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ORIGINAL ARTICLE

Development of octanol membranes for drug screening

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Abstract Most drugs permeate biological membranes via passive diffusion and it is generally assumed that the main barrier is the lipophilic structure of the membranes. However, it has been observed that an unstirred water layer adjacent to the membrane surface can in some cases be just as effective barrier as the lipophilic membrane itself. Hydrophilic cyclodextrins can enhance drug delivery through biological membranes by increasing the availability of dissolved drug molecules immediate to the membrane surface, i.e., by increasing drug delivery through the unstirred water layer. We have developed an artificial octanol membrane that is cheap and simple to prepare. The novel membrane consists of a hydrated semi-permeable cellophane membrane with a molecular weight cut off (MWCO) of 12,000–14,000 that mimics the unstirred water layer and a lipophilic membrane of pure *n*-octanol in a nitrocellulose matrix. The membrane was used to investigate the effects of 2-hydroxypropyl-*β*-cyclodextrin (HP*β*CD) on the flux of hydrocortisone through the membrane. In aqueous HP*β*CD saturated with hydrocortisone the drug concentration gradient, over the unstirred water layer, increased with increasing HP*β*CD concentration which resulted in enhanced drug delivery through the membrane. When excess HP*β*CD was present in the donor phase the octanol/water partition coefficient decreased with increasing HP*β*CD concentration that lead to decreased drug delivery through the membrane.

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Introduction

The chemical structure of hydrophilic cyclodextrins (i.e., the large number of hydrogen donors and acceptors), their molecular weight (i.e., greater than 970 Dalton) and their very low octanol/water partition coefficient ($\log K_{o/w}$ less than –3) are all characteristics of compounds that do not readily permeate biological membranes [1, 2]. Based on these observations it has been suggested that hydrophilic cyclodextrin enhance drug delivery through biological membranes by increasing the availability of dissolved drug molecules in an aqueous layer immediate to the lipophilic membrane surface [3–5]. Cyclodextrins solubilize the lipophilic water-insoluble drug molecules in the aqueous vehicle and enhance their permeation through an aqueous diffusion layer at the membrane surface. According to this model cyclodextrins can only act as penetration enhancers if permeation through an unstirred water layer at the membrane surface contributes to the overall barrier function of the biological membrane [5]. Furthermore, the physicochemical properties of the drug (e.g., its solubility in water), the drug:cyclodextrin concentration ratio and the composition of the drug formulation (e.g., aqueous or non-aqueous) will also determine whether cyclodextrins will enhance or hamper drug delivery through a biological membrane. Cyclodextrins are in most cases unable to enhance drug permeation through a lipophilic membrane barrier and excess cyclodextrin (more than is needed to dissolve the drug) will hamper drug

permeation through the membrane [6]. Since cyclodextrins can both enhance and hamper drug delivery through biological membranes it is of uttermost importance to optimize cyclodextrin containing drug formulations with regard to drug delivery from the formulations [3]. Too much or too little cyclodextrin can result in less than optimum drug bioavailability. The purpose of this study was to standardize the thickness and the viscosity of the unstirred water layer by fusing together a hydrated semi-permeable cellophane membrane and the lipophilic octanol membrane. The membrane was used to investigate the effects of cyclodextrins on drug permeability through biological membranes.

Experimental

Materials

2-Hydroxypropyl-β-cyclodextrin of molar substitution 0.64 (HPβCD) was purchased from Roquette (Lestrem, France), hydrocortisone from ICN Biomedicals Inc (Ohio, USA), *n*-octanol (99%) from Sigma (St. Luis, USA), and collodion (containing 4–8% nitrocellulose) and diethyl ether from Fluka (Hamburg, Germany). The cellophane membrane used was a Spectra/Pore membrane from Spectrum Laboratories Inc. (Rancho Dominguez, USA) with molecular weight cut off (MWCO) 12,000–14,000 Dalton. All other reagents were of analytical or special regent grade.

Membrane preparation

Ten grams of the collodion solution was diluted with 6 g of ether-ethanol (vol. ratio 8.5:1.5) solution and 4 grams of *n*-octanol added to this solution. The dry cellophane membrane (4.4 × 4.5 cm) was fixed with miniature washing clips to a string in a vertical position, and the octanol/nitrocellulose solution was poured three times (approx. 1.5 ml each time) on to the upper ridge of the cellophane membrane at 15 min intervals and the membrane left to dry over night. The weight ratio of *n*-octanol and nitrocellulose in the dry lipophilic octanol/nitrocellulose membrane was approximately 10:1 [7].

