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Research paper

Investigating how the attributes of self-associated drug complexes influence the passive transport of molecules through biological membranes

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ABSTRACT

Relatively little is known about how drug self-association influences absorption into the human body. This study presented two hydrophobic membranes with a series of solutions containing different types of tetracaine aggregates with the aim of understanding how the attributes of supramolecular aggregate formation influenced passive membrane transport. The data showed that aqueous solutions of the unprotonated form of tetracaine displayed a significantly higher ($p < 0.05$) passive membrane transport compared to solutions with mixtures of the unprotonated and protonated drug microspecies (e.g. transport through the skin was 0.96 ± 0.31 μg cm^{-2} min^{-1} and 1.59 ± 0.26 μg cm^{-2} min^{-1} respectively). However, despite an enhanced rate of drug transport and a better membrane partitioning the unionised molecules showed a significantly longer ($p < 0.05$) lag time to membrane penetration compared solutions rich in the ionised microspecies. Analytical characterisation of the solutions applied to the apical surface of the membranes in the transport studies showed that larger tetracaine aggregates with smaller surface charge gave rise to the longer lag times. These large aggregates demonstrated more extensive intermolecular bonding and therefore, it was suggest that it was the enhanced propensity of the unionised species to form tightly bound drug aggregates that caused the delay in the membrane penetration.

1. Introduction

Pharmacologically active compounds can display amphiphilic properties and this can lead to molecular aggregation in solution. Aggregate formation can have a significant impact on the biological function of a molecule because it can influence the passage through membranes. These changes are driven by alterations in diffusion speed and modification of the interactions with other molecules [1–3]. However, the physical interactions between the aggregated and non-aggregated entities, the multiple routes that molecules can take through a barrier and the potential for both the unaggregated and aggregated drug to pass through a membrane mean that the influence of molecular aggregation upon transport is a complex field of study that warrants further investigation.

One example of a molecule that is known to aggregate in solution is tetracaine [4]. Its self-association is thought to be driven by intermolecular tertiary amine hydrogen bonding and amine–ester hydrogen bonding [4]. Tetracaine has a good bioavailability when applied topically to biological membranes [5–7], but it can have a slow onset of topical anaesthesia (30–60 min), which hinders its effective clinical use in certain contexts [8–10]. Attempts to enhance tetracaine diffusion across the membranes have been reported in the literature [11–13], but there has been very little investigation into how molecular aggregation influences the passive diffusion of this drug and thus the consequences of molecular aggregation on its speed of onset remain unknown.

Tetracaine is commercially available as a 4% gel (formulation pH ~ 9) and can exist in solution as three different microspecies depending on the pH. Tetracaine base (TC) is thought to be the efficient in crossing biological barriers [14], and it is the dominant microspecies above pH 9. Below pH 6.5 both the tertiary (TCH^+) and secondary amine (TCH_2^{2+}) are protonated and the microspecies TCH^+ prevails at the physiological pH of the skin (4.2–6.5). It is generally accepted that the tetracaine tertiary amine binds to the sodium channels, blocking the sodium influx, inhibiting nerve cell depolarization and preventing the propagation of nerve cell impulses [15], but which is the most effective species to gain rapid penetration into the skin remains less clear.

The aim of this work was to use tetracaine as a model drug in order to gain a better understanding of how the physical attributes

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of supramolecular masses formed as a consequence of drug aggregation influence hydrophobic membrane transport. Tetracaine was chosen as the model agent due to the fact that its amphiphilic characteristics could be manipulated by simply altering the pH of aqueous solutions containing the drug when it was above its critical aggregation concentration (CAC). This allowed a series of aqueous vehicles where the degree of ionization of both the tertiary and secondary amine was varied to be presented to two hydrophobic membranes, porcine skin and silicone, in the anticipation that different types of drug aggregates and different transport rates could be recorded and analysed. Skin was chosen as an example of a biological barrier as there was evidence in the literature that tetracaine could pass through this membrane [5–7]. Photon correlation spectroscopy was employed to determine the critical aggregation concentration at which nanosized aggregates were formed and zeta potential measurements provided details of the electrostatic interactions. Fourier transform infrared spectroscopy (FTIR) and ^{1}H NMR were employed to investigate the molecular arrangement and intermolecular bonding between the different microspecies in solution.

2. Materials and methods

2.1. Materials

Acetonitrile and methanol both HPLC grade, grade A glass pipettes, clear glass high performance liquid chromatography (HPLC) vials with crimpable lids and 0.45 μm nylon filter papers were purchased from Fischer Scientific (Leicester, UK). Tetracaine base BP grade (99.9%) and deuterium oxide (99.9 atom%) were supplied by Sigma Aldrich (Dorset, UK). Concentrated hydrochloric acid and sodium hydroxide were from Fluka (Dorset, UK). Sodium acetate, potassium dihydrogen phosphate and 1-Octanol were provided by Alfa Aesar (Heysham, UK). Silicone membranes with a thickness of 0.25 mm were purchased from GBUK Healthcare (Selby, UK).

