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RESEARCH ARTICLE

PROTECTIVE EFFECT OF TAURINE AGAINST MERCURY INDUCED TOXICITY IN RATS

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ABSTRACT

The aim of the present study was to estimate the protective role of taurine (2-aminoethanesulfonic acid), a sulfur containing conditionally essential amino acid, against mercury -induced cardiac dysfunction in rats. Mercuric chloride was administered orally at a dose of 2 mg/kg body weight for 30 days. Mercury exposure caused significant accumulation of the mercury and rat's hearts tissue. Levels of serum specific markers related to cardiac impairments, e.g. total cholesterol, HDL cholesterol and triglyceride were altered due to mercury toxicity. Reduction in the activities of antioxidant enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathioneperoxidase (GPx) have been observed in mercury exposed rats. Mercury intoxication also decreased the cardiac glutathione (GSH) and lipid peroxidation end products. Oral administration of taurine at a dose of 50 mg/kg body weight for 15 days, however, prevented all the toxin-induced oxidative impairments. The present results suggest that taurine protects the heart tissue against mercury induced toxicity.

INTRODUCTION

Mercury is one of the oldest chemical elements used in human applications. Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders (Risher and Amler, 2005). Its wide industry- related effects on human and animal biosystems have been well documented (WHO, 1991) and general exposure to this biologically-active chemical agent has been shown to be exacerbated through contaminated water and food (Magos and Clarkson, 2006). Nowadays, large populations worldwide are exposed to relatively low levels of Hg, especially via the use of pesticides in agriculture and of fluorescent light bulbs as well (El-Shenawy and Hassan, 2008).

In its elemental state, mercury is a silver-white liquid, being also known as metallic mercury (Hg◦). However, mercury may also be present in two oxidized forms [mercurous ion (Hg^{2+2}) and mercuric ion (Hg^{+2})] and as different organo metallic species (alkyl mercury, alkoxy mercury and phenylmercury), being the short chain alkyl mercury species, as methylmercury (CH_3Hg) and dimethylmercury ($(CH_3)2Hg$), the most dangerous compounds in terms of their toxicological effects. These organometallic compounds have a higher solubility in lipids when compared to inorganic species, making it easier to diffuse through the lipidicmatrix of the cellular membrane, therefore increasing its toxicity potential (USEPA, 1997; NRC, 2001; Baird and Cann, 2004). Mercury versatility as a metal explains its numerous applications in areas as different as industry, odontology, pharmacology, primary gold mining and agriculture. Many of these applications are based on the unusual ability of mercury to bind other metals making amalgams. In odontology, for example, mercury–silver amalgams are used to fill dental cavities. Heart disease is a leading cause of death worldwide heavy metal toxicity. There are a number of risk factors associated with different types of heart diseases (Patrick, 2003; Lee et al., 2001). Heavy metal exposure has been linked to increased incidence of cardiovascular diseases (Bhatnagar, 2006). Subramanyam et al. (1992) have reported atherosclerotic changes in rabbits due to mercury exposure. Sroczynski et al. (1990) suggested that heavy metal affects the circulatory system. Smetana et al.(1987) have observed higher blood heavy metal concentrations inpatients with idiopathic dilated cardio myopathy. Increased evidence suggests that oxidative stress might play a crucial role in different types of cardiac diseases. Generation of reactive oxygen species

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(ROS) is the hallmark of mercury toxicity (Szuster et al., 1990). Oxidative stress may, therefore, be one of the reasons for mercury -induced cardiac damages. Considering the relationship between mercury exposure and oxidative stress, attention has been focused on compounds having antioxidant properties in order to combat against mercury-induced cardiac damage.

Taurine (2-aminoethanesulfonic acid), a derivative of cysteine, is the most abundant free amino acid in many tissues, like brain, heart, liver, etc. It is not incorporated into proteins, but it does play many important roles in the body, like bile acid conjugation, detoxification, membrane stabilization, osmoregulation, and modulation of excitatory neurotransmission and intracellular calcium levels (Wright et al., 1986; Wessberg et al., 1983). Although its precise metabolic role is still undefined, considerable evidence shows that it can act as a direct antioxidant by scavenging ROS or as an indirect antioxidant by preventing changes in membrane permeability due to oxidative insult (Wright et al., 1986). It has been used clinically as a protective agent in cardiovascular diseases; it protects the heart from ischemic damage by acting as an antioxidant and membrane stabilizer (Takahashi et al. 1986; Cahane and Feng 1998). Taurine also reduces arrhythmias induced by chemicals or ischemia (Birdsall, 1998). Chronic taurine depletion in heart leads to severe cardiomyopathy (Takahashi et al., 2003). It also reduces the development of atherosclerosis in animals (Kondo et al., 2000). The present study has been undertaken to determine the protective role of taurine against mercury -induced cardiac damages in rats.

