Pre-existing renal failure worsens the outcome after I/I-R in rats

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Abstract

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Introduction

Pre-existing chronic kidney disease (CKD) is a risk factor in the development of acute kidney injury (AKI) [1–4]. Clinical studies have shown that AKI superimposed on CKD in patients requiring renal replacement therapy (RRT) has an overall mortality of ~25% during index hospitalization [5–8]. Furthermore, the period of RRT is longer in AKI patients with pre-existing CKD compared with those without CKD [6,7]. The latter is supported by various clinical studies, which suggest that AKI per se may contribute to CKD progression and incidence of end-stage renal disease (ESRD) [5,9].

Most recent estimates indicate that the population prevalence of CKD exceeds 10% in the United States and Europe [10–12]. Moreover, progression to ESRD has rapidly increased in the past two decades, owing to the rising number of older patients and the exponential growth of diabetic nephropathy [10,13–15]. If this tendency persists, the requirement for RRT will be immense and difficult to meet. The poor outcome of patients developing post-surgical AKI is also highly prevalent in cardiac surgery with reported rates of AKI after any type of cardiac surgery between 7 and 30%. This is particularly frequent in cardiac surgery patients who have prolonged time on cardiopulmonary bypass (e.g., combined coronary artery bypass graft and valve surgery) and those with pre-existing impairment of renal function. Patients with impaired renal function (defined as CKD Stage III or worse, i.e. having a GFR < 60 mL/min/1.73 m²) constitute ~30% of the overall population undergoing cardiac surgery and up to 30% develop AKI postoperatively, and the 90-day mortality, co-morbidity and increased hospital/ intensive care unit (ICU) stay is 8–30 times higher in patients developing post-surgical AKI [16,17].

Therefore, an experimental model mimicking superimposed AKI on animals with CKD is needed for investigating the pathophysiology and developing therapeutic strategies. We developed a two-stage rat model of 5/6 nephrectomy (i.e. remnant kidney model) [18–20] followed by lethal clamping of the superior mesenteric artery (SMA) for 45 min and reperfusion. A 2-week follow-up period was taken after the induction of 5/6 nephrectomy to produce mild CKD and to allow significant hypertrophy of the remnant kidney [18,20–22]. Thereafter, AKI was achieved by intestinal ischaemia and reperfusion (I-I/R), which leads to multiple organ failure (MOF) due to the massive inflammation after I/R injury within the injured tissue as well as in remote organs [23–27]. Particularly, in the kidneys, MOF-induced AKI is associated with a decreased renal blood flow (RBF), compromised tubular function and microvascular injury [28–31]. The purpose of the present study was to examine whether CKD increases the morbidity and mortality of AKI induced by I-I/R in an experimental animal model.

Materials and methods

Experimental protocols
The study was performed on 68 adult male Munich-Wistar rats initially weighing 255 ± 1.6 g (Taconic, Eiby, Denmark). The rats were housed in pairs at room temperature (21°C) with alternating 12:12-h light–dark cycles and fed standard rat chow (Altromin, Lage, Germany) with free access to tap water.

The rats were allocated into four groups. Group N-IR, 5/6 nephrectomy with I-I/R (n = 14); group S-IR, sham nephrectomy with I-I/R (n = 14); group N-C, 5/6 nephrectomy without I-I/R (n = 11) and group S-C, sham nephrectomy without I-I/R (n = 10) (see Figure 1). The experiment was approved by the Danish Ministry of Justice and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication no. 85-23, revised 1996).

Induction of CKD by surgical reduction of renal mass
Experimental CKD was induced by excision of approximately two-thirds of the left kidney and right total nephrectomy using the excision remnant kidney model [32]. The protocols used in this study are depicted in Figure 1. The rats were anaesthetised with isoflurane 2% (Abbott Scandinavia) and atmospheric air (2 L/min). During surgery, the rats were placed on a heated table to maintain rectal temperature at 37–38°C. Under aseptic conditions, the left kidney was exposed through a left flank incision. The kidney was gently dissected free from the adrenal gland, and approximately two-thirds of the kidney including the upper and lower pole was excised. The wound was closed with 5-0 Vicryl and metal clamps. One week later, using the same procedure, the entire right kidney was removed. In the sham-operated groups (S groups), the same procedures were performed, except that entire kidneys or poles of the kidneys were not removed. The sham groups (S groups) were monitored for 2 weeks in parallel with the nephrectomy groups (N groups). Immediately after each surgical procedure, buprenorphine 0.1 mg/kg (Temgesic; Reckitt and Colman) was injected subcutaneously and thereafter administered in the drinking water at a similar dose to relieve pain for the following 2 days. After surgery, the rats were allowed to recover from anaesthesia in cages with free access to water and standard rat chow.
Clearance studies