2.2. Tetracaine pKa determination

The automatic titration system used in this study comprises an autoburette (Dosimat 765 liter ml syringe, Metrohm, Buckingham, UK) and pH meter (MP230 Mettler Toledo, Leicester, UK) with a pH electrode (Metrohm, Buckingham, UK). A 0.1 M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25 °C ± 0.1 °C by using a temperature controller (Techne TE-8J, Sigma–Aldrich, Dorset, UK). The solution under investigation was stirred vigorously during the experiment. A pump with speed capability of 20 mL min^{-1} (Mini-plus, Gilson, Luton, UK) was used to circulate the test solution through a quartz flow cuvette using a cuvette with a path length of 0.1 cm. The flow cuvette was mounted on an UV–visible spectrophotometer (HP 8453, Agilent, Cheadle, UK). All instruments were interfaced to a computer and controlled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: the pH of a solution was increased by 0.1 pH unit by the addition of KOH from the autoburette; when pH readings varied by <0.001 pH unit over a 3 s period the spectrum of the solution was then recorded. The cycle was repeated automatically until the defined end point pH value was achieved. All the titration data were analysed with the pHab program [16]. The microspecies plot was generated with the HYSS program [17].

2.3. Tetracaine aggregate analysis

2.3.1. Photon correlation spectroscopy characterisation

Changes in derived count rate and zeta potential of the donor solutions were tracked using photon correlation spectroscopy (PCS) (Malvern Nanoseries Zetasizer, Malvern Instruments Ltd., Malvern, UK). Measurements were taken at a scattering angle of 173°. Refractive index and viscosity constants were set at 1.33 and 0.88 mPa s, respectively. Samples were filtered through a 0.45 μm cellulose nitrate filter prior to the analysis. The scattering information was determined at increasing tetracaine molar concentrations in acetate buffer (0.1 M) at pH 4, 6, 7.6, 9 and 10. The critical aggregation concentration was determined from the data discontinuity in the linear model applied to the derived count rate data; this was confirmed by the application of a second derivative function (OriginPro 9.1 Software, OriginLab, Northampton, USA). The size of the molecular aggregates was detected by converting the signal into a hydrodynamic radius using the Stokes–Einstein equation given in (Eq. (1)), where k is the Boltzmann constant, T is the absolute temperature and η is the solvent viscosity. The size of the molecular aggregates was determined above critical aggregation concentration ca. 87–95% of tetracaine saturation in each aqueous vehicle at pH 4, 6, 7.6, 9 and 10. The pH was adjusted to the required value when necessary by adding NaOH (1 M) or acetic acid. Zeta potential magnitude was determined at pH 4, 7.6 and 9 using the same solutions described above.

$$R_H = \frac{kT}{6\pi\eta D} \quad (1)$$

2.3.2. Molecular dynamic studies

Tetracaine self-assembly was generated from the crystal structure reported by Nowell et al. [18]. The atomic co-ordinates and unit cell parameters were obtained from the Cambridge Structural Database; entry XISVOK [19]. The assembly was generated ($2 \times 2 \times 2$ unit cells) using the Mercury software [20] and then visualised using Accelrys Viewerlite v5.0 (Biovia, San Diego, USA).

2.3.3. Apparent distribution coefficient

The apparent drug distribution coefficients were measured using tetracaine saturated solutions at room temperature as previously described, using n-octanol in acetate buffer (0.1 M) at pH 4, 6, 7.6, 9 and 10 [21]. After phase separation the aqueous phase was withdrawn and samples were centrifuged at 13,000 rpm (Biofuge, Heraeus, Germany) and aliquots of the liquid phase were then transferred into vials. The samples were analysed using HPLC. The apparent distribution coefficient (D) was defined as the ratio of the drug concentration in octanol (C_0) to the total concentration of ionised (C_i) and unionised (C_u) drug in the aqueous phase (Eq. (2)).

$$D = \frac{C_0}{(C_i + C_u)w} \quad (2)$$

2.3.4. Fourier transform infrared spectroscopy (FTIR) characterisation

Tetracaine solutions at pH 4, 6, 7.6, 9 and 10 were prepared in deuterium oxide at the same concentrations used for molecular aggregate size analysis. Deuterium oxide (D_2O) was employed in the solutions as it dampened the solvent signal in the 1700–1300 cm^{-1} range. The pH was adjusted with NaOH (1 M) or acetic acid. The samples were loaded into a demountable universal transmission cell system (Omni-Cell, Specac Ltd., Kent, UK) fitted with CaF_2 windows and a 25 μm mylar spacer (Specac Ltd., Kent, UK). The infrared spectra were recorded from 4500 to 1000 cm^{-1} using a Spectrum One spectrometer (Perkin Elmer Ltd., Bucks, UK) and spectral analysis was performed with Spectrum software version 5.3.1 (Perkin Elmer Ltd., Bucks, UK). After normalisation of transmittance, background subtraction and baseline correction of the spectra, hydrogen bonding interactions in the carbonyl group region were determined by analysing spectral shifts of the C=O stretching peak as described previously [22].