The Protective Role Of N-Acetylcysteine Against Acrylamide-Induced Genotoxicity And Oxidative Stress In Rats

Research Article

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**Summary**

Acrylamide is neurotoxic, genotoxic and highly carcinogenic in humans and animals. The aim of this study was to research the protective role of N-acetylcysteine against acrylamide-induced genotoxicity and oxidative stress in rats. In the present study, male wistar albino rats were divided into four groups, each containing 10 as follows: Control (C) group, Acrylamide (AA) group, N-acetylcysteine (NAC) group, Acrylamide + N-acetylcysteine (AA+NAC) group. At the end of 21 days, Malondialdehyde (MDA) and Glutathione (GSH) levels were measured in plasma and DNA damage was observed in lymphocytes. Plasma GSH level decreased significantly in AA group compared to C group (P < 0.05). However, GSH level increased significantly in AA+NAC group compared to AA group (P< 0.05). MDA levels increased significantly in AA group compared to C group (P< 0.05). But AA+NAC administration decreased MDA levels as compared to AA group (P< 0.05). Comet analysis results showed that lymphocyte DNAs were normal appearance in C and NAC groups. DNA damage was observed at a highest level in AA group. Furthermore some lymphocytes exhibited apoptotic appearance. However this damage was prevented by NAC administration on lymphocyte DNAs significantly. Our results demonstrated that high level of AA caused oxidative stress and DNA damage, but NAC could have a protective role on acrylamide-induced toxicity.
I. Introduction:
Acrylamide (AA), an α, β unsaturated carbonyl compound with high chemical activity, may lead to cancer and neurotoxicity when human and animal nervous systems are exposed to exceed certain doses of this chemical agent (Shipp A et al, 2006). In the epidemiologic studies on human beings, it has reported that neurotoxicity is advanced extremely in the societies which are exposed to AA into food intensively, but the cancer risk has not been reported (Collins J et al, 1989; Swaen G et al, 2007; Hogervorst JG et al, 2008b).

It has been proven that cooking the foods at high temperatures leads to high level of acrylamide to form and this fact has been an important milestone for the studies related to acrylamide (Tareke et al., 2002; Stadler et al., 2002). AA is neurotoxic, genotoxic and highly carcinogenic in humans and animals. Lee et al. showed that AA induced several neurotoxicity like axonal degeneration in the spinal gracile fasciculus and sciatic nerve (Lee et al, 2005).

The studies have showed that AA and its metabolites are both genotoxic and carcinogenic (Segerback D et al, 1995; Gamboa da Costa G et al, 2003). Upon AA is taken into the body, it is oxidized and converted into glycidamide (GA) which is a genotoxic metabolite (Callemann CJ et al, 1990). The mutagenic effect of AA on human is lower than animals, because the level of GA formation is lower in human (Dearfield KL et al, 1995; Favor J and Shelby MD 2005). International Agency for Research on Cancer (IARC) stated that AA is included in group 2A carcinogen (IARC) (International Agency for Research on Cancer, 1994). IARC reported that AA caused the chromosomal abnormalities and gene mutations on germ and somatic cells of rodent in vivo studies. Moreover, in vitro studies showed that AA caused gene mutations and chromosomal abnormalities in cultured mouse embryonic fibroblast cells (Besaratinia A and Pfeifer GP, 2003). In vitro studies showed that AA reacts with amino groups of adenine and guanine bases of DNA and thus, many different compounds occur (Solomon JJ et al, 1985). It was indicated that this genotoxic effect occurring on DNA mainly resulted from GA, metabolite of AA, rather than AA (Paulsson B et al, 2003). The experimental studies showed that metabolism of AA to GA occured greatly in rats and mice and GA reacted with purine bases of liver, pulmonary and renal DNAs of these animals and caused to genotoxic effects (Segerback D et al, 1995; Gamboa da Costa G et al, 2003).

Oxidative stress is the condition that oxidant/antioxidant balance is spoiled in favor of the oxidants as a result of significant increase of reactive oxygen radicals (ROS) and decrease in antioxidant levels in cells. In this situation, oxidative damages occur in the structural macromolecules of cells such as lipid, protein, carbohydrate and DNA, because ROS cannot be detoxified in an adequate manner (Bast A et al, 1991). It has been showed that oxidative stress is among the main reason of many diseases (Akkuş I, 1995; Speit G et al, 2002).

