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Modulatory effects of *N*-acetylcysteine on hyperoxaluric manifestations in rat kidney

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ABSTRACT

Hyperoxaluria is a condition where excessive oxalate is present in the urine. Many reports have documented free radical generation followed by hyperoxaluria as a consequence of which calcium oxalate deposition occurs in the kidney tissue. The present *in vivo* study was designed to investigate the potential of *N*-acetylcysteine in modulating hyperoxaluric manifestation induced by sodium oxalate in the rat kidneys. Male wistar rats in one group were administered single dose of sodium oxalate (70 mg/kg body weight) intraperitoneally to induce hyperoxaluric conditions and in the other group, rats were injected *N*-acetylcysteine (NAC) (200 mg/kg body weight) intraperitoneally, half an hour after sodium oxalate dose. The treatment is for a period of 24 h. *N*-acetylcysteine significantly reduced hyperoxaluria caused oxidative stress by reducing lipid peroxidation, restoring antioxidant enzymes activity in kidney tissue, followed by reduction in impairment of renal functioning. In addition, NAC administration reduced the number of calcium oxalate monohydrate (COM) crystals in the urine as observed under polarization microscope. Histological analysis depicted that NAC treatment decreased renal epithelial damage, inflammation and restored normal glomeruli morphology. Thus, it shows that use of an extraneous antioxidant may prove beneficial for combating the conditions of oxidative stress produced by hyperoxaluria.

1. Introduction

About 70–80% of all kidney stones are composed of calcium, usually combined with oxalate (Coe et al., 2005). Although their cause is still not clearly known, abnormally elevated urinary calcium (hypercalciuria) or elevated urinary oxalate (hyperoxaluria) increases the risk of developing calcium stones. Hyperoxaluria is more important risk factor for calcium oxalate stone formation than hypercalciuria (McConnell et al., 2002). Even though urinary oxalate is directly related to diet but still majority of urinary oxalate is produced by metabolism (glyoxylic acid cycle) (Conyers et al., 1990). Hyperoxaluria also results due to an inherited genetic disorder i.e. primary hyperoxaluria. This occurs due to deficiency of a liver-specific peroxisomal enzyme alanine/glyoxylate aminotransferase (Danpure and Jennings, 1986). Many authors have found that hyperoxaluria causes serious damage to the renal tissue by oxidative stress, which further results in stone formation (Khan, 2005; Thamilselvan and Menon, 2005). But recently a report by Green (Green et al., 2005) showed that lipid peroxidation results due to progression of calcium oxalate stone instead of direct renal injury by oxalate exposure. So it becomes even more important to study oxidative stress caused by hyperoxaluria by an acute dose instead of chronic exposure.

Recently, *N*-acetylcysteine (NAC) has been extensively studied, with some reports indicating its outstanding efficacy in the renal protection. Prophylactic administration of NAC is found be effective in preventing contrast agent-associated nephrotoxicity induced by a low dose of non-ionic, low-osmolality contrast dye (Briguori et al., 2004). There are several reports indicating prevention of radiocontrast nephropathy by administration of NAC (Itoh et al., 2005a). NAC have also been shown to reverse hyperoxaluric manifestations in rat liver (Bijarnia et al., 2007). The purpose of the present work is to review the pathogenesis of hyperoxaluria by an acute dose and to explore the biologic effects of NAC on hyperoxaluric manifestations. To study the potential benefit of NAC, hyperoxaluric conditions were induced and then *N*-acetylcysteine was exploited to study its free radical scavenging property.

Abbreviations: NAC, *N*-acetylcysteine; COD, calcium oxalate dihydrate; COM, calcium oxalate monohydrate; MDA, malondialdehyde; HCl, hydrochloric acid; SOD, superoxide dismutase; CAT, catalase; w.r.t., with respect to; NF-κB, nuclear factor- κB.

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2. Material and methods

2.1. Test material

N-acetylcysteine was purchased from Sigma-Aldrich, sodium oxalate from Merck, urea and creatinine were purchased from Himedia Laboratories Pvt. Limited.

2.2. Animals and treatments

Healthy male wistar rats were obtained from central animal house of Panjab University Chandigarh. Animal grouping was performed on the basis of their body weight (weighing between 150–175 g). The animals were acclimatized for one month in polypropylene cages under hygienic conditions and were provided standard animal feed and water *ad libitum*. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals and were approved by the local care of Experimental Animals Committee.

For the present experiment, a total of 15 animals were divided into three groups of five animals each. One group having control animals (group I) was given normal saline intraperitoneally. Group II was administered single dose of sodium oxalate (70 mg/kg body weight) intraperitoneally and third group (group III) was given intraperitoneal injection of *N*-acetylcysteine (200 mg/kg body weight) 30 min after sodium oxalate injection.

Just after treatments, the rats were placed in metabolic cages and urine was collected for 24 h period in 20 μl of 20% sodium azide as a preservative. After measuring urinary volume, the urine was centrifuged at 1500 g for 5 min to remove all debris and acidified by the addition of 3 N HCl (hydrochloric acid) for the determination of urinary oxalate and creatinine.

The rats were anaesthetized with diethyl ether and sacrificed by decapitation after 24 h of above treatment. Before sacrificing, the blood was taken from orbital sinus into a centrifuge tube without anticoagulant and allowed to clot at room temperature. It was then centrifuged for 15 min at 1814 g and supernatant was collected as serum.

