Quantitative structure-retention relationship of selected imidazoline derivatives on α_1-acid glycoprotein column

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ABSTRACT

The retention behaviour of 22 selected imidazoline drugs and derivatives was investigated on α_1-acid glycoprotein (AGP) column using Sørensen phosphate buffer (pH 7.0) and 2-propanol as organic modifier. Quantitative Structure-Retention Relationships (QSRR) models were built using extrapolated $\log k_w$ values as well as isocratic retention factors ($\log k_5$, $\log k_8$, $\log k_{10}$, $\log k_{12}$, $\log k_{15}$ obtained for 5%, 8%, 10%, 12%, and 15%, of 2-propanol in mobile phase, respectively) as dependant variables and calculated physicochemical parameters as independant variables. The established QSRR models were built by stepwise multiple linear regression (MLR) and partial least squares regression (PLS). The performance of the stepwise and PLS models was tested by cross-validation and the external test set prediction. The validated QSRR models were compared and the optimal PLS-QSRR model for $\log k_w$ and each isocratic retention factors (PLS-QSRR($\log k_5$), PLS-QSRR($\log k_8$), PLS-QSRR($\log k_{10}$), MLR-QSRR($\log k_{12}$), MLR-QSRR($\log k_{15}$)) were selected. The QSRR results were further confirmed by Linear Solvation Energy Relationships (LSER). LSER analysis indicated on hydrogen bond basicity, McGowan volume and excess molar refraction as the most significant parameters for all AGP chromatographic retention factors and $\log k_w$ values of 22 selected imidazoline drugs and derivatives.

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1. Introduction

Serum-protein binding is crucial factor for drug distribution, pharmacokinetic profile and pharmacological effects. Strong protein binding may cause low clearance, low brain penetration, drug–drug interactions, loss of efficacy and influence on drug safety [1–4]. Human serum albumin (HSA) and α_1-acid glycoprotein (AGP) are two most abundant serum proteins presenting plasma binding capacity for drugs [5,6]. For AGP has been reported lower isoelectric point than for HSA [7–9]. Therefore, AGP exerted significant affinity to basic and neutral drugs, while HSA dominantly bounded acidic and neutral drugs [9]. Drug binding with the plasma proteins involve formation of electrostatic and hydrophobic interactions as well as specific enantiomer recognition at the binding domain of the proteins [10,11]. Pathological processes in infection, cancer, inflammation, influence on the AGP plasma levels and glycosylation status and therefore influence on affinity and extent of drug-protein binding [12–14]. Therefore extent of a drug binding with AGP should be considered as one of crucial factors in initial phases of drug discovery and development.

Previously correlated lipophilicity indices with protein binding [15,16] was not sufficient to define plasma protein binding [17,18] for structurally diverse compounds. Previously reported equilibrium dialysis and ultrafiltration are time and cost consuming methods to determine AGP binding of drugs [19,20]. Also, there are no specific *in silico* studies for predictions of imidazoline derivatives binding with AGP [21–23].

The protein-based human serum albumin and α_1-acid glycoprotein HPLC columns, packed with chemically immobilized HSA and AGP, respectively on a silica stationary phase [24–26], provide very efficient experimental tool for studying drug-proteins interaction [22,23]. Since the HSA is the most abundant protein in plasma, HSA chromatography has been proposed for rapid plasma protein binding screening [22,27]. This type of studies were underscoring very important role of AGP for binding of basic and neutral drugs [23].

Several displacement studies have investigated consistency of the immobilized AGP binding properties and those of the protein in solution [28–31], while the molecular factors ruling the retention mechanism were examined for HSA chromatography [22,27,32,33]. Only few chemometric studies have compared HSA and AGP retention and defined complexity of the retention mechanism [23,34–36].

Based on the important role of AGP in plasma protein binding of basic and neutral drugs and the advantages of HPLC, we examined

AGP retention of 22 imidazoline derivatives under different chromatographic conditions. The selected imidazoline derivatives are basic/neutral compounds and therefore predominantly bind AGP in plasma.

Most of the examined compounds (clonidine, moxonidine, rilmenidine, xylometazoline, amiloride, phentolamine, metformin, brimonidine, guanfacine and oxymetazoline) are approved drugs, while the other imidazoline derivatives are ligands used in medicinal chemistry and experimental pharmacology as lead compounds. Group of selected imidazoline drugs belong to the family of imidazoline and the alpha adrenergic receptors ligands with diverse pharmacological effects, such as centrally acting antihypertensives (rilmenidine, moxonidine, clonidine, phentolamine, brimonidine, guanfacine) [37–39], diuretics (amiloride) [40], and nasal decongestives (xylometazoline, oxymetazoline).

The retention behaviour of 22 selected imidazoline drugs and derivatives was investigated on AGP column and used for quantitative structure-retention relationships (QSRR) modeling for each isocratic retention factors ($\log k_5$, $\log k_8$, $\log k_{10}$, $\log k_{12}$, $\log k_{15}$ obtained for 5%, 8%, 10%, 12%, and 15%, of 2- propanol in mobile phase, respectively) and for extrapolated $\log k_w$ values in order to explore the crucial interactions of selected drugs with AGP. Further goals of our study were to define essential molecular factors for the retention mechanism expressed by Abraham's solvation parameters and to evaluate the potential of AGP retention to simulate drug-AGP protein binding. The QSRR modeling was performed by use of stepwise Multiple Linear Regression (MLR) and Partial Least Squares (PLS) regression.

