Indian Journal of Experimental Biology
Vol. 40, February 2002, pp. 181-186

Protective effect of N-acetylcysteine against gamma ray induced damages in rats – Biochemical evaluations

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Received 20 July 2001; revised 11 October 2001

The effect of N-acetylcysteine (NAC) (1g/kg body weight in saline for 7 days) against the damages induced by gamma ray was studied. Whole body exposure of rats to γ-rays (3.5 Gy) caused increases in lipid peroxides ($P<0.01$). Reduced glutathione (GSH) ($P<0.01$) and total sulphydryl groups (TSH) ($P<0.05$), were found to be increased probably to counteract the damages produced by the lipid peroxides. The plasma antioxidant vitamins E, C and A were reduced. The activities of antioxidant enzymes, superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) were enhanced, which might be to eliminate the superoxide radical and H_2O_2 and accompanied by a fall in glutathione-s-transferase (GST) and glutathione reductase (GR) activity. The excessive production of free radicals and lipid peroxides might have caused the leakage of cytosolic enzymes such as aminotransferases (AST and ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and phosphatases. Membrane damage is quite evident from histological studies undertaken in the intestinal tissue, which is susceptible to radiation damage. Intragastric pretreatment of NAC (1g/kg body weight in saline for 7 days) prevented the radiation induced damage to an appreciable extent. From the results it may be concluded that NAC is effective in protecting from the damages caused by γ-ray radiations and its prospects as an adjuvant to radiotherapy should be considered.

The interaction of ionizing radiation with biological systems results in generation of free radicals, H and OH radicals, H_2 and H_2O_2[1]. Radiation induced free radicals in turn impair the antioxidant defense mechanism leading to an increased membrane lipid peroxidation which results in the damage of the membrane structure and inactivation of membrane bound enzymes[2]. Antioxidant enzymes are among the endogenous systems that are available for the removal or detoxification of these free radicals and their products formed by ionizing radiation[3].

Endogenous cellular thiol-dependent enzymes play an important role in the radiation response. Many of the thiol-dependent enzymes depend on reduced Glutathione (GSH), in particular Glutathione-S-transferase (GST) and Glutathione peroxidase (GPx) are important against radiation damage[4]. Hence, post irradiation damage to normal tissue might be prevented by the administration of GSH and endogenous thiols, which may involve induction, or activation of antioxidant enzymes[5].

Thiols are among the best radioprotectors and are thought to protect DNA irradiated in aqueous solutions by two mechanisms (1) scavenging of hydroxy radicals and (2) chemical repair of DNA radicals[6]. Biological compounds containing thiol groups are essential for the maintenance of cellular structure and function. GSH is the most studied and abundant endocellular aminothiol, which plays a key role in physiological detoxication.

NAC (N-Acetylcysteine), a synthetic aminothiol possesses antioxidative and cytoprotective properties. It exerts a protective effect on DNA and cytoplasmic enzyme activities from the damaging activity of X-rays[6,7]. It also forms disulphide bridges with protein sulphydryl groups and protects the membrane proteins against oxidative and destructive effects[8].

The present study was focussed to investigate the effect of NAC on the perturbances in the structure of the intestinal membrane, activities of antioxidant enzymes in liver cells, the leak of cytosolic enzymes and level of antioxidants in blood serum and plasma during gamma ray induced damage in rats.

Materials and Methods

Chemicals—N-Acetylcysteine, epinephrine, 1-chloro-2,4-dinitrobenzene (CDNB), 2-thiobarbituricacid, tetraphenyl butadiene, reduced glutathione (GSH), oxidized glutathione (GSSG), Nicotinamide adenine dinucleotide phosphate (NADP) and glucose-6-phosphate were purchased from Sigma chemical company, St. Louis, MO. USA. Other chemicals were of analytical grade.

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Animals—Wistar adult male albino rats weighing 150-200 g were purchased from Fredrick Institute of Plant Protection and Toxicology, Padappai, India. The animals were housed in well aerated room and maintained on rat pellet diet (Lipton India Animal Feed, Bangalore) and water *ad libitum*. The animals were divided into four groups. Each group constituted of six animals.

Group I : Control rats receiving 1.0ml saline intragastrically for 7 days.

Group II : Rats receiving N-Acetylcysteine in saline for 7 days (1g/kg body weight) intragastrically.

Group III : Rats receiving 1.0ml saline intragastrically for 7 days and exposed to γ-ray radiation (7.5 Gy) on the 8th day.

