Evaluation of protective effect of N-acetyl cysteine on arsenic-induced hepatotoxicity

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Abstract

Objective: The present study was aimed to study protective role of N-acetyl cysteine (NAC) was assessed against arsenic (As)-induced hepatotoxicity in rats. Methods: Twenty four male Wistar rats were divided into 4 groups of 6 animals each and treated as follows: Group 1: sham control, 2: arsenic control (sodium arsenite @ 10 mg/kg b. wt orally for 4 wks), 3: Pre-treatment with NAC (@ 300 mg/kg orally for 2 wks) followed by sodium arsenite along with NAC (as per above doses) and 4: Sodium arsenite + NAC (as per above doses for 4 wks). Results: The concentration of thiobarbituric acid reacting substances (TBARS) and protein carbonyls was significantly (P<0.05) increased, while the concentration of reduced glutathione (GSH), and the activity of CYP450, Na⁺ -K⁺ ATPase and Mg2⁺ ATPase in liver were significantly (P<0.05) reduced in group 2 as compared to control. Groups 3 and 4 revealed improvement in the parameters in study. Conclusion: The study revealed that arsenic induces hepatotoxicity by inducing oxidative stress and supplementation of NAC is beneficial in countering the adverse effects.

Key words: Arsenic, hepatotoxicity, N-acetyl cysteine

INTRODUCTION

Arsenic is a major global health concern due to its wide distribution and adverse health effects on humans, animals, birds, aquatic life and plants through polluted ground water and food chains. It is a known carcinogen that has been associated with cancers of the skin, lung, urinary bladder, and possibly liver, kidney and prostate in humans. Besides the natural sources, arsenic-contaminated herbicides, insecticides and rodenticides are also potential vehicles of arsenic toxicity.[1] Organic arsenicals are generally considered non-toxic, whereas inorganic forms are toxic. Inorganic arsenic exists predominantly in trivalent (As³⁺) and pentavalent (As⁵⁺) forms, where trivalent compounds are more toxic than pentavalent ones.[2] Liver is the target of arsenic-induced carcinogenesis.[3] Arsenic-induced global DNA hypomethylation was also seen in mouse livers chronically exposed to inorganic arsenic.[4-7] The present work was undertaken in male Wistar Kyoto rats to study the protective effect of NAC on arsenic-induced hepatotoxicity.

MATERIALS AND METHODS

Male albino rats of Wistar Kyoto strain weighing about 200-220 g were procured from National Institute of Nutrition (NIN), Hyderabad, India. The animals were housed in solid bottom polypropylene cages. Animals were placed on commercial standard mash feed for rats (NIN, Hyderabad) and provided water ad libitum. Experimental protocol was approved by the Institutional Animal Ethics Committee. Rats were divided into four groups of 6 in each. Group 1 was kept as sham control, 2 was arsenic control (sodiumarsenite@10 mg/kg b. wt orally for 4 weeks), 3 was pre-treated with N-Acetyl cysteine (@ 300 mg/kg orally for 2 weeks) followed by sodium arsenite along with N-Acetyl cysteine (as per above doses for 4 weeks) and 4 was given sodium arsenite + N-acetyl cysteine (as per above doses for 4 weeks). The animals were then euthanized on 29th day and livers were immediately excised, rinsed...
with ice-cold physiological saline and stored at −20°C for further homogenization to estimate the concentration of thiobarbituric acid reacting substances (TBARS), protein carbonyls and reduced glutathione (GSH), and the activity of CYP450, Na⁺–K⁺ ATPase and Mg²⁺ ATPase.

The data were subjected to statistical analysis by applying one way ANOVA using SPSS (version 15.0) and the means were compared by Duncan’s multiple comparison test. Significance was set at $P < 0.05$.

