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Solubility and permeability of steroids in water in the presence of potassium halides

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Water forms a network of hydrogen bonded water molecules that gives liquid water unique physicochemical properties. Ions that affect the network structure, e.g. potassium halides, are known to either increase or decrease aqueous solubilities of drugs. Most biological membranes consist of hydrophilic exterior and a lipophilic interior. Mathematically they can be treated as two-layer membranes, i.e. a hydrophilic water layer that is referred to as unstirred water layer (UWL) and a lipophilic membrane. The purpose of this study was to investigate if and then how ions affect drug permeation through the UWL. The effects of potassium halides on the solubility and permeability of dexamethasone and hydrocortisone was investigated. The potassium halides had either increasing or decreasing effect on their aqueous solubility but did not have any effect on their permeability through UWL.

1. Introduction

Life as we know it is based on water and water plays an important role in all biological processes. The main reasons of its importance is the complex and dynamic three-dimensional hydrogen-bonding network of liquid water, which gives it numerous unique physical and chemical properties (Eisenberg and Kauzmann 1969; Franks 1972). One of the best known anomalies of water is the decrease in density upon cooling at atmospheric pressure below 4 °C. This and all the other anomalous, like melting or boiling point, can be explained by transitions in the hydrogen-bond water network.

The complex and not yet well understood hydrogen-bond network is created by the polarity of the water molecules. The network can be disturbed by polar and non polar additives. Ions for example are such polar additives, which are known to be either structure-making (kosmotropic) or structure-braking (chaotropic) agents. The influence of ions on the hydration shell of dissolved molecules can be regarded as a balance between water-water and water-ion interactions (Hribar et al. 2002). However, the means of kosmotropic and chaotropic ions is only empirical and it remains unclear how and why a particular ion acts as either structure-maker or as a structure-breaker. Furthermore it is not possible to completely resolve arrangement of hydrogen bonds in the vicinity of a solvated ion, at least not by currently available experimental techniques. The hydrogen-bond water network is not only affected by the presence of ions, but also by interfaces with gases (Du et al. 1993) and other solvents (Benjamin 2005; Luo et al. 2006; Hore et al. 2008).

Almost 60% of our body is water and thus the structural properties of water are very important for drug development and drug delivery. Hydrogen-bond networking strongly affects the aqueous solubility of drugs and ability of the dissolved drug molecules to permeate biological membranes (Lipinski et al. 1997). According to the Biopharmaceutics Classification System (BCS) drugs and new drug candidates can be classified into four groups based on their aqueous solubility and ability to permeate biological membranes (Amidon et al. 2004). This classification is based on Fick's first law. Most biological membranes consist of a hydrophilic exterior and a lipophilic interior. Such membranes can be treated mathematically as a two-layer membrane barrier where one layer forms an aqueous membrane barrier and the other lipophilic membrane barrier:

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

Where J is the drug flux through the membrane (mass/area/time), P is the permeability coefficient of the drug through the membrane and C_{Aq} is the drug concentration in the aqueous exterior. The aqueous membrane barrier is frequently called unstirred water layer (UWL). Depending on the physicochemical properties of the permeating drug molecule, such as its size and lipophilicity, as well as on the membrane barrier, such as the relative thickness of the two layers, either the UWL or the lipophilic membrane can become the rate-determining membrane barrier (Loftsson et al. 2006, 2007). The total permeation resistance of the membrane barrier is the sum of the resistance of the UWL (R_{Aq}) and the resistance of the lipophilic membrane barrier (R_M). The permeability coefficient is the reciprocal of the resistance and thus the resistance can be replaced by the permeability constant in Eq. (1) where P_{Aq} is the permeability coefficient of the drug molecule through the UWL and P_M is the permeability coefficient through the lipophilic membrane. Most permeation enhancers increase drug permeability through membranes by making the lipophilic membrane more permeable, i.e. by decreasing R_M (increasing P_M). We would like to know if drug permeation can be enhanced by decreasing R_{Aq}