Group IV : Rats receiving N-Acetylcysteine in saline for 7 days (1g/kg body weight) intragastrically and exposed to γ-ray radiation (3.5 Gy) on 8th day.

Source of radiation—The animals were exposed to whole body radiation at MERADO LABS, CSIR Complex, Taramani, Chennai-600 113. γ-ray was given at a dose of 0.23 Gy/min from the source ^{60}Co at 6 cm distance for 15 min.

Blood and liver preparation—After 24 hr post irradiation, the animals were killed by cervical dislocation. Blood was collected in heparinized and non-heparinized tubes to separate plasma and serum respectively. The liver was removed quickly and placed in chilled 0.9% saline solution. A known weight was quickly weighted and then homogenized in ice-cold 0.1*M* Tris-HCl buffer (*pH* 7.4) at 4°C in a Potter Elvehjem Homogenizer with a teflon pestle to give a 10% homogenate. The homogenization procedure was performed as quickly as possible under standard conditions. The homogenate was centrifuged and the supernatant was kept on ice for the enzyme assays.

A portion of intestine was removed, washed with saline and was preserved in 10% formal saline for histological examination.

Biochemical analysis—Total protein was estimated by Lowry's method using bovine serum albumin as a standard. In liver homogenate, the levels of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and total sulphydryl groups (TSH) were estimated by the method of Okhawa *et al.*[9] Moron *et al.*[10] and Sedlack and Lindsay[11] respectively. Superoxide dismutase (SOD) was assayed according to Misra and Fridovich[12] based on the inhibition of epinephrine auto-oxidation by the enzyme. Catalase activity was measured by following the breakdown of hydrogen peroxide as substrate according to the method of Claiborne *et al.*[13]. Activities of Glutathione peroxidase (GPx)[14], Glutathine-s-transferase (GST) [15], Glutathione reductase (GR) [16] and Glucose-6-phosphate dehydrogenase (G6PD)[17] were also assayed.

Activities of serum pathophysiological enzymes such as aspartate aminotransferase (AST)[18], alanine aminotransferase (ALT)[18], lactate Dehydrogenase (LDH)[19], creatine kinase (CK)[20], alkaline phosphatase (ALP) [21], and acid phosphatase (ACP) [21]were also determined in the liver homogenate. Estimation of antioxidants in plasma such as vitamin A, vitamin C and vitamin E were done by the method of Bessey *et al.*[22] Kyaw[23] and Varley *et al.*[24] respectively.

Statistical analysis—Results were expressed as mean ± SD and statistical analysis by use of Student's *t* test was performed to determine significant differences between groups.

Results

Lipid peroxidation, antioxidants and antioxidant enzymes in liver and plasma of experimental rats—Whole body γ-ray irradiation causes an increase in the amount of lipid peroxidation products ($P<0.01$), GSH ($P<0.01$) and TSH ($P<0.05$) (Table 1). Intragastric administration of NAC (1g/kg body weight) suppressed the rate of lipid peroxidation and conserved the GSH and TSH in the system. A decrease in the plasma vitamins E, C and A levels, which was observed in animals after irradiation, was maintained at near normal levels with NAC pretreatment ($P<0.001$) (Table 2).

The activities of antioxidant enzymes SOD, catalase and GPx was more enhanced, whereas a fall in the activities of GST and GR was observed in liver tissue of irradiated animals. Significant restoration of the activities of all the above enzymes ($P<0.001$) was seen in the NAC supplemented group (Table 3).

Activities of pathophysiological enzymes in experimental rats—Whole body irradiation elevated the levels of the pathophysiological enzymes ACP, ALP, aminotransferases, LDH and CK in liver and serum. Pre-administration of NAC protected the damages by decreasing the activities of all these enzymes ($P<0.001$) (Table 4).

Histological analysis on intestinal tissues of experimental animals—The radioprotection offered by NAC was observed in the intestine of the pretreated

animals than the animals without supplementation of NAC (Fig. 1).

Discussion

Ionizing radiation is toxic to organisms since it induces deleterious structural changes in essential macromolecules[25]. The whole body irradiation in our study increased the lipid peroxide levels in the circulation that led to an increase in the free radicals and a decrease in antioxidant potential of the liver after irradiation, which may be the reason for the membrane peroxidation[26]. Enhanced GSH[27] and TSH levels were seen in NAC pretreated animals which offer protection to the irradiated animals by scavenging the free radicals and decreasing lipid peroxidation thereby, facilitating the animals to tolerate the insult of radiation damage. Similar reports also suggest that there was enhanced synthesis of GSH in liver due to radiation[28], which affirms the present study. These findings indicated that glutathione synthesis in the liver is induced by radiation to eliminate free radicals as a compensatory mechanism.

