Reversing the reno-cardiac perspective
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Myocardial Infarction Enhances Progressive Renal Damage in an Experimental Model for Cardio-Renal Interaction


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Abstract

Studied were the effects of myocardial infarction (MI) on mild renal function loss in unilateral nephrectomized (UnX) rats. UnX was performed, followed after 1 week by a variable MI (UnX + MI; \(n = 24\)). Rats with only UnX (\(n = 15\)) or MI (\(n = 9\)) and double sham animals (CON, \(n = 15\)) served as controls. Renal outcome was measured by proteinuria and plasma creatinine. Renal histology was evaluated by the incidence of focal glomerulosclerosis. Cardiac function and systolic blood pressure were also measured. A division into small and large infarcts after UnX was made \textit{a priori}, resulting in two groups, one with a mild MI (<20%; \(n = 15\)) and one with a moderate MI (>20%; \(n = 9\)). Mild proteinuria up to 55.5 mg/d was observed in the UnX + mild MI group, whereas proteinuria rose significantly higher to 124.5 mg/d in the UnX + moderate MI group. The incidence of focal glomerulosclerosis was significantly increased in both UnX + MI groups compared with all other groups. The average MI size was 18%, 17%, and 25% in the MI, UnX + mild MI, and UnX + moderate MI group, respectively. Left ventricular pressure in both UnX + MI groups was correlated with proteinuria, indicative of a cardio-renal interaction. It is concluded that myocardial infarction markedly accelerates the mild state of renal damage, as evidenced by increased levels of proteinuria and focal glomerulosclerosis. Putative extrapolation of the model to the human setting would mean that when patients are struck by a myocardial infarction, their chance of progressive renal damage increases, rendering them more vulnerable for subsequent cardiovascular events and closing a vicious circle.

Key words

Cardio-renal interaction, myocardial infarction, unilateral nephrectomy, proteinuria.
**Introduction**

Recent data from clinical studies have fueled the interest in the interaction between kidney and heart, and in particular the interaction between progressive function loss of both organs. The effect of renal impairment on cardiovascular function has been demonstrated in various studies. Patients with mild to severe renal insufficiency, including those patients starting renal replacement therapy, show an increased prevalence of cardiovascular disease\(^1\)-\(^3\) and Mann *et al.*\(^4\) showed that impaired renal function increased the risk of cardiovascular events in the HOPE trial. Hillege *et al.*\(^5\) found that impaired renal function is a stronger predictor of mortality than impaired cardiac function in advanced chronic heart failure. In addition, microalbuminuria is an independent risk factor for cardiovascular disease\(^6\)-\(^9\). Although the mechanism behind the association between compromised renal function and the increased risk for cardiovascular disease remains unknown, it does not appear to involve common risk factors such as hypertension, diabetes or hyperhomocysteinemia\(^10\). While the effects of renal function impairment on cardiovascular mortality and morbidity have been extensively described, it is unknown whether the opposite is also true - that is, whether cardiac function affects the outcome of mild renal function loss. The aim of the present study was to investigate whether cardiac damage could aggravate a mild state of chronic renal function loss. For this purpose, we used the rat model of unilateral nephrectomy (UnX) as this model shows a very mild state of chronic, mild renal function loss. In this model we introduced a myocardial infarction (MI) and determined the effect on renal function using intermediate (proteinuria and plasma creatinine) and hard endpoints (glomerulosclerosis), taking into account the size of the MI. In addition, we studied several cardiac, renal and neurohumoral parameters to study the underlying mechanism.
Materials and Methods

Experimental animals. Animal experimentation was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The Animal Experimental Committee approved all protocols. Male Wistar rats (250 – 275 g; n=72) were housed under standard conditions with free access to food and drinking water. Rats received a normal salt diet (0.3 % NaCl) throughout the study.

Surgical procedures

Unilateral nephrectomy. Laparotomy was performed under anesthesia with 3% isoflurane in N\textsubscript{2}/O\textsubscript{2} (2:1). The right kidney was carefully separated from the adrenal gland and the surrounding tissue. The renal artery and vein, as well as the urethra, were ligated with a 4.0 silk suture and cut followed by removal of the right kidney. For analgesic purposes, rats received a subcutaneous injection of 10 mg/kg Temgesic® (Schering & Plough, England) postoperatively and were allowed to recover from surgery in a warmed cage for 1 to 2 hours.

