IJPT
1735-2657/05/42-118-123
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 4:118-123, 2005

RIDR

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

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Received August 12, 2005; Revised October 29, 2005; Accepted November 5, 2005

This paper is available online at *http://ijpt.iums.ac.ir*

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 (CCl_4) (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, $CCl_3^{\cdot}$ through the action of the mixed function cytochrome P450 oxygenase system of the endoplasmic reticulum [2]. The CCl_3 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 CCl_3 radical is converted to the trichloromethyl peroxy radical ($CCl_3OO^{\cdot}$). This radical is more reactive and is capable of abstracting hydrogen from polyunsaturated fatty acids (PUFA) to initiate the process of lipid peroxidation [3].

Modulation of cellular thiols has been used to protect the hepatocytes against attack by reactive oxygen intermediates and is currently being investigated as a novel therapeutic strategy in different liver pathologies. 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 CCl_4-induced toxicity in patients [9-11] and in rats [12-15]. But there are no detailed reports on the antioxidant defense of NAC in CCl_4-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 CCl_4-induced hepatotoxicity in rats.

Table 1. Effect of NAC on the activities of marker enzymes in plasma of normal and CCl_4-treated rats.

Groups	ALP (IU/L)	GGT (IU/L)	AST (IU/L)
Normal	72.61±5.13[a]	0.57±0.04 [a]	73.12±6.47[a]
Normal + NAC	70.18 ±7.49[a]	0.54±0.06[a]	71.24±5.33[a]
CCl_4	193.1±16.28[b]	1.65±0.11[b]	139.0±9.42[b]
CCl_4 + NAC	89.04±5.44[c]	0.70±0.04[c]	83.39±5.49[a]

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

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. CCl_4 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 CCl_4 (3 mL/kg body weight/week) [17].
- Group IV. Rats orally administered with NAC (150 mg/kg body weight) along with subcutaneous injection of CCl_4 (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. After the last treatment, the animals were fasted overnight and killed by cervical dislocation. Blood was collected in heparinised tubes. Plasma was separated and used for various biochemical estimations. Liver and kidney were collected in ice-cold containers, washed with saline, homogenised with appropriate buffer and used for various estimations.

In plasma, the levels of marker enzymes such as AST [18], ALP [19], GGT [20] and the levels of TBARS [21], hydroperoxides [22], GSH [23], vitamin C [24] and vitamin E [25] were estimated by standard procedures.

In liver and kidney, the concentration of TBARS [21], HP [22], GSH [23], superoxide dismutase [26], catalase [27] and glutathione peroxidase [28] were also estimated.

For histopathological studies, livers from animals of different groups were perfused with 10% neutral formalin solution. Paraffin sections were made and stained using hematoxylin-eosin (H&E) stain. After staining, the sections were observed under light microscope and photographs were taken.

Statistical Analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± S.D. for 6 rats in each group. p-Values < 0.05 were considered as significant.

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 CCl_4-induced rats is presented in Table 1. A significant increase in the activities of these marker enzymes were observed in CCl_4-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 CCl_4-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 CCl_4-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 CCl_4-administered rats (Table 3). Oral administration of NAC significantly decreased the concen-

Table 2. Effect of NAC on the levels of TBARS and hydroperoxides and nonenzymic antioxidants in plasma of normal and CCl_4-treated rats.

Groups	TBARS (nM/mL)	Hydroperoxides (values × 10^{-5} mM/dL)	GSH (mg/dL)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
Normal	0.14 ± 0.01[a]	7.13 ± 0.52[a]	23.25 ± 1.89[a]	1.70 ± 0.88[a]	1.33 ± 0.09[a]
Normal + NAC	0.11 ± 0.01[a]	6.34 ± 0.33[a]	24.30 ± 1.64[a]	1.88 ± 0.92[a]	1.46 ± 0.09[a]
CCl_4	0.52 ± 0.06[b]	28.64 ± 1.84[b]	10.71 ± 1.29[b]	0.81 ± 0.10[b]	0.67 ± 0.04[b]
CCl_4 + NAC	0.27 ± 0.04[c]	13.58 ± 1.30[c]	20.43 ± 2.17[a]	1.52 ± 0.74[c]	1.14 ± 0.08[c]

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 CCl_4-treated rats.

