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THE PROTECTIVE ROLE OF N-ACETYLCYSTEINE IN AN EXPERIMENTAL RENAL ISCHEMIA-REPERFUSION MODEL ON WISTAR RATS

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ABSTRACT. Many studies have demonstrated that oxidative stress plays an important role in ischemic acute renal failure. The aim of the present study was to investigate the effects of N Acetyl-cystein (ACC) on renal function and the oxidative stress in a rat model of renal ischemia-reperfusion (IR) injury. Histological analysis of kidney tissue was performed. Renal IR was performed by clamping the unilateral renal artery for 20 min followed by 24 h of reperfusion. ACC was administered 72, 24 and 3h prior to IR and the same dose was given 3 h, 24 and 72 h after IR. Treatment groups were: control group, IR group and IR-ACC group. Renal function was assessed by measuring plasma creatinine on days 1, 3 and 7 after IR. Malondialdehyde (MDA) was measured on days 1, 3, 7 after IR. Superoxide dismutase (SOD), catalase and gluthatione were measured as antioxidative markers. IR produced elevated levels of MDA and increased creatinine compared with control group ($p<0.05$). SOD and catalase levels were lower in IR compared with control group. ACC reduced elevated levels of MDA, increased SOD, catalase and gluthatione levels ($P<0.05$) and attenuated renal dysfunction. These results demonstrate the antioxidant effect of ACC in attenuating renal IR injury.

Keywords: ischemia reperfusion model, acetylcystein, oxidative stress, creatinine, histological analysis

INTRODUCTION

Acute renal failure is a severe clinical condition with an increased mortality especially in intensive care patients and yet there is no pharmacological intervention which can convincingly improve the outcome of these patients (Schrier RW et al, 2004, Himmelfarb et al, 2004). There are a lot of clinical trials studying different therapeutic agents with positive role in treating acute renal failure. One of this drugs is N-acetylcysteine (ACC), an attractive drug as it has few side-effects and there is much experience from its use in critically ill patients (Birck R et al., 2003, Efrati et al, 2005). On the other hand, as it has been suggested that oxidative stress is linked to increased morbidity and mortality in ESRD (Galle et al, 2001), oxidative stress may be an important target for therapy also in ARF. ACC 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 and the hydroxyl radical (OH•) These reactive oxygen species are powerful mediators of renal endothelial and tubular cell injury, and ACC has proven to be renoprotective in experimental models of both toxic and ischaemic ARF, although results have not been conclusive (Zhu J et al, 2007,Paromov V.et al 2008, Nittescu N et al,2006, Nath KA et al, 2000). Thus, anti-oxidants such as ACC 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, plasma levels of the anti-oxidant glutathione and markers of systemic oxidative stress: malondialdehyde (MDA), as marker of lipid peroxidation, superoxiddismutase (SOD), catalase and gluthatione as antioxidants 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 (Birck R. et al., 2003).

MATERIALS AND METHODS

Male Wistar rats, weighing approximate 200 g, were divided in three groups: control group - 11 rats, a group who underwent ischemia reperfusion (IR) model - 22 rats and a group treated with ACC(200 mg i.p.) with 72, 24 and 3 hours prior IR and 3, 24, 72 hours after IR - 25 rats. The animals were anaesthetized with a mixture of xylazine (10 mg/kg, i.p.) and ketamine (75 mg/kg, i.p.). The operatory technique was the following: through flank incisions, the left renal artery was clamped for 30 min by a non-traumatic microvascular clip, and a right-sided nephrectomy was performed. 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. On days 1, 3 and 7 venous blood samples were collected from all the three groups for measurements of plasma creatinine, MDA, SOD, catalase and gluthathione concentrations. At the end of the study animals were sacrificed and kidney tissue was examined in optic microscopy. Statistic analysis of

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data was performed, using Excel, Sigma Stat and Sigma Plot v. 11.

RESULTS AND DISCUSSIONS

Plasma creatinine concentrations were significantly increased in rats subjected to renal IR, compared with control group, on study days 1, 3 and 7 ($P<0.05$, figure 1). Treatment with NAC significantly reduced plasma creatinine levels on days 1, 3 and 7 after renal IR injury ($P<0.05$, figure 1).

