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The effect of N- acetyl cysteine on oxidative stress during renal ischemic-reperfusion

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Abstract

Background: Oxidative stress is implicated in the patho-physiology and complications of renal ischemic-reperfusion (I/R).

Objectives: The aim of the present study is to evaluate the protective effect of N-acetyl cysteine (NAC), a thiol-containing anti-oxidant, on some renal functions and biomarkers of oxidative stress in rats subjected to renal ischemic-reperfusion.

Methodology: In the present study, 32 adult male albino rats were used. The rats underwent unilateral renal I/R (45 min of clamping of renal artery followed by renal reperfusion for 90 min), or were sham-operated. They were divided into 4 equal groups. Group 1 (sham-saline group). Group 2 (sham-NAC group), Group 3 (I/R-saline group). Group 4 (I/R-NAC group). NAC was administered in a bolus dose of 250mg/kg BW; dissolved in 0.5 ml normal saline intravenously 5 min. before reperfusion. Renal tissue malondialdehyde (MDA, a marker of oxidative stress and lipid peroxidation) and renal tissue superoxide dismutase (SOD, a marker of tissue antioxidant enzyme defensive activity) were measured. Also, glomerular filtration rate (GFR, by creatinine clearance) as a marker of glomerular dysfunction and serum aspartate transaminase (AST, as a marker of tubular dysfunction) were measured.

Results: Renal tissue MDA was significantly higher while SOD was lower in the reperfusion homogenate in rats subjected to renal I/R (I/R-saline group) when compared to the sham-operated groups ($p<0.05$). In addition, GFR was significantly lower and serum AST was higher in I/R rats compared to the sham-operated groups ($p<0.05$). The treatment with NAC ameliorated the above-mentioned post-ischemic changes ($p<0.05$).

Conclusion: The deleterious effects of renal I/R were attenuated by administration of NAC probably via its free radical scavenger ability in a rat model

Keywords: Oxidative stress, renal ischemia, N-acetyl cysteine, albino rats

Introduction

Renal ischemia, whether caused by shock, renal artery stenosis or during kidney transplantation, is the major cause of acute renal failure (ARF). The patho-physiological changes that are responsible for post-ischemic renal injury and depressed renal function remain incompletely understood and are multifactorial (1).

Ischemia per se is known to cause oxygen deprivation-cell injury and the reperfusion, despite it is beneficial in restoring the tissue level of oxygen yet, it increases the risk of oxidant formation in the ischemic renal tissue. Interestingly, ischemic reperfusion (I/R) is reported to be accompanied by excessive generation of reactive oxygen species (ROS). These ROS are linked to the patho-physiology and the high mortality rate in critically ill patients with ARF **(2).**

ROS are reported to be highly generated in the ischemic kidney at the onset of reperfusion **(3).** It has been proposed the main sources of such ROS during I/R are endothelial dysfunction with subsequent decrease of nitric oxide (NO) level **(4).** Also, polymorphonuclear leukocytes (PMNs) that are activated during ischemia, presumably by cytokines, enter the kidney at the onset of reperfusion causing the release of ROS **(5).**

Despite, renal transplantation is considered the live-saving choice for patients with ARF yet, renal I/R injury is a frequent consequence and remains one of the biggest facing obstacles (6). I/R-induced ROS generation was found to be the main cause of immediate post-operative graft nephritis and deterioration of graft microcirculation, increase cell apoptosis, and at a later stage, cell necrosis (7).

Irrespective of the origin of ROS, it is clear that I/R-induced ROS are involved in the development of post-ischemic renal injury of not only ARF but also during the renal transplantation. Therefore, ROS are considered real challenges and are important indirect targets to be scavenged in such cases. Thus, one would anticipate that treatment with antioxidants would minimize I/R damage. In that issue, different therapeutic strategies, of free radical scavengers, like SOD, GSH and many antioxidant vitamins have been much tried in the literature in limiting the consequences of renal I/R injury (8). However, N-acetyl cystenie (a thiol-containing anti-oxidant), is little studied.

