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Original Article

Beneficial Effect of N- acetylcysteine on Certain Oxidative Stress Parameters during Reperfusion following Renal Ischemia in Rats

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ABSTRACT *Background:* **A major internal threat to cellular homoeostasis arises from reactive oxygen species (ROS) formed as a result of normal physiological and metabolic processes that are essential to life which are overcome by the presence of body's natural antioxidant system. In the present study we evaluated the efficacy of N-acetylcysteine (NAC) against the damage caused by oxygen free radical (OFR) during reperfusion following renal ischemia using biochemical parameters. Wistar Albino rats were used in the present study. Animals were subjected to 60 minutes of unilateral ischemia followed by 10 minutes of reperfusion keeping the contralateral kidney intact. N- acetylcysteine was administered just before the reperfusion period. Biochemical parameters such as malnoaldehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) were estimated from the tissue homogenate to assess the degree of damage.**

Results: **The tissue lipid peroxidation, (MDA) was significantly enhanced but simultaneous decrease in GSH, SOD in reperfusion period when compared to control ($p<0.0001$). These results indicate that free radical formation involves in renal injury. Administration of NAC could reverse the alterations ($p<0.0001$)**

Conclusion: **The above results suggest that NAC has protective effect against damage caused by oxygen free radicals.**

Key words: **N- acetylcysteine, kidney ischemia reperfusion, lipid peroxidation, glutathione, superoxide dismutase.**

INTRODUCTION

Renal ischemia is the most common cause for acute renal failure occurring in nearly 5% of hospitalized patients. Acute renal failure following ischemia is a complex syndromes involving renal vasoconstriction, a sudden decrease in glomerular filtration rate, and destruction of renal tubules and back flow of glomerular filtrate[1]. It accounts for nearly 50% mortality among the elderly people with multiple problems, despite prompt therapeutic interventions. A number of processes have been implicated in the pathogenesis of oxygen deprivation induced cell injury. These include decrease in ATP, increase in cellular calcium concentration, disruption of the generation of free radicals, activation of phospholipases with production of lipid metabolites and loss of cell volume[2,3]. The effect of reperfusion in ischemic tissue also includes the irreversible cellular damage that is collectively known as "Reperfusion Injury".[4] Thus, the mechanisms underlying ischemia/reperfusion (I/R) damage to kidney are multifactorial and interdependent[5]. Therefore, restoration of tissue levels of oxygen, although

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beneficial, increases the risk of oxidant formation in the damaged tissue.

The protective enzyme like SOD, catalse(CAT) and GSH present in the tissue prevent the damage caused by oxygen free radicals. N-acetyl cystenie is a small molecule containing a thiol group, which has antioxidant properties[6]. Although the effects of many antioxidants such as SOD, GSH, vitamins etc, have been studied in limiting the consequences of ischemic-reperfusion injury(7-15); only very few studies have been done with NAC. It has been suggested that NAC replenishes GSH stores, increases SOD activity, scavenges hydroxyl free radical and interferes autocatalytic lipid peroxidation(16). As all the free radical scavengers having protective role in renal injury, the purpose of this study was to evaluate the protective effect of NAC against oxidative stress on the natural antioxidant biochemical parameters occurred during reperfusion.

MATERIAL AND METHODS

Inbred Wistar rats weighing 200-300 gm were used in the present study. Animals were housed 4-5 rats per cage and fed adlib. All experimental protocols were approved by the ethical committee of Manipal Academy of Higher Education (MAHE), Manipal, Karnataka, India. For the experimental purpose, the animals were divided into following groups

Group I: (normal control). These normal animals were neither undergone ischemia nor reperfusion, served as normal control.

Group II: (sham). In this group the surgical procedure was performed but ischemia and reperfusion procedure were not performed.

Group III: (untreated experimental group; 60 min.ischemia+ 10 min reperfusion) In this group, the animals were induced renal ischemia and followed by reperfusion

Group III a: (NAC treated group 60 min. of ischemia + N-acetylcysteine +10 min.of reperfusion) In this group, the animals were subjected to renal ischemia and NAC was infused intravenously just before reperfusion and then followed by reperfusion for 10 minutes.