Myocardial infarction. One week after nephrectomy, rats were intubated, connected to a ventilator (Amsterdam Infant Ventilator, Hoek/Loos, Schiedam, The Netherlands) and anesthetized by administration of 3% isoflurane in N\textsubscript{2}/O\textsubscript{2} (2:1). Myocardial infarction (MI) was induced by ligation of the left anterior descending coronary artery (LAD) as described previously\textsuperscript{11-13}. In short, after left sided thoracotomy, the left coronary artery was occluded 2-5 mm from its origin using a 6.0 silk suture. The wound was closed, anesthetics were shut off and 100 % oxygen was administered until the rat was able to breathe sufficiently. As data on MI mortality in UnX animals are lacking, we initially aimed to keep infarct size as small as possible by putting the ligature most distal around the LAD. When the combination of UnX and MI did not result in an increased mortality compared to only MI, we performed a second series shortly thereafter, in which the ligature was placed more proximal, resulting in larger infarctions.

Study groups

The following groups were studied: UnX + MI (n = 24), MI (n = 9), UnX (n = 15) and controls (CON; n = 15). MI, UnX and CON rats underwent appropriate sham operations. Within the UnX + MI group, a division in small and large infarcts was made \textit{a priori} (on purpose) by varying the location of the suture around the LAD coronary artery. Differences were confirmed by histology at autopsy: UnX + mild MI (<20%; n = 15) and UnX + moderate MI (>20%; n = 9).
**Functional parameters**

Systolic blood pressure (SBP) was measured using tail-cuff plethysmography (IITC Life Science, Woodland Hills, CA) in awake, restrained animals. Measurements of water and food intake as well as 24-h urine collection were obtained on a weekly basis during the follow-up period of 16 weeks by placing the animals in polycarbonate metabolic cages (Tecniplast Gazzada, Buguggiate, Italy). The animals were allowed to adapt for one day. Measurements were performed on 2 consecutive days and values for proteinuria were averaged. At the end of the follow-up, cardiac performance was measured by means of a pressure transducer catheter under isoflurane anesthesia. To this end, the right carotid artery was cannulated and the transducer inserted (Micro-Tip 3French, Millar Instruments Inc., Houston, TX, USA) was connected to a personal computer equipped with an analog-to-digital converter and appropriate software (Millar Instruments, Germany). After a 3-min period of stabilization, maximal left ventricular pressure (LVP), left ventricular end-diastolic pressure (LVEDP), left ventricular end-systolic pressure (LVESP), and heart rate were recorded. Thereafter, the catheter was withdrawn into the aortic root to measure mean arterial pressure (MAP). As a parameter of global contractility and relaxation, we determined the maximal rates of increase and decrease in LVP (systolic dP/dt and diastolic dP/dt), corrected for peak systolic LVP.

**Autopsy**

Laparotomy was performed under isoflurane anesthesia (see above) and a blood sample was taken from the abdominal aorta. After bleeding the animals, heart and kidneys were removed, flushed with saline and weighed.

**Histology – Kidney**

*Tissue handling.* Kidneys were fixed by immersion for 48 h in a 3.6% buffered formaldehyde solution (Lommerse Pharma, Oss, The Netherlands) after longitudinal bisection. Subsequently, they were processed for paraffin embedding according to standard procedures.

*Focal glomerulosclerosis.* Sections of 3 µm were stained with periodic acid Schiff (PAS) and microscopically evaluated by determining the incidence of focal glomerulosclerosis (FGS). For each animal, 100 glomeruli were examined in the inner and outer cortical region and the number of sclerotic glomeruli was counted. Criteria on which glomeruli were designated as sclerotic consisted of adhesion of the glomerulus to Bowman’s
capsule, thickening of Bowman’s capsule, the presence of increased amounts of PAS-positive material in the mesangial region, and/or folding of the glomerular basement membrane with entrapment of amorphous material. An examiner blinded for the groups evaluated all sections. Evaluating media hypertrophy and/or intimal hyperplasia of intrarenal arterioles in the PAS-stained slides qualitatively assessed intra renal vascular damage.

