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Alteration upon Oral Ingestion of Monosodium Glutamate in Various Lipid and Lipoprotein Fractions in Serum of Adult Male Rat

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KEYWORDS Monosodium Glutamate (MSG). Low Density Lipoproteins (LDL). High Density Lipoproteins (HDL). Very Low Density Lipoproteins (VLDL)

ABSTRACT Monosodium glutamate, a sodium salt of glutamic acid is commonly used as food additive in Chinese, Japanese and ready to serve foods all over the world as a flavor enhancer. Concomitantly, there is a tremendous increase in the incidences of coronary heart disease and atherosclerosis. So, the present study was conducted to elucidate the effect of oral ingestion of monosodium glutamate at dose levels of 4 and 8 mg/g body weight for 7-consecutive days to normal adult male rats by evaluating the changes in serum lipid and lipoprotein fractions, glucose and protein levels. A significant increase was observed in serum total lipids, phospholipids and free fatty acids in monosodium glutamate ingested rats with respect to normal healthy control animals whereas cholesterol levels were remained normal. Monosodium glutamate ingestion produced hyperglycemia by significantly increasing the glucose levels in 4 and 8mg/g. body weight ingested MSG rats and a nominal increase was seen in serum protein levels in MSG ingested rats. The oral ingestion of monosodium glutamate at dose levels of 4 mg g^{-1} body weight and above significantly increased the levels of low density lipoproteins, very low density lipoproteins whereas a significant decrease was observed in high density lipoproteins. All the above observations suggested that oral ingestion of monosodium glutamate at dose levels of 4mg/g body weight and above for 7 consecutive days produced hyperlipidemia, hyperlipoproteinemia and hyperglycemia and thereby could be responsible for the initiation of atherosclerosis.

INTRODUCTION

Cardiovascular deceases (CVD) remained one of the main causes of death all over the world and several developing countries like India. The underlying causes of this disease is atherosclerosis. According to American Heart Association, around 79.4 million people are suffering from one or more types of CVDs like coronary heart disease (CHD), angina, high blood pressure and stroke (Dalla 2007). Current projections suggested that by the year 2020, India will have the largest coronary artery disease (CAD) burden in the world (Surekha 2007). CAD, is the most common form of heart disease, CAD is a disease affecting the arterial blood vessel and is commonly referred to as "hardening or furring" of the arteries. It is caused by formation of multiple plaques within the arteries. Based on demographic trends, it has been estimated that in India, deaths attributable to CAD/ atherosclerosis will probably double in both sexes, in the period 1985-2015. The risk of CAD in Indians is three time higher than in the White Americans, six times higher than in the Chinese and twenty times higher than in the Japanese. At the threshold of this millennium, CAD is looming large as a new epidemic afflicting Indians at a relatively younger age (Subramanian et al. 2003; Surekha 2007).

Healthy eating habits are more essential for maintaining rich and good quality of life. Taste and flavor are important to enjoy food. To improve the nutritional value, of food additives like flavor enhancer and coloring agent are added to food (Pavolic 2007). Concomitantly, in the present era, India is undergoing an industrial revolution which has led to the use of many chemicals as additives during food manufacturing and processing. Approximately 3000 different chemicals are added intentionally to foods during their manufacturing to improve taste, flavor etc. (Meadows 2003). Monosodium Glutamate (MSG), a sodium salt of glutamic acid is the most widely used flavor enhancer in all Chinese, Japanese, ready to serve food

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like 2'minute noodles, soups, sauces etc. all over the world (Farombi and Onyema 2006). However, its use has become controversial because of its association with Chinese Restaurant Syndrome and obesity (Dolnikoff et al. 2001; Wang et al. 2005; Kim et al. 2005; Nagata et al. 2006). In the past few years, the consumption of MSG has increased manifold in India due to the craze for Chinese, Japanese, ready to serve, especially in the younger generation along with increase in the number of people suffering from CHD. So, in the present work, we wanted to study the effect of oral ingestion of MSG on classical markers of coronary heart disease like various fractions of lipid and lipoprotein in serum of adult male rat.

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 rats (Wistar), 140-150g in body weight, procured from animal house, Punjab 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, 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 samples were drawn from the eye of rats into two tubes, with and without anticoagulant. Each blood sample was centrifuged for 10 minutes at 1000rpm to collect plasma and serum. The plasma and serum samples were stored at 4°C and used for various biochemical assays.

