Tissue ACE inhibition and sodium status in left ventricular dysfunction
Westendorp, Bart

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Chapter 3

Beneficial Effects of Add-on Hydrochlorothiazide in Rats with Myocardial Infarction Optimally Treated with Quinapril.

Bart Westendorp
Regien Schoemaker
Hendrik Buikema
Dick de Zeeuw
Frans Boomsma
Wiek van Gilst
Dirk Jan van Veldhuisen
Abstract

Background: The antihypertensive and renoprotective effects of ACE inhibitor (ACE-I) therapy are enhanced by inducing a negative sodium balance. Whether this strategy also improves outcome of chronic ACE-I treatment after myocardial infarction (MI) is unknown. Therefore, we investigated whether hydrochlorothiazide (HCTZ) or dietary sodium restriction further improves survival in ACE-I-treated rats with MI.

Methods: MI was induced by coronary ligation. After 2 weeks rats were randomised to quinapril (QUI), HCTZ added to quinapril (QUI-HCTZ), or low sodium diet added to quinapril (QUI+LS). Survival was monitored for 62 weeks, after which left ventricular (LV) pressures were measured and blood for neurohumoral characterisation was collected. A separate group of rats, subjected to the same procedure, was evaluated after 35 weeks.

Results: After 62 weeks, mortality was comparable in all groups. However, survival was improved by HCTZ until 35 weeks. This effect on survival was paralleled by decreased proteinuria and LV end-diastolic pressures in QUI-HCTZ rats at 35, but not 62 weeks. Plasma renin activity was significantly decreased in QUI+HCTZ rats at 35 weeks. Contrary to HCTZ, LS added to QUI caused no benefit.

Conclusions: Adding HCTZ, but not LS, to quinapril improved survival, neurohumoral status, and proteinuria during the early chronic phase of experimental post-MI LV dysfunction. Since no adverse effects were observed, HCTZ may safely be used to improve ACE-I therapy.
**Introduction**

The progression of left ventricular (LV) dysfunction towards overt chronic heart failure (CHF) after myocardial infarction (MI) is associated with progressive cardiac remodelling. Renin angiotensin aldosterone system (RAAS) activation plays a central role in this process, and blocking the RAAS with angiotensin-converting enzyme inhibitors (ACE-I) effectively reduces remodelling and prolongs survival after MI. Still, prognosis post-MI is grim, and optimisation of therapy is necessary. Antihypertensive and renoprotective effects of ACE-I can be enhanced by dietary sodium restriction or co-treatment with diuretics. For heart failure treatment, this has not been investigated. Sodium depletion itself has never been shown to affect the progression of LV dysfunction in terms of LV remodelling or LV hemodynamic parameters. However, induction of a negative sodium balance may provide a strategy to enhance the therapeutic efficacy of ACE-I in heart failure. We previously showed that sodium restriction added to ACE-I treatment further attenuated LV hypertrophy in MI-rats, which was associated with augmented inhibition of LV tissue ACE activity. Whether this also results in improved cardiac function and reduced mortality was addressed in the current study. We evaluated the long-term effects of additional diuretic treatment or a low sodium diet on mortality, cardiac function, neurohumoral activity, and urinary protein excretion in rats with experimental MI on optimal ACE-I therapy. We hypothesised that diuretics and dietary sodium restriction 1) augment the beneficial effects of ACE-I therapy and thus positively affect cardiac function and survival in rats with MI, and 2) exert no adverse effects and can be safely applied in post-MI ACE-I therapy.

**Methods**

*Study design*

The investigation conforms to the Guide for the Care and Use of Laboratory Animals (Published by the US National Institutes of Health, NIH publication No. 85-23, revised 1996). The animal research committee of the University of Groningen approved this study protocol.

Male, Sprague Dawley rats (Harlan, Zeist, The Netherlands), weighing 280±25g were subjected to coronary ligation as previously described. Since the study was aimed at the chronic phase after myocardial infarction, and not at interfering with the early healing process and scar formation, treatment was started 14 days post-MI. Rats were randomly assigned to: quinapril alone (QUI), quinapril + hydrochlorothiazide (QUI+HCTZ), quinapril + low sodium (QUI+LS). Since HCTZ or LS alone do not affect post-MI LV remodelling and function, we did not include treatment arms on only HCTZ or LS. As the effects of ACE-I on MI rats are well established, we also refrained from including a non-treated MI-group in the survival study.

