Tissue ACE inhibition and sodium status in left ventricular dysfunction
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Chapter 2

Dietary Sodium Restriction Specifically Potentiates Left Ventricular ACE Inhibition by Zofenopril, and is Associated with Attenuated Hypertrophic Response in Rats with Experimental Myocardial Infarction.

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Abstract

Background: In patients with myocardial infarction-induced heart failure, angiotensin converting enzyme inhibitors (ACE-I) are proven effective therapy by inhibiting the progression towards overt heart failure. However, prognosis is still very poor, and optimization of therapy is warranted. Antihypertensive and renoprotective effects of ACE-I can be substantially enhanced by dietary sodium restriction. In line with the latter, the aim of the present study was to explore if dietary sodium restriction enhances the cardioprotective efficacy of ACE-I after myocardial infarction.

Methods: Rats with myocardial infarction-induced left ventricular dysfunction received ACE-I therapy with zofenopril (5.5 mg kg\(^{-1}\) day\(^{-1}\) orally), either with or without dietary sodium restriction. ACE activity was measured in non-infarcted left ventricular tissue, kidneys, and plasma. Effects on cardiac hypertrophy were examined by means of organ weight/body weight ratios. After blood pressure measurements, functional consequences of therapy were evaluated as left ventricular pressure development in isolated perfused hearts.

Results: Blood pressure was reduced after infarction, and further reduced by zofenopril, but not affected by sodium diet. Dietary sodium restriction alone had no effect on any of the measured parameters, zofenopril alone significantly reduced plasma and kidney ACE activity, but not left ventricular ACE activity, nor left ventricular weight/body weight. However, only when ACE-inhibitor therapy was combined with dietary sodium restriction, left ventricular ACE activity was significantly reduced. This effect was paralleled by inhibition of left ventricular hypertrophy. Neither treatment could be associated with effects on the myocardial infarction-induced reduction of in vitro left ventricular function.

Conclusions: Effects of ACE inhibition with zofenopril can be potentiated by additional dietary sodium restriction. However, effects were tissue specific, since left ventricular - but not kidney or plasma- ACE activity was affected by the additional dietary sodium restriction. Effects on left ventricular ACE activity were paralleled by reduced left ventricular hypertrophy. Since measured parameters did not indicate adverse side effects, dietary sodium restriction may provide a safe strategy to improve ACE-inhibitor efficacy in patients with infarction-induced left ventricular dysfunction.
**Introduction**

Chronic activation of the renin-angiotensin-aldosterone system (RAAS) is regarded as one of the major causes of progressive deterioration of left ventricular pump function after acute myocardial infarction (MI). Accordingly, RAAS-inhibition with ACE-inhibitor therapy is associated with better cardiac function\(^1\), improved prognosis\(^2\), and increased quality of life\(^3\). Although ACE-inhibition slows the gradual progression of myocardial dysfunction towards established chronic heart failure, it does not prevent it\(^4-6\). Hence, morbidity and mortality remain high, and further optimization of therapy is warranted. One area which has not been explored yet in this context is optimization of ACE-I efficacy by dietary sodium restriction. A substantial load of clinical and experimental evidence shows that dietary sodium restriction can improve the antihypertensive and renoprotective effects of ACE-inhibition in patients with hypertension and renal dysfunction, respectively\(^7-10\). Vice versa, sodium loading may completely annihilate the effect of ACE-inhibitors\(^11\).

In view of the fact that dietary sodium restriction is quite commonly advised to patients with LV dysfunction to reduce fluid retention, surprisingly little has been published about its effect on the efficacy of ACE-inhibitor therapy in post-AMI left ventricular (LV) dysfunction. The aim of the present study, therefore, was to explore the influence of dietary sodium restriction on the therapeutic effects of ACE-inhibitor treatment in rats with myocardial infarction. To this end, we studied the effects on blood pressure and cardiac hypertrophy, as well as ACE activity in plasma, and renal and cardiac tissue after 10 weeks of ACE-I treatment, with and without dietary sodium restriction.

