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The effect of candesartan on the pharmacokinetics of enalaprilat in nephrotic rats

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Abstract. – BACKGROUND AND OBJECTIVES: The adverse reactions in combination of angiotensin-converting enzyme inhibitors (ACEIs) and Ang II receptor blockers (ARBs) were severer than that in monotherapy for patients with nephropathy. The effect of candesartan on pharmacokinetics of enalaprilat in nephrotic rats was investigated to make references for the clinical therapy in patients with nephropathy to avoid related adverse effects.

MATERIALS AND METHODS: Nephrotic rats were prepared by adriamycin injection. Control group and one nephrotic group received enalapril alone, another nephrotic group received enalapril and candesartan simultaneously. Blood samples were drawn at time points after a single oral administration. The concentration of enalaprilat was determined using LC-MS/MS.

RESULTS: Compared with control group and nephrotic group received enalapril alone respectively, T_{max} of enalaprilat in nephrotic group received both enalapril and candesartan cilexetil prolonged about 21.43% and 6.224%, respectively; $AUC_{(0-t)}$ increased by 185.3% and 60.63%, respectively; C_{max} increased by 219.4% and 56.64%, respectively; $t_{1/2}$ incerased by 163.7% and 30.05%, respectively; CL/F reduced by 65.12% and 40.78%, respectively. There were no significant differences of the V1/F of enalaprilat between three groups. The CL/F and $t_{1/2}$ of enalaprilat showed significant correlations with serum creatinine (Scr) respectively ($r = -0.7502$; $r = 0.5626$).

DISCUSSION: The combination with candesartan in nephrotic rats significantly changed the pharmacokinetics of enalaprilat, showing increased accumulation and decreased elimination. In view of these findings, we should lower dosage and prolong dosing interval for nephrotic patients in the combination of enalapril and candesartan.

Key Words:
Angiotensin II, Candesartan, Enalaprilat, Nephrosis, Pharmacokinetics.

Introduction

The renin-angiotensin system (RAS) plays an important role in the progress of renal disease. The principal bioactive peptide in RAS is angiotensin II (Ang II), which may contribute to vascular endothelial dysfunction, atherosclerosis, remodeling and hypertrophy of renal tissue, and cell death. These changes represent a pathophysiologic basis of renal diseases[1,2].

Evidences have pointed out that blockade of RAS can delay the progress of renal diseases[3,4]. The general RAS blockers in clinical application include angiotensin-converting enzyme inhibitors (ACEIs) and Ang II receptor blockers (ARBs). ACEIs are able to reduce Ang II production by inhibiting angiotensin-converting enzyme (ACE), and ARBs can bind the specific receptor of Ang II, arresting its biological effects[5]. Because of the emerging "angiotensin escape phenomenon" in the use of ACEIs alone[4], the combination of ACEIs and ARBs has been proposed for clinical application. Studies found that the combination of ACEIs and ARBs was more effective than the single entity alone in depressing systemic blood pressure and treating albuminuria in diabetic nephropathy[6,7]. The joint use of them would lead to a dual RAS blockade and a greater clinical benefit theoretically[3,5-7]. However, clinical observations revealed that adverse reactions in the combined therapy were severer than that in monotherapy, including hypotension, hyperkalemia, and renal function deterioration, even if consciously reduced the dosages[8-11]. Pharmacodynamic studies indicated that these adverse reactions were related to the excessive inhibition of RAS. However, the pharmacokinetic changes in the combined use of both drugs remain unclear.

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Enalapril is a widely-used ACEI. Its active metabolite enalaprilat is mainly excreted by the kidneys. Studies found elevated concentrations of enalaprilat in nephrotic patients with conventional dosages[12]. Candesartan is a commonly used ARB. It is cleared via kidneys, biliary and intestinal route. Whether the physiological disposition of enalaprilat and candesartan would be changed in their combined use for nephrotic patients is unknown. Compared with ARBs, ACEIs have been used more generally, but can result in more adverse effects[13]. Accordingly, in this study we chose enalaprilat as the monitoring object, and investigate the effect of candesartan on pharmacokinetics of enalaprilat in nephrotic rats to make references to the clinical application for patients with nephropathy for avoiding related adverse effects.

Material and Methods

Chemicals

Adriamycin was purchased from Main Luck Pharmaceuticals Inc, ShenZhen, China. Kit for measuring creatinine was purchased from Shanghai Mind Bioengineering Co. Ltd., Shanghai, China. Enalapril and candesartan cilexetil were purchased from YiBang Pharmaceutical Technology Development Co. Ltd., GuangZhou, China. Enalaprilat was purchased from USPC Inc., Rockville, MD, USA. Benazepril was supplied by the National Institute for the Control of Pharmaceutical and Biological Products. All chemicals were at least analytical grade.

