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Short communication

QRAR models for cardiovascular system drugs using biopartitioning micellar chromatography

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Abstract

The capability of biopartitioning micellar chromatography (BMC) to describe and estimate pharmacological parameters of cardiovascular system drugs has been studied. The retention of cardiovascular system drugs was studied using different pH of Brij-35 as micellar mobile phase in modified C_{18} stationary phase. Quantitative retention–activity relationships (QRAR) in BMC were investigated for these compounds. An adequate correlation between the retention factors (log k) and the toxicity (LD_{50}) of cardiovascular system drugs was obtained.

Keywords: Biopartitioning micellar chromatography (BMC); Cardiovascular system drugs; QRAR model

1. Introduction

Today with the development of combinatorial chemistry hundreds and hundreds of drugs that show potential biological activity are synthesized. The studies of drugs from discovery to market are very expensive and time consuming and include the selection of drug candidates and the study of their pharmacological properties. In the early stages of drug discovery, pharmacological studies have traditionally been conducted in living systems such as mice, rabbits, dogs, etc. For ethical and/or economic reasons, a great deal effort is currently being made to develop in vitro systems in order to avoid or reduce the use of experimental animals. The aim of this paper is to take a look at the biopartitioning micellar chromatography (BMC) approach, which is estimating of the pharmacological properties of a set of drugs acting on the cardiovascular system. These structures–activity relationships have been proposed by modern medicinal chemistry as an alternative to "in vivo" measurements. The usual physicochemical parameter employed in quantitative structure–activity relationships (QSAR) studies is the octanol–water partition coefficient (log p). In some cases, the steric factor and/or electronic effects are also important to describe the biological behavior of drugs. The pharmacological processes of drug action are considered to have much in common with the processes on which chromatographic separations are based. The same molecular features (hydrophobicity, electrical charge, and steric effects) affect not only transport processes and drug–biological target interactions, but also the compound retention in a chromatographic system under specific experimental conditions.

Sagrado [1–3] has demonstrated that the chromatographic system comprising a hydrophobic stationary phase and saline solutions of Brij-35 micelles as mobile phase can be used as a system to model drug biopartitioning process and have named this methodology BMC.

This methodology has been applied for describing and predicting the biological activity of different pharmacological kinds of drugs [4–6], permeability across the intestinal barriers, blood–brain barrier, and cornea [7].

We focused our attention on cardiovascular system drugs in this paper. The selective calcium channel antagonists share a similar anti-hypertensive mechanism of action: They inhibit the influx of extracellular calcium through the L-type channel,

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resulting in relaxation of vascular smooth muscle and reduction in vascular resistance. And alpha-adrenergic blockers, which are present on the muscle in the walls of blood vessels, and which are relaxing the vascular smooth muscle, it will produce vasodilation and lower the blood pressure.

In this paper, quantitative retention–activity relationships of cardiovascular system drugs were established. Finally, quantitative retention–activity relationships between the retention of cardiovascular system drugs and LD_{50} were examined.

2. Experimental

2.1. Instruments and measurements

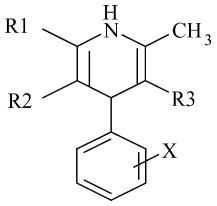
The chromatography system consisted of JASCO (JASCO, Japan) PU-1580 and PU-1586 pumps and an UV-1570 UV-VIS detector. Data processing was carried out with a JASCO LC-1500 chromatography workstation. The column used was a Spherisorb C_{18} column (5 μm, 125 mm × 4.6 mm i.d.). Mobile phase flow-rate was 1.0 mL min^{-1}. Detection was performed at 237 nm. The column temperature was kept at 36.5 °C for all assays. The k_{BMC} values determined in this study, were averages of at least triplicate determinations. The retention date were highly reproducible, the relative standard deviation (R.S.D.) values were <1.0% for intra-day and <5.0% for inter-day assays.