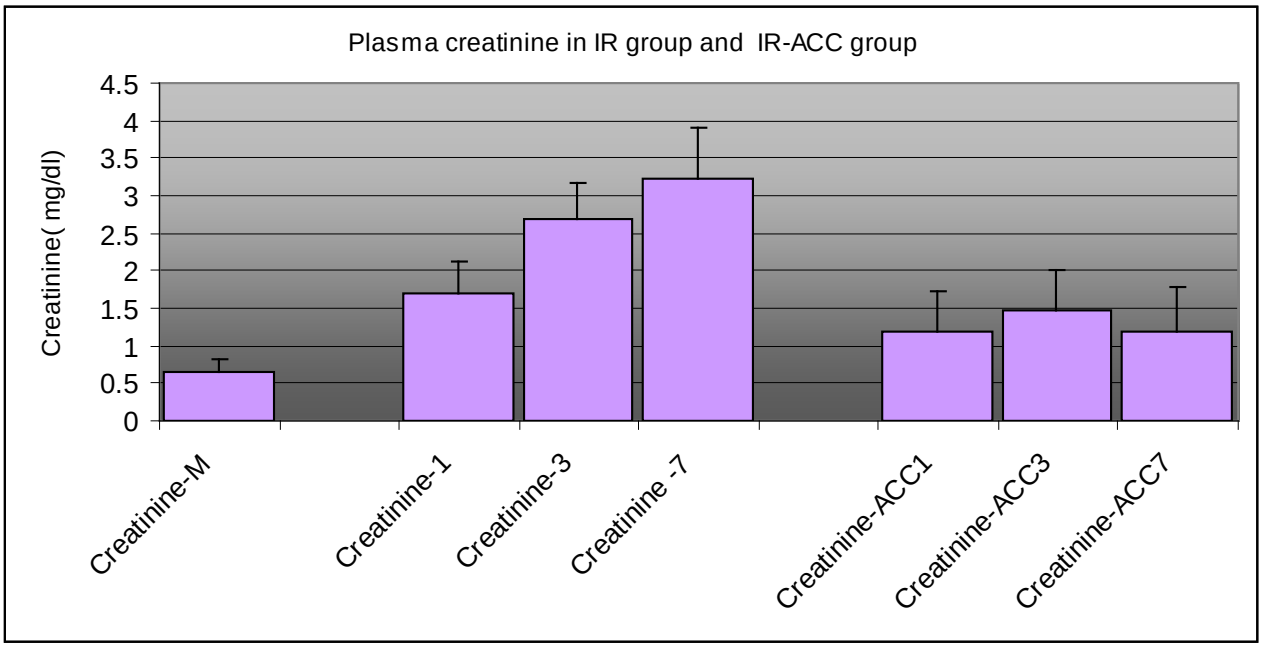

Fig. 1 Plasma creatinine in control group, IR group and ACC group.