At the time of right nephrectomy and death, 1–2 mL of blood was collected in heparinized tubes for the determination of plasma electrolytes, urea and creatinine (Vitros 950; Johnson & Johnson). Urine was collected over the final 24 h of each protocol. The urinary concentrations of sodium and potassium were determined by standard flame photometry (Eppendorf FCM6341). Furthermore, the urinary concentration of creatinine, urea and albumin were determined (Vitros 950; Johnson & Johnson). Urine and plasma osmolality were measured with a vapour pressure osmometer (Osmomat 030; Genotec, Berlin, Germany).

Enzyme-linked immunosorbent assay for neutrophil gelatinase-associated lipocalin in urine

Direct ELISA was performed to detect urinary neutrophil gelatinase-associated lipocalin (NGAL). Briefly, urine samples were centrifuged to remove debris and the supernatant was analysed (Bioporto Diagnostics, Gentofte, Denmark). Urinary antigens were bound to the wells of microtiter plates by incubation of 100 µL urine samples for 1 h at room temperature. The primary antibody was biotinylated rat NGAL. This incubation was followed by treatment with 100 µL of HRP-conjugated streptavidin. Tetramethylbenzidine substrate (100 µL) was added for colour development, which was read after 30 min at 450 nm with a Benchmark Plus microplate reader. Between each step, the microwells were washed three times with 300 µL diluted wash solution. All measurements were performed in triplicate.

Intestinal ischaemia and reperfusion

Animals were anaesthetized with isoflurane 2% (Abbott Scandinavia) and atmospheric air 2 L/min. Surgery was performed under aseptic conditions. Initially, a micro-tip pressure catheter was inserted into the femoral artery (see below). Thereafter, the small intestine was exteriorized by midline laparotomy and the SMA was occluded at its origin of the abdominal aorta by use of a vascular clamp. Care was taken not to occlude the superior mesenteric vein. Ischaemia was confirmed by cessation of the mesenteric pulsations and paleness of the intestine. After 45 min of ischaemia, the vascular clamp was released. Reperfusion was verified by return of pulsation in the mesenteric arcade and the reestablishment of the pink colour of the loops of the intestine. Before closing the abdominal wall, 1 mL of 37°C saline was instilled in the peritoneal cavity and 1 mL was administered subcutaneously. During I-I/R, the rats were placed on a heated table to maintain rectal temperature at 37–38°C. The laparotomy was closed with 5-0 Vicryl, both during ischaemia and reperfusion. Death during reperfusion was defined as lack of blood pressure and respiration.

Statistical analysis

Quantitative data were tested for normality and homogeneity of variance and, when these conditions were fulfilled, parametric analyses were applied. Otherwise, nonparametric analyses were used. Results are expressed as the means ± SEM. Differences between groups were tested by ANOVA or Kruskal–Wallis tests. In cases in which differences occurred, all pairwise multiple comparisons procedures were applied (unpaired t-test or Mann–Whitney rank sum test). These calculations were performed with SigmaStat v.3.0 (Systat Software Inc., Richmond, CA). The null hypothesis was rejected when $P < 0.05$.

Results

Surgical removal of renal mass for 5/6 nephrectomy

On average, 75% (data not shown) of the kidney mass was removed in the rats with CKD. This calculation was based...
on the assumption that both kidneys had the same weight. The following equation was used: \[
\frac{\text{(total nephrectomy weight + partial nephrectomy weight)}}{2 \times \text{total nephrectomy weight}} \times 100
\]
A significant hypertrophy of the remnant kidney also occurred during the 14 days of follow-up. The weight of the left kidney, 15 days after 2/3 nephrectomy, was 0.24 ± 0.01 g/100 g body weight, whereas the weight of the right total kidney was 0.33 ± 0.01 g/100 g body weight (n.s.) (Table 1).

**Increased urine production and decreased urinary concentration in rats with CKD**

Urine production was significantly higher in group N-IR (118.5 ± 14.3 µL/min/kg) and group N-C (104.1 ± 8.4 µL/min/kg) compared with group S-IR (39.1 ± 3.6 µL/min/kg) and group S-C (32.8 ± 2.7 µL/min/kg) (P < 0.05). In contrast, there were no significant differences between the groups N-IR and N-C (n.s.) or S-IR and S-C (n.s.).

