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Development of predictive quantitative retention-activity relationship models of HMG-CoA reductase inhibitors by biopartitioning micellar chromatography

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Abstract

Biological fluid cell membranes are barriers for the uptake of many kinds of drugs and their metabolites, along with passive transport across membranes and bioaccumulation. Biopartitioning micellar chromatography (BMC) is a mode of micellar liquid chromatography that uses micellar mobile phases of Brij35 under adequate experimental conditions and can be useful to simulate the drug's passive absorption and the transport in biological systems. The use of micellar aqueous solutions of Brij35 as mobile phases in reversed-phase liquid chromatography has proven to be valid to predict the biological activities of barbiturates, benzodiazepines, catecholamines, local anesthetics, non-steriodal anti-inflammatory drugs and tricyclic antidepressants. In this study, the relationships between the capacity factor in BMC and some pharmacokinetic and pharmacodynamic parameters of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are studied. Predictive quantitative retention-activity relationship (QRAR) models describing some of the biological activities and pharmacokinetic properties of HMG-CoA reductase inhibitors are obtained. The results indicate that QRAR model may be a useful tool during the drug discovery process.

Keywords: Quantitative retention–structure relationship; Quantitative retention–activity relationship; HMG-CoA reductase inhibitors; Biopartitioning micellar chromatography

1. Introduction

Traditional pharmacokinetic and pharmacodynamic studies probably prevent the evaluation of many compounds in the early phase of drug discovery because of the difficulties and costs associated with experimental animals as well as the ethical problems. To circumvent the problems associated with screening new drugs in animal, many in vitro models for the prediction of pharmacokinetic and pharmacodynamic parameters have been set up including the use of physicochemical parameters of drugs, the permeability data from cell culture lines and chromatography models [1–4]. Quantitative structure–activity relationship (QSAR) studies play an important role in the research. The application of chromatographic parameters in QSAR gives rise to a new field, quantitative retention-activity relationship (QRAR) [5–7]. A great deal of efforts have been made to develop biological chromatographic models such as immobilized artificial membranes chromatography (IAMs chromatography [8]), immobilized liposomes chromatography (ILs chromatography) [9] and biopartioning micellar chromatography (BMC) [10].

BMC is a chromatographic modality that uses reversed stationary phases and polyoxyethylene (23) lauryl ether (Brij35) solution above the critical micellar concentration (CMC) as mobile phases under adequate experimental conditions [11]. BMC's system could describe the biological behavior of many kinds of drugs and simulate biopartitioning process. The success of BMC in describing drugs' biological behavior can be

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attributed to the fact that the characteristics of the BMC systems are similar to biological barriers and extracellular fluids [12]. In the first place, the stationary phase modified by the hydrophobic adsorption of Brij35 surfactant monomers structurally resembles the ordered array of the membranous hydrocarbon chains. Meanwhile, the hydrophilic/hydrophobic character of the adsorbed Brij35 monomers resembles the polar membrane regions. Secondly, BMC micellar mobile phases prepared at physiological conditions could also mimic the environment of drug biological partitioning. The extracellular and intracellular fluids are basically composed of water, salts, glucose, amino acids, cholesterol, phospholipids, fatty acids and proteins. Phospholipids, cholesterol, fatty acids and triglycerides form micellar complexes with proteins (lipoproteins) (critical micelle concentration, CMC $< 10^{-6}$ M) [12].

HMG-CoA Reductase is a natural compond that helps the liver to produce cholesterol. The HMG-CoA reductase inhibitors, commonly referred to as "statins", get in the way of that process, thus reducing the amount of cholesterol being produced [13–16]. All statins can effectively lower LDL cholesterol (LDL-C or "bad cholesterol"), total cholesterol, and triglycerides; and each drug in this class can also raise HDL cholesterol (HDL-C or "good cholesterol"), which is desirable [16]. Statins are considered as a first-line therapy for the treatment of hypercholesterolemia and have showed remarkable activity in preventing cardiovascular morbidity and mortality [13–15].

2. Experimental

2.1. Instrumental and measurement

An Agilent 1100 series HPLC from Agilent Technologies (Waldbronn, Germany) comprised of G1312A binary pump, G1313A auto sampler, G1314A variable wavelength UV detector, G1322A degasser, G1316A thermostatted column compartment. Data acquisition and processing were performed on HP-Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 μl loop. The HPLC column was a Luna C_{18} (phenomenex, Torrance, CA, USA) 150 mm × 4.6 mm, 5 μm particle size, with a phenomenex securityGuard™ C_{18} guard cartridge. The mobile phase flow rate was 1.0 ml/min, and the detective wavelength was 240 nm. Temperature of the eluent was maintained at 36.5 °C by pre-heating the container of the eluent buffer in a thermostat-controlled water bath (PolyScience, Niles, USA). Column temperature was also maintained at 36.5 °C for simulating human body temperature. The retention data in BMC were calculated as capacity factors, $k = (t_r - t_0)/t_0$, where t_r is the retention time of the test compound and t_0 is the column dead time. The k values used in this study were the average value of triplicate injections.

2.2. Materials and methods

Mobile phases were aqueous solutions of polyoxyethylene (23) lauryl ether (Brij35, Acros, New Jersey, USA). The pH value of mobile phases was adjusted to 7.4 with 0.05 M phosphate buffer, which was prepared with sodium dihydrogenphosphate and sodium hydroxide (analytical-reagent grade, Kelong, Chengdu, China). NaCl (analytical-reagent grade, Kelong, Chengdu, China) was added to mobile phases for simulating the osmotic pressure of biological fluids.

Fluvastatin sodium, mevastatin, lovastatin and simvastatin were kindly donated by Sichuan Industrial Institute of Antibiotics, Chengdu, China. Other HMG-CoA reductase inhibitors were obtained in terms of bulk drug or pharmaceutical preparations as follows: rosuvastatin calcium (Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China), pravastatin sodium tablet (Squibb, Shanghai, China), atorvastatin calcium tablet (Godecke GmbH, Freiburg, Germany), cerivastatin sodium tablet (Bayer, Leverkusen, Germany).

Atorvastatin, cerivastatin, fluvastatin, pravastatin and rosuvastatin are administered as active compounds (acid form), whereas lovastatin and simvastatin are applied as inactive forms (lactone), which have to be enzymatically hydrolyzed to generate active forms [17].

Stock standard solutions were prepared by dissolving 10 mg of the bulk compound in 10 ml of mobile phase solution. Working solutions were prepared by dilution of the stock standard solutions using mobile phase solution. For pharmaceutical preparations, working solutions were prepared as follows: tablet powders of the HMG-CoA reductase inhibitors studied and mobile phase were taken into a mortar, and ground thoroughly, then sonicated for 10 min in a sonxi CQ-250 sonicator (Shanghai, China). The mixtures were transferred to a brown volumetric flask. The samples were centrifuged at 4000 × g for 5 min, and the supernatant was filtered through 0.45 μm microporous membrane. The solutions were stored at 4 °C before injection.

Water was from a Millipore (Billerica, MA, USA) synergy™ 185 system and was degassed before HPLC. The mobile phase and the solutions injected into the chromatograph were filtered through 0.45 μm microporous membrane.

2.3. Software and data processing

Matlab 6.0 of the MathWorks Incorporation and Excel 2003 of Microsoft office software were used to accomplish the statistical analysis of the multiple linear regression (MLR).

2.4. Evaluation of the QRAR models predictive ability

To estimate the predictive ability of the QRAR models, three important parameters were proposed, which were root mean squared error of calibration (RMSEC), root mean squared error of cross-validation (leave-one-out) (RMSECV), and root mean squared error of cross-validation (leave-one-out) for interpolated data (RMSECVi) [12], respectively.

RMSEC displays the fit error, whereas RMSECV and RMSECVi indicate the prediction error. RMSEC value informs