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Hydrochlorothiazide increases plasma or tissue angiotensin-converting enzyme-inhibitor drug levels in rats with myocardial infarction: Differential effects on lisinopril and zofenopril

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Abstract

Sodium depletion with diuretics augments the efficacy of angiotensin-converting enzyme-inhibitor therapy for hypertension and renal dysfunction, and possibly for left ventricular dysfunction after myocardial infarction. Underlying mechanisms may involve altered angiotensin-converting enzyme-inhibitor pharmacokinetics. We hypothesized that the diuretic hydrochlorothiazide causes increased steady-state levels of the angiotensin-converting enzyme-inhibitors lisinopril and zofenopril in rats with myocardial infarction. Rats were subjected to coronary ligation to induce myocardial infarction. After 1 week, rats were randomized to 50 mg/kg/day hydrochlorothiazide or control treatment for 3 weeks. The last week, rats received lisinopril or zofenopril in equipotentent dosages (3.3 and 10 mg/kg/day, respectively). Rats were sacrificed at T_{max} after the last dose of angiotensin-converting enzyme-inhibitor, and tissues were collected for analysis of drug concentrations. Lisinopril concentrations in plasma were significantly increased by hydrochlorothiazide, at unchanged tissue concentrations. This increase could be fully explained by decreased renal function, as evidenced by increased plasma creatinine levels (lisinopril + hydrochlorothiazide 82 ± 5 μM versus lisinopril 61 ± 5 μM, $P < 0.001$). In contrast, zofenoprilat levels in kidney and non-infarcted left ventricle were markedly increased by hydrochlorothiazide, whereas plasma concentrations were unchanged. Although hydrochlorothiazide tended to increase plasma creatinine in zofenopril-treated rats as well, this increase was less pronounced (zofenopril + hydrochlorothiazide 61 ± 3 μM versus zofenopril 54 ± 2 μM, $P = 0.15$). Hydrochlorothiazide increases steady-state angiotensin-converting enzyme-inhibitor drug levels, most likely by affecting their renal clearance. Notably, the lipophilic angiotensin-converting enzyme-inhibitor zofenopril accumulated in tissue, whereas the hydrophilic lisinopril increased in plasma. Whether combining different angiotensin-converting enzyme-inhibitors with hydrochlorothiazide translates into distinct clinical profiles requires further study.
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Keywords: Myocardial infarction; Angiotensin-converting enzyme inhibition; Diuretic; Pharmacokinetics

1. Introduction

Left ventricular dysfunction after myocardial infarction is characterized by progressive cardiac remodeling eventually leading to chronic heart failure. Activation of the renin angiotensin aldosterone system is thought to play a central role in this process. Consequently, angiotensin-converting enzyme inhibitor therapy effectively prevents this remodeling and reduces mortality, although the therapeutic effects of angiotensin-converting enzyme-inhibitors may be at least partially independent from inhibition of the angiotensin-converting enzyme in itself. Angiotensin II levels can even be elevated during angiotensin-converting enzyme inhibition therapy (van Kats et al., 2000; Jorde et al., 2002).

Diuretic-induced sodium restriction can enhance the effects of angiotensin-converting enzyme inhibition in renoprotective and antihypertensive therapy (Navis et al., 1987; Heeg et al., 1989; Gansevoort et al., 1992; Buter et al., 1998). Whether this can be extended to cardioprotection by angiotensin-

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converting enzyme inhibition is less well established, but experimental data from animal studies suggest that adding a diuretic may improve angiotensin-converting enzyme-inhibitor treatment in the early chronic phase after myocardial infarction (Westendorp et al., in press; Seeland et al., 2003; Seeland et al., 2002). The mechanism underlying diuretic-induced enhanced efficacy of angiotensin-converting enzyme-inhibitor therapy is unknown, but a pharmacokinetic interaction may play a role. Interestingly, we previously observed in rats with myocardial infarction augmented inhibition of cardiac angiotensin-converting enzyme activity by zofenopril during dietary sodium restriction, whereas dietary sodium intake in itself had no effect on angiotensin-converting enzyme activity (Westendorp et al., 2004). Potentially, sodium restriction affected pharmacokinetics of the angiotensin-converting enzyme-inhibitor for instance by affecting its renal clearance. Accordingly, we studied the influence of hydrochlorothiazide on steady-state plasma, cardiac and renal tissue angiotensin-converting enzyme-inhibitor drug levels and angiotensin-converting enzyme activity in an experimental setting of left ventricular dysfunction after myocardial infarction in rats.

2. Methods

2.1. Study design

The present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health. The animal research committee of the University of Groningen approved the study protocol. Male Sprague-Dawley rats were subjected to coronary artery ligation ($n=163$) or sham operation ($n=9$), as described before (Pinto et al., 1993).

