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The Use of Bile Salt Micelles for the Prediction of Human Intestinal Absorption

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ABSTRACT

Human intestinal absorption (HIA) will dictate biopharmaceutical performance through its influence on absorption, distribution, metabolism, and elimination and can vary significantly depending upon the nature of the compound under consideration. In this study, an *in vitro* assay method is proposed for the prediction of HIA through the measurement of drug solubility in an aqueous phase containing micellar bile salt, namely sodium deoxycholate. A series of twenty compounds, displaying a range of physicochemical properties and known HIA values, were analyzed using UV spectroscopy to determine a solubilization ratio for each compound. A micelle/water partition coefficient ($K_{xm/a}$) was calculated and then used to develop an equation through simple linear regression; logit HIA $= -0.919 + 0.4618 \log K_{xm/a}$ ($R^2 = 0.85$). From this equation, a value for % HIA was determined which compared well with literature. Furthermore, 4 additional drugs were then analyzed using the developed equation and found to match well with literature, confirming the suitability of the method. Using a simple, economic, and robust UV bile salt assay allows prediction of HIA and avoids many of the disadvantages of other techniques, such as animal-based methods.

Introduction

Human intestinal absorption (HIA) is the mechanism through which drugs traverse from the intestine into the bloodstream. The vast majority of active pharmaceutical ingredients are administered orally; thus, it is essential that they are absorbed within the intestine to reach the intended site of action. Although it is possible to measure the percent HIA (% HIA) during clinical studies, it is far more useful to be able to predict the value much earlier on during drug development. It is for this reason that a significant amount of research has been undertaken in an attempt to develop a reliable, robust, and accurate method to predict % HIA.

Several different predictive approaches have been undertaken, including computational (*in silico*) methods,[1,2] such as quantitative structure-activity relationships[3,4] and physiologically based pharmacokinetic modelling.[5] These techniques have a clear advantage in that they remove the need for costly laboratory-based experimental measurement yet their predictive ability can be limited.

In vitro models for the prediction of absorption include the application of dissolution analysis,[6] chromatographic analysis,[7] and dynamic gastric models.[8] Many of these *in vitro* models have included the presence of physiologically relevant solvent compositions, mainly because it is known that solvent composition dictates intestinal drug solubility which, in turn, is an important factor in determining the rate, and extent, of absorption.[9] The specific components within human intestinal fluids that dramatically alter drug solubility are bile salts. The main biological function of bile salts is to solubilize lipids and vitamins in the intestine with a similar effect encountered for orally administered drugs. For a full review of the absorption-enhancing effects of bile salts.[10]

In humans, the composition of bile salts is rather complex and for the purposes of this study was simplified to consider 1 bile salt in particular, namely sodium deoxycholate (NaDC). NaDC is a well-characterized amphiphilic molecule which can undergo micellar aggregation,[11,12] stabilized by polar interactions,[13] with comparatively small aggregation numbers as a result of the rigid molecular structure.[14] Previous research within our group has shown that NaDC, when in the presence of drugs, will exhibit modified physicochemical properties, for example, a variable (drug-specific) reduction in critical micellar concentration.[15]

When quantifying (or comparing) enhancement in solubility for a specific drug, or series of drugs, it is possible to evaluate the solubilization ratio (SR), where SR is equal to the moles of drug solubilized per mole of bile salt. One study in particular calculated

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SR for a series of steroids and then used these data to calculate micelle/water partition coefficients ($K_{m/w}$) which were then correlated with octanol/water partition coefficients ($P_{o/w}$).[16] Using this same theory as a basis for drug-NaDC measurement, this article describes the evolution of measuring SR and then using these values as the basis to form an equation to permit prediction of % HIA, thereby presenting an *in vitro* method to predict *in vivo* behavior.

Materials and Methods

Materials

Aqueous solutions of NaDC (97%), used as purchased from Sigma-Aldrich (Dorset, UK), were prepared by dilution from a 20 mM stock solution with distilled water as necessary to achieve concentrations of 7, 9, 11, 13, 17, and 20 mM (i.e., always at concentrations greater than the stable micelle critical micellar concentration of NaDC[12]). The following 24 compounds considered in this work were used as purchased: acetaminophen (99%; Sigma-Aldrich), acetyl salicylic acid (99%; Acros Organics, Geel, Belgium), alprenolol (98%; Sigma-Aldrich), amitriptyline (98%; Sigma-Aldrich), carbamazepine (99%; Sigma-Aldrich), cimetidine (Sigma-Aldrich), diclofenac (98%; TCI Europe, Zwijndrecht, Belgium), diphenhydramine (98%; TCI Europe), fenoprofen (97%; Fluka, Dorset, UK), fluconazole (98%; Sigma-Aldrich), flurbiprofen (98%; TCI Europe), gemfibrozil (98%; TCI Europe), ibuprofen (98%; BASF, Cheshire, UK), indomethacin (99%; Sigma-Aldrich), ketoprofen (98%; Sigma-Aldrich), lidocaine (98%, Sigma-Aldrich), mannitol (98%; Sigma-Aldrich), meloxicam (98%; TCI Europe), naproxen (98%; Sigma-Aldrich), phenylbutazone (99%; Sigma-Aldrich), piroxicam (98%, Sigma-Aldrich), propranolol (99%; Sigma-Aldrich), quinine (96%; Fluka), and terbutaline (96% Sigma-Aldrich). All experimental work was conducted without altering the pH or ionic strength to avoid the formation of a surfactant-gel hydropolymer.

