

ELIMINATION OF ANGIOTENSIN – CONVERTING ENZYME INHIBITORS BY PERITONEAL DIALYSATE

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Angiotensin-converting enzyme (ACE) inhibitors are a significant group of drugs that are primarily used in the treatment of hypertension and congestive heart failure. Even though they belong to the same group of drugs and have similar efficacy, ACE inhibitors exhibit different pharmacological characteristics. The lipophilicity is one of the most important properties of biologically active substances. It influences their absorption, distribution, tissue penetration, action, elimination. In this paper, the elimination of ACE inhibitors by peritoneal dialysate in patients on peritoneal dialysis was investigated. The influence of ACE inhibitors' hydrophobicity in this way of elimination was discussed. *Acta Medica Medianae* 2011;50(2):12-17.

Key words: angiotensin-converting enzyme inhibitors (ACE inhibitors), peritoneal dialysis, elimination, lipophilicity

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Introduction

Angiotensin - converting enzyme (ACE) inhibitors represent significant group of drugs widely used in the treatment of hypertension, congestive heart failure and renal failure, especially in patients with diabetes mellitus and / or proteinuria. They were introduced in clinical practice three decades ago and today are the most commonly prescribed antihypertensive drugs (1).

ACE inhibitors are complex organic molecules. Based on the differences in chemical structure, they can be classified into three groups: those with the sulfhydryl group (captopril), those with a carboxyl group (enalapril, cilazapril, ramipril, lisinopril and others) and those with the phosphoric group (fosinopril) (2).

The ACE inhibitors are esterified prodrugs. Following administration, *in vivo*, they undergo hydrolysis into di-acid active metabolites, with exception of lisinopril, which is already in di-acid form, and captopril, which forms disulfide. In the biological materials, plasma, serum or urine, ACE inhibitors can be found as their metabolites just several hours after administration (2,3).

Active metabolites of ACE inhibitors demonstrate their antihypertensive effect by modulation

of the renin-angiotensin-aldosterone enzymatic system and selective dilation of efferent renal arterioles. In addition to antihypertensive, ACE inhibitors exhibit many other effects – anti-proliferative, antiatherosclerotic, fibrinolytic (4). In hypertensive patients with renal failure, particularly of diabetic etiology, ACE inhibitors are used as drugs of choice because, in addition to antihypertensive effects, they slow the progression of microalbuminuria and proteinuria (5-7). They are also often applied in patients with end-stage renal failure treated with renal replacement therapy - hemo or peritoneal dialysis.

Pharmacological and pharmacodynamic properties of ACE inhibitors (absorption, distribution, activity, elimination) differ depending on their lipophilicity. The lipophilicity of drug molecules is defined by its distribution between the aqueous and non-aqueous phase and can be expressed as the logarithm of n-octanol/water partition coefficient (log P). It plays an important role in drugs absorption, distribution, binding to plasma proteins and elimination. Lipophilic molecules show better absorption, penetration into tissues and have a higher degree of distribution compared to less lipophilic ones with similar properties. Also, the lipophilicity affects the duration of action of a drug, as well as efficiency of its elimination. Namely, weakly lipophilic drugs are eliminated by the urine, while the highly lipophilic ones can be eliminated by feces. Also, lipophilicity affects drugs elimination via the dialysate in renal patients on peritoneal dialysis. The very lipophilic drugs will be poorly eliminated by dialysate than the less lipophilic ones (8-14).

Pharmacological properties (absorption, protein binding, distribution, activity, duration of action

and elimination), as well as their relationship with the lipophilicity of ACE inhibitors, were investigated in numerous studies by HPLC, thin layer chromatography, capillary electrophoresis, spectrophotometry and spectrofluorimetry. ACE inhibitors were determined in pharmaceutical formulations and biological material, but there is little data on the elimination of ACE inhibitors by peritoneal dialysate (8-21).

In our previous studies, we examined the lipophilicity of several ACE inhibitors using thin-layer chromatography (22-24). The aim of this study is to monitor the elimination of ACE inhibitors by peritoneal dialysate as well as the influence of their lipophilicity on this way of elimination.

Materials and methods

Materials

In this paper, the elimination of two ACE inhibitors of different lipophilicity: cilazapril (log P = 1,04) and fosinopril (log P = 8,93) and their active metabolites cilazaprilat (log P = 0,46) and fosinoprilat (log P = 6,38) were investigated (22-24). The structure of the investigated drugs are shown in Table 1.

Peritoneal Dialysate Samples

The dialysate samples were collected from 16 hypertensive patients on peritoneal dialysis, 12 h after oral administration of fosinopril 20 mg (8 patients) or cilazapril 10 mg (8 patients). All patients were on continuous treatment with fosinopril or cilazapril and no other antihypertensive drugs. Blank dialysate samples were obtained from several patients on peritoneal dialysis, but not treated with ACE inhibitors.