Rats with myocardial infarction were randomly allocated to one of five experimental groups. One group of rats with myocardial infarction, as well as the group of sham-operated rats, received no active treatment (i.e. untreated control groups). The other four groups of rats with myocardial infarction were allocated to one of four different treatment regimens, as summarized in Fig. 1. We chose to design a protocol with established hydrochlorothiazide treatment preceding the start of angiotensin-converting enzyme-inhibitor treatment. Thus, after a recovery period of 1 week, rats were randomized to either hydrochlorothiazide or control treatment. Hydrochlorothiazide was dissolved in the drinking water to achieve a final dosage of 50 mg/kg/day. We and others previously showed that in rats with myocardial infarction this dose results in diuresis and activation of the renin angiotensin aldosterone system without blood pressure reduction (Kohzuki et al., 1996; Westendorp et al., in press). As initiation of hydrochlorothiazide affects water intake, hydrochlorothiazide was initiated 2 weeks before angiotensin-converting enzyme-inhibitor therapy, to ensure stable water intake and hydrochlorothiazide dosing at the onset of angiotensin-converting enzyme inhibition.

Against this background of established hydrochlorothiazide-treatment, angiotensin-converting enzyme-inhibitor treatment with either lisinopril or zofenopril was started at day 21, i.e. 3 weeks after induction of myocardial infarction. The treatment regimen of zofenopril (10 mg/kg/day) and lisinopril (3.3 mg/kg/day) was based on previous experiments in our laboratory (Buikema et al., 2000; van Wijngaarden et al., 1991). The dose of lisinopril was chosen relative to zofenopril based on the clinical defined daily doses for both angiotensin-converting enzyme-inhibitors (10 and 30 mg, for lisinopril and zofenopril, respectively; ATC index, 1998). Until day 26, a steady-state of both angiotensin-converting enzyme-inhibitors was achieved by dissolving appropriate amounts of drug in the drinking water. Steady-state is achieved after 4–5 half-lives, which is in total 20–35 h for zofenopril and 48–60 h for lisinopril (Subissi et al., 1999). The required amounts of food and water were determined as guided by measurements of body weight and daily water intake. This was done by measuring water intake

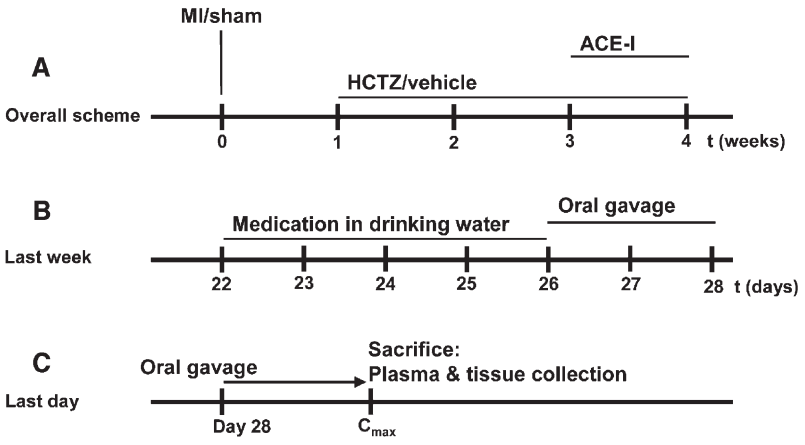

Fig. 1. Study scheme. (A) One week after recovery of myocardial infarction, rats were instituted on hydrochlorothiazide (50 mg/kg/day in drinking water) or vehicle. Two weeks thereafter, additional angiotensin-converting enzyme-inhibitor treatment was started with either lisinopril or zofenopril (3.3 and 10 mg/kg/day) in drinking water. (B) During the last 2 days before sacrifice, drugs were administered by means of oral gavage to ensure synchronized and accurate drug intake in all rats. (C) After receiving the last dose of angiotensin-converting enzyme-inhibitor at 4 weeks after induction of myocardial infarction, rats were sacrificed at T_{max}, i.e. 0.5 and 4 h for zofenopril and lisinopril, respectively. MI, myocardial infarction; HCTZ, hydrochlorothiazide; ACE-I, angiotensin-converting enzyme inhibitor.

and average body weight per cage weekly, and calculating the required drug concentration in food and water (per cage) for the week after.

On days 27 and 28 of the study protocol, the angiotensin-converting enzyme-inhibitors as well as hydrochlorothiazide were administered by means of oral gavage (total volume of 1 μl/g body weight nitrocellulose containing the drugs, or without the drugs in case of untreated controls) as to ensure a synchronized, accurate drug intake in all rats. During the last night before sacrifice, all rats were fasted.

2.2. Harvesting of tissues and plasma

At the day of sacrifice, rats were terminated exactly at time of maximum plasma concentration T_{max} after administration of zofenopril (0.5 h) or lisinopril (4 h) (Subissi et al., 1999). T_{max} was found to be unaffected by hydrochlorothiazide, both for zofenoprilat (unpublished studies at Menarini Ricerche, Firenze) and lisinopril (Swaisland, 1991).

Rats were anaesthetized with isoflurane (2.0–2.5%), heparin (1000 IU) was injected into the tail vein for anticoagulation, arterial blood was drawn from the abdominal aorta and collected in separate tubes for analysis of plasma angiotensin-converting enzyme activity and angiotensin-converting enzyme-inhibitor drug levels; one ml of arterial blood was mixed with *N*-ethylmaleimide (5 mg/ml) to prevent oxidation of zofenoprilat. Tubes were immediately centrifuged at 1600×*g* for 10 min at 4 °C. Subsequently, plasma was frozen in liquid nitrogen, and stored at −80 °C until assay.