**Immunohistochemistry - interstitial alpha-smooth muscle actin.** Interstitial alpha-smooth muscle actin (α-SMA) was determined as a pro-fibrotic marker and detected in paraffin sections using a mouse monoclonal α-SMA antibody (clone 1A4; Sigma Chemical, St. Louis, MO). First, the antibody was incubated for 60 min and its binding detected using sequential incubations with peroxidase (PO)-labelled rabbit anti-mouse and PO-labelled goat anti-rabbit antibody (both from Dakopatts, DAKO, Glostrup, Denmark) for 30 min. The expression of interstitial α-SMA was measured using computerized morphometry. Therefore, 50 fields were scored; glomeruli and vessels were excluded from measurement by tracing them with a cursor along Bowman’s capsule and the vessel wall over the surface of a graphic tablet connected to the computer. Renal sections were evaluated moving from cortex to medulla and *vice versa* at 20X magnification. Total staining was divided by the total area surface and expressed as percentage. The average score was calculated per cortical section and an examiner blinded for the groups evaluated all sections.

**Immunohistochemistry - glomerular macrophages number.** The number of glomerular macrophages was determined as an indication of the degree of inflammation. Therefore, a mouse monoclonal anti-rat monocyte and macrophage IgG1 (ED1, Serotec, Oxford, England) was used and primary antibodies were dissolved in PBS (pH 7.4) supplemented with 1% bovine serum albumin (BSA). Following incubation with the antibody, the sections were incubated in appropriate dilutions of peroxidase labelled second step antibodies. All second step antibodies were dissolved in 1% BSA/PBS containing 5% normal rat serum. Peroxidase activity was visualized using AEC. Thirty glomeruli were counted to determine the average number of glomerular ED-1 positive cells using light microscopy.

**Histology – Heart**

**Infarct size.** The heart was arrested in diastole in a cold 2M KCl solution and weighed. The ventricles were dissected from the atria and large vessels, the right free wall (RV) was separated from the left ventricle, and left- and right ventricular weights were obtained. A left ventricular mid sagittal slice (of approximately 2 mm) was fixed in Bouin’s solution, imbedded in paraffin and cut into 5 µm slices, which were stained
with 0.1% Fast Green FCF. Endo- and epicardial circumference of the left ventricle and of scar tissue was determined by means of a computerized planimeter (Quantimed 520, Cambridge Instruments, Cambridge, UK). Infarct size was expressed as the sum of scar lengths divided by the total left ventricular circumference and an examiner blinded for the groups evaluated all sections.

**Analytical procedures and statistical analysis**

Plasma and urinary samples were analyzed using colorimetric assays for total protein with molybdate red, albumin with bromocresol green, and creatinine with the Jaffé method without deproteinization. Brain natriuretic peptide (BNP) as a marker of left ventricular damage was measured using a radio immuno assay kit (DRG Instruments GmbH, Marburg, Germany).

**Calculations and statistical analysis**

All data are presented as mean ± SEM. Differences in mean values between groups were compared using one-way analysis of variance (ANOVA) and a Student-Newman-Keuls test to identify the groups that were different. Repeated measures ANOVA was used to identify which proteinuria curves were different. Correlations were calculated using linear regression analysis. In all tests, P < 0.05 was considered statistically significant. Regarding the level of proteinuria, initial calculations were made in the total combined UnX + MI group, and after confirmation by cardiac histology in the UnX + mild MI (<20%) and UnX + moderate MI (>20%) groups. Differences for all other parameters were subsequently calculated according to these groups.
Results

Overall condition and survival. Bodyweights and overall food and water intake did not differ between the groups. Baseline characteristics (i.e., body weight and proteinuria) were not different between the groups. After UnX, there were 30 animals that received an additional MI. Of these 30 animals, 6 died acutely (20%). In the group of 12 two-kidney animals, we induced a MI, after which 3 animals died acutely (25%). Therefore, on average 21.4% of the animals that received an MI died acutely. These animals were excluded from the study. Baseline characteristics of dropouts ($n = 9$) did not differ from those animals included in the final analysis ($n = 63$).

Renal parameters (see Table 1)

Kidney weight. The wet weight of the remaining left kidney (LKW) in the three nephrectomized groups at autopsy was significantly higher compared to the non-nephrectomized groups. Within these groups, there were no significant differences.