NAC is an anti-oxidant that acts by increasing intracellular glutathione levels, and also by the direct scavenging of ROS, such as hypochlorous acid (HOCl), hydrogen peroxide (H2O2), superoxide $(O_2^-\cdot)$ and the hydroxyl radical (OH·) (9). Also, it is available in the clinical field as a mucolytic agent and plays an important role in the treatment of paracetamol overdose (10). Thus, the aim of this study was to evaluate the beneficial role of NAC against the damage caused by I/R in a rat model.

Material and methods

Animals:

In the present study, 32 adult male albino rats of local strain, weighing 150-200 gram each, were used. They were kept on laboratory diet and had free access to water throughout the whole study period. The rats were divided into 4 equal groups.

Group 1 (sham-saline group), in this group, the surgical procedure was performed but ischemia and reperfusion were not done then a bolus dose of 0.5 ml normal saline was infused intravenously.

Group 2 (sham-NAC group), in this group, the surgical procedure was performed but ischemia and reperfusion were not done then NAC (Acetylcysteine NM Pharma, Sweden) was administered in a bolus dose of 0.5 ml of NAC (250mg/kg BW; dissolved in normal saline) was infused intravenously.

Group 3 (I/R-saline group), in this group, the rats were subjected to 45 min. renal ischemia followed by 90 min reperfusion. A bolus dose of 0.5 ml normal saline was infused intravenously 5 min before the opening of the occluded renal artery (i.e before the onset of reperfusion) (11).

Group 4 (I/R-NAC group), in this group, the rats were subjected to 45 min. renal ischemia then renal reperfusion was allowed for 90 min. A bolus dose of 0.5 ml of NAC (250mg/kg BW; dissolved in normal saline) was infused intravenously 5 min before the onset of reperfusion.

Experimental procedure

1-Renal ischemic-reperfusion (11)

On the day of the experiment, the rats of each group were weighted and subjected to overnight fasting, after which they were anesthetized intraperitoneally by sodium pentobarbital, 40mg/kg BW. Thereafter, the abdomen was opened by left flank incision. Left renal vessels identified and dissected free from the surrounding fat and tissues. Left renal artery was clamped by a by a non-traumatic microvascular bulldog clip for 45 minutes to produce renal ischemia (keeping the contralateral kidney intact). Occlusion was verified visually by change in the color of the kidney to a paler shade and the onset of reperfusion was confirmed by observing the development of reactive hyperemia.

After 90 minutes of reperfusion, the abdominal aorta was exposed and cannulated for collecting blood samples. Then the perfused kidney was rapidly dissected free from adherent fats, connective tissues and peritoneum and rapidly excised out of the abdomen to be blotted dry and minced then homogenized for 5 min. in a glass homogenizer containing 10 ml of ice-cold phosphate buffer saline (PBS) (pH 7.4) of the following composition, KCl 140 mmol/L, phosphate 20 mmol/L and of pH 7.4.

Thereafter, the homogenized renal tissue was centrifuged at 3000 round per minute for 30 min at 4 ◦C and the resulting supernatant was separated, frozen in liquid nitrogen and stored at -70 ◦C until measuring the renal tissue content of MDA and SOD. Sham-operated rats (groups 1 and 2) underwent identical surgical procedures but without renal clamping and were maintained under anesthesia for the duration of the experiment.

2- Analytical study during the reperfusion period

a- Determination of the renal tissue MDA level (nmol/gm wet renal tissue) **(12).**

b- Determination of the renal tissue SOD level (unit/ mg protein) **(13).**

c- Determination of GFR by creatinine clearance as a marker of reperfusion glomerular dysfunction (Serum and urine creatinine levels and urine flow rate were measured then creatinine clearance was calculated **(2).**

d- Determination of serum AST as a marker of reperfusion tubular dysfunction (2).

Statistical analysis:

All results were presented as mean ± Standard Error of Mean (SEM).The results were statistically analyzed using the Student "t" test. The statistical criterion of significance was set at P value <0.05 (14).

Results

Table 1-3 and fig. 1-6 showed that NAC administration to the sham-operated group had no effect on all the measured parameters (p >0.05).