Animals and Treatment

Male and female Sprague-Dawley rats (250~280 g) in this study were obtained from the Experimental Animal Center, Southern Medical University, Guangzhou, China. The animals were housed in at 25 ± 2°C with water and standard diet accessible *ad libitum*. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. After one week of acclimatization, the rats were divided into control group (n=8) and model group (n=18). As adriamycin (ADR) has been reported to be able to induce renal impairment[14-16], rats in model group received injection of ADR (3 mg/kg, once every two days, thrice in total) through the tail vein. Control rats received injection of saline (2 mL/kg). After confirming the nephropathy rat model in biochemistry and histology, the normal rats and nephrotic rats were treated as the following: control group (n=6) and one model group (n=6) received enalapril (15 mg/kg, p.o.) alone, and another model group (n=6) received simultaneously enalapril (15 mg/kg, p.o.) and candesartan cilexetil (3 mg/kg, p.o.). Blood samples were drawn under ether anesthesia from orbital venous plexus before administration and 1, 2, 3, 4, 6, 8, 12, 24, and 48 h after single dosing. Blood samples were collected using heparinized tubes, centrifuged for 20 min at 450 × *g* at 4°C. Plasma samples were stored at -20°C before analysis.

Determination of Serum Creatinine

Before injection with ADR or saline and 7 weeks after injection, blood and serum collection was carried out. Avoiding hemolysis during the collection process, the Scr was measured using corresponding kit with enzymatic method.

Sample Preparation

The preparation of samples was carried out as described[17] with adjusted. A 200 μL of plasma sample was mixed with 20 μL internal standard (3 μg/ml benazepril) and 10 μL formic acid. The mixture was vortexed with 370 μL methanol by oscillator followed by centrifuging for 15 min at 10,000 × *g* at 4°C. The supernatant was collected and filtered. 3-μL aliquot of supernatant was injected to LC-MS/MS system for analysis.

LC-MS/MS

LC-MS/MS was carried out as described[18] with adjusted. The mobile phase composed of methanol-water-formic acid (70:30:0.03, v/v/v) at a flow rate of 0.3 mL/min. An API 4000 Q TRAP LC-MS/MS system (Applied Biosystems, Foster City, CA, USA) was used for analyses. The mass spectrometer was operated in positive ion detection mode. Nitrogen was used as the ion source gas at 55 psi, the collision gas at medium, and the curtain gas at 25 psi. The ion spray voltage was adjusted to 5,500 V and the temperature of vaporizer was at 350°C. The declustering potentials were set at 63 V and 100 V for enalaprilat and benazepril, respectively.

Pharmacokinetic Analysis

Non-compartmental pharmacokinetic parameters were calculated from the plasma concentrations of enalaprilat in each time point of each rat using DAS 2.0 Program registrated from Mathematical Pharmacology Professional Committee

Table I. Precision, recovery and accuracy of enalaprilat (n = 3 days, five replicates per day).

Concentration (ng/ml)		R.S.D. (%)		R.E. (%)	Extraction recovery (%)
Added	Found	Intra-day	Inter-day		
50	59.63 ± 3.05	11.44	2.03	19.26	85.80
500	599.9 ± 12.23	5.00	1.12	19.98	84.26
5000	5277 ± 65.74	2.79	0.80	5.54	74.53

of China. This program has been applicated in other pharmacokinetic studies[19-21]. The non-compartmental pharmacokinetic parameters including area under the concentration-time curve from zero to last quantifiable time ($AUC_{(0-t)}$), half-life of elimination ($t_{1/2}$), total clearance (CL/F), volume of distribution (V1/F) were calculated using statistical moments[22-24]. The maximum plasma concentration (C_{max}) and the time to maximum concentration (T_{max}) were measured according to the actual levels of enalaprilat.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA). Values were expressed as mean ± SEM. The comparison of Scr between control group and model group was performed by unpaired *t*-test. One-way Analysis of Variance followed by LSD multiple comparison or Dunnett's multiple comparison tests was performed for the comparison of pharmacokinetic parameters. Correlation analysis was used to investigate the relationships of factors. Significant difference was set at $p < 0.05$.

Results

Confirmation of Nephropathy Rat Model

To confirm the nephropathy rat model with ADR induction, we determined Scr and examined nephrons under optical microscopy. Before ADR administration, it was no difference of the Scr level between the two groups. 7 weeks after ADR administration, the concentration of Scr increased by 25.69% ($p < 0.05$). When compared to control group in which rats received saline injection, Scr increased by 41.24% ($p < 0.05$) (Figure 1). The kidneys of rats were examined for their histological changes with Hematoxylin-Eosin staining. 7 weeks after ADR administration, the kidneys of model group showed glomerular distention, glomerular syncretism to capsule, and nephric tubule dilatation in comparison to the rats in control group (Figure 2). These biochemical and histological changes indicated that ADR administration successfully induced a nephropathy model.