Proteinuria. Proteinuria remained stable at baseline levels around 15 mg/day in the UnX, MI and CON group during follow-up. In contrast, proteinuria in the total UnX + MI group gradually increased from week 6 onwards to a level of $89.3 \pm 22.9$ mg/day at week 16, which was significantly higher compared to all other groups ($P < 0.05$). Moreover, after dividing this group into two subgroups with mild (<20%) and moderate (>20%) MI sizes, the increase in proteinuria was significantly different between these two groups. Proteinuria in the UnX + mild MI group gradually increased from week 6 onwards to a level of $55.5 \pm 9.7$ mg/day, whereas it rose significantly higher to a level of $124.5 \pm 36.5$ mg/day in the UnX + moderate MI group (Figure 1A). Both curves of the UnX + MI groups were significantly different from the other curves and the curve of the UnX + moderate MI was significantly different from that of the UnX + mild MI group ($P < 0.05$). To exclude that the observed increased proteinuria was subject to bias resulting from a dropout of animals with MI, we performed a worst-case scenario analysis. Here, we included the basal level of proteinuria of dropout animals, as measured before induction of MI, as data in all subsequent time points (Figure 1C). This analysis demonstrates that there is still a significant increase in proteinuria in the UnX + MI group compared with the other groups.

Plasma creatinine. Plasma creatinine, as an estimation of glomerular filtration rate, was significantly lower in the CON group compared to all other groups. The level of plasma creatinine was not significantly different between the UnX + mild MI and the UnX + moderate MI group.
Immunology. The number of macrophages in the CON group was significantly lower compared with all other groups. The highest number (although not significant) of macrophages was observed in both UnX + MI groups.

Smooth muscle actin. For a more subtle measure of renal damage, the amount of interstitial α-SMA was determined by morphometry. Renal interstitial damage was significantly less severe in the CON group compared to all other groups, while the most extensive renal interstitial damage was observed in both UnX + MI groups. The values in the latter group were similar compared with MI.

Focal glomerulosclerosis. At autopsy, the incidence of FGS was significantly highest in both UnX + MI groups compared to all other groups, while the incidence in the UnX + moderate MI group was significantly higher compared to the UnX + mild MI group. In the CON group, almost no FGS was found. Figure 2 shows representative photomicrographs of glomeruli of rats with the highest proteinuria in each group. No evidence of increased intraglomerular pressure, i.e. media hypertrophy and intimal hyperplasia of intrarenal arterioles was found. Although the micrograph in Figure 2A shows capillary widening or perhaps mesangiolysis, this seems to be a unique change of a few glomeruli, rather than a general feature of this animal model.

Cardiac parameters (see Table 2)

Blood pressure. At the end of the 16 week follow-up, systolic blood pressure (SBP) was significantly higher in both UnX + MI groups compared to all other groups, but not significantly different between both UnX + MI groups. The levels of mean arterial pressure (MAP) were not different in the four experimental groups, but significantly higher in the healthy control group.

Myocardial infarction size. The post-mortem MI size determined by histology was significantly higher in the UnX + moderate MI group versus the UnX + mild MI and MI groups. MI sizes in the latter two groups were not significantly different.

Heart weight. Wet total heart weight was significantly higher in the cardiac compromised groups compared to the other groups, and was significantly higher in the UnX + moderate MI group compared to the UnX + mild MI group. As expected, the values between both groups without MI (CON and UnX) were not significantly different.
**Cardiac function.** Cardiac function was significantly impaired in all renal and cardiac compromised groups. LVP and maximal rate of increase and decrease of ventricular pressure, +dP/dt and –dP/dt, were significantly higher in CON versus the other groups. Surprisingly, there were no differences in the end diastolic pressures between the groups, which are a major determinant of cardiac dysfunction. The end systolic and mean arterial pressures however showed a significantly higher value in the control versus the other groups.

**Brain natriuretic peptide.** Plasma brain natriuretic peptide (BNP) was measured after 16 weeks to evaluate the amount of left ventricular damage in the groups, and averaged 46.83 ± 1.36 pg/ml in the UnX + moderate MI group, 47.56 ± 1.55 pg/ml in the UnX + mild MI group, 45.51 ± 1.76 pg/ml in the UnX group, 51.49 ± 3.51 pg/ml in the MI group, and 43.76 ± 2.55 pg/ml in the control group. Although these levels showed a trend towards higher levels in the groups with MI compared to the groups without MI, differences were insignificant.