After blood collection, rats were perfused with saline to remove remnant blood, as to avoid contribution from the blood compartment in assessment of tissue drug levels and tissue angiotensin-converting enzyme activity. To this end, 10 ml cold 0.9% NaCl solution was gently injected into the aorta, and a hole was pinched into the vena cava inferior to let out the rinsing solution. Thereafter, organs were quickly removed, weighed, divided and frozen in liquid nitrogen for measurement of either tissue concentrations of zofenopril/zofenoprilat or lisinopril, or tissue angiotensin-converting enzyme activity. For measurement of renal drug levels and angiotensin-converting enzyme activity, small pieces of cortical tissue were used. In case of cardiac measurements, both angiotensin-converting enzyme activity and drug concentrations were measured in small pieces of non-infarcted free left ventricular wall. Moreover, a mid-ventricular slice of the left ventricle was stored in 2% paraformaldehyde for histological assessment of infarct size using planimetry on Sirius Red/Fast Green-stained slides as described previously (Pinto et al., 1993). Only rats with infarct sizes comprising over 20% of the left ventricle were included for analysis.

2.3. Drug measurements

Before shipment, tissues were homogenized on dry ice in a 0.5 mol/l K_2PO_4 buffer containing 5 mg/ml *N*-ethylmaleimide to prevent oxidation of zofenoprilat. For measurement of lisinopril concentrations, homogenates were shipped on dry ice to Analytical Laboratories R&D, Berlin-ChemieLab (Berlin, Germany) for HPLC analysis. The determination of lisinopril and internal standard (Enalapril-Diketopiperazin DKP) in rat plasma was performed by means of a validated high performance liquid chromatography (HPLC)–mass spectroscopy (MS) analytical method. The lower limit of quantification (LOQ) for lisinopril was 2.0 ng/ml of rat plasma.

The lisinopril and the internal standard were extracted from rat plasma by a solid-phase extraction method. Briefly, after addition of 0.2 M HCl to the samples, the samples were vortexed and transferred into a solid phase extraction cartridge (Waters Oasis 30 mg) that had been conditioned with about 1 ml methanol and 1 ml HPLC water. After loading, the cartridge was washed with about 1 ml water and 1 ml methanol/water (5:95 v/v) then, both drugs were eluated with 3×1 ml methanol. The methanolic eluates were evaporated to dryness; the residue was redissolved in mobile phase and injected into the chromatographic system.

The HPLC-MS/MS system consisted of a model 200 solvent delivery pump (Perkin Elmer) equipped with an autosampler Gilson 234, a column oven Agilent (G1316A), an HPLC-column (Symmetry Shield RP-8, 2.1×150 mm and 5 μm particle size (Waters)), an HPLC precolumn (Symmetry Shield RP-8, 2.1×10 mm and 3.5 μm particle size (Waters), an API 2000 tandem mass spectrometer (PE Biosystems)) and a computer equipped with Analyst 1.3 Software.

For measurement of zofenopril and zofenoprilat samples were shipped on dry ice to the research lab of Menarini Richerche (Rome, Italy). The assay was performed by liquid chromatography coupled with tandem mass spectrometry as described previously (Marzo et al., 1999). Briefly, analytes were extracted by liquid–liquid extraction with toluene. The organic phase was separated, dried, reconstituted with 200 μl of a methanol/water mixture (1:1), and injected through an autosampler. The extract was chromatographed on a reverse phase column coupled to a triple quadripole mass spectrometer.

2.4. Angiotensin-converting enzyme activity

Angiotensin-converting enzyme activity in the plasma and spared myocardial tissue was determined according to the Hip-His-Leu method, as has been described before (Pinto et al., 1993). In short, tissues were homogenized in a 50 mM KPO_4 buffer. Of the homogenates 100 μl was pipetted in a 0.5 M K_2PO_4 buffer. Then the angiotensin-converting enzyme substrate Hippury-His-Leu 12.5 nM (Sigma) was added and incubated at 37 °C for exactly 10 min. The conversion of the substrate was stopped by adding 1.45 ml 280 mM NaOH. Thereafter, 100 μl phtaldialdehyde was added for the labeling of free His-Leu. The amount of labeled His-Leu was fluorimetrically determined at excitation and emission wavelengths of 364 and 486 nm, respectively. Control samples were included in which the conversion of substrate was prevented by adding NaOH before the substrate Hippuryl-His-Leu.