Biochemical Assays

1. *Total Lipids:* Serum total lipid levels were estimated by applying the method of Frings and Fendly (1972).
2. *Phospholipids:* The levels of phospholipids in serum were assayed by the method of Fiske and Suba Row (1925).
3. *Triglycerides:* Serum triglyceride levels were determined by using the method of Mc Gowan et al. (1983)
4. *Free Fatty Acids:* The contents of serum free fatty acids were estimated by applying the method of Lowry and Tinsley (1976).
5. *Total Cholesterol:* Serum total cholesterol levels were evaluated by the method of Allain et al. (1974).
6. *HDL- Cholesterol:* HDL- Cholesterol was estimated by the method of Grillo and Izzo (1985).
7. *LDL- Cholesterol:* Serum LDL- Cholesterol was estimated by using the empirical equation [Total Cholesterol - (HDL+ VLDL)] of Friedewald et al. (1972).
8. *VLDL-Cholesterol:* VLDL-Cholesterol in serum was evaluated by the formula as; VLDL = Triglyceride/5
9. *Glucose:* Plasma glucose levels were estimated using orthotouidine method of Hyvavria and Nikkila (1962).
10. *Serum Protein:* The protein contents were estimated by Lowry et al. (1951) method.

Statistical Analysis: Results of biochemical analyses are presented as mean value ± standard deviation (S.D.). The difference between control and test groups was analyzed by using Student "t" test (significant difference at $p < 0.05$ confidence level). Correlation between the investigated groups was performed using test ONE-WAY ANOVA (one-way variance analysis).

RESULTS AND DISCUSSION

The oral ingestion of MSG at dose level of 4 and 8 mg per gram body weight, significantly increased the level of serum total lipids by 19.93 percent and 29.37 percent, triglycerides by 4.97 percent and 9.27 percent, phospholipids by 21.18 percent and 37.52 percent and free fatty acids by 18.37 percent and 23.26 percent respectively with respect to control rats. However, a nominal increase in the levels of total serum cholesterol by 0.66 percent and 7.26 percent was observed in group-2 and group-3 animals as compared to group-1 (Table 1 and Table 2). Hyperlipidemia observed in the present study, upon MSG ingestion at dose levels of 4mg/g body weight and above could be due to the fact that glutamate favors lipogenesis by converting to glutamine (Malik and Ahluwalia 1989,1994). In 2000, Sacks and their collegues reported that in men, 30 percent change in plasma lipid concentration corresponds to a change in coronary risk of 7 percent for triglycerides versus 30 per-

cent for LDL-cholesterol or HDL cholesterol. In the present work, we observed 29.4 percent increase in serum total lipids, which suggests that ingestion of MSG could be a risk factor for CHD.

Table 1: Effect[a] of oral ingestion of MSG at different dose levels (0, 4 and 8mg/g body weight) for consecutive 7 days on various fractions of lipids in serum of normal adult male rat

Lipid fractions	*Group-1 (Control)*	*Group-2 (4 mg/g b wt)*	*Group-3 (8 mg/g b wt)*
Total lipids (mg/dl)	205.71± 3.23[b]	246.62±4.59 (+ 19.93)[c*]	266.14±6.44 (+ 29.37)[**]
Phospholipids (mg/dl)	49.70 ± 4.70	60.23 ± 2.16 (+ 21.18)[**]	68.35±1.7 (+ 37.52)[***]
Free fatty acids (ìg/ dl)	142.33 ± 2.95	168.49 ± 4.16 (+ 18.37)[*]	175.44±2.77 (+ 23.26)[**]

a - on 8th day of first dose
b- values are expressed as mean ± S.D of 6 observations
c- Values in parentheses represent percentage changes compared to control
* P < 0.05, ** P < 0.01, *** P < 0.001

Table 2: Effect[a] of oral ingestion of MSG at different dose levels (0, 4 and 8mg/g body weight) for consecutive 7 days on various lipoprotein fractions in serum of normal adult male rat

Lipoprotein fractions	*Group-1 (Control)*	*Group-2 (4 mg/g b wt)*	*Group-3 (8 mg/g b wt)*
Total cholesterol (mg/dl)	68.78 ± 3.22	69.24 ± 2.94 (+ 0.66)[NS]	73.78 ± 3.41 (+ 7.26) [NS]
Triglycerides (mg/dl)	94.13 ± 4.68	98.79 ± 3.39 (+4.95)	102.86 ± 5.7 (+ 9.27)[*]
VLDL (mg/dl)	18.82 ± 3.01	19.76 ± 3.41 (+ 5.00)	20.57 ± 3.83 (+ 9.30)[*]
HDL (mg/dl)	11.79 ± 1.29[b]	8.82 ± 1.99 (-25.19)[c**]	7.70 ± 1.64 (-34.52)***
LDL (mg/dl)	38.17 ± 2.56[a]	40.66 ± 2.86 (+6.52)*	45.49 ± 2.93 (+ 19.17)**

a - on 8th day of first dose
b- values are expressed as mean ± S.D of 6 observations
c- Values in parentheses represent percentage changes compared to control
* P < 0.05, ** P < 0.01, NS: Not significant