In parallel, a significantly higher daily water intake were seen in group N-IR (144.7 ± 9.6 µL/min/kg) and group N-C (143.5 ± 7.7 µL/min/kg) compared with group S-IR (64.7 ± 4.3 µL/min/kg) and group S-C (57.9 ± 4.4 µL/min/kg) (P < 0.05). However, no significant differences were seen between the groups N-IR and N-C (n.s.) or S-IR and S-C (n.s.). This is depicted in Figure 2.

**Serum and urine biomarkers reflecting the development of CKD and AKI in rats**

At the time of right total nephrectomy (Day 0), blood was collected from tail veins in all of the four groups. This was just 1 week after 2/3 left nephrectomy, demonstrating that serum urea was already increased significantly in the N groups compared with the S groups. In contrast, serum creatinine, sodium, potassium and osmolality showed more contradictory results (Table 2). Fourteen days after 5/6 nephrectomy or sham operation, blood was collected by cardiac puncture at time of death in the IR groups and at sacrifice in the S-C and N-C groups, respectively. In the following, comparison is performed within the N groups or the S groups in order to verify the development of AKI. Moreover, group N-C and group S-C were compared in order to verify the development of CKD. Serum creatinine was significantly higher in the group N-IR compared with the group N-C (126.79 ± 10.23 vs 41.46 ± 1.84, P < 0.05), which reflected the development of AKI in rats with CKD. The same was seen between the group S-IR and

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**Table 1. Weight data**

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight initial (g)</th>
<th>Weight 5/6 nephrectomy (g)</th>
<th>Weight I-I/R (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-IR (n = 14)</td>
<td>250 ± 2.4</td>
<td>256 ± 5.0</td>
<td>264 ± 9.3</td>
</tr>
<tr>
<td>S-IR (n = 14)</td>
<td>259 ± 3.0</td>
<td>266 ± 4.2</td>
<td>307 ± 7.1*</td>
</tr>
<tr>
<td>N-C (n = 11)</td>
<td>255 ± 2.9</td>
<td>267 ± 3.7</td>
<td>270 ± 6.6</td>
</tr>
<tr>
<td>S-C (n = 10)</td>
<td>257 ± 4.8</td>
<td>265 ± 3.4</td>
<td>292 ± 7.7*</td>
</tr>
</tbody>
</table>

Values are calculated as means (±SE). Rats are weighed before each of the three procedures during the study.

*P < 0.05 compared to group N-IR.

**P < 0.05 compared to group N-C.

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**Fig. 2.** Urine output and water intake. Collected urine and measured water intake in the last 24 h in metabolic cages are shown. There were no significant differences between groups S-IR and S-C or groups N-IR and N-C regarding urine output and water intake. Values are presented as the mean ± SE. *P < 0.05 vs group S-C, **P > 0.05 vs group S-IR.
the group S-C (133.00 ± 8.50 vs 37.10 ± 1.23, P < 0.05), reflecting the development of AKI in sham-operated control rats. Furthermore, serum creatinine and urea were significantly higher in the group N-C compared with the group S-C as an evidence of CKD induced by 5/6 nephrectomy in the group N-C. An increase in serum creatinine in the group N-C (41.46 ± 1.84 vs 71.64 ± 4.19, P < 0.05) was seen during the 14 days of follow-up after 5/6 nephrectomy (Tables 3 and 4).

Urine was collected in all four groups during the last 24 h of the study period before I-I/R or sacrifice. The groups N-C and S-C were compared to confirm the development of CKD. Fourteen days after 5/6 nephrectomy, creatinine clearance was significantly lower in group N-C compared with group S-C (0.72 ± 0.09 vs 1.54 ± 0.27, P < 0.05; Table 2). NGAL was measured in urine, a marker of kidney injury, and were significantly higher in the rats that belonged to the 5/6 nephrectomy groups (groups N-

