Protective effect of N-acetylcysteine in isoniazid induced hepatic injury in growing rats

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Status of oxidative/antioxidative profile was the mechanistic approach to inumerate the nature of protection by N-acetylcysteine (NAC) in isoniazid (INH) exposed experimental animals. Analysis of lipid peroxidation, thiol levels, cytochrome P_{450}, superoxide dismutase (SOD), catalase, glutathione peroxidase, reductase and transferase were estimated in liver along with the body and liver weight of animals and histological observations. Isoniazid exposure to animals resulted in no change in body and liver weights. Thiois, lipid peroxidation, catalase, SOD glutathione peroxidase, reductase, transferase and cytochrome P_{450} levels were altered with INH exposure. Supplementation of NAC with INH protected the animals against hepatotoxic reactions by minimizing the free radical induced tissue injury and overall maintenance of the endogenous scavengers of free radicals.

The aminothiol, N-acetyl-L-Cysteine (NAC), an analogue and one of the precursors of glutathione (GSH) is capable of rapidly replenishing the depleted intracellular GSH levels\(^1\). The intracellular effects of NAC are amenable to the fact that it enriches the thiol pool in the cellular environment and by acting as a precursor of GSH, triggers many of the enzymatic and non-enzymatic protective mechanisms of cellular defense system\(^2\). Most of the circulating GSH originates from liver\(^3\) and hepatocytes are known to contain the highest concentrations (5-10 mmol/l). GSH homeostasis is disturbed in excessive conjugation reactions of GSH with xenobiotic or endogenous reactive intermediates formed during lipid peroxidation\(^4\). Formation of reactive oxygen species (ROS) can occur during xenobiotic metabolism and could be a mechanism for initiation of hepatotoxicity. ROS appear to have broader significance in the production of tissue injury under conditions of oxidative stress\(^5,6\).

N-acetylcystine at the cellular level suppresses the production of reactive oxygen metabolites from human neutrophils during their oxidative burst in vitro\(^7\). The ability of NAC to support the GSH system and to scavenge ROS represents one of the major mechanisms explaining its multiple therapeutic properties and the antioxidant, antigenotoxic and anticarcinogenic effects\(^2,6\).

Isoniazid (INH), a sheet anchor of antitubercular therapy, induced hepatic injury and was oxidative in nature\(^8\). The role of ROS, thus the oxidative stress, has been recently highlighted in INH-induced hepatotoxicity in experimental animals\(^9,10\).

Keeping above in view, the present study has been designed to understand the protective effect of NAC on INH induced oxidative hepatic injury in young rats.

**Materials and Methods**

Young male wistar rats (24) of about 40g body weight were divided into 4 groups of 6 rats each. Purified powder of INH was administered ip to animals, at a dose of 50 mg/kg body weight daily in 4 ml/kg sterile distilled water for 3 weeks. NAC (Sigma, USA) was administered ip either alone or simultaneously alongwith INH, at a dose of 100 mg/kg body weight\(^11\) daily in 4 ml/kg sterile distilled water for 3 weeks. Similarly, sterile water (4 ml/kg) was administered by the same route to control animals.

Rats were fasted for 12 hr before sacrificing by cervical dislocation under light anaesthesia. Liver tissue was excised immediately and quickly cooled in...
a beaker resting in an ice bath followed by perfusion with ice-cold normal saline. Histopathological investigations of tissue of control and treated rats were carried out on H & E stained sections.

Liver tissues were homogenised in 100 mM potassium phosphate buffer as per Huang and Iwu. Total, protein and non-protein thiols were estimated in crude homogenate. Lipid peroxidation was measured in crude homogenate as per Ohkawa et al.

Enzyme assays—All enzyme assays were carried out in post-mitochondrial supernatant (PMS). Cytochrome P₄₅₀ (Ref. 15) superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase were assayed. Glutathione-S-transferases were assayed using 1-chloro-2,4-dinitrobenzene (CDNB), 2,3-dinitro-4-chlorobenzene (DCNB) and ethacrynic acid (EA) as substrates following Habig et al. Lowry’s method was employed for estimation of tissue proteins.

Statistical analysis—Statistical analysis was applied as reported earlier.

Results and Discussion

Effect of INH and NAC on various biochemical parameters are given in Tables 1-4.

INH and NAC treatments, either alone or in combination had no effect on body weights of animals. Similarly, relative liver weights were also not affected by any of the drug treatments.