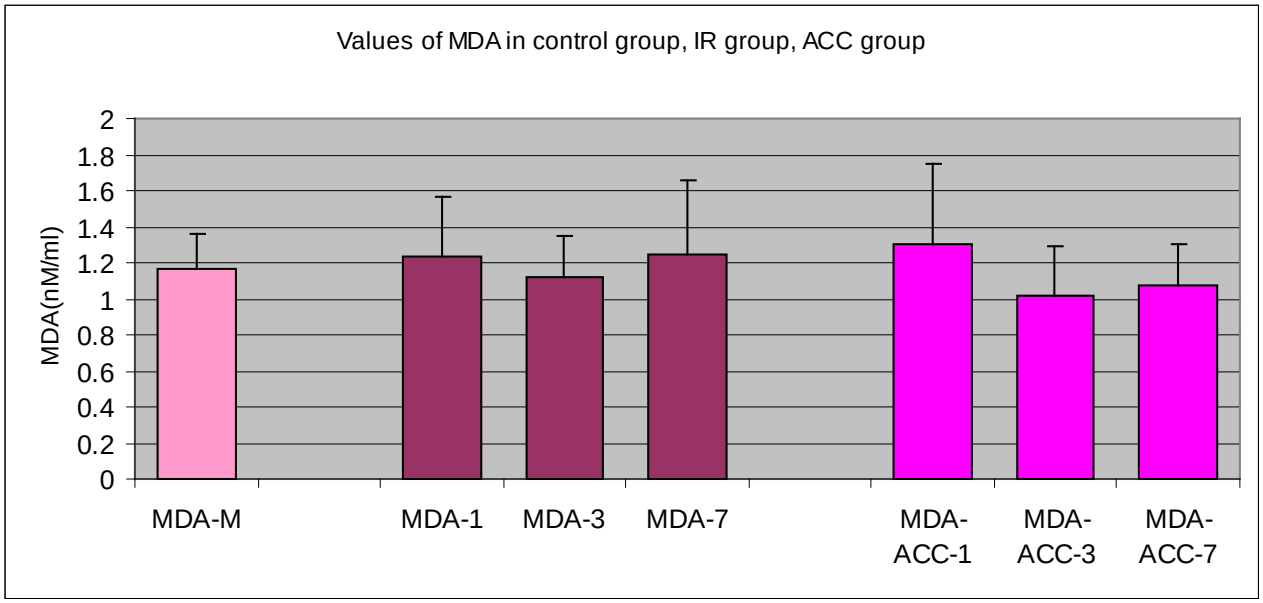

Fig. 2 Values of MDA in control group, IR group , ACC group

Malondialdehyde concentrations were increased in IR rats but without statistical signification. In the group treated with ACC MDA concentrations were significantly lower compared with IR in days 3 and 7 after the administration of ACC (fig. 2).

Glutathione values were similar in the IR group compared with control group. Treatment with NAC completely prevented the drop in glutathione ($p<0.05$) (fig. 3).

Superoxiddismutase had a significant decrease in days 3 and 7 after IR ($p<0.05$). After administration of ACC the values of SOD were significantly increased ($p<0.05$) (fig.4).

Catalase had a significant decrease in days 3 and 7 after IR ($p<0.05$). After administration of ACC the

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values of SOD were significantly increased ($p<0.05$) (fig. 5).

Histological analysis of kidney tissue showed severe tubular necrosis, medullar congestion, proteinaceous debris; intratubular hyaline casts (fig. 6, 7).