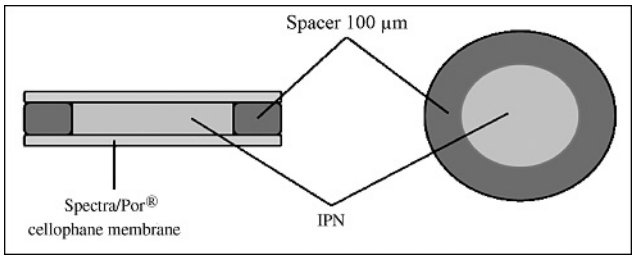

Fig. 1: Profile (left) and top view (right) of the membrane – a double cellophane layer enveloping the IPN

(increasing P_{Aq}). The purpose of this study is to investigate how kosmotropic and chaotropic ions affect drug solubility and drug permeability through UWL.

2. Investigations and results

The effects of potassium halides on the aqueous solubilities of two lipophilic steroids, dexamethasone and hydrocortisone, was investigated. Both drugs are known for their low solubility in pure water, which makes them good candidates for this study. Potassium halide salts are known to decrease the density of water. The intensity of the potassium halide effect is related to the position of the halide ion within the periodic table and corresponding position within the Hofmeister series (Wiggins 1990; Chaplin 2008). Furthermore, the effects of the salts on the permeability of hydrocortisone through an UWL, formed by semi-permeable cellophane membrane, were determined and compared with those of maltose and 2-hydroxypropyl-β-cyclodextrin (HPβCD). The semi-permeable cellophane membrane enveloped 100 μm thick interpenetration network (IPN). The IPN-layer was chosen to provide highly structured water channels, similar to the UWL next to a biological membrane (Fig. 1). The IPN consisted of 5% (w/v) polymer, hydroxypropyl methyl cellulose, and 95% water containing KI, KF, maltose or HPβCD at concentrations corresponding to that of the donor phase.

The phase solubilities of dexamethasone and hydrocortisone (Fig. 2) are almost identical, the only difference being the intrinsic solubility, 84.76 ± 0.96 μg/ml for dexamethasone and 321.03 ± 14.40 μg/ml for hydrocortisone (mean ± standard deviation). KF decreases solubility of both drugs by nearly 90% at a concentration of 15% (w/v) (2.58 M). KCl decreased it by roughly 50% at 15% (w/v) (2.01 M). The large iodide ion increased the solubility by roughly 50% at 15% (w/v) (0.90 M) KI.

Based on the phase solubility studies we selected KI and KF for the permeability studies, i.e. the ions that had the largest increasing and decreasing effects, respectively, on the solubility. Maltose and HPβCD were use as reference excipients. Maltose increases solubility of hydrocortisone by 13% at 15% (w/v) (0.44 M) maltose, and HPβCD increases solubility by 3067% at 15% (w/v) (0.10 M) HPβCD.

Results of Franz diffusion cell experiments were evaluated by plotting the amount of drug that had permeated the membrane versus the time the sample was taken, which gave a linear permeation profile. The slope of the profile is the flux (J) of the drug through the membrane (i.e. the UWL), as previously described (Loftsson et al. 2006). The following equation was used to correct for drug concentration differences in the donor phase, which should according to Fick's equation be constant:

$$P = \frac{J}{c_D} \text{ with } \quad c_D = \frac{c_0 + c_{120}}{2} \tag{2}$$

The subscripts of the donor phase concentration refer to the time the sample was taken. Assumption on this calculation is that the donor phase concentration decreases linear over the measured time period of 120 min. The total drug loss in the donor phase concentration over the whole experiment was between 5 and 8%. KI, KF and maltose did not affect the permeability coefficient (P) (see Fig. 3). However HPβCD decreased the permeability coefficient, most probably due to aggregate formation. In a preliminary investigation the ions did not either have any effect on the permeation of dexamethasone.