Table 1
Effects of experimental myocardial infarction and of 10 mg/kg/day zofenopril and 3.3 mg/kg/day lisinopril on general characteristics

	No treatment		MI-Lisinopril		MI-Zofenopril	
	Sham	MI	Vehicle	HCTZ	Vehicle	HCTZ
N	9	7	14	12	15	12
Bodyweight at sacrifice (g)	358±6	357±9	336±6[a,b]	317±3[a,b,c]	344±8	297±4[a,b]
Δbodyweight (g)	21± 5	34±8	15±2[b]	2±1[a,b,c]	13±2 [b]	5±3 [a,b,d]
Infarct size (%)	–	30±3	26±1	32±2[c]	29±2	28±2
Plasma K^+	3.8±0.1	3.8±0.1	4.4±0.1[a,b]	4.6±0.1[a,b]	4.3±0.1 [a,b]	3.9±0.1[d]
LV:Body weight (mg/g)	2.4±0.1	2.9±0.1[a]	2.5±.0[b]	2.5±0.1[b]	2.5±0.0 [b]	2.5±0.1[b]

Data are shown as mean±S.E.M. The Δbody weight is the difference in bodyweight (g) between time of operation and sacrifice, indicating body weight development. Infarct size is expressed as percentage of infarct-to-total left ventricular circumference. [a]$P<0.05$ versus Sham; [b]$P<0.05$ versus MI, [c]$P<0.05$ versus MI-lisinopril; [d]$P<0.05$ versus MI-zofenopril. MI, myocardial infarction; HCTZ, hydrochlorothiazide; LV, left ventricle.

2.5. Statistical analysis

For comparison of zofenopril and lisinopril effects in the infarct model, analysis of variance (ANOVA) with post hoc least square difference correction was performed. For analysis of drug levels, group averages of hydrochlorothiazide+angiotensin-converting enzyme-inhibitor were compared to angiotensin-converting enzyme-inhibitor monotherapy with a Student's *t*-test in case of normal distribution. If distribution was not normal, logarithmic transformation was used to achieve normality. If logarithmic transformation did not result in a normal distribution, a non-parametric Mann-Whitney test was used.

Levels of zofenoprilat in some of the left ventricle and kidney tissue samples were undetectably low. In case of detection problems, zofenoprilat levels were given the value of the detection limit in statistical analysis, to be sure not to overestimate any difference.

3. Results

3.1. General characteristics

Mortality during the first 24 h was 33% after induction of myocardial infarction; during the rest of the follow-up period, none of the rats died. None of the rats died after sham operation.

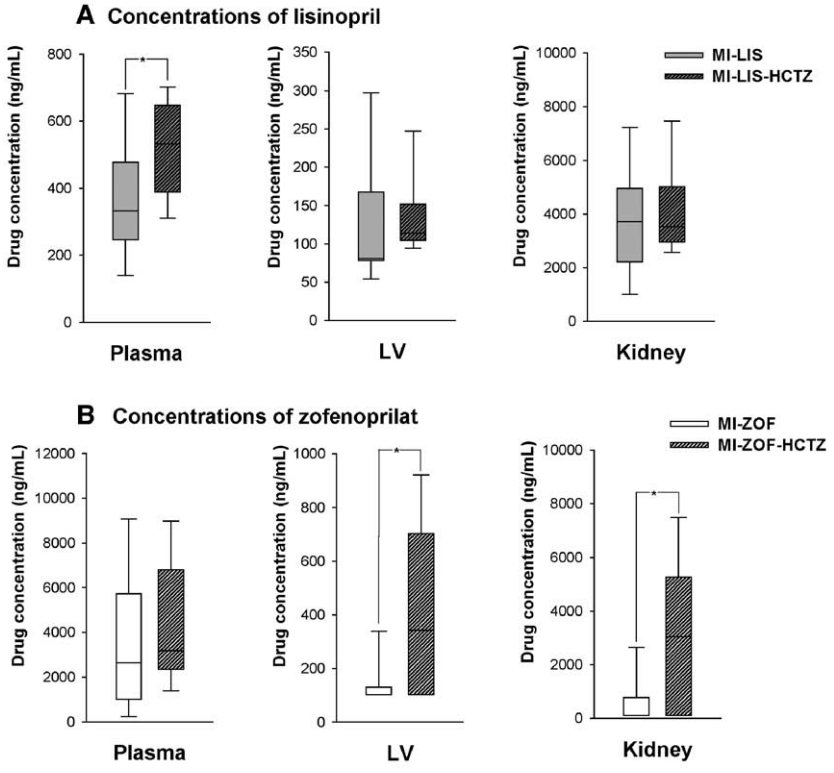

Fig. 2. Plasma, left ventricle and kidney tissue angiotensin-converting enzyme-inhibitor drug concentrations in myocardium infarcted rats instituted either or not with hydrochlorothiazide, after treatment with either (A) lisinopril (3.3 mg/kg/day) or (B) zofenopril (10 mg/kg/day). Boxes delineate 25th and 75th percentiles, lines within boxes represent medians, and whiskers represent 10th and 90th percentiles, respectively. *$P<0.05$ as indicated. LV, left ventricle; MI, myocardial infarction; HCTZ, hydrochlorothiazide; ZOF, zofenopril; LIS, lisinopril.