MATERIALS AND METHODS

Chemicals

Mercuric chloride ($HgCl_2$) and all other necessary reagents of analytical grade were bought from HiMedia laboratories Ltd. Mumbai, India.

Animals

The Wister strain rats (45 days old) of the Wister strain weighing ranging from 200±5g were used in this experiments. They were divided at random into four groups (each of six rats). All the animals were fed on a standard rat feed and water. Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of RMMCH, Annamalai University.

Wistar albino rats were divided into four groups each consisting of six animals:

Group-I Saline (0.9% NaCl)-treated control group;

Group-II Mercuric chloride (2 mg/kg orally.,for 30 days single dose)-treated group (Hg);

Group-III Mercuric chloride (2 mg/kg orally single dose) + Taurine (50 mg/kg daily orally. for 15 days) treated group (Hg +taurine),

Group-IV Taurine (50 mg/kg daily for 15 days)-treated control group.

The animals were sacrificed under light ether anesthesia and hearts were collected and after decapitation, trunk blood was collected; the serum was separated and measured the Total cholesterol HDL cholesterol, tryglecerides by using the standard kids and the heart tissue was used for the estimation of various biochemical parameters.

Assessment of Total cholesterol, HDL cholesterol and Triglyceride in serum

For assessment of serum specific markers (total cholesterol, HDL cholesterol and triglyceride levels) related to cardiac dysfunction, blood samples were collected by puncturing mice hearts of all experimental animals, kept overnight for clotting and then centrifuged at 3000×g for 10min. Total cholesterol, HDL cholesterol and triglyceride levels in the sera were estimated by using standard kits.

Preparation of heart homogenate

Heart samples were homogenized using glass homogenizer in 100mM potassium phosphate buffer containing1mM EDTA, pH 7.4 and centrifuged at 12,000×g for 30 minat 4 °C. The supernatant was collected and used for the experiments.

Estimation of lipid peroxidase

The level of lipid peroxidase was estimated with the method of Nichens and Samuelson (1968). The homogenate was prepared in Tris – HCL buffer (pH 7.5). 1 ml of the tissue homogenate was taken in a clean test tube and 2.0 ml of TBA-TCA-HCL reagent was added and then mixed thoroughly. The mixture was kept in a boiling water bath (60°C) for 15 minutes. After cooling, the mixture was centrifuged at 1000 rpm for 10 minutes and the supernatant was taken to read the absorbance of the chromophore at 535 nm against the reagent blank in a UV visible spectrophotometer (Spectronic –20, Bausch and Lamb). 1, 1', 3, 3' tetra methoxy propane was used to construct the standard graph.

Estimation of reduced glutathione

The glutathione (reduced) was determined according to the method of Beutler and Kelley (1963). The homogenized in PBS buffer solution and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the sample (supernatant) was taken clean test tube and 1.8 ml of EDTA solution was added. To this 3.0 ml of precipitating reagent was added and mixed thoroughly and kept for 5 minutes before centrifugation at 3000 rpm for 10 minutes. In each test tube, 2.0 ml of the filtrate was taken and to this 4.0 ml of 0.3M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added. The appearance of yellow colour was read at 412 nm in UV-visible spectrophotometer (Spectronic-20, Bausch and Lamb). A set of standard solution containing 20-100 μg of reduced glutathione was treated similarly.

Estimation of glutathione peroxidase

The activity of glutathione peroxidase was assayed using the method of Rotruck *et al.* (1973). The homogenized in PBS buffer and centrifuged at 2500 rpm for 5 minutes. 0.2 ml of the enzyme preparation (supernatant) was taken in a clean test tube, and then was added the following enzyme mixture: The enzyme assay mixture contained 0.2 ml of phosphate buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide. In the reaction, the mixture was mixed well and kept at 37°C for two minutes in an incubator. Then 0.2 ml of reduced glutathione and 0.1 ml of H_2O_2 were again added to the above mixture and incubated

at 37°C exactly for 10 minutes. The reaction was arrested by the addition of 0.5 ml of 10% TCA. Reduced glutathione content was estimated in the supernatant obtained after centrifugation at 3000 rpm for 10 minutes. A blank was prepared similarly to which 0.2 ml of the enzyme was added after the incubation period. Reduced glutathione used to construct the standard graph.

