Effects of Monosodium Glutamate (MSG) on Neuron Damages in Hippocampus in Sprague-Dawley rats

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Abstract

Introduction: Monosodium glutamate (MSG) is a flavor enhancer commonly used worldwide. Studies have shown that high dose of MSG could act as neurotoxic or excitotoxic agent for neurons in the central nervous system. The aim of this study was to determine the effect of MSG on neuron changes of hippocampusanimal model.

Materials and Methods: A total of 25 white male Sprague-Dawleyrats, aged 8-10 weeks,were dividedinto 5 groups (2 control groups (i.e., none and solvent group) and3 treatment groups that received2, 4and 6 mg/gram MSG orallyfor 30 days). Afterfour weeks on treatment, all animals were sacrificed and the entire brain tissueswere removed and immediately fixed in formalin for hematoxylin and eosin (H&E) staining.

Results:The percentage of damaged neurons in three *Cornuammonis*areas of hippocampus was higher in animal supplemented with MSG compared to controls. At the highest MSG concentration (6 mg/gram), 52.1%, 55.2% and 66.0% of neurons from *Cornuammonis* 1, 2, and 3, respectively were damaged. The percentage of neuron damages in hippocampus was in dose-dependent manner.

Conclusion: Our data suggested that high dose of MSG increased thehippocampus neuron damages in dose-dependent effect. This suggests the neurotoxicity effect of high dose of MSG.

Keywords:*Monosodium glutamate (MSG), histology,hippocampus, neurotoxic effects, animal model*

Introduction

Monosodium glutamate (MSG) is a substance that is added to foods as a flavor enhancer. In 2014, Asia was the largest MSG manufacturer region, accounting for around 94% of the production of MSG worldwide.[1]High demand, economic and ample workforce, and its use in feed stocks may be the reasons behind its large-scale production in Asia.[1]MSG is used in many countries including Indonesia and has been became one of the most investigated research topics in toxico-pharmacology.[2, 3]Glutamate, the main composition of MSG, is the excitatory neurotransmitter and most commonly found in human body. It is extensively metabolized in enterocytes that is further metabolized as carbon. If glutamate is elevated to 3-4-folds, then it will be primarily used in ATP production or in transformation of many other amino acids.[1]Glutamate is responsible for 75% of the excitatory communication in the brain. Although it has been used asa food ingredient, the excessive presence of glutamate could lead to receptors overstimulation that continue to irreversible cell damages and cell death.[4]

The abundance of glutamate in cerebral cortex and hippocampal dentate gyrus and striatum indicating that it has an important role in cognitive functions, including learning and memory.[2, 3, 5-8]Therefore, the effects of MSG on neurons become a concerning issue. A study in early life period of animals shown that high concentration of MSG may act as neurotoxic or excitotoxic agent.[9]MSG also possibly damagesthe central nervous system cells resulting abnormal histologicalexaminations of the cerebral cortex and hippocampus, depletion of cerebral cortex layer, damage to neurons of primary sensory area of cerebral cortex, and damage to the paraventricular nucleus of neonates whose parents fed with MSG.[2, 5, 10, 11]Since glutamate is an essential amino acid, more thorough and vigorous studies are necessary to validate

the root cause of the vivid health consequences in particular in neuropathology.The aim of this study was toassess the effects of MSG on neuron damages in the hippocampus areas in animal model.

Method

Study setting

In this study, 25 male rats of Sprague-Dawley strain, aged8-10 weeks with weight150-200 g, were used to assess the effect of MSG on neuron damages. MSG in the form of sodium L - glutamate monohydrate ($C_5H_8NNaO_4$) was used. The changes of histological structures of hippocampus in each animal were assessedand compared among the groups.

Procedures

Two weeks prior to study, animals were adapted to laboratory condition for acclimation. The animals were randomly divided into five groups: pure control group (C1), solvent control group (C2), MSG treatment group of 2 mg/g BW/day (T1), MSG treatment group of 4 mg/g BW/day (T2), and MSG treatment group of 6 mg/g BW/day (T3). The MSG was administered orally daily for 4 weeks.

