Cardiorenal interaction
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CHAPTER 3

ACE inhibition attenuates the interaction of renal and cardiac damage in 5/6 nephrectomized rats with myocardial infarction

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Submitted
ABSTRACT

INTRODUCTION Recent studies have implicated a mutual interaction between renal and cardiac function loss, in which failure of either organ accelerates function loss of the other organ. ACE inhibition is accepted as an optimal therapeutic strategy in renal and cardiac disease. However, in cardiac patients with high serum creatinine levels, ACE inhibitors (ACEi) tend to be underused. Therefore, we investigated the effectiveness of the ACEi lisinopril on cardiorenal interaction. To this end, we employed the rat model of severe renal failure (5/6 nephrectomy) plus myocardial infarction (MI).

METHODS AND RESULTS Male Wistar rats underwent MI, sham MI, 5/6 nephrectomy (5/6NX), or 5/6NX + MI. Six weeks later, the nephrectomized rats were randomized on proteinuria and treated with lisinopril (2.5mg/kg/day) or vehicle for 6 weeks thereafter. In vehicle treated rats, cardiorenal interaction in the 5/6NX + MI group was evidenced by a significant increase in heart weight (3.9 ± 0.2 versus 3.3 ± 0.1 and 2.9 ± 0.1 mg/gBW) and a decrease in creatinine clearance (2.1 ± 0.3 versus 2.9 ± 0.3 and 5.2 ± 0.3 ml/min/kg) compared to 5/6NX and MI groups, respectively. Moreover, in 5/6NX + MI renal blood flow was decreased compared to 5/6NX (2.2 ± 0.6 versus 3.6 ± 0.4 ml/min/kg). ACEi therapy restored heart weight, creatinine clearance and renal blood flow to similar levels in 5/6NX + MI and 5/6NX.

CONCLUSION We conclude that cardiorenal interaction is clear from accelerated deterioration of both kidney and heart function, even in a model which features gross renal impairment. ACEi therapy effectively attenuates specific features of cardiorenal interaction in the rat. Therefore, treatment with ACEi therapy should be considered after MI in patients with chronic, severe kidney disease.
INTRODUCTION
An intriguing interaction is present between the heart and the kidney when either organ function is compromised. Clinical studies indicate that high serum creatinine levels, as an intrinsic measure for renal damage, and decreased filtration ability are independent risk factors for the development of cardiovascular disease. Conversely, a myocardial infarction (MI) may lead to decreased renal function and therefore an increase in serum creatinine levels. Both mechanisms are thought to induce a vicious circle of cardiorenal interaction, which may account for increased morbidity and mortality in patients with cardiovascular and renal disease. Various mechanisms have been hypothesized to cause this vicious circle, such as elevated blood pressure, hypervolemia, anemia, and activation of the renin angiotensin aldosteron system (RAAS) and endothelin system. RAAS intervention by angiotensin converting enzyme inhibitors (ACEi) might be able to interrupt this vicious circle.

The cardiorenal interaction has hitherto been studied mainly from the perspective of isolated renal function loss in both experimental and clinical studies, showing impaired cardiac outcome in both humans and rats. We recently reported an interaction model in the rat, in which progressive proteinuria and focal sclerosis was induced by myocardial infarction in rats with mild renal function loss due to uninephrectomy. In this model, ACEi therapy proved effective in protection against accelerated renal function loss. However, probably because of the mild renal function impairment, the uninephrectomy plus MI model did not feature an obvious cardiac deterioration in access of cardiac impairment inflicted by a sole MI.

The aim of the present study was to investigate the effectiveness of ACEi on specific features of the cardiorenal interaction as heart weight, proteinuria, and creatinine clearance in an animal model with obvious renal and cardiac function impairment. To this end, we studied the effect of the ACEi, lisinopril, on various renal and cardiac parameters in a rat model of 5/6 nephrectomy (5/6NX) plus MI.

MATERIALS AND METHODS
Experimental protocol
Male Wistar rats (275-350 g; n=95) were housed under standard conditions with free access to food and drinking water. Rats received a standard chow diet. Animal experiments were approved by the institutional animal ethical committee.