Groups	TBARS (mM 100/g tissue)		Hydroperoxides (mM 100/g wet tissue)	
	Liver	Kidney	Liver	Kidney
Normal	0.66±0.04[a]	1.13±0.08[a]	83.81±7.09[a]	69.08±6.31[a]
Normal + NAC	0.61±0.04[a]	1.37±0.08[a]	88.83±6.27[a]	65.50±6.07[a]
CCl_4	3.80±0.24[b]	5.27±0.44[b]	249.07±22.61[b]	209.51±18.18[b]
CCl_4 + NAC	1.22±0.07[c]	2.83±0.20[c]	112.67±8.07[c]	97.68±7.44[c]

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

tration of TBARS and hydroperoxides in all the tissues.

Effect of NAC on Tissue Enzymic Antioxidants

Table 4 presents the activities of antioxidant enzymes (superoxide dismutase [SOD] and catalase) in normal and CCl_4-treated rats. The activities of these enzymes were significantly decreased in the tissues of CCl_4-administered rats. On treatment with NAC, the decreased activities of these enzymes were brought back to near normal.

Table 5 shows the concentration of GSH and the activity of glutathione peroxidase in the tissues of CCl_4-administered rats. The concentration of GSH and the glutathione peroxidase (GPx) activity were found to be decreased upon CCl_4 administration. Oral administration of NAC restored the changes brought about by the administration of CCl_4.

Histological Examination of the Liver

Histopathological examination of the liver sections from normal rats showed normal parenchymal architecture (Fig 1-A). The liver of rats treated with NAC alone did not show any noticeable alterations (Fig 1-B). In rats treated with CCl_4 alone, the liver sections showed thickening of blood vessels (Fig 1-C) and microvesicular fatty changes around portal triad (Fig 1-D). In rats treated with CCl_4 + NAC, only mild sinusoidal dilatation was observed (Fig 1-E).

Oral administration of NAC to normal rats did not show significant effect in any of the parameters studied.

DISCUSSION

Carbon tetrachloride induced lipid peroxidation results in changes of structures of the endoplasmic reticulum and other membranes, loss of metabolic enzyme activation and reduction of protein synthesis leading to liver damage [29]. In this study, CCl_4 administration to rats lead to a marked elevation in the levels of plasma AST, GGT and ALP. This might be due to the release of these enzymes from the cytoplasm, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage [30]. Treatment with NAC significantly reduced the levels of these marker enzymes in CCl_4 treated rats. This implies that NAC tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels.

Lipid peroxidation as well as altered levels of some endogenous scavengers are taken as indirect *in vivo* reliable indices for oxidative stress [31]. Increased levels of TBARS and hydroperoxides were observed in plasma and tissues of CCl_4-treated rats. Lowered levels of TBARS and hydroperoxides by oral administration of NAC could be related to its antioxidant capacity to scavenge reactive oxygen species. NAC contains free sulfhydryl groups and it may directly react with electrophilic compounds such as free radicals [32].

Excessive liver damage and oxidative stress caused by CCl_4 depleted the levels of GSH, vitamin C and vitamin E in our study. Oxidative stress induced by CCl_4 results in the increased utilisation of GSH and subsequently the levels of GSH is decreased in plasma and tissues. Utilisation of vitamin E is increased when oxidative stress is induced by CCl_4 and this shows the protective role of vitamin E in mitigating the elevated oxidative stress. Vitamin C scavenges and destroys free radicals in combination with vitamin E and glutathione [33]. It also functions cooperatively with vitamin E by regenerating tocopherol from the tocopheroxyl radical [34]. A decrease in the levels of vitamin C may indicate increased oxidative stress and free radical formation in CCl_4-induced liver injury.

