Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats

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Monosodium glutamate (MSG), administered to rats (by gavage) at a dose of 0.6 mg/g body weight for 10 days, significantly \(P<0.05\) induced lipid peroxidation (LPO), decreased reduced glutathione (GSH) level and increased the activities of glutathione-s-transferase (GST), catalase and superoxide dismutase (SOD) in the liver of the animals; these were observed 24 hr after 10 days of administration. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and \(\gamma\) glutamyl transferase (GGT) were also significantly increased in the serum, on MSG administration. Vitamin E (0.2 mg/g body wt) co-administered with MSG, significantly reduced the LPO, increased the GSH level and decreased the hepatic activities of GST, catalase and SOD. The activities of ALT, AST and GGT in the serum were also significantly reduced. The results showed that MSG at a dose of 0.6 mg/g body wt induced the oxidative stress and hepatotoxicity in rats and vitamin E ameliorated MSG-induced oxidative stress and hepatotoxicity.

Keywords: Monosodium glutamate, vitamin E, antioxidants, hepatotoxicity, oxidative stress.

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substantiate whether MSG induces oxidative stress at an appreciably lower dose and study the effect of nutritional antioxidants on it.

In this study, the effect of antioxidant vitamin E (0.2 mg/g body wt) on MSG (0.6 mg/g body wt)-induced hepatic oxidative stress was investigated. The choice of MSG dose was based on earlier report. Antioxidants such as vitamin E sometimes act as pro-oxidant. Since vitamin E together with MSG may be present in human diet, it is, therefore, necessary to examine the possible effect of their interaction, in order to establish whether vitamin E would exacerbate or ameliorate the adverse effects of MSG.

We, therefore, investigated the effect of concomitant administration of MSG and vitamin E on some makers of oxidative stress and hepatocellular injury in rats. The effect on thiobarbituric acid-reactive substances (TBARS) (an index of lipid peroxidation), reduced glutathione (GSH) and the activities of superoxide dismutase (SOD) and catalase, the enzyme markers of oxidative stress and glutathione-s-transferase (GST), a phase II enzyme of xenobiotic metabolism and a secondary oxidative stress enzyme, was examined. In addition, the effect on the enzyme markers of hepatocellular injury —alanine aminotranferase (ALT) and aspartate aminotransferase (AST) and γ glutamyl transferase (GGT), was also studied.

Materials and Methods

Chemicals

Monosodium glutamate (MSG), vitamin E (α tocopherol), reduced glutathione (GSH), hydrogen peroxide, adrenaline, bovine serum albumin (BSA), trichloroacetic acid (TCA), thiobarbituric acid (TBA), 5,5′-dithiobis-[2-nitrobenzoic acid] (Ellman’s reagent), sulfosalicylic acid, 1-chloro-2, 4-dinitrobenzene (CDNB), 2,4-dinitrophenyl hydrazine (DNPH), DL-alanine, L- aspartic acid, 2-ketoglutaric acid, glycyglycine, and γ glutamyl-p-nitroanilide were obtained from Sigma Chemical Co., St. Louis, MO, USA. Other chemicals were of certified analytical grade.

Animals and treatment

Thirty male albino Wistar rats (180-200 g), 3-months old were used for the experiment. The animals were kept in neat metallic cages in the well-ventilated animal house of the Dept. of Biochemistry, University of Ibadan till the study lasted. They had access to 12 hr of darkness and 12 hr of daylight and were provided with standard rat feed (from Ladokun Feed Mill, Ibadan) and tap water ad libitum. All the animals received humane care in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals.

The animals were divided into the following five groups: Group 1, MSG; group 2, MSG + vitamin E; group 3, vitamin E; group 4, water; and group 5, corn oil. These substances were administered to the rats by gavage for 10 days. MSG and vitamin E were administered at a dose of 0.6 mg/g and 0.2 mg/g body wt using water and corn oil as the vehicle, respectively. MSG was dissolved in water at a concentration of 120 mg/ml, whereas vitamin E was dissolved in corn oil at concentration of 200 mg/ml. The animals were weighed before administration started and before they were sacrificed and the change in weight noted.

The animals were sacrificed 24 hr after the 10 days of administration by cervical dislocation. The blood was taken by heart puncture and centrifuged at 3000 rpm for 10 min to obtain the serum. The liver was removed, washed in ice-cold 1.15% KCl to remove blood and other extraneous substances, dried in a filter paper and weighed. It was later homogenized in four parts of ice cold buffer containing 50 mM tris-HCl and 1.15% KCl, pH 7.4. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant obtained was the fraction used for all the analysis on the liver tissue.

Protein concentration of the liver tissue fraction was determined by the Lowry’s method using bovine serum albumin (BSA) as standard.

Determination of oxidative stress parameters

The extent of lipid peroxidation in the liver fractions was determined by measuring the level of TBARS formed. The results were expressed as MDA formed using an extinction coefficient of 1.56 × 105/Mcm. A deproteinized sample of the liver fraction (using TCA) reacted with 52 mM TBA to form a pink-colored complex that absorbed at 532 nm.

