Original Article

N-acetylcysteine attenuates kidney injury in rats subjected to renal ischaemia-reperfusion

Nicoletta Nitescu1, Sven-Erik Ricksten1, Niels Marcussen4, Börje Haraldsson2, Ulf Nilsson2, Samar Basu5 and Gregor Guron2,3

1Department of Anaesthesiology and Intensive Care, Institute of Surgical Sciences, 2Department of Nephrology, Institute of Internal Medicine, 3Department of Physiology, Institute of Physiology and Pharmacology, The Sahlgrenska Academy at Göteborg University, Sweden, 4University Institute of Pathology, Aarhus Kommunehospital, Denmark and 5Section of Geriatrics and Clinical Nutrition, Department of Public Health and Caring Sciences, Faculty of Medicine, Uppsala University, Sweden

Abstract

Background. The aim of the present study is to examine the effects of N-acetylcysteine (NAC), a thiol-containing anti-oxidant, on renal function and morphology, and biomarkers of oxidative stress, in rats subjected to renal ischaemia-reperfusion (IR).

Methods. Sprague–Dawley rats underwent unilateral nephrectomy and either contralateral renal IR (40 min of renal arterial clamping), or sham manipulation. Treatment groups were: (1) IR-Saline, (2) IR-NAC, (3) Sham-Saline and (4) Sham-NAC. The N-acetylcysteine was administered in a dose of 200 mg/kg intraperitoneally at 24, 12 and 2 h before, and 24, 48 and 72 h after, renal IR. Plasma creatinine was measured on days 1, 3 and 7 after IR, and kidney histology was assessed on day 7. In separate groups of animals we measured renal levels of the anti-oxidant glutathione, markers of systemic oxidative stress (plasma ascorbyl radical, urinary 8-iso-prostaglandin F2α), and glomerular filtration rate (GFR) by 51Cr-EDTA clearance, on day 1 after renal IR.

Results. Treatment with NAC ameliorated the decline in GFR and reduced hyperkalaemia on day 1 (P < 0.05), lowered plasma creatinine levels on days 1 and 3 (P < 0.05), and decreased renal interstitial inflammation on day 7 (P < 0.05), after renal IR. Kidney glutathione levels decreased significantly in group IR-Saline in response to IR (P < 0.05), but were completely repleted in group IR-NAC. Groups with renal IR injury and acute renal failure showed increased plasma ascorbyl radical levels, and elevated urinary 8-iso-prostaglandin F2α excretion, compared with sham (P < 0.05). N-acetylcysteine treatment reduced plasma ascorbyl concentrations 24 h after renal IR (P < 0.05), but had no effect on the rate of urinary 8-iso-prostaglandin F2α excretion.

Conclusions. N-acetylcysteine improves kidney function, and reduces renal interstitial inflammation, in rats subjected to renal IR. These effects were associated with increased renal glutathione levels, and decreased plasma ascorbyl concentrations, suggesting that NAC attenuates renal and systemic oxidative stress in this model.

Keywords: acute renal failure; ischaemia reperfusion injury; lipid peroxidation; N-acetylcysteine; oxidative stress; reactive oxygen species

Introduction

Acute renal failure (ARF) affects 5–20% of patients in the intensive care unit (ICU) and imposes a risk for death that is independent of other complications and co-existing diseases [1]. Despite this, there is as yet no effective pharmacological intervention convincingly improving outcome in patients with ARF [1]. Recent clinical trials suggest that N-acetylcysteine (NAC) may be effective in preventing radiocontrast-induced ARF [2]. From a clinical viewpoint, NAC is an attractive drug as it has few side-effects and as there is much experience from its use in critically ill patients. NAC is an anti-oxidant that acts by increasing intracellular glutathione levels, and also by the direct scavenging of reactive oxygen species (ROS), such as hypochlorous acid (HOCl), hydrogen peroxide (H2O2), superoxide (O2•−) and the hydroxyl radical (OH−) [3].
Interestingly, Himmelfarb et al. [4] have shown that plasma protein oxidation is markedly increased in critically ill patients with ARF compared with patients with multiorgan failure without ARF, or with patients with end-stage renal disease (ESRD). As it has been suggested that oxidative stress is linked to increased morbidity and mortality in ESRD [5], oxidative stress may be an important target for therapy also in ARF. In addition, ROS are powerful mediators of renal endothelial and tubular cell injury [3], and NAC has proven to be renoprotective in experimental models of both toxic [6–8], and ischaemic [9] ARF, although results have not been conclusive [10]. Thus, anti-oxidants such as NAC may be effective both in the prevention of renal injury, and in limiting systemic oxidative stress, in ARF. The aim of the present study is to examine the effects of NAC on kidney function and morphology, renal levels of the anti-oxidant glutathione, and markers of systemic oxidative stress (i.e. plasma ascorbyl radical and urinary 8-isoprostaglandin F₂α), in a model of renal ischaemia-reperfusion (IR) injury in rats. For this purpose, treatment with NAC was initiated prior to the ischaemic insult, to mimic protocols in clinical studies demonstrating beneficial effects of NAC in the prevention of radiocontrast nephropathy.