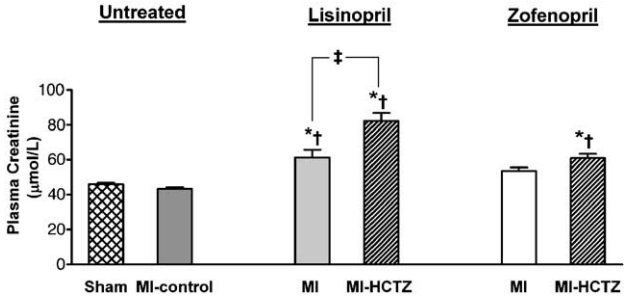

Fig. 3. Renal function in rats with myocardial infarction, and effects of lisinopril and zofenopril, alone or treated with the diuretic hydrochlorothiazide. Renal function was measured by plasma creatinine concentration. Data are shown as mean±S.E.M. *$P<0.05$ versus sham, †$P<0.05$ versus MI, ‡$P<0.05$ versus lisinopril alone. MI, myocardial infarction; HCTZ, hydrochlorothiazide.

After exclusion of data from rats with infarcts smaller than 20% of the left ventricular circumference, infarct sizes were comparable in all groups, except for a small, yet significant difference between rats with lisinopril+hydrochlorothiazide and lisinopril only (Table 1).

Both zofenopril and lisinopril significantly reduced body weight gain, as compared to untreated rats with myocardial infarction. Hydrochlorothiazide further reduced body weight gain, in both zofenopril- and lisinopril-treated rats (Table 1).

Both lisinopril and zofenopril treatment alone caused a moderate, but highly significant increase in plasma K^+ concentrations. Hydrochlorothiazide treatment did not alter this increase in lisinopril-treated rats, whereas hydrochlorothiazide in zofenopril-treated rats normalized plasma K^+ concentrations (Table 1).

Myocardial infarction resulted in left ventricular hypertrophy, as indicated by increased left ventricle: body weight ratios in untreated myocardial infarction compared to sham-operated rats. Zofenopril and lisinopril treatment similarly reduced left ventricle: body weight ratios, without additional effects of hydrochlorothiazide (Table 1).

3.2. Drug concentrations

Plasma lisinopril concentrations were significantly higher (60%, $P=0.03$) in rats instituted with hydrochlorothiazide as compared to those not, whereas lisinopril concentrations in cardiac and renal tissue were not different (Fig. 2A).

Plasma levels of the prodrug zofenopril were very low (approximately 4.5% of total plasma zofenopril plus zofenoprilat levels) compared to the plasma levels of zofenoprilat, but neither plasma zofenopril (152±14 vs. 125±15 ng/ml, $P=0.3$) nor plasma zofenoprilat levels (Fig. 2B) differed between rats treated with hydrochlorothiazide and vehicle, respectively. Contrary to lisinopril, however, tissue levels of the active metabolite zofenoprilat were significantly higher in rats treated with hydrochlorothiazide as compared to those not; 3-fold and

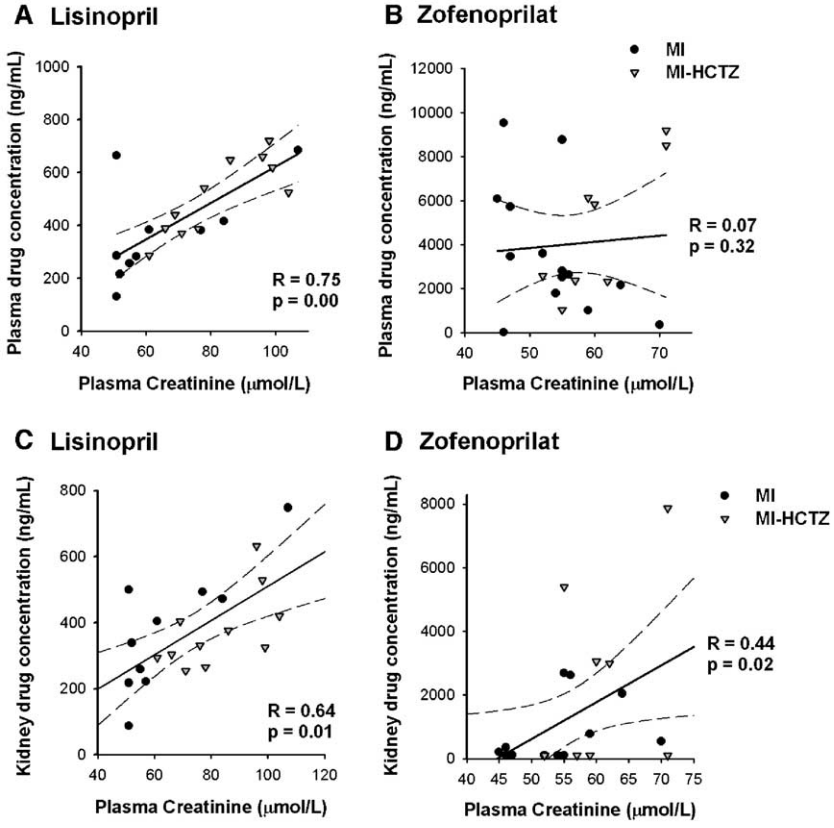

Fig. 4. Relation between kidney function and plasma angiotensin-converting enzyme-inhibitor concentrations in rats with myocardial infarction, either or not treated with hydrochlorothiazide. (A) Lisinopril concentrations in plasma were highly correlated with plasma creatinine concentrations. (B) Zofenoprilat and creatinine concentrations in plasma did not correlate significantly. (C, D) Correlation between plasma creatinine and (kidney) tissue concentrations of zofenoprilat. MI, myocardial infarction; HCTZ, hydrochlorothiazide.

