The Anti-Genotoxic Effect of Taurine on Aluminum Sulphate-Induced DNA Damage in Human Peripheral Lymphocytes

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Abstract

Aluminum sulphate $Al_2(SO_4)_3$ is commonly used as a coagulant in the purification of drinking water in many regions of the world. Aluminum (Al) is also involved in several pharmaceutical and cosmetic products. However, Al is suspected to cause serious disorders such as Alzheimer's by oxidative DNA damage. On the other hand, taurine (TA) is an amino acid found in mammalian tissues and it has been suggested to play a role in the defense against cellular damage. The objective of this study was to determine whether the supplemetation of a natural antioxidant, TA conferred the protection against Al exposure in human blood cultures. For this aim, sister chromatid exchange (SCE) and micronucleus (MN) rates were assessed. Present results showed that 10 μg/ml of $Al_2(SO_4)_3$ has no effect on SCE and MN rates, but 20 μg/ml of this compound increased the rates of SCEs and MN. Besides, the TA at all studied concentrations (25, 50 and 100 μg/ml) did not alter the mean SCE and MN values as compared to controls. Moreover, the negative cytogenetic alterations induced by Al could be significantly ($P<0.05$) reduced by the application of TA. This study reveals for the first time the ameliorative role of TA against Al-induced DNA damage *in vitro*.

Keywords: Aluminum sulphate, Micronucleus, Sister-chromatid exchange, Taurine, Human lymphocytes.

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Introduction

Al is involved in the environment in a number of chemical forms, including aluminum sulphate, aluminum hydroxide, aluminum fluoride, aluminosilicates (Testolin et al. 1996; Gauthier et al. 2000). The progressive increase in the use of these metal compounds has enhanced the possibility of human exposure (Roy et al. 1990). However, there is considerable debate as to whether chronic exposure to Al salts may be involved in neurodegenerative disorders such as Alzheimer's disease (Flaten 2001; Ribes et al. 2008), Parkinson's dementias (Hirsch et al. 1991), hepatotoxicity and nephrotoxicity (Chinoy and Patel 1999; Reinke et al. 2003; Sushma and Rao 2007)

Al compounds have very high affinities for DNA, RNA and many mononucleotides (Ganrot 1986) and complexes with DNA (Matsumoto 1988). However, information on the effects of these metallic compounds on chromosomes in mammalian systems is relatively meagre (Sharma and Talukder 1987; Leonard and Garber 1988). There are few studies relating the genotoxic activities of Al. Mutagenic potential of different Al compounds

have been studied by mammalian MN, SCE, chromosomal aberration (CA) and bacterial assays (Varella et al. 2004; Banasik et al. 2005; Geyikoglu et al. 2005; Lima et al. 2007). Several studies have implicated oxidative stress as one of the molecular mechanisms underlying Al toxicity (Bondy et al. 1998).

Nowadays, extensive efforts are being made to investigate therapeutic substances capable of reducing the genotoxicity of various natural and man-made mutagens in human life. These include vitamins, sulfhydryl substances and plant products (Edenharder et al. 1999; Rao et al. 2001). Concomitant treatment with the antioxidants with mutagens provided protection against oxidative damage in experimental animals (Abubakar et al. 2003; Esparza et al. 2003). As a matter of fact, Sinha et al. (2008) reported that, TA was abundant in the tissues of many animals and protected many of the body's organs against toxicity and oxidative damage induced by several heavy metals. Although, the mechanisms of its protective actions were not completely understood, it was suggested that, the useful effects could be viewed as a direct antioxidant due to its radical scavenging properties or an indirect antioxidant because of its preventative activities against changes in membrane permeability caused by oxidative stress (Wright et al. 1986; Timbrell et al. 1995).

To our the best knowledge, there is no investigation about the effect of TA on $Al_2(SO_4)_3$-induced cytogenetic damage in human lymphocytes. Thus, the aim of the present study was to elucidate the potential beneficial role of TA against Al-induced genotoxicity. To do this, the frequencies of SCEs and MNs were assessed in human lymphocytes following treatments with TA and $Al_2(SO_4)_3$ by using the well established two cytogenetic assays. SCE and MN are inexpensive, rapid and sensitive tests for evaluating the presence and extent of DNA damage in human populations that are exposed to genotoxic substances in environments and lifestyles.