**Methods**

*Experimental protocol*

Male Wistar rats (Harlan, Zeist, The Netherlands) were subjected to experimental myocardial infarction (MI) by coronary ligation, or were sham operated (t=0). Two weeks after MI-induction (t=2), rats were maintained on a normal sodium diet or switched to a low sodium diet, either with or without zofenopril. Thus, MI-rats were allocated to one of the following treatment regimes: normal sodium (n=10), low sodium (n=6), normal sodium + zofenopril (n=10), low sodium + zofenopril (n=6). Sham-operated rats (n=10) were kept on a normal sodium diet and functioned as control rats for untreated MI. Treatment was maintained for 10 weeks (t=12), after which period rats were anaesthetized with isoflurane (2.0-2.5%). Subsequently, aortic blood pressure was obtained through a cannula in the carotid artery. A blood sample was drawn from the abdominal aorta before hearts were isolated and perfused according to Langendorff for *in vitro* assessment of ventricular function. Renal and cardiac tissues were washed and weighed, snap frozen in liquid nitrogen and stored at ~80°C for future analysis. The University of Groningen committee on animal experiments approved the above study design.
Myocardial infarction
Rats were anesthetized with isoflurane (2.0-2.5%) in a mixture of $N_2O$ (2:1), intubated and mechanically ventilated. Coronary artery ligation was performed as described in detail elsewhere. Briefly, a left side thoracotomy was performed and the left anterior descending coronary artery was occluded with a 6-0 silk suture, 1-2 millimeters from its origin. In sham-operated animals the suture was placed but not tightened. Subsequently, the thorax was closed and rats were extubated upon spontaneous respiration. The coronary occlusion procedure results in extensive transmural MI, comprising a major part of the LV free wall. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices, as the percentage of LV circumference. Rats with infarctions of less than 20% of the LV tissue were excluded from further analysis, since these infarcts are found to be hemodynamically fully compensated.

Diet and medication
Dietary sodium restriction was achieved by treating rats with food pellets containing 0.05% NaCl. Rats on a normal sodium diet were given food pellets with 0.3% NaCl. Zofenopril treatment was achieved by mixing the drug with rat chow during preparation (Hope Farms, Woerden, The Netherlands). Drug content and distribution in the rat chow were checked at the I.P.A.S. institute (Ligornetto, Switzerland). Analysis of drug content of the food together with the assessment of food intake and body weight revealed that drug intake was similar (on average 5.5 and 5.3 mg. kg\(^{-1}\) body weight. day\(^{-1}\)) in both zofenopril groups.

Blood pressure measurements
After anesthesia with isoflurane (2.0-2.5%), the right carotid artery was catheterized with a polyethylene catheter filled with 0.9% saline with heparin, 5000 U. L\(^{-1}\), connected to a pressure transducer (Statham 23 Db, Gould Instruments, Cleveland, OH). The catheter was advanced into the thoracic aorta, and after stabilization aortic blood pressure was recorded.

In vitro left ventricular function
In all rats, LV function was measured in on a Langendorff setup, as described previously. After 500 IU of heparin i.v., the heart was isolated and perfused according to Langendorff. For perfusion, a modified Tyrode solution was used (composition in mmol. L\(^{-1}\): glucose 10, NaCl 128.3, KCl 4.7, NaHCO\(_3\) 20.2, CaCl\(_2\) 1.35, NaH\(_2\)PO\(_4\) 0.42, MgCl\(_2\) 1.05, equilibrated with 95% O\(_2\) and 5% CO\(_2\)). Perfusion pressure was maintained at 60 mm Hg, and temperature was kept between 38.0 and 38.5 °C. After 10 minutes of stabilization, LV pressure was measured by means of a catheter placed into LV via the mitral valves and connected to a pressure transducer. Coronary flow was measured through a microprocessor, which controlled the perfusion pressure by adjusting the peristaltic perfusion pump. LV pressure, dP/dt, and heart rate were measured and stored in a computerized database system.
In order to ensure that induction of MI resulted in LV dysfunction, LV function was evaluated in sham rats and rats with untreated MI\textsuperscript{12}. Although effects of ACE inhibitors on in vivo LV function are undisputed\textsuperscript{15-17}, they cannot be shown on this in vitro setup\textsuperscript{18,19}. Therefore functional data of the treatment groups were not evaluated. Decreased LV systolic pressure, and maximum rates of contraction and relaxation were significantly reduced in all MI rats as compared to sham operated rats, showing indeed MI-induced LV dysfunction.