20-fold increases in median values for left ventricular and renal tissue, respectively (Fig. 2C). Finally, the prodrug zofenopril could not be detected in these tissues.

3.3. Kidney function

To asses whether alterations in drug levels could be explained by altered renal drug clearance, kidney function was determined, as measured by plasma creatinine concentrations. Myocardial infarction alone did not alter plasma creatinine levels. Lisinopril alone caused a moderate increase in plasma creatinine, and treatment with hydrochlorothiazide caused a significant further increase (Fig. 3). Treatment with zofenopril alone did not significantly increase plasma creatinine. Zofenopril treatment in rats treated with hydrochlorothiazide caused a moderate increase in plasma creatinine ($P<0.05$ versus untreated sham and untreated myocardial infarction).

The hydrochlorothiazide-induced increase in plasma lisinopril was related to a decrease in renal function, as is shown by the highly significant correlation between plasma creatinine and lisinopril concentrations (Fig. 4A). Tissue concentrations of lisinopril were positively correlated with plasma creatinine as well (Fig. 4C). Trends were similar for left ventricle and kidney. Plasma concentrations of zofenoprilat were not significantly associated with creatinine (Fig. 4B). However kidney as well as left ventricular concentrations of zofenoprilat were significantly correlated with plasma creatinine (Fig. 4D).

3.4. Angiotensin-converting enzyme activity

Both angiotensin-converting enzyme-inhibitors caused a nearly complete reduction (±90%) in plasma angiotensin-converting enzyme activity compared to untreated infarcted and

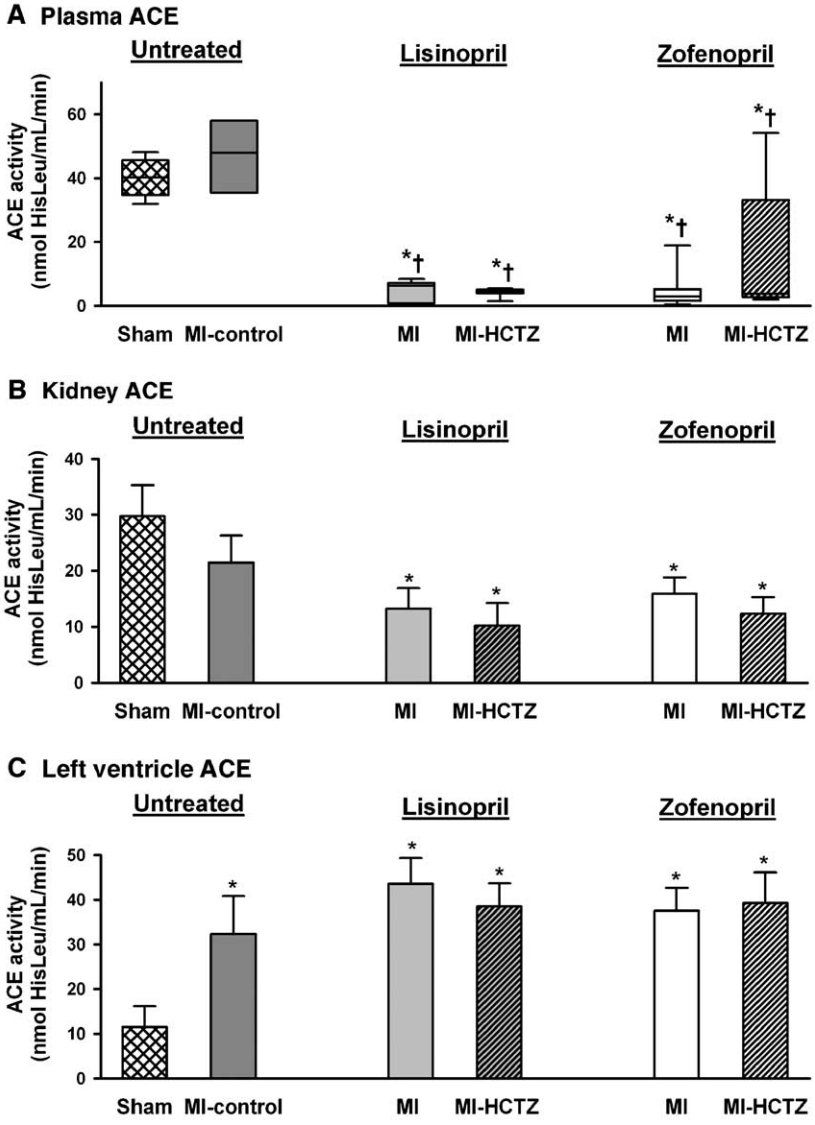

Fig. 5. Angiotensin-converting enzyme activity in plasma, left ventricle and kidney in rats with myocardial infarction, and effects of the angiotensin-converting enzyme-inhibitors zofenopril and lisinopril, alone or combined with hydrochlorothiazide. Data are shown as mean±S.E.M. in case of normal distribution, and in boxplots in case data were not normally distributed. *$P<0.05$ versus sham; $^{\dagger}P<0.05$ versus MI control. MI, myocardial infarction; HCTZ, hydrochlorothiazide.

sham rats, and this effect did not differ between rats treated with or without hydrochlorothiazide (Fig. 5).