Table 1 and fig. 1 showed no statistical significant difference in the renal tissue MDA between the two sham-operated groups. However, it was significantly increased in rats subjected to renal I/R (I/R-saline group), compared to the sham-operated groups (sham-saline and sham-NAC groups) (66.22±4.14 vs 6.78 ± 1.39 and 6.24 ± 1.02 nmol/gm wet tissue, p<0.05). In comparison, treatment with NAC in I/R-NAC group, significantly decreased renal tissue MDA to be 18.54

± 2.33 nmol/gm wet tissue ($p < 0.05$).

Table 1 and fig. 2 showed no statistical significant difference in the renal tissue SOD between the two sham-operated groups. However, it was significantly decreased in rats subjected to renal I/R (I/R-saline group), compared to the sham-operated groups (sham-saline and sham-NAC groups) (5.84 ± 0.11 vs 14.16±1.65 and 14.10±1.02 unit/mg protein; $p<0.05$) On the other hand, treatment with NAC in I/R-NAC group, significantly increased the renal tissue SOD to be 12.83±1.17 unit/mg protein ($p < 0.05$).

Table 2-3 and fig 3-5 showed no statistical significant difference in the serum creatinine, urine flow rate and creatinine clearance (GFR) between the sham-operated groups. However, serum creatinine increased and urine flow rate & creatinine clearance (GFR) decreased in rats subjected to renal I/R (I/R-saline group), compared to the sham-operated groups (sham-saline and sham-NAC groups).

Table 2 and fig. 3 showed that serum creatinine in I/R-saline group was significantly higher than sham-saline and sham-NAC groups (260.55±12.41, vs 51.24±3.57 and 50.39±4.25 (µmol/L), $p<0.05$). Table 2 and fig. 4 showed that urine flow in I/R-saline group was significantly lower than sham-saline and sham-NAC groups (4.17 ± 0.01 vs 7.13±0.26 6.97±0.30 µl/min. $p<0.05$). Table 3 and fig. 5 sowed that clearance (GFR) in I/R-saline group was significantly lower than sham-saline and sham-NAC groups (0.40±0.01 vs 0.92±0.01 and 0.86±0.01 ml/min. $p<0.05$). On the contrary, in the I/R-NAC animals, the administration of a single intravenous bolus of NAC produced a significant reduction in serum level of creatinine to be 148.22±8.57 (µmol/L) ($p<0.05$) associated and raised flow rate to be 6.25±0.36 µl/min. ($p < 0.05$) and increase clearance (GFR) to be 0.71±0.01 ml/min. ($p < 0.05$).

Table 3 and fig. 6 showed that no statistically significant difference in the serum AST between sham-operated groups. However, I/R-saline group exhibited a significant increase serum AST, compared with sham-saline and sham-NAC group (1200.96±53.41 vs 230.19±12.54 and 231.41±13.78 ($p < 0.05$).

However, treatment with NAC in I/R-NAC group, significantly decreased the serum AST level to be 500.66±32.45 ($p < 0.05$).

Discussion

Severe renal ischemia is reported to cause irreversible renal damage that involves cell apoptosis and/or necrosis depending on the duration of oxygen deprivation (2). A number of processes have been implicated in the pathogenesis of this oxygen-deprivation cell injury. Theses include decrease ATP, increase cellular calcium concentration, generation of free radicals with increased lipid peroxidation (15).

It has been assumed that restoration of tissue level of oxygen, if started before the irreversible renal damage, could limit ischemic insult to the kidney. Although reperfusion is essential for the survival of ischemic tissue, there is a good evidence that reperfusion itself causes additional cellular injury that is collectively known as reperfusion injury (11). Reperfusion initiates a complex and interrelated sequence of events that results in injury and the eventual death of renal cells as a consequences of apoptosis and necrosis (16).

In the present study, a significant increase in renal MDA (a good indicator of increased lipid peroxidation) during I/R was observed. This increase in lipid peroxidation was in agreement with a previous study though the duration of reperfusion period was different (17). The lipid peroxidation is an autocatalytic process leading to oxidative destruction

of cell membrane with subsequent production of toxicmetabolites and cell death (18).