Material and Methods

Experimental Design

Human blood was obtained by veinpuncture from three donors with no history of exposure to toxic substances. Questionnaires were obtained for each blood donor to evaluate exposure history and informed consent forms were signed by each donor. For all the volunteers hematological and biochemical parameters were analyzed and no disease was detected. This study was approved by *Atatürk University, Medical Faculty Ethical Review Board*. TA (2-aminoethanesulfonic acid) and $Al_2(SO_4)_3$ were purchased from Sigma–Aldrich® Chemical Company (St. Louis, MO, USA). The doses of chemicals were selected according to the reports by Chesney (1985) and Roy et al. (1990). After supplementation with TA (25, 50 and 100 µg/ml) and $Al_2(SO_4)_3$ (10 and 20 µg/ml), the blood was incubated for 72 h at 37 °C to adjust body conditions. The control samples of each volunteer were incubated, and they were treated equally as the samples, but without chemical addition.

SCE Assay

Human peripheral blood lymphocyte cultures were set up following a slight modification of the protocol described by Evans and O'Riordan (1975). The 0.5 ml of heparinized blood was cultured in 5 ml of culture medium (Biochrom®) with phytohemagglutinin. In order to provide successive visualization of SCEs, 5-bromo-2'-deoxyuridine (Sigma®) was added after culture initiation. The cultures were incubated in complete darkness for 72h at 37°C. At exactly 70h and 30 min after beginning incubations, colcemid (Sigma®) was added to the cultures. After hypotonic treatment (0.075 M KCl) followed by three repetitive cycles of fixation in methanol/acetic acid solution (3: 1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged, and then differentially stained for the inspection of SCE rate according to fluorescence plus Giemsa (FPG) procedure (Perry and Wolff 1974). For

each treatment condition, 25 well-spreaded second division metaphases were scored bu single observer, and the values obtained were calculated as SCEs per cell.

MN Assay

The MN test was performed by adding cytochalasin B (Sigma®) after 44 h of culture as previously described by Fenech and Morley (1985). At the end of the incubation period, the lymphocytes were fixed with ice-cold methanol: acetic acid (1:1). The fixed cells were put directly on slides using a cytospin, and stained with May Grünwald-Giemsa. All slides were coded before scoring. The slides were scored according to criteria reported by Fenech (1993). At least 2000 binucleated lymphocytes were examined per concentration (two cultures per concentration) for the presence of one, two or more micronuclei.

Statistical Analysis

The results are expressed as mean ± standard deviation (S.D). Comparison between groups were carried out by one-way analysis of variance followed by Duncan multiple range test with the level of significance set at $P < 0.05$.

Results

The results of the present study showed that, 10 μg/mL $Al_2(SO_4)_3$ did not effect the frequency of SCEs. Similarly, three TA doses alone (25, 50 and 100 μg/ml) also didn't change the rate of SCEs (data not shown). The unprotected cultures treated with 20 μg/mL $Al_2(SO_4)_3$ showed a significant increase in the SCE frequencies ($P<0.05$). However, high statistical significances in the reduction of SCE frequencies were found in the cultures concomitantly treated with TA and high dose of $Al_2(SO_4)_3$ as compared to the group Al treated alone (Fig. 1).