Permeation experiments

Drug flow through our membranes was measured in Franz-diffusion cells (FDC400 15 FF) from Vangard International Inc. (Neptune, USA) where the donor phase (2 ml) is unstirred but the receptor phase (12 ml) is stirred with a magnetic stirrer. The area of the exposed membrane is 1.77 cm^2. The donor phases consisted of water or aqueous 1–15% (w/v) HPβCD solution containing hydrocortisone in a solution or suspension. The receptor phase contained 10% (v/w) HPβCD octanol saturated aqueous solution. The hydrocortisone flux through the membrane was determined at room temperature (22–23 °C). Samples were withdrawn from the receptor phase at various time points and the flux was calculated from each permeability profile. The results presented are the means and the standard deviation of from 3 to over 6 separate experiments. High performance liquid chromatography (HPLC) was used for quantitative determination of hydrocortisone in the donor and receptor phases [8]. The octanol concentration in the aqueous cyclodextrin solutions was also monitored [7, 8]. Maximum amount of octanol that a 2 ml of aqueous 10% (w/v) HPβCD donor phase could extract from the membrane is 3 mg or about 2% of the octanol membrane. It is unlikely that this small amount could have effect on drug permeability through the membrane.

Determination of partition coefficients

The procedure for determining the partition coefficients for drug distribution between *n*-octanol and a cyclodextrin containing aqueous solutions has previously been described [8]. Briefly a 3 ml aliquot of an octanol solution containing 2 mg/ml of hydrocortisone was transferred to 10 ml vials containing 3 ml octanol saturated aqueous cyclodextrin solution. The vials were shaken, with a mechanical shaker, for 5–20 h at room temperature. The phases were then separated by centrifugation and samples from each phase were analyzed be HPLC to determine the drug concentrations. The partition coefficient is the drug concentration ratio between octanol and the aqueous phase.

Results and discussion

Figure 1 shows the effect of HPβCD concentration on the flux of hydrocortisone through the artificial membrane. In the diffusion cell the hydrated cellophane membrane (thickness up to 230 μm) is on the donor side and the lipophilic octanol membrane (thickness about 120 μm) on the receptor side [7]. The donor phase contained constant amount of hydrocortisone (16 mg/ml) in a suspension at HPβCD concentrations below 10% (w/v) but in solution at HPβCD concentration of 10% or higher. In aqueous HPβCD solutions saturated with hydrocortisone the amount of free