Table (1): Renal tissue MDA and SOD in all studied groups

• Group / Parameters	Sham-saline group	Sham-NAC group	I/R-saline group	I/R-NAC group
Renal tissue MDA (nm/gm)	6.78±1.39	6.24±1.02	66.22±4.14*	18.54±2.33*●
Renal SOD (unit/mg protein)	14.16±1.56	14.10±1.02	5.84±0.11*	12.83±1.17●

- Values are represented as mean ±SEM
- Significant when compared with sham-saline group
- Significant when compared with I/R-saline group

Fig. (1): Histogram of renal tissue MDA in all studied groups

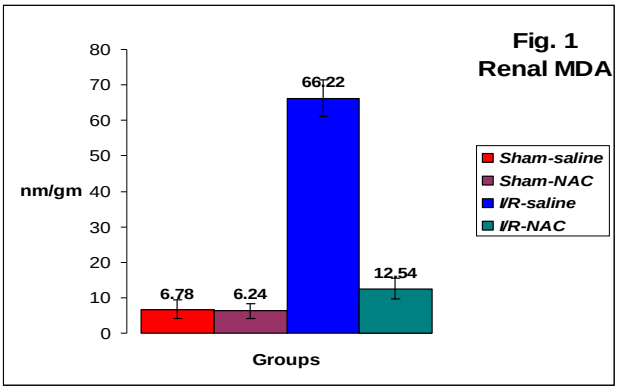


Fig. (2): Histogram of renal tissue SOD in all studied groups

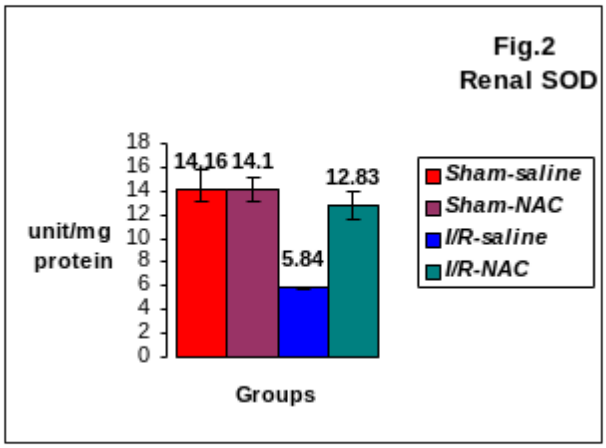


Table (2): Serum creatinine and urine flow in all studied groups

• Group Parameters	Sham-saline group	Sham-NAC group	I/R-saline group	I/R-NAC group
Serum creatinine (µmol/L)	51.24±3.57	50.39±4.25	260.55±12.41*	148.22±8.57*●
Urine flow (µl/min)	7.13±0.26	6.97±0.30	4.17 ± 0.01*	6.25±0.36●

Values are represented as mean ±SEM

- Significant when compared with sham-saline group
- Significant when compared with I/R-saline group