Hepatic injury—Histological observations are shown in Figs 1-4. Portal triaditis of mild to moderate degree was the main finding on histopathology (Fig. 2). The infiltration was observed in hepatic lobules in INH treated animals (Fig. 3). Co-administration of NAC with INH was found to have completely ameliorated the induction of hepatic lesions by INH as evidenced by the normal morphology of rat liver (Fig. 4).

By augmenting cellular antioxidative system, cells can be protected against the oxidative injuries produced by various drugs and chemicals. NAC happens to be least toxic with best ability to be a precursor of GSH.

In the present study INH and NAC, either individually or in combination, did not have any effect on body weights and relative liver weights. These results are in accordance with earlier studies reporting no change in relative liver weights of animals exposed to INH.

Hepatotoxicity produced by INH was revealed by histological changes wherein mild to moderate degree of portal triaditis with occasional collection of inflammatory cells in the hepatic lobules was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of INH and NAC on Total, protein bound and non protein thiols. [Values are mean ± SD for 6 rats]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Hepatic thiols (µ mol/g tissue)</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Control (C)</td>
<td>13.32 ± 0.54</td>
</tr>
<tr>
<td>INH</td>
<td>12.01 ± 0.41*</td>
</tr>
<tr>
<td>INH+NAC</td>
<td>13.20 ± 0.49</td>
</tr>
<tr>
<td>NAC</td>
<td>13.44 ± 0.46</td>
</tr>
</tbody>
</table>

P value *<0.01 between control & INH

Table 2—Effect of INH and NAC on lipid peroxidation, catalase and superoxide dismutase. [Values are mean ± SD for 6 rats]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid peroxidation (nmol MDA/g tissue/10min)</th>
<th>Catalase (µmol/min/mg protein)</th>
<th>Superoxide dismutase (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>117 ± 7</td>
<td>312 ± 33</td>
<td>14.7 ± 2.1</td>
</tr>
<tr>
<td>INH</td>
<td>133 ± 9*</td>
<td>181 ± 22**</td>
<td>17.7 ± 1.86*</td>
</tr>
<tr>
<td>INH+NAC</td>
<td>113 ± 17</td>
<td>289 ± 28</td>
<td>18.2 ± 1.76**</td>
</tr>
<tr>
<td>NAC</td>
<td>101 ± 13*</td>
<td>303 ± 31</td>
<td>14.2 ± 2.64</td>
</tr>
</tbody>
</table>

P values; *<0.05 between control vs INH and Control vs NAC; **<0.001 control vs INH Controls vs INH+NAC.

Table 3—Effect of INH and NAC on Glutathione peroxidase, glutathione reductase and cytochrome P₄₅₀ [Values are mean ± SD for 6 rats]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glutathione peroxidase (nmol/min/mg protein)</th>
<th>Glutathione reductase (nmol/min/mg protein)</th>
<th>Cytochrome P₄₅₀ (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>460 ± 67</td>
<td>25.3 ± 2.1</td>
<td>0.42 ± 0.05</td>
</tr>
<tr>
<td>INH</td>
<td>294 ± 39**</td>
<td>29.0 ± 1.5**</td>
<td>0.485±0.052*</td>
</tr>
<tr>
<td>INH+NAC</td>
<td>438 ± 86</td>
<td>32.3 ± 1.73***</td>
<td>0.468±0.052*</td>
</tr>
<tr>
<td>NAC</td>
<td>471 ± 58</td>
<td>28.0 ± 1.72*</td>
<td>0.408±0.053</td>
</tr>
</tbody>
</table>

P values; *<0.05 between control vs INH and control vs NAC and Control vs INH+NAC; **<0.01 control vs INH; ***<0.001 control vs INH+NAC.

Table 4—Effect of INH and NAC on Glutathione-S-transferases using CDNB, DCNB and EA as substrates [Values are mean ± SD for 6 rats]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glutathione-S-transferases (nmol/min/mg protein)</th>
<th>CDNB</th>
<th>DCNB</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>626 ± 62</td>
<td>20.2 ± 2.9</td>
<td>14.2 ± 2.32</td>
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<tr>
<td>INH</td>
<td>551 ± 65*</td>
<td>18.0 ± 3.2</td>
<td>16.8 ± 1.47*</td>
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</tr>
<tr>
<td>INH+NAC</td>
<td>609 ± 46</td>
<td>19.8 ± 1.7</td>
<td>17.5 ± 2.6*</td>
<td></td>
</tr>
<tr>
<td>NAC</td>
<td>678 ± 58*</td>
<td>22.17</td>
<td>14.8 ± 2.32</td>
<td></td>
</tr>
</tbody>
</table>

P values; *<0.05 between control vs INH and control vs NAC and Control vs INH+NAC.
observed. The histopathological pattern of liver injury in the present study was similar as reported by Mitchell et al. 23.