### Table 2. Urinary values

<table>
<thead>
<tr>
<th></th>
<th>Group N-IR (n = 14)</th>
<th>Group S-IR (n = 14)</th>
<th>Group N-C (n = 11)</th>
<th>Group S-C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAlbumin (g/L)</td>
<td>0.30 ± 0.37</td>
<td>&lt;0.036</td>
<td>0.35 ± 0.24</td>
<td>&lt;0.036</td>
</tr>
<tr>
<td>V-UNa/BW (mmol/day/kg)</td>
<td>5.16 ± 0.55</td>
<td>5.55 ± 0.42</td>
<td>5.28 ± 0.70</td>
<td>4.96 ± 0.55</td>
</tr>
<tr>
<td>V-UK/BW (mmol/day/kg)</td>
<td>15.72 ± 1.52</td>
<td>17.31 ± 1.02</td>
<td>16.47 ± 1.90</td>
<td>14.71 ± 1.63</td>
</tr>
<tr>
<td>V-UCrea/BW (mmol/day/kg)</td>
<td>0.25 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.26 ± 0.03</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>UC (mL/min)</td>
<td>47.02 ± 4.84</td>
<td>57.03 ± 3.25</td>
<td>48.02 ± 4.69</td>
<td>14.37 ± 4.55</td>
</tr>
<tr>
<td>uNGAL (U/day/kg)</td>
<td>176 444.4 ± 96 641.04</td>
<td>22 914.43 ± 3030.13</td>
<td>51 292.84 ± 4901.91</td>
<td>22 024.30 ± 6689.18</td>
</tr>
</tbody>
</table>

Values are calculated as means ± SE. Urine is collected during the last 24 h of the study period. V-UNa/BW, urinary sodium excretion per kilo body weight; V-UK/BW, urinary potassium excretion per kilo body weight; V-UCrea/BW, urinary creatinine excretion per kilo body weight; Clcre, creatinine clearance; uNGAL, neutrophil gelatinase-associated lipocalin in units per day per kilo body weight.

*P < 0.05 compared to group N-IR.
**P < 0.05 compared to group S-IR.
***P < 0.05 compared to group N-C.
****P < 0.05 compared to group S-C.

### Table 3. Serum values at Day 0

<table>
<thead>
<tr>
<th></th>
<th>Group N-IR (n = 14)</th>
<th>Group S-IR (n = 14)</th>
<th>Group N-C (n = 11)</th>
<th>Group S-C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNa (mmol/L)</td>
<td>141.93 ± 1.25</td>
<td>128.57 ± 2.33</td>
<td>138.18 ± 1.72</td>
<td>125.90 ± 1.30</td>
</tr>
<tr>
<td>PCr (mmol/L)</td>
<td>5.69 ± 0.16</td>
<td>6.04 ± 0.01</td>
<td>5.50 ± 0.14</td>
<td>6.10 ± 0.17</td>
</tr>
<tr>
<td>PCarb (mmol/L)</td>
<td>20.71 ± 0.97</td>
<td>6.31 ± 0.20</td>
<td>20.16 ± 1.06</td>
<td>6.15 ± 0.31</td>
</tr>
<tr>
<td>PCrea (µmol/L)</td>
<td>126.79 ± 10.23</td>
<td>76.14 ± 11.05</td>
<td>34.50 ± 2.14</td>
<td>27.26 ± 4.66</td>
</tr>
<tr>
<td>Posm (mosmol/kg H2O)</td>
<td>318.86 ± 9.02</td>
<td>328.79 ± 2.18</td>
<td>270.55 ± 10.39</td>
<td>272.60 ± 4.66</td>
</tr>
</tbody>
</table>

Values are calculated as means ± SE. Plasma is collected at the time of right nephrectomy (Day 0) for all groups. PNa, plasma sodium; PCrea, plasma creatinine; PCr, plasma potassium; PCarb, plasma carbamide; Posm, plasma osmolality.

*P < 0.05 compared to group N-IR.
**P < 0.05 compared to group S-IR.
***P < 0.05 compared to group N-C.
****P < 0.05 compared to group S-C.

### Table 4. Serum values at Day 15

<table>
<thead>
<tr>
<th></th>
<th>Group N-IR (n = 14)</th>
<th>Group S-IR (n = 14)</th>
<th>Group N-C (n = 11)</th>
<th>Group S-C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNa (mmol/L)</td>
<td>134.17 ± 1.09</td>
<td>135.39 ± 1.12</td>
<td>136.91 ± 3.22</td>
<td>133.80 ± 2.53</td>
</tr>
<tr>
<td>PCr (mmol/L)</td>
<td>5.16 ± 0.32</td>
<td>5.16 ± 0.32</td>
<td>5.68 ± 4.05</td>
<td>6.08 ± 0.59</td>
</tr>
<tr>
<td>PCarb (mmol/L)</td>
<td>18.40 ± 1.58</td>
<td>18.40 ± 1.58</td>
<td>37.10 ± 1.23</td>
<td>37.10 ± 1.23</td>
</tr>
<tr>
<td>PCrea (µmol/L)</td>
<td>139.59 ± 7.65</td>
<td>133.00 ± 8.50</td>
<td>71.64 ± 4.19</td>
<td>71.64 ± 4.19</td>
</tr>
<tr>
<td>Posm (mosmol/kg H2O)</td>
<td>337.79 ± 9.94</td>
<td>332.14 ± 2.38</td>
<td>265.91 ± 9.82</td>
<td>265.91 ± 9.82</td>
</tr>
</tbody>
</table>