Subjects and methods

General procedures

Male Sprague–Dawley rats (Scanbur BK, Sollentuna, Sweden) weighing ~250 g, were divided into four treatment groups: (1) IR-Saline (n = 20), (2) IR-NAC (n = 17), (3) Sham-Saline (n = 8) and (4) Sham-NAC (n = 8). N-acetylcysteine (Acetylcysteine NM Pharma, Sweden) was administered in a dose of 200 mg/kg intraperitoneally (i.p.) at 24h, 12h and 2h before induction of renal IR or sham surgery. Following surgery, NAC was administered once daily (200 mg/kg i.p.) for three consecutive days. Control rats similarly received isotonic saline in equivalent volumes.

Renal IR was carried out in animals anaesthetized with xylazine (10 mg/kg, i.p.) and ketamine (75 mg/kg, i.p.). Through flank incisions, the left renal artery was clamped for 40 min by a non-traumatic microvascular clip, and a right-sided nephrectomy was performed. During sham surgery the right kidney was excised and the left renal artery was dissected and manipulated but no clip applied. Rectal temperature was kept at 37–38°C throughout. After surgery, fluid losses were replaced by administration of 5 ml of warm (37°C) isotonic saline i.p., and rats were returned to their cages. The rats had free access to normal rat chow and tap water throughout. All experiments were approved by the regional ethics committee in Göteborg. Chemicals were from Sigma (St Louis, MO, USA) if not stated otherwise.

Plasma creatinine and kidney histology, 7-day protocol

On days 1 and 3 after renal IR, venous blood samples (~200μl) were collected from tail veins under brief isoflurane anesthesia (Isofluran, Baxter International Inc., Deerfield, IL, USA) for measurements of plasma creatinine concentrations using an enzymatic assay (Roche Diagnostics GmbH, Mannheim, Germany). Seven days after IR, animals were anaesthetized (pentobarbital sodium, 60 mg/kg, i.p.) and blood samples were taken from the aorta. Kidneys were excised, decapsulated, weighed, and immersion-fixed in 4% formaldehyde in phosphate buffer (pH 7). The kidneys were processed for semiquantitative histological assessments by light microscopy as previously described [11]. The following variables were quantified in the renal cortex and in the outer medullary zone: tubular atrophy, tubular dilatation, tubular calcification, and interstitial inflammation and fibrosis. Analyses were made by an investigator blinded for treatment group using an arbitrary scale where 0 = no changes, 1 = mild focal changes, 2 = modest diffuse changes, and 3 = severe diffuse changes, as described [11]. In addition, the degree of inflammation was analysed separately in the cortex, outer stripe of the outer medullary zone (OSOMZ), and in the inner stripe of the outer medullary zone (ISOMZ), using a scale where 0 = no inflammation, 1 = few scattered inflammatory cells, 2 = inflammation in many interstitial areas and 3 = severe diffuse inflammation. Furthermore, we semiquantitatively characterized the type of leukocytes (i.e. lymphocytes, plasma cells and granulocytes) in the interstitial infiltrate of the cortex, OSOMZ and ISOMZ, by light microscopy. For this purpose an arbitrary scale was utilized where 0 = no or only a few scattered cells, 1 = cell type present but in minority, 2 = cell type dominating and in majority, 3 = cell type heavily dominating.