Fig. (3): Histogram of serum creatinine in all studied groups

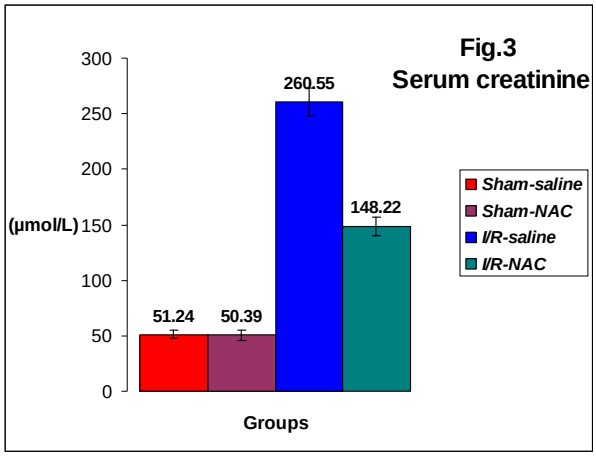


Fig. (4): Histogram of urine flow in all studied groups

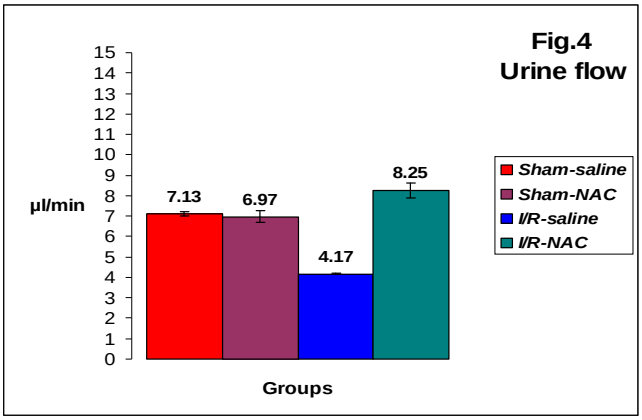


Table (3): GFR and serum AST in all studied groups

Group Parameters	Sham-saline group	Sham-NAC group	I/R-saline group	I/R-NAC group
GFR (ml/min)	0.92±0.01	0.86±0.01	0.40±0.01*	0.71±0.01*●
Serum AST (Iu/L)	230.19±12.54	231.41±13.78	1200.96±53.41*	500.66±32.45*●

Values are represented as mean ±SEM

- Significant when compared with sham-saline group
- Significant when compared with I/R-saline group

Fig. (5): Histogram of GFR in all studied groups

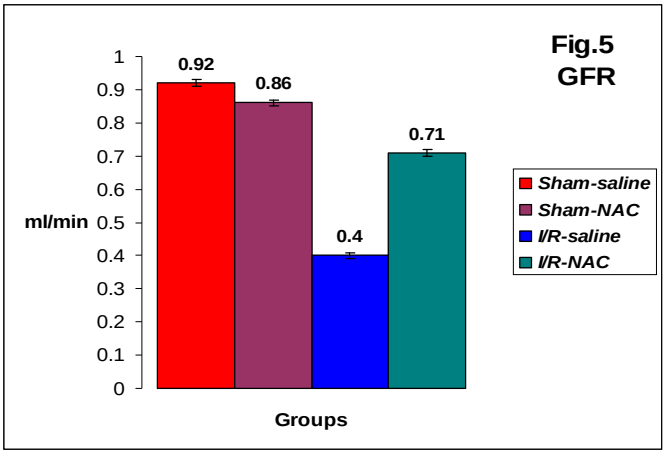
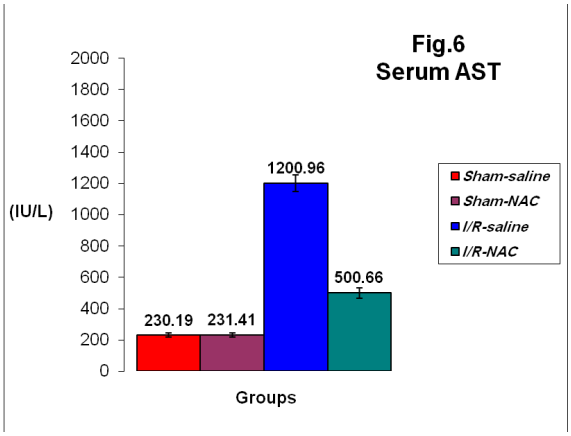


Fig. (6): Histogram of serum AST in all studied groups



The mechanisms proposed to explain increased MDA in rats subjected to is mostly the anoxia that is followed by release of oxygen-derived free radicals during reperfusion. These free radicals led to endothelial cell dysfunction with decreased NO release, leukocyte-endothelial adhesion and leukocyte activation (19). Superoxide anion, one of these free radicals, can interact with NO to generate peroxynitrite ($ONOO^-$), a potent and cytotoxic oxidant that could produce renal vasoconstriction and medullary ischemia, thus eliciting a vicious circle of I/R, superoxide production, ischemia and so on (20). In addition, it is reported that ROS produced during I/R inactivate local renal NO (21).

