Original article

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CORRELATION BETWEEN ANGIOTENSIN-CONVERTING ENZYME INHIBITORS LIPOPHILICITY AND PROTEIN BINDING DATA

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Angiotensin-converting enzyme (ACE) inhibitors represent a significant group of drugs primarily used in the treatment of hypertension and congestive heart failure. In this research, seven ACE inhibitors (enalapril, quinapril, fosinopril, lisinopril, cilazapril, ramipril, benazepril) were studied to evaluate the relationship between their protein binding and calculated (logP values) or ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS) and reversed-phase thin-layer chromatography (RP-TLC) lipophilicity data (φ_0, CHI or C_0 parameters, respectively). Their protein binding data varied from negligible (lisinopril) to 99% (fosinopril), while calculated $logP_{KOWWIN}$ values ranged from -0.94 (lisinopril) to 6.61 (fosinopril). The good correlations were established between protein binding values and $logP_{KOWWIN}$ data (R^2=0.7520) as well as between protein binding and chromatographic hydrophobicity data, φ_0, CHI or C_0 parameters (R^2 were 0.6160, 0.6242 and 0.6547, respectively). The possible application of hydrophobicity data in drugs protein binding evaluation can be of great importance in drug bioavailability. *Acta Medica Medianae 2012;51(4):13-18.*

Key words: *angiotensin-converting enzyme inhibitors (ACE inhibitors), protein binding, lipophilicity*

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Introduction

Absorption, distribution, metabolism and elimination (ADME) properties play a critical role in clinical success of drug candidates. Lipophilicity is one of the most important properties that significantly influence drugs absorption, distribution, binding to plasma proteins and elimination due to hydrophobic interactions of the drug with biological targets and its penetration across biological membranes during transport. Lipophilic molecules exhibit better absorption, penetration into tissues and higher degree of distribution. Also, it is well-known that more lipophilic drugs exert a higher degree of protein binding in comparison to less lipophilic ones with similar properties (1-3).

The plasma protein binding (PPB) degree significantly influences drugs efficiency. The less bound drug more efficiently passes through cell membranes or diffuses. Mainly, the unbound fraction actually exhibits pharmacologic effects. It is also the fraction that may be metabolized and/or excreted (4).

Angiotensin-converting enzyme (ACE) inhibitors represent significant group of drugs widely used in the treatment of hypertension, congestive heart failure and renal failure. They were introduced in clinical practice three decades ago and today represent the most commonly prescribed antihypertensive drugs (4-10).

According to the available literature, a number of authors investigated the relationship between lipophilicity and ACE inhibitors pharmacological activity, duration of action and absorption (11-13). In our previous studies of ACE inhibitors, we reported their lipophilicity under different chromatographic conditions (14-16), a relationship between chromatographic and in silico hydrophobicity parameters (17), as well as the correlation between UHPLC-MS and RP-TLC hydrophobicity data and ACE inhibitors absorption values (18). Conducting these researches, the aim of this study was to investigate the relationship between ACE inhibitors lipophilicity data (both calculated and chromatographically obtained) and their protein binding data. The main topic was to establish the approach capable for protein binding prediction of selected ACE inhibitors as well as the new synthesized drugs.

Materials and methods

Materials

The following ACE inhibitors (Figure 1) were investigated:

1. enalapril maleate, 2. quinapril hydrochloride, 3. fosinopril sodium, 4. lisinopril dihydrate, 5. cilazapril monohydrate, 6. ramipril and 7. benazepril hydrochloride.