Reduced glutathione (GSH) is the most important non-enzymatic antioxidant molecule of the mammalian cells. GSH detoxifies ROS in a non-enzymatic way and protects the tissues against the harmful effects of oxidative stress. GSH is present in cells in a free or protein-bound manner. It has been reported that decreased GSH level in human beings has a vital role in pathophysiology of many diseases including cancer, neurodegenerative and cardiovascular diseases (Cotgreave IA and Gerdes RG, 1998; Klatt P and Lamas S, 2000).

Recently, malondialdehyde (MDA) is the most studied parameter in relation to lipid peroxidation in many diseases. The relationship between cancer etiology and lipid peroxidation is the most accurate proof for this
condition. Therefore, MDA is both a biomarker of lipid oxidation and one of the potential causes of cancer. MDA was first described as a carcinogenic by administering topically in rats in 1972 (Shamberger RJ et al, 1974). Many cancer studies emphasized the increasing MDA levels in cancer (Gonenc A et al, 2001; Akbulut H et al, 2003). While AA administration increased MDA levels in many tissues, it also decreased GSH level (Srivastava SP et al, 1983; Yousef MI and El-Demerdash FM, 2006).

NAC is a precursor molecule of GSH. It shows antioxidant effect and eliminates hydrogen peroxide (\(H_2O_2\)), hydroxyl radicals and hypochloric acid (Aruoma OI et al, 1989). The studies have shown that NAC acts as both antioxidant and pro-oxidant in accordance with the dose and application duration. While it protects the rats against oxidative damage when administered in low doses, it may cause lung damage and deaths when applied in high doses (Sprong RC et al, 1998).

In this study, we aimed to examine the protective role of NAC against AA-induced oxidative stress and genotoxicity in rats.

II. Materials and Methods:

A. Experimental Animals

In the present study, 40 male Wistar albino rats (Experimental Research Unit of Inonu University Faculty of Medicine, Malatya, Turkey), weighing between 225-250 g, were housed in a room with a mean constant temperature of 21°C, moisture at the rate of 55-60% and a light/ dark photoperiod of 12:12 (08:00 and 20:00). Animals were fed with standard rat chow and tap water ad libitum. The experiments were performed in accordance with the Guide for the Care and Use of Laboratorary Animals of Inonu University Animal Ethic Committee. At the end of 21 days, MDA and Glutathione GSH levels were measured in plasma and DNA damage was observed in lymphocytes.

B. Experimental Design

Control (C) Group (n=10): 1 mL 0.9 % saline solution was administered via gavage. Acrylamide (AA) Group (n=10): AA (Merck, 800830) dissolved in saline solution [25 mg/kg body weight (bw)] was administered via gavage (LoPachin RM et al, 2003). N-acetylcysteine (NAC) Group (n=10): NAC (Sigma, A-7250) dissolved in saline solution (250 mg/kg bw) was administered via gavage (Sprong RC et al, 1998; Wang AL et al, 2006).

Acrylamide + N-acetylcysteine (AA+NAC) Group (n=10): AA (25 mg/kg bw) and NAC (250 mg/kg bw) were administered via gavage. The administrations were continued regularly at the same hours every day for 21 days. At the end of 21 days, the rats were anesthetized by xylazine-ketamine anesthesia. The intracardiac blood was collected by laparotomy and transferred into heparinized tubes. The heparinized blood samples were centrifuged at 1500 rpm for 10 minutes and the blood plasma was separated. Plasma samples were used for GSH and MDA analysis, while buffy-coat layer was used in comet analysis in order to observe the genotoxic effects of AA. GSH and MDA levels were analyzed by the spectrophotometric measurement methods.

C. Biochemical Analyses

Reduced Glutathione (GSH) Analysis

Plasma GSH levels were measured by the Ellman method (Elman GL, 1979). Trichloroacetic acid solution was added to the plasma. They were mixed and centrifuged again at 3000 rpm at 4 °C for 20 minutes, and protein sedimentation was precipitated. The light-colored supernatant samples were used in GSH analysis. Distilled water was used as a blank. Standard graphic was plotted recording the absorbance of serial standards at 410 nm. Results are expressed as \(\mu\)mol/L.
D. Malondialdehyde (MDA) Analysis
Plasma MDA levels were measured by using the method of Ohkawa (Ohkawa H et al, 1979). 0.5 mL plasma was mixed with 3 mL 1% H_3PO_4 and 1 mL 0.6% thiobarbituric acid. This mixture was heated in boiling water for 60 minutes. It was extracted in 4 mL n-butanol upon cooling. n-butanol was used as a blank. The absorbents were recorded at 535 and 520 nm. Using tetramethoxypropane as a standard, plasma lipid peroxidation level was measured as μmol/L.