A significant increase (p<0.001) in the levels of glucose from 56.12 ± 1.12 mg percent to 87.72 ± 3.40 mg percent and a nominal increase in serum protein levels was observed in MSG ingested animals (Group-2 and Group-3) with respect to control animals (Table 3). It is well reported in literature that increased levels of cholesterol and triglycerides indicate hyperlipedemia, which in turn was associated with insulin resistance and type-2 diabetes mellitus (Nikkila 1984; Huponen et al. 1984; Schummer et al. 2008) and thereby produced hyperglycemia (Diniz et al. 2005). In 1993, Machado et al. reported that MSG treated mice showed hyperinsulinemia due to insulin resistance. Insulin increases glucose uptake in cells by stimulating the translocation of the glucose transporter GLUT4 from intracellular sites to the cell surface. Up to 75 percent of insulindependent glucose disposal occurs in skeletal muscle, whereas adipose tissue accounts for only a small fraction (Saltiel and Kahn 2001). So, hyperglycemia might be due to impaired glucose uptake by tissues due to decreased GLUT-4 expression despite hyperinsulinemia (Machado et al. 1993; Saltiel and Kahn 2001).

Table 3: Effect[a] of oral ingestion of MSG at different dose levels (0, 4 and 8mg/g body weight) for consecutive 7 days on glucose and protein content in serum of normal adult male rat

Biochemical analyses	*Group-1 (Control)*	*Group-2 (4 mg/g b wt)*	*Group-3 (8 mg/g b wt)*
Blood glucose (mg/dl)	56.12 ± 1.12[b]	82.39 ± 3.37 (+ 46.82)[c***]	87.73 ± 3.40 (+ 56.33)[***]
Protein (mg/dl)	52.30 ± 0.18	56.49 ± 0.59 (+ 8.03) [NS]	57.09 ± 0.18 (+ 9.17) [NS]

a - on 8th day of first dose
b- values are expressed as mean ± S.D of 6 observations
c- Values in parentheses represent percentage changes compared to control
*** P < 0.001, NS: Not significant

MSG ingestion resulted in significant increased levels of LDL by 6.52 percent and 19.17 percent and VLDL by 5.00 percent and 9.30 percent in group-2 and group-3 respectively as compared to group-1, whereas a significant decrease in HDL levels by 25.19 percent and 34.52 percent was observed upon MSG ingestion at dose levels of 4 and 8 mg/g body weight (Table 2). Hyperlipoproteinemia observed in present work might be due to hyperinsulinemia, which caused a significant increase in VLDL levels in MSG treated rats (Oida et al. 1984). Hyperglycemia has been shown to increase the activity of lipoxygenase and lipid peroxidation products. Lipoxygenase metabolizes arachidonic acid to produce leukotriene and products that play an important role for initiating atherosclerosis by inducing oxidation of LDL and stimulating growth and migration of vascular smooth muscle cells (Antonipillai et al. 1996; Natarajan et al.

1997; Sacks et al. 2000). Previously, we have also reported from our lab (Ahluwalia and Singh 2002; Singh and Ahluwalia 2003, 2005) that MSG increased oxidative stress and hence could induce oxidation of LDL. Most of the cholesterol in the mature lesion originates from circulating LDL particles, the circulating LDL particles cross the endothelium into the intimal of blood vessels. In their native form they are unfavorable for uptake into intimal macrophages and most return to the circulation. However, some particles may be oxidized by local cells possibly facilitated by the presences of transition metal ions and binding to proteoglycanes. After oxidative modification the LDL particles are rapidly taken up into macrophages via the scavenger receptor. Subsequent loading with cholesteryl esters forms so called foam cells (Simon and Gregory 1997), which could be responsible for the initiation of atherosclerosis.

So, all the above observations suggested that oral ingestion of MSG at dose levels of 4 mg/g body weight and above produced hyperlipidemia, hyperlipoproteinemia and hyperglycemia, which could play an important role for the onset of atherosclerosis.

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