Renal clearance experiments 24 h after renal IR

Twenty-four hours after renal IR, or sham surgery, separate groups of rats (IR-Saline, n = 10; IR-NAC, n = 11; Sham-Saline, n = 6; Sham-NAC, n = 6) were anaesthetized with thiobutabarbital (Inactin, Sigma-Aldrich, St Louis, MO, USA, 100 mg/kg i.p.), placed on a heating table, and catheterized for renal clearance experiments as described [11]. An arterial line was connected to a pressure transducer (Smiths Medical, Kirchseeon, Germany) for monitoring of mean arterial pressure (MAP) and heart rate (HR) using a data acquisition program (Biopac MP 150, Biopac Systems, Santa Barbara, CA, USA). The urinary bladder was catheterized for urine collection into pre-weighed vials. Rectal temperature was kept at 37°C. Throughout the experiment, rats received isotonic saline in a total volume of 10 ml/kg/h. After completion of the surgical preparation, a 40 min equilibration period was allowed before measurements during three consecutive 20 min clearance periods. Glomerular filtration rate (GFR) was measured by the renal clearance of ⁵¹Cr-EDTA (⁵¹Cr-ethylenediaminetetraacetic acid, Amersham Laboratories, Buckinghamshire, UK) as previously described [11]. Arterial blood samples (0.3 ml) were replaced by equivalent volumes of 4% bovine serum albumin (BSA) in isotonic saline. Arterial blood gases were analysed at the end of each experiment (ABL 510 blood-gas analyzer, Radiometer, Copenhagen, Denmark). Urine and plasma samples were analysed for sodium, potassium and radioactivity, as previously described [11]. Fractional urinary excretion rates of sodium (FENa, %), potassium (FEK, %) and water (FEH₂O, %), were estimated as the ratio of their respective clearances to that of ⁵¹Cr-EDTA, ×100.
Markers of oxidative stress and kidney glutathione

Separate groups of rats (IR-Saline, n = 10; IR-NAC, n = 10; Sham-Saline, n = 6; Sham-NAC, n = 6) were subjected to renal IR, or sham manipulation, following a protocol identical to the one described above. The rats were kept in metabolic cages from 48 h before, to 24 h after, surgery, to enable collection of urine over 24 h periods. Urine was collected in vials kept on ice containing 0.01% butylated hydroxytoluene and was stored at −70°C until analysed for 8-iso-prostaglandin F2α (8-iso-PGF2α) using a highly specific, and sensitive radioimmunoassay [12]. Twenty-four hours after IR, rats were anaesthetized (pentobarbital sodium, 60 mg/kg, i.p.), blood sampled from the aorta, and left kidneys were excised for measurements of renal glutathione concentrations (vide infra). Plasma was immediately snap-frozen in liquid nitrogen and stored at −70°C until analysed for plasma ascorbyl radical concentrations by electron spin-resonance (ESR) spectroscopy (Bruker ECS 106 EPR spectrometer), as described [13]. A free radical standard solution of 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-Hydroxy-TEMPO) was used to calculate the absolute concentration of the ascorbyl radical.

Whole kidney glutathione concentrations were measured on contralateral, normal, right kidneys excised immediately prior to the induction of IR, and on injured left kidneys from the same animal 24 h after IR. Total glutathione was measured with an assay based on a reaction using Ellman’s reagent, according to the manufacturer’s instructions (Cayman Chemicals, Ann Arbor, MI, USA).

Statistics

Values are means±SEM except for semiquantitative data which are presented as the median with 25th and 75th percentiles. Differences between groups were analysed by one-way, and two-way analyses of variance (ANOVA), followed by Bonferroni’s post-hoc test. Histological data were analysed by non-parametric Kruskal–Wallis and Mann–Whitney tests. Cumulative survival was examined by Kaplan–Meier analysis. A two-sided value of $P<0.05$ was considered statistically significant. The statistical software program SPSS 11.5.1 was used (SPSS Inc., Chicago, IL, USA).

Results

Mortality and body weights

The number of deaths in the respective groups were: Sham-Saline, 0/8 (0%); Sham-NAC, 0/8 (0%); IR-Saline, 8/20 (40%) and IR-NAC, 3/17 (18%). All deaths occurred during the first three days after renal IR. Cumulative survival tended to be higher with NAC treatment in rats subjected to renal IR ($P=0.13$, Figure 1). Rats in groups IR-Saline and IR-NAC showed a similar weight loss of approximately 10% during the study period ($P<0.05$ vs sham groups, data not shown).