Estimation of Catalase

Catalase activity was determined according to calorimetrically method of Sinha (1972). It is based on the principle that the homogenized in phosphate buffer solution 0.1 ml of the homogenate was taken in a test tube and 1.0 ml of phosphate buffer was added. 0.4 ml of hydrogen peroxide was added to the above mixture. After 30 and 60 seconds 2.0 ml of dichromate acetic acid reagent was added. Test tubes were kept in boiling water bath (60°C) for 10 minutes. The mixture was cooled immediately in tap water and the colour was read at 620 nm against a reagent blank in UV-visible spectrophotometer (Spectronic-20, Bausch and Lamb).20-100μ moles of H_2O_2 is used as standard

Estimation of superoxide dismutase

SOD activity was assayed according to the method of Kakkar et al. (1984). The homogenized with 3 ml of 0.25 M sucrose solution and centrifuged at 10,000 rpm in cold condition for 30 minutes. The supernatant was dialysed against Tris HCL buffer (0.0025M, pH 7.4). The supernatant, thus, obtained was used as an enzyme source. The assay mixture (2.0 ml) contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, 0.1 ml of enzyme preparation (tissue homogenate) and 0.3 ml of water. The reaction was started by the addition of 0.2 ml of NADH solution and then it was incubated at 30°C for 90 seconds. After incubation the reaction was arrested by the addition of 1.0 ml of glacial acetic acid. The reaction reaction mixture was stirred and shaken with 4.0 ml of n-butanol. The mixture was allowed to stand for 10 minutes and then centrifuged for 15 minutes at 3000 rpm. After centrifugation, the butanol layer was separated. The colour intensity of the chromogen was measured at 560 mm in UV visible spectrophotometer (Spectronic-20, Bausch and Lamb).Water was used as blank.

Statistical analysis

Statistical significance was evaluated using ANOVA followed by Duncan Multiple Range Test (Duncan1957).

RESULTS

Assessment of serum specific markers related to cardiac impairment

The Illustrates levels of serum specific markers namely total cholesterol, HDL cholesterol and triglyceride respectively (Fig. 1, 2 and 3). Mercury administration caused a significant increase in total cholesterol as well as triglyceride levels ($P < 0.05$) in the experimental animals although decreased HDL cholesterol level was observed in the same treatment. Treatment with taurine prior to toxin administration, however, could protect these alterations significantly.

Activities of antioxidant enzymes

The illustrate activities of antioxidant enzymes GPx, CAT, and SOD measured from cardiac homogenates of all experimental animals. Mercury administration reduced the activities of all the antioxidant enzymes to a significant extent from the normal control ($P < 0.05$). Treatment with taurine prior to toxin administration, however, could prevent the reduction in activities of all the antioxidant enzymes (Table 1).

Estimation of Lipid peroxidase (LPO) and glutathione

Level of Lipid peroxidase and protein carbonyl content has been represented in Table 1. Elevation in the levels of both the parameters has been observed in mercury intoxicated animals compared to that of normal ($P < 0.01$). Taurine post treatment could ameliorate the elevation and retains the values close to normal.

DISCUSSION

Free radicals are highly reactive chemical species that, in addition to an important physiological role, can also cause DNA damages to DNA and, consequently, to cells leading, eventually, to carcinogenic processes (Halliwel, 2007). Mercury compounds enter the cell through plasmatic membrane or transport proteins (grey cylinder). (1) Inside the cell, they may produce reactive oxygen species (ROS) which react directly with DNA or, indirectly, induce conformational changes in proteins responsible for the formation and maintenance of DNA (DNA repair enzymes, proteins of microtubules).Mercury compounds may be also able to bind directly to: (2) DNA molecules, forming mercury species-DNA adducts,(3) “zinc fingers” core of DNA repair enzymes (white large arrow), affecting their activity and (4) microtubules, avoiding mitotic spindle formation and chromosome segregation. Chemical species easily react with nearby molecules with consequent energy liberation. Free radicals are also responsible for initiating auto-catalytic reactions through which proteins, lipids and carbon hydrates are also converted to free radicals, thus propagating a chain reaction. Reactive oxygen species (ROS) constitute the main type of free radicals implicated in pathogenic mechanisms. The most important species are: superoxide radical, hydrogen peroxide, hydroxyl radical, oxygen singlet, alkyl radical, peroxyl radical and nitric oxide (Ercal et al., 2001). ROS may be playing a potential pro-cancer role by promoting proliferation, invasiveness, angiogenesis, metastasis and suppressing apoptosis (Halliwel, 2007).Mercury compounds have the ability to induce cellular damage through an increase of ROS levels (Ercal et al., 2001) and therefore ROS production was already proposed as a molecular mechanism involved in mercury genotoxicity (Di Pietro et al., 2008; Lee et al., 1997; Rao et al., 2001; Schurz et al., 2000).