Enalaprilat Detection and Method Validation

The detection of enalaprilat was carried out by LC-MS/MS. Figure 3 shows the mass spectrums of enalaprilat and the internal standard benazepril of rat plasma. Quantification was performed using multiple reaction monitoring of the transitions of m/z 349.2 → m/z 206.3 for enalaprilat and m/z 425.4 → m/z 351.3 for benazepril. The peak area ratios of enalaprilat to benazepril varied linearly over the concentration range tested. Equation for calibration curve ($1/\chi^2$ weighting) was y = 0.000675 × + 0.0145 (r = 0.9978) for the extent of 50~5000 ng/mL. The lower limit of quantification was 0.785 ng/mL. As shown in Table I, the intra-day and inter-day precisions at concentrations of 50, 500, and

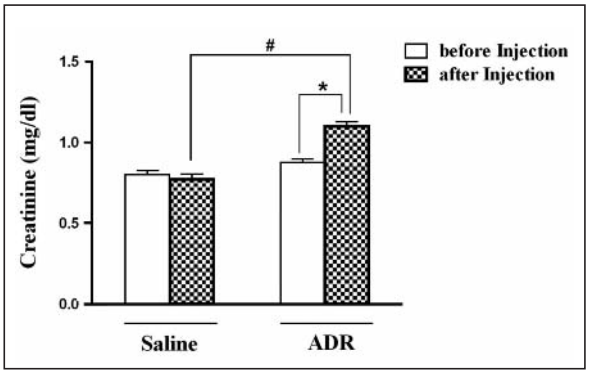

Figure 1. Concentrations of serum creatinine before and 7 weeks after injection of ADR. Rats in control group received saline injection (n=8, 2 mL/kg) and in model group received ADR injection (n=18, 3 mg/kg) once every two days, thrice totally through the tail vein. Serum creatinine was determined by commercial kit. Values are expressed as mean ± SE. $^{*}p < 0.05$ vs. model group before injection, $^{\#}p < 0.05$ vs. control group after injection.

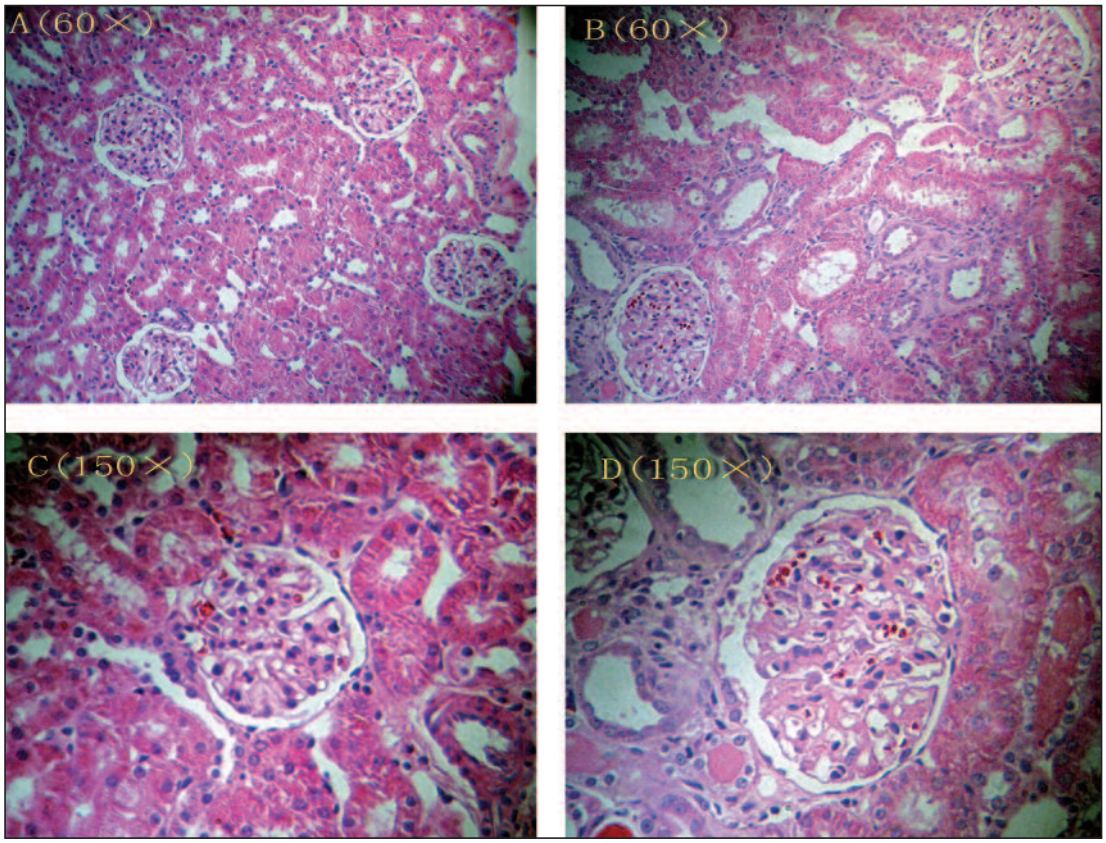

Figure 2. The histology of kidneys in rats received saline or ADR. Compared to control group ***(A)*** and ***(C)***, ADR administration ***(B)*** and ***(D)*** induced glomerulus distention, glomerular syncretism to capsule, tubule dilatation, tubule epithelial cell vacuolization, and nephric tubule cast. Magnification: (A) 60×; (B) 60×; (C) 150×; (D) 150×.