Kidney function after renal ischaemia-reperfusion injury

Plasma creatinine concentrations were significantly increased in rats subjected to renal IR, compared with sham, on study days 1, 3 and 7 ($P<0.05$, Figure 2). Treatment with NAC significantly reduced plasma creatinine levels on days 1 and 3 after renal IR injury ($P<0.05$, Figure 2). There was no statistically
significant difference in plasma creatinine between sham-operated groups (Figure 2).

Renal IR produced significant reductions in GFR and plasma sodium concentrations (P-Na), and significant increases in $\text{FE}_{\text{Na}}$, $\text{FE}_K$, $\text{FE}_{\text{H}_2\text{O}}$ and plasma potassium concentrations (P-K), compared with sham, on day 1 after renal IR ($P < 0.05$, Table 1). The N-acetylcysteine treatment significantly attenuated the decline in GFR, and reduced hyperkalaemia, in rats subjected to IR ($P < 0.05$, Table 1). In addition, NAC treatment tended to lower $\text{FE}_{\text{Na}}$ and $\text{FE}_K$, and to diminish the fall in MAP, in rats with renal IR injury ($P = \text{ns}$, Table 1). Urinary potassium excretion during clearance experiments was $0.25 \pm 0.04$ vs $0.14 \pm 0.04 \mu\text{mol/min/100 g BW}$ in groups IR-NAC and IR-Saline, respectively ($P = 0.20$).

Kidney weights and histology 7 days after renal ischaemia-reperfusion

Macroscopically, kidneys subjected to IR were clearly enlarged, pale and appeared oedematous. Both IR-groups had significantly increased kidney weights compared with sham ($P < 0.05$, data not shown). Kidney weights were significantly reduced in group IR-NAC compared with IR-Saline (2.00 ± 0.15 g vs 2.41 ± 0.11 g, $P < 0.05$).

Both groups subjected to renal IR showed significant tubular atrophy and dilatation, accompanied by interstitial inflammation and fibrosis ($P < 0.05$ vs sham, Table 2, Figure 3). Histopathological changes were generally more pronounced in the OSOMZ, but scarce in the ISOMZ and inner medulla. Glomeruli and the vasculature appeared normal. Treatment with NAC significantly reduced interstitial inflammation in the cortex and whole outer medulla of kidneys subjected to IR ($P < 0.05$, Table 2, Figure 3). However, when analysed separately in the cortex, OSOMZ, and ISOMZ, there were no statistically significant differences between groups IR-NAC and IR-Saline in the degree of interstitial inflammation (Table 3). Characterisation of the inflammatory infiltrate revealed that lymphocytes were the predominant inflammatory cell type in all kidney zones ($P < 0.05$ vs plasma cells and granulocytes, Table 3). There were no significant histopathological changes in sham-operated groups (Tables 2 and 3, Figure 3).

Markers of oxidative stress

Both groups subjected to renal IR demonstrated an increased urinary excretion of 8-iso-PGF$_{2\alpha}$ compared with baseline values, and to sham, during the first 24 h after IR ($P < 0.05$, Figure 4). However, there were no effects of NAC on the rate of urinary 8-iso-PGF$_{2\alpha}$ excretion either before, or after IR (Figure 4).

Plasma levels of the ascorbyl radical, measured 24 h after IR, were significantly increased in rats subjected to renal IR, compared with sham ($P < 0.05$, Figure 5). N-acetylcysteine treatment caused a significant reduction of plasma ascorbyl concentrations in group IR-NAC compared with IR-Saline (219 ± 14 nmol/l vs 300 ± 19 nmol/l, $P < 0.05$, Figure 5).

Kidney glutathione

Kidney glutathione concentrations decreased significantly in group IR-Saline in response to IR ($P < 0.05$, Figure 6). Treatment with NAC completely prevented

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### Table 1. Renal clearance data 24 h after renal ischaemia-reperfusion