Rats were divided into four groups: 1. 5/6NX + MI (n=28), 2. 5/6NX (n=29), 3. MI (n=14), and 4. sham MI (n=9). At T= -2 weeks 5/6NX, and at T =0 weeks MI or sham MI were performed. After 6 weeks, rats with 5/6NX (groups 1 and 2) were randomized based on proteinuria into a vehicle group (VEH n=13 or 14) and a group treated with lisinopril (Merck Sharp & Dohme, Haarlem, The Netherlands) 2.5mg/kg/day in the drinking water (ACEi n=15). In all groups, the experiment was ended at T=12 weeks.

At the end of the experiment, functional cardiac parameters were measured under 2.5% isoflurane anesthesia, laparotomy was performed, renal blood flow was measured, and afterwards the rats were exsanguinated by taking blood samples from the abdominal aorta for plasma measurements. The remaining kidney was flushed with saline and the heart and kidney were removed and weighed.

Surgical interventions
5/6 Nephrectomy was performed by taking out one kidney and by ligation of two of the three branches of the contralateral kidney under anesthesia with 2.0% isoflurane in N₂O/O₂ (2:1) as described
before\textsuperscript{13-15}. Before the induction of MI, rats were intubated, ventilated (AIV, Hoek/Loos, The Netherlands) and anesthetized using 2.0% isoflurane in \textsuperscript{1}O\textsubscript{2}. MI was induced by ligation of the left anterior descending coronary artery (LAD) as described previously\textsuperscript{16-18}.

**Functional parameters**

*Urine, plasma, and tissue measurements*

For the measurement of urinary total protein excretion, 24-hour urine samples were collected in metabolic cages at baseline, before start of therapy and at the end of the experiment. Urinary total protein was analyzed using endpoint measurement with TCA (Nephelometer Analyzer II, Dade Behring, Marburg, Germany). As a measure for renal function, creatinine clearance was investigated. For the calculation of creatinine clearance, urinary creatinine and plasma creatinine levels were measured at baseline, before start of therapy and at the end of the experiment. Creatinine was determined using photometric determination with the Jaffe method (Ecoline Mega, DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

*Hemodynamic, cardiac, and renal characteristics*

Systolic blood pressure (SBP) was measured using tail-cuff plethysmography (IITC Life Science, Woodland Hills, CA, USA) in trained awake, restrained animals. SBP was measured at baseline, before start of therapy and at the end of the experiment. Cardiac performance was measured with a pressure transducer catheter under anesthesia, (2.5% isoflurane in \textsuperscript{1}O\textsubscript{2}) inserted through the right carotid artery (Micro-Tip 3French, Millar Instruments Inc., Houston, TX, USA), connected to a personal computer equipped with an analog-to-digital converter and appropriate software (Millar Instruments, Germany). After a 3-minutes period of stabilization, left ventricular end diastolic pressure (LVEDP) and left ventricular peak systolic pressure (LVPSP) were recorded. Thereafter, the catheter was withdrawn into the aortic root to measure systolic blood pressure (SBP). As a parameter of global myocardial contractility and relaxation, we determined the maximal rates of increase and decrease in left ventricular pressure (systolic +dP/dt\textsubscript{max} and diastolic -dP/dt\textsubscript{max}) which were normalized to left ventricular pressure change (i.e., LVPSP-LVEDP) for individual rats.

Renal blood flow was measured at the end of the study to investigate the effects of ACEi therapy in the 5/6NX and 5/6NX + MI group on renal blood flow using a 1mm flow probe around the left renal artery (1RB; Transonic, Ithaca, NY, USA), connected to a flow meter (T106 Small Animal Research Flow meter Transonic, Ithaca, NY, USA).

**Histology**

*Kidney*

Kidneys were fixed by immersion for 48 hours in a 4% buffered formaldehyde solution (Klinipath, Duiven, The Netherlands) after longitudinal bisection and subsequently embedded in paraffin according to standard procedures. Sections of 3 µm were stained with periodic acid Schiff (PAS). The degree of focal glomerulosclerosis was assessed in 50 glomeruli by scoring semi-quantitatively on a scale of 0 to 4. Focal glomerulosclerosis was scored positive when mesangial matrix expansion and adhesion to Bowman’s capsule was present in the same quadrant. When 25% of the glomerulus was affected, a score of 1+ was adjudged, 50% was scored as 2+, 75% as 3+ and 100% as 4+. Overall focal...
glomerulosclerosis score is expressed as arbitrary units (AU) with a maximum of 200. An examiner blinded for the groups evaluated all sections.