**ACE activity**

ACE activity in plasma, LV (non-infarcted free wall) and kidney were determined using a method previously described in detail\textsuperscript{20}. In short, tissues were homogenized in a 50 mmol. L\textsuperscript{-1} K$_2$PO$_4$ buffer at pH 7.5. Of the homogenates 100 µl was pipetted in a 0.5 mol. L\textsuperscript{-1} K$_2$PO$_4$ buffer. Then the ACE substrate Hippuryl-His-Leu (12.5 nmol. L\textsuperscript{-1}, Sigma) was added and incubated at 37\textdegree{}C for exactly 10 minutes. The conversion of the substrate was stopped by adding 1.45 ml of 280 mmol. L\textsuperscript{-1} NaOH. Thereafter, 100 µl of 1% phtaldialdehyde was added for the labeling of free His-Leu product. 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. Moreover, the substrate was added after the incubation period in these control samples.

**Data analysis**

Data are presented as mean±S.E.M. Untreated MI-rats on normal sodium were compared to sham control rats using student’s unpaired $t$-test, as to study the effect of CHF after experimental MI.

Subsequently, all groups were compared among each other using oneway-analysis of variances (ANOVA) in combination with least significant difference post-hoc analysis for multiple comparisons (SPSS for Windows Standard version 10.0). Differences were considered statistically significant at a level of p<0.05 (two-tailed).

**Results**

**General characteristics**

Acute mortality after MI-induction was 26%; none of the sham-operated animals died. After post hoc exclusion of rats with infarcts smaller than 20% from further analysis (15 out of 59), infarct sizes were evenly balanced between the MI groups (table 1). Body weights (BW) were comparable at the start of the protocol, but significantly lower at the end of treatment period in the groups receiving zofenopril.
Table 1. General characteristics of the experimental groups

<table>
<thead>
<tr>
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<th>Sham Control</th>
<th>Myocardial infarction MI</th>
<th>MI-LS</th>
<th>MI-ZOF</th>
<th>MI-ZOF-LS</th>
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<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>6</td>
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<tr>
<td>Infarct size (%)</td>
<td>34.3±3.5</td>
<td>35.4±3.5</td>
<td>35.3±3.6</td>
<td>38.0±3.8</td>
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<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>at t=0 weeks</td>
<td>273±6</td>
<td>278±3</td>
<td>266±5</td>
<td>277±3</td>
<td>268±4</td>
</tr>
<tr>
<td>at t=12 weeks</td>
<td>497±16</td>
<td>482±17</td>
<td>460±25</td>
<td>380±9*</td>
<td>386±13*</td>
</tr>
<tr>
<td>Organ weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>2.03±0.07</td>
<td>2.71±0.24*</td>
<td>2.56±0.16</td>
<td>2.09±0.14*</td>
<td>1.62±0.06*</td>
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<tr>
<td>Left ventricle</td>
<td>1.23±0.03</td>
<td>1.63±0.13*</td>
<td>1.59±0.07</td>
<td>1.28±0.07*</td>
<td>1.01±0.03*</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.26±0.01</td>
<td>0.37±0.06*</td>
<td>0.37±0.05</td>
<td>0.25±0.02*</td>
<td>0.20±0.01*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Infarct-size and organ weights were determined at t=12 weeks after infarction when rats were sacrificed. Abbreviations: myocardial infarction, MI; dietary sodium restriction; LS, zofenopril; ZOF. # p<0.05 for MI versus control; * p<0.05 treated versus untreated MI.

Blood pressure
Mean arterial blood pressure was lower after MI (73±4 mmHg), as compared to sham control rats (87±4 mmHg, p=0.008). Dietary sodium restriction per se did not affect blood pressure (71±4 mmHg, p=NS versus untreated MI). Zofenopril treatment significantly reduced blood pressure, independent of sodium diet; 44±3 and 48±5 mmHg for zofenopril + normal sodium and zofenopril + low sodium, respectively (both p<0.001 versus untreated MI).