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>HR (bpm)</th>
<th>GFR (ml/min/100 g BW)</th>
<th>UV (µl/min/100 g BW)</th>
<th>$\text{FE}_{\text{Na}}$ (%)</th>
<th>$\text{FE}_K$ (%)</th>
<th>$\text{FE}_{\text{H}_2\text{O}}$ (%)</th>
<th>P-Na (mmol/l)</th>
<th>P-K (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-Saline ($n=6$)</td>
<td>146 ± 8</td>
<td>437 ± 10</td>
<td>0.47 ± 0.02</td>
<td>6.6 ± 2.2</td>
<td>0.8 ± 0.5</td>
<td>16 ± 2</td>
<td>1.5 ± 0.6</td>
<td>146 ± 1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Sham-NAC ($n=6$)</td>
<td>137 ± 11</td>
<td>435 ± 11</td>
<td>0.56 ± 0.06</td>
<td>4.5 ± 1.7</td>
<td>1.2 ± 0.6</td>
<td>16 ± 4</td>
<td>0.9 ± 0.3</td>
<td>149 ± 1</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>IR-Saline ($n=10$)</td>
<td>128 ± 3</td>
<td>404 ± 7</td>
<td>0.01 ± 0.00</td>
<td>4.5 ± 1.3</td>
<td>12.5 ± 11.8</td>
<td>170 ± 12</td>
<td>33.8 ± 4.1</td>
<td>142 ± 1</td>
<td>8.0 ± 0.4</td>
</tr>
<tr>
<td>IR-NAC ($n=11$)</td>
<td>145 ± 5</td>
<td>431 ± 13</td>
<td>0.05 ± 0.01</td>
<td>7.6 ± 1.3</td>
<td>18.9 ± 3.7</td>
<td>139 ± 15</td>
<td>29.5 ± 5.4</td>
<td>147 ± 2</td>
<td>6.4 ± 0.6</td>
</tr>
</tbody>
</table>

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### Table 2. Renal histopathological scores 7 days after renal ischaemia-reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Sham-Saline ($n=8$)</th>
<th>IR-Saline ($n=12$)</th>
<th>IR-NAC ($n=14$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular atrophy</td>
<td>0 (0–0)</td>
<td>1 (1–1.75)$^a$</td>
<td>1 (0.75–1)$^a$</td>
</tr>
<tr>
<td>Tubular dilatation</td>
<td>0 (0–0)</td>
<td>2 (2–2)$^a$</td>
<td>2 (0–2)$^a$</td>
</tr>
<tr>
<td>Tubular calcification</td>
<td>0 (0–0)</td>
<td>0 (0–2.75)</td>
<td>0 (0–2)</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>0 (0–0)</td>
<td>2 (1.25–2)$^a$</td>
<td>1 (1–2)$^b$</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0 (0–0)</td>
<td>1 (1–2)$^a$</td>
<td>1 (1–2.5)$^a$</td>
</tr>
</tbody>
</table>

Renal histopathological scores 7 days after renal ischaemia-reperfusion (IR) in rats treated with N-acetylcysteine (IR-NAC) or isotonic saline (IR-Saline). Histological abnormalities in the cortex and outer medulla were scored using an arbitrary scale from 0–3 (see ‘Methods’). Data are presented as median and 25th and 75th percentiles. Kruskal-Wallis and Mann–Whitney tests for non-parametric data were used. $^aP < 0.05$ vs sham. $^bP < 0.05$ vs group IR-Saline.
the drop in kidney glutathione levels (Figure 6). Kidney glutathione concentrations were significantly elevated in group IR-NAC, compared with IR-Saline, 24 h after IR (80±9 vs 38±10 nmol/g kidney weight, *P* < 0.05, Figure 6). There were no significant differences in renal glutathione content at baseline between saline-treated, and NAC-treated, rats (Figure 6).

Table 3. Inflammation and leukocytes in the kidney 7 days after renal ischaemia-reperfusion(12,621),(683,738)

<table>
<thead>
<tr>
<th></th>
<th>Sham-Saline (n=8)</th>
<th>IR-Saline (n=12)</th>
<th>IR-NAC (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial inflam.</td>
<td>0.5 (0–1)</td>
<td>1 (1–2)*</td>
<td>1 (1–2)*</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>1.5 (0–3)</td>
<td>3 (3–3)*</td>
<td>3 (3–3)*</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0 (0–0)</td>
<td>0 (0–1)</td>
<td>0 (0–0.25)</td>
</tr>
<tr>
<td>OSOMZ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial inflam.</td>
<td>0.5 (0–1)</td>
<td>2 (2–3)*</td>
<td>2 (1–2.25)*</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>0 (0–0)</td>
<td>3 (3–3)*</td>
<td>3 (3–3)*</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0 (0–0)</td>
<td>0 (0–1)</td>
<td>0 (0–0.25)</td>
</tr>
<tr>
<td>ISOMZ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial inflam.</td>
<td>0 (0–0)</td>
<td>2.5 (2–3)*</td>
<td>2 (1.75–3)*</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>0 (0–0)</td>
<td>3 (3–3)*</td>
<td>3 (3–3)*</td>
</tr>
<tr>
<td>Plasma cells</td>
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<td>0 (0–0)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>0 (0–0)</td>
<td>0 (0–1)</td>
<td>0 (0–0.25)</td>
</tr>
</tbody>
</table>