N-acetyl cysteine treatment effectively restored the depleted levels of these nonenzymic antioxidants. NAC could significantly interfere with the pathophysiology of free radical producing drug induced oxidative stress [5]. Wong et al. have reported the ability of NAC in regulating GSH concentration and thus protect liver damage from reactive metabolites formed from CCl_4 [15]. Increase in GSH levels could also contribute to the recycling of other antioxidants such as vitamin E and vitamin C [35].

A major defense mechanism involves the antioxidant enzymes including SOD, catalase and GPx which

Table 4. Effect of NAC on the activities of superoxide dismutase and catalase in the tissues of normal and CCl_4-treated rats.

Groups	SOD (Units/mg protein)		Catalase (μmole of H_2O_2 consumed/min/mg protein)	
	Liver	Kidney	Liver	Kidney
Normal	15.64±1.29[a]	16.11±1.07[a]	61.31±4.23[a]	64.64±5.25[a]
Normal + NAC	16.94±1.31[a]	16.81±1.39[a]	57.62±5.04[a]	63.20±5.99[a]
CCl_4	6.17±5.32[b]	9.06±0.85[b]	44.36±4.97[b]	38.06±4.06[b]
CCl_4 + NAC	13.90±1.04[c]	15.22±1.41[c]	57.61±4.17[a]	59.81±5.24[c]

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 $p < 0.05$.