3. Discussion

As expected the halide anions did affect the aqueous solubility of both hydrocortisone and dexamethasone as predicted by Hofmeister series (Wiggins 1990; Chaplin 2008). The smallest ion provides the highest decrease in solubility, whereas the largest ion tested, the iodine ion, increases the solubility. Bromide does not really change the solubility of these drugs. Unexpectedly we did not observe any effect of these ions and maltose on in the permeability of hydrocortisone through the UWL. It is possible that the method we applied is not sensitive enough to detect such changes, but most likely their effect is negligible and too small to have any effect on drug delivery through biological membranes. It appears that the ions only

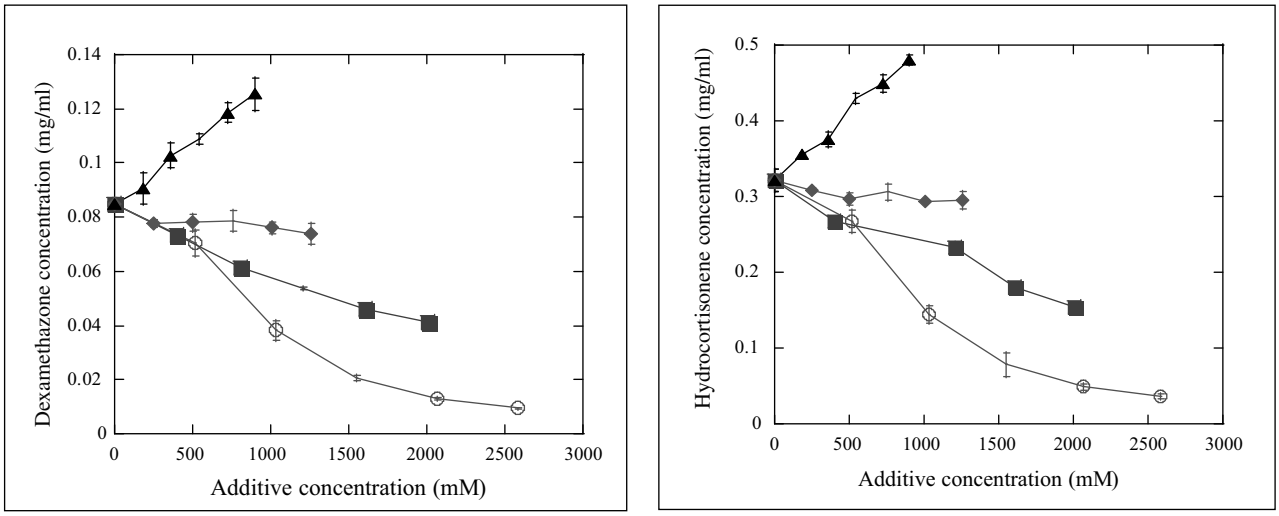

Fig. 2: The effect of KF (◯), KCl (■), KBr (♦) and KI (▲) on the solubility profile of dexamethasone (left) and hydrocortisone (right) in pure water at ambient temperature

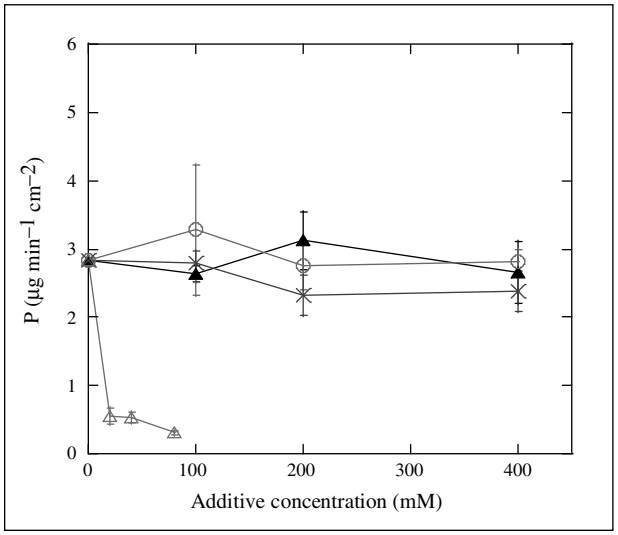

Fig. 3: Permeability coefficient of hydrocortisone through a double layer cellophane membrane (MWCO 12-14.000) enveloping a 100 μm thick IPN-layer, representing the UWL, containing KI (▲), KF (◯), maltose (×) or HPβCD (△)