E. Comet Analysis
The analysis method of Singh et al. (Singh NP et al, 1993) was used in the study. The microscope slides (75 x 26 mm) were coated with agar layer with a thickness of 1 mm which is obtained from liquid NMP (Normal Melting Point) agar solution at 45 °C and were gelated for keeping at + 4 °C for 10 minutes. Then, 5 μL from liquid LMP (Low Melting Point) agar solution and 15 μL from lymphocyte solution (2x10^3/μL) were collected and mixed at 37 °C and agar was gelated upon being kept at + 4 °C for 10 minutes in such a manner that a second layer in the thickness of 1 mm was formed. This microscope slides were kept in cooled lysis solution at + 4 °C for one hour and lymphocyte membranes were melted and DNA was revealed. The lysed microscope slides were subjected to electrophoresis procedure for 20 minutes at 25 volts and 300 mA in horizontal agar electrophoresis tank. At the end of the procedure, the microscope slides were maintained in neutralization buffer for 5-10 minutes in a dark medium. During the period, neutralization buffer was replaced three times. The slightly dried microscope slides were kept in ethidium bromide solution and DNAs were stained. The DNAs stained with ethidium bromide were analyzed in x400 zoom with 515-560 filters in fluorescence microscope (Nikon E800) and the analyzed sections were photographed.

F. Statistical Analysis
Statistical analyses were performed via SPSS program (SPSS for Windows version 11.0). All the results were expressed as mean ± standard error of the mean (mean±SE). Whether the data showed normal distribution was determined by means of Shapiro Wilk test and it was found that the data did not show normal distribution (p<0.05). Therefore, Mann-Whitney U test was used in the comparison of the groups. The value (p<0.05) was accepted as statistically significant.

III. Results
3.1 Biochemical Results:
Figure 1 presents the plasma levels of MDA. It was found that AA administration increased MDA levels statistically significant in AA group compared to C group (p<0.05). However, administration of NAC+AA decreased the plasma MDA level significantly and reached a level close to the values of C group (p<0.05) when compared to AA group.

Figure 2 presents the plasma levels of GSH. AA administration decreased the GSH levels significantly (p< 0.05) in AA group as compared to C group. But administration of NAC+AA increased GSH levels significantly and reached a level close to the values of C group in AA+NAC group as compared to AA group (P<0.05).

Figure 3 presents comet analysis results. In accordance with comet analysis results of the groups, lymphocyte DNAs of C and NAC groups were normal appearance; lymphocyte DNAs fragmented at a highest level in AA group and even some lymphocytes exhibited apoptotic appearance.

However, NAC administration together with AA prevented the damages on lymphocyte DNAs significantly.
*p < 0.05 vs C; ** p < 0.05 vs AA; *** p < 0.05 vs A
Data are expressed as mean ± SE

**Figure 1.** Plasma MDA levels.

* p < 0.05 vs C; ** p < 0.05 vs AA; *** p < 0.05 vs A
Data are expressed as mean ± SE

**Figure 2.** Plasma GSH levels.

**Figure 3.** Comet analyses results of all groups.
IV. Discussion

AA, of which the genetic risks have not been clear yet (Favor J and Shelby MD, 2005), is a substance that must be paid attention in terms of human health. While cooking the foods containing carbohydrates and proteins at higher temperatures, AA is formed through the maillard reaction by interaction of amino acids, especially asparagine, with reducing sugars like glucose (Biederman M and Grob K, 2003). The studies demonstrate that AA administration increases the oxidative damage (Srivastava SP et al, 1983; Yousef MI and El-Demerdash FM, 2006).

Previous study revealed that AA decreased Glutathione-S-Transferase (GST) and GSH levels in the brain tissue and thus, AA-induced oxidative damages (Srivastava SP et al, 1986). Puppel et al. (Puppel N et al, 2005) investigate whether intracellular GSH levels are important factors in terms of genotoxicity levels that can be created by AA and GA. The high levels of intracellular GSH decreased the formation of GA in conjugation with AA and significantly prevented the GA induced genotoxic damages. In addition, in the animals were administered with GSH synthesis inhibitor, even low AA dose (1 mM) administration caused severe chain breaks, base shifts and severe damages on cell DNAs. These results have shown that intracellular GSH levels have utmost importance in preventing the genotoxic effects based on both AA and GA.

In present study, upon AA administration GSH levels in the rat plasma decreased significantly. However, administration of NAC together with AA equalized the GSH levels to the levels of C group. Because, NAC both increases intracellular GSH level as being its precursor molecule and detoxifies ROS as a reductant by means of thiol group (Zafarullah M et al, 2003).