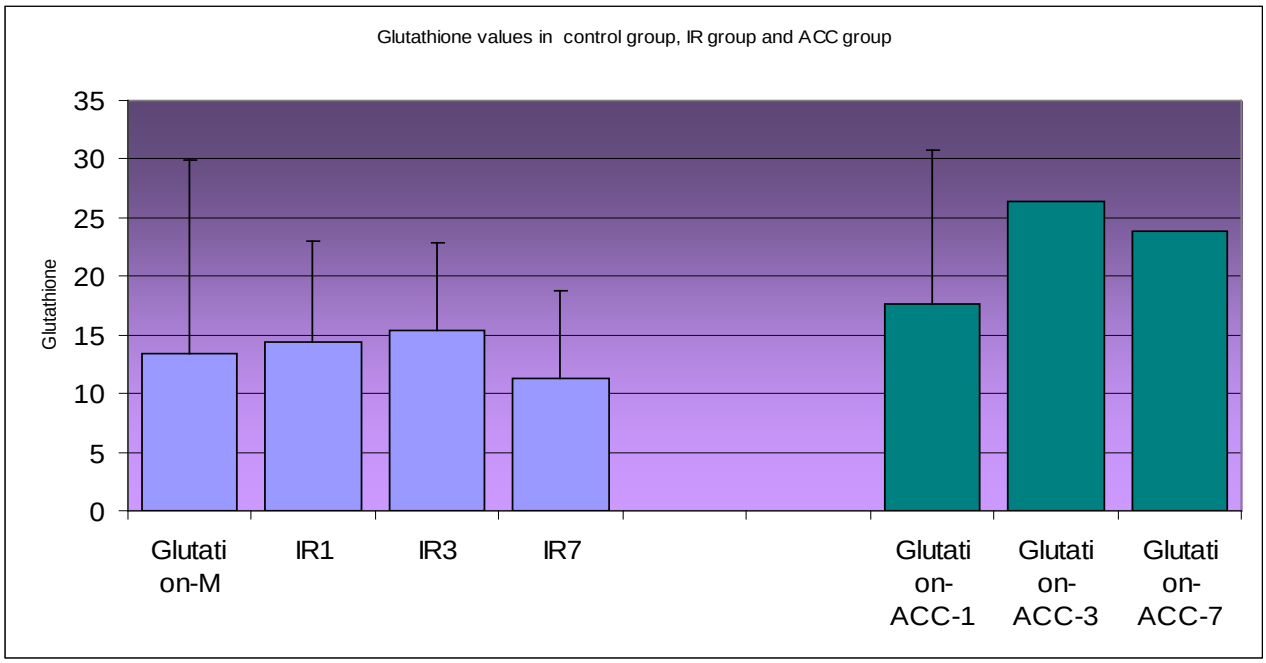

Fig. 3 Gluthatione values in control group, IR group and ACC group

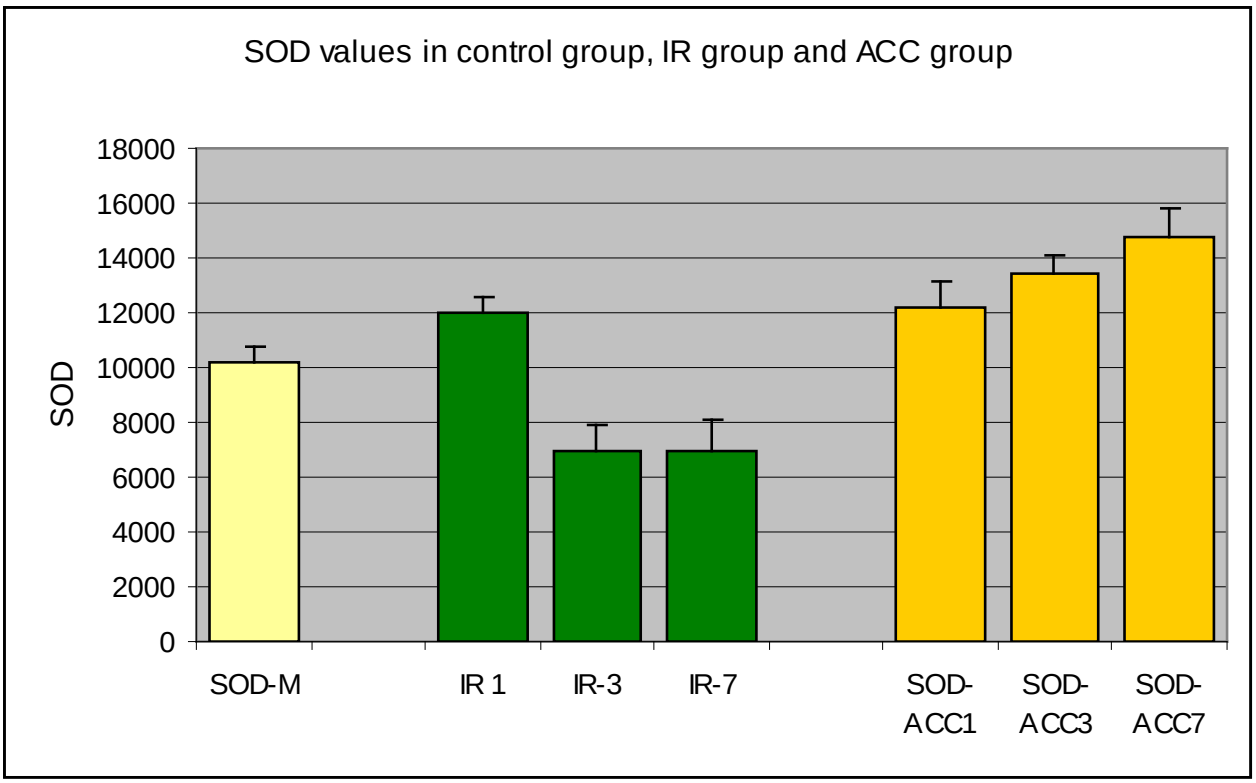

Fig. 4 SOD values in control group, IR group and ACC group

The main findings of the present study were that treatment with NAC diminished the reduction in GFR, and reduced plasma creatinine in rats subjected to 30min 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 malondialdehyde concentrations and decreased levels of

superoxiddismutase and catalase. Treatment with ACC reduced malondialdehyde concentrations, and restored glutathione levels, in rats with renal IR injury, suggesting that NAC reduced systemic and renal oxidative stress. The histological analysis of renal tissue revealed severe lesions of acute tubular necrosis, indicating that the IR model was correctly performed. As we know, the reproduction of this experimental model is a premiere in our country.