Interstitial alpha-smooth muscle actin (α-SMA) was determined as a profibrotic marker and detected in paraffin-embedded sections by means of a mouse monoclonal α-SMA antibody (Sigma Chemical, St. Louis, MO, USA). First, the antibody was incubated for 60 min and its binding detected by sequential incubations with peroxidase (PO)–labeled rabbit anti-mouse and PO-labeled goat anti-rabbit antibody (both from Dakopatts, DAKO, Glostrup, Denmark) for 30 min. The expression of interstitial α-SMA was measured by computerized morphometry. Therefore, 40 fields were scored at x20 magnification in the cortical region; glomeruli and vessels were excluded from measurement along Bowman's capsule and the vessel wall. Total staining was expressed as percentage of total area surface.

### Heart

The heart was arrested in diastole in a cold 1M KCl solution and weighed. The ventricles were dissected from the atria and the right free wall was separated from the left ventricle. A left ventricular mid-sagital slice (of approximately 2 mm) was fixed in 4% buffered formaldehyde solution, embedded in paraffin, cut into 5 µm slices and stained with 0.1% Sirius Red F3B and 0.1% Fast Green FCF (Klinipath, Duiven, The Netherlands). Endo- and epicardial circumference of the left ventricle and of scar tissue was determined by means of a computerized planimeter (Image-Pro plus, Media Cybernetics Inc. Silver Spring, MD, USA). Myocardial infarction size was expressed as the sum of scar lengths divided by the total left ventricular circumference and all sections were evaluated by an examiner blinded for the groups.

### Calculations and statistical analysis

All data are presented as mean ± SEM. If normally distributed, differences between the groups were compared using a one-way ANOVA, followed by a Fisher’s protected LSD post hoc test to identify the groups that were different from each other, otherwise a non-parametric test was used. The effect of ACEi was tested with an independent sample t-test compared to the vehicle treated group, or if the data were not normally distributed with a non-parametric Mann-Whitney test. To identify differences in one animal between two time-points, a paired sample t-test was used, when the data not normally distributed, a Wilcoxon signed ranks test was used. In all tests, P< 0.05 was considered statistically significant.

### Results

#### Survival and overall condition

All animals survived the nephrectomy. In the 5/6NX group, out of 39 animals that additionally received an MI, 28 survived and 11 animals died within one day after MI. In the group receiving only an MI, 4 out of 18 animals undergoing surgery died within one day. All sham operated animals recovered well after surgery. At the end of the 12-weeks follow up, the animals in the 5/6NX + MI group had a significant lower body weight compared to the other groups (table 1). Treatment with ACEi did not influence bodyweight in 5/6NX and 5/6NX + MI (table 2).
**Cardiorenal interaction: effects on the kidney**

Renal effects were assessed by creatinine clearance, proteinuria, kidney weight and focal glomerulosclerosis. Compared to sham operated animals, MI did not induce a change in these renal parameters (*table 1 and figure 1B and 2*). 5/6NX alone resulted in renal damage, as demonstrated by a significantly decreased creatinine clearance from 5.5 ± 1.3 ml/min/kg at baseline to 2.9 ± 0.3 ml/min/kg (*table 1*), an increased proteinuria from 19 ± 3 mg/24h at baseline to 265 ± 24 mg/24h, and increased kidney weight, focal glomerulosclerosis and interstitial α-SMA (*figures 1B and 2*).

In the 5/6NX + MI group, additional changes in renal parameters were noted compared to 5/6NX. While kidney weight, proteinuria, focal glomerulosclerosis, and interstitial α-SMA were comparable between these groups, a further decline in creatinine clearance (*P= 0.05*) and renal blood flow (2.2 ± 0.6 versus 3.6 ± 0.4 ml/min/kg) was observed in the 5/6NX + MI group compared to sole 5/6NX. Thus, cardiorenal interaction resulted in an additional change in renal hemodynamic function as demonstrated by reduced clearance and reduced renal blood flow.