2. Materials and methods

2.1. Chemicals and reagents

The examined data set consisting of 22 imidazoline derivatives and related compounds were purchased from Sigma–Aldrich, St. Louis, MO, USA (clonidine hydrochloride, moxonidine hydrochloride, guanfacine hydrochloride, brimonidine hydrochloride, efaroxan hydrochloride, idazoxan hydrochloride, rilmenidine hemifumarate, harman, harmine hydrochloride, tizanidine hydrochloride, xylometazoline hydrochloride, tetrahydrozoline hydrochloride, oxymetazoline hydrochloride, antazoline phosphate, phentolamine hydrochloride, benazoline oxalate, cirazoline hydrochloride, detomidine hydrochloride, metformin hydrochloride, and RX 821002 hydrochloride) or provided by Zdravlje-Actavis, Leskovac, Serbia (trimazoline hydrochloride) and Galenika, Belgrade, Serbia (amiloride hydrochloride).

Methanol (J.T. Baker, Deventer, Netherlands) and 2-propanol (Sigma–Aldrich, St. Louis, MO, USA) of HPLC grade and deionized water (TKA water purification system, Niederelbert, Germany) were used throughout this study. Potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Merck (Darmstadt, Germany).

2.2. Chromatographic conditions

The HPLC analysis was performed on a Dionex Ultimate 3000 system (Thermo Fisher Scientific, Germering, Germany) equipped with autosampler, Dionex Ultimate 3000 quaternary pump, and photodiode array detector. The Chromeleon 7 software (Thermo Fisher Scientific, Germering, Germany) implemented in chromatographic system was used for data acquisition. The retention behaviour of selected compounds were examined on CHIRALPAK®AGP column 100 mm × 2 mm I.D. packed with α_1-acid glycoprotein chemically bound to silica particles size of 5 μm (DAICEL CORPORATION, France). The mobile phases consisted of

Sørensen phosphate buffer (pH 7.0; 0.01 M) and 2-propanol added in different concentrations in the range 5–15% [41]. The flow rate was set to 0.2 mL/min and temperature to 25 °C. The UV detection was performed at 225 nm.

Standard solutions of all tested compounds were prepared in methanol in concentration 1 mg/mL and subsequently diluted with the mobile phase (Sørensen phosphate buffer (pH 7.0; 0.01 M)-2-propanol (90:10, v/v)) to final concentrations 0.1 mg/mL. The 5 μL of final solution was injected into the column.

Retention time t_r of each compound was measured from three separate injections and used for calculation of logarithm with base 10 of retention factor ($\log k$) according to Eq. (1):

$$\log k = \log \left(\frac{t_r - t_0}{t_0} \right) \tag{1}$$

where t_0 is the retention time of KNO_3.

Isocratic $\log k$ values obtained in the presence of different concentration of 2-propanol in mobile phase were further linearly extrapolated to $\log k_w$ values corresponding to 100% of buffered eluent (Table S1, Supplementary material).

2.3. Computational method

Among 22 analyzed compounds clonidine, moxonidine, tizanidine, brimonidine and rilmenidine can exist in two major tautomeric forms (amino and imino). Based on the obtained Self Consistent Field (SCF) energy calculated by using B3LYP/6–31 G (d,p) level of the Density Functional Theory (DFT) [42,43] included in ChemBio3D Ultra 13.0 program [44] it has been shown that moxonidine and rilmenidine exist as more stable imino tautomers, while predominant tautomeric form of tizanidine is amino form. The SCF energies obtained for tautomeric forms of brimonidine and clonidine were equivalent and therefore the both tautomeric forms were included in the models building. Therefore, selection of dominant molecules/cations species at pH 7.4 has been performed for set of 24 structures using the MarvinSketch 6.1.0, ChemAxon program [45]. All dominant forms of 24 structures were pre-optimized with semiempirical PM3 method [46,47] using Gaussian09 software [48] included in ChemBio3D Ultra 13.0 program [44] and then refined by using more precise Hartree-Fock/3–21 G method for energy minimization [49].

2.3.1. Calculation of molecular descriptors

For all optimized molecular structures molecular descriptors were calculated using ChemBio3DUltra 13.0 [44], Dragon 6.0 [50], and Gaussian09 software [48]. Energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were calculated by Gaussian09 software [48] using B3LYP/3–21 G theory. The global reactivity parameters, such as hardness (η), global softness (S), chemical potential (μ), electronegativity (χ) and electrophilicity index (ω) were calculated according to formulas described in references [51–53]. Hardness is calculated as $\eta = (E_{LUMO} - E_{HOMO})/2$, global softness is defined as the inverse of global hardness, $S = 1/2\eta = 1/(E_{LUMO} \times E_{HOMO})$. Chemical potential is calculated as $\mu = (E_{LUMO} + E_{HOMO})/2$, electronegativity is expressed as negative value of chemical potential, $\chi = -\mu$. Electrophilicity index is calculated by formula $\omega = \mu2/2\eta$ [51–53]. Plasma Protein Binding data (PPB) values were calculated by use of ADMET Predictor v. 6.5.0 software [54]. Physico-chemical and Biopharmaceutical Module of ADMET Predictor program is used for estimation of the percentage of free drug in plasma.

Descriptors with intercorrelation lower than 0.99 were used for PLS analysis while descriptors with intercorrelation lower than 0.90 were used for MLR study. All molecular properties with constant values were excluded from the data set for QSRR modeling.