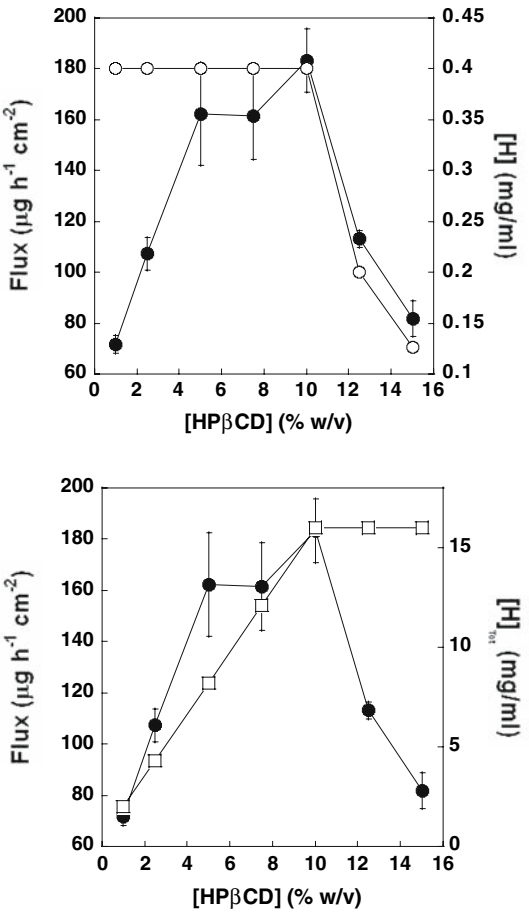

Fig. 1 The effect of HPβCD concentration on the flux of hydrocortisone from an aqueous HPβCD solution (•), containing 16 mg/ml of hydrocortisone, trough the cellophane/octanol membrane, and the concentrations of free hydrocortisone ([H], *o*) and the total concentration of dissolved hydrocortisone ($[H]_{Tot}$, □). At HPβCD concentrations below 10% the drug was in suspension in the donor phase but in solution at HPβCD concentrations of 10% and higher. Excess HPβCD was present in the donor phase at HPβCD concentrations greater than 10%. Mean values and standard deviations of at least three experiments

hydrocortisone is constant and equal to the intrinsic solubility (S_0) of hydrocortisone in water. The stability constant ($K_{1:1}$) of the water-soluble 1:1 hydrocortisone/HPβCD complex (H/HPβCD) is expressed as:

$$K_{1:1} = \frac{[H/HP\beta CD]}{[H] \times [HP\beta CD]} = \frac{[H/HP\beta CD]}{S_0 \times [HP\beta CD]} \quad (1)$$

where [H] is the concentration of free hydrocortisone in the aqueous complexation medium. The total concentration of dissolved hydrocortisone ($[H]_{Tot}$) will then be:

$$[H]_{Tot} = S_0 + [H/HP\beta CD] \quad (2)$$

In the donor phase $[H]_{Tot}$ increases with increasing HPβCD concentration up to 10% (w/v), i.e., until all hydrocortisone (i.e., 16 mg/ml) is in solution, but it remains constant at higher HPβCD concentrations (Fig. 1). The amount of free dissolved hydrocortisone ([H]) is constant and equal to the intrinsic solubility (S_0 = 0.4 mg/ml) up to 10% HPβCD but decreases at higher HPβCD concentrations when excess HPβCD (more than is needed to solubilize the drug) is present in the donor phase (Fig. 1). Thus, the flux increase with increasing HPβCD concentration appears to correlate with $[H]_{Tot}$ but the decrease at higher HPβCD concentrations with [H]. These observations can be explained by Fick's first law of diffusion. The flux (J_w) of drug molecules through the unstirred water layer is expresses by Fick's first law as:

$$J_w = -D_w \cdot \frac{C_w - C_{Aq}}{h_w} \quad (3)$$

where D_w is the diffusion coefficient of the drug molecule through the layer, C_w is the drug concentration in the donor phase, C_{Aq} is the drug concentration immediate to the lipophilic (e.g., octanol) membrane surface and h_w is the thickness of the unstirred water layer. The concentration difference (C_w–C_{Aq}) is the concentration gradient that drives the drug permeation through the unstirred layer. The fundamental equation describing passive drug transport through a lipophilic membrane is also based on Fick's first law:

$$J_M = P_M \cdot C_{Aq} \quad (4)$$

where J_M is the drug flux through a membrane (mass/area/time) and P_M is the permeability coefficient of the drug through the membrane. The permeability coefficient is defined as:

$$P_M = \frac{D_M \cdot K_{o/w}}{h_M} \quad (5)$$

where D_M is the diffusion coefficient of the drug within the membrane, $K_{o/w}$ is the partition coefficient of the drug from the aqueous exterior into the octanol membrane and h_M is the effective thickness of the membrane. Figure 2 shows the relationship between the observed $K_{o/w}$ and the HPβCD concentration in the aqueous phase. It shows that $K_{o/w}$ is strongly influenced by the HPβCD concentration, decreasing with increasing HPβCD concentration.

In this type of membrane the total drug permeation resistance is the sum of resistance within the aqueous cellophane membrane that represents the unstirred