At the end of fourth week, the animals were sacrificed; hippocampal tissues were preserved and stained with hematoxylin and eosin (H&E) staining. Three hippocampus areas, *cornuammonis* 1 (CA1), CA2, and CA3 were examined and damaged neurons from each *CA* area of each animal were counted. Damaged neurons indicated as pyknotic cells and cells with condensed chromatin compared. Pyknotic nuclei have a nucleus diameter of about 1/3 of the standard nucleus, with a high density of evenly dispersed but deeply stained nuclear content. Condensed chromatin is the sign when intensively stained nuclei represent the aggregation of chromatin in some nucleus regions. The examinations were conducted using Image Raster software and OptiLab Camera.

Statistical analysis

The mean percentage of damaged neurons in hippocampus areas, CA1, CA2, and CA3,among groups were compared using one-way ANOVA, followed by theLeast Significant Difference (LSD) post-hoc test. The data was considered statisticallysignificant at $p<0.05$.

Results

Our data suggested that the percentage of damaged neurons were significantly different among MSG groups and control. The percentage of damaged neurons in hippocampus areas was higher among animals within high MSG dose group (**Table 1**). Our data suggested that the effect of MSG on neuron damages was in dose-dependent manner. In CA1 areaof hippocampus for example, around 36.2% of neurons were damaged in animals with 2 mg/g BW MSG per day and this percentage increased to 44.8% and 52.1% in animals who reactive 4 mg/g and 6 mg/g MSG, respectively. Our analysis suggested that the damaged neurons were significantly higher in all MSG groups compared to both control groups in all three CA with $p<0.05$ for all comparations.

Table 1. Neuron damages in each hippocampus area after MSG supplementation in different concentration for 4 weeks

Group	Percentage of damaged neurons (mean ± SD)		
	Cornuammonis 1	Cornuammonis2	Cornuammonis3
C1(none)	25.92 ± 10.21	23.95 ± 11.42	26.98 ± 10.04
C2(solvent)	17.50 ± 3.88	22.27 ± 6.45	21.74 ± 3.47
T1(2 mg/g)	36.21 ± 7.38*	37.87 ± 7.50*	41.84 ± 4.50*
T2(4 mg/g)	44.89 ± 4.00*	44.48 ± 5.90*	50.75 ± 6.96*
T3(6 mg/g)	52.13 ± 5.95*	55.29 ± 8.15*	66.00 ± 5.23*

* Significant at p=0.05 compared to C1 and C2

Based on histological examinations, whose who treated with the highest MSG dose (6 mg/g BW), had more pyknotic cells and condensed chromatin compared to other groups (**Figure 1**). This finding was dose-dependent manner where the lower MSG dose, the less pyknotic cells and condensed chromatin found in hippocampus of the animals.