Values are calculated as means ± SEM. Plasma from group N-IR and S-IR are collected after I-I/R (Day 15). Groups 3 and 4 did not undergo I-I/R and, therefore, plasma is collected at the time of sacrifice (Day 15). PNa, plasma sodium; PCrea, plasma creatinine; PCr, plasma potassium; PCarb, plasma carbamide; Posm, plasma osmolality.

*P < 0.05 compared to group N-IR.
**P < 0.05 compared to group S-IR.
***P < 0.05 compared to group N-C.
****P < 0.05 compared to group S-C.
IR and N-C) compared with the sham-operated groups (groups S-IR and S-C; Table 2).

**Early death in rats with AKI superimposed on CKD compared with rats with AKI**

The average time to death during reperfusion was shorter in group N-IR compared with group S-IR (71.0 ± 7.1 vs 112.4 ± 11.0 min, P < 0.05; Figure 3). MAP and HR were measured in sedated rats before and during I-I/R. Because of the earlier death of animals in group N-IR compared with group S-IR, values for MAP and HR are only calculated until 40 min of reperfusion. At the beginning of the ischaemic period, an increase in MAP and HR was observed in both groups. In contrast, there was a slow but steady decline in MAP and HR toward baseline values until reperfusion. Ten minutes after reperfusion, MAP decreased by 16 and 32% from the baseline in group S-IR and group N-IR, respectively. Thereafter, MAP remained constant, although significantly different, in both groups during the next 30 min of reperfusion. HR decreased 10 min after reperfusion by 5 and 7% from the baseline in S-IR and N-IR, respectively. Again, there was a compensatory increase during the next 30 min of reperfusion, which were 8 and 4% higher than the baseline value in group S-IR and group N-IR, respectively (Figure 4).

**Discussion**

It is well established clinically that post-surgical AKI is associated with increased mortality, co-morbidity and length of hospital/intensive care unit (ICU) stay and that the outcome is even worse if the patient suffers from pre-surgical CKD. However, the underlying mechanisms are only partly understood and the importance of, e.g. milder forms of CKD/dysfunction on the outcome of post-surgical AKI is less well understood [16,17]. Moreover, only few experimental models have been presented regarding establishing pre-surgical CKD followed by surgically induced AKI. In the present study, we present a model with chronic (or sub-acute) kidney disease established by 5/6 nephrectomy (for 2 weeks) followed by AKI induced by intestinal ischaemia/reperfusion.

The main finding of the present study was that rats with AKI superimposed on pre-existing CKD had a poorer outcome than rats with AKI superimposed on normal kidney function. Furthermore, that only 14 days of follow-up after 5/6 nephrectomy was sufficient to induce mild chronic kidney dysfunction (confirmed by urinary concentration defects and biomarkers for kidney function in serum and urine) and that this mild CKD is sufficient to worsen the outcome of superimposed AKI experimentally.

*The ‘two-hit’ model*

In this two-stage model, CKD was induced by 5/6 nephrectomy and AKI by lethal clamping of the SMA. Doi et al. [33] demonstrated similar findings in a murine model where CKD was induced by injection of folic acid and MOF by sepsis due to sub-lethal caecal ligation and puncture. In terms of differences between the two studies, the induction of renal failure in our study allowed compensatory adaptations within the renal tissue after nephron loss. Moreover, AKI was induced by MOF through ischaemia and reperfusion injury and not by direct bacteraemia.
5/6 nephrectomy caused a severe loss of renal mass, resulting in a combination of glomerular and haemodynamic alteration [18,19,34]. As mentioned, we chose a 2-week follow-up period after 5/6 nephrectomy, which allowed significant hypertrophy of the remnant kidney and development of azotaemia, polyuria and proteinuria, whereas no systemic hypertension was observed. About 75% of the total kidney mass was removed; this might be underestimated because of the possible hypertrophy of the right kidney during the 7 days after contralateral 2/3 nephrectomy (see calculations in the Materials and methods section). Moreover, the rats with CKD revealed mild growth retardation during the development of kidney failure. All things considered, a mild chronic renal failure was achieved after 5/6 nephrectomy [21,32].

