Protective Effect of N-Acetyl Cysteine in Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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ABSTRACT
The present study determines the efficacy of N-acetyl cysteine (NAC) on marker enzymes, lipid peroxidation and antioxidants in carbon tetrachloride induced hepatotoxicity in rats. Carbon tetrachloride (CCl₄) (3 mL/kg/week) administered subcutaneously to albino Wistar rats for a period of three months significantly increased the activities of marker enzymes in plasma such as aspartate transaminase, γ-glutamyl transferase and alkaline phosphatase and increased the levels of thiobarbituric acid reactive substances and hydroperoxides in plasma and tissues (liver and kidney). A significant decrease in the levels of plasma antioxidants (glutathione, vitamin C and vitamin E) was also noted. Further, a decrease in the concentration of glutathione and the activities of superoxide dismutase, catalase and glutathione peroxidase in the tissues were observed. N-acetyl cysteine (150 mg/kg) was orally administered to normal and carbon tetrachloride-treated rats for a period of three months. N-acetyl cysteine decreased the activities of marker enzymes, lipid peroxidation and improved the antioxidant status in carbon tetrachloride-treated rats. But there were no significant alterations in these parameters in normal rats treated with N-acetyl cysteine. Histopathological observations of the liver also showed the protective effect of N-acetyl cysteine in carbon tetrachloride-induced hepatotoxicity in rats. The results of this study show the protective action of N-acetyl cysteine in carbon tetrachloride-induced hepatotoxicity in rats. This is mainly due to the effective antioxidant potential of N-acetyl cysteine.

Keywords: N-acetyl cysteine, Hepatotoxicity, Carbon tetrachloride

Carbon tetrachloride is commonly used as a model to evaluate hepatotoxicity [1]. Carbon tetrachloride metabolism begins with the formation of the trichloromethyl free radical, CCl₃ through the action of the mixed function cytochrome P450 oxygenase system of the endoplasmic reticulum [2]. The CCl₃ radical reacts with various biologically important substances such as amino acids, nucleotides and fatty acids, as well as proteins, nucleic acids and lipids. In the presence of oxygen, the CCl₃ radical is converted to the trichloromethyl peroxy radical (CCl₃OO⁺). This radical is more reactive and is capable of abstracting hydrogen from polyunsaturated fatty acids (PUFA) to initiate the process of lipid peroxidation [3].

One of the most extensively studied agents is N-acetyl-L-cysteine, a sulfur-containing amino acid that possesses many biological properties. It is credited as a drug with multiple therapeutic applications [4]. NAC could significantly interfere with the pathophysiology of free radicals producing drug induced oxidative stress [5]. Reports have shown that NAC treatment protects against acetaminophen hepatotoxicity in patients [6] and in rats [7, 8]. Also, there are few reports on the protective role of NAC in CCl₄-induced toxicity in patients [9-11] and in rats [12-15]. But there are no detailed reports on the antioxidant defense of NAC in CCl₄-induced hepatotoxicity in a long run. Hence, we considered it worthwhile and carried out this investigation to assess the effect of NAC on marker enzymes, nonenzymic and enzymic antioxidants in CCl₄-induced hepatotoxicity in rats.
N-Acetyl Cysteine in Carbon Tetrachloride-Induced Hepatotoxicity

Table 1. Effect of NAC on the activities of marker enzymes in plasma of normal and CCl₄-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (IU/L)</th>
<th>GGT (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>72.61 ± 15.3³</td>
<td>0.570 ± 0.04³</td>
<td>73.12 ± 6.47³</td>
</tr>
<tr>
<td>Normal + NAC</td>
<td>70.18 ± 7.49³</td>
<td>0.540 ± 0.06³</td>
<td>71.24 ± 5.3³</td>
</tr>
<tr>
<td>CCl₄</td>
<td>193.1 ± 16.28³</td>
<td>1.650 ± 0.11³</td>
<td>139.0 ± 9.42³</td>
</tr>
<tr>
<td>CCl₄ + NAC</td>
<td>89.04 ± 5.44³</td>
<td>0.700 ± 0.04³</td>
<td>83.39 ± 5.49³</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a,b,c) differ significantly. p < 0.05.

