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Effects of Monosodium Glutamate on Unscheduled DNA Synthesis and DNA Single-Strand Breaks in Primary Cultures of Rat Hepatocytes

Dong-Hyun Kim and K.H. Yang

Department of Biological Science and Engineering
Korea Advanced Institute of Science and Technology
Seoul, Korea

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Cytotoxic and genotoxic potential of monosodium glutamate (MSG) were evaluated in primary cultures of rat hepatocytes. When exposed to liver cell culture continuously for 24 hr, MSG did not show any cytotoxic effects up to 0.5% (w/v) level as determined by Tryphan Blue exclusion and lactic dehydrogenase release test. MSG also did not induce unscheduled DNA synthesis or DNA single-strand breaks in hepatocyte cultures up to 1% level. No synergistic effects of MSG were observed on aflatoxin B_1-induced DNA damage when 1% MSG was treated to liver cell culture along with aflatoxin B_1.

INTRODUCTION

Glutamic acid occurs as a common constituent of protein and monosodium glutamate (MSG) has been widely used as a flavor agent. The Joint FAO/WHO Expert Committee on Food Additives estimated the acceptable daily intake of MSG for man as 0 to 120 mg per kg of body weight (WHO, 1974). Although the toxicological effects of MSG has been well established over years and the U.S. Food and Drug Administration listed MSG as a GRAS ingredient, the safety of MSG as a food additive has been a public issue from time to time due to its wide use.

The carcinogenic effects of MSG were studied in rat and mouse and proved no evidence of malignancy (Ebert, 1979 a,b). Although these long term animal studies provide the safety of MSG, few informations are available on *in vitro* cytotoxicity and mutagenic effects on cultured mammalian cells. Recently, primary culture of rat hepatocytes has been widely used as an *in vitro* screening system for detecting carcinogenic chemicals since hepatocytes not only contain sufficient level of enzymes necessary for activation of the carcinogen to a chemically reactive form but also serve themselves as the target of the reaction. The measurement of chemically induced DNA repair as unscheduled DNA synthesis (UDS) and determination of DNA single-strand breaks using alkaline elution technique have been extensively studied (Nicolini *et al.*, 1982; Williams, 1982).

The present study was undertaken to evaluate cytotoxicity and mutagenicity of MSG on primary rat hepatocytes culture. We used MMS and aflatoxin B_1 as a positive control in DNA single strand breaks and unscheduled DNA synthesis assay, respectively.

MATERIALS AND METHODS

Primary Hepatocyte Cultures

Male Sprague-Dawley rats (200-250g) were used. Hepatocytes were isolated by a collagenase perfusion technique as reported previously (Yang *et al.*, 1983). Modified Waymouth's medium supplemented with hormones as described by Salocks *et al.* (1981) was used. The hepatocyte suspension was diluted to 1×10^6 cells per ml of medium and 3 ml were pipetted into 60 x 15 mm plastic petri dishes which were precoated with rat tail collagen. The cultures were then incubated at 37°C in a humidified 5% CO_2/95% air incubator for the time indicated. The medium was changed 3 hr after initial plating to remove unattached cells and cellular debris.

Viability and Lactic Dehydrogenase (LDH) Release Test

At 6 hr after initial plating, the medium was replaced with fresh medium containing various doses of MSG. For the viability test, cells were treated with MSG for 24 hr. After incubation, the medium was aspirated off and 0.4% Tryphan Blue in saline was added to culture and allowed to stand for 5 min. The number of viable and nonviable cells was then counted under microscope.

For LDH release test, cells were treated with MSG for 4 hr and LDH activity released into the medium was measured by the method of Lindastrom *et al.* (1978) using the lactate dehydrogenase kit No. 340-UV (Sigma). Triton X-100 was added to one sample to lyse the hepatocytes. The percentage release of LDH was calculated by dividing the enzyme activity in the nonlysed samples by the activity in the lysed sample and multiplying by 100.

DNA Single Strand Breaks Assay

At 6 hr after initial plating, the medium was replaced with fresh medium containing hydroxyurea (10 mM). 1-β-arabinofuranosylcytosine (ara-C, 10 μM) and various doses of MSG. MMS (2 mM) was also used as a positive control. After 24 hr incubation, cells were harvested and the alkaline elution assay was performed as described by Bradley and Sina (1984).

Unscheduled DNA Synthesis Assay

The experimental protocol for UDS assay was similar to that of DNA single strand breaks assay. At 6 hr after the initial plating, the fresh medium containing hydroxyurea (10 mM), ^{3}H-thymidine (1 μCi/ml) and MSG was added. Aflatoxin B_1 (10^{-6} M) was also used as a positive control. After 24 hr incubation, the medium was aspirated off and the plate was washed twice with phosphate-buffered saline (PBS) containing cold thymidine (0.5 mg/ml) and the hepatocytes were harvested.

The filter elution method used for the detection of DNA single-strand breaks was used to measure unscheduled DNA synthesis according to the modification of Hsia *et al.* (1983). The plate which was rinsed with PBS containing 0.5 mg/ml cold thymidine was treated with EDTA solution for 10 min at 37°C and the cells were detached by gentle scrapping with a rubber policeman and dispersed in cold PBS solution. About 6×10^5 cells were loaded onto a 2.0 μm pore size polyvinylchloride filter in a 25 mm filter holder. The cells were lysed in the dark with 10 ml of lysis solution (2% sodium dodecylsulfate, 0.025M EDTA, 0.1 M

glycine, pH 10.0) supplemented with thymidine (0.5 mg/ml) and proteinase K (0.2 mg/ml). The lysis solutoin allowed to flow by gravity. When 1 ml of the lysis solution remained on the filter, the outflow tube from