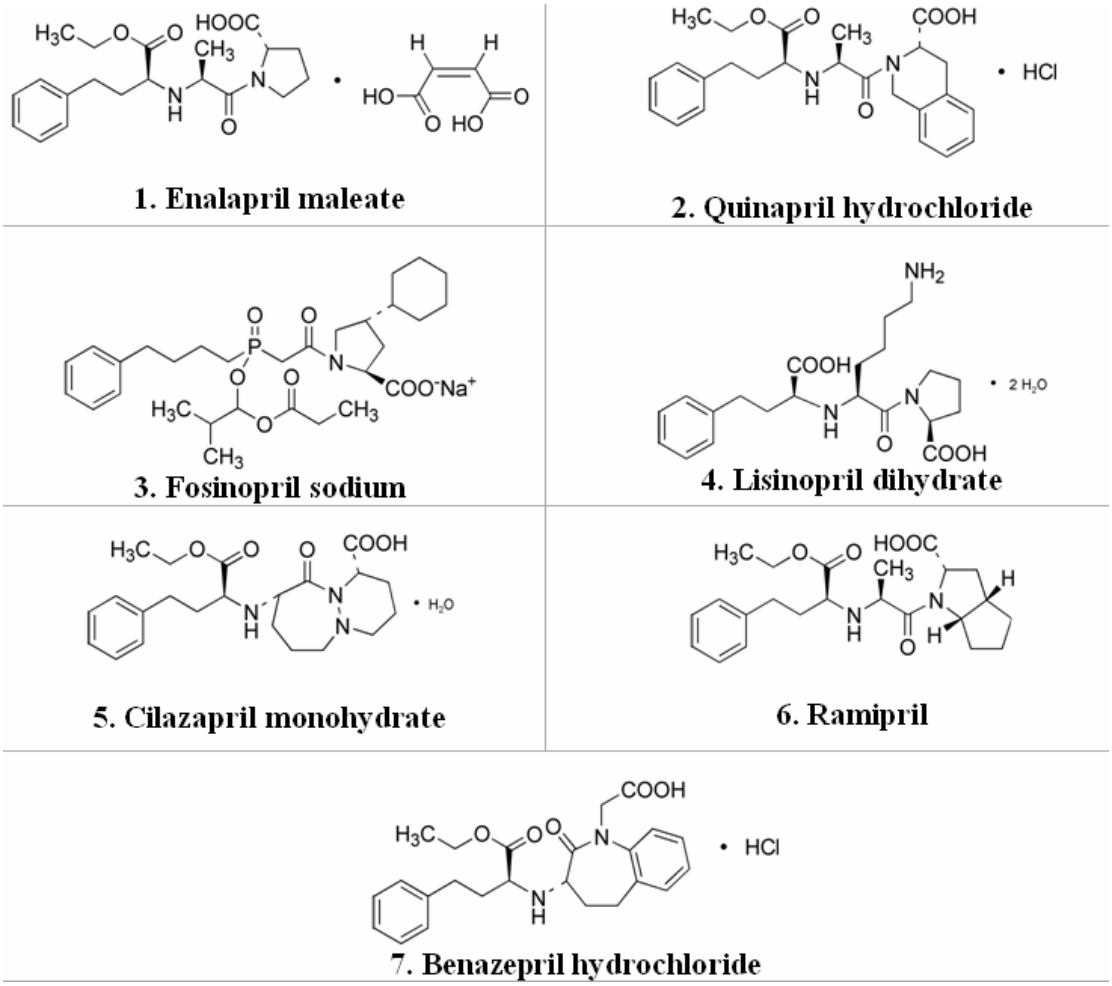

Figure 1. The chemical structure of investigated ACE inhibitors

Methods

The chromatographic conditions, ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS) and reversed phase thin-layer chromatography (RP-TLC) for ACE inhibitors examination were reported previously (18). The UHPLC-MS investigations were carried out, as previously described (18) on a UHPLC-MS/MS system consisting of a Thermo ACCELA UHPLC (Thermo Scientific, Waltham, Massachusetts, USA) coupled to a triple quad Mass Spectrometer Thermo TSQ Quantum Access Max (Thermo Scientific, Waltham, Massachusetts, USA) with a heated electrospray ionization (HESI) interface. Samples were placed in thermostated autosampler at 4°C. A 10 μl samples were injected onto a Thermo Hypersil Gold column (1.9μm, 50x2.1mm) and eluted at a temperature of 25°C and a flow rate of 300μl min^{-1} by the use of binary solvent system (mobile phase A was 0.1% $CH3COONH_4$ aqueous solution (pH=6.85), while mobile phase B was methanol) (18). The RP-TLC examinations were performed, as previously described on RP-18 silica gel plates with water-methanol binary solvent system (18).

Reagents: ammonium acetate (Analytika, Ltd., Prague, Czech Republic), methanol (LC-MS Chromasolv, Sigma-Aldrich Steinhem, Germany) and deionized water (Gen Pure Ultrapure, Germany) where used throughout. Uracil (Merck, Darmstadt, Germany) was used for dead time determination (18).

