) HPβCD MS 0.6 aqueous solution	0.075 ± 0.027
6% (w/v) HPβCD MS 0.6, 3.5% (w/v) CMC hydrogel	0.092 ± 0.017
Uniderm® cream	0.116 ± 0.042

permeates about ten to fifteen times slower through the membrane than the free hydrocortisone molecules, increased complexation leads to smaller total flux of hydrocortisone through the membrane[11]. Similar results were obtained when the flux of hydrocortisone was measured through a semi-permeable cellophane membrane from an oil-in-water cream containing 0 to 10% (w/v) HPβCD (Table 1). Maximum total flux of hydrocortisone through the membrane was obtained when just enough HPβCD was used to dissolve all hydrocortisone in the cream vehicle. For example, in a cream vehicle containing 0.5% (w/v) hydrocortisone, and various lipophilic excipients, about 5% (w/v) HPβCD was needed to dissolve all hydrocortisone (Table 1). For this hydrocortisone concentration the maximum flux of hydrocortisone through the membrane was obtained at 5% (w/v) HPβCD. As the HPβCD concentration was increased above this value the flux became smaller.

Table 2 shows the effect of HPβCD on the flux of hydrocortisone through hairless mouse skin. The flux of hydrocortisone from Uniderm® cream is reported for comparison. As for the semi-permeable cellophane membrane a maximum flux was obtained when just enough HPβCD was added to the vehicle to keep all hydrocortisone, or almost all of it, in solution. In fact, maximum flux from an aqueous HPβCD vehicle containing 1% (w/v) hydrocortisone, through excised

hairless mouse skin, was obtained when the HPβCD concentration was kept low so that only 97% of the drug was in solution (at 6% HPβCD). The flux from HPβCD solutions was comparable to that obtained from Uniderm®. The flux of hydrocortisone from the HPβCD MS 0.6 containing hydrogel through the semi-permeable cellophane membrane was 1.73×10^{-3} mg min^{-1} cm^{-2} but from Uniderm® it was only about 0.03×10^{-3} mg min^{-1} cm^{-2}. Thus, the hydrogel released hydrocortisone at almost 60 times faster rate than the cream. However, since the transdermal flux was controlled by the permeability through the skin barrier, and not by the hydrocortisone release from the vehicle, the transdermal flux of hydrocortisone was almost identical from the two vehicles (Table 2).

CONCLUSIONS

In aqueous vehicle systems hydrophilic CDs like HPβCD can be used to promote dermal and transdermal delivery of lipophilic water-insoluble drugs like hydrocortisone. HPβCD keeps the drug molecules in solution and delivers them to the surface of the barrier where they partition into and then through the barrier. Maximum flux of the drug through the barrier was obtained when just enough HPβCD was added to the vehicle to keep all the drug in solution. Too much HPβCD reduced the flux.

ACKNOWLEDGMENT

This work was supported by the University of Iceland Research fund.

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