

TOXICITY OF MONOSODIUM GLUTAMATE ON ARTICULAR CARTILAGE IN YOUNG MALE AND FEMALE ALBINO RATS: OXIDATIVE STRESS, PRO-INFLAMMATORY CYTOKINES AND FREE AMINO ACIDS

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Abstract

Monosodium glutamate (MSG) is a widely used flavor enhancer with a number of adverse effects such as cartilage inflammation. In the current study, we have tested the inflammation in articular cartilages following MSG administration, which might be a contributor to the changes in the markers of oxidative stress and alteration in articular cartilage free amino acids observed in the animals. Thirty two young male and female albino rats divided into two main groups. Rats of the first group, served as male and female control groups received distilled water orally. The second group, male and female groups of eight rats received monosodium glutamate orally at a high dose of 8g/kg b.wt. for long duration (three months) daily. Monosodium glutamate-treated rats showed significant alterations in serum pro-inflammatory cytokines, interleukin-1 β (IL-1 β) and tumor necrosis factor alpha (TNF α) with an increment in cartilage homogenate nitric oxide concentration. Also, significant differences in number of oxidative stress parameters like malondialdehyde (MDA), glutathione (GSH), and total antioxidant activity. In addition, there was significant increases in some free amino acids (Glu, GABA, Asp) contents and significant decreases recorded in the others (Ala, Gly, Tau) in cartilage homogenate following MSG administration at (P<0.05). Moreover, significant increases in serum calcium and phosphorus levels followed the increment of glutamic acid were shown in MSG-treated rats as compared to control animals. The pattern of induction of oxidative stress and activity of pro-inflammatory cytokines in the animals is an indication that oxidative stress induced by MSG in the cartilage tissues of rats, which might be contributed to cartilage matrix degradation, inflammation and may be osteoarthritis.

Keywords:

monosodium glutamate, articular cartilage, oxidative stress, pro-inflammatory cytokines, free amino acids

I. Introduction

Monosodium glutamate (MSG) is a food additive used to preserve flavor and enhance the taste [1-3]. Glutamate is non-essential amino acid presented in many proteins and most tissues [4]. The three ionotropic Glu receptors are N-methyl-D-Aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and Kainate receptors [4-6]. Glutamate receptors have been demonstrated in central nervous system, liver, kidney, spleen, lungs and testicles [4].

Monosodium glutamate induces several physiological alterations such as liver enlargement, changes in protein profile and glucose metabolism, renal abnormalities, ovary and testes lesions and endocrine disturbance [2, 7-

11]. Several studies investigated the effect of monosodium glutamate on the CNS of rodents and recorded that MSG induces neuronal necrosis and Alzheimer disease [2, 3]. Administration of MSG in high doses, repetitive and systemic ways causes neuronal death through excitotoxicity mechanism in which glutamate receptors activation induced intracellular Ca^{++} increment [3, 5, 7].

Also, many studies stated that, Glutamate receptors and transporters are expressed in connective tissues such as chondrocytes, osteoblasts, osteocytes, osteoclasts and fibroblasts [12-14]. In human arthritic tissue and rat antigen-induced arthritis diseased areas of bone and cartilage expressed AMPA and KA GluRs [14] and NMDA receptor may be involved in biomechanical responses [15]. Rat costal chondrocytes, rat knee joints and human articular cartilage express different glutamate receptor subunit mRNA, NMDA and non-NMDA and excitatory amino acid transporters (EAATs), including the glial subtype glutamate transporter-1 (GLT1) and glutamate/aspartate transporter (GLST) [15, 16].

In the present study, we investigated if MSG has any side effects on articular cartilage when administered in a high and long term manner.

II. Materials and Methods

Drugs

Monosodium glutamate was purchased from Fluka Chemika. The standards of amino acids were from Sigma and all are HPLC grade.

