Alteration in Serum Homocysteine Levels upon Ingestion of Monosodium Glutamate (MSG) to Adult Male Rats

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ABSTRACT The study was conducted to evaluate the alterations in serum homocysteine levels upon ingestion of monosodium glutamate at the dose levels of 4 mg and 8 mg per gram body weight for consecutive 7 days. A study revealed a significant increase in serum homocysteine levels by 27.06% ($p < 0.01$) and 73.98% ($p < 0.001$) in 4 mg monosodium glutamate per gram body weight (Group-2) and 8 mg per gram body weight monosodium glutamate (Group-3) treated animals compared to normal animals. These observations suggested that ingestion of monosodium glutamate at dose level of 4 mg/g body weight and 8 mg/g body weight produced hyperhomocysteinemia and thereby could initiate atherothrombosis.

INTRODUCTION

Cardiovascular diseases are increasing in prevalence and represent a major public health burden of 21st century. Adiels et al. (2008). Atherosclerosis is profoundly affected by diet and can be approached from a nutritional point of view also. Getz and Reardon (2007). The craze for Chinese, Japanese, fast food and ready served foods like 2-minute noodles, soups etc. all containing Monosodium glutamate (MSG) is increasing tremendously especially in Indian younger generation. Previously, we have reported that MSG induces hyperlipidemia, hyperglycemia and oxidative stress in blood, erythrocyte, liver and heart, a well known risk factor for atherosclerosis (Ahluwalia et al. 1996; Choudhary et al. 1996; Kuldip and Ahluwalia 2002, 2003, 2005, 2007).

According to American Heart Association, around 79.4 million people are suffering from one or more types of cardiovascular diseases like coronary heart disease (CHD), angina, high blood pressure and stroke. Dalla (2007). This epidemic has the potential to place a large social and economic burden on the developing countries like India. In 2008, Kaul reported that about 60

million people in India are suffering from blockages of coronary artery, which is an alarming data. Recently, this situation warrants the need to understand new risk factors responsible for this problem. New emerging independent risk factors of cardiovascular diseases (CVD) include elevated level of homocysteine in serum (Yusuf et al. 2004; Humphrey et al. 2008). Altered homocysteine metabolism is implicated as a pathogenic factor in atherosclerosis, may be due to its export from endothelial cells, contributing to the generation of extra cellular hydroxyl radicals associated with oxidative modification of LDL-cholesterol and its incorporation into macrophages, a key step for initiation of atherosclerosis. Nurk et al. (2002). So in the present work, we wanted to study the effect of oral ingestion of MSG at dose levels of 4 and 8mg/g body weight for consecutive 7 days on the levels of serum homocysteine, reported to be altered in coronary heart disease.

MATERIALS AND METHODS

Chemicals: Monosodium glutamate was purchased from SRL (Sisco Research Laboratories Pvt. Ltd., Mumbai). All other chemicals used were of analytical grade.

Animals and Treatment: Normal adult male rat (Wistar), 140-150gm in body weight, procured from animal house, Panjab University, Chandigarh were divided into three groups of 6 rats each and MSG was given orally at dose level of 4 and 8 mg per gram body weight for 7 consecutive days,

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using canola. Animals were maintained on a rat pellet diet (Hindustan Lever Ltd., Mumbai) and had free access to water.

Sample Preparation: Animals were fasted overnight and on 8th day blood was drawn from the eye of rat and collected in the plain vial. The blood samples were centrifuged by using cold centrifuge machine at 4^o C for 10 minutes at 3000rpm and supernatant (serum) obtained, was used for the estimation of homocysteine levels.

Biochemical Assay

Homocysteine Assay: Homocysteine was qualified by using reverse phase high performance liquid chromatography (HPLC - Water India Limited) and fluorescence detections by applying the method of Ueland et al. (1993). This assay was measure total homocysteine (both reduced and oxidized forms). Homocysteine was dissociated from non relevant proteins and other disulfides by reduction with sodium borohydride. The proteins was precipitated by using 0.6M perchloric acid. The sulfhydryl amino acids so, obtained will reacts with fluorescent reagent 7-fluorobenzo-2-oxa-1, 3-diazole 4-sulfonic acid reagent.

Statistical Analysis

Numerical data from study groups (4 and 8 mg MSG/g body weight treated groups) and control group (Group-1) were compared using Students "t" test. Values were expressed as mean values $\pm$ standard deviation (SD). Sigma stat version 3.0 was used for statistical analysis. "P" value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Oral ingestion of MSG at dose level of 4mg and 8mg per gram body weight was found to be significantly increased the levels of homocysteine by 27.067% and 73.98% in 4mg MSG (from $9.57 \pm 1.08 \mu\text{mol/lit}$ to $12.16 \pm 1.26 \mu\text{mol/lit}$) and 8mg MSG per gram body weight (from $9.57 \pm 1.08 \mu\text{mol/lit}$ to $16.65 \pm 1.02 \mu\text{mol/lit}$) respectively compared to control animals (Table 1). A significant increase in homocysteine observed in MSG ingested animals could be responsible for the induction of atherothrombosis either via the formation of homocysteine thiolactone, which reacts with LDL to form LDL-homocysteine thiolactone aggregates. Theses aggregates are taken up by

Table 1: Changes in serum Homocysteine level upon ingestion of MSG at different dose levels (0, 4 and 8mg/g body weight) for 7 consecutive days to adult male rat.

Groups	Homocysteine (mmol/litre)
Group-1 (0 mg/g b.wt.)	9.57 ± 1.08^a
Group-2 (4 mg/g b.wt.)	$12.16 \pm 1.26^{**}(+27.06)^b$
Group -3 (8 mg/g b.wt)	$16.65 \pm 1.02^{***}(+73.981)$

a- values are expressed as mean $\pm$ S.D of 6 observations
b- Values in percentages represent change with respect to control.

*p<0.05, **p<0.01, ***p<0.001

macrophages and subsequently incorporated into foam cells in early atherosclerotic plaques. Within these plaques homocysteine thiolactone accelerates proteins and modifies the oxidative processes of the vessel, thereby promoting atherothrombosis (McCully and Wilson 1975; Angeline et al. 2007). In addition, homocysteine contain sulfhydryl group, which undergoes oxidation to the disulfide at physiological pH. The reaction is catalyzed by transition metals and a variety of reactive oxygen species (ROS) such as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2). The oxygen derived molecules may contribute oxidation of LDL and endothelial dysfunction and promote proliferation of vascular smooth muscle cells (Loscaizo 1996; Wald et al. 2002) and ultimately cause tissue damage and eventual loss.

In conclusion, the aforementioned observations suggested that ingestion of MSG at dose levels of 4 mg and 8 mg per gram body weight produced hyperhomocysteinemia and hence could be responsible for the initiation of atherothrombosis.

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