Clean – up procedure

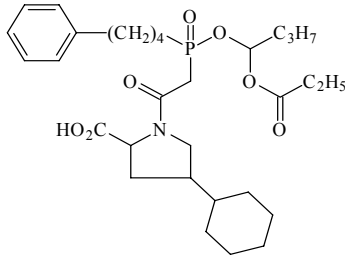
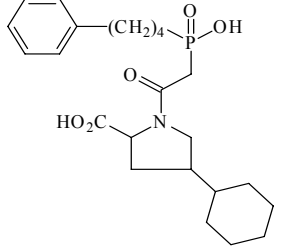
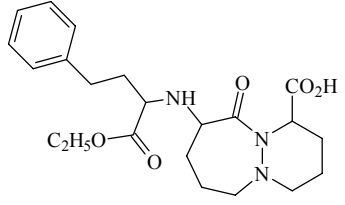
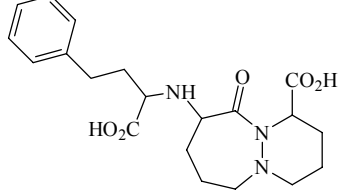
The investigated substances were extracted from prepared standard solution and dialysate samples obtained from patients, by SPE extraction on Bakerbond speTM Octadecyl (C18) columns, using the procedure established for urine samples by Prieto et al. (19).

The extraction columns for cilazapril and cilazaprilat were activated by solution of methanol, water and borate buffer, pH=9. The examined samples (25 ml) with the added borate buffer were missed through the activated columns. The columns were rinsed with phosphate buffer and water, dried under vacuum and n-hexane solution. After rinsing and drying columns, the examined substances were eluted with methanol. The methanol solution was matched to dry and dissolved in a solution of mobile phase to volume of 200 µL.

The extraction columns for fosinopril and fosinoprilat were activated by the use of methanol, water and phosphoric acid. The samples (25ml)

with the added phosphoric acid were missed through the activated columns. The columns were rinsed with phosphoric acid and ammonium acetate and dried under vacuum. After rinsing and drying columns, the investigated substances were eluted with methanol. The methanol solution was matched to dry and dissolved in a solution of mobile phase to volume of 250µL.

Table 1. Chemical structure of investigated substances

No	Structure	Name
1		Fosinopril
2		Fosinoprilat
3		Cilazapril
4		Cilazaprilat

HPLC analysis

The separations were performed on Hewlett Packard 1100 HPLC system with a spectrophotometry detection and chromatographic column Zorbax Eclipse RP - 8, 5µm, 150 • 4,6mm. The determination of fosinopril and fosinoprilat were performed with methanol 66% (v/v), water 34% (v/v), triethanolamine 0.1% (v/v) and pH 5.5 (acetate buffer) while cilazapril and cilazaprilat were determined with methanol 41% (v/v), water 59% (v/v), triethanolamine 0.1% (v/v) at pH 2.3 (fosforic acid). The mobile phase was filtered through a 0.45 µm membrane filter. The

injection volume was 100 μ L, flow rate was 1.0 mL/min. The column was maintained at 25°C. The chromatograms were recorded at 210 nm using DAD. Under these conditions the retention time for investigated substances was: cilazapril 30 min., cilazaprilat 9 min., fosinopril 90 min. and fosinoprilat 10 min.

The methanol of HPLC grade were Merck (Darmstadt, Germany). The deionized water was purified by Simplicity 185 system (Millipore, Billerica, MA). All other chemicals were of analytical grade of purity.

Calibration curves

Solutions for calibration curves were made by spiking blank dialysate samples with known amounts of examined substances and extracting them in the same way as unknown samples. The seven solutions of different concentrations for each calibration curve were prepared. The concentration range of calibration solutions were in the range 2.5–10.0 μ g/mL.

Results

The elimination of ACE inhibitors with different hydrophobicity, fosinopril and cilazapril, and their active metabolites fosinoprilat and cilazaprilat, by dialysate in patients on peritoneal dialysis was examined by the use of HPLC method.

Calibration graph was established by plotting average area of peak with drug concentration in prepared solutions. The obtained calibration curves parameters are for cilazaprilat: $Y = 19.3259 X - 2.9756$; $R = 0.9883$ and for fosinoprilat: $Y = 37.8883 X - 5.0457$; $R = 0.9967$.

The chromatograms of blank dialysate, blank dialysate spiked with examined ACE inhibitors and their metabolites, as well as the chromatograms of a dialysate samples obtained from patients after intake of fosinopril 20 mg or cilazapril 10 mg and after extraction procedure are presented on Figures 1, 2 and 3.