The Excel 2003 from Microsoft Office and Origin 7.0 PRO (Origin Lab Corporation, USA) were used to perform the statistical analysis of the regression.

Results

In this research, seven ACE inhibitors (enalapril, quinapril, fosinopril, lisinopril, cilazapril, ramipril, benazepril) were studied to evaluate the relationship between their protein binding and calculated (logP values) or ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS) and reversed-phase thin-layer chromatography (RP-TLC) determined lipophilicity data (φ_0, CHI or C_0 parameters, respectively). Their protein binding data vary from negligible (lisinopril) to 99% (fosinopeil), while calculated $logP_{KOWWIN}$ values ranged from -0.94 (lisinopril) to 6.61 (fosinopril).

*Table 1.*The lipophilicity and protein binding data of selected ACE inhibitors

ACE inhibitors	log$P_{O/W}$	log P_{KOWWIN}	Protein binding %
Enalapril	2.45	2.45	55
Quinapril	3.72	3.72	97
Fosinopril	6.61	6.61	99
Lisinopril	-1.22	-0.94	0
Cilazapril	/	2.27	24
Ramipril	3.32	3.32	73
Benazepril	3.50	3.50	94

Table 2. The hydrophobicity parameters of investigated ACE inhibitors obtained in UHPLC-MS and RP-TLC

ACE inhibitors	UHPLC-MS		RP-TLC
	φ_0	CHI	C_0
Enalapril	0.533	0.537	0.596
Quinapril	0.642	0.637	0.673
Fosinopril	0.773	0.800	0.802
Lisinopril	0.201	0.213	0.429
Cilazapril	0.636	0.620	0.655
Ramipril	0.615	0.610	0.660
Benazepril	0.623	0.606	0.670

The ACE inhibitors PPB data were (Table 1) were collected from relevant references (4).

The experimentally determined log$P_{O/W}$ values (Table 1) of examined ACE inhibitors were obtained from Clarke's Analysis of drugs and Poisons (19). The software packages Molinspiration (20), Virtual Computational Chemistry Laboratory (21) and CS Chem Office, version 7.0 (22) were used to calculate different ACE inhibitors lipophilicity descriptors, (in silico hydrophobicity parameters) computed logP values.

The ACE inhibitors hydrophobicity parameters, φ_0, CHI, and C_0 values (Table 2) were chromatographically obtained in UHPLC-MS and RP-TLC investigations (18).

Discussion

This study included the most often prescribed ACE inhibitors. In the first stage the selection of appropriate logP values was evaluated. The applied software packages (20-22) could calculate different logP values (milogP, AlogP, AClogP, XlogP, logP_{KOWWIN}) of investigated ACE inhibitors.

The absolute calculated logP values were significantly different. In our previous paper the relationships between all collected logP values were studied. The selection of appropriate logP values was estimated on the basis of their agreement with experimentally determined partition coefficients log$P_{O/W}$ values (19). The logP_{KOWWIN} values were selected for this study due to its best correlation (R^2=0.999) with the experimentally obtained ones log$P_{O/W}$ values (17). The logP_{KOWWIN} values were also selected, since the best correlations were obtained between these values and chromatographically obtained hydrophobicity parameters, C_0 (RP-TLC) as well as φ_0 or CHI (UHPLC-MS) (18).

In the next stage of this study, the relationship between calculated lipophilicity (logP_{KOWWIN} values) and PPB data of examined ACE inhibitors was investigated and the following correlation was obtained:

logP_{KOWWIN}=0.0501(±0.0129)PPB–0.1734(±0.9351) **...(1)**
n=7, R^2=0.7520

The good correlation (Figire 2) was obtained as proposed (the range of R^2 0.49–0.79) in literature (23).