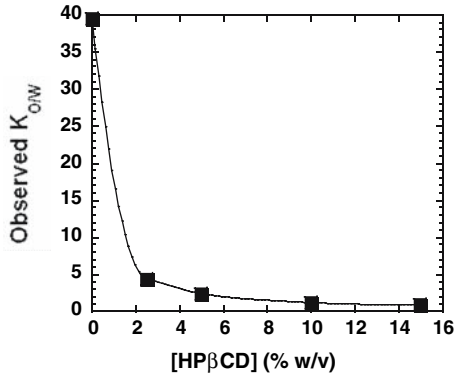

Fig. 2 The effect of HPβCD concentration on the partition coefficient of hydrocortisone between aqueous HPβCD solution and *n*-octanol at room temperature

water layer (R_{Aq}) and the resistance within lipophilic octanol membrane (R_M), and their relative importance depends on the physicochemical properties of both the drug and the membrane. Since the permeability constants are the reciprocals of the resistance the following equation is obtained [9]:

$$J = P \cdot C_w = (R_{Aq} + R_M)^{-1} \cdot C_w = \left(\frac{1}{P_{Aq}} + \frac{1}{P_M}\right)^{-1} \cdot C_w \quad (6)$$

At HPβCD concentrations below 10%, when the donor phase is saturated with hydrocortisone, the flow of drug molecules through the membrane is diffusion controlled and the flux through artificial membrane

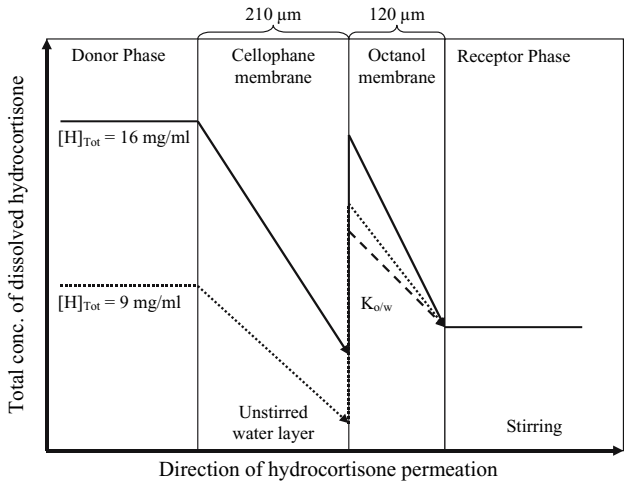

Fig. 3 The effect of HPβCD concentration on permeation of hydrocortisone through the artificial membrane. Aqueous 5 and 10% (w/v) HPβCD solutions containing respectively 9 mg/ml (▪▪▪▪) and 16 mg/ml (—) of hydrocortisone, and hydrocortisone solution containing 16 mg/ml hydrocortisone and excess HPβCD (e.g., 15% w/v) (- - - -)

increases with increasing HPβCD concentration, i.e., with increasing $[H]_{Tot}$ (Fig. 3). The hydrocortisone flux through the unstirred water layer (i.e., the cellophane membrane) increases with increasing hydrocortisone concentration gradient over the membrane (i.e., the difference in $[H]_{Tot}$ between the donor phase and immediate surface of the octanol membrane (C_w and C_{Aq} in Eq. 3)). Increase in C_{Aq} will offset the decrease in the observed $K_{o/w}$ with increasing HPβCD concentration (Fig. 3). When excess HPβCD is present in the donor phase then the observed $K_{o/w}$ (in Eq. 5) will continue to decrease with increasing HPβCD concentration whereas there will be no further increase in the flux through the unstirred aqueous layer. This will result in sharp decrease in the hydrocortisone concentration gradient over the octanol membrane and hydrocortisone permeation through the artificial membrane (the flux (J) in Eq. 6) becomes membrane controlled. Permeation patterns similar to that shown in Fig. 1 have been observed when permeation of lipophilic drugs from aqueous cyclodextrin solutions through various biological membranes were investigated [5, 6, 10]. Hydrophilic cyclodextrins will not enhance drug permeation through a biological membrane if the permeation through the membrane is very slow or if the unstirred water layer is very thin (and/or non-viscous), i.e., if $R_M >> R_{Aq}$. Conventional penetration enhancers, such as fatty acids, fatty amines and fatty alcohols, enhance drug delivery through biological membranes by permeating into the membrane and disrupting its barrier properties [11] without affecting the unstirred aqueous layer. Hydrophilic cyclodextrins, on the other hand, increase drug delivery through the unstirred water layer without affecting the membrane barrier. However, since cyclodextrins can both enhance and decrease drug permeation through biological membranes it is of uttermost importance to optimize cyclodextrin concentrations in pharmaceutical formulations with regard to drug availability. Too much or too little cyclodextrin can result in less than maximum drug release from the formulation.

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