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Human serum albumin-mimetic chromatography based hexadecyltrimethylammonium bromide as a novel direct probe for protein binding of acidic drugs

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ABSTRACT

Human serum albumin (HSA) is the most important drug carrier in humans mainly binding acidic drugs. Negatively charged compounds bind more strongly to HSA than it would be expected from their lipophilicity alone. With the development of new acidic drugs, there is a high need for rapid and simple protein binding screening technologies. Biopartitioning micellar chromatography (BMC) is a mode of micellar liquid chromatography, which can be used as an in vitro system to model the biopartitioning process of drugs when there are no active processes. In this study, a new kind of BMC using hexadecyltrimethylammonium bromide (CTAB) as micellar mobile phases was used for the prediction of protein binding of acidic drugs based on the similar property of CTAB micelles to HSA. The use of BMC is simple, reproducible and can provide key information about the pharmacological behavior of drugs such as protein binding properties of new compounds during the drug discovery process. The relationships between the MLC retention data of a heterogeneous set of 17 acidic and neutral drugs and their plasma protein binding parameter were studied and second-order polynomial models obtained in two different concentrations (0.07 and 0.09 M) of CTAB. However, the developed models are only being able to distinguish between strongly and weakly binding drugs. Also, the developed models were characterized by both the descriptive and predictive ability (R^2 = 0.885, R^2_{CV} = 0.838 and R^2 = 0.898, R^2_{CV} = 0.859 for 0.07 and 0.09 M CTAB, respectively). The application of the developed model to a prediction set demonstrated that the model was also reliable with good predictive accuracy.

1. Introduction

In drug discovery, the knowledge of drug-plasma protein binding is valuable to evaluate adsorption, distribution, metabolism and excretion (ADME) related properties and the whole pharmacokinetic profile of drug candidates. It is widely accepted that only the free concentration rather than the total drug concentration is pharmacologically active [1]. The serum proteins favor the solubility of the drugs and also act as drug carriers to the drug's specific target. Since only the free drug is able to interact efficiently with the target, to become of therapeutical interest, the interaction between the drug and the carrier should be strong enough to facilitate the transport but also weak enough to release the drug to the target. Therefore, the extent of protein–drug binding can have a significant impact on pharmacokinetic parameters such as clearance rates and volume of distribution [2–4]. All these considerations have to be taken into account for new drug candidates and highlight the importance of determining the binding affinity to HSA in order to optimize the pharmacokinetic behavior of the drug.

Human serum albumin (HSA) and α_1-acid glycoprotein (AGP) are the two major binders of acidic and basic drug molecules in plasma, respectively. Neutral lipophilic drug molecules can bind to both HSA and AGP as well as to other plasma proteins [4]. HSA, the most abundant circulating protein in the blood, has multiple lipophilic binding sites and binds a diverse set of drugs, especially neutral and negatively charged hydrophobic compounds [5]. This implies the formation of ionic bonds, although non-specific hydrophobic interactions are considered to be essential in binding [6]. In early years, the solute hydrophobicity as measured by its partition behavior between octanol and water ($\log P_{ow}$) has been used to correlate protein binding [7,8]. However, such correlations are unfortunately unreliable, because recognition forces like ionic bonds are not encoded in this biphasic system [9].

Chromatography is a powerful technique for measuring the physicochemical parameters of drugs. A variety of chromatographic approaches have been used to evaluate protein–drug binding such

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as size-exclusion chromatography [10], high performance frontal analysis [11], and affinity chromatography using HSA or bovine serum albumin (BSA) columns [12–14].

Recently, biopartitioning micellar chromatography (BMC) with polyoxyethylene 23 lauryl ether (Brij-35) micellar mobile phases and C18 reversed stationary phase in adequate experimental conditions has been proposed as a noncell-based and high-throughput primary screening tool and attracted considerable attention as an in vitro model to predict the different pharmacological behaviors of drugs such as oral absorption [15], toxicity [16], blood–brain barrier behavior [17], skin permeability [18] and protein drug binding [19]. This methodology is positioned under the umbrella of quantitative retention–activity relationships (QRARs) and has the important advantage that it requires only one descriptor (the retention factor) to construct the QRAR models and consequently it is possible to obtain validated models from a smaller number of compounds than the QSAR approach [19]. The adequacy of QRAR models is due to the fact that the characteristics of the compounds such as hydrophobicity, electrical charge and steric effects determine both their retention in BMC and their pharmacokinetic and pharmacodynamic behavior [20]. Here, it should be remarked that the physicochemical properties of drugs (i.e. hydrophobicity and electrical charge) play an important role in ADME processes. However, there are other specific metabolic and active processes where these properties become less important [19]. Distribution is a process that is mediated by carrier proteins (mainly serum albumin and α_1-glycoprotein). The percentage of a drug bound to these proteins strongly depends on the hydrophobicity and electrical charge of the drug [21]. However, stereochemistry also plays a role in drug-protein binding and the information cannot be considered to result only from the binding of drugs to specific binding sites. Although BMC has demonstrated its capability of predicting different properties of many different families of compounds, it is necessary to note its limitations in order to clarify the situations where it is useful. BMC can neither describe active and metabolic processes nor determine enantioselective differences between the enantiomers of a chiral drug [19]. Despite the mentioned limitation of present method, it has some important advantages over other in vitro techniques used to protein binding assay (e.g. equilibrium dialysis [22], ultrafiltration [22], biosensors and 96-well fluorescence plate readers [23,24]): the preparation of the chromatographic system is rapid, simple and economical, the reproducibility intra- and inter-day of the retention data is very high that permits the protein binding estimation with high accuracy [25]. Also, this approach can provide key information about the potential protein binding properties of new acidic drugs during the drug discovery processes and so, can be very useful in medicinal chemistry and pharmaceutical research.