Experimental design

Thirty two young male and female albino rats divided into two main groups. Rats of the first group, served as male and female control groups. Control groups administered 1ml distilled water orally. The second group, male and female groups of eight rats for each received monosodium glutamate orally at a high dose of 8g/kg b.wt. [17] for long duration (three months) daily. The rats fasted overnight and sacrificed by fast decapitation. Blood samples collected and serum were separated and frozen until biochemical analysis of interleukin- 1β (IL- 1β), tumor necrosis factor - α (TNF- α), calcium and phosphorus. Articular cartilage separated from the knee of experimental and control animals, served in liquid nitrogen and cartilage homogenate prepared according to the modified method of [18]. The joints homogenized by cooling homogenizer at 4°C in 100 mM potassium phosphate buffer.

Biochemical analysis

Interleukin- 1β (IL- 1β) and tumor necrosis factor - α (TNF- α) were determined using ELISA kits. The kits were used according to the manufacturer's recommendation (Bio Source, International, Inc. Camarill, California USA).

Cartilage homogenates were used for the determination of Malondialdehyde (MDA) content as an index for lipid peroxidation (LPO) according to the method of [19], assay modified according to the suggestions of [20]. Nitric oxide (NO) concentrations estimated by the determination of total nitrate and nitrite concentration in samples [21]. Method of [22] used to assessment of glutathione (GSH) contents. In addition, total antioxidant capacity was detected in control and treated samples according to the method of [23-25]. Methods used in estimation of serum calcium and phosphorus respectively. Cartilage free amino acids were determined by HPLC according to [26].

III. High Performance Liquid Chromatography (HPLC)

The AGILENT HPLC system with a Rheodine injector, 20 μ l loop and a UV variable wavelength detector was used for amino acids (Glu, GABA, Asp, Ala, Tau, Gly) assays where the samples were injected directly into the PICO-TAG column (Waters) was used for free-amino acid analysis 3.9 x 30 cm. Eluent (1), Phenylisothiocyanate (PITC), Triethylamine and Amino acids standard (Standards and Eluents are Waters chemistry package for free amino acids). The assay conditions were as follows: temperature: 46 °C; wave-length: 254 nm; flow rate: 1ml/min.

Preparation of the sample

The first step in determination of free amino acids by HPLC method involved weighing and homogenization of the tissue on liquid nitrogen then we added 1/10 weight/volume of 75% aqueous HPLC grade methanol. The homogenate was spun at 4000 r.p.m. for 10 min, part of each sample was then dried using vacuum (70 millipores) at room temperature for derivatizing procedure.

IV. Statistical analysis

All values were expressed as mean \pm standard error of mean (SEM), and the data were analyzed using an independent test for significant differences between the two groups. A level of $p < 0.05$ was accepted as statistically significant (SPSS software -17). Two-way ANOVA used to express sex vs. treatment at $p < 0.05$.

V. Results And Discussion

The current study examined the effect of daily oral administration of high dose 8g/kg b.wt. MSG for long duration (three months) on young male and female albino rats. The data illustrated in figure (1) reported that serum tumor necrosis factor α (TNF- α) and interleukin-1 β (IL1- β) levels increased significantly in all treated groups. In addition, MDA and NO contents in articular cartilage homogenate increased significantly.