**Cardiorenal interaction: effects on the heart**

Compared to sham, the MI group displayed cardiac hypertrophy and a decrease in cardiac function. Left ventricular end diastolic pressure (LVEDP) was higher compared to sham operated animals (*P= 0.06*), and cardiac contractility (+dP/dtmax, *P= 0.1*) and cardiac dilation (-dP/dtmax, *P< 0.01*) were lower. Heart weight was significantly higher after MI compared to sham (*figure 1A*).

Following 5/6NX, both systolic and diastolic blood pressure was significantly higher compared to sham operated animals (*table 1*). Further, 5/6NX impaired cardiac contractility and cardiac dilation and increased heart weight (*figure 1A*) to a level comparable to the cardiac hypertrophy after MI.

**Table 1. Renal and hemodynamic characteristics at the end of follow up**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>5/6NX</th>
<th>5/6NX + MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>500 ± 12</td>
<td>493 ± 8</td>
<td>485 ± 9</td>
<td>456 ± 8(^d)</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>40 ± 10</td>
<td>31 ± 6</td>
<td>265 ± 24(^ab)</td>
<td>303 ± 46(^ab)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/kg)</td>
<td>4.8 ± 0.3</td>
<td>5.2 ± 0.3</td>
<td>2.9 ± 0.3(^ab)</td>
<td>2.1 ± 0.3(^abc)</td>
</tr>
<tr>
<td>MI size (%)</td>
<td>-</td>
<td>34 ± 2</td>
<td>-</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123 ± 3</td>
<td>110 ± 4</td>
<td>146 ± 6(^ab)</td>
<td>143 ± 6(^ab)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82 ± 3</td>
<td>83 ± 4</td>
<td>104 ± 5(^ab)</td>
<td>105 ± 4(^ab)</td>
</tr>
<tr>
<td>LVPSP (mmHg)</td>
<td>127 ± 3</td>
<td>115 ± 3</td>
<td>151 ± 6(^ab)</td>
<td>142 ± 6(^b)</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>10.4 ± 1.1</td>
<td>13.8 ± 1.1</td>
<td>11.5 ± 0.9</td>
<td>14.6 ± 1.5</td>
</tr>
<tr>
<td>+ dP/dt_max ((s^{-1}))</td>
<td>112 ± 2</td>
<td>106 ± 2</td>
<td>94 ± 3(^ab)</td>
<td>102 ± 3(^a)</td>
</tr>
<tr>
<td>- dP/dt_max ((s^{-1}))</td>
<td>-97 ± 1</td>
<td>-86 ± 2(^a)</td>
<td>-85 ± 2(^a)</td>
<td>-85 ± 3(^a)</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. MI, myocardial infarction; 5/6NX, 5/6th nephrectomy; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVPSP, left ventricular peak systolic pressure; LVEDP, left ventricular end diastolic pressure. \(^a\): *P< 0.05 versus sham, ^b^: *P< 0.05 versus MI, ^c^: *P= 0.05 versus 5/6NX, ^d^: *P< 0.05 versus all groups.*
Table 2. Animal and renal characteristics before and after treatment with ACEi

<table>
<thead>
<tr>
<th>Time point</th>
<th>5/6NX</th>
<th>5/6NX + MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>VEH</td>
<td>ACEi</td>
</tr>
<tr>
<td>Bodyweight (∆g)</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Randomization</td>
<td>48 ± 3</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Autopsy</td>
<td>125 ± 14</td>
<td>131 ± 17</td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>265 ± 24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303 ± 46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Autopsy</td>
<td>3.5 ± 0.4</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/kg)</td>
<td>115 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>136 ± 28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. MI, myocardial infarction; 5/6NX, 5/6th nephrectomy; VEH, vehicle treated rats; ACEi, angiotensin converting enzyme inhibitor treated rats. Body weight given as difference between randomization and autopsy.  
<sup>a</sup>: P< 0.05 versus randomization,  
<sup>b</sup>: P< 0.05 versus VEH,  
<sup>c</sup>: P< 0.05 versus 5/6NX.

**Figure 1.** Effect of cardiac and renal damage on heart weight and kidney weight. MI, myocardial infarction; 5/6NX, 5/6th nephrectomy; VEH, vehicle treated rats; ACEi, angiotensin converting enzyme inhibitor treated rats. * : P< 0.05 versus sham and in panel B versus sham and MI, ** : P< 0.05 versus all other groups, # : P< 0.05 versus VEH.