The determination of reduced glutathione (GSH) level of tissue was based on the measurement of the absorbance of 2-nitro-5-thiobenzoic acid formed, at 412 nm, when Ellman’s reagent reacted with GSH. An aliquot of the liver fraction was deproteinized in 4% sulphosalicylic acid and centrifuged at 17,000 rpm for 15 min at 4°C. The supernatant was reacted with Ellman’s reagent and the absorbance of the complex formed read at 412 nm. The activity of GST in the liver fraction was determined as described.
The 1.0 mM GSH and 1.0 mM CDNB were reacted with the tissue fraction and the change in optical density (OD) at 340 nm within 30 sec interval for 3 min was taken. The activity was calculated with an extinction coefficient of 9.6 mM/cm.

The superoxide dismutase (SOD) activity was determined as described\(^\text{27}\). The liver fraction was reacted with adrenaline solution and the rate of inhibition of adenochrome formation from the auto-oxidation of adrenaline was measured spectrophotometrically at 480 nm. The catalase oxidation of adrenaline was measured in inhibition of adenochrome formation from the auto-determined as described, with an extinction coefficient of 9.6 mM/cm.

Measurement of activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and \(\gamma\)-glutamyl transferase (GGT)

ALT and AST activities were determined as reported previously\(^\text{31}\). For determination of ALT activity, the serum sample was added to the buffered solution containing DL-alanine and 2-ketoglutарате (pH 7.4) and incubated for 30 min at 37°C. After incubation, 1.0 mM, DNPH was added, followed by the addition of 0.4 M NaOH. The absorbance was read at 500 nm and the ALT activity deduced. For determination of AST activity, L-aspartic acid was used in place of the DL-alanine and the incubation time was 1 hr. For determination of GGT activity\(^\text{32}\), the serum sample was added to a substrate solution containing glycylglycine, MgCl\(_2\) and \(\gamma\)-glutamyl-\(p\)-nitroanilide in 0.05 M tris (free base), pH 8.2. The mixture was incubated at 37°C for 1 min and the absorbance read at 405 nm at 1 min interval for 5 min. The activity of GGT was calculated from the absorbance values.

Statistical analysis

Results obtained from the experiment were analysed using analysis of variance (ANOVA), while comparisons were made using the Dunnet’s test at \(P<0.05\) level of significance.

Results and Discussion

In this study, we looked at the possibility of MSG to induce oxidative stress at a dose of 0.6 mg/g body wt. The modulatory effect of vitamin E, a nutritional antioxidant on MSG-induced oxidative stress was also studied. A significant increase in the liver weight of the animals was observed on administration of MSG (Table 1), which could be attributed to an increase in the activity of inflammatory agents that could have led to inflammation of liver tissues\(^\text{33}\). The total protein concentration was not significantly affected on MSG administration (Table 1), supporting the fact that the increase in liver weight might have resulted from inflammation. Vitamin E reduced the MSG-induced increase in liver weight, possibly via its action as a radical scavenger. By scavenging the radicals that contributed to oxidative stress, vitamin E could help in reducing inflammation.

MSG administration significantly induced lipid peroxidation (LPO), decreased the level of GSH and increased the activities of GST, SOD and catalase in the liver of the experimental animals (Table 2). The activities of the serum enzyme markers of hepatocellular injury — ALT, AST and GGT were also significantly increased on MSG administration (Table 3).

Earlier, induction of LPO\(^\text{12}\) and decrease in the level of GSH\(^\text{6,11-13}\) was reported on MSG administration, at a dose of 4 mg/g body wt. Lipid peroxidation is a marker of oxidative stress. MSG generated the reactive oxygen species (ROS) that caused LPO. Glutathione depletion is an indicator of tissue degeneration/damage\(^\text{34}\). Glutathione can function as a direct radical scavenger and can also stabilize membrane structure, through the removal of acyl peroxides, formed by lipid peroxidation\(^\text{35}\). Thus, MSG-induced LPO contributed to the depletion of tissue level of GSH.
The increase in GST activity in MSG-treated rats was correlated with the decrease in GSH level. GSH is a substrate for GST and, therefore, should decrease with increased GST activity. GST is required for reducing the oxidative stress through the detoxification of ROS. Thus, the generation of ROS such as lipid peroxides by MSG administration could have contributed to the increased GST activity in the animals. GSTs possess unique property against oxidative stress, with the GSTA exhibiting a potent antioxidant activity. The increased activities of GSTs have contributed to the increased GST activity in the serum of MSG-treated rats.

Vitamin E when co-administered with MSG significantly reduced LPO and the activities of SOD, GST, and catalase and significantly increased the level of GSH in the tissues. The recycling of vitamin E contributes to glutathione depletion. When vitamin E is depleted due to its oxidation, glutathione reduces the tocopheroxyl radicals to tocopherol, and is itself oxidized. In the presence of an exogenous supply of vitamin E, glutathione is maintained in its reduced state.

Vitamin E when administered together with MSG reduced the activities of the markers of hepatocellular injury ALT, AST, and GGT. The ALT and AST are also elevated in cases of injury to other organs like kidney, heart, and muscle. GGT is also elevated in most cases of liver disorders. Therefore, the increased activities of ALT, AST and GGT in the sera of MSG-treated animals might have resulted from the liver injury caused by the MSG-induced oxidative stress.

In conclusion, the results of present study showed that MSG induced oxidative stress and hepatic toxicity in the experimental animals at a dose of 0.6 mg/g body weight. Vitamin E significantly reduced the oxidative stress and hepatic toxicity induced by MSG.
References