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ALTERATION IN SOME ANTIOXIDANT ENZYMES IN CARDIAC TISSUE UPON MONOSODIUM GLUTAMATE [MSG] ADMINISTRATION TO ADULT MALE MICE

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ABSTRACT

4mg and 8mg monosodium glutamate per gram body weight was administered subcutaneously for 6 consecutive days to normal adult male mice and its effect was seen on 31st day after the last injection on some antioxidant enzymes in heart. A significant dose dependent increase in lipid peroxidation and xanthine oxidase level was observed, whereas the activity of free radical scavenging enzymes such as superoxide dismutase and catalase was decreased in both monosodium glutamate treated groups (Group-2 and Group-3). So, the present work suggested that monosodium glutamate at dose level of 4mg/g body weight and above induced oxidative stress in the cardiac tissue by changing the activity of free radical initiating enzyme such as xanthine oxidase and scavenging enzymes like superoxide dismutase and catalase.

KEY WORDS

Monosodium glutamate (MSG), Coronary heart disease (CHD), Atherosclerosis, oxidative stress, Hyperlipidemia and Lipid Peroxidation (LPO)

INTRODUCTION

Monosodium glutamate (MSG) [$C_5H_8NO_4NaH_2O$] a sodium salt of naturally occurring (non-essential) L-form of glutamic acid, is one of the main flavor enhancers used as an ingredient in various food products. Its palatable and favorite flavor is a must in almost all Chinese and South-Asian dishes, where it is known by the names of *Ajinomoto, Sasa, Ve- tsin, Miwon and Weichaun* etc. (1). The interest in the toxicity of MSG, a flavor enhancer increased due to its association with Chinese restaurant syndrome (2). In our previous work, we have reported that MSG used as a flavor enhancer in all Chinese, Japanese and ready served foods like 2- minute noodles, soups, sauces etc. induced hyperlipidemia, hyperglycemia and hence oxidative stress (3, 4, 5, 6, 7, 8, 9 and 10). Oxidative stress is reported to be responsible for the pathophysiology of many diseases like cancer, diabetes, coronary heart disease (CHD) etc (11 and 12). Increased oxidative stress brings change in the membrane lipids and proteins, which could be responsible for the initiation of CHD.

In the present era, there is a considerable increase in the number of deaths due to CHD all over the World. At the threshold of this millennium, CHD is looming large as a new epidemic, afflicting Indians at a relatively younger age. Therefore an immediate attention is needed to prevent this disease. So, in the present work, we wanted to study whether MSG an inducer of oxidative stress is an additional factor responsible for the onset of *"myocardial infarction"*, by studying its effect on some antioxidant enzymes like xanthine oxidase (XOD), superoxide dismutase (SOD) and catalase (CAT) in cardiac tissue of adult male mice.

MATERIALS AND METHODS

Animals and treatment: Normal adult mice LAKA – UK mice weighing 25-30g procured from the Central Animal House, Panjab University Chandigarh were divided into three groups of 6 mice each and injected subcutaneously with 1 ml water containing MSG at dose level of 0, 4 and 8mg/g body weight. Animals were maintained on a rat pellet diet (Hindustan Lever Ltd., Bombay) and free access to water.

Sample preparation: Animals were fasted overnight and sacrificed by decapitation on 31st day after the last injection of MSG because obesity was established after a month's of MSG administration (13 and 14). The hearts were removed washed with normal saline and 10% homogenate was prepared in potassium

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phosphate buffer (100mM, pH 7.5). Homogenate was centrifuged at 1000 xg for 15 minutes at 4°C and supernatant was used for various biochemical assays.

Biochemical Assays: The lipid peroxidation level was estimated by measuring the pink color chromophore formed by the reaction of thiobarbituring acid with malondialdehyde (MDA) according to the method of Hochestein *et al.* (15).

XOD activity was measured by the method of Fried and Fried (16) using nitro blue tetrazolium (NBT), which formed farmazan. The increase in the intensity of color with time was measured spectrophotometrically at 540 nm for 10 minutes. SOD activity was assayed by applying the method of Kono (17). The activity of SOD was measured by monitoring the rate of inhibition of NBT reduction. One unit is defined as the amount of enzyme, which caused half-maximal inhibition of NBT reduction. CAT activity was estimated by the method of Luck (18) in which decomposition of H_2O_2 catalyzed by this enzyme was measured by decrease in absorbance at 240 nm, taking 0.0394 $mM^{-1}cm^{-1}$ extinction coefficient and enzyme activity was expressed as K/mg protein. Here "K" is the first order rate constant. Total protein content was estimated by the method of Lowry et al (19).