The enhanced generation of reactive oxygen species, as in case of radiation, results in 'oxidative

Table 1—Effect of N-acetylcysteine on the levels of lipid peroxides, reduced glutathione and total sulphydryl groups in the liver of rats irradiated with γ-ray

[Values are mean ± SD for six animals in each group]

Parameters	Group I (Normal)	Group II (NAC treated)	Group III (γ-ray)	Group IV (NAC + γ-ray)
Lipid peroxides (nmole of TBARS/mg protein)	153.52 ± 7.8	144.24 ± 10.2^{NS}	168.21 ± 12.3**	148.02 ±10.4^{NS++}
Reduced glutathione (nmoles/g wet tissue)	8.21 ± 0.66	10.82 ± 0.78***	9.02 ± 0.4**	8.11 ± 0.62^{NS++}
Total sulfhydryl groups (TSH) (μg/mg protein)	5.02 ± 0.37	6.98 ± 0.32***	5.39 ± 0.31*	4.99 ± 0.28^{NS**}

*Statistical significant variations when compared to control
+Statistical significant variations when compared to group IV Vs group III
P values: +/*- <0.05; ++/**- <0.01; +++/***- <0.001; NS- Non significant

Table 2—Effect of N-acetylcysteine on the levels of plasma vitamins E, C and A in rats irradiated with γ-ray

[Values are mean ± SD for six animals in each group]

Parameters	Group I (Normal)	Group II (NAC treated)	Group III (γ-ray)	Group IV (NAC+γ-ray)
Vitamin E (mg/dl)	6.92 ± 0.35	8.34 ± 0.50***	3.93 ± 0.21***	6.53 ±0.51^{NS+++}
Vitamin C (mg/dl)	6.72 ± 0.48	6.95 ± 0.50^{NS}	5.92 ± 0.21***	6.91 ± 0.46^{NS+++}
Vitamin A (mg/dl)	19.3 ± 1.3	21.6 ± 1.6**	13.1 ± 0.97***	19.7 ± 1.2^{NS+++}

*Statistical significant variations when compared to control
+Statistical significant variations when compared to group IV Vs group III
P values: +/*- <0.05; ++/**- <0.01; +++/***- <0.001; NS-Non significant

Table 3—Effect of N-acetylcysteine on the activities of antioxidant enzymes in the liver of rats irradiated with γ-ray

[Values are mean ± SD for six animals in each group]

Parameters	Group I (Normal)	Group II (NAC treated)	Group III (γ-ray)	Group IV (NAC+γ-ray)
Superoxide dismutase (Units/mg ptn)	5.26 ± 0.25	5.09 ± 0.31^{NS}	6.77 ± 0.28***	5.21 ±0.31^{NS++}
Catalase (nmoles of H_2O_2 decomposed/min/mg ptn)	56.6 ± 3.51	53.2 ± 4.27^{NS}	61.4 ± 2.98**	57.1 ± 2.32^{NS++}
Glutathione peroxidase (nmole of GSH oxidized/min/mg ptn)	58.05 ± 3.39	60.12 ± 4.72^{NS}	63.03 ± 3.73*	58.14 ± 4.81^{NS+}
Glutathione-S-transferase (nmole of CDNB) conjugated/min/mg ptn)	2.25 ± 0.18	2.10 ± 0.15^{NS}	2.01 ± 0.07**	2.23 ± 0.14^{NS+++}
Glutathione reductase (nmole of GSSG reduced/hr/mg ptn)	40.6 ± 2.1	43.5 ± 2.8*	37.8 ± 2.9*	38.9 ± 2.3^{NS}

*Statistical significant variations when compared to control
+Statistical significant variations when compared to group IV Vs group III.
P values: +/*- <0.05; ++/**- <0.01; +++/***- <0.001; ^{NS}- Non significant

stress'[29] which was observed in the present study with a poor antioxidant status in the plasma of irradiated animals. Radioprotection was offered by NAC with an increase in the tocopherol, ascorbic acid and vita-

Table 4—Effect of N-acetylcysteine on the activities of acidphosphatase and alkalinephosphatase in liver, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and creatine kinase in serum of rats irradiated with γ-ray

[Values are mean ± SD for six animals in each group]