Quinapril (15 mg kg⁻¹ day⁻¹) was mixed through food (Hope Farms, Woerden, The Netherlands). This dose results in optimal ACE-I therapy. HCTZ (50 mg kg⁻¹ day⁻¹) was dissolved in drinking water. This dose causes an increase in diuresis and RAAS activation, but no blood pressure reduction in normotensive rats. The LS-group was fed...
with food pellets containing 0.05% NaCl instead of 0.3% (normal diet) (LS-diet; Hope Farms, Woerden, The Netherlands). To ensure constant drug intakes during the entire study period, concentrations of quinapril and HCTZ were adjusted weekly. This was done by measuring food/water intake and average body weight per cage weekly, and calculating the required drug concentration in food and water (per cage) for the week after. Rats were fed *ad libitum*, and housed group-wise in clear polyethylene cages in temperature (22°C)- and humidity (50%)-controlled rooms with a 12h light/dark cycle.

**Study 1: 62 weeks survival**
Animals surviving surgical procedures (159 out of 320) were allocated to one of three active treatment groups, and monitored - in a blinded fashion - until death or for 62 weeks after onset of therapy. Use of colour-tags ensured appropriate housing and treatment by caretakers. Cages were checked for dead animals at least once daily; tissues of dead rats were collected, weighed and stored for analysis.
At 17, 35 and 52 weeks of treatment 24h urine samples were collected for measurement of total urinary protein using a nephelometer (Dade Behring Diagnostic, Marburg, Germany). After the 62-week follow-up period, subgroups of randomly chosen surviving rats were sacrificed for assessment of LV function and neurohumoral activity.

**Study 2: 35 weeks of treatment**
In total 90 rats were used for this protocol, designed to study neurohormones and cardiac function during the course of the study period in relation to untreated MI rats. Rats were operated and treated according to the same procedures as in the above study, except that they were sacrificed and studied at 35 weeks after onset of treatment.

**Assessment of cardiac function**
Rats were anaesthetised with isoflurane (2%) in a mixture of O₂ and N₂O (1:2), the carotid artery was cannulated and a pressure tip catheter (Micro-Tip 3French, Millar instruments Inc., Houston TX, USA) advanced into the LV. After registration of LV systolic and diastolic pressures (LVSP and LVEDP), and maximal rates of increase and decrease in LV pressure (+dP/dt and –dP/dt), the catheter was retracted into the aortic arch, and arterial pressures and heart rate were recorded. Subsequently, arterial blood and tissues were collected for further analysis.

**Neurohumoral measurements**
Arterial blood was anti-coagulated with EDTA and N-terminal atrial natriuretic peptide (N-ANP), plasma renin activity, and aldosterone, were measured in the plasma as previously described¹⁷. Plasma for ACE activity determination was collected separately, and not anti-coagulated with EDTA. ACE activity in the plasma and spared myocardial tissue was determined as described before¹².

**Tissue collection and histology**
Hearts were rinsed with ice cold NaCl (0.9%), and the atria and right ventricle were removed on ice for determination of LV weights. The apical 1/3 part of the LV was cut-off, spared and infarcted tissues were separated, and frozen in liquid nitrogen for
measurement of left ventricular ACE activity. The mid-ventricular slice of the LV was stored in 2% paraformaldehyde for histologic assessment of infarct size on slices stained with Sirius Red/Fast Green, as described previously\textsuperscript{12}. Infarct size was determined as percentage of LV circumference. The mid-ventricular section provides adequate estimation of total LV infarct size\textsuperscript{18}. Only rats with MI sizes larger than 20% of LV were included for analysis, since smaller infarcts do not result in LV dysfunction\textsuperscript{19,20}.