Statistical Analysis: Data was analyzed using Bonferoni "t" test following one-way analysis of variance (ANOVA) using MINITAB computer software package. The difference from the control not receiving MSG was considered significant at $p< 0.05$.

RESULT AND DISCUSSION

Subcutaneous administration of MSG at a dose level of 4mg/g and 8 mg/g body weights significantly increased the level of malondialdehyde (representing lipid peroxidation) by 10% -19% (Table-1) in heart tissue of both MSG treated groups (Group -2 and Group -3). MSG has been reported to induce hyperglycemia (10), 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 P_{450}. The increase in lipid peroxidation level could be due to increased level of glutamine following MSG administration (9). Glutamine could also initiate the lipid peroxidation by changing the redox potential of cell and thus favoring the lipogenesis (9)

The activity of XOD, a superoxide-generating enzyme was found to be significantly increased by 9% to 21% in group -2 and group-3 respectively (Table-1). XOD is a highly versatile enzyme that is widely distributed among species from bacteria to human (20). Interest in XOD has increased recently because of its ability to generate free radicals in the cell. XOD exists predominantly as NAD^+ dependent xanthine dehydrogenase (XDH) that itself has no role in the initiation or potentiation of oxidative damage in cells. However, in many pathological conditions XDH is converted into XOD. There is evidence that suggests the involvement of the conversion of XDH into XOD in ischemia/ reperfusion (21, 22 and 23). The conversion of XDH into XOD is suggested to occur in damaged cells (24 and 25). XOD catalyses the oxidation of hypoxanthine/ xanthine to uric acid and generates superoxide radicals (O_2^-). Hydrogen peroxide formed

Table 1. Effect of subcutaneous administration of MSG for 6 days on lipid peroxidation and xanthine oxidase level in heart tissue of adult male mice (31st day after the last injection)

Groups	Lipid Peroxidation (n mol of MDA formed/ mg protein	Xanthine Oxidase (U/mg protein)
1 Control (0mg MSG/g wt)	3.540 ± 0.252	0.0100 ± 0.0003
2 (4mg MSG/g wt)	3.897 ± 0.261 (+10.08) *	0.0109 ± 0.0004 (+9.00)*
3 (8mg MSG/g wt)	4.228 ± 0.268 (+19.43) **	0.0121 ± 0.0003 (+21.11) **

Values in parentheses represent percentage changes compared to control.
*p < 0.05, **p < 0.01,

Table 2. Effect of subcutaneous administration of MSG for 6 days on superoxide dismutase and catalase activity in heart tissue of adult male mice (31st day after the last injection)

Groups	Superoxide dismutase (U/mg protein)	Catalase (n mol H_2O_2 decomposed / min / mg protein)
1 Control (0mg MSG/g wt)	3.861± 0.291	3.83 ± 0.261
2 (4mg MSG/g wt)	3.403 ± 0.310 (-11.86)*	3.474 ± 0.221 (-9.30)*
3 (8mg MSG/g wt)	2.992 ± 0.279 (-22.51)**	2.995 ± 0.260 (-21.80)**

Values in parentheses represent percentage changes compared to control.
*p < 0.05, **p < 0.01,

from O_2 could be converted into highly reactive hydroxyl radical (HO·) leading to oxidative stress as a result of oxidation of biological molecules. Therefore increased level of XOD in both MSG treated groups may produce a burst of free radicals and induced oxidative stress and hence may be responsible for the tissue injury.

Superoxide dismutase (SOD), one of the important intracellular antioxidant enzymes, present in all aerobic cells has an antitoxic effect against superoxide anion. The presence of SOD in various fractions such as cytosol (CuZn-SOD), mitochondria (Mn-SOD) and plasma (EC-SOD) in our body enables SOD to dismutate superoxide radicals immediately and protect the cell from oxidative damage (26 and 27). It is well documental that SOD activity can be decreased by ischemia or hypoxia (28 and 29). Concomitantly, we also observed that increase in the oxidative stress was accompanied by a significant decrease in the activity of SOD by 12% and 23% in group-2 and group-3 respectively (Table-2).

A significant decrease by 9% to 22% was observed in the activity of catalase, another antioxidant enzyme, upon MSG administration (Table –2). Catalase protects cells from the accumulation of H_2O_2 by dismutating it to form H_2O and O_2 or by using it as an oxidant in which it works as a peroxidase (28). So, decrease in the activity of catalase in the present work could be due to less availability of NADPH as MSG favor lipogenesis by increasing the level of glutamine (10).

All the above observations, suggested that administration of MSG at dose level of 4 mg/g body weight and 8mg/ g body weight could induce oxidative stress in heart tissue by altering the activities of XOD, SOD and CAT, thereby being responsible for the initiation of coronary heart disease/ atherosclerosis.

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