**Figure 2.** Renal histology. Panel A. focal glomerulosclerosis, panel B. α-smooth muscle actin staining. MI, myocardial infarction; 5/6NX, 5/6th nephrectomy; VEH, vehicle treated rats; ACEi, angiotensin converting enzyme inhibitor treated rats. * : P< 0.05 versus sham and MI, # : P< 0.05 versus VEH.
In the 5/6NX + MI group, additional changes in cardiac parameters were observed compared to the 5/6NX group and the MI group. While cardiac function parameters did not differ, 5/6NX + MI featured a significant increase in cardiac hypertrophy (figure 1A) compared to sole 5/6NX or sole MI. Thus, cardiorenal interaction resulted in an excess cardiac hypertrophy.

Effect of RAAS intervention on the vicious circle after cardiac and renal function loss
To investigate whether intervention in the RAAS by ACE inhibition is effective in interrupting the vicious circle of cardiac and renal impairment, the above mentioned parameters were studied in 5/6NX and 5/6NX + MI groups treated for 6 weeks with ACEi or vehicle. Treatment with ACEi effectively normalized the blood pressure in 5/6NX from 146 ± 6 mmHg to 115 ± 4 mmHg (P< 0.001), and in the 5/6NX + MI from 143 ± 6 to 122 ± 7 mmHg (P= 0.02). Furthermore, while functional cardiac parameters were unaffected by the therapy (table 3), ACEi treatment significantly reduced cardiac hypertrophy in 5/6NX and 5/6NX + MI groups (figure 1A).

ACEi treatment prevented renal damage to a similar extend in 5/6NX and 5/6NX + MI, as demonstrated by a similar reduction in proteinuria (table 2) and a significantly lower interstitial α-SMA (figure 2B), while a trend towards lower focal glomerulosclerosis was observed (figure 2A; 5/6NX: P= 0.07, 5/6NX + MI: P= 0.1). Moreover, while ACEi treatment did not affect creatinine clearance and renal blood flow in the 5/6NX group, it normalized the decrease in both parameters observed in the 5/6NX + MI (figure 3).

### Table 3. Hemodynamic characteristics after treatment with ACEi

<table>
<thead>
<tr>
<th></th>
<th>5/6NX</th>
<th>5/6NX + MI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VEH</td>
<td>ACEi</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>MI size (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>146 ± 6</td>
<td>115 ± 4⁺</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>104 ± 5</td>
<td>81 ± 4⁺</td>
</tr>
<tr>
<td>LVPSP (mmHg)</td>
<td>151 ± 6</td>
<td>125 ± 4⁺</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>11.5 ± 0.9</td>
<td>12.5 ± 0.8</td>
</tr>
<tr>
<td>+ dP/dt_max (s⁻¹)</td>
<td>94 ± 3</td>
<td>106 ± 3⁺</td>
</tr>
<tr>
<td>- dP/dt_max (s⁻¹)</td>
<td>-85 ± 2</td>
<td>-91 ± 2</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM. MI, myocardial infarction; 5/6NX, 5/6th nephrectomy; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVPSP, left ventricular peak systolic pressure; LVEDP, left ventricular end diastolic pressure. VEH, vehicle treated rats; ACEi, angiotensin converting enzyme inhibitor treated rats. ⁺: P< 0.05 versus VEH.
DISCUSSION

This is the first experimental study in which the effectiveness of ACEi intervention was studied with regard to the effects of severe renal and cardiac function loss in rats. The main findings of this study are that the cardiorenal interaction model comprising of 5/6NX + MI features specific additional damage to both kidney and heart compared to 5/6NX or MI alone. Moreover, treatment with an ACEi effectively attenuates these specific features of cardiorenal interaction in the rat.

Recent studies have disclosed a complex relationship between cardiovascular and renal disease. Impaired renal function is detrimental to the heart both in clinical\textsuperscript{1,11,19–24} and experimental\textsuperscript{7,9} settings. Conversely, impaired cardiac function is detrimental to the kidney, although the latter is less well established\textsuperscript{3,25}. In our experiment and in the time span we used, a myocardial infarction did not cause a decrease in renal function, nor did it cause renal damage in previously healthy rats. This is in line with earlier experiments\textsuperscript{26}. Subtle renal alterations might be present in response to hemodynamic adaptation after the MI. However, with the means applied in this study, these tenuous alterations were not detected.