The generation of free radicals can result on a widespread variety of effects, but two of them are especially important to classify free radicals as genotoxic. First of all, direct action of free radicals on nucleic acids may generate genetic mutations (Schurz et al., 2000). Curiously, although mercury compounds were not mutagenic in bacterial an assay, inorganic mercury was able to induce mutational events in eukaryotic cell lines

Schurz et al., 2000). The present study describes the effect of taurine against mercury (in the form of $HgCl_2$) induced cardiac impairment in rats. Experimental results showed that taurine treatment up to a dose of 50mg/kg body weight could prevent that impairment in mice hearts when applied for 30days prior to mercury intoxication. Although several mechanisms have been postulated, the precise one in heavy metal -induced cardiac oxidative impairment is still undefined. Earlier investigations suggested that having a pro-oxidant catalytic activity, Hg^{2+} can initiate peroxidation of membrane poly unsaturated fatty acids by generating excessive free radicals followed by inhibiting the intracellular antioxidant defense system in tissues (Casalino et al., 2002).

indicators of cardiac dysfunction. Taurine administration, prior to toxin exposure could reverse the alterations of those indices in serum suggesting its prophylactic role in mercury -induced cardiac dysfunction. Antioxidant enzymes are considered to be the first line of cellular defense that prevents cellular ingredients from oxidative damage.

Among them SOD and CAT mutually function as important enzymes in the elimination of ROS. Reduction in cardiac SOD activity has been observed in the mercury exposed animals. Since SOD is a metallo-enzyme reduction in its activity may be attributed to dysfunctional

Table.1. Level of Lipid peroxidation, antioxidants, and biochemical studies in the heart tissue of rats treated with mercuric chloride followed by taurine

Parameters	**Group-I** Control	**Group-II** Mercuric chloride	**Group-III** Mercuric chloride+Taurine	**Group-IV** Taurine
LPO (nmolo/mg protein)	3.54 ± 0.31	12.56 ± 0.38*	3.36 ± 0.27**	3.56 ± 0.23
GSH (nmole/mg peotein)	18.89 ± 1.82	9.65 ± 0.58*	16.58 ± 1.20**	18.92 ± 1.07
GPx (nmole/min./mg protein)	380.45 ± 3.40	220.87 ± 4.80*	360.45 ± 2.70**	378.24 ± 1.90
SOD (Unit/mg/protein)	123.12 ± 1.67	67.68 ± 1.64*	118.12 ± 1.31**	125.81 ± 1.62
CAT (μmole/min./mg protein)	342.12 ± 2.60	186.89 ± 2.40*	278 .68 ± 4.30**	344.89 ± 2.70

Mean ± S.D of six individual observations;
*Significance ($p<0.05$) Group II compared with groupI;
*Significance ($p<0.05$) group III compared with group II

Casalino et al., (1997) reported that cadmium intoxication released free iron from membrane and participation of this free iron in Fenton type reactions produces reactive oxygen species (ROS). In other reports, it has been suggested that mercury exposure may increase or decrease the nitric oxide (NO) level in endothelial cells and that may, in turn, increase the generation of ROS leading to lipid peroxidation (Chen et al., 2000; Cavicchi et al., 2000). Mercury intoxication induces the expression of a number of stress genes (Wang et al., 1998) and this may cause an important role in affecting the activities of some antioxidant enzymes (Jay et al., 1991; Watjen et al., 2001). After an oxidative stress, ROS can stimulate myocyte hypertrophy, re-expression of fetal gene programs and apoptosis in cardiac myocytes in culture (Lefer et al., 2000). Reiter and Tan (2003) reported that the production of ROS increased significantly in the myocardium tissue of patients with ischemic-reperfused hearts. In addition to oxidative stress, cadmium toxicity can also include: alteration in metabolic processes (Muller 1986), modification in thiols containing proteins (Li et al., 1993), alteration in membrane structure and function (Shukla et al., 1987). Treatment with taurine prior to mercury exposure, however, effectively inhibited the metal toxicity by decreasing the levels of lipid peroxidation and protein carbonylation. The status of intracellular ferric reducing antioxidant power has been significantly impaired due to mercury toxicity and that could be prevented by taurine treatment before the toxin administration. The triglycerides,

conformational change which may be due to the replacement of Zn2+ present in SOD by mercury leading to loss of enzymatic activity (Casalino et al., 2002; Confod et al., 1992). Like SOD, another antioxidant enzyme, CAT also experienced significant reduction in activity in the cardiac tissue of the mercury exposed animals under experimental set of conditions. The treatment with taurine significantly elevated the activities of all these antioxidant enzymes indicating that mercury -induced cardiac injury can be prevented by taurine treatment. Thiol based antioxidant system plays second line of cellular defense against reactive free radicals and other oxidant species mediated oxidative damage. The levels of GSH, GSSH and total thiols are an additional indicator of cardiac oxidative injury.