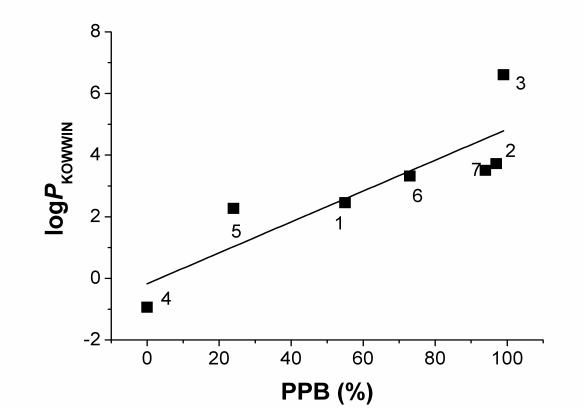

Figure 2. The relationships between protein binding (%) and logP_{KOWWIN} values (R^2=0.7520). The numbers denote substances used for linear regression

As a final point, the possible correlations between chromatographically (UHPLC-MS or RP-TLC) obtained hydrophobicity parameters (φ_0, CHI or C_0) and protein binding data were studied.

φ_0=0.0036(±0.0013)PPB+0.3461(±0.0929)**...(2)**
n=7, R^2=0.6160
CHI=0.0036(±0.0013)PPB+0.3454(±0.0915)**...(3)**
n=7, R^2=0.6241
C_0=0.0023(±0.0007)PPB+0.4934(±0.0550)**...(4)**
n=7, R^2=0.6547

All the correlations obtained can be considered as good, with acceptable F values due to limited number of compounds. The lipophilicity data both calculated as well as obtained with different chromatographic techniques, are capable of evaluating ACE inhibitors protein binding. However, the best relationship was observed between protein binding data and calculated $logP_{KOWWIN}$ values. The best correlation was found between in silico hydrophobicity parameters and protein binding data, thus confirming the calculation of logP as high-throughput screening technique for the evaluation of selected compounds protein binding degree.

It is generally accepted that increase in drugs lipophilicity led to increase of their absorption, distribution, activity and duration of action, but also to increase of drugs protein binding. Since high protein binding degree may cause decrease of drugs action and activity, the good balance between lipophilicity and protein binding should be established, especially in new synthesized drugs, for the patients' benefits.

Conclusion

The discovery of new pharmacologically active substances and drugs modeling led to necessity of predicting drugs properties and its ADME data.

It was established that all ACE inhibitors lipophilicity data (calculated hydrophobicity parameters $logP_{KOWWIN}$ as well as chromatographically obtained parameters C_0, φ_0 and CHI) correlate well with protein binding values. The proposed model based on hydrophobicity data, calculated or chromatographically obtained, is capable of evaluating ACE inhibitors protein binding values. The possible application of computed logP values in drugs protein binding evaluation is of great importance in drug research.

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ZAVISNOST LIPOFILNOSTI I VEZIVANJA ZA PROTEINE PLAZME INHIBITORA ANGIOTENZIN KONVERTUJUĆEG ENZIMA

Jadranka Odović i Jasna Trbojević-Stanković

Inhibitori angiotenzin konvertujućeg enzima (ACE inhibitori) predstavljaju veliku grupu lekova koji nalaze primenu u lečenju hipertenzije. U ovom radu analizirano je sedam ACE inhibitora (enalapril, kvinapril, fosinopril, lizinopril, cilazapril, ramipril i benazepril) kako bi se ispitala zavisnost između njihovog vezivanja za proteine plazme i lipofilnosti. Korelisane su vrednosti izračunatih ($logP_{KOWWIN}$) ili hromatografski (UHPLC-MS i RP-TLC) dobijenih (φ_0, CHI ili C_0) hidrofobnih parametara. Procenat vezivanja za proteine plazme ispitivanih ACE inhibitora kretao se u opsegu od 0% do 99%, dok su vrednosti izračunatih $logP_{KOWWIN}$ vrednosti iznosile od -0.94 do 6.61. Dobijene su zadovoljavajuće korelacije između vrednosti vezivanja ACE inhibitora za proteine plazme i izračunatih $logP_{KOWWIN}$ vrednosti (R^2=0,7520) kao i hromatografski dobijenih parametara hidrofobnosti, φ_0, CHI, C_0 (R^2: 0,6160; 0,6242; 0,6547). *Acta Medica Medianae 2012;51(4):13-18.*

Ključne reči: *inhibitori angiotenzin konvertujućeg enzima (ACE inhibitori), vezivanje za proteine plazme, lipofilnost*