Treatment with NAC significantly decreased the renal MDA production probably in part by scavenging the very reactive hydroxyl radicals and in turns attenuates the lipid peroxidation. NAC is an acetylated derivative of the amino acid cysteine. It reacts with and deactivates hydroxyl radicals yielding NAC- thiol radical intermediates that are not oxidants **(22).**

In this model of I/R, renal tissue SOD level was significantly lower in rats subjected to renal I/R compared to the sham operated groups. On the other hand, treatment with NAC restores the SOD level nearly to its normal level. The renal tissue SOD enzyme provides protection against ROS by eliminating peroxides released during I/R injury **(19).**

Therefore, in this study, analysis of renal SOD level was significant to reveal the mechanisms by which NAC protects the kidney during I/R injury.

In the present work, serum creatinine increased while urine flow rate decreased in rats subjected to renal I/R suggesting a significant degree of glomerular dysfunction. Treatment with NAC ameliorated such deleterious changes. These results were consistent with that obtained by (Sharples et al (2) who proposed that renal I/R was accompanied by structural alterations in renal glomeruli and tubular epithelia represented by, increased serum creatinine with decrease urine flow in rats. Also, Yang et al., (23) found significant decrease of urine flow in rats subjected to I/R.

NAC did not alter serum creatinine in sham-operated animals. This disagree with Hoffman et al. (16) who previously demonstrated that NAC treatment decreases plasma creatinine levels in healthy volunteers. However, they found that oral administration of NAC significantly decreased the raised serum creatinine in persons subjected to radiographic-contrast-agent. This protective role of NAC was paralleled to its effect on creatinine in rats subjected to renal IR injury in this work.

It was reported that the renoprotective effect of NAC is dependent not only on free radical scavenging but also on NO potentiation, tripling endothelial NO expression, increasing NO bioavailability and protect NO from inactivation. NO is known to promote vasodilatation and preventing leukocyte activation and leukocyte-endothelial adhesion and, ultimately, attenuates the endothelial dysfunction (19 & 24).

Moreover, it was found that the biological activity and decomposition of peroxynitrite is very much dependent on the cellular level of thiols that influence its toxic potential (25). Thus, NAC being a thiol-containing anti-oxidant can attenuate the activity of this peroxynitrite and therefore could provide protection against I/R injury.

In the present study, renal I/R led to glomerular dysfunctions, as evidenced by significant decreased GRF and tubular dysfunctions, as evidenced by a significant increased serum AST,. These finding were in agreement with

the results obtained by Sharples et al., (2) who found a significant decreased GFR and increase AST and Yang et al., (23) who reported decrease of GRF in rats subjected to I/R.

The beneficial effects of NAC in partially restoring GFR and AST levels in this study NAC is mostly due to interference with ROS production by inhibiting active neutrophils (22). In accordance with this mechanism, it was found that the thiol-containing amino acid taurine reduced the ischaemic damage caused by PMNs in the ischaemic-reperfused heart (11).

In a recent clinical trial, it was found that NAC prevents radiocontrast-induced nephropathy in high-risk patients. This renoprotective mechanism of NAC was attributed to the direct scavenging of ROS specially the hydroxyl radical (OH·) (16). Furthermore, it was reported that renal I/R led to depletion of kidney glutathione (GSH) stores. GSH is an important intracellular anti-oxidant that plays a central role in the body's defense against cellular oxidative damage and acts directly by scavenging ROS. Lack of GSH therefore renders renal tissue susceptible to oxidative stress (26).

The relation of NAC to the GSH is biochemically explained where after its deacetylation in the intestines and liver, NAC yields L-cysteine as one of its metabolites. This circulating L-cysteine enters renal cells and combines with glycine and glutamate forming glutathione (27). Interestingly, NAC was authorized to exert its antioxidant effects through preventing GSH depletion and induction of GSH synthesis (21). In consistent with this it was found that NAC replenished tissue GSH level after renal I/R (28) and hepatic I/R in rats (29).

Conclusion, NAC is found to be a reno-protective agent in a model of renal I/R injury probably through its antioxidant activity. Further studies are still needed to support these findings in humans.

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