**Statistical methods**

The survival study was designed to detect a difference of 20% in 1-year mortality with a power of 0.80, with an expected mortality of 60% in the control group that received quinapril only, based on the early captopril studies\textsuperscript{21}. Survival analysis was done using log rank analysis with pairwise comparison over strata. Functional and neurohumoral parameters were compared using one-way analysis of variances (ANOVA) with least square difference post hoc analysis for multiple comparisons in case of normal distribution of data. Otherwise, a Kruskal-Wallis test was used. Differences were considered significant at the level of 0.05 (two tailed). Bodyweights, food and water intakes were analysed with ANOVA for repeated measurements (general linear model).

Data are presented as means ± SEM in case of normal distribution, otherwise in boxplots.

**Results**

*Study 1: 62 weeks survival*

Of the 159 rats studied, 35 were excluded from survival analysis: MI-size was <20% in 26 rats, infarct size could not be determined in 3 rats; 6 rats developed tumours and were sacrificed prematurely. Remaining group sizes at the onset of treatment were n=44 for quinapril, n=45 for quinapril + HCTZ, and n=35 for quinapril + low sodium diet. Infarct sizes were evenly distributed over the different treatment groups (table 1). Comparison of subgroups based on infarct size irrespective of treatment showed that total mortality was significantly higher in rats with extensive infarcts (>35% of LV) than in rats with moderate (20-35% of LV) infarcts (fig. 1, p=0.02). This confirms that mortality in this study is related to myocardial infarction, in a MI-size-dependent way.

The time course of mortality suggests different phases, with a low mortality during the first 35-40 weeks of treatment, followed by a steep increase in mortality in rats with large MI in the late phase of follow-up.

Survival was similar in the three treatment groups after the complete follow-up period of 62 weeks (figure 2A). However, the data suggest two different periods with distinct effects of additional treatment (figures 2B and C. During the first 35 weeks survival was significantly improved by additional hydrochlorothiazide (table 1, Figure 2B). Mortality in the groups treated with quinapril only and quinapril + HCTZ was parallel between week 35 and 62 (figure 2C). Survival in the group receiving a low sodium diet in addition to quinapril was intermediate and never significantly different from the other groups.
Table 1. General characteristics of rats included in the survival study, and general characteristics and hemodynamics of MI rats after chronic ACE-I therapy, and additional effects of HCTZ or dietary sodium restriction after 35 and 62 weeks.

<table>
<thead>
<tr>
<th></th>
<th>MI control</th>
<th>QUI</th>
<th>QUI+HCTZ</th>
<th>QUI+LS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Survival study:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (start treatment)</td>
<td></td>
<td>44</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>Infarct size (% of LV)</td>
<td></td>
<td>33.7±1.1</td>
<td>32.0±1.1</td>
<td>34.8±1.4</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 35 weeks (%)</td>
<td></td>
<td>11</td>
<td>0 *</td>
<td>3</td>
</tr>
<tr>
<td>at 62 weeks (%)</td>
<td></td>
<td>23</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td><strong>Sacrificed rats:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>wk35</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk62</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Infarct size (% of LV)</td>
<td></td>
<td>wk35</td>
<td>26.9±2.5</td>
<td>24.7±1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk62</td>
<td>-</td>
<td>34.0±2.2</td>
</tr>
<tr>
<td><strong>Hemodynamic parameters:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td>wk35</td>
<td>98.3±7.2</td>
<td>72.1±3.0 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk62</td>
<td>-</td>
<td>71.3±4.5</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td></td>
<td>wk35</td>
<td>14.0±2.3</td>
<td>10.4±1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk62</td>
<td>-</td>
<td>12.4±2.6</td>
</tr>
<tr>
<td>+dP/dt (10³ mmHg. s⁻¹)</td>
<td></td>
<td>wk35</td>
<td>10.3±0.8</td>
<td>8.4±0.2 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk62</td>
<td>-</td>
<td>8.5±0.3</td>
</tr>
<tr>
<td>-dP/dt (10³ mmHg. s⁻¹)</td>
<td></td>
<td>wk35</td>
<td>-8.6±0.6</td>
<td>-6.9±0.6 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk62</td>
<td>-</td>
<td>-7.1±0.3</td>
</tr>
<tr>
<td><strong>Electrolytes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Na⁺ (mmol. L⁻¹)</td>
<td></td>
<td>wk35</td>
<td>142.8±4.4</td>
<td>137.4±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wk35</td>
<td>3.9±0.2</td>
<td>4.4±0.1 *</td>
</tr>
</tbody>
</table>