Parameters	Group I (Normal)	Group II (NAC treated)	Group III (γ-ray)	Group IV (NAC+γ-ray)
ACP (μmoles of phenol liberated/min/mg protein)	4.71 ± 0.26	4.62 ± 0.32^{NS}	$5.13 \pm 0.32^{**}$	$4.51 \pm 0.28^{NS+++}$
ALP (μmoles of phenol liberated/min/mg protein)	9.68 ± 0.44	9.32 ± 0.64^{NS}	$10.71 \pm 0.91^{**}$	$9.01 \pm 0.83^{NS+++}$
ALT (IU/1)	12.8 ± 0.93	12.6 ± 1.11^{NS}	$14.1 \pm 0.63^{**}$	$12.4 \pm 0.53^{NS+++}$
AST (IU/1)	22.9 ± 1.09	22.6 ± 1.42^{NS}	$24.3 \pm 1.06^{**}$	$22.0 \pm 1.23^{NS+++}$
LDH (IU/1)	131.02 ± 7.9	130.52 ± 10.9^{NS}	$148.21 \pm 7.5^{***}$	$128.63 \pm 8.3^{NS+++}$
CK (IU/1)	304 ± 17.2	296 ± 14.5^{NS}	$397.4 \pm 21.5^{***}$	$312.3 \pm 21.8^{NS+++}$

*Statistical significant variations when compared to control
+Statistical significant variations when compared to group IV Vs group III
P values: +/*- <0.05; ++/**- <0.01; +++/***- <0.001; NS- Non significant