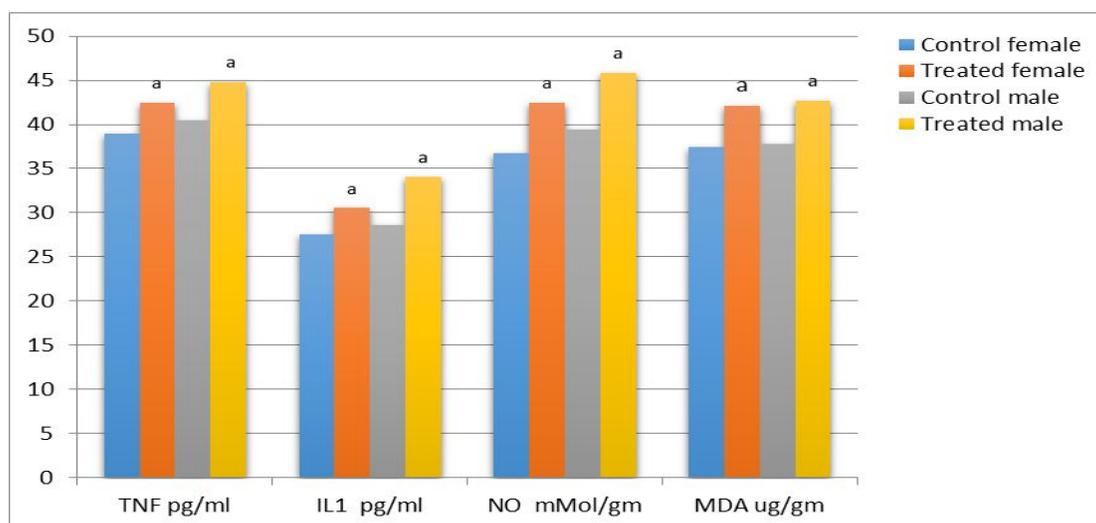


Figure 1. TNF- α , IL1- β levels and MDA and NO contents in articular cartilage homogenate after oral administration of MSG (8g/kg b.wt.). Values are means \pm SE of 8 rats in all groups at $p \leq 0.05$, a=significant change vs. same-sex controls. Sex vs. treatment: TNF- α (F= 40.50, $P \leq 0.05$), IL1- β (F=80.34, $P \leq 0.05$), NO (F=31.59, $P \leq 0.05$), MDA (F=9.32, $P \leq 0.05$).

Repeated high doses of MSG produced oxidative stress in various tissues of experimental animals [1, 27-29]. Excessive ROS production can lead to mitochondrial DNA damage, increased lipid peroxidation and cytokines and activation of signaling pathways which promote cartilage degeneration [30].

Articular cartilage is connective tissue which consisted of chondrocytes and extracellular matrix such as collagen, hyaluronan, aggrecan and proteoglycans [18]. Chondrocytes of human articular cartilage are produce reactive oxygen species such as nitric oxide, O_2^- , $\cdot OH$ and H_2O_2 induced apoptotic cell death in chondrocytes [31]. The treatment of articular cartilage with sub-millimolar concentrations of H_2O_2 leads to lipid peroxidation, apoptotic cell death and proteoglycan degeneration [32].

Non-enzymatic inflammatory mediators played important role in cartilage degeneration along with associated immunomodulation and oxidative stress. In addition, the increment of MDA and NO may be attributed to the elevation in intracellular Ca^{++} triggered by glutamate receptors activation by high dose of MSG. Then, the pathway of glutamate changed to glutamate release stimulating NO production and calcium overload. The elevation in Ca^{++} concentration starts chains of extravagant free radical production [5].

Nitric oxide converted to peroxynitrite radical (ONOO \cdot) inducing physiological damage within joints, including modulation of interleukin-1 [33].

Tumor necrosis factor- α and IL1- β act as stimulators of mesenchymal cells, such as synovial fibroblasts, osteoclasts and chondrocytes that release tissue-destroying matrix metalloproteinases[18]. Tumor necrosis factor - α initiates a cascade of inflammatory reactions through the production of ILs such as IL1 and IL6 to inflammation and organ injury at high concentrations and initiates the defense mechanisms against infection at low concentrations. TNF- α is responsible for neutrophil migration and production of the nuclear factor NF-kB in endothelial cells during inflammation, neutrophils attack tissues and cause severe cell necrosis. NF-kB is responsible for the activation of a number of pro-inflammatory genes, including nitric oxide synthase [34]. The present data agreed with [35], they stated that, there is a positive relationship between Pro-inflammatory cytokines and NO in articular cartilage, when TNF- α and interleukin-1 increased the NO increased.

Sex vs. treatment was estimated by two-way ANOVA in all tested parameters in response to oral treatment of high and repeated dose of MSG. The elevation in proinflammatory cytokines (TNF- α and IL-1), Malondialdehyde and nitric oxide in treated male group was significantly ($P < 0.05$) greater than in female group. Kataranovski et al. [36] investigated the gender differences in pro-inflammatory response of cadmium in rats and stated that gonad hormones are the influenced factor because estrogen can suppress interleukin-6 production in mice. Also, [37] attributed the elevation in oxidative damage in male than in the female to the elevation in oxidative damage to DNA in males than in females.

