Studies on the Effect of Monosodium Glutamate [MSG] Administration on Some Antioxidant Enzymes in the Arterial Tissue of Adult Male Mice

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(Received February 25, 2002)

Summary The subcutaneous administration of monosodium glutamate to normal adult male mice at dose levels of 4 and 8 mg/g body weight caused a significant increase in lipid peroxidation level in the arterial tissue. The levels of total sulfhydryl and protein-bound sulfhydryl groups were found to be significantly increased, whereas non-protein-bound sulfhydryl, representing the glutathione level, was significantly decreased. It was also observed that the administration of monosodium glutamate at a dose level of 4 mg/g body weight and above induced oxidative stress by significantly lowering the activities of antioxidant enzymes like superoxide dismutases, catalase, and glutathione metabolizing enzymes like glutathione reductase and glutathione peroxidase in the arterial tissue.

Key Words monosodium glutamate (MSG), catalase (CAT), lipid peroxidation (LPO), atherosclerosis, oxidative stress.

Monosodium glutamate (MSG) [$C_5H_8NO_4NaH_2O$], a sodium salt of naturally occurring (non essential) L-form glutamic acid, has been used for many years as a flavor enhancer for a variety of foods prepared at home, in restaurants, and by food processors. Its palate pleasing favorite flavor is a must in almost all Chinese, Japanese, and South-Asian dishes (1, 2). The interest in the toxicity of MSG as a flavor enhancer has increased due to its association with Chinese restaurant syndrome in human beings (3, 4). We have reported previously from our laboratory that the administration of MSG produced hyperlipidemia, hyperglycemia, and hence obesity, which is one of the major factors responsible for the onset of atherosclerosis (5–9).

Atherosclerosis is one of the major causes of death in developing and under-developed countries and is reported to be responsible for about 10% of the deaths all over the world. This trend has increased about 3-fold in the last decade (10, 11). At the threshold of this millennium, atherosclerosis is looming large as a new epidemic. Therefore, immediate attention is needed to prevent this disease.

We have reported previously that MSG induced obesity along with oxidative stress in tissues like erythrocyte and liver. In the present work, we wanted to study the change in oxidative stress upon MSG administration into arterial tissue by studying its effect on some of the antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), and glutathione metabolizing enzymes like glutathione reductase (GR) and glutathione peroxidase (GPx).

MATERIALS AND METHODS

Animals and treatment. Adult male LAKA-UK mice

weighing 25–30 g were procured from the animal house of Panjab University, Chandigarh, India, and divided into three groups of 25 mice each. MSG was dissolved in water and injected subcutaneously at dose levels of 0, 4, and 8 mg/g body weight for six consecutive days. Animals were maintained on a rat pellet diet (Hindustan Lever Ltd., Bombay, India) with free access to water. This experimental design was approved by the Animal Experiment Committee of Panjab University, Chandigarh.

Sample preparation. The mice were fasted overnight and sacrificed by decapitation on day 31 after the last injection of MSG because obesity was established after MSG administration for 1 mo (12, 13). The arteries were removed, kept on ice, and washed with ice-cold saline. Arteries from five mice were pooled and a 10% homogenate was prepared in potassium phosphate buffer (100 mM, pH 7.5). The homogenate was centrifuged at $1000\times g$ for 15 min in a cold centrifuge (4°C). The supernatant was stored at 4°C and used for various biochemical assays.

Procedures. The lipid peroxidation (LPO) level in the homogenate was assayed by measuring the pink color chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) according to the method of Hochstein et al. (14). The activities of various antioxidant enzymes like SOD, CAT, GR, and GPx were assayed by the methods of Kono (15), Luck (16), William and Arscott (17), and Necheles et al. (18), respectively. The contents of the total sulfhydryl (T-SH) and non-protein-bound sulfhydryl (NPB-SH) groups were measured using Ellman's reagent by the method of Sedlak and Lindsay (19), and the content of the protein-bound sulfhydryl (PB-SH) group was calculated by subtracting the NPB-SH group from the T-SH group. The level of total protein in the arterial tissue homogenate was assayed by the method of Lowry et al. (20).

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Statistical analysis. Data were analyzed using the Bonferoni “t”-test following ANOVA using MINITAB computer software package. The difference from the control not receiving MSG was considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The subcutaneous administration of MSG at dose levels of 4 and 8 mg/g body weight significantly increased the level of malondialdehyde (representing lipid peroxidation) in arterial tissue by 19.5–35.5% (Table 1). MSG has been reported to induce hyperglycemia (9), which can result in the peroxidation of membrane lipids by increasing the events responsible for glucose oxidation, which in turn promotes NADPH-dependent thiobarbituric acid reactive substances (TBARS) in the presence of cytochrome P₄₅₀ (9, 21).

The increase in LPO level was accompanied by a proportional decrease in the level of NPB-SH group (representing the level of glutathione). In the present study,

the level of NPB-SH (representing the glutathione level) was significantly decreased by 18–25% in MSG-treated animals (Table 2). This observation is in agreement with the report that an inverse relationship exists between lipid peroxidation and glutathione status (22). Glutathione depletion to approximately 20–30% can impair cell defense against the toxic action of xenobiotic, and may lead to cell injury and death (23, 24). In the present study, however, animals remained healthy. Thus, it seemed likely that the body tried to protect the cells as the level of T-SH and PB-SH groups was increased by 28–45% and 27–44% in groups II and III, respectively (Table 2); glutathione (represented by NPB-SH) might be used for maintaining the activity of various enzymes, as the level of the NPB-SH group significantly decreased, but the level of the T-SH and PB-SH groups significantly increased.