In our previous work, we have used sodium dodecyl sulfate (SDS) as a mobile phase to predict protein binding of basic drugs [26]. To the best of our knowledge, the protein binding prediction of acidic drugs using hexadecyltrimethylammonium bromide (CTAB) in BMC system has not been described in the literature.

The aim of this work is to evaluate the usefulness of CTAB as a mobile phase in BMC to predict protein–drug binding for a heterogeneous set of 17 acidic and neutral drugs (training set). The use of CTAB as a cationic surfactant leads to the formation of biomimetic protein similar to HSA and can be used to emulate both the ionic and hydrophobic interactions of protein and acidic drugs. In addition, CTAB micellar mobile phases prepared at physiological conditions could also mimic the environment of protein–drug binding. The retention of compounds in this chromatographic system depends on their interactions with the modified reversed stationary phase and micelles present in the mobile phase. These interactions are governed by hydrophobic, electronic and steric properties of compounds. Regression models for the prediction of protein–drug binding is derived from the training set using the backward-multiple linear regression (MLR) analysis and compared at two CTAB concentrations, 0.07 and 0.09 M. Then, the predictive ability of models was evaluated by external and internal (leave-one-out method) [27–29] validations.

2. Materials and methods

2.1. Instruments

The HPLC system consisted of a model 515 solvent delivery system equipped with model 7725i injector fitted with a 20 μL loop, all from Waters (Milford, MA, USA) and a Perkin-Elmer LC-95 UV detector (Norwalk, CT, USA) set at 220 nm for non-steroidal anti-inflammatory drugs (NSAIDs), 287 nm for quinolones, 240 nm for hypnotic and antiepileptic drugs and 300 nm for other compounds. The analytical column used was Perfectsil Target ODS-3 (5 μm, 150 × 4.6 mm i.d.) from MZ-Analysentechnik (Mainz, Germany). All experiments were performed using isocratic elution at the flow rate 1.0 ml min^{-1} during chromatography. Also, the column temperature was kept at stable 36.5 °C to approach normal human body temperature.

2.2. Reagents and standards

Mobile phases were prepared by aqueous solutions of 0.07 and 0.09 M CTAB (Merck, Darmstadt, Germany). The pH of the micellar eluent was adjusted to 7.4 with 0.05 M phosphate buffer, prepared with disodium hydrogenphosphate and sodium dihydrogenphosphate (analytical grade, Fluka, Buchs, Switzerland). It should be remarked that, the CMC value of CTAB in water (9×10^{-4} M) is depressed by the presence of added salts [30]. Consequently, the effect of salts on the CMC of a surfactant should be kept in mind when using MLC. However, the concentrations of CTAB in the studied mobile phases (0.07 and 0.09 M) are considerably above the CMC value of CTAB in the presence of salts. So, the micelles are definitely formed. To reproduce the osmotic pressure of biological fluids, NaCl (9.2 g L^{-1}, Merck, Darmstadt, Germany) was added to the micellar mobile phase. The training set of drugs used for building the QRAR models were obtained from the internal pharmaceutical laboratories in Iran (Sobhan, Pars-Daru and Abidi). The structures of the compounds studied are shown in Fig. 1. Stock standard solutions of 1 mg ml^{-1} of the model drugs were prepared separately in methanol and then diluted in proper concentrations when they were needed. The solutions were stored at 4 °C. The micellar solutions were prepared in double-distilled, deionized water and vaccum-filtered through a 0.45 μm Millipore solvent filter.

2.3. Data sources, software and data processing

Plasma protein binding data values of 22 acidic drugs were taken from the handbooks of Goodman and Gilman's [31] and of Martindale [32]. Of the 22 compounds, 17 compounds were chosen as the training set (Table 1) and the remaining ones were used for the prediction set. The logarithm of octanol–water partition coefficients ($\log P_{ow}$) and acidity constants (pK_a) in Table 1 were taken from the Ref. [17,33–37]. The retention data in BMC were calculated as a retention factor, $k_{BMC} = (t_R - t_0)/t_0$ where t_R is the retention time of the test compound and t_0 corresponds to column dead time. The dead time value was determined for each injection as the first perturbation in the chromatogram and the average of these values (average t_0 = 1.481 min) was used for all 17 drugs. The logarithm of the retention factor values ($\log k_{BMC}$) calculated for modeling, was the average of at least triplicate measurements. The retention data were highly reproducible, the relative standard deviation (R.S.D.)