have short range effect on the water structure. Some studies have shown that the influence of dissolved ions on the hydrogen bond geometries is quite weak and that each ion only induces rearrangement of water molecules in the first solvation shell (Smith 2007; Riemenschneider 2008). But then again it is reported that of the ions tested fluoride is the strongest hydrated anion and iodide the weakest hydrated, i.e. fluoride has stabilizing effect on the water structure and iodine destabilizing effect (Chaplin 2008). However, with regard to drug permeation our studies do not support the theory of structure makers and structure brakers. These effects might by strong enough to induce changes in the solubility of the steroids but not strong enough to affect their permeation though UWL. The permeability coefficient for hydrocortisone was not affected by maltose, not even at relatively high maltose concentrations. The permeation studies show that the additives do not have the effect on the water structure in a range and intensity, which would be needed to affect the permeability coefficients. For HPβCD the decrease observed in the permeability coefficient is due to hydrocortisone/HPβCD complex formation and consequent complex aggregation to form aggregates that have total molecular weight greater than 14 kDa exceeding the molecular-weight-cutoff of the membrane (Loftsson et al. 2004).

4. Experimental

4.1. Phase solubility studies

An excess amount of hydrocortisone or dexamethazone (both Fagron, NL) was added to an aqueous solution, containing 0, 3, 6, 9, 12 and 15% (w/v) of potassium iodide (KI), potassium bromide (KBr), potassium chloride (KCl), potassium fluoride (KF), maltose (all purchased from Sigma Aldrich, U.S.A.) or HPβCD (2-hydroxypropyl-β-cyclodextrin with degree of substitution 0.6, from Wacker, Germany). The suspensions formed, were heated in an autoclave (Astell MXN 472, UK) in sealed containers to 121 °C for 20 min. The suspensions were allowed to cool to room temperature (22–23 °C), small amount of solid drug was then added and the sealed containers equilibrate for 7 days, while agitated on a shaking plate (EB Edmund Bühler GmbH, Germany) at 250 rpm. After equilibrium was attained, the suspension was filtered through a 0.45 μm RC media filter (Spartan 13/Whatman, Germany), diluted and analyzed by HPLC.

4.2. Permeability studies

Permeability studies were carried out in Franz diffusion cells (SES GmbH – Analysesysteme, Germany), 12 ml receptor phase, 2.22 cm^2 diffusion area) using double layer cellophane membranes enveloping an IPN-layer. Dry cellophane membranes (Spectra/Por® MWCO 12-14.000, USA) were soaked in aqueous solution containing the studied concentration of additive. IPN was prepared by dissolving 5% (w/v) hydroxypropyl methylcellulose (viscosity of 2% w/v solution is given to be 40-60 cp at 20 °C, Sigma, USA) in aqueous solution containing the studied concentration of additive (i.e. potassium halide, maltose or HPβCD) by stirring (Variomag Poly 15, USA) for 1 h. The membrane was built like a sandwich starting from single cellophane membrane, followed by a 100 μm thick ring spacer filled with the IPN and covered with another single cellophane membrane. Donor phase was prepared like described previously under phase solubility studies and filtrated through a 0.45 μm RC media filter (Spartan 13/Whatman, Germany). An aqueous solution with identical additive and at identical concentration, as in the donor phase or in IPN, was used as receptor phase. In other words, the composition of the donor phase, the IPN and the receptor phase were identical except the donor phase contained the drug, hydrocortisone, and the IPN contained 5% of the cellulose. Samples were taken from the receptor compartment every 10 min starting 30 min after adding the donor phase until 120 min and analyzed by HPLC.

4.3. Quantitative determination of the drugs

Dionex Ultimate 3000 HPLC system (Dionex, Germany) was used under isocratic conditions for quantitative determinations of the drugs using Phenomenex Luna C18 150 × 4.60 mm, 5 micron column (Phenomenex, UK) with a matching 4.0 × 10 mm pre-column. The mobile phase consisted of acetonitrile, water and tetrahydrofuran 33:66:1 (volume ratios) at flow of 1.5 ml/min and UV detection at 244 nm (hydrocortisone) and 241 nm (dexamethasone). The retentiontimes were 3.1 and 6.1 min for hydrocortisone and dexamethasone, respectively.

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