Co-administration of NAC alongwith INH protected experimental animals against hepatotoxic reactions. These results are supported by earlier studies 16,27. The hepatoprotection by NAC observed in the present study may provide an additional support to the oxidative stress mechanism of INH induced hepatic injury.

Exposure of animals to INH resulted in depletion of hepatic thiols (protein as well as non-protein bound) which are critical components of antioxidant defense system and made the animals susceptible to oxidative stress (Table 1). This was evident from increased lipid peroxidation observed in these animals (Table 2). N-acetylcysteine was found to significantly prevent the alteration in antioxidative profiles and protected the animals against drug-induced lipid peroxidation. As NAC has been shown to inhibit lipid peroxidation in the present study, it is likely that protein thiol groups were spared from the attack of lipid radicals or reactive lipid soluble aldehydes.

Total cytochrome P450 levels were induced by INH treatment indicating more production of reactive metabolites and superoxide radicals. NAC did not reduce levels of cytochrome P450 in INH exposed animals in the present study. This is in accordance with the results observed in the present study.

Figs 1-4—Photomicrograph of rat liver showing (1) normal morphology of control animal including one portal tract and one central vein; (2) portal tract inflammation (arrows) in INH treated animal; (3) infiltration in hepatic lobules (arrow heads) in INH treated animal; and (4) normal morphology in INH and NAC treated animals (all Figs H & E, x<520).
with experimental study by De Flora et al. The present study, INH exposed animals exhibited significantly enhanced SOD activity. This may be, as the liver is trying to remove more superoxide radicals formed due to INH. Supplementation of NAC did not alter the INH induced SOD activity because NAC may be unable to remove superoxide radicals formed due to INH.

The activities of both H_2O_2 scavengers i.e. catalase and glutathione peroxidase showed a severe decline with INH treatment which is in accordance with the previous investigations. The decreased activities of these enzymes can be explained on the basis that superoxide radicals when produced in excess may inactivate these H_2O_2 scavengers. Simultaneous administration of NAC with INH prevented the decrease of catalase and glutathione peroxidase activities either directly or via GSH.

Supplementation of NAC significantly prevented the depletion of non-protein thiols (GSH) in INH exposed animals (Table 1). Similar kind of mechanism has been proposed for NAC hepatoprotection in case of acetaminophen.

A significant decrease in protein thiols has been observed in INH treated animals in the present study (Table 1). The depletion of protein thiols has been proposed as a critical event in the lethal injury of hepatocytes exposed to menadione or adriamycin induced acute oxidative stress. Supplementation of NAC prevented the loss of protein thiols in INH treated animals (Table 1). INH exposure significantly enhanced glutathione reductase activity, an enzyme that regenerates reduced glutathione from oxidised glutathione (Table 3). Increase in this enzyme activity may be associated with GSH depletion which is in accordance with Joshi et al. Supplementation of NAC to INH treated animals and to controls also stimulated significantly the glutathione reductase enzyme activity. Similar effect of NAC has also been reported.

The present results of alteration in glutathione transferase enzyme (CDNB) activity shown in Table 4 are in accordance with Aniya and Naito who observed that the microsomal GST (CDNB) activity was decreased after 80 min perfusion of liver with H_2O_2. The enzyme activity against EA as substrate was found to be stimulated by treatment with INH as reported earlier also. Supplementation of NAC prevented the decrease of GST (CDNB) activity in INH treated rats probably by providing adequate GSH for functioning of GST.

The present study indicates that simultaneous administration of NAC alongwith INH protected the experimental animals against hepatotoxic reactions. It can be concluded that NAC is a valuable tool for protection against hepatotoxicity induced by INH in an animal model by increasing the antioxidant defense systems, minimizing the free radical induced tissue injury and overall maintenance of the endogenous scavengers of free radicals.

Acknowledgement

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