GSH with its sulfhydryl group acts as a catalyst in disulfide exchange reaction. It functions by scavenging free radicals as well as detoxifying various xenobiotics and consequently converted to its oxidized form, glutathione disulfide (GSSG). In the present study following mercury intoxication, cardiac glutathione red-ox status was greatly impaired, as indicated by a significant decrease in the levels of total thiols, GSH along with the increased level of its metabolite GSSG. Treatment with taurine prior to the mercury administration could prevent that alteration indicating that it might play an important role in the metabolism of GSH which in turn increase the concentration of thiol groups and thus increases intracellular antioxidant power.

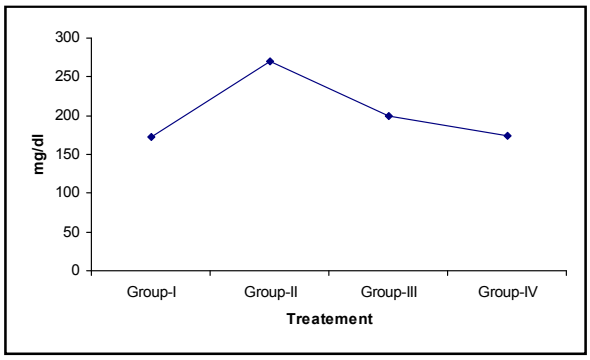

Fig.1. Level of total cholesterol in serum of rats treated with mercury followed by taurine

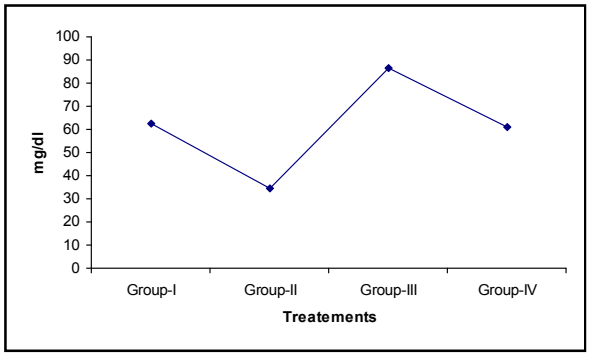

Fig.2. Level of HDL cholesterol in serum of rats treated with mercury followed by taurine

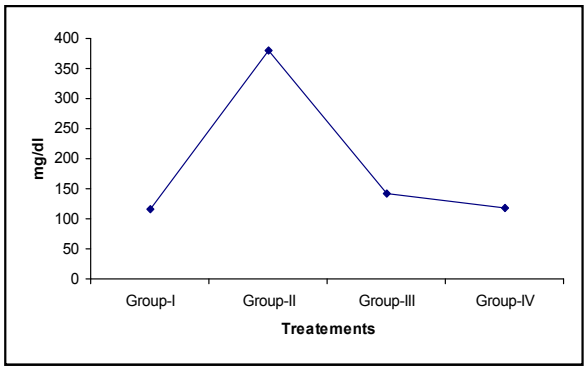

Fig.3. Level of trygleceride in serum of rats treated with mercury followed by taurine

The following mechanisms have been proposed for the antioxidant effects of taurine: (a) being a direct antioxidant, taurine could quench and detoxify several reactive intermediates, like hypochlorous acid (HOCl) generated bymyeloperoxidase (Huxtable, 1992; Timbrell,1995), nitric oxide (Redmond et al., 1996), H_2O_2 (Cozzi et al., 1995) and hydroxyl radical (•OH) (Aruoma et al., 1988); (b) as an indirect antioxidant, taurine could prevent the changes in membrane permeability due to oxidative injury via intercalating into the membrane and stabilizing it (Wessberg et al., 1983, Gordon and Heller,1992).

Timbrell et al. (1992) and Wright et al. (1986) reported that the membrane stabilizing effect of taurine is linked to an action on permeability of ions and water. In conclusion, we could protect cardiac tissue against mercury -induced oxidative impairment. At this stage the precise mechanism of protection played by taurine is not fully clear. Further studies are necessary to investigate the detailed protective mechanism played by taurine against mercury -induced cardiac dysfunction and that is in progress

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