Semiquantitative assessment of inflammation and leukocytes in the cortex, outer stripe of the outer medullary zone (OSOMZ), and in the inner stripe of the outer medullary zone (ISOMZ), of kidneys subjected to ischaemia-reperfusion (IR) or sham surgery. The degree of interstitial inflammation, and the relative amount of leukocytes (i.e. lymphocytes, plasma cells and granulocytes) in the interstitial infiltrate, were graded using arbitrary scales from 0–3 (see ‘Methods’). Data are presented as median and 25th and 75th percentiles. Kruskal–Wallis and Mann–Whitney tests for non-parametric data were used.

*P* < 0.05 vs sham.

*P* < 0.05 vs other leukocyte types within the treatment group.

**Discussion**

The main findings of the present study were that treatment with NAC diminished the reduction in GFR, and reduced plasma creatinine and hyperkalaemia, in rats subjected to 40 min of renal IR. In addition, animals with ARF due to renal IR showed elevated systemic oxidative stress compared with controls with normal kidney function, as indicated by increased urinary 8-isoprostaglandin F<sub>2α</sub> excretion and plasma ascorbyl concentrations. Treatment with NAC reduced plasma ascorbyl levels, and restored renal glutathione levels, in rats with renal IR injury, suggesting that NAC reduced systemic and renal oxidative stress. Moreover, NAC-treated animals with renal IR injury showed a significant reduction in renal interstitial inflammation 7 days after the ischaemic insult.

In the present study, treatment with NAC ameliorated the decline in GFR on day 1, and reduced plasma creatinine by ~40% on days 1 and 3, after renal IR. A similar reduction in plasma creatinine was demonstrated by DiMari et al. [14] using high intravenous doses of NAC (1 g/kg) immediately before and after,
bilateral renal IR in rats. Notably, the model of bilateral renal IR is a much less severe model of ischaemic ARF, and GFR values were ~5 times higher in the study by DiMari et al. [14], compared with in the present study. Thus, our findings indicate that NAC improves kidney function also in a more severe form of ischaemic ARF. In addition, our results suggest that repeated administration of lower doses of NAC may be as effective in attenuating renal IR injury as much higher doses given in immediate conjunction to the ischaemic insult.

Hoffman et al. [15] have previously demonstrated that NAC treatment decreases plasma creatinine levels in healthy volunteers without affecting GFR. However, in the present study the reduction in plasma creatinine concentration was paralleled by a significant increase in GFR, in NAC-treated rats with renal IR injury. In addition, NAC did not alter plasma creatinine concentrations in sham-operated animals. Thus, it appears that plasma creatinine can be used as a reliable surrogate marker for renal function in rats, when effects of NAC are examined.

In support of previous studies [16], we found that renal IR depleted kidney glutathione stores. Glutathione is an important intracellular anti-oxidant which acts by directly scavenging ROS and also by being a substrate for glutathione peroxidase catalysed reactions that degrade ROS. Lack of glutathione therefore renders cells susceptible to oxidative stress. Interestingly, we found that treatment with NAC completely replenished renal glutathione levels 24 h after IR in the present study, suggesting that NAC attenuated renal IR injury, at least partly, by preventing intrarenal glutathione depletion. However, NAC might also exert anti-oxidative effects by direct scavenging of ROS [17].