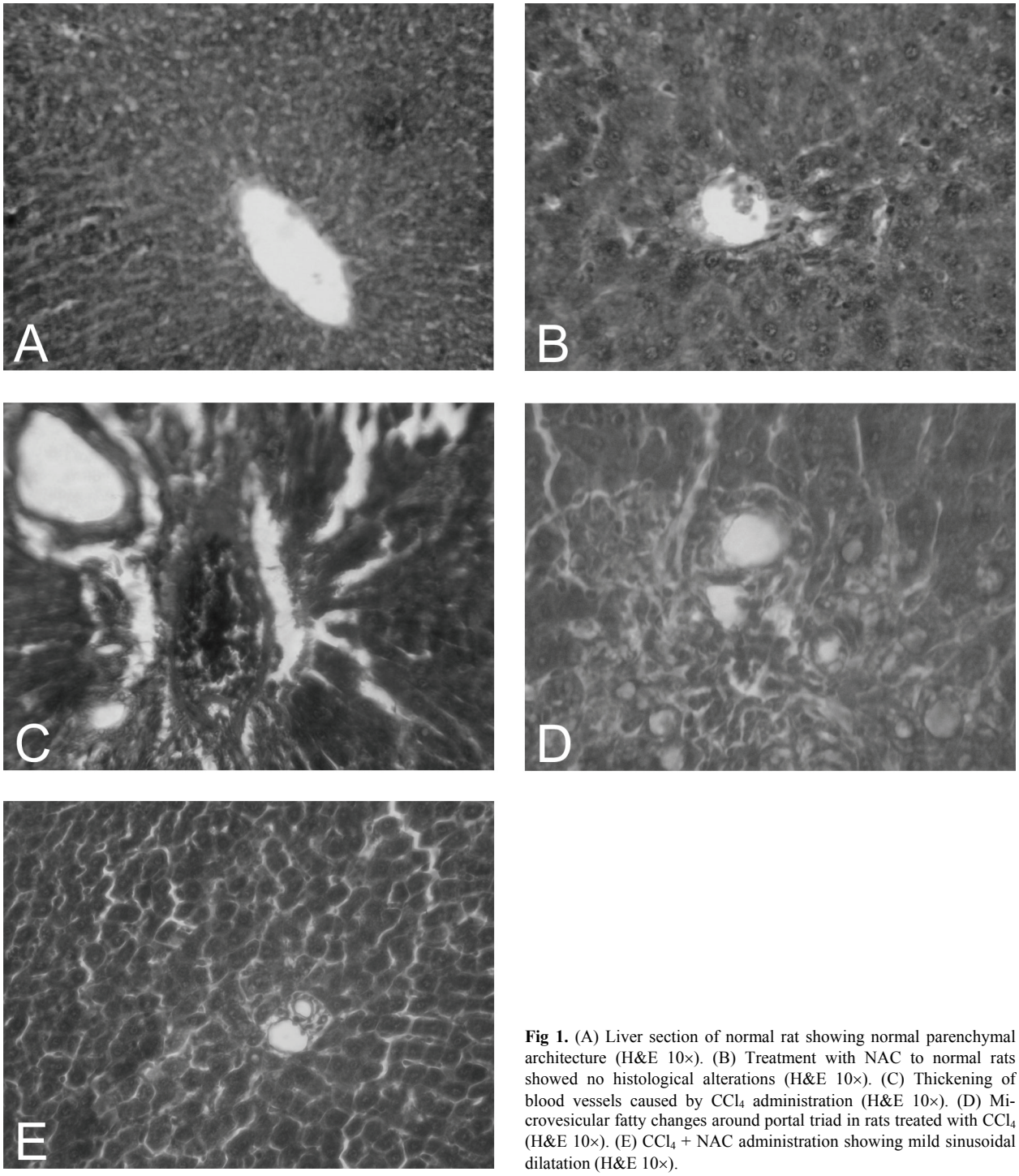

Fig 1. (A) Liver section of normal rat showing normal parenchymal architecture (H&E 10×). (B) Treatment with NAC to normal rats showed no histological alterations (H&E 10×). (C) Thickening of blood vessels caused by CCl_4 administration (H&E 10×). (D) Microvesicular fatty changes around portal triad in rats treated with CCl_4 (H&E 10×). (E) CCl_4 + NAC administration showing mild sinusoidal dilatation (H&E 10×).

convert active oxygen molecules into non-toxic compounds.CCl_4-administration decreased the activities of these antioxidant enzymes and GSH concentration in the tissues. Oral administration of NAC restored the activities of these enzymes and glutathione in rats treated with CCl_4. NAC contributes significantly to the intracellular antioxidant defense system by acting as a powerful consumer of superoxide, singlet oxygen and hydroxyl radicals [36].

NAC induces its beneficial effect mainly through maintaining –SH groups of enzymes and membrane proteins in the reduced state [37]. NAC can prevent the hepatic GSH depletion as well it can slow the decrease of hepatic GSH. The hepatoprotective effects of NAC may also be due to its ability to enhance glutathione production by providing more substrate for reactive intermediates that promote detoxification mechanisms [38]. This also may be the reason for the restoration of other antioxidant enzymes such as SOD and catalase.

Histopathological examination of the liver also provided supporting evidence for our study. Rats treated with NAC reduced the damage caused by CCl_4 administration. This clearly indicates the membrane stabilizing effect of NAC by scavenging free radicals and preserving the integrity of the membranes.

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 CCl_4-treated rats.

Groups	GSH (mM 100/g tissue)		Glutathione peroxidase (µg of GSH consumed/min/mg protein)	
	Liver	Kidney	Liver	Kidney
Normal	160.30±12.23[a]	132.85±12.46[a]	10.65±0.85[a]	8.93±0.71[a]
Normal + NAC	168.18±14.60[a]	139.67±11.49[a]	11.12±0.94[a]	9.10±0.87[a]
CCl_4	80.98±9.04[b]	68.46±7.11[b]	3.84±0.30[b]	4.11±0.44[b]
CCl_4 + NAC	142.37±11.67[c]	127.48±10.28[c]	8.90±0.81[c]	7.78±0.62[c]

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

effect of N-acetyl cysteine in CCl_4-induced hepatotoxicity in rats by its ability to stabilize cell membranes, scavenge free radicals and antioxidant properties. The present investigation has also confirmed the usefulness of NAC as an effective hepatoprotectant.

ACKNOWLEDGEMENT

We thank UGC for sanctioning a project. The first author is a Junior Research Fellow in the project.

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