Experimental procedure:

On the day of the experiment the rats were anesthetized intraperitoneally with pentabarbitone sodium (40 mg/kg/bw) under strict aseptic conditions. The abdomen was opened by left flank incision. Left renal artery and vein were identified and dissected free from the surrounding fat and tissues. Left renal artery and vein were occluded by a microvessel occluder for 60 minutes to produce ischemia. The group IIIa animals were received a loading dose of 0.5 ml of NAC (250mg/kg bw; dissolved in normal saline) was infused intravenously just before the opening of the occluded vessels. The onset of reperfusion was confirmed by observing the development of reactive hyperemia. After 10 minutes of reperfusion the kidneys were removed and kept in cold phosphate buffered saline (PBS, 0.9%). The reperfused kidney was blotted dry and minced. The minced tissues were transferred to a glass homogenizer containing 10 ml of cold PBS (pH 7.4) and were centrifuged at 3000 rpm for 30 minutes to obtain the supernatant. The supernatant obtained were used to estimate the biochemical parameters such as MDA, GSH and SOD.

Estimation of MDA: MDA was estimated by the method described by Kartha & KrishnaMurthy(18). Thiobabutric acid reactive material was expressed in terms of nanomoles of MDA /gm wet tissue.

Estimation of GSH: GSH was estimated standard protocol (Beaulter et al[19]) and glutathione content was expressed as (g/gm protein.

Estimation of SOD: SOD was estimated by the technique explained by Fridovich[20]. The activity was expressed as unit/ mgprotein

Protein Estimation:

Protein content of the tissue samples was determined by Lowry et al method[21].

Statistical analysis: All data are expressed as mean ± SD. Data were analyzed by using non-parametric (Kruskal- Wallis) test followed by multiple comparison test with $p < 0.05$ considered as being significant.

RESULTS

Tissue MDA: Reperfusion after ischemia caused a significant increase in the level of lipid peroxidation compared to normal or sham respectively. (Fig. 1) (57.30 ± 7.32 nmol/gm wet tissue $p<0.0001$) However, the treatment with NAC significantly reduced tissue lipid peroxidation to be 29.29 ± 6.25 nmol/gm wet tissue ($p <0.0001$) in group IIIa compared to the untreated group

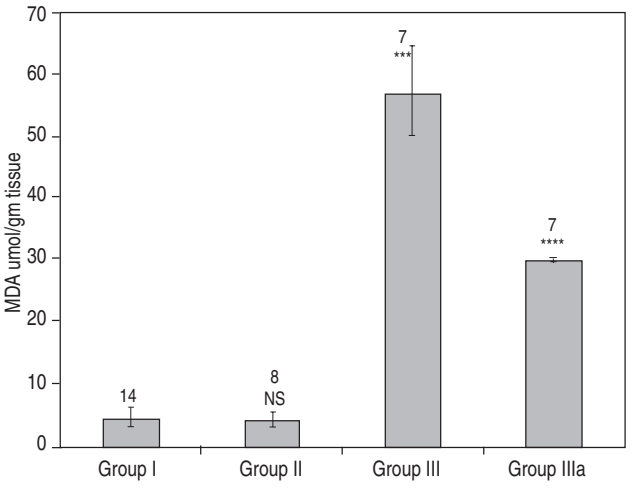

Fig. 1 Effect of NAC on lipid peroxldation affer ischemia reperfusion. The values are SD. Gr.III vs Gr.II and Gr.IIIa *p<0.001

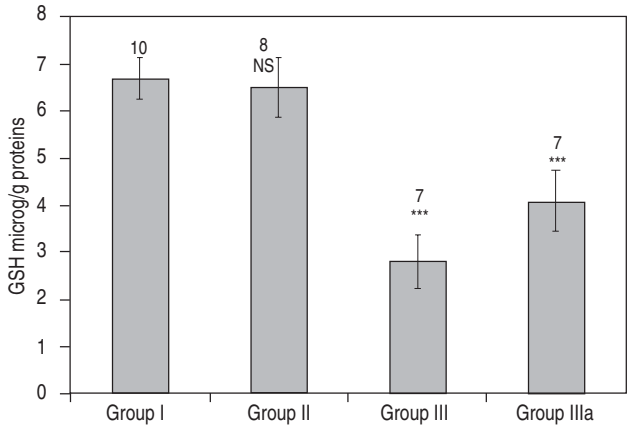

Fig. 2 Effect of NAC on tissue GSH level after ischemia reperfusion. The values are mean ± SD. Gr.III vs Gr.I & Gr.II *p<0.0001, Gr.IIIa vs Gr.III *p<0.0002 Gr.II vs Gr.I NS Not significant

(group III). No statistical significance was observed between normal control and sham (4.35 ± 1.59; 4.09 ± 1.12 nmol/gm wet tissue).

b) Tissue GSH: From figure 2, I/R, in group III, significantly suppressed GSH level to be 2.8 ± 0.56 μg/gm protein (p<0.0001). Treatment with NAC (group IIIa) increased the level to be 4.07 ± 0.66 (g/gm protein; P<0.0001) the compared to untreated experimental control (gr. III). There was no statistical significance between normal control and sham (6.74 ± 0.453; 6.509