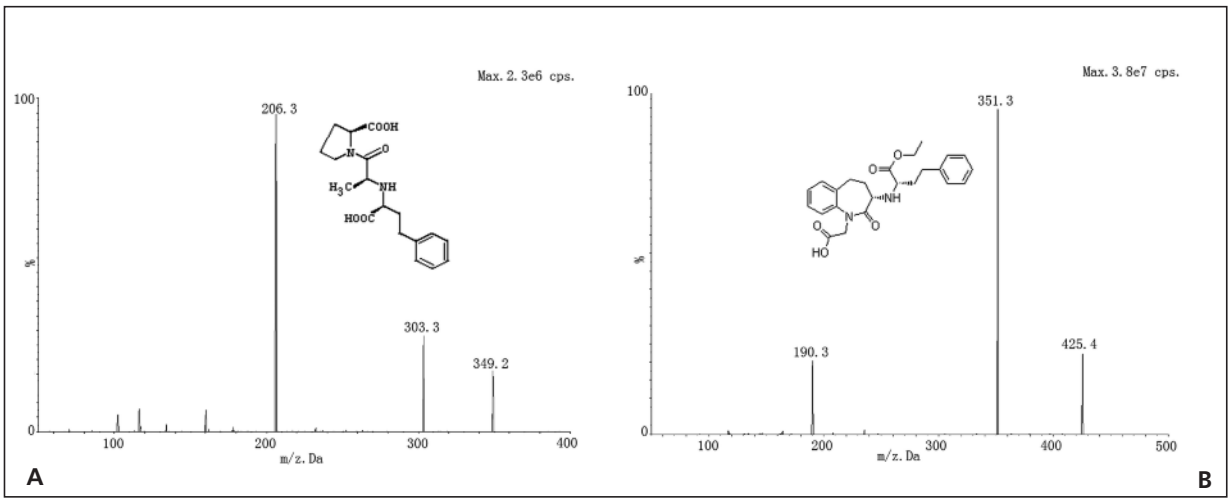

Figure 3. Mass spectra of enalaprilat and internal standard benazepril in rat plasma. The spectra showed multiply charged ions of enalaprilat ***(A)*** at m/z 206.3, 303.3, 349.2 and benazepril ***(B)*** at m/z 190.3, 351.3, 425.4. Quantification was performed using multiple reaction monitoring of the transitions of m/z 349.2 → m/z 206.3 for enalaprilat and m/z 425.4 → m/z 351.3 for benazepril.

5,000 ng/mL expressed in terms of relative standard deviation (R.S.D.) were within 15%; the relative error (R.E.) of accuracies were within 20% (n=3 days, five replicates/d). The extraction recoveries at concentrations of 50, 500, and 5,000 ng/mL were 85.80%, 84.26%, and 74.53%, respectively.

Pharmacokinetics of Enalaprilat in Nephrotic Rats

Concentration-time curves of enalaprilat were shown in Figure 4, and corresponding non-compartmental pharmacokinetic parameters were listed in Table II. As revealed in Figure 4, the concentrations of enalaprilat increased rapidly after a single oral administration. The non-compartmental pharmacokinetic analysis shown that compared with the rats in control group, the time to maximum concentration (T_{max}) of enalaprilat in nephrotic rats received enalapril alone prolonged about 14.32% (p = 0.251). The area under concentration-time curve ($AUC_{(0-t)}$) increased by 77.62% (p = 0.006), and the maximum plasma concentration (C_{max}) increased by 103.9% (p = 0.031). Moreover, the elimination half-life ($t_{1/2}$) in nephrotic rats prolonged by 102.8% (p = 0.024), and enalaprilat clearance (CL/F) reduced by 41.10% (p = 0.003).