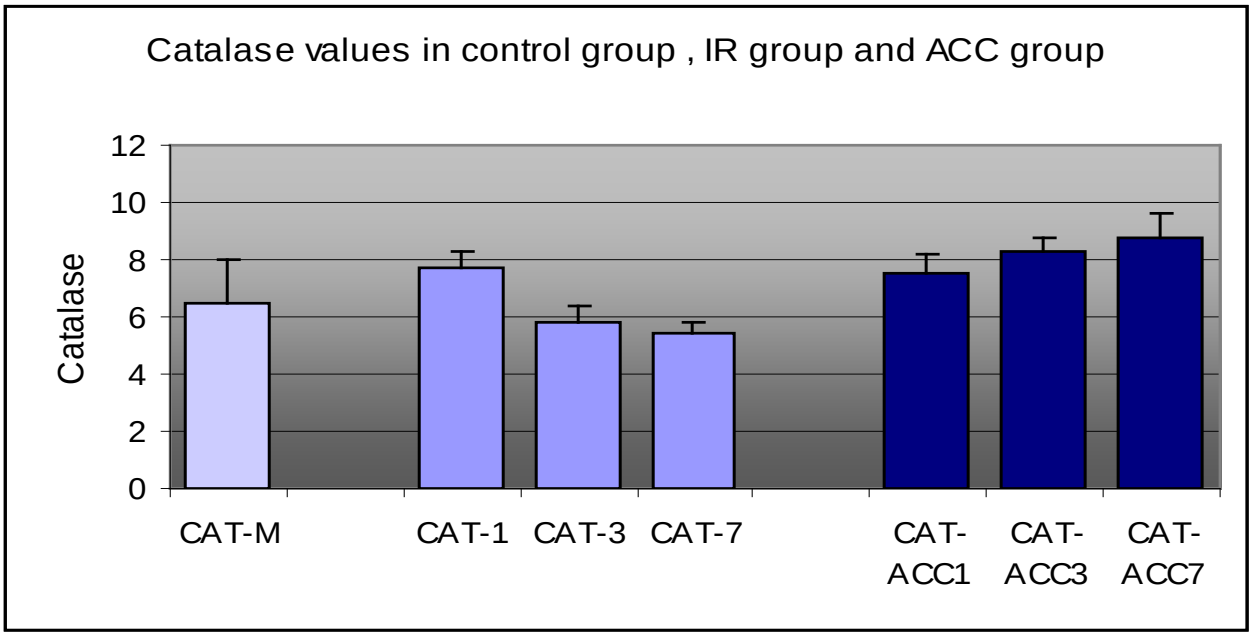

Fig. 5 Catalase values in control group, IR group and ACC group

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. using high intravenous doses of NAC (1 g/kg) immediately before and after, bilateral renal IR in rats. It has previously demonstrated that NAC treatment decreases plasma creatinine levels in healthy volunteers without affecting GFR (Hoffman et al., 2004). 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. 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.

Also a lot of drugs were studied for their potential benefic effect on ARF; a few of them were really effective. In this respect, NAC has many advantages as it is already in clinical use, well tolerated, and it is easy to administer.

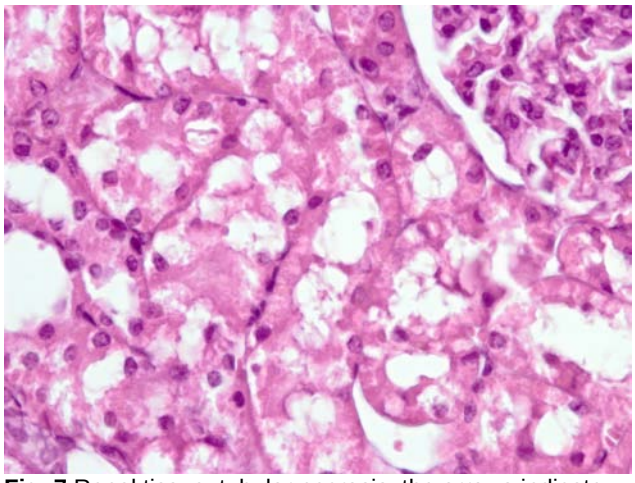

Fig. 7 Renal tissue: tubular necrosis, the arrows indicate necrotized renal cells, HE col., x400