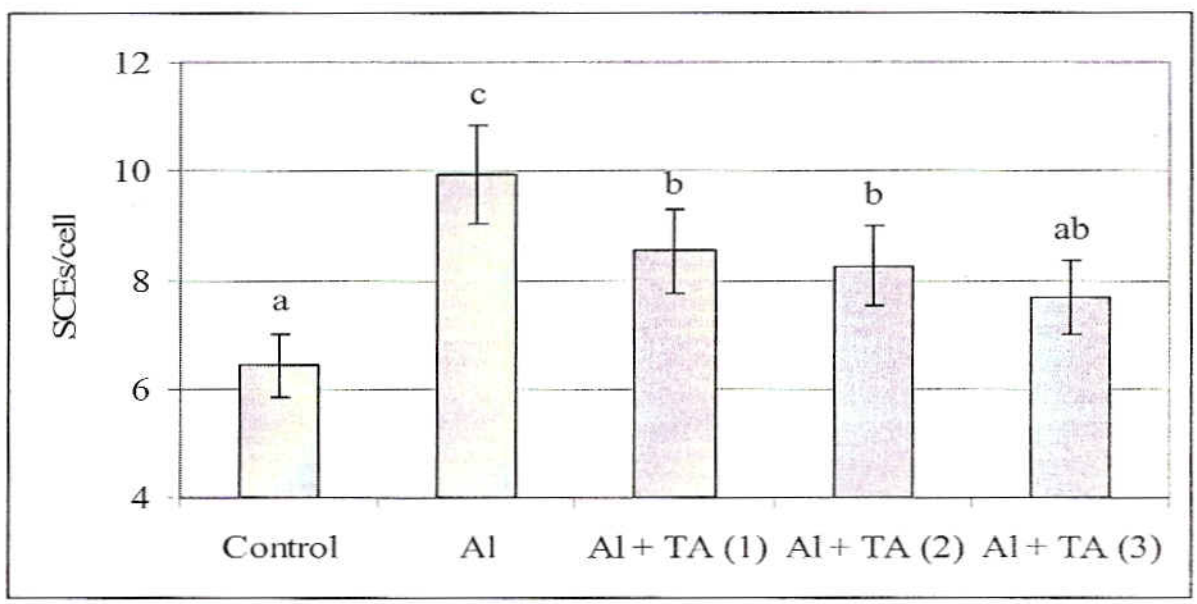

Figure 1. Frequency of SCEs in cultured human lymphocytes simultaneously exposed to 20 μg/ml $Al_2(SO_4)_3$ and TA. TA (1) = 25 μg/ml of TA, TA (2) = 50 μg/ml of TA, TA (3) = 100 μg/ml of TA, means in the figure followed by the different letters present significant differences at the $P<0.05$ level. Values are means ± standard deviation.

In the treatment for 72 h a significant increase in induced MN was found at the highest concentration of $Al_2(SO_4)_3$ ($P<0.05$). TA at tested concentrations did not increase the rate of MNs (data not shown). However, the positive effect of TA in decreasing the incidence of MNs in comparison with an unprotected level was attained when cultures were treated simultaneously with Al and TA (Fig. 2)

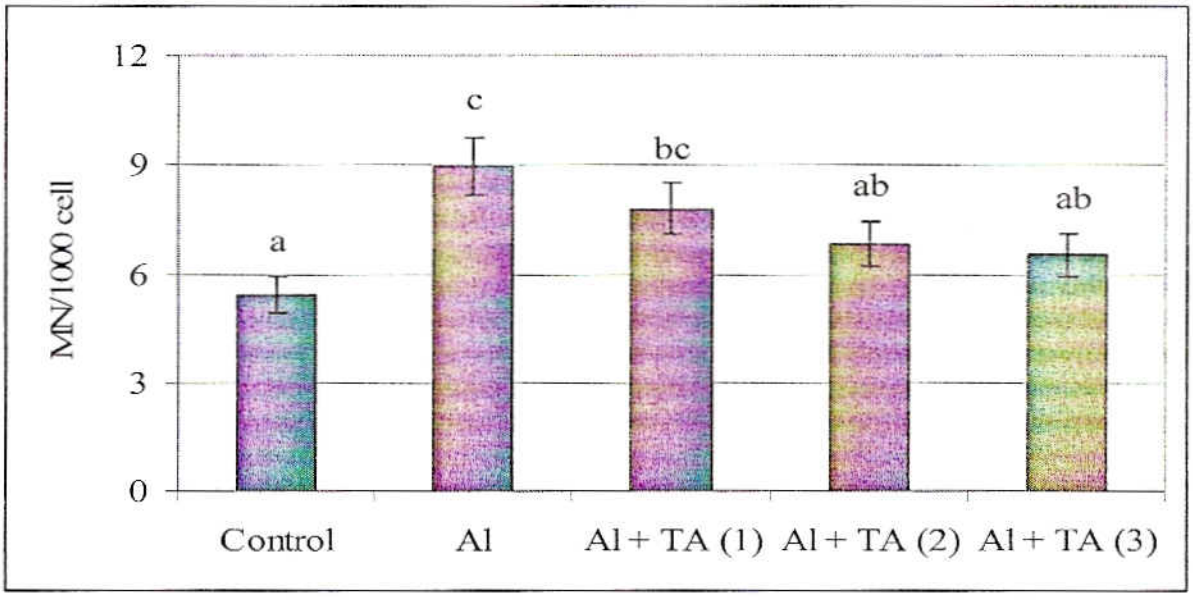

Figure 2. The rates of MNs (‰) in cultured human lymphocytes simultaneously exposed to 20 μg/ml $Al_2(SO_4)_3$ and TA. Abbreviations are as in Figure 1.