* indicates p<0.05 versus MI-control. # LVEDP at 35 weeks: QUI+HCTZ p=0.07 versus control. wk; week of treatment.
Body weight gain was significantly reduced during the first 3 weeks in rats treated with quinapril + HCTZ. Thereafter, the difference remained approximately 20 g compared to quinapril only throughout the study (fig. 3A). The reduced weight gain in rats on quinapril + HCTZ was paralleled by a combination of markedly increased drinking and slightly decreased food intake (figs. 3B and C). Water intake remained significantly increased in the HCTZ group during the entire treatment period (fig. 3C). Body weights in rats additionally receiving low sodium diets as compared to quinapril only were almost identical during the first 45 weeks of treatment, but became significantly lower thereafter.

After 62 weeks of treatment, no differences in mean arterial blood pressure (MAP), cardiac function (LV systolic and diastolic pressures, +dP/dt and -dP/dt), and LV hypertrophy (cardiac weights) were seen (tables 1 and 2).

At comparable urine production during all time points (see legend figure 4), urinary protein excretion was significantly lower in rats treated with quinapril + HCTZ than in rats receiving quinapril only at 17, but not at 35 and 52 weeks. Contrary to HCTZ, dietary sodium restriction did not influence urinary protein excretion. Interestingly, comparison of survival curves of MI-rats based on urinary protein excretion at week 17 irrespective of treatment, suggests a trend of improved survival in the group with protein excretion below average (Figure 4B).

Study 2: 35 weeks treatment
Mortality within 24 hours after MI-induction in this group was 38%. Of the remaining 53 MI-rats that entered the study, 33 were excluded from analysis for infarct sizes below 20%, leaving the following groups: n=4 untreated MI controls, n=6 for QUI, n=5
for QUI+HCTZ and n=5 for QUI+LS. MI-sizes were similar in all groups in study 2, but on average slightly smaller than in study 1 (table 1).

![Graph showing survival rates over time](image)

**Figure 2.** Survival after experimental MI in rats treated with quinapril, and effects of additional hydrochlorothiazide or low sodium diet. A) Survival after 35 weeks was significantly improved by HCTZ added to quinapril, as denoted by * . B) and C) exhaustion analysis of mortality between week 0-35 and 35-62, with number of rats alive after 35 weeks reset to 100% in C).

### Table 2. Organ weights of MI rats after long term quinapril therapy, and the additional effects of hydrochlorothiazide therapy or dietary sodium restriction after 35 and 62 weeks.

<table>
<thead>
<tr>
<th></th>
<th>MI- control</th>
<th>QUI</th>
<th>QUI+HCTZ</th>
<th>QUI+LS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk35</td>
<td>520±16</td>
<td>457±8 *</td>
<td>440±16 *</td>
<td>460±19 *</td>
</tr>
<tr>
<td>wk62</td>
<td>-</td>
<td>487±19</td>
<td>452±10</td>
<td>466±11</td>
</tr>
<tr>
<td><strong>Heart weight (mg g⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk35</td>
<td>3.51±0.09</td>
<td>2.98±0.07 *</td>
<td>3.04±0.12 *</td>
<td>2.96±0.10 *</td>
</tr>
<tr>
<td>wk62</td>
<td>-</td>
<td>3.27±0.10</td>
<td>3.26±0.08</td>
<td>3.13±0.07</td>
</tr>
<tr>
<td><strong>Left ventricle weight (mg g⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk35</td>
<td>2.38±0.04</td>
<td>1.97±0.05 *</td>
<td>2.00±0.10 *</td>
<td>1.90±0.09 *</td>
</tr>
<tr>
<td>wk62</td>
<td>-</td>
<td>2.03±0.04</td>
<td>2.07±0.05</td>
<td>2.06±0.04</td>
</tr>
</tbody>
</table>

*All organ weights were corrected for body weight. * indicates p<0.05 versus MI-control.