Materials and Methods

Animals

Male albino Wistar rats of body weight 150-180 g were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University and were maintained there. The rats were housed in polypropylene cages lined with husk. They were fed on a standard pellet diet (Agro Corporation Private Ltd., Bangalore, India) and water ad libitum.

Materials

N-acetyl-L-cysteine was obtained from Sigma Chemical Company, St. Louis, MO, USA. CCl₄ was purchased from Merck Ltd., Mumbai, India. All other chemicals and biochemicals used in our study were of high analytical grade.

Experimental Design

In our study, a total of 24 rats were used. The rats were divided into 4 groups of 6 rats each.

- Group I: Normal control rats.
- Group II: Normal rats orally administered with NAC (150 mg/kg body weight) [16].
- Group III: Rats subcutaneously injected with CCl₄ (3 mL/kg body weight) [17].
- Group IV: Rats orally administered with NAC (150 mg/kg body weight) along with subcutaneous injection of CCl₄ (3 mL/kg body weight/week).

The experiment was carried out for a period of three months. All the experimental protocols were approved by the Ethical Committee of Annamalai University.

RESULTS

Effect of NAC on Marker Enzymes

The effect of oral administration of NAC on plasma AST (aspartate transaminase), GGT (γ-glutamyl transferase) and ALP (alkaline phosphatase) activities in normal and CCl₄-induced rats is presented in Table 1. A significant increase in the activities of these marker enzymes was observed in CCl₄-treated rats. On treatment with NAC, the activities of these enzymes were found to be significantly decreased.

Effect of NAC on Lipid Peroxidative Products and Nonenzymic Antioxidants in Plasma

Table 2 shows the changes in the levels of plasma TBARS (thiobarbituric acid reactive substances), HP (hydroperoxides), vitamin C, vitamin E and GSH (glutathione) in normal and CCl₄-treated rats. There was a significant increase in the levels of TBARS and hydroperoxides and a decrease in vitamin C, vitamin E and GSH in CCl₄-treated rats. Treatment with NAC significantly decreased the elevated levels of TBARS and hydroperoxides and increased the levels of vitamin C, vitamin E and GSH in plasma.

Effect of NAC on Lipid Peroxidative Products in the Tissues

There was a significant increase in the concentration of TBARS and hydroperoxides in the tissues (liver and kidney) of CCl₄-administered rats (Table 3). Oral administration of NAC significantly decreased the concentra-

