STUDIES ON THE EFFECT OF MONOSODIUM GLUTAMATE (MSG) ADMINISTRATION ON THE ACTIVITY OF XANTHINE OXIDASE, SUPEROXIDE DISMUTASE AND CATALASE IN HEPATIC TISSUE OF ADULT MALE MICE

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ABSTRACT

Subcutaneous administration of monosodium glutamate (MSG), to normal adult male mice, for six consecutive days at dose levels of 4 and 8 mg/g body weight, significantly increased the level of free radical initiating enzyme, xanthine oxidase, whereas the activity of free radical scavenging enzymes, like catalase and superoxide dismutase was significantly decreased in hepatic tissue. These observations suggested that ingestion of MSG at dose level of 4 mg/g body weight and above, induced oxidative stress in the hepatic tissue of adult male mice.

KEY WORDS :

Monosodium glutamate (MSG), Xanthine oxidase (XOD), Superoxide dismutase (SOD), Catalase (CAT) and Oxidative stress.

INTRODUCTION

Monosodium glutamate (MSG), a sodium salt of L-glutamic acid, is used as a flavor enhancer in Chinese, Japanese and ready served foods like 2'noodles, sauces, soups, etc (1, 2). The interest in the toxicity of MSG has increased greatly because of the association of this compound with *Chinese restaurant syndrome* (3). We have previously reported that administration of MSG at dose level of 4 and 8 mg/g b.wt. caused a significant increase in the level of lipid peroxidation in hepatic tissue (4) alongwith significant increase in blood glutamate and glutamine level (5) and hence induced hyperlipidemia and hyperglycemia, which are important factors for the onset of oxidative stress (4, 5, 6 and 7).

Liver plays a major role against the damage by free radicals by virtue of having a variety of antioxidants, free radical scavenging molecules and enzymes like superoxide dismutase, catalase

and glutathione peroxidase etc. which under normal circumstances, help the hepatic cells to maintain a reducing environment, preventing the potentially deleterious effects of free radicals on cell membranes and organelles (8). So, in the present work, we wanted to study the change in the activity of free radical initiating and free radical scavenging enzymes like xanthine oxidase (XOD), superoxide dismutase (SOD) and catalase (CAT) in the hepatic tissue upon MSG administration.

MATERIALS AND METHODS

Normal adult male mice (LAKA, UK 25-30 gm in body weight procured from the animal house, Panjab University, Chandigarh), were divided in three groups of 6 mice each and MSG was injected subcutaneously at dose level of 0, 4 and 8 mg/g body weight for 6 consecutive days. Animals were maintained on a rat pellet diet (Hindustan Lever Ltd., Bombay) and had free access to water. The mice were fasted overnight and sacrificed by decapitation, on 31st day after last injection, as obesity was established after 30th day of MSG administration (9, 10).

Liver were removed, washed with ice-cold normal saline, to remove the blood and 10% homogenate was prepared in potassium

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phosphate buffer (100mM, pH 7.5) containing 0.15M KCl. Homogenate was centrifuged at 1000 x g for 15 minutes in a cold centrifuge (4°C), the supernatant was stored at 4°C and used for various biochemical assays.

Catalase activity was estimated by the method of Luck, (11) in which decomposition of H_2O_2 catalyzed by this enzyme was measured by decrease in absorbance at 240 nm, taking $0.0394 \text{ mM}^{-1} \text{ cm}^{-1}$ extinction coefficient and enzyme activity was expressed as K/mg protein, where K is the first order rate constant. Superoxide dismutase activity was assayed by applying the method of Kono (12). Total-SOD activity was estimated by monitoring the rate of inhibition of nitrobluetetrazolium (NBT) reduction. One unit is defined as the amount of enzyme, which caused half-maximal inhibition of NBT reduction. The enzyme concentration was determined by using $E = 9920 \text{ M}^{-1} \text{ cm}^{-1}$ at 258 nm. The activity of xanthine oxidase (XOD) was estimated by the method of Fried and Fried (13), using nitrobluetetrazolium (NBT), which formed formazan. The increase in the intensity of color with time was measured spectrophotometrically at 540 nm for 10 minutes and total protein content was assayed by applying the method of Lowry *et al* (14).

Statistical analysis was done using one way analysis of Variance (ANOVA) and Bonferoni t-test by using MINITAB computer software package.

RESULTS AND DISCUSSION

In our previous work, we have shown that subcutaneous administration of monosodium glutamate (MSG), at dose levels of 4 and 8 mg/g body weight, significantly increased the level of lipid peroxidation. So in the present work, we wanted to study the change in the activity of free radical initiating and scavenging enzymes like xanthine oxidase, catalase, and superoxide dismutase.