After 35 weeks, mean arterial blood pressure was markedly decreased by quinapril treatment, but not further affected by hydrochlorothiazide or dietary sodium restriction. LVEDP in rats treated with HCTZ in addition to quinapril was decreased by
50% compared with untreated MI controls and 30% compared with quinapril alone (table 1). Both $+dP/dt$ and $-dP/dt$ were similar in the 3 groups treated with quinapril. At 35 weeks, heart and left ventricular weights were significantly reduced by quinapril, and additional hydrochlorothiazide or low sodium diet (table 2) did not modify this reduction.

![Graphs of body weight, water intake, and food intake](image)

Figure 3. Body weights, water and food intake of MI rats treated with quinapril (QUI), and effects of additional HCTZ or dietary sodium restriction. Error bars in C) are omitted for reasons of clarity. nd: no data. * $p<0.05$ vs. QUI.

**Neurohormones in studies 1 and 2**

Neurohormones were studied after 35 and 62 weeks of treatment (figures 5 and 6). At 35 weeks, plasma N-ANP (indicative for LV stress), ACE activity, and aldosterone were significantly decreased in quinapril treated rats compared to untreated MI-rats. From 35 to 62 weeks, N-ANP, ACE activity, and aldosterone showed an increasing trend in all groups, suggesting progression of LV dysfunction despite ACE inhibition therapy.
Additional treatment with hydrochlorothiazide or dietary sodium restriction did not modify these effects of quinapril, except for plasma aldosterone being significantly higher in rats co-treated with low sodium than with quinapril alone at 62 weeks. LV ACE activity was comparable in all groups both at 35 and 62 weeks of treatment. Quinapril caused a profound increase in plasma renin activity at 35 weeks, which tended to be decreased again after 62 weeks treatment. Interestingly, this increase was markedly reduced after co-treatment with hydrochlorothiazide, but not with dietary sodium restriction. After 62 weeks, plasma renin activity no longer differed among the treatment groups.

Slightly increased plasma K⁺ levels in quinapril-treated rats at 35 weeks were not affected by co-treatment. In contrast, plasma Na⁺ concentrations were not significantly affected at 35 weeks in rats treated with quinapril alone, as compared to those untreated, but significantly decreased in those rats additionally treated with...
Beneficial Effects of Add-on Hydrochlorothiazide

hydrochlorothiazide or low sodium – which is in accordance with our study aim to induce a negative sodium balance.

Discussion

The aim of this study was to investigate whether a negative sodium balance enhances cardioprotective effects of high-dose ACE inhibitor therapy after MI. We studied long-term effects of additional hydrochlorothiazide or dietary sodium restriction on survival, cardiac function, and neurohormones in rats with MI-induced LV dysfunction receiving quinapril.

Hydrochlorothiazide

Overall mortality during ACE-I therapy was low compared to similar survival studies with experimental heart failure. Since only high-dose ACE-I treatment exerts a profound effect on long-term survival after MI, the low mortality rate in the present study indicates ACE-I treatment in accordance with our aim; to study sodium depletion on top of optimal ACE-I therapy.

Survival was not improved by HCTZ at the complete follow up period of 62 weeks. However, when looking at the survival curves in figures 1 and 2 in more detail, different phases could be identified. Survival curves based on infarct size (figure 1) show two distinct phases: comparable mortality in rats with moderate and extensive MI in the early phase (± week 1-40), whereas curves diverged rapidly in the late phase (± week 40-62). Add-on HCTZ therapy significantly improved survival until 35 weeks, but had no beneficial effect on survival between week 35 and 62. Interestingly, several other animal studies on RAAS inhibition with long follow-up periods show a biphasic pattern as well. Captopril only improved early survival in rats with large infarcts, but predominantly late survival in rats with moderate infarct sizes. In a clinical setting, the beneficial effects of ACE-I therapy with enalapril were most pronounced during the early treatment phase, and may decrease over time. Taken together, these findings suggest different stages of post-MI dysfunction, both time- and infarct-size related, with distinct effects of ACE-I treatment. It may hence be not surprising that in our study effects of additional HCTZ treatment follow these phases.