Effect of Candesartan on the Pharmacoinetics of Enalaprilat in Nephrotic Rats

The concentration-time curve of enalaprilat in nephrotic rats received the combination of enalapril and candesartan cilexetil was shown in Figure 4, and corresponding non-compartmental pharmacokinetic parameters were listed in Table II. As shown in Table II, although it was not significant differences for all of pharmacokinetic parameters between the nephrotic rats with single or combined administration, we found that the enalaprilat accumulation and its slower elimination were obvious in the combination group. In contrast to control group and nephrotic group received enalapril alone, the time to maximum concentration (T_{max}) in nephrotic rats received the combination of enalapril and candesartan cilexetil prolonged about 21.43% (p = 0.093) and

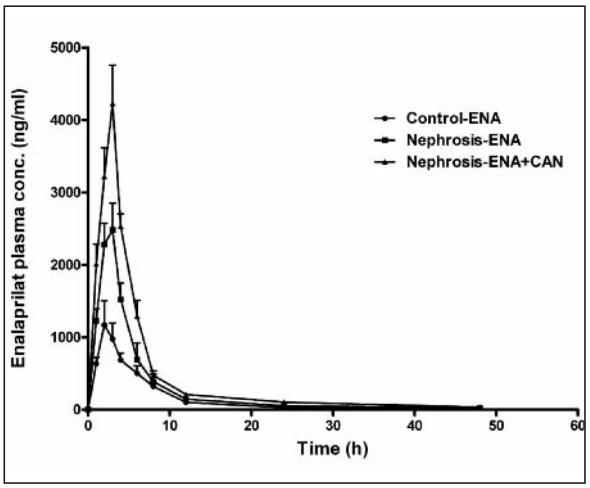

Figure 4. Mean plasma concentrations of enalaprilat over time after single dosing. Control rats and one group of nephrotic rats received enalapril (ENA) (15 mg/kg, p.o.) alone, another group of nephrotic rats received enalapril (ENA) (15 mg/kg, p.o.) and candesartan cilexetil (CAN) (3 mg/kg, p.o.) simultaneously. Values are expressed as mean ± SE (n = 6/group).

Table II. Non-compartmental pharmacokinetic parameters of enalaprilat in control and nephrotic rats with or without combined use of candesartan cilexetil.

Group (Enalapril) (Candesartan)	Control (+) (–)	Nephrosis (+) (–)	Nephrosis (+) (+)
$AUC_{(0-t)}$ (ng/ml·h)[a]	7458 ± 329.4	13247 ± 1683*	21278 ± 1388*†
C_{max} (ng/ml)[b]	1329 ± 331.4	2710± 342.5*	4245 ± 530.4*†
T_{max} (h)[c]	2.333 ± 0.2108	2.667 ± 0.2108	2.833 ± 0.1667
$t_{1/2}$ (h)[d]	4.501 ± 0.3835	9.127 ± 1.726*	11.87 ± 1.680*
CL/F (L/h/kg)[e]	2.029 ± 0.08942	1.195 ± 0.1412*	0.7077 ± 0.05499*†
V1/F (L/kg)[f]	13.20 ± 1.281	15.74 ± 3.485	16.01 ± 1.718

[a]$AUC_{(0-t)}$: area under the concentration-time curve from zero to last quantifiable time; [b]C_{max}: maximum plasma concentration; [c]T_{max}: time to maximum concentration; [d]$t_{1/2}$: half-life of elimination; e CL/F: total clearance; [f]V1/F: volume of distribution of enalaprilat. Values are expressed as mean ± SE (n = 6/guoup); *p < 0.05 vs. control group, †p < 0.05 vs. model group without the combined use of candesartan cilexetil.

6.224% (p = 0.559), respectively; $AUC_{(0\text{-}t)}$ increased by 185.3% (p = 0.001) and 60.63% (p = 0.001), respecitively; and C_{max} increased by 219.4% (p = 0.001) and 56.64% (p = 0.019), respectively; $t_{1/2}$ incerased by 163.7% (p = 0.003) and 30.05% (p = 0.198), respectively; CL/F reduced by 65.12% (p = 0.001) and 40.78% (p = 0.044), respectively. There were no significant differences of the volume of distribution (V1/F) of enalaprilat between three groups in the study ($p > 0.05$).

The Relationship Between the Scr and the Cl/F and $T_{1/2}$ of Enalaprilat

To investigate the impact of renal function on the CL/F of enalaprilat, we analyzed the relationship between the Scr and the CL/F of enalaprilat. As shown in Figure 5(A), the enalaprilat CL/F showed an obvious negative correlation with Scr (r = –0.7502, p = 0.0003). Meanwhile, the relationship between the Scr and the $t_{1/2}$ of enalaprilat was analyzed. As shown in Figure 5(B), the enalaprilat $t_{1/2}$ showed a positive correlation with Scr (r = 0.5626, p = 0.0151).

Discussion

Clinical observations revealed that the adverse reactions from the combination of ACEIs and ARBs usually happen to the patients with mild to severe decrease in glomerular filtration rate (GFR)[8-11]. Therefore, the present study need to establish a nephropathy model in rats to aim directly at simulating this clinical phenomena. ADR has been reported to be able to induce renal impairment[14-16]. The early pathological changes induced by ADR are mainly podocyte injury and tubulointerstitial lesions with the concomitant symptom of proteinuria and electrolyte disturbances, but it is not reflect the abnormal GFR in this stage[14-16]. GFR, a measurement of renal function, will decrease after the further progression of renal impairment. Measuring Scr is a simple test and it is the most commonly used indicator of GFR[25-27]. A rise of Scr is observed only with marked damage to functioning nephrons. Therefore, this study carried out Scr test which is relative suitable for detecting dysfunction-stage of kidney disease. Meanwhile, histological observation revealed that ADR induced glomerular distention, glomerular syncretism to capsule, and nephric tubule dilatation. The biochemical and histological changes observed in the present study indicated that ADR administration successfully induced a nephropathy model.