The activity of xanthine oxidase, a superoxide generating enzyme was found to be significantly increased by 13.63% and 27.27% in group II and III respectively (Table-1). XOD has the ability to promote peroxidation of biological membrane by the production of superoxide radicals and is implicated as a possible mediator

of tissue damage (15,16). So, increase in the activity of XOD in MSG treated animals play a role in the tissue injury.

The activity of SOD, capable of dismutating superoxide ion was decreased significantly by 14%-34% in both group -II and III respectively (Table -2). The amount of SOD is organ specific and it is abundant in hepatic tissue. Three types of SOD have been purified; CuZn-SOD, Mn-SOD (17) and extracellular-SOD (EC-SOD) (18). CuZn-SOD consists of two protein subunits each unit has an active site containing one Cu ion and one Zn ion (17). Cu ion serves as an active redox site and Zn ions maintain the protein structure (19). CuZn-SOD is found abundantly in cytosol. Mn-SOD is located in mitochondrial matrix (20). It has four subunits with Mn in each subunit. EC-SOD is present in plasma, bound to heparin sulfate on the surface of endothelial cells (21). EC-SOD is a tetrameric glycoprotein, which contains Cu and Zn. The presence of SOD in various compartments of our body enable SOD to dismutate superoxide radicals immediately. In the present work, we estimated total-SOD but not CuZn-SOD and Mn-SOD individually, so decrease in the activity of SOD in the present work, suggested that MSG neither induce cytosolic CuZn-SOD nor mitochondrial Mn-SOD.

In the present study, there was a significant decrease in the activity of catalase, the enzyme responsible for detoxification of H_2O_2 in the hepatic tissues, by 29% and 47% in group-II and III respectively was observed (Table-2). Hepatic catalase, is concentrated in the peroxisome surrounded by uricase core, so the H_2O_2 produced by uricase reaction is decomposed exclusively by catalase (22). Catalase has four subunits, each of which contains a heme group as an active site and NADPH as a stabilizing compartment (23). Catalase bound NADPH, prevents and reverses the accumulation of compound-II (inactive form of catalase). The binding site of catalase have strict order of affinity for nicotinamide adenine dinucleotide ($NADPH > NADH > NADP^+ > NAD^+$) (24). Our findings of significant decrease in the activity of catalase in the MSG treated groups could be due to less availability of NADPH as MSG favor lipogenesis (4).

So all the above observations suggested

that MSG at dose level of 4 and 8 mg/g body weight induced oxidative stress by changing the activity of free radical initiating and free radical scavenging enzymes like XOD, SOD and CAT in the hepatic tissue of adult male mice.

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TABLE 1

Effect^a of subcutaneous administration of MSG at different dose levels (0, 4 and 8 mg/g body weight) for six days, on the level of xanthine oxidase and protein in hepatic tissue of adult male mice.

Groups	Protein (mg/g tissue)	Xanthine oxidase (U/mg protein)
I (0 mg MSG/g b.wt.)	172.00 ± 8.90 ^b	0.022 ± 0.0012
II (4 mg MSG/g b.wt.)	195.00 ± 12.40 (+13.37) ^c	0.025 ± 0.0011 (+13.63)
III (8 mg MSG/g b.wt.)	234.60 ± 18.30 (+36.39)*	0.028 ± 0.0016 (+27.27)*

a- 31st day after the last injection.

b- Mean ± S.D. of 6 observations.

c- Values in parentheses represent percentage change w.r.t. group I.

* $p < 0.05$

TABLE 2

Effect^a of subcutaneous administration of MSG at different dose levels (0, 4 and 8 mg/g body weight), for six days, on the activity of catalase and superoxide dismutase in the hepatic tissue of adult male mice.

Groups	Catalase (K/mg protein)	Total -Superoxide dismutase (U/mg protein)
I (0 mg MSG/g b.wt.)	0.125 ± 0.009 ^b	4.43 ± 0.195
II (4 mg MSG/g b.wt.)	0.089 ± 0.01 (-28.8) ^{c*}	3.82 ± 0.14 (-13.76)*
III (8 mg MSG/g b.wt.)	0.066 ± 0.009 (-47.2)**	2.93 ± 0.192 (-33.86)**

a- 31st day after the last injection.

b- Mean ± S.D. of 6 observations.

c- Values in parentheses represent percentage change w.r.t. group I.

* $P < 0.05$, ** $P < 0.01$

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