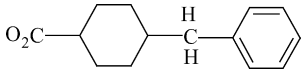
2.2. Reagents and standards

The quinazoline derivatives were obtained from Institute of Medicament, Hebei Province, Shijiazhuang, China. The selective calcium channel antagonists were obtained from different sources: nitrendipine, nifedipine, nicardipine L-levoamlodipine, and diltiazem were from Zhongnuo, Shijiazhuang Pharmaceutical Group. *m*-Nisoldipine, nisoldipine, and nimodipine were from College of Pharmacy, Hebei Medical University. Their molecular structures were shown in Tables 1 and 2. Mobile phases were prepared by aqueous solutions of polyoxyethylene (23) lauryl ether (Brij-35, Acros, Geel, Belgium). Micellar eluent pH was adjusted with 0.01 M phosphate buffer, which was prepared with disodium hydrogenphosphate and potassium

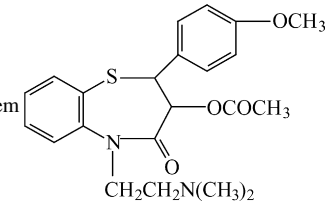
Table 1
Structure of the selective calcium channel blockers

General structure



Compounds	R_1	R_2	R_3	X
Nitrendipine	CH_3	$CO_2CH_2CH_3$	CO_2CH_3	3-NO_2
Nifedipine	CH_3	CO_2CH_3	CO_2CH_3	2-NO_2
Nisoldipine	CH_3	$CO_2CH_2CH(CH_3)_2$	CO_2CH_3	2-NO_2
m-Nisoldipine	CH_3	$CO_2CH_2CH(CH_3)_2$	CO_2CH_3	3-NO_2
Nicardipine	CH_3	$CO_2CH_2CH_2NHCH_2C_6H_5$ (CH3 branch)	CO_2CH_3	3-NO_2
Nimodipine	CH_3	$CO_2CH_2CH_2OCH_2$	CO_2CH_3	3-NO_2
L-Levoamlodipine	CH_3	$CH_2OCH_2CH_2NH_2$	CO_2CH_3	2-Cl
Felodipine	CH_3	$CO_2CH_2CH_3$	CO_2CH_3	2,3-2Cl
Benidipine	CH_3		CO_2CH_3	3-NO_2

Diltiazem



Veraparil

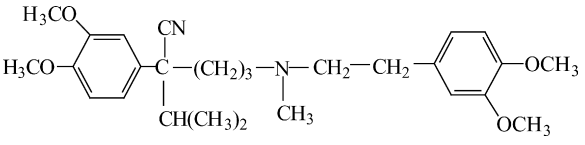
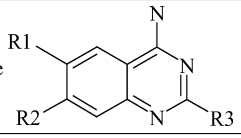
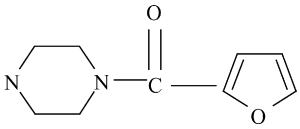
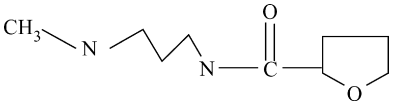
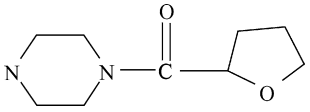
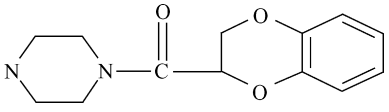


Table 2
Structure of the quinazoline derivatives

Compounds	R_1	R_2	R_3
General structure			
Prazosin	OCH_3	OCH_3	
Alfuzosin	OCH_3	OCH_3	
Terazosin	OCH_3	OCH_3	
Doxazosin	OCH_3	OCH_3	

dihydrogenphosphate. In order to reproduce the osmotic pressure of biological fluids, 9.20 g L^{-1} NaCl was added to the micellar mobile phase. This NaCl concentration was close to physiological concentration of biological fluids.

Stock standard solutions at 1.00 g L^{-1} of the compounds were prepared using methanol as solvent. Working solutions were prepared by dilution of the stock standard ones using mobile phases. The solutions were stored in the refrigerator at 4 °C. MILLIPORE-pure water was used throughout. The mobile phase and the solutions injected into the chromatograph were filtered through 0.45 μm nylon membranes, respectively.

2.3. Software and data processing

SPSS 12.0 for windows program was used to perform the statistical analysis of the regressions and drawing. The retention data in BMC were calculated as retention factors, $k_{MBC} = (t_R - (t_0/t_0))$ where t_R is the retention time of the test compound and t_0 corresponds to column dead time. Namely, the dead time is t_0 corresponds to column retention time of an unretained compound.