In accord with earlier studies [10,14], we observed a discrepancy between the improvement of kidney function, and lack of significant renal morphological recovery, in NAC-treated animals with renal IR injury. Thus, we found no significant reduction of tubular atrophy or dilatation, or interstitial fibrosis, in NAC-treated animals 7 days after IR. This could indicate that NAC improves kidney function without affecting the renal structural injury. However, it should be pointed out that in the present study renal histological analyses were only performed at a late time-point (i.e. 7 days after IR), when plasma creatinine approached normal values. Still, renal interstitial inflammation, which mainly consisted of lymphocytes, was significantly reduced in the cortex and outer medulla of NAC-treated animals 7 days after the ischaemic insult. Notably, ROS are considered to be important components of inflammatory processes as they are produced in several cell types, including neutrophils, in response to cytokines and act as second messengers by stimulating redox-sensitive inflammatory gene expression [18]. Accordingly, NAC has been shown to exert anti-inflammatory effects, e.g. by inhibiting cytokine-stimulated NF-κB activation, and by down-regulating the expression of vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin [17]. One could therefore speculate that NAC treatment reduced recruitment of lymphocytes into the renal interstitium of ischaemic kidneys in the present study, through the above mentioned mechanisms. In support of this notion, de Araujo et al. [19] recently found that NAC reduced the interstitial infiltration of macrophages, and lymphocytes, in rat kidneys 48 h after IR.

It is plausible to hypothesize that the less severe hyperkalaemia in NAC-treated rats with renal
IR-injury could contribute to the tendency towards an increased survival rate in this group of animals. However, other mechanisms, e.g. reduced systemic oxidative stress, could also be involved. Interestingly, we found that systemic biomarkers of oxidative stress (i.e. plasma ascorbyl levels and urinary excretion of 8-iso-PGF$_{2\alpha}$) were increased in rats with renal IR injury and ARF. Although several studies have demonstrated a local increase of ROS in the kidney immediately after IR, little is known about systemic oxidative stress in animals with established renal IR injury and ARF [3,4]. The ascorbyl radical, an oxidation product of ascorbic acid that can easily be detected by ESR, has been used as a marker of free radical production in many experimental and clinical settings [20]. As it is quickly metabolized into ESR-silent species (e.g. ascorbate and dehydroascorbate), plasma levels of the ascorbyl radical appear to be independent of renal function [20]. The isoprostanes are reliable biomarkers of in vivo lipid peroxidation [20]. Additionally, 8-iso-PGF$_{2\alpha}$ is a bioactive molecule that exerts potent renal vasoconstriction [20]. Thus, increased generation of 8-iso-PGF$_{2\alpha}$ could be of pathophysiological importance in ARF by reducing renal blood flow. In the present study, NAC treatment reduced plasma ascorbyl concentrations 24 h after induction of renal IR, but had no significant effect on the rate of urinary 8-iso-PGF$_{2\alpha}$ excretion during the first 24 h after the ischaemic insult. The discrepancy between these results may be partly explained by the fact that measurements were not undertaken at the same time-point. In addition, different compartments (i.e. plasma and urine) were analysed. However, urinary 8-iso-PGF$_{2\alpha}$ excretion is believed to reflect systemic, whole body lipid peroxidation [20]. One might speculate that although NAC inhibited oxidation of ascorbic acid, NAC treatment in the doses used was ineffective in preventing lipid peroxidation. Our finding of increased systemic oxidative stress in rats with ARF due to renal IR, is in accordance with observations by Himmelfarb et al. showing increased plasma protein oxidation in patients with ARF [4]. The pathophysiological role, and clinical relevance, of increased systemic oxidative stress in ARF, clearly needs to be investigated further.

Although several drugs have been shown to be effective in attenuating renal abnormalities in different experimental models of ARF, few if any are in clinical use due to lack of efficacy, or serious side effects. In this respect, NAC has many advantages as it is already in clinical use, well tolerated, and is easy to administer. Moreover, clinical trials suggest that NAC may be effective in preventing radiocontrast nephropathy [2]. In view of the results in the present study, we speculate that prophylactic administration of NAC may protect renal integrity in patients at risk of developing ischaemic ARF, e.g. in patients undergoing elective aortic aneurysm repair, or cardiac surgery. In addition, and as suggested by Himmelfarb et al. [4], treatment with NAC might also be beneficial in reducing oxidative stress in patients with established ARF.

In conclusion, NAC improves kidney function, and reduces renal interstitial inflammation, in rats subjected to renal IR. These effects were associated with replen of renal glutathione, and decreased plasma ascorbyl levels, suggesting that NAC attenuated renal, and systemic oxidative stress.

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