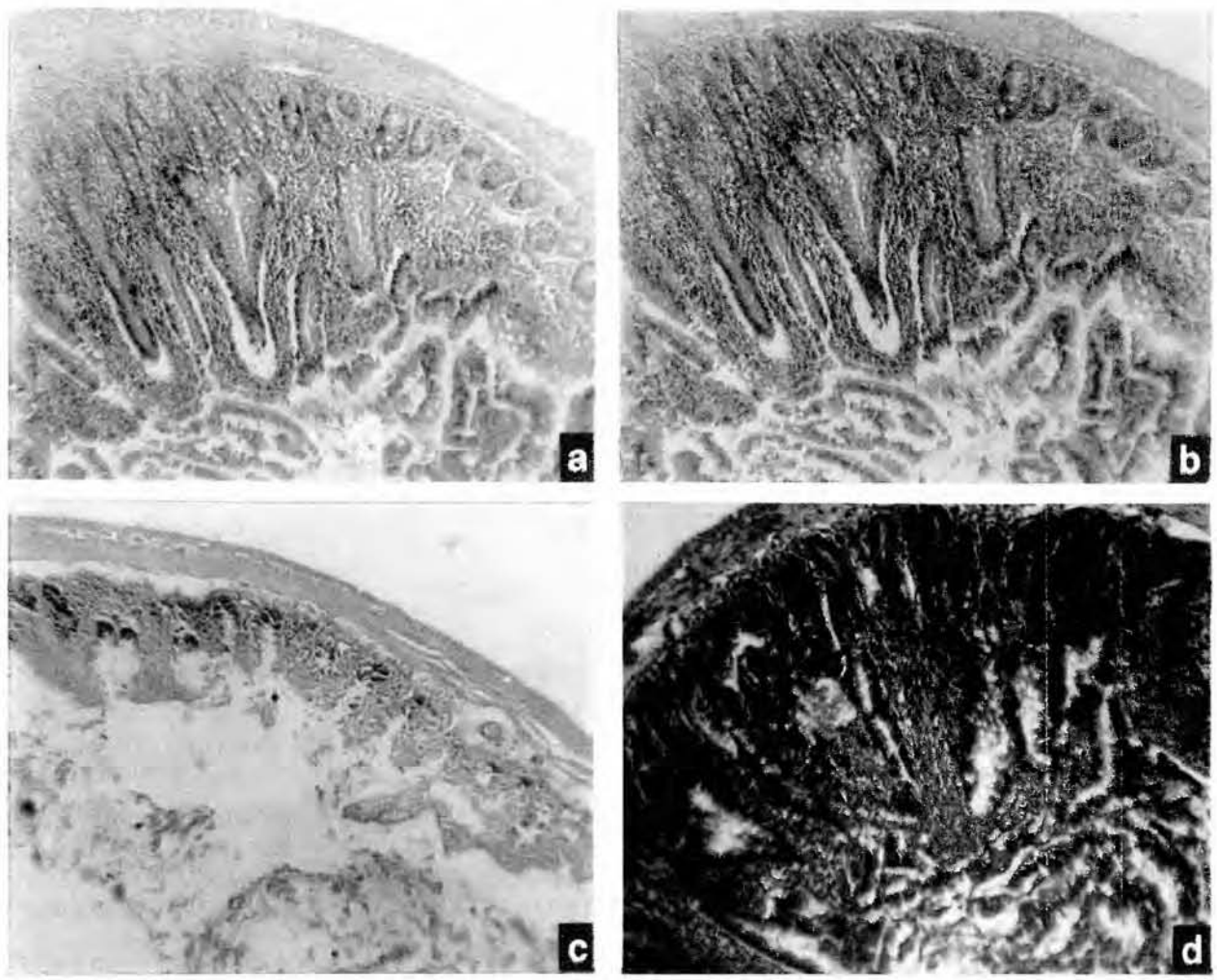

Fig. 1—Section of intestine (H&E × 100) (a) From control rats showing normal architecture; (b) From NAC treated rats showing architecture; (c) From γ-ray irradiated rats revealing destruction of villi and crypts; (d) From NAC pretreated and γ-ray irradiated rats showing extensive protection to intestinal villi and crypts.

min A levels with a reduction in the production of lipid peroxides as compared to normal controls, whereas the radiation exposed animals showed depleted levels of these vitamins. Since vitamin E and glutathione are involved in the termination mechanisms of lipid peroxidation, the rate of reaction of vitamin E with lipid hydroperoxy radicals is much faster than the rate of reaction of lipid hydroperoxy radicals with neighbouring polyunsaturated fatty acids, thus preventing further damage as lipid radical scavenger[30].

It has been reported that low dose and high doses of radiation caused SOD activity to be increased inorder to eliminate superoxide radical in the liver[31] and the resulting H_2O_2 has a higher reactivity which caused the increased activity of GPx and catalase that eliminates H_2O_2. In the present study, irradiation increased the SOD, catalase, and GPx activities in the liver. The circulatory highly reactive H_2O_2 produced by radiation propagated and forwarded other free radicals, which caused cell damage. In order to protect from this lethal damage the liver synthesized more endogenous GSH, thereby increasing the activities of GPx and catalase. Thus, catalase plays a dual role in providing radiation protection and detoxifying enzymatically generated H_2O_2[32].

Cellular GPx and GR are a part of redox system of glutathione and remove hydrogen peroxide generated by SOD in cytosol and mitochondria[33] by oxidizing the tripeptide glutathione (GSH) in to the oxidised form GSSG. The influence of radiation in the animals was well reflected in our study by decreased levels of GST and GR, which are indicative of oxidative damage.

The hepatocellular damage due to radiation were quite evident from the pronounced activities of aminotransferases, lactate dehydrogenase, phosphatases and creatine kinase, which are all indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver and erythrocytes[34]. In the present study, the most intensive ultrastructural changes were observed after 24hr of irradiation. The time of these changes was consistent with the time of an increase in liver lipid peroxidation products. This was the reason for the increase in cell membrane permeability and leakage of enzymes from cells into the intercellular space and into the blood. Serum lactate dehydrogenase concentration was found to be directly proportional to the extent of radiation injury. The activities of serum aminotransferases are a measure of the integrity of the cells[35] since the animals were exposed to whole body radiation, the damage was found to act directly on the skeletal muscles to enhance the leakage of creatine kinase, especially of the slow twitch oxidative type that repeated the muscle stress, which potentiated the radiation effect. Treatment of animals with NAC enhanced the GSH stores in the lives thereby protecting it from the damage caused by radiation exposure. Therefore the levels of the pathophysiological enzymes were maintained at near normal levels.

Histological studies show damage to the intestine because of its susceptibility to radiation. Thus radiation damaged the intestinal villi and crypts[36].

The results, which were obtained in the present investigation, indicate the protective effect of N-acetylcysteine against damages produced by γ-radiation due to the generation of free radicals and consequently to subsequent peroxidative disintegration of cell membranes. Since N-acetylcysteine metabolism is related to reduced glutathione, at least part of the beneficial effect of N-acetylcysteine may be ascribed to the inhibition of lipoperoxidative processes and restoration of the membrane properties, which would further add to the protective effect of N-acetylcysteine[37].

Acknowledgement

The authors are thankful to Dr. Ramakrishnan, Director, MERADO Labs, Department of Radiation, CSIR Complex, Taramani, Chennai for providing radiation facilities.

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