Several studies on diabetic and non-diabetic nephropathy demonstrated the beneficial effects of ACEIs with a number of adverse reactions, including hypotension and deterioration of renal function at low or moderate doses[28,29]. These phenomenon have raised interest in studying the relationship between adverse effects and *in vivo* accumulation of ACEIs. It has been reported that patients with chronic renal failure given small or

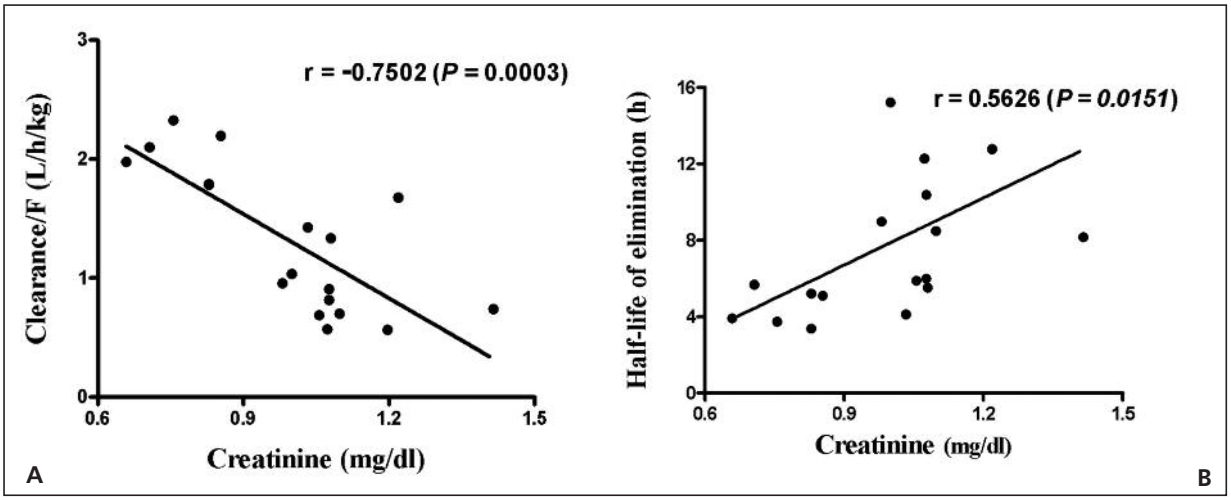

Figure 5. Correlation of serum creatinine levels with enalaprilat clearance and half-life of elimination. ***(A)*** Enalaprilat clearance was negatively correlated with serum creatinine levels (r = –0.7502, p = 0.0003). ***(B)*** The elimination half-life of enalaprilat was positively correlated with serum creatinine levels (r = 0.5626, p = 0.0151). Each point represents one experimental rat.

moderately high doses of enalapril may have markedly elevated enalaprilat *in vivo*[30]. The pharmacokinetic analytic results in the present study also showed an significantly increased concentration of enalaprilat and decreased clearance of enalaprilat in nephrotic rats with a single oral dose of enalapril, and the CL/F and $t_{1/2}$ of enalaprilat was significantly correlated with Scr which reflects the deteriorated degree of renal function. Given previous study observed that the decline of renal function in high-concentration enalaprilat group was faster than that in low-concentration group during a 12-month medication of enalapril for renal failure patients[12], the above findings suggest that the dosage of enalapril in clinical application for patients with nephrosis should be carefully adjusted according to their renal function because of the easier accumulation of enalaprilat which might induce the deterioration of renal function.