Discussion

In the present study we observed that $Al_2(SO_4)_3$ caused genotoxicity in human lymphocytes. The mechanism of Al induced DNA damage is not known (Lankoff et al. 2006) although genotoxic mechanisms seem to be related to the generation of oxidative damage and to the interference with DNA repair and replication processes (Hartwig 1995). Likewise, Al exposure induced generation of reactive oxygen species (ROS) and lipid peroxidation (Roy et al. 1990; Zatta et al. 2002; Abubakar et al. 2003). Similarly, a recent report suggested that Al compounds could induce morphological and functional alterations in erythroid cells by a direct action on circulating erythrocytes (Vittori et al. 1999), suggesting erythrocyte fragility due to oxidative damage (Sibmooh et al. 2000). Matsumoto et al. (2004) have found that the Al salts might bind to DNA and RNA. Moreover Al influenced gene expression, altered protein phosphorylation and inhibited some cellular enzymes (Li et al. 1998; Lankoff et al. 2006). In this context, it was reported that $Al_2(SO_4)_3$ caused increases in SCE formations and decreases in the activities of main antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutahione peroxidase (GSH-Px) in blood (Yousef 2004; Geyikoglu et al. 2005). In fact, oxidative stress develops when the levels of antioxidants are lowered (Bukowska and Kowalska 2004). It has been assumed that SOD has a central role in the defence against oxidative stress (Kakarla et al. 2005). The activity of GSH-Px eliminates ROS (Bukowska 2004). Endogenous H_2O_2 may be converted to H_2O by CAT (Svistunenko 2005). Damage to any of these enzymes could significantly affect the defensive mechanisms against free radicals in the living cell

(Bukowska and Kowalska 2004). It seems that the suppressing of above mentioned enzyme activities by $Al_2(SO_4)_3$ is very important and this situation might lead to the formation of SCEs and MN in the present study.

Our study also suggested that TA could have important biological and pharmaceutical consequences, because the induction of SCEs and MNs here observed were also ameliorated by concurrent administration of TA. This modulating effects of TA against Al-induced genotoxicity could be explained with two hypotheses. First, TA generally exhibited protective action *in vitro* at different concentrations depending on the nature of toxicity and the cells (Das et al. 2008). TA which was presented in high amounts in inflammatory cells, seems to be uniquely capable of modifying both target and receptor cell homeostasis through antioxidant pathways (Nahashima et al. 1990; Satoh 1994, Fennesy et al. 2003). TA supplementation showed normalization of the activities of SOD, CAT and GSH-Px and the normalization of antioxidants was implicated in the reduced levels of lipid peroxidation (Pushpakiran et al. 2004). Secondly, TA could stabilize receptor proteins in membranes, reduced protein carbonyl formation after free radical damage and inhibited oxidative damage to DNA (Eppler and Dawson 2002). In support of these hypotheses, Cozzi et al. (1995) have found that TA protected the DNA by acting as a scavenger of ROS. Besides, TA was established as an antimutagen in the Salmonella Ames tester strain assay against several mutagens (Laidlaw et al. 1989).

In conclusion, Al exposure is unavoidable during the entire human life and increasing evidence from *in vivo* and *in vitro* studies indicates that the adverse effects of Al are mediated by oxidative stress. In the light of the findings obtained in the present study, it is suggested that TA supplements in foods could protect blood tissue against Al-induced oxidative DNA damage. However, further studies are necessary to to find out the exact mechanism of anti-genotoxic action of TA against Al-induced genotoxicity.

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Abbreviations

Al, aluminum; CA, chromosomal aberration; CAT, catalase; GSH-Px, glutahione peroxidase; MN, micronucleus; ROS, reactive oxygen species; SCE, sister chromatid Exchange; SOD, superoxide dismutase; TA, taurine.