Cardiac hypertrophy
MI-induced hypertrophy was observed by a marked increase in total heart weight, which could be attributed to increases in left ventricular as well as right ventricular weight (table 1). Zofenopril treatment, but not dietary sodium restriction per se, prevented this cardiac response. However, when zofenopril treatment was combined with a dietary sodium restriction, left ventricular weight was reduced by an additional 21%. Right ventricular weights showed the same treatment effects (20%), though statistical significance was not reached.

Left ventricular hypertrophy induced by MI was only effectively prevented by the combination of zofenopril and sodium restriction (figure 1). When left ventricular hypertrophy, represented by LVW-to-BW ratio, of MI-rats was compared to sham-operated animals, all groups except the low sodium-zofenopril group showed a significant increase. Moreover, LVW-to-BW ratio was significantly lower in rats that received zofenopril + dietary sodium restriction compared with rats on zofenopril alone (fig. 1).
Dietary Sodium Restriction Potentiates LV ACE Inhibition

Figure 1. Effect of treatment with zofenopril (ZOF) and/or low sodium diet (LS) on left ventricular ACE activity.

Induction of CHF did not result in significantly elevated ACE activity in plasma and tissue (figure 2). However, plasma ACE activity was almost completely inhibited by zofenopril, irrespective of sodium intake. Although absolute values were much higher, a similar pattern was seen in the kidneys: marked inhibition by zofenopril but no additional effects by sodium intake.

Interestingly, ACE activity in LV tissue was not reduced by zofenopril in rats that were fed on a normal sodium diet. Only in combination with a low sodium diet, ACE activity was significantly decreased. ACE activity in RV tissue showed a similar pattern (data not shown). LV ACE activity and LVW-to-BW ratio showed a significant correlation (figure 3).

Discussion

We studied the effect of dietary sodium restriction on efficacy of ACE-inhibitor therapy after experimental myocardial infarction in rats. The most important observations were: 1) LV ACE activity was inhibited only when zofenopril treatment was combined with dietary sodium restriction; 2) this effect occurred in heart, but not in plasma or in kidney tissue; 3) the significant reduction of LV ACE activity during combined ACE inhibitor/low sodium diet treatment could be associated with a significant reduction in LVW-to-BW ratio, indicating prevention of hypertrophy; 4) A low sodium diet per se had no effects on any of the measured parameters.
Figure 2. Effect of treatment with zofenopril (ZOF) and/or low sodium diet (LS) on plasma (top panel), left ventricular (middle panel) - and renal tissue ACE activity (bottom panel) in rats with experimental myocardial infarction (MI). Data are mean ± SEM and bars represent the formation of His-Leu from Hippuryl-His-Leu. * p<0.05 as indicated.
In the present study, MI did not increase ACE activity in plasma, nor in renal and LV tissue. Cardiac ACE may be activated in the early stage after induction of heart failure and is related to the degree of LV dysfunction. Moreover, LV ACE activity appears also related to MI size. The relatively moderate MI size in the present study may therefore not have increased ACE activity. Moreover, the major area in which ACE activity is elevated post-MI is the infarcted area itself, where mainly fibroblasts display increased activity, whereas analysis in the present study was restricted to the viable part of the LV free wall.

In accordance with the lack of activation after MI, there was no significant inhibition of LV ACE activity due to zofenopril treatment. In previous studies zofenopril did significantly inhibit cardiac ACE activity, but the applied dose turned out to be lower in the current study. This confirms the importance of optimal dosing, as has been reported before.

Dietary sodium restriction alone did not show any effect on the parameters measured in the present study. This is in general accordance with a previous study of Hodsman and coworkers in rats with myocardial infarction. The lack of effect of dietary sodium restriction alone on ACE activity in the present study suggests absence of a direct influence of sodium restriction on ACE activity and/or expression. However, other components of the renin angiotensin system may have been affected. In this respect it is interesting to mention that both ACE gene expression, and angiotensin II type 1 (AT-1) receptor expression may be regulated by sodium intake. The AT-1 receptor is regarded the primary mediator of angiotensin II induced cardiac remodeling. Although not affecting ACE activity when applied alone, when dietary

\[ R^2 = 0.58 \]
\[ p = 0.01 \]