The pharmacodynamics of ACEIs and ARBs have been extensively studied in patients with nephropathy[3,8-11,13], but there is a paucity of data on the pharmacokinetic interactions of these two types of drugs. In this study, we found no significant differences of the V1/F of enalaprilat between three groups in the study, indicating no differences of the free fraction of enalaprilat in plasma between the groups. However, it was contradicted that the T_{max} of enalaprilat did not significantly change in three groups of this study, given that T_{max} should rather be increased with decreased clearance and unchanged absorption in pharmacokinetic theory. There is no obvious explanation for this finding at present. Notably, the $AUC_{(0-t)}$ and C_{max} of plasma enalaprilat increased and the CL/F decreased when used together with enalapril and candesartan cilexetil, compared to administration of enalapril alone in nephrotic rats, suggesting that combining with candesartan cilexetil aggravated the accumulation of enalaprilat, which probably related to the lower total clearance of enalaprilat. Similar to enalaprilat, candesartan in the systemic circulation is mainly cleared by kidney. It was reported that patients with decreased creatinine clearance would have a decreased clearance of candesartan[31]. The results in recent studies supported the important role of tubular transporters in the renal excretion of many drugs, for example, organic anion transporters display a role in enalaprilat and candesartan excretion[32]. In this study, the nephric tubule impairment of nephropathy rat model is obvious, thus the accumulation of enalaprilat may be resulted from the two drugs competition for tubular excretion in the impaired kidney. Given that its metabolite enalaprilat is more difficult to be excreted, the dosage of enalapril for nephrotic patients combined with candesartan cilexetil should be further reduced. Based on the findings of this study that $AUC_{(0-t)}$ and C_{max} increased by 60.63% and 56.64% respectively, we recommend at least for the nephrotic patients that the reduction of the dosage of enalapril should be no less than 50%, when combining with candesartan cilexetil in conventional dosage. Further studies are needed to verify the dosage adjustment of candesartan cilexetil for the nephrotic patients when combining with enalapril.

In fact, the dosage of ACEIs have often been reduced in the therapy for nephrotic patients, but this approach is still difficult to prevent the adverse reactions, especially hypotension and renal function deterioration[10]. In this study, we observed that the $t_{1/2}$ of enalaprilat in nephrotic rats received enalapril alone prolonged by 102.8% compared to control group, suggesting that the dosing interval of enalapril for nephrotic patients should be prolonged based on the individual $t_{1/2}$ of enalaprilat. Actually, monitoring plasma drug concentration is an effective strategy to establish the individual $t_{1/2}$ to obtain the optimal dosing interval, but it is difficult to be carried out extensively in clinical practice. Interestingly, we found an significant correlation between $t_{1/2}$ of enalaprilat and Scr, suggesting that Scr of nephrotic patients should be considered in the establishment of dosing interval to prevent adverse reactions from enalapril administration. Notably, compared to nephrotic group with enalapril alone, we found that the $t_{1/2}$ of enalaprilat in nephrotic rats received enalapril and candesartan prolonged by 30.05%. This exact $t_{1/2}$ of enalaprilat in the combination of enalapril and candesartan, which has never been reported, is a valuable reference for the enalapril-candesartan combination regimen in patients with renal disease, indicating the necessity of prolonging dosing interval. Further studies are needed to quantify the relationships between $t_{1/2}$ of enalaprilat, Scr and enalapril-candesartan combination regimen.

In conclusion, we found in the present study that the pharmacokinetic changes of enalaprilat depended on the renal function and drug regimens, especially combined with candesartan cilexetil administration. In view of these findings, we should lower dosage and prolong dosing interval for nephrotic patients in the combination of enalapril and candesartan cilexetil.