In contrast to the absence of renal effects of an MI, severe nephron number loss caused by 5/6NX induced a reduction in cardiac contractility and an increase in heart weight to a similar extent as observed by MI. This observed decrease in cardiac contractility could be explained by left ventricular hypertrophy\textsuperscript{27–29}, which is associated with an increase in cardiomyocyte diameter and volume\textsuperscript{8} and a decrease in capillary length density\textsuperscript{30,31} in experimental renal failure.

Cardiac function loss caused by myocardial infarction led to a further decrease in renal blood flow and creatinine clearance in rats with already impaired renal function after 5/6NX. Apparently, reduced cardiac output after myocardial infarction may lead to reduced renal perfusion, which in turn would lead to compensatory RAAS activation. This is in line with the decrease in renal blood flow observed in the nephrectomized rats with an additive MI, which is though to be associated with increased angiotensin II levels\textsuperscript{32}, causing efferent vasoconstriction in the glomerulus. This RAAS activation in turn can be detrimental to both heart and kidney. An elevated angiotensin II level is known to interact with cardiac function leading to progressive cardiac function loss\textsuperscript{33}. In addition, elevated angiotensin II may lead to progressive renal damage\textsuperscript{34} as well, resulting in a vicious circle.
Beside the effects of ACEi on renal blood flow and creatinine clearance, cardiorenal interaction is evidenced in this study by an increase in cardiac hypertrophy in rats with impaired renal function, despite similar levels of blood pressure in groups with and without MI. These data are consistent with previous studies showing that renal failure induces left ventricular hypertrophy, which is partly independent of elevated blood pressure\textsuperscript{27-29}. Besides hemodynamic alterations, sympathetic overactivity and activation of the RAAS are held responsible for the cardiac hypertrophy. Following MI, similar mechanisms are held responsible for cardiac hypertrophy\textsuperscript{35}. Thus, the increase in cardiac hypertrophy in 5/6NX + MI may be explained by a synergy between the renal dependent hypertrophy due to increased blood pressure and the hypertrophy caused by the reduced cardiac function due to the MI. Indeed, a similar interaction was shown in the spontaneously hypertensive rat\textsuperscript{36}. In summary, our data demonstrate that specific features of cardiorenal interaction are present both in the heart and kidney of the 5/6NX + MI model at 12 weeks after MI.

Thusfar, experimental data on the effects of RAAS-intervention with ACEi on cardiorenal interaction are lacking. In the current study, ACEi therapy was able to attenuate renal and cardiac effects in 5/6NX and 5/6NX + MI as shown by effects on proteinuria, focal glomerulosclerosis, interstitial α-SMA, blood pressure, LVPSP and cardiac hypertrophy. More importantly, ACEi also restored the specific features of cardiorenal interaction in this model by normalizing the creatinine clearance, renal blood flow and cardiac hypertrophy in 5/6NX + MI to the levels of sole 5/6NX. These results are in favor of the important role of adjuvant RAAS stimulation in cardiorenal interaction, and add to the supposition that cardiorenal interaction in this model is driven by increased renal angiotensin II production after myocardial infarction, in turn decreasing renal function and renal blood flow and augmenting cardiac hypertrophy.

**Conclusion**

A cardiorenal interaction is evident from accelerated deterioration of kidney and heart function, even in a model which features gross renal impairment. Furthermore, ACEi therapy effectively attenuates the specific renal as well as cardiac damage features of cardiorenal interaction in the rat. These data support the well-known concepts that intervention of the RAAS should be applied both in renal patients to prevent renal function loss, and in cardiac patients to prevent further cardiac function loss. In addition, these data indicate that cardiac patients with renal dysfunction appear to be at great risk for both renal and cardiac progression, and appear to need treatment with RAAS intervention to prevent both renal and cardiac progression. The natural anxiety of cardiologist to treat a cardiac patient with high serum creatinine levels with RAAS-intervention should thus be re-evaluated\textsuperscript{37}, since it is in fact those subjects that are at the highest risk and need these therapies the most.

**Acknowledgements**

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