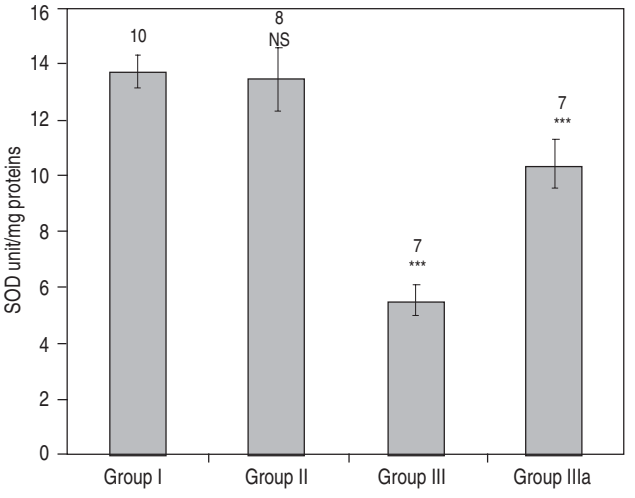

Fig. 3 Effect of NAC on tissue SOD level after ischemia reperfusion. The values are mean ± SD. Gr.III vs Gr.I & Gr.II *p<0.0001, Gr.IIIa vs Gr.III *p<0.0001 Gr.II vs Gr.I NS Not significant

± 0.622 μg/gm protein respectively).

c) Tissue SOD: The tissue SOD level after IR (group. III) was significantly decreased (Fig. 3; 5.55 ± 0.54 unit/mg protein; p (0.0001) There was no significant difference in SOD levels between group. I and group. II. (13.47±0.621; 13.452±1.12 unit/mg protein; p=0.1442). NAC (group IIIa) could restore the SOD level (10.4±0.877 unit/mg protein, p< 0.0001) when compared to untreated group (group III).

DISCUSSION

The pathophysiology of acute renal failure continues to elude the scientists for its complexity and challenging nature. For decades they have been trying to decipher the precise mechanism of tubular necrosis following acute renal failure (ARF). Once a clear picture regarding the cellular and molecular aspects of the disease process has been understood, the findings may be useful in day-to-day clinical practice. Such therapeutic interventions may help understand graft versus host reactions in patients receiving renal transplantations and formulate drugs that can either reduce or prevent the intensity of such reactions, for that evaluation of new drug is essential. Therefore, in this present study we used examined the role of NAC a thiol containing antioxidant In this model of renal ischemia reperfusion, we observed a significant increase in MDA content of the

tissue, an induced oxidant product of lipid peroxidation. Ischemia in the kidney is associated with lipid peroxidation, which is an autocatalytic mechanism leading to oxidative destruction of cell membrane leading to the production of toxic metabolites and cell death[22, 23]. Lipid peroxidation, as a free radical generating system, may be closely related to I/R induced tissue damage, and MDA is a good indicator of the degree of lipid peroxidation.The increase in lipid peroxidation observed in the present study is in agreement with previous studies though the duration of reperfusion period was different[1, 24]. Treatment with NAC showed a significant decrease in MDA production probably in part by scavenging the very reactive OH^- and ROO^-, indicating a reduction in lipid peroxidation and cellular injury[25]. Though the NAC treatment showed a decrease in MDA level but the value have not reach exactly back to normal. GSH provides a major protection in oxidative injury by participating in the cellular defence system against oxidative damage[26]. Several reports indicate that tissue induced by various stimuli is coupled with GSH depletion[27,28].

In the present study a decrease in GSH level was observed in reperfusion following ischemia, is in agreement with previous study[29]. The decrease in GSH level during I/R was probably due to consumption during oxidative stress. However, the NAC administration at the time of reperfusion increased the GSH level. GSH scavenges O_{2^-} and protect the thiol groups from oxidation[30]. GSH also has a major role in restoring other free radical scavengers and antioxidant such as vitamin E ascorbic acid to their reduced state[30]. An increase in intracellular GSH has been reported after NAC administration[31]. NAC may also exert its antioxidant effect indirectly by facilitating GSH synthesis and supplying GSH for GSH peroxidase catalyzed reaction. Elevated intracellular GSH, a direct free radical scavenger and indirect antioxidant, protects rat renal proximal tubules from in vitro stimulated reperfusion injury[32].

The tissue level of SOD also showed a significant decrease after I/R, and a significant increase in the level was observed after the administration of NAC. Therefore, the results of the present study show that NAC play a protective role in reducing lipid peroxidation and restoration of GSH and SOD during I/R. This may be a beneficial effect of NAC to prevent tissue damages if I/R condition persist.

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