The present findings recorded significant decreases in glutathione content and total antioxidant activity in cartilage homogenate of animals treated by 8g/kg b. wt. monosodium glutamate (Figure 2). Insignificant increment in GSH and total antioxidant capacity was recorded in the female group as compared with a male group ($F = 3.315$ and 0.522 respectively).

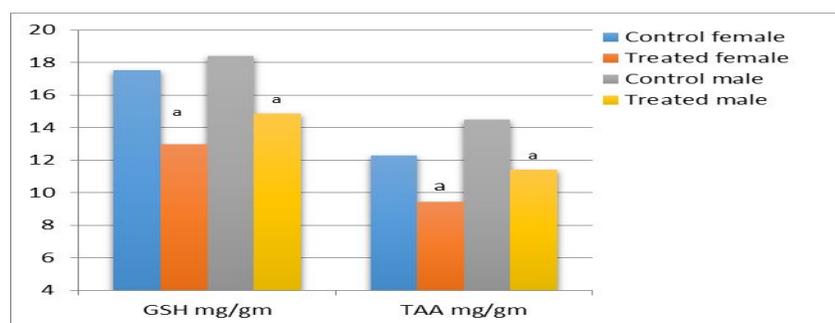


Figure 2. Glutathione content and TAA in articular cartilage homogenate after oral administration of MSG (8g/kg b.wt.). Values are means \pm SE of 8 rats in all groups at $p \leq 0.05$, a = significant change vs. same-sex controls. Sex vs. treatment: GSH ($F = 3.32$, $P \leq 0.05$), TAA ($F = 0.522$, $P \leq 0.05$).

The intracellular thiol-containing compound glutathione participates in the defense mechanism of cells against oxidative stress. Glutathione acts as a substrate for various enzymatic reactions and as non-enzymatic free radical

scavenger. Reactive oxygen species decrease glutathione content and activate matrix metalloproteinase resulted in catabolism of matrix metalloproteinase such as proteoglycan, aggrecan presented in the extracellular matrix of articular cartilage [31]. Total antioxidant activity is a biomarker of nutritional and food diseases [38]. The reduction in TAA may be attributed to the decrease in the antioxidant pool and oxidative stress elevation.

In this study, serum calcium and phosphorus recorded significant increases in all treated groups (Figure 3). The glutamate activation related with phosphoinositides hydrolysis and calcium release from intracellular stores [39]. In addition, the treatment with high amount of MSG over activates glutamate receptors and increased intracellular Ca^{++} concentration [5]. Glutamate receptor subunit mRNA were expressed in rat knee joints. Glutamate, NMDA or kainite (KA) stimulated intracellular calcium contents in rheumatoid arthritis fibroblast-like synoviocytes indicating the functional role of specific ionotropic glutamate receptors [12]. Ingestion of large amounts of MSG increases blood lactate [9]. Acidosis promotes shifts of phosphate from the intracellular to extracellular pool [33].

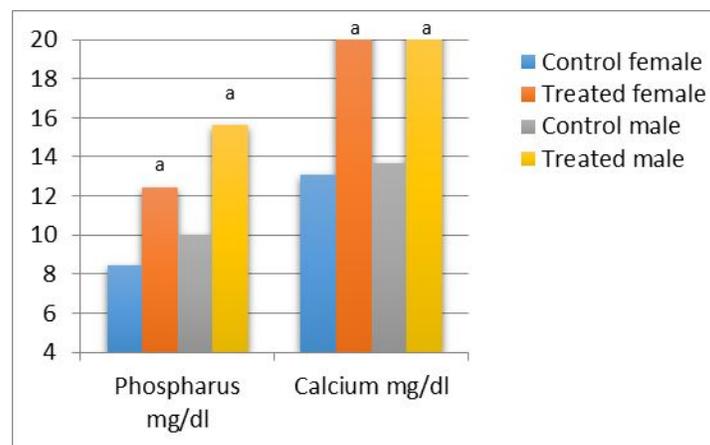


Figure 3. Serum calcium and phosphorus after oral administration of MSG (8g/kg b.wt.). Values are means \pm SE of 8 rats in all groups at $p \leq 0.05$, a = significant change vs. same-sex controls. Sex vs. treatment: phosphorus ($F = 86.16$, $P \leq 0.05$), calcium ($F = 14.17$, $P \leq 0.05$).