**Correlations**

To obtain insight into possible mechanisms of cardio-renal interaction, cardiac and renal parameters in the experimental groups were (inter) correlated. LVP in the UnX + mild MI group correlated positively with proteinuria at 16 weeks (r = 0.57, P = 0.02). This correlation was also present (but borderline significant) in the UnX + moderate MI group (r = 0.51, P = 0.06). Correlations between LVP and FGS were also significant in these groups (r = 0.52, P = 0.05 for moderate MI and r = 0.57, P = 0.02 for mild MI). Moreover, a significant correlation between heart and kidney weight was present in these groups (r = 0.63, P = 0.01 for moderate MI, and r = 0.65, P = 0.005 for mild MI). The above correlations were not present in the other groups. LVEDP, MAP and SBP did not correlate with proteinuria or FGS. Although there were differences in MI size and proteinuria on a group level, individual correlation between MI size and proteinuria was borderline significant (r = 0.36, P = 0.063).
Table 1. Renal characteristics at 16 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Proteinuria (mg/day)</th>
<th>LKW (g)</th>
<th>Creat (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UnX + mod. MI</td>
<td>9</td>
<td>124.5 ± 36.5(^a)</td>
<td>2.22 ± 0.14</td>
<td>8.91 ± 0.87</td>
</tr>
<tr>
<td>UnX + mild MI</td>
<td>15</td>
<td>14.0 ± 3.1(^b)</td>
<td>1.96 ± 0.06</td>
<td>8.01 ± 0.33</td>
</tr>
<tr>
<td>UnX</td>
<td>15</td>
<td>2.22 ± 0.14</td>
<td>1.96 ± 0.06</td>
<td>10.2 ± 0.63</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>13.2 ± 2.9</td>
<td>2.49 ± 0.25</td>
<td>2.5 ± 1.7</td>
</tr>
<tr>
<td>CON</td>
<td>15</td>
<td>16.7 ± 2.8</td>
<td>2.22 ± 0.14</td>
<td>2.5 ± 1.7</td>
</tr>
</tbody>
</table>

Mod: moderate, LKW: left kidney weight, Creat: plasma creatinine.

Significant differences \((P < 0.05)\):

\(^a\)UnX + moderate MI groups versus other groups.
\(^b\)UnX + mild MI versus UnX, MI and CON.
\(^c\)MI and CON versus other groups.
\(^d\)CON versus other groups.

Table 1. Renal characteristics at 16 weeks (continued)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MØ (#/glomerulus)</th>
<th>α-SMA (%)</th>
<th>FGS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UnX + mod. MI</td>
<td>9</td>
<td>2.91 ± 0.19</td>
<td>3.10 ± 0.60</td>
<td>17.7 ± 3.4(^f)</td>
</tr>
<tr>
<td>UnX + mild MI</td>
<td>15</td>
<td>2.77 ± 0.23</td>
<td>1.82 ± 0.24</td>
<td>3.6 ± 2.1</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>2.49 ± 0.25</td>
<td>2.62 ± 0.44</td>
<td>2.5 ± 1.7</td>
</tr>
<tr>
<td>CON</td>
<td>15</td>
<td>1.71 ± 0.14(^d)</td>
<td>1.08 ± 0.15(^d)</td>
<td>0.8 ± 0.5(^d)</td>
</tr>
</tbody>
</table>

Mod: moderate, MØ: number of macrophages per glomerulus, α-SMA: interstitial alpha smooth muscle actin, FGS: focal glomerulosclerosis (incidence).