MSG administration significantly decreased the level of glutathione-metabolizing enzymes like GR and GPx. GPx (a selenium-containing enzyme) inhibits the peroxidation of membrane phospholipids by converting hydroperoxides groups in the phospholipids into corresponding alcohols (25, 26). It is conceivable that low activity of GPx may render the tissue more susceptible to LPO damage (27). We observed a significant decrease in the activity of GPx, by 8–15%, in MSG-treated animals upon the increase in LPO level (Table 3). These observations could be in line with the hypothesis that tissue LPO and GPx may play a role in the etiology of atherosclerosis.

A significant decrease of 18–35% in the activity of GR, a cytosolic enzyme having more affinity for NADPH than NADH, was observed after MSG administration (Table 3). GR is mainly responsible for maintaining glutathione in the reduced state, as it effectively reduces GSSG to GSH by NADPH:GSSG reductase, and hence protects cell against oxygen toxicity (28). The decrease in the activities of GR and GPx could be due to the utilization of more NADPH or increased oxidative stress in the arterial tissue after MSG administration.

A significant decrease in the activity of SOD, a super-

Table 1. Effect of subcutaneous administration of MSG for six consecutive days on the level of lipid peroxidation in the arterial tissue of adult male mice (31st d after last injection).

Group	Lipid peroxidation (nmol MDA formed/min/mg protein)
I Control (0 mg MSG/g b.wt.)	4.17±0.17 ^a
II (4 mg MSG/g b.wt.)	4.99±0.22 (+19.48)*
III (8 mg MSG/g b.wt.)	5.66±0.16 (+35.53)***

^a Mean ± SD of five observations.
Values in parentheses represent percentage change as compared to the control; * $p<0.05$ and *** $p<0.001$.

Table 2. Effect of subcutaneous administration of MSG for six consecutive days on the content of total-sulphydryl, protein bound-sulphydryl, and non-protein bound-sulphydryl groups in the arterial tissue of adult male mice (31st d after last injection).

Group	Total-sulphydryl (mmol/g tissue)	Protein bound-sulphydryl (mmol/g tissue)	Non-protein bound-sulphydryl (mmol/g tissue)
I Control (0 mg MSG/g b.wt.)	4.11±0.16 ^a	3.72±0.122	0.170±0.0035
II (4 mg MSG/g b.wt.)	5.26±0.120 (+28.1)**	4.74±0.134 (+27.41)**	0.139±0.0038 (-18.2)*
III (8 mg MSG/g b.wt.)	5.94±0.132 (+45.2)***	5.37±0.132 (+44.3)***	0.127±0.0034 (-25.2)**

^a Mean ± SD of five observations.
Values in parentheses represent percentage change as compared to the control; * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Table 3. Effect of subcutaneous administration of MSG for six consecutive days on the activities of glutathione reductase and glutathione peroxidase in the arterial tissue of adult male mice (31st d after last injection).

Group	Glutathione reductase (nmol NADPH oxidized/min/mg protein)	Glutathione peroxidase (nmol GSH oxidized/min/mg protein)
I Control (0 mg MSG/g b.wt.)	22.0±0.9 ^a	20.8±0.7
II (4 mg MSG/g b.wt.)	18.0±0.8 (-18.18)*	19.0±0.5 (-8.56)
III (8 mg MSG/g b.wt.)	14.4±0.8 (-35.45)***	17.6±0.5 (-15.38)*

^a Mean±SD of five observations.
Values in parentheses represent percentage change as compared to the control; **p*<0.05 and ****p*<0.001.

Table 4. Effect of subcutaneous administration of MSG for six consecutive days on the activities of superoxide dismutase and catalase in the arterial tissue of adult male mice (31st day after last injection).

Group	Total-SOD (U/mg protein)	Catalase (nmol of H ₂ O ₂ decomposed/min/mg protein)
I Control (0 mg MSG/g b.wt.)	2.95±0.11 ^a	4.135±0.152
II (4 mg MSG/g b.wt.)	2.50±0.09 (-15.50)*	3.735±0.146 (-9.67)
III (8 mg MSG/g b.wt.)	2.25±0.08 (-23.72)*	3.404±0.121 (-17.67)**

^a Mean±SD of five observations.
Values in parentheses represent percentage change as compared to the control; **p*<0.05 and ***p*<0.01.

oxide scavenging enzyme, by 15.5–23.72%, was observed in MSG-treated groups II and III (Table 4). The presence of SOD in various fractions such as cytosol (CuZn-SOD), mitochondria (Mn-SOD), and plasma (EC-SOD) of our bodies enables SOD to dismutate superoxide radicals immediately and protects the cells from oxidative damage (29–31). Although we have not estimated CuZn-SOD and Mn-SOD individually, the decrease in SOD activity suggests that MSG induced neither cytosolic CuZn-SOD nor mitochondrial Mn-SOD.

Catalase, another potent antioxidant enzyme, especially against the superoxide radical and singlet oxygen, was also decreased significantly upon MSG administration (Table 4). Catalase protects cells from the accumu-

lation of H₂O₂ by dismutating it to form H₂O and O₂, or by using it as an oxidant, in which it works as a peroxidase. Therefore, the decrease in the activity of catalase by 10–18% observed in the present study could be due to less availability of NADPH as MSG favors lipogenesis by increasing the level of glutamine (5–8).

All the above observations suggest that MSG administration could cause oxidative stress in the arterial tissue by lowering the activities of SOD, CAT, GR, and GPx, thereby being responsible for the initiation of atherosclerosis.

Acknowledgement

The authors are grateful to the University Grants Commission for providing financial assistance to conduct this study.

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