Renal angiotensin-converting enzyme activity tended to be decreased in rats with myocardial infarction compared to sham operated rats. Angiotensin-converting enzyme-inhibitor treatment further decreased angiotensin-converting enzyme activity in the kidneys, and consequently all angiotensin-converting enzyme-inhibitor-treated groups displayed significantly decreased renal angiotensin-converting enzyme activity compared to the sham-group. As all angiotensin-converting enzyme-inhibitor-treated groups displayed very low renal angiotensin-converting enzyme activity no differences were observed between rats with myocardial infarction treated with or without hydrochlorothiazide (Fig. 5).

Left ventricular angiotensin-converting enzyme activity was significantly increased in rats with myocardial infarction compared to sham-controls, but both angiotensin-converting enzyme-inhibitors failed to reduce left ventricular angiotensin-converting enzyme activity, regardless whether or not rats were treated with hydrochlorothiazide (Fig. 5).

No significant correlation between angiotensin-converting enzyme-inhibitor drug levels (neither zofenoprilat nor lisinopril) and angiotensin-converting enzyme activity was observed in plasma or tissue.

4. Discussion

Aim of the present study was to investigate the effects of hydrochlorothiazide on steady-state plasma, cardiac and renal tissue angiotensin-converting enzyme-inhibitor drug levels and angiotensin-converting enzyme activity after angiotensin-converting enzyme-inhibitor therapy in an experimental setting of left ventricular dysfunction after myocardial infarction in rats.

4.1. Angiotensin-converting enzyme-inhibitor accumulation by diuretic treatment

Lisinopril, the lysine derivate of enalaprilat, is highly hydrophilic, does not require metabolic transformation to become active, and is cleared unchanged via the kidneys. We found that plasma drug levels of this angiotensin-converting enzyme-inhibitor were higher in rats treated with hydrochlorothiazide. This was explained by decreased renal clearance of lisinopril, as plasma concentrations of lisinopril showed a highly significant relation with plasma creatinine, a measure for glomerular filtration rate. Most likely, volume depletion accounted for this decrease in glomerular filtration rate, thereby resulting in drug accumulation. Others have reported no or marginal interactions in terms of clearance between hydrochlorothiazide and lisinopril (Laher et al., 1991; Swaisland, 1991), but note that these were all studies testing effects of hydrochlorothiazide on plasma lisinopril concentrations after one single dose. To our knowledge, this study is the first to address the effects of hydrochlorothiazide on steady-state angiotensin-converting enzyme-inhibitor levels in a setting of repeated drug dosing.

Infarct sizes were significantly different between lisinopril- and lisinopril+hydrochlorothiazide-treated rats. However this difference in infarct size between the two lisinopril groups does not interfere with our findings, as we found no relation between infarct size and angiotensin-converting enzyme-inhibitor concentrations. We also tested effects of hydrochlorothiazide on angiotensin-converting enzyme-inhibitor concentrations in sham-operated rats (data not shown for reasons of clarity), and results were very similar, confirming that presence of myocardial infarction did not play a role in the observed effects of hydrochlorothiazide.

To compare angiotensin-converting enzyme-inhibitors with different kinetic properties, we also investigated the effects of hydrochlorothiazide on zofenopril/zofenoprilat drug levels. Zofenopril is a lipophilic prodrug which undergoes hydrolyzation to its active metabolite zofenoprilat in tissue. Plasma prodrug levels represented only 4.5% of the total circulating levels of zofenopril plus zofenoprilat in the present study, indicating a near total conversion of zofenopril into its active metabolite. Zofenoprilat is hydrophobic, and cleared predominantly via the kidneys (±70%), but also via the bile and feces (Subissi et al., 1999).

In contrast to lisinopril, plasma zofenoprilat levels were only marginally increased by hydrochlorothiazide, whereas tissue zofenoprilat levels increased markedly, indicating angiotensin-converting enzyme-inhibitor drug accumulation in the tissue. These findings may be related to volume depletion by addition of hydrochlorothiazide to zofenopril treatment. Rather than leading to increased plasma levels, the lipophilic nature of zofenopril would favor drug penetration of the angiotensin-converting enzyme-inhibitor into the tissue, with a subsequent tissue accumulation of the active metabolite zofenoprilat (Ranadive et al., 1992). Note that we observed a significant correlation between plasma creatinine and tissue zofenoprilat concentrations, potentially as both depend on volume status. Surprisingly, zofenoprilat concentrations in plasma did not increase significantly along with tissue concentrations during hydrochlorothiazide treatment. Thus, mechanisms independent of volume status may be involved as well in the observed accumulation of zofenoprilat in renal and cardiac tissue.