Significant differences \((P < 0.05)\):

\(^a\)UnX + mild MI versus UnX, MI and CON.
\(^d\)CON versus other groups.
\(^f\)UnX + moderate MI versus UnX + mild MI.
Table 2. Cardiac characteristics at 16 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart weight (g)</th>
<th>SBP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>MI size (%)</th>
<th>LVPSP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UnX + mod. MI</td>
<td>1.71 ± 0.07</td>
<td>131 ± 7</td>
<td>94.6 ± 2.5</td>
<td>24.9±2.1</td>
<td>117.9 ± 2.5</td>
</tr>
<tr>
<td>UnX + mild MI</td>
<td>1.46 ± 0.03</td>
<td>130 ± 6</td>
<td>88.7 ± 3.6</td>
<td>16.7±2.2</td>
<td>116.5 ± 2.5</td>
</tr>
<tr>
<td>UnX</td>
<td>1.21 ± 0.04</td>
<td>119 ± 3</td>
<td>93.1 ± 3.5</td>
<td>108.8 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>1.51 ± 0.04</td>
<td>117 ± 4</td>
<td>92.6 ± 3.8</td>
<td>120.7 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.23 ± 0.03</td>
<td>114 ± 6</td>
<td>98.6 ± 2.2</td>
<td>126.9 ± 6.0</td>
<td></td>
</tr>
</tbody>
</table>

Mod: moderate, SBP: systolic blood pressure, MAP: mean arterial pressure, LVPSP: left ventricular peak systolic pressure. Significant differences (P < 0.05):

a Both groups with UnX + MI versus other groups.
b UnX + moderate MI versus other groups.
c UnX + mild MI and MI versus UnX and CON.
d CON versus the groups with an MI.

Table 2. Cardiac characteristics at 16 weeks (continued)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LVEDP (mmHg)</th>
<th>LVESP (mmHg)</th>
<th>+dP/dt(_{\text{max}}) (mmHg/s)</th>
<th>-dP/dt(_{\text{max}}) (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UnX + mod. MI</td>
<td>9</td>
<td>9.9 ± 1.4</td>
<td>112.4 ± 2.9</td>
<td>11,358 ± 272</td>
<td>-9,558 ± 297</td>
</tr>
<tr>
<td>UnX + mild MI</td>
<td>15</td>
<td>7.2 ± 1.0</td>
<td>114.9 ± 3.3</td>
<td>10,972 ± 304</td>
<td>-9,729 ± 376</td>
</tr>
<tr>
<td>UnX</td>
<td>15</td>
<td>9.9 ± 0.6</td>
<td>120.5 ± 2.3</td>
<td>10,404 ± 537</td>
<td>-9,046 ± 555</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>9.1 ± 1.7</td>
<td>110.6 ± 6.5</td>
<td>11,910 ± 279</td>
<td>-10,226 ± 376</td>
</tr>
<tr>
<td>CON</td>
<td>15</td>
<td>8.1 ± 0.5</td>
<td>130.7 ± 5.7</td>
<td>12,388 ± 527(^d)</td>
<td>-11,436 ± 649(^d)</td>
</tr>
</tbody>
</table>

Mod: moderate, LVEDP: left ventricular end diastolic pressure, LVESP: left ventricular end systolic pressure, +dP/dt\(_{\text{max}}\): maximum rate of isovolumic pressure development, -dP/dt\(_{\text{max}}\): maximum rate of isovolumic pressure decay. Significant differences (P < 0.05):

d CON versus the groups with an MI.
* CON versus other groups.
Figure 1. Panel A: Proteinuria in rats with or without unilateral nephrectomy (UnX) with or without a myocardial infarction (MI) during a follow-up of 16 weeks. The UnX + MI group is regarded as one group. Values are given as mean ± SEM. *$P<0.05$ UnX + MI compared to the other groups. Panel B: Proteinuria in rats with or without UnX with or without an MI and two-kidney control rats during a follow-up of 16 weeks with a subdivision in both UnX + MI groups into a group with a mild (<20%) and moderate (>20%) MI. Panel C: The same curve as in panel A including pre-surgery proteinuria levels in the rats that died within 24 h after induction of MI. Values are given as mean ± SEM. *$P<0.05$ UnX + moderate MI compared to other groups. *$P<0.05$ UnX + mild MI compared to UnX, CON and MI groups.
Figure 2. Representative photomicrographs (magnification 40x) of rats with the highest proteinuria from every experimental group. **A:** UnX + moderate MI, **B:** UnX + mild MI, **C:** UnX, **D:** MI, **E:** control.