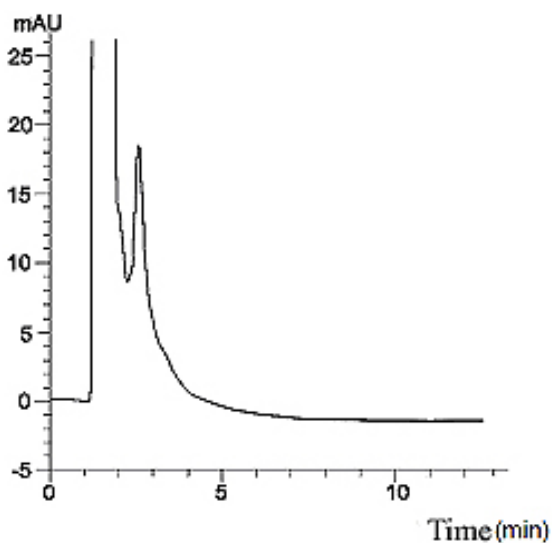


Figure 1. Chromatograms of blank dialysate sample

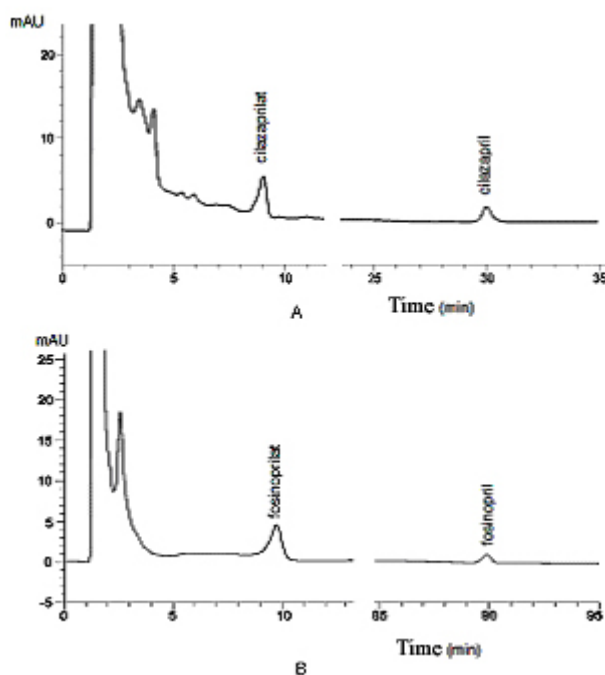


Figure 2. Chromatograms of blank dialysate spiked with cilazapril and cilazaprilat (A), blank dialysate spiked with fosinopril and fosinoprilat (B)

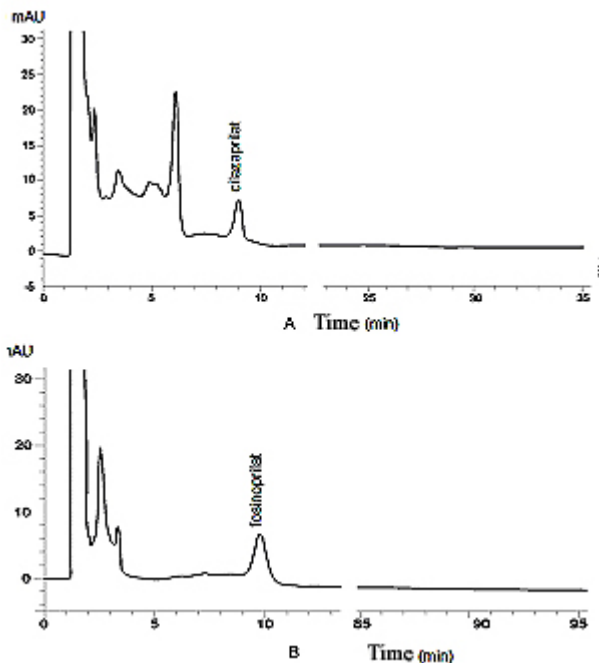


Figure 3. Chromatograms of dialysate sample obtained from patient after intake of cilazapril 10 mg (A) and fosinopril 20 mg (B)

The samples of peritoneal dialysate contained only metabolites of examined ACE inhibitors, consistent to fast in vivo hydrolysis of ACE inhibitors. The results are presented in Table 2.

Both studied metabolites are eliminated via the dialysate in a very low percentage: 0.4153% of administered fosinopril in the form of its metabolite fosinoprilat, and 0.6557% of administered cilazapril in the forms of cilazaprilat.