The exact mechanism underlying improvement of ACE-I therapy with hydrochlorothiazide is not clear. As dietary sodium restriction had no beneficial effects, despite similar reduction in plasma Na+ levels, favourable effects of HCTZ cannot be explained by sodium depletion only. A hemodynamic effect may play a role, since hydrochlorothiazide has direct vasodilator effects on vascular smooth muscle. LVEDP after 35 weeks tended to be lower in rats treated with HCTZ on top of quinapril, which could reflect decreased venous return. Interestingly, parallel to the observed survival benefit at 35 but not 62 weeks, plasma renin activity after 35 but not 62 weeks of treatment was markedly reduced by HCTZ added to quinapril. From the point of view that diuretic therapy generally causes volume depletion and consequently increased plasma renin activity, this observation is surprising. An association between increased plasma renin activity and mortality in post-MI patients was shown in several studies.
The most important regulators of renin activity post-MI are decreased cardiac output and sympathetic activation, which both are associated with LV dysfunction and remodelling. Renin itself is not known to have direct cellular effects during post-infarct cardiac remodelling. However, HCTZ alone decreases sympathetic activation in rats with MI. Thus, the decrease in plasma renin is likely to be an indicator for improved therapy outcome rather than a mediator of HCTZ effects.

Figure 5. Circulating and tissue RAAS after 35 weeks treatment with QUI, and effects of additional hydrochlorothiazide or low sodium diet. * p<0.05 vs. MI untreated, † p<0.05 vs. QUI.
In addition to improved survival during the first phase of the study, total urinary protein excretion was significantly decreased in rats treated with HCTZ. This further supports the early beneficial effects of additional HCTZ treatment. MI causes a progressive loss of renal function which affects prognosis. Furthermore in humans urinary albumin excretion, even at non-proteinuric levels, is predictive for cardiovascular mortality. As the degree of proteinuria reduction by ACE inhibition
was found to be predictive for clinical long-term cardioprotection, we postulate that any reduction in urinary protein excretion may be considered to be indicative for improved ACE-I therapy.

**Dietary sodium restriction**

Dietary sodium restriction did not positively affect quinapril treatment in this study, despite a reduction in plasma Na⁺. We previously reported that addition of dietary sodium restriction to ACE-I therapy resulted in significantly reduced cardiac hypertrophy and ACE activity. However, this previous study was performed using zofenopril at a relatively lower (possibly sub-optimal) dose, which could explain differences between the two studies.

One concern is that dietary sodium restriction caused a twofold increase in plasma aldosterone levels in survivors at 62 weeks of treatment. This may be considered deleterious, since high aldosterone levels were associated with clinical events in heart failure patients.

**Clinical safety / feasibility**

Although often applied in clinical practice, the use of diuretics in patients with LV dysfunction is controversial. Major concerns are severe hypotension and hypokalemia causing arrhythmia-associated deaths in patients with LV dysfunction. Hence, we chose to treat rats with hydrochlorothiazide, which has less pronounced effects than loop diuretics, and indeed adding hydrochlorothiazide did not affect blood pressure or plasma K⁺ levels. Thus, our results indicate that HCTZ may be safely added to long-term quinapril treatment.

In summary, we observed that additional hydrochlorothiazide treatment further improved survival during quinapril therapy in the first 35 weeks of chronic treatment after experimental MI in rats. This improvement in survival was associated with decreased urinary protein excretion, and improved parameters of left ventricular filling pressure and decreased RAAS activation. In contrast, dietary sodium restriction added to quinapril did not show any beneficial effects. As the rise in circulating aldosterone levels by dietary sodium restriction may be detrimental, the benefit of long-term dietary sodium restriction on post-MI outcomes could be questioned. Since no adverse effects of the combined therapy were observed, diuretics added to ACE inhibition can be a safe strategy to improve prognosis of post-MI LV dysfunction.

**References**