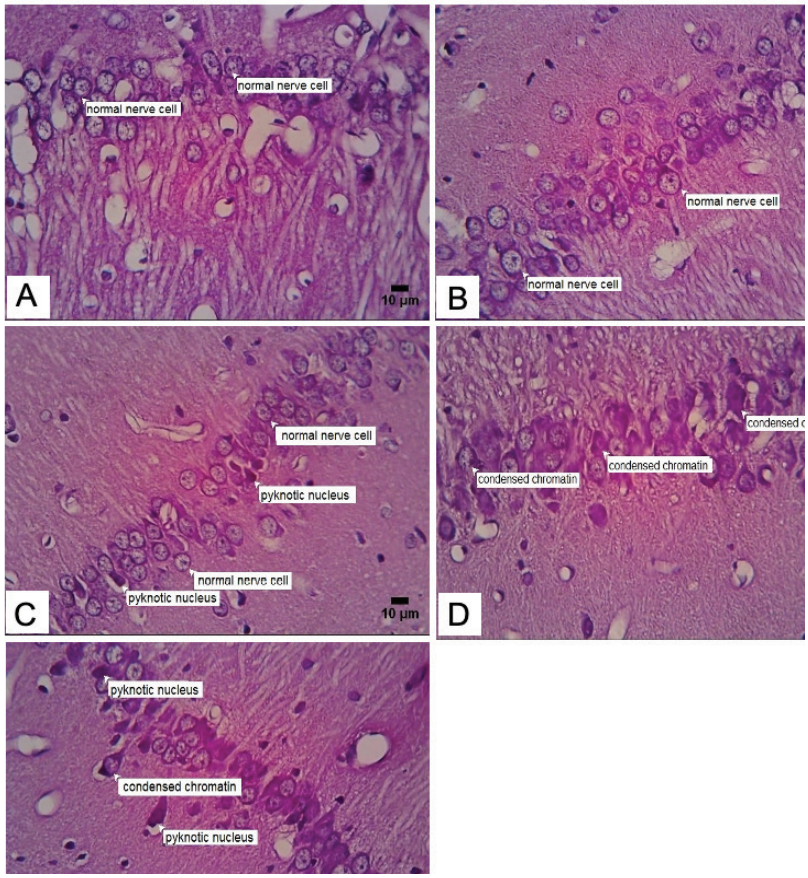

Figure 1. **Hippocampus histological examination of animals within control groups $C1_{(none\ added)}$ (A) $C2_{(solvent)}$ (B) as well as MSG treatment groups $T1_{(2\ mg/g)}$ (C), $T1_{(4\ mg/g)}$ (D), and $T1_{(6\ mg/g)}$ (E). All figures were H&E stained and magnified 400 times.**

Discussion

Glutamate is essential component for neurotransmitter, but excessive glutamate levels would result the accumulation in synaptic cleftsthatwould be excitotoxic to nerve tissues.This accumulation would lead to overstimulation of glutamate receptors, in particular the N-methyl-D-aspartate (NMDA) receptors andactivate multiplepathways thatcause neuron damages and therefore affect the function of nerve tissues.[3, 5, 12, 13] Our data suggested that the percentage of damaged neurons wasincreased in all MSG treatment groupsand wasin dose-dependent effect. The damaged nerve cells werecharacterized by chromatin condensation and pyknotic nucleus. The finding of this study is similar with several other studies which also showed that MSG could cause nervedamage.[14-16]Previous studies found that MSG intake could cause several changes in hippocampus in particular in pyramidal neuronssuch as contracted hyperchromatic nuclei and widening of Virchow - Robin space.[14, 15]

A previous study reported that consumption of MSG 2.4 g/kg/day orally associated with depletion layer of cortex and increase of neuronal damage in animal model.[16]Another study suggested that MSG-associated neurons damages is most likely due to the effects of glutamate excitotoxicity triggered by elevated levels of Ca^{2+} in the cytosol and is mediated through

the process of necrosis and apoptosis mechanism of neuronal excitotoxicity.[11, 17] The existence of excessive stimulation of glutamate receptors will be able to initiate a variety of potential cascades to induce cell damages and death. Moreover, MSGcould also activate glutamate receptors metabotropic causing increased release of Ca^{2+}, stored in the reticulum endoplasm.[18]This condition could trigger a variety of pathways that will eventually cause damages to the synapse and cell death,that can be either apoptosis or necrosis.[19, 20] This occurs due to the formation of free radicals resulted in mitochondrial dysfunction and activation cascade degradation pathway intracellular proteins.[19, 20]

A previous study also suggested that administration of 1 and 1.5 mg/kg MSG for 14 days was associated with decrease spatial learning and memory.[22] Another study suggested that MSG consumption could affected the behavioral of animal included increase the anxiety and caused memory retention.[23]A study found high MSG was associated with high chromatin condensation and pycnoticnuclei of neuron cells in hippocampus[11] suggesting that the reduce of spatial memory probably due to neurotoxicity effect of MSG.

There are some limitations of our study. The number of animals for each group was relatively small. To reduce this effect, all assessments and measurements in this study were conducted in triplicate. At the present study, the histopathology changes were assessed using H&E staining and therefore further specific immunohistochemical examination might could refine the findings. Nevertheless, our study enabled to provide the evidence the effects of MSG on neuron damages in hippocampus.

Conclusion

High dose of MSG for four weeks could increase the number of neuron damagesin rat hippocampus areas, in dose-dependent manner. Thisfinding highlightsthe neurotoxicity effect of MSG in high dose and long-term consumption of this food flavor enhancer.

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Conflict of Interest: Nil

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