**RESULTS AND DISCUSSION**

The concentration of TBARS (n mol MDA/mg protein) and protein carbonyls (n mol/mg protein) in liver showed a significant ($P < 0.05$) rise in Group 2 (3.06 ± 0.16 and 4.07 ± 0.25, respectively) as compared to group 1 (1.47 ± 0.09 and 1.19 ± 0.03, respectively). Groups 3 and 4 showed a significant ($P < 0.05$) decrease as compared to Group 2. The concentration of GSH in liver revealed a significant ($P < 0.05$) reduction in Group 2 (14.12 ± 1.47 µmol/mg protein) as compared to the remaining groups [Table 1].

Oxidative stress mediated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) is the cause for arsenic toxicity. In the present study, concentration of TBARS and protein carbonyls were increased in the liver of arsenic toxic group suggesting an ongoing oxidative stress. Similar results were obtained by Demerdash et al., Flora et al., and Sharma et al. Arsenic produces oxidative damage by disturbing the prooxidant–antioxidant balance, because it has very high affinity for sulfhydryl groups in GSH (non-enzymatic antioxidant), which might have implications in the maintenance of thioldisulfide balance. Arsenic also induces oxidative tissue damage through interference with GSH utilization. N-Acetylcysteine (NAC) is a thiol-containing antioxidant that has been used to reduce various conditions of oxidative stress. Its antioxidant action is attributed to GSH synthesis; therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS). It is also known as potent metal chelator. NAC has a strong ability to restore the impaired pro-oxidant/antioxidant balance in metal poisoning.

The activity of CYP450 (n mol/mg microsomal protein), Na⁺–K⁺ ATPase (µ mol Pi liberated/mg microsomal protein/30 min) and Mg²⁺ ATPase (µ mol Pi liberated/mg microsomal protein/30 min) in liver revealed a significant ($P < 0.05$) reduction in Group 2 (14.12 ± 1.47 µmol/mg protein/30 min) as compared to Group 1 (5.0 ± 0.30 µmol/mg protein/30 min) [Table 1]. Groups 3 and 4 showed a significant ($P < 0.05$) increase in the activity of CYP450 and ATPases as compared to Group 2. The altered activity of ATPases may be attributed to the membrane lipid peroxidation resulting in structural derangement. Earlier reports confirm that low levels of ATP in hepatic cells with arsenic and treatment with NAC improved the activity of Na⁺/K⁺ ATPases. The activity of CYP450 was significantly reduced in Group 2 as compared to control in this study, which is in agreement with the findings of Noreault et al.

**CONCLUSION**

The study revealed that arsenic induces toxic effects in liver due to generation of reactive oxygen species with eventual oxidative damage. Supplementation of NAC is beneficial in preventing these toxic effects to certain extent. Pre-treatment with NAC was found more protective as compared to NAC co-treatment against arsenic-induced toxicity.

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Table 1: Results of oxidative stress and enzymes in liver tissue

| Group | TBARS (nmol MDA/mg protein) | Protein carbonyls (nmol/mg protein) | GSH (µmol/mg protein) | CYP450 activity (nmol/mg microsomal protein) | Na⁺/K⁺ ATPase activity (µmol of Pi liberated/mg microsomal protein/30 min) | Mg²⁺ ATPase activity (µmol of Pi liberated/mg microsomal protein/30 min) |
|-------|-----------------------------|-------------------------------------|-----------------------|---------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| 1     | 1.47±0.09                   | 1.91±0.03                           | 31.84±4.05            | 3.55±0.39                                   | 16.64±1.25                                                              | 13.96±1.11                                                              |
| 2     | 3.06±0.16                   | 4.07±0.25                           | 14.12±1.47            | 1.16±0.19                                   | 5.16±0.28                                                               | 4.94±0.42                                                               |
| 3     | 1.94±0.10                   | 2.50±0.14                           | 26.68±3.29            | 2.55±0.20                                   | 14.62±0.92                                                              | 11.54±0.97                                                              |
| 4     | 2.26±0.12                   | 2.30±0.30                           | 22.45±2.48            | 2.37±0.23                                   | 13.51±0.91                                                              | 9.41±0.78                                                               |

Values are Mean±SE, $n=6$; Means with different alphabets as superscripts differ significantly ($P<0.05$), ANOVA (SPSS version 15).
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