The ‘second hit’ was made by I-I/R. This model has been widely used to induce MOF [23,24]. Ischaemia itself induces only little damage, whereas reperfusion of the pre-
viously ischaemic organ can result in remote organ injury and MOF [35]. This is because of the systemic release of several pro-inflammatory cytokines that occurs during the reperfusion period [36]. The common theory is that, during ischaemia, intestinal mucosal damage occurs, which results in translocation of bacteria or bacterial products, such as endotoxin, from the intestinal lumen. The influx of these antigenic products and spreading to the body beyond the barrier of the bowel can trigger the activation of innate immune cells and release of cytokines (TNFα, IL-1, IL-6, etc.). This vicious circle initiates and perpetuates inflammation in remote tissues as well as vascular dysfunction and thereby MOF [35,37]. AKI occurs because of decreased RBF, compromised tubular function and microvascular injury [28–31].

Neutrophils gelatinase-associated lipocalin as a biomarker for renal injury

Conventional biochemical indicators in serum and urine confirmed the induction of CKD and AKI. In addition, NGAL was measured in the urine, which is a small 25-kD protein belonging to the ‘lipocalins’ superfamily. This protein has been shown to be released in the blood as well as in the urine from injured tubular cells after various acute or chronic conditions potentially detrimental to the kidney, as well as in experimental and in human models [38–40]. It has been hypothesized that urinary NGAL levels are indicative of real-time active kidney damage and not simply one of the many markers of kidney function [41]. Thus, urinary NGAL is a host defence protein that can indicate renal injury rapidly and noninvasively [42]. Consistent with this, we showed that, after 14 days after 5/6 nephrectomy, the urinary NGAL increased significantly in the N groups compared with the S groups, after adjusting for urinary output and body weight. To our knowledge, this has not been shown in rats with kidney failure of a more chronic sort.

Intestinal ischaemia and reperfusion was associated with altered haemodynamic parameters

Haemodynamic parameters were changed significantly during I-IR. During ischaemia, a steep rise in MAP and HR occurred, and then they gradually diminished, reaching pre-occlusion values just before reperfusion. Similar observations have been described before in this I-IR model [36,43,44]. It has been proposed that the abrupt rise in MAP induced by intestinal ischaemia may be mediated via a decrease in baroreceptor input to the medullary vasomotor centre in response to reduced splanchnic perfusion. The return of MAP to baseline values toward the end of ischaemia may be due to the transduction of fluid across the microcirculation [44]. Shortly after clamp removal, an abrupt and sustained decrease in MAP, indicating severe shock, was observed. This may be in part mediated by the release of the platelet-activating factor (PAF) from the post-ischaemic intestine [44,45]. This could be supported by evidence that the PAF antagonist has been shown to prevent the circulatory collapse from accompanying reperfusion [45].

Reperfusion resulted in an abrupt decrease in HR in both I-IR groups. This was followed by a gradually rise to pre-occlusion values, and at the end of the 40-min reperfusion period, it eventually increased. The initial bradycardia may be mediated by vagal stimulation and tachycardia as a response to decreased MAP or hypovolaemia due to ischaemia and reperfusion.

Summary and limitations

This study describes an increased mortality of rats with CKD that are exposed to AKI and MOF. Furthermore, we showed that the ability to compensate haemodynamically during the early period of intestinal reperfusion were impaired to a larger extent in rats with CKD compared with rats with normal kidney function prior to the development of AKI. Finally, that the development of CKD and AKI was confirmed by conventional as well as a novel biomarkers in serum and urine.

Further studies are needed to characterize the pathophysiology behind this increased mortality. This might then help us to develop strategies to prevent or improve the outcome in CKD patients exposed to AKI and MOF. However, the present study has some limitations that should be mentioned. The time following of 5/6 nephrectomy (2 weeks) only produces mild chronic (or subacute) renal failure. In terms of mimicking later stages of CKD where systemic hypertension, glomerular sclerosis and interstitial fibrosis are evident, a follow-up period of 5–10 weeks are needed to achieve moderate to severe form of CKD [18,19,21,34]. Again, in the present study, we showed that even mild CKD is associated with increased mortality during the development of AKI/MOF.

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Conflict of interest statement. None declared.

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Pre-existing renal failure worsens the outcome after I-I/R in rats


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