Data represented in figure (3) illustrated the free amino acids contents (Asp, Glu, Gly, Tau, GABA, Ala) in articular cartilage homogenate in treated and control animals. Glu, GABA, Asp contents were increased significantly as compared with corresponding control groups.

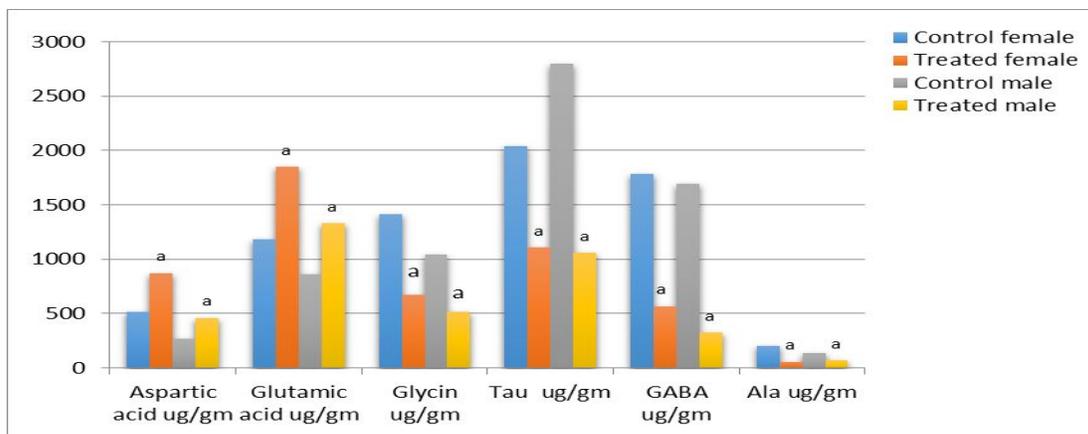


Figure 4. Free amino acids contents (Asp, Glu, Gly, Tau, GABA, Ala) in articular cartilage homogenate after oral administration of MSG (8g/kg b.wt.). Values are means \pm SE of 8 rats in all groups at $p \leq 0.05$, a = significant change vs. same-sex controls. Sex vs. treatment: Asp ($F=0.39$, $P \leq 0.05$), Glu ($F=0.26$, $P \leq 0.05$), Gly ($F=32.41$, $P \leq 0.05$), Tau ($F=8.16$, $P \leq 0.05$), GABA ($F=30.09$, $P \leq 0.05$), Ala ($F=14.22$, $P \leq 0.05$).

Treatment of MSG increases glutamate extracellular concentrations in plasma and various tissues [5, 9] mentioned that when large amount of MSG ingested, an increase in liver glutamate metabolism occurred leading to the release of glucose, lactate and other amino acids in the blood stream.

In general, glutamate dehydrogenase, glutamine synthase and transaminases play a central role in amino acid biosynthesis. In instance, the formation of L-alanine and L-aspartate by transamination in which transfer of glutamate α -amino group to pyruvate and oxaloacetate occurred [40]. It may be concluded that, the elevation in glutamate concentration increases other amino acids. Also, Glutamic acid can be converted to gamma-aminobutyric acid (GABA) and *vice versa* can be formed from GABA [41-43].

In this study, Ala amino acid decreased significantly in female treated group and insignificantly in male treated group and these results may be attributed to amino acid metabolism disturbance. In addition, there are significant decreases in Tau and Gly amino acids in treated groups. Taurine acts as antioxidant through the regulation of calcium level [44, 45]. Alarcon-Aguilar et al. [46] stated that, glycine, an amino acid that has anti-oxidant effects, protects against endotoxic shock, liver injury, and nephrotoxicity by inhibiting apoptosis and recorded that, glycine can block the expression of pro-inflammatory cytokines in vitro and affect in vivo cytokine production in monosodium glutamate treated mice.

Sex differences in calcium, phosphate and some tested free amino acids (GABA, Ala, Tau, Gly) were significantly occurred. There were significant decreases in calcium and phosphorous in the female group than in male one. [15] reported that, the differences between male and female in pathophysiological changes seem to be based on sexual hormones such as testosterone and estrogen.

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