Table 2. Effect of NAC on the levels of TBARS and hydroperoxides and nonenzymic antioxidants in plasma of normal and CCl₄-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nM/mL)</th>
<th>Hydroperoxides (values x 10⁻⁴ M/mL)</th>
<th>GSH (mg/dL)</th>
<th>Vitamin C (mg/dL)</th>
<th>Vitamin E (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.14 ± 0.01⁵</td>
<td>7.13 ± 0.52⁵</td>
<td>23.25 ± 1.89⁵</td>
<td>1.70 ± 0.88⁵</td>
<td>1.33 ± 0.09⁵</td>
</tr>
<tr>
<td>Normal + NAC</td>
<td>0.11 ± 0.01⁵</td>
<td>6.34 ± 0.33⁵</td>
<td>24.30 ± 1.64⁵</td>
<td>1.88 ± 0.92⁵</td>
<td>1.46 ± 0.09⁵</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.52 ± 0.06⁶</td>
<td>28.64 ± 1.84⁶</td>
<td>10.71 ± 1.29⁶</td>
<td>0.81 ± 0.10⁶</td>
<td>0.67 ± 0.04⁶</td>
</tr>
<tr>
<td>CCl₄ + NAC</td>
<td>0.22 ± 0.04⁴</td>
<td>13.58 ± 1.30⁴</td>
<td>20.43 ± 2.17⁴</td>
<td>1.52 ± 0.74⁴</td>
<td>1.14 ± 0.08⁴</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p < 0.05.
Table 3. Effect of NAC on TBARS and hydroperoxides in the tissues of normal and CCl₄-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (mM 100/g tissue)</th>
<th>Kidney (mM 100/g tissue)</th>
<th>Liver (mM 100/g wet tissue)</th>
<th>Kidney (mM 100/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.66±0.04⁴</td>
<td>1.13±0.08⁴</td>
<td>83.81±7.09⁴</td>
<td>69.08±6.31⁴</td>
</tr>
<tr>
<td>Normal + NAC</td>
<td>0.61±0.04⁴</td>
<td>1.37±0.08⁴</td>
<td>88.83±6.27⁴</td>
<td>65.50±6.07⁴</td>
</tr>
<tr>
<td>CCl₄</td>
<td>3.80±0.24⁴</td>
<td>5.27±0.44⁴</td>
<td>249.07±22.61⁴</td>
<td>209.51±18.18⁴</td>
</tr>
<tr>
<td>CCl₄ + NAC</td>
<td>1.22±0.07⁴</td>
<td>2.83±0.20⁴</td>
<td>112.67±8.07⁴</td>
<td>97.68±7.44⁴</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p < 0.05.

Table 4. Effect of NAC on the activities of superoxide dismutase and catalase in the tissues of normal and CCl₄-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units/mg protein)</th>
<th>Catalase (umole of H₂O₂ consumed min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal</td>
<td>15.64±1.29⁴</td>
<td>61.31±4.23⁴</td>
</tr>
<tr>
<td>Normal + NAC</td>
<td>16.94±1.31⁴</td>
<td>62.65±5.04⁴</td>
</tr>
<tr>
<td>CCl₄</td>
<td>6.17±5.32⁴</td>
<td>44.36±4.97⁴</td>
</tr>
<tr>
<td>CCl₄ + NAC</td>
<td>13.90±1.04⁴</td>
<td>57.61±4.17⁴</td>
</tr>
</tbody>
</table>

SOD units: Enzyme concentration required to inhibit the O.D at 560nm of chromogen production by 50% in 1 min. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p < 0.05.
convert active oxygen molecules into non-toxic compounds. CCl₄-administration decreased the activities of these antioxidant enzymes and GSH concentration in production by providing more substrate for reactive the tissues. Oral administration of NAC restored the intermediates that promote detoxification mechanisms activities of these enzymes and glutathione in rats. This also may be the reason for the restoration of treated with CCl₄. NAC contributes significantly to the other antioxidant enzymes such as SOD and catalase.

intracellular antioxidant defense system by acting as a powerful consumer of superoxide, singlet oxygen and provided supporting evidence for our study. Rats treated hydroxyl radicals. NAC induces its beneficial effect mainly through scavenging free radicals and preserving the integrity of the membranes.

hepatic GSH depletion as well it can slow the decrease. The overall results of our study confirm the protective
Table 5. Effect of NAC on the concentration of glutathione and glutathione peroxidase in the tissues of normal and CCl4-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mM 100/g tissue)</th>
<th>Glutathione peroxidase (µg of GSH consumed min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal</td>
<td>160.30±12.23*a</td>
<td>132.85±12.46*a</td>
</tr>
<tr>
<td>Normal + NAC</td>
<td>168.18±14.60*a</td>
<td>139.67±11.49*a</td>
</tr>
<tr>
<td>CCl4</td>
<td>80.98±9.04*a</td>
<td>68.46±7.11*a</td>
</tr>
<tr>
<td>CCl4 + NAC</td>
<td>142.37±11.67*a</td>
<td>127.48±10.28*a</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a,b,c) differ significantly at p < 0.05.

Acknowledgement

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References

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