4.2. Relevance/implications of increased drug levels

Increased angiotensin-converting enzyme-inhibitor levels were not associated with a significant reduction in angiotensin-converting enzyme activity in the current study. In case of plasma and kidney, this may be explained by maximal inhibition as a result of high dosing: both zofenopril and lisinopril caused nearly complete inhibition of circulating and renal angiotensin-converting enzyme. For cardiac angiotensin-converting enzyme activity, we observed no effect of zofenopril or lisinopril, and also no effects of hydrochlorothiazide treatment. This finding is consistent with previous studies employing the rat coronary ligation model of left ventricular dysfunction after (Wollert et al., 1994; Hirsch et al., 1992; Westendorp et al., 2004). An explanation may be upregulation of cardiac angiotensin-converting enzyme expression under

angiotensin-converting enzyme inhibition (Schunkert et al., 1993), although this has not unambiguously been shown for cardiac tissue (Kelly et al., 1997; Samani et al., 1994; Seeland et al., 2003).

It has long been recognized that the local rather than the circulating renin angiotensin aldosterone system is involved in pathophysiology of cardiovascular disease (Dzau et al., 2002). Thus, increasing the tissue penetration of the angiotensin-converting enzyme-inhibitor may potentiate its beneficial effects. Although we found no further reduction of angiotensin-converting enzyme activity, the hydrochlorothiazide-induced increase in tissue zofenoprilat levels may have local beneficial effects independent from angiotensin-converting enzyme inhibition per se. Firstly, increased levels of tissue angiotensin-converting enzyme-inhibitor could lead to further reduction in oxidative stress. Zofenoprilat contains a sulphydryl-group, which may scavenge reactive oxygen species, thereby reducing inflammation and increasing bioavailability of nitric oxide (Buikema et al., 2000; Cominacini et al., 2002; Evangelista and Manzini, 2005). Secondly, angiotensin-converting enzyme-inhibitors have zinc-chelating properties, which may interfere with cardiac remodeling via reduction of matrix metalloproteinase activity (Sorbi et al., 1993; Hayashidani et al., 2003; Reinhardt et al., 2002; Sakata et al., 2004).

4.3. Renal effects of hydrochlorothiazide on lisinopril versus zofenopril

We used equipotent dosages of angiotensin-converting enzyme-inhibitors, based on previous results (Buikema et al., 2000), and the ratio between clinically used dosages. Dose equivalence was illustrated by similar reductions in left ventricular hypertrophy and angiotensin-converting enzyme activity, and a comparable rise in serum K^+. Notably, effects of hydrochlorothiazide treatment on zofenopril and lisinopril were differential, substantiating the view that not all angiotensin-converting enzyme-inhibitors are by definition interchangeable within their class (Furberg and Psaty, 2003;Furberg and Pitt, 2001).

Firstly, we observed differential effects of hydrochlorothiazide on lisinopril and zofenoprilat concentrations, as discussed above. Furthermore, we observed that hydrochlorothiazide decreased (i.e. normalized) plasma K^+ levels in zofenopril- but not lisinopril-treated rats. We have no explanation for this finding, but as plasma electrolyte disturbances are of great importance in patients with heart failure (Macdonald and Struthers, 2004), this matter deserves further study.

Combining hydrochlorothiazide with lisinopril increased plasma creatinine concentrations. Although plasma creatinine may not be the most accurate indicator of renal function, it corresponds with a substantial drop in glomerular filtration rate by ±25% compared to lisinopril monotherapy and ±50% compared to untreated rats. Importantly, decreased renal function by combining angiotensin-converting enzyme-inhibitors and angiotensin-2 receptor antagonists with diuretic treatment has also been reported in humans (Esnault et al., 2005). This effect of combining angiotensin-converting enzyme inhibition with hydrochlorothiazide treatment could have clinical implications, as even mildly impaired renal function is strongly and independently associated with worsened prognosis after myocardial infarction (Sorensen et al., 2002).

4.4. Conclusion

In the present study diuretic treatment with hydrochlorothiazide significantly influenced steady-state plasma and tissue angiotensin-converting enzyme-inhibitor drug levels in rats with experimental myocardial infarction. The effect of hydrochlorothiazide differed for the different angiotensin-converting enzyme-inhibitor employed, resulting in increased angiotensin-converting enzyme-inhibitor drug levels in the plasma in case of the hydrophilic angiotensin-converting enzyme-inhibitor lisinopril versus increased angiotensin-converting enzyme-inhibitor drug levels in renal and cardiac tissue in case of the lipophilic angiotensin-converting enzyme-inhibitor zofenopril. Increased tissue angiotensin-converting enzyme-inhibitor drug levels may contribute to the enhanced organ-protective effects of angiotensin-converting enzyme-inhibitor therapy. However, decreased renal function by combining hydrochlorothiazide with angiotensin-converting enzyme inhibition may have adverse effects.

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