Method

A calibration plot was established at each of the 6 bile salt concentrations using the Agilent Cary 60 UV-Vis Spectrophotometer set at wavelength of maximum absorbance for each drug as follows: acetaminophen λ_{max}. 243 nm, acetyl salicylic acid λ_{max}. 295 nm, alprenolol λ_{max}. 270 nm, amitrityline λ_{max}. 240 nm, carbamazepine λ_{max}. 284 nm, cimetidine λ_{max}. 218 nm, diclofenac λ_{max}. 276 nm, diphenhydramine λ_{max}. 221 nm, fenoprofen λ_{max}. 271 nm, fluconazole λ_{max}. 260 nm, flurbiprofen λ_{max}. 247 nm, gemfibrozil λ_{max}. 274 nm, ibuprofen λ_{max}. 272 nm, indomethacin λ_{max}. 320 nm, ketoprofen λ_{max}. 261 nm, lidocaine λ_{max}. 262 nm, mannitol λ_{max}. 295 nm, meloxicam λ_{max}. 362 nm, naproxen λ_{max}. 230 nm, phenylbutazone λ_{max}. 264 nm, piroxicam λ_{max}. 355 nm, propranolol λ_{max}. 292 nm, quinine λ_{max}. 332 nm, terbutaline λ_{max}. 280 nm; also, the sample cell was thermostated at 37°C. Separately, an excess of drug was added to 1 mL of each bile salt concentration in a microcentrifuge tube and placed in a shaking water bath for 48 h at 37°C, then centrifuged at 13,000 rpm, filtered, and diluted using the corresponding bile salt concentration. Using the regression equation obtained from the established calibration plot of each drug at each bile salt concentration, the concentration of solubilized drug was determined. A plot of the amount solubilized with bile salt concentration facilitated calculation of the SR whereby the mole fraction solubilized (X_m) is equal to SR/(1 + SR) and can be combined with the literature-based calculated mole fraction aqueous solubility (X_a) to determine the micelle/water partition coefficient ($K_{xm/a}$) as follows[17]:

$$K_{xm/a} = X_m/X_a$$

Results from the UV analysis permitted the development of a dataset that contained $\log K_{xm/a}$ values for 20 compounds along with their physicochemical parameters (e.g., molecular weight, rotatable bonds, molar volume, number of hydrogen bond acceptors) and published HIA values, facilitating development of an equation to relate $\log K_{xm/a}$ with HIA using simple linear regression in combination with the established equation:

$$Logit\ HIA = \log\left[\%\ HIA/(100 - \%\ HIA)\right]$$ [18]

A further 4 compounds were then similarly analyzed by measuring $\log K_{xm/a}$ to predict % HIA. A comparison was then made between the predicted values and those published in literature. Simple linear regression analysis was carried out using Minitab 17® (Minitab Inc., State College, PA; licensed to the University of Huddersfield) where the previously mentioned dataset was imported into it. The final model was obtained by excluding molecular descriptors which were not statistically significant (p value > 0.05), and those with unacceptably high levels of variance inflation factor, which is considered as a multicollinearity indicator, were not included in the final model. Cook's distance and residuals were used to detect whether any of the model variables had high leverage. The optimal final model was then obtained including only $\log K_{xm/a}$ as a predictor for logit HIA, and the model was then validated using 4 compounds.

Results and Discussion

In total, 24 drugs were analyzed to determine the concentration of drug in solution as a function of NaDC concentration; these were selected to cover a range of physicochemical properties, such as reported HIA, log $P_{o/w}$, and other properties. All data were then plotted to determine a SR value for each drug (i.e., the slope), a selection of which can be seen in Figure 1.

Figure 1 clearly shows a linear relationship between the concentration of drug and the concentration of NaDC. Only linear sections of the plots were incorporated to calculate SR, some were deemed to be nonlinear, such as the lower concentrations of quinine and the higher concentrations of acetaminophen (data not shown). These nonlinear relationships may be due to preferential drug-drug interactions rather than drug-NaDC interactions as such

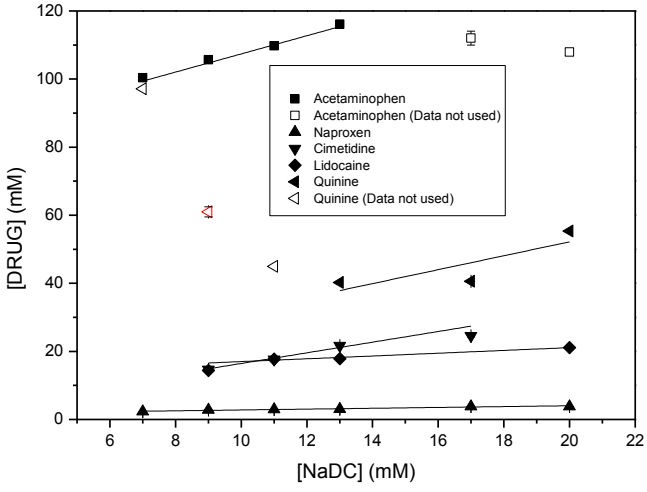

Figure 1. Plots of the concentration of a selection of analyzed compounds in solution with NaDC concentration to determine the SR. Each data point = mean, n = ≥3, ±SD.