It was stated in another study conducted in parallel with the results of present study that AA administration in rats lead to an increase in reagents of thiobarbituric acid in the plasma and a significant decrease in GSH levels (Yousef MI and El-Demerdash FM, 2006).

The experimental studies on rats and mice showed that a great amount of GA was formed from AA and GA reacted with purine bases of liver, pulmonary and renal DNAs of this animals and created genotoxic effects (Segerback D et al, 1995; Gamboa da Costa G et al, 2003; Paulsson B et al, 2003). Koyama et al. (Koyama N et al, 2006) analyzed the damages of AA and GA on human lymphoblastoid cells by comet analysis. It was demonstrated that AA caused moderate genotoxic damage on lymphoblastoid cells. However, when they performed the same experiment with GA, it was showed that a stronger genotoxic effect occurred, many chromosomes were damaged significantly and also, GA induced mutagenic effect may lead to cancer in human beings.

Bjorge et al. (Bjorge C et al, 1996) showed that AA administration had an important role in the AA-induced damages on DNA by increasing free oxygen radicals. However, the administration of antioxidant vitamins together with AA eliminated free oxygen radicals and decreased AA-induced DNA damages significantly.

Blasiak et al. (Blasiak J et al, 2004) reported that AA decreased intracellular GSH level significantly and caused significant genotoxic damages on lymphocyte DNAs when healthy adults were treated with 0.1-0.5 μmol AA. Moreover, it was also detected that the administration of AA together with antioxidant N-tert-butyl-phenylnitron decreased AA-induced DNA damage significantly.

In another study, Mani`ere et al. (Mani`ere I et al, 2005) administered 18, 36 or 54 mg/kg AA to rats.

In the following 24th hour, the rats were decapitated and the blood, brain, liver, bone marrow and testicular tissues were collected.
as samples. The examinations performed with comet analysis showed that significant genotoxic damages occurred on the DNAs of lymphocytes and the other tissues. Comet analyses of the germ cells of rats and mice showed that AA lead to severe mutagenic damages on DNAs of germ cells (Sega GA et al, 1989; Cao J et al, 2008).

In a study performed on human cell cultures, the addition of AA into the culture medium increased the production of free oxygen radicals and lead to oxidative stress and cellular damages (both oxidative and genotoxic damages). However, the addition of antioxidant substances together with AA into culture medium decreased both oxidative stress and genotoxic effects significantly (Cao J et al, 2008; Zhang X et al, 2008). In our study, on the other hand, the administration of NAC, a precursor molecule of GSH, together with AA suppressed oxidative stress in rats and decreased AA-induced genotoxic damages in lymphocytes significantly, which shows similar results with the studies performed.

We investigated that NAC increased GSH levels significantly in NAC group and decreased the MDA levels significantly. Moreover, GSH levels increased in AA+NAC group when compared to AA group, whereas MDA levels increased when compared to NAC group. These findings also show that AA increases MDA level by leading to oxidative stress and NAC creates a defense against increased MDA. Alturfan et al. (Alturfan EI et al, 2012) also demonstrate that AA increases MDA and myeloperoxidase activity (MPO), decreases GSH level in rats tissues. However, NAC inhibits oxidative stress and tissue damage.

In present study, while MDA levels increased in AA group (p<0.05), GSH levels decreased significantly. These results show that AA causes a significant decrease in antioxidant parameters. AA especially decreases GSH levels in the cells and spoils the oxidant/antioxidant balance against the oxidants and thus, leads to oxidative damages on the cells (Biswas SK et al, 2005; Park J et al, 2002; Bjorge C et al, 1996).

It has been reported that NAC is a thiol compound (Cotgreave IA, 1997) and clears the free radicals by reacting with the ROS (Zafarullah M et al, 2003). In our study, AA administration decreased the GSH level in the plasma and increased the MDA level. When NAC was administered together with AA, these values were approximated to that of the C group. NAC shows this effect as a result of increasing GSH level and GST enzyme activity.

Eskiocak et al. (Eskiocak S. et al, 2008) found that NAC decreased lipid peroxidation when taken in pharmacological doses and caused a significant increase in GSH levels by reducing oxidative stress. This finding is also consistent with the findings of the present study.

In conclusion, we demonstrate that AA administration causes oxidative stress and genotoxicity; however, NAC could play a protective role against AA-induced oxidative and DNA damages.

References:
Altinox et al: Protective role of N-acetylcysteine against acrylamide toxicity