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References

1) Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. Circulation 2002; 105: 393-396.
2) Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: Part II: AT(1) receptor regulation. Circulation 2002; 105: 530-536.
3) Taal MW, Brenner BM. Renoprotective benefits of RAS inhibition: from ACEI to angiotensin II antagonists. Kidney Int 2000; 57: 1803-1817.
4) Werner C, Baumhakel M, Teo KK, Schmieder R, Mann J, Unger T, Yusuf S, Bohm M. RAS blockade with ARB and ACE inhibitors: current perspective on rationale and patient selection. Clin Res Cardiol 2008; 97: 418-431.
5) Sonkodi S, Mogyorosi A. Treatment of diabetic nephropathy with angiotensin II blockers. Nephrol Dial Transplant 2003; 18(Suppl 5): v21-23.
6) Parving HH, Andersen S, Jacobsen P, Christensen PK, Rossing K, Hovind P, Rossing P, Tarnow L. Angiotensin receptor blockers in diabetic nephropathy: renal and cardiovascular end points. Semin Nephrol 2004; 24: 147-157.
7) Fried LF, Duckworth W, Zhang JH, O'Connor T, Brophy M, Emanuele N, Huang GD, McCullough PA, Palevsky PM, Seliger S, Warren SR, Peduzzi P. Design of combination angiotensin receptor blocker and angiotensin-converting enzyme inhibitor for treatment of diabetic nephropathy (VA NEPHRON-D). Clin J Am Soc Nephrol 2009; 4: 361-368.
8) Ferrari P, Marti HP, Pfister M, Frey FJ. Additive antiproteinuric effect of combined ACE inhibition and angiotensin II receptor blockade. J Hypertens 2002; 20: 125-130.
9) Kim HJ, Ryu JH, Han SW, Park IK, Paik SS, Park MH, Paik DJ, Chung HS, Kim SW, Lee JU. Combined therapy of cilazapril and losartan has no additive effects in ameliorating adriamycin-induced glomerulopathy. Nephron Physiol 2004; 97: 58-65.
10) Mangrum AJ, Bakris GL. Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in chronic renal disease: safety issues. Semin Nephrol 2004; 24: 168-175.
11) Takaichi K, Takemoto F, Ubara Y, Mori Y. Analysis of factors causing hyperkalemia. Intern Med 2007; 46: 823-829.
12) Elung-Jensen T, Heisterberg J, Sonne J, Strandgaard S, Kamper AL. Enalapril dosage in progressive chronic nephropathy: a randomised, controlled trial. Eur J Clin Pharmacol 2005; 61: 87-96.
13) Tang SC, Leung JC, Chan LY, Eddy AA, Lai KN. Angiotensin converting enzyme inhibitor but not angiotensin receptor blockade or statin ameliorates murine adriamycin nephropathy. Kidney Int 2008; 73: 288-299.
14) Venkatesan N, Punithavathi D, Arumugam V. Curcumin prevents adriamycin nephrotoxicity in rats. Br J Pharmacol 2000; 129: 231-234.
15) El-Shitany NA, El-Haggar S, El-desoky K. Silymarin prevents adriamycin-induced cardiotoxicity and nephrotoxicity in rats. Food Chem Toxicol 2008; 46: 2422-2428.
16) He L, Rong X, Jiang JM, Liu PQ, Li Y. Amelioration of anti-cancer agent adriamycin-induced nephrotic syndrome in rats by Wulingsan (Gorei-San), a blended traditional Chinese herbal medicine. Food Chem Toxicol 2008; 46: 1452-1460.
17) Lu S, Jiang K, Qin F, Lu X, Li F. Simultaneous quantification of enalapril and enalaprilat in human plasma by high-performance liquid chromatography-tandem mass spectrometry and its application in a pharmacokinetic study. J Pharm Biomed Anal 2009; 49: 163-167.
18) Gu Q, Chen X, Zhong D, Wang Y. Simultaneous determination of enalapril and enalaprilat in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 813(1-2): 337-342.
19) Xu H, He L, Nie S, Guan J, Zhang X, Yang X, Pan W. Optimized preparation of vinpocetine proliposomes by a novel method and in vivo evaluation of its pharmacokinetics in New Zealand rabbits. J Control Release 2009; 140: 61-68.
20) Yan Z, Zhu ZL, Wang HQ, Li W, Mi YX, Liu CX. Pharmacokinetics of panaxatrol disuccinate sodium, a novel anti-cancer drug from Panax notoginseng, in healthy volunteers and patients with advanced solid tumors. Acta Pharmacol Sin 2010; 31: 1515-1522.
21) Liang YG, Liu P, Gao HZ, Li HY, Hao GT, Wang XF, Zhang XG, Meng QY, Liu ZY. Pharmacokinetics and safety of a new bioengineered thrombolytic agent, human tissue urokinase type plasminogen activator in Chinese healthy volunteers. Eur J Drug Metab Pharmacokinet 2011; 35: 97-102.
22) Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm 1978; 6: 547-558.
23) Piotrovskii VK. The method of statistical moments and the integral model-independent parameters of pharmacokinetics. Farmakol Toksikol 1986; 49: 118-127.
24) Dunne A. Statistical moments in pharmacokinetics: models and assumptions. J Pharm Pharmacol 1993; 45: 871-875.

25) Mogensen CE, Heilskov NS. Prediction of GFR from serum creatinine. Acta Endocrinol Suppl (Copenh) 1980; 238: 109.

26) Lewis J, Greene T, Appel L, Contreras G, Douglas J, Lash J, Toto R, Van Lente F, Wang X, Wright JT. A comparison of iothalamate-GFR and serum creatinine-based outcomes: acceleration in the rate of GFR decline in the African American Study of Kidney Disease and Hypertension. J Am Soc Nephrol 2004; 15: 3175-3183.

27) Jafar TH, Schmid CH, Levey AS. Serum creatinine as marker of kidney function in South Asians: a study of reduced GFR in adults in Pakistan. J Am Soc Nephrol 2005; 16: 1413-1419.

28) Maschio G, Alberti D, Janin G, Locatelli F, Mann JF, Motolese M, Ponticelli C, Ritz E, Zucchelli P. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. N Engl J Med 1996; 334: 939-945.

29) GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet 1997; 349(9069): 1857-1863.

30) Elung-Jensen T, Heisterberg J, Kamper AL, Sonne J, Strandgaard S, Larsen NE. High serum enalaprilat in chronic renal failure. J Renin Angiotensin Aldosterone Syst 2001; 2: 240-245.

31) Gleiter CH and Morike KE. Clinical pharmacokinetics of candesartan. Clin Pharmacokinet 2002; 41: 7-17.

32) Yamashita F, Ohtani H, Koyabu N, Ushigome F, Satoh H, Murakami H, Uchiumi T, Nakamura T, Kuwano M, Tsujimoto M, Sawada Y. Inhibitory effects of angiotensin II receptor antagonists and leukotriene receptor antagonists on the transport of human organic anion transporter 4. J Pharm Pharmacol 2006; 58: 1499-1505.