Table 2. The elimination of fosinoprilat and cilazaprilat by dialysate at hypertensive patients 12h after intake of single dose of fosinopril (20mg) or cilazapril (10mg)

Patient No.	Volume	Total eliminated metabolits (µg)	The percentage of eliminated drugs
1.*	2000	82,9	0,4145
2.*	2080	79,4	0,3968
3.*	2010	84,1	0,4203
4.*	1980	84,4	0,4220
5.*	2020	83,5	0,4175
6.*	1990	80,4	0,4020
7.*	2110	85,7	0,4285
8.*	2050	84,3	0,4215
9.**	1850	67,7	0,6766
10.**	2050	63,5	0,6354
11.**	2030	65,4	0,6536
12.**	1970	64,3	0,6432
13.**	2120	66,1	0,6614
14.**	2090	65,9	0,6589
15.**	1990	65,8	0,6577
16.**	2000	65,9	0,6592

*Patients who ingested 20mg of fosinopril

**Patients who ingested 10mg of cilazapril

Discussion

Pharmacological properties of ACE inhibitors may be very different due to differences in chemical properties of their molecules.

Resorption of ACE inhibitors from the gastrointestinal tract shows significant differences. Enalapril, cilazapril and quinapril are relatively well absorbed, by about 60%, fosinopril shows lower absorption (36%), while lisinopril shows the lowest one of about 25%, although it can range from 6-60% (8).

Metabolites of ACE inhibitors are bound to plasma proteins. However, the degree of their binding is very different and vary from 0% for lisinopril to 98% for fosinoprilat and quinaprilat (8).

ACE inhibitors and their metabolites are mostly eliminated by the urine. The exception is fosinoprilat that has a double (compensatory) way of elimination (urine as well as feces). This

makes fosinopril especially suitable for patients with renal failure (8).

The elimination of ACE inhibitors by dialysate is very different. The lisinopril has good elimination by dialysis, while the elimination cilazaprilat and fosinoprilat is minimal (8). The elimination of substances through the dialysate is a complex process influenced by many factors, such as molecular weight, molecules size, binding to plasma proteins, volume of distribution, water solubility (hydrophilicity or lipophilicity), plasma clearance. The molecules with higher molecular weight, lipophilicity and protein binding show lower elimination through the dialysate (9).

In previous studies, there is little data on the elimination of ACE inhibitors by peritoneal dialysate (8,9). In this paper, the elimination of fosinopril, cilazapril and their metabolites by peritoneal dialysate in patients on peritoneal dialyse was studied. The results suggest that fosinopril and cilazapril are eliminated by dialysate in the form of their active metabolites fosinoprilat and cilazaprilat, and that the degree of their elimination is low. Slightly higher elimination of cilazaprilat compared to fosinoprilat is consistent with the fact that increase of drugs lipophilicity leads to decrease of its elimination by the dialysate. Another reason for fosinoprilat poor elimination can be the lower molecular weight of cilazaprilat (389.45), compared to fosinoprilat (434.49), as well as high degree of fosinopril plasma protein binding.

Conclusion

Lipophilicity significantly affects the pharmacokinetics and pharmacodynamics of drugs, their absorption, metabolism, distribution, activity and elimination. In this paper, the elimination of two ACE inhibitors - cilazapril and fosinopril, and their active metabolites - cilazaprilat and fosinoprilat was examined by the HPLC method. It was found that both ACE inhibitors are eliminated in the form of their metabolites in very small percent. Also, the obtained results show that the elimination of very lipophilic fosinoprilat by peritoneal dialysate is lower than the elimination of less lipophilic cilazaprilat.

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ELIMINACIJA INHIBITORA ANGIOTENZIN KONVERTUJUĆEG ENZIMA PUTEM PERITONEUMSKOG DIJALIZATA

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Inhibitori angiotenzin–konvertujućeg enzima (ACE inhibitori) najčešće su propisivani antihipertenzivni lekovi. Iako pripadaju istoj grupi lekova i pokazuju sličnu kliničku efikasnost, pojedini ACE inhibitori imaju različite farmakološke osobine, što može biti posledica njihovih različitih hemijskih karakteristika. Jedna od najznačajnijih osobina biološki aktivnih supstanci je njihova lipofilnost. Ona utiče na njihovu apsorpciju, raspodelu u tkiva, aktivnost, eliminaciju. U ovom radu proučavana je eliminacija ACE inhibitora fosinopрила i cilazaprila dijalizatom kod bolesnika na peritoneumskoj dijalizi i uticaj lipofilnosti na ovaj put njihove eliminacije. *Acta Medica Medianae 2011;50(2):12-17.*

Ključne reči: *Inhibitori angiotenzin–konvertujućeg enzima (ACE inhibitori), peritoneumska dijaliza, eliminacija, lipofilnost*