**Discussion**

The main finding of the present study is that myocardial infarction (MI) after unilateral nephrectomy (UnX) results in accelerated and mild renal damage in the rat, as evidenced by increased levels of proteinuria and focal glomerulosclerosis (FGS). In contrast, no increase in proteinuria or FGS was observed in UnX, MI alone and CON group during this study. Correlations were found between renal and cardiac parameters in the combined UnX + MI groups that were not present in the other groups. This is the first time that an interaction model is presented in which cardiac damage aggravates the mild state of chronic renal function loss in rats. The UnX model is a relatively mild model of renal damage, as evidenced by the lack of proteinuria and histological damage. However, in the MI groups two findings support our statement that myocardial infarction accelerates damage, i.e. proteinuria and FGS. In fact, in the MI group with large infarct size, these parameters are both increased compared to animals with a small infarct size. Therefore, as the combination of FGS and proteinuria represents a very strong indicator of renal damage, we conclude that that myocardial infarction size determines the extent of renal damage.

**Heart and kidney**

The influence of cardiac damage on kidney function has never been thoroughly examined before. There is however data suggesting that cardiac impairment influences renal vascular function, which fits into the known classical cardiorenal relationship (fall in cardiac output with eventual fall in GFR). Mento et al.\textsuperscript{14} found statistically significant reductions in renal blood flow, one month after induction of MI by ligation of the left coronary artery in rats. This post-MI intrarenal vasoconstriction contributed to a reduced renal excretory function, which is responsive to RAS modulating pharmacotherapy\textsuperscript{14-16}. Less specific to the kidney, several authors have demonstrated that MI results in endothelial dysfunction in both large conduit and resistance arteries in rats\textsuperscript{17-22}. The extent of endothelial dysfunction appeared to be related to the size of the MI and was also preserved by RAS modulating therapy\textsuperscript{23-25}. Interestingly, Gschwend et al.\textsuperscript{26} found a significant inverse correlation between individual renal vascular endothelial function and renal susceptibility to damage in rats by means of 5/6 nephrectomy (5/6 Nx). Considering the observations of the present study, it appears that cardiac, endothelial and renal functions are interlinked. One may infer that impairment of one of these functions seriously affects the other two components, and that dysfunctional components might even interact in a synergistic way contributing to the pathogenesis of reno-cardio-vascular disease.
Possible mechanisms

As this study was not primarily designed to investigate potential mechanisms for the observed cardio-renal interaction, no final conclusions can be drawn on the underlying mechanism involved. Nevertheless, several mechanisms can be hypothesized. The first possible mechanism implies that the effect on the kidney is induced by the impairment of cardiac function, as MI is a well-known cause of heart failure in rats. After 16 weeks, there was no statistically significant difference in cardiac function loss between the UnX + MI groups and the UnX (and MI) group accounting for the pronounced renal function loss observed in the UnX + MI groups. Interestingly, UnX leads to (mildly) impaired cardiac function. However, any degree of cardiac impairment may have had more impact on kidney function in case of less kidney tissue being present. In addition, an initially more severe impairment of cardiac function after recent MI may have triggered neurohumoral activation, eventually leading to increased blood pressure, increased intraglomerular pressure and subsequent renal function loss. However, regarding the role of the increased systolic blood pressure in both UnX + MI, we have no evidence that this might have contributed to the increased levels of proteinuria and FGS. First, UnX + MI included animals with normal blood pressure, which also developed proteinuria. Moreover, we found similar blood pressures in small and large infarct groups, while there was more proteinuria and FGS in the latter. Finally, mean arterial pressure was not increased in the experimental groups compared with the healthy control group and there was no correlation between blood pressure and renal damage. Also, no evidence of intrarenal vascular damage representative for increased intraglomerular pressure was found. However, we can also not totally exclude the opposite, i.e. the possibility that subtle kidney changes may be due to subtle elevations in blood pressure. A more detailed analysis comprising measurements of local and systemic plasma neurohumoral substances at multiple time points could further elucidate the role of the RAS system in the observed cardio-renal interaction.