Figure 3. Correlation between LV tissue ACE activity and LV weight in zofenopril (ZOF)-treated rats with experimental myocardial infarction. The solid line represents linear regression line through individual points.
sodium restriction is added to the zofenopril treatment, LV ACE activity was significantly reduced. Notably, this effect was specific for the LV since no additional ACE inhibition could be shown in plasma or kidneys. It remains unclear why ACE activity in the kidney homogenates was not further inhibited by the combination of dietary sodium restriction and zofenopril, as it was in LV. This may seem paradoxical, since renoprotective effects of ACE inhibition can be enhanced by dietary sodium restriction. However, this improved renoprotection, as measured by proteinuria reduction, has not yet been shown to be associated with a further decrease in renal ACE activity. Moreover, a possible effect of sodium on ACE activity may be only be present in a situation of tissue damage, i.e. effects becoming only apparent when the tissue RAAS is activated by organ damage. In the current study, kidneys were considered to be intact. Thus, the exact mechanisms underlying the additional cardio- or renoprotective effects of combined dietary sodium restriction and zofenopril treatment is not yet clear and will be subject of further investigation.

Cardiac hypertrophy
LVW was significantly increased after MI, and neither dietary sodium restriction, nor zofenopril treatment alone did have any effect. Only when the two treatments were combined, LV hypertrophy was effectively prevented. Interestingly, the effects of therapy on LV ACE activity paralleled the effects on LV hypertrophy. Indeed, regression analysis revealed that LV ACE activity in zofenopril-treated rats was linearly related to left ventricular weight (Figure 3), suggesting that LV ACE activity is a major determinant of LV hypertrophy in presence of an ACE inhibitor. A previous study, using a 3 times higher dose of zofenopril and significantly decreasing LV ACE activity as well as LV weight, support this suggestion. In contrast, high sodium diet abolished the effect of perindopril on blood pressure and cardiac hypertrophy, but cardiac ACE inhibition was found unaltered. Although in the latter study blood pressure rather than cardiac ACE activity was regarded the determinant of cardiac hypertrophy, in the present study, the effects on hypertrophy were found to be independent of blood pressure – i.e. blood pressure was similarly reduced in the zofenopril groups without additional effects of dietary sodium. It has been reported before that RAAS activation rather than blood pressure determines cardiac hypertrophy after MI.

Clinical implications
Dietary sodium restriction is commonly recommended and/or applied to heart failure patients, who most often are on ACE-inhibitor therapy as well. Theoretically this dietary sodium restriction may have a dual effect. On the one hand it may (further) stimulate the RAAS, with its well-known deleterious effects. On the other hand it may improve efficacy of inhibition of the RAAS, either by a similar or by different mechanisms. In a recent study, this dual effect was illustrated by improved survival, but at the same time substantial potentiation of plasma renin activity, in spontaneously hypertensive rats with MI, treated with furosemide in addition to ramipril.
Dietary Sodium Restriction Potentiates LV ACE Inhibition

Furosemide, by causing sodium depletion and increased plasma renin activity may have similar effects as dietary sodium restriction. Data from the present study suggest that dietary sodium restriction may be safely applied to MI patients on ACE-inhibitors, in that it specifically potentiates cardiac ACE inhibition leading to further inhibition of cardiac remodeling, and without adverse side effects such as hypotension. Whether dietary sodium restriction will lead to further improvement of cardiac function and/or prognosis when added to ACE-inhibitor treatment in patients with MI-induced heart failure, needs further investigation.

Conclusion

The present study was aimed to explore whether dietary sodium restriction would influence the effects of ACE-inhibitor therapy with zofenopril in rats with myocardial infarction. Results show that left ventricular-, but not plasma or renal ACE inhibition, was potentiated by additional dietary sodium restriction, indicating a tissue specific effect. Effect on ventricular ACE inhibition was paralleled by effects on cardiac hypertrophy, without effects on blood pressure, suggesting left ventricular ACE activity as a primary determinant of cardiac hypertrophy. Additional studies are needed to examine whether this effect is drug specific, and whether the observed effects can be associated with beneficial effects on in vivo cardiac function and prognosis.

References