Urine from the urinary bladder was directly obtained by puncturing with a 5/8 in. needle attached to a 1 ml tuberculin syringe. The kidneys were perfused through heart with 5 ml syringe containing ice-cold isotonic saline (0.9%). A 10% (w/v) kidney tissue homogenate was prepared in ice-cold 10 mM PBS (phosphate buffer saline, 0.15 M NaCl, pH 7.4). The homogenate was centrifuged at 1000 g for 10 min at 4 °C and the supernatant was used for biochemical assays. For the superoxide dismutase assay, the supernatant was further centrifuged at 12,000 g for 20 min.

2.3. Biochemical assays in urine and serum

Urinary oxalate level was quantified by the colorimetric method as described by Hodgkinson and Williams (1972). Serum urea level was determined by diacetylmonoxime method as described by Marsh et al. (1965). The creatinine in both serum and urine was estimated by the method of Bonsnes and Taussky (1945). Creatinine clearance was calculated according to standard clearance formula $C = U/S \times V$, where U is the urinary concentration of creatinine, S is the concentration of creatinine in the serum and V is the urinary volume in mL/min.

2.4. Parameters for oxidative stress and antioxidant status in renal tissue

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) level in kidney tissue homogenate using the method of Buege and Aust (1987). For the estimation of phospholipids content, lipids from renal tissue were first extracted using the method of Folch et al. (1957). Then the content of phospholipids was analysed by the method of Bartlet (1954). Catalase (CAT) activity was determined by UV spectrophotometer method described by Luck (1971) and expressed as μ moles of H_2O_2 decomposed/min/mg protein. The assay for superoxide dismutase (SOD) was performed according to the method of Kono (1978). The activity of enzyme was expressed as units per milligram of protein, where one unit of enzyme is defined as the amount inhibiting the rate of reaction by 50%. To calculate the activity of CAT and SOD, protein content in the homogenate was estimated by Lowry method (1951).

2.5. Urinary crystal study

A drop of urine obtained from bladder is spread on a glass slide and visualized under polarized light using Leica DM3000 light microscope.

2.6. Histopathological studies

The kidneys were removed and its transverse sections were fixed in 10% buffered formalin solution (pH 7). The tissues were dehydrated and embedded with paraffin wax (M.P. 68 °C). The paraffin sections were than cut and finally stained with Delafield's Hemotoxylin and Eosin staining (H & E staining).

2.7. Statistical analysis

The data were expressed as mean ± SD and analyzed by Student's *t*-test.

3. Results

3.1. Evaluation of renal functioning

To determine induction of hyperoxaluria, urinary excretion of oxalate ions was measured. 24 h after sodium oxalate dose, excretion of oxalate in urine increased tremendously, amounting to 289% more as compared to control animals (Table 1). In group III again the oxalate excretion is high as compared to control animals but there is a significant ($p < 0.01$) decrease in urinary oxalate excretion of this group animal as compared to group II animals. Animals of group I show increased urine volume (62%) as compared to control animals. In addition group II animals also showed no significant change in their urine volume in comparison to group I animals (Table 1).

The urea content in the serum of group II animals increased significantly ($p < 0.001$) in comparison to control group I animals. However, administration of NAC significantly ($p < 0.001$) reduced its level in serum. A significant increase ($p < 0.001$) in creatinine level in serum, after sodium oxalate treatment was also observed. NAC treatment brings the level of creatinine comparable to the control animals. Creatinine excretion in the urine increased significantly ($p < 0.001$) after oxalate exposure and NAC significantly ($p < 0.001$) lowered its content.

On examining creatinine clearance as shown in Table 1, animals of group II (hyperoxaluric) represented a significant ($p < 0.001$) decline of 120% as compared to control group animals. On the other hand supplementation of NAC, normalized creatinine clearance significantly ($p < 0.001$) as compared to hyperoxaluric animals.

3.2. Renal oxidative stress

Lipid peroxidation in the kidney tissue was increased significantly after oxalate exposure (Table 2). Malondialdehyde (MDA) status in this group was found to increase by 27%. After treatment with NAC, a significant decrease ($p < 0.01$) in MDA status was observed as compared to oxalate exposed rats. As depicted by Table 2, the amount of phospholipids in the kidney of sodium oxalate treated rats is significantly decreased ($p < 0.001$) as compared

Table 1
Alterations in the serum level of urea, creatinine, urinary volume, oxalate excretion, and creatinine clearance in control (group I), oxalate exposed rats (group II) and *N*-acetylcysteine treated hyperoxaluric rats (group III)

		Control (group I)	Oxalate exposed (group II)	*N*-acetylcysteine treated (group III)
Serum	Urea (mg/dl) mean ± SD	189.45 ± 28.7	$414.7 \pm 24.93^{***}$ (+118%)	$384.59 \pm 15.24^{***}$ ### (+103%)
	Creatinine (mg/dl) mean ± SD	0.66 ± 0.02	$1.01 \pm 0.04^{***}$ (+53%)	0.84 ± 0.072### (+27%)
Urine	Urine volume (ml/24 h)	15.56 ± 1.2	$25.29 \pm 3.1^{***}$ (+62%)	$24.73 \pm 1.8^{***}$ (+59%)
	Oxalate (mg/24 h) mean ± SD	1.8 ± 0.07	$7.01 \pm 0.61^{***}$ (+289%)	4.71 ± 0.11## (+161%)
Creatinine clearance (ml/min)		2.07 ± 0.11	$0.98 \pm 0.07^{***}$ (−120%)	$1.71 \pm 0.08^{**}$ ## (−22%)

Values in brackets are % increase (+) or % decrease (−) as compared to control (group I). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$: Indicates significant change in comparison to control group I. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$: indicates significant change between group II and group III.