Another possible mechanism concerns systemic and/or renal inflammation as a factor aggravating renal function loss. Indeed, inflammation is an important feature of acute MI. Berton et al. have speculated that this inflammation could involve the renal vascular system, thereby increasing glomerular permeability and the leak of urinary albumin. However, we found no significant differences at 16 weeks, but there might have been a role of inflammatory mediators in the acute phase after UnX and/or MI. Several studies show an increase in several (neuro)humoral and inflammatory parameters in the first few weeks after MI. However, in the chronic phase thereafter, when heart failure (further) develops, these parameters return back to normal values. Measuring plasma levels after more than 10 weeks might therefore not detect important differences. It is true that, in contrast to FGS, macrophages influx and SMA following
MI only show trends. This is not surprising as quantification depends on morphometry (low sensitivity) and both stainings only reflect specific processes (as opposed to overall renal damage by FGS). Therefore, these observations are in line with development of proteinuria and increase in FGS. However, the CON group showed significantly less renal inflammation compared to all other groups. Thus, our data suggest that MI as well as UnX induces a mild renal inflammatory reaction, but no clear additive effect was seen after applying both interventions. This renal inflammatory reaction may be part of a generalized inflammatory reaction that accompanies cardiac necrosis and/or nephrectomy.

**Limitations**

There were substantial individual differences in renal damage after MI, in terms of intra group differences in values for proteinuria and FGS. Proteinuria is a very early marker of a decrease in renal function, which can occur before or without changes in renal function (increase in serum creatinine). More so, plasma creatinine can even decrease when the kidney enters a stage of hyperfiltration. Increased burden to the kidney almost always means maintaining filtration fraction with an increase in GFR. The question is whether changes in serum creatinine represent a functional difference in rodents. However, the significant higher serum creatinine in the four experimental groups compared with the control group might reflect mild deterioration in renal function. The fact that none of the rats in the groups with only MI or UnX showed significant increase in proteinuria or glomerulosclerosis compared to the CON group makes our results quite conclusive. While heart weight at autopsy was significantly increased in the MI groups, demonstrating the effectiveness of the procedure, MI resulted only in a limited change in cardiac function compared to non-MI groups. Specifically, LVEDP was not increased in rats with MI. A possible explanation for absence of increase in LVEDP may be the relative small size of MI, even in the group with moderate MI (25%). In cardiac failure studies, typically only animals with MI sizes over 20% are included as these animals develop heart failure in a timeframe similar to our study. Thus, a likely explanation is that these animals are not (yet) in heart failure. Furthermore, LVEDP is calculated from the pressure curve obtained in the ventricle at Millar catheterization and not directly measured, which also introduces an additional source of variation. Finally, subtle hemodynamic changes in case of mild heart failure may become less prominent when measured under anesthesia.
Clinical impact

An important issue that needs to be addressed is how this animal model can be translated to the clinical setting. Ample clinical evidence exists for the interaction between kidney and heart, in particular for the implications of impaired renal function on cardiovascular morbidity and mortality, as shown in the HOPE study. In addition, microalbuminuria (as an indicator of renal function loss) is an important independent risk factor for cardiovascular disease, and patients with chronic renal insufficiency presenting with MI appear to have a relatively poor outcome compared to patients presenting with MI without chronic renal insufficiency. However, the consequences of impaired cardiac function on the course of renal function are far less clear. The limited data available, shows that microalbuminuria is present in patients after MI and that a first anterior MI is associated with progressive loss in renal function during the first year of follow up, in terms of a marked deterioration in GFR. Although these studies may point to an effect of cardiac function on renal outcome, interpretation is hampered by the fact that the time sequence of events is hard to establish. A chronic, mild deterioration in renal function prior to the occurrence of MI may have altered chronic systemic (cardiac) vascular function. In contrast, a high degree of certainty about renal and cardiac function before and after MI was obtained in this experimental rat model. Cardiac and renal (vascular) function were normal prior to the UnX and MI, while renal function was measured after UnX and cardiac function was measured after MI in these rats. The fact that clinical studies show similar phenomena is supportive for the potential clinical impact of the presented experimental model.
Conclusion

This experimental study clearly showed that cardiac damage markedly accelerates the mild state of chronic renal damage. There is cumulating evidence that silent (subclinical) renal dysfunction is far more present in the ‘general’ population than was anticipated before 34. Putative extrapolation of the model to the human setting would therefore not only mean that more patients are at risk for cardiovascular events, but would also mean that when these individuals are struck by a myocardial infaction, their chance of more progressive renal function loss increases, rendering them again more vulnerable for cardiovascular events and closing a vicious circle. In this respect, it is important to further unravel the mechanism behind cardio-renal and reno-cardiac interaction, enabling improved protection of both kidneys and heart in future.
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Reference List