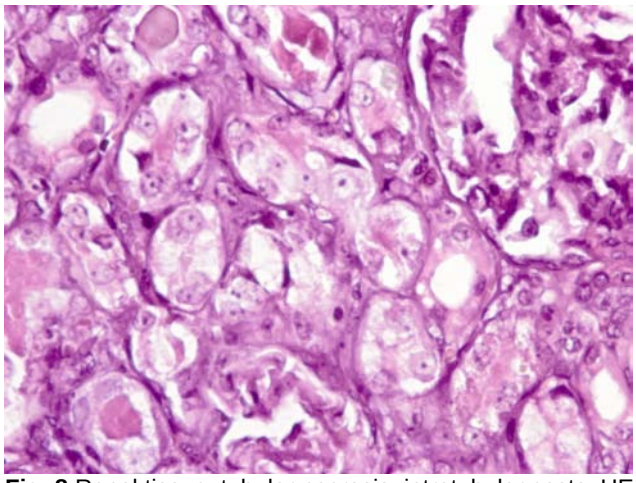

Fig. 8 Renal tissue: tubular necrosis, intratubular casts, HE col., x400

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CONCLUSIONS

In conclusion, NAC improves kidney function in rats subjected to renal IR. These effects were associated with repletion of glutathione, and increased superoxiddismutase and catalase values, suggesting that NAC attenuated renal, and systemic oxidative stress. More studies are needed in order to evaluate the positive role of ACC in diminuating oxidative stress.

REFERENCES

Birck R, Krzossok S, Markowetz F et al. Acetylcysteine for prevention of contrast nephropathy: meta-analysis. Lancet 2003; 362: pp. 598–603

DiMari J, Megyesi J, Udvarhelyi N, Price P, Davis R, Safirstein R. N-acetyl cysteine ameliorates ischemic renal failure. Am J Physiol Renal Physiol 1997; p. 272: F292–F298

Himmelfarb J, McMonagle E, Freedman S et al. Oxidative stress is increased in critically ill patients with acute renal failure. J Am Soc Nephrol 2004; 15: pp. 2449–2456

Hoffmann U, Fischereder M, Kruger B, Drobnik W, Kramer BK. The value of N-acetylcysteine in the prevention of radiocontrast agent-induced nephropathy seems questionable. J Am Soc Nephrol 2004

Jan Galle. Oxidative stress in chronic renal failure Nephrol. Dial. Transplant., Nov 2001; 16: pp. 2135 - 2137.

Nath KA, Norby SM. Reactive oxygen species and acute renal failure. Am J Med 2000; 109: pp. 665–678

Nicoletta Nitescu, Elisabeth Grimberg , Sven-Erik Ricksten , Gregor Guron .Effects Of N-Acetyl-L-Cysteine on renal haemodynamics and function in early ischaemia–reperfusion injury in rats. Clinical And Experimental Pharmacology And Physiology, Volume 33 Issue 1-2, pp. 53-57

Schrier RW, Wang W, Poole B, Mitra A. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. J Clin Invest 2004; 114: pp. 5–14

Slusser SO, Grotyohann LW, Martin LF, Scaduto RC Jr. Glutathione catabolism by the ischaemic rat kidney. Am J Physiol Renal Physiol 1990

Victor Paromov, Min Qui, Hongsong Yang, Milton Smith, and William L Stone. The influence of N-acetyl-L-cysteine on oxidative stress and nitric oxide synthesis in stimulated macrophages treated with a mustard gas analogue. BMC Cell Biol. 2008; 9: p. 33.

Zhu J, Yin R, Shao H, Dong G, Luo L, Jing H.N-acetylcysteine to ameliorate acute renal injury in a rat cardiopulmonary bypass model. J Thorac Cardiovasc Surg. 2007 Mar;133(3): pp. 696-703.