3. Results and discussion

3.1. Retention behavior and relation of structure–activity in compounds

The retention of the compounds was measured using 0.04 M (pH 6.5 and 7.4) Brij-35 mobile phases. The pH was adjusted to 7.4 to obtain experimental conditions as close as possible to physiological ones, and the pH 6.5 was considered as the average pH of the small intestine. At physiologic pH, quinazoline

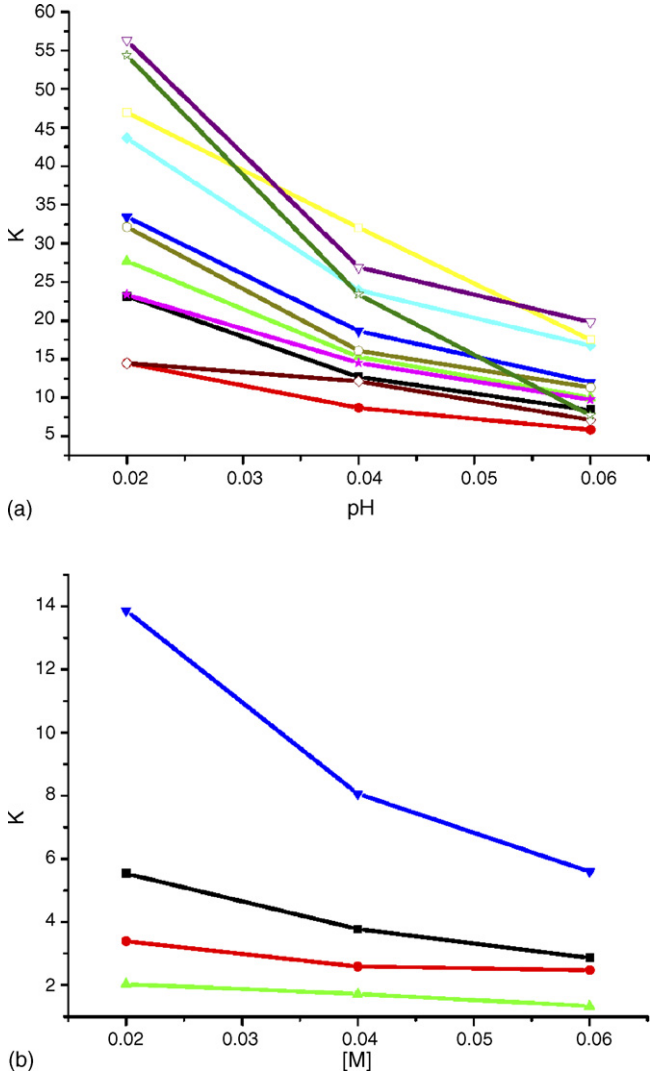


Fig. 1. Effect of Brij35 concentration in mobile phase on the retention of cardiovascular system drugs: (a) nitrendipine (■), nifedipine (●), nisoldipine (▲), *m*-nisoldipine (▼), nicardipine (♦), nimodipine (⋆), L-levoamlodipine (□), felodipine (◯), benidipine (▽), diltiazem (◊), verapamil (☆). (b) Prazosin (■), alfuzosin (●), terazosin (▲), doxazosin (▼).

derivatives, verapamil and diltiazem are primarily ionized, while 1,4-DHPs are primarily nonionized. There are two exceptions to this. L-Amlodipine and nicardipine contain basic amine groups as part of the side chains connected to the 1,4-DHP ring. While the 1,4-DHP ring of these compounds is nonionized, the side chain amines will be primarily ionized at physiologic pH. Since ionic attraction is often the initial interaction between a drug and its receptor, the differences in basicity between the 1,4-DHP ring and the tertiary amines of verapamil and diltiazem are consistent with the previously noted fact that the binding site for the 1,4-DHPs is distinct from those for verapamil and diltiazem. Fig. 1 shows the effect of the Brij-35 concentration in the mobile phase (pH 7.4) on the retention of cardiovascular system drugs. As can be observed for the highly hydrophobic compounds (verapamil, felodipine, benidipine, nicardipine, and doxazosin), large changes in the retention were obtained upon increasing the surfactant concentration in the mobile phase, while for those with

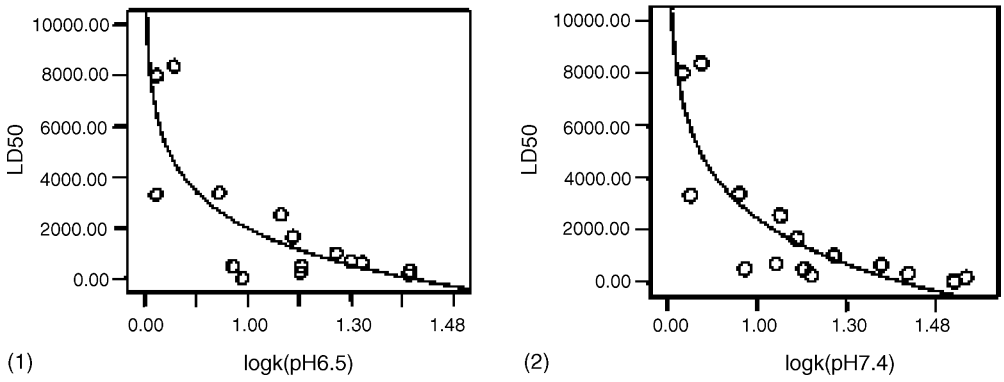


Fig. 2. LD_{50} vs. log k obtained using pH 6.5 mobile phase (1), pH 7.4 mobile phase (2).

Table 3
Statistical analysis and predictive features of the QRAR models: $LD_{50} = a + b\log k$ corresponding to the retention data obtained using different Brij-35 mobile phases coefficients[a]

Model	Brij-35 (pH)	Unstandardized coefficients, $B \pm$ SE	T	Sig$T$(L)[b]	R	SE	F	Sig$F$(L)[*]	DW
1	Constant	6772.4599 ± 1086.9911	6.2300	0.0000					
	6.5	−2077.7195 ± 440.7885	−4.7140	0.0004	−0.7943	1708.1972	22.2184	0.0004	2.7861
2	Constant	8302.5659 ± 1180.6367	7.0320	0.0000					
	7.4	−2542.4898 ± 455.4585	−5.5820	0.0001	0.8400	1525.4579	31.1617	0.0001	2.2830

[a] Dependent variable: LD_{50}; unstandardized coefficients B: deflection regression coefficients; unstandardized coefficients SE: standard error of deflection regression coefficients.
[b] Statistically significant. L: 95% confidence interval for coefficients estimates; R: correlation coefficients; SE: standard error of the estimate; F: F-ratio; DW: Durbin–Watson statistic.

low hydrophobicity (i.e., nifedipine and terazosin) the retention was scarcely modified. This fact indicates, the modification of the surface of the stationary phase with monomers of Brij-35 is continued even above the critical micelle concentration (CMC), i.e., the number of adsorbed monomers on surface unit of stationary phase rises [8]. Therefore, stationary phase becomes more hydrophilic and retention of highly hydrophobic compounds decreases sharply with an increase of concentration of Brij-35 in the mobile phase. The polyoxyethylene chains of monomers retain compounds with low hydrophobicity, hence their retention is scarcely modified. The monolayer of Brij-35 investigated by Quiñones-Torrelo et al. [4,5] reflects this suggestion.

3.2. Retention-LD_{50}-relationships

BMC is used to predict oral drug acute-toxicity parameter model. As the molecular features of drugs, mainly hydrophobicity, electronic and steric properties, determine the drug-carrier protein (in biological fluids) and drug–receptor interactions and consequently their biological activity, therefore, it could be expected that retention–activity relationships exist.

In order to obtain predictive and interpretative models, the retention data of cardiovascular system drugs and the corresponding biological activities were adjusted to the logarithmic model: activity = $a + b\log k$. The results shown below were obtained using different Brij-35 mobile phases. Similar QRAR models were achieved using the retention data corresponding to 0.04 M (pH 6.5 and 7.4) Brij-35 mobile phases (see Fig. 2). Moreover, as oral drug delivery is the preferred route of drug administration, it is well known that the major absorption barrier to drugs taken orally is the intestinal mucosa membrane and generally drugs are absorbed by a passive diffusion mechanism. Therefore, pH 6.5 was adopted to build the model, too. The results indicate that the retention of compounds in BMC is capable of described and predicting in vitro the acute toxicity of cardiovascular system drugs.

Table 3 contains the statistical analysis and the predictive features of the QRAR models obtained when 0.04 M and pH 6.5 and 7.4 Brij-35 mobile phases were used. Since the P-values for QRAR models are less than 0.05, there is a statistically significant relationship between the acute toxicity parameters studied and log k values at the 95% confidence level.

4. Conclusion

The results showed that the BMC approach seems to be an attractive tool for estimating the potential activity of new drugs, e.g., a newly synthesized compound from a generic molecular structure, which justifies the development of predictive LD_{50}QRAR models. It has shown that the adequate conditions the chromatographic system can reproduce the drug biopartitioning. The use of the retention in BMC, which encompasses the main interactions between a drug and its corresponding biological target (hydrophobic, electronic, and steric contributions to the free energy change in the biological response), should guarantee a progressive incorporation of BMC into the drug discovery scheme.

The BMC methodology proposed is probably one of the most accessible, economical, robust, and stable of the HPLC-based

methodologies employed in QRAR analysis. The use of only one descriptor (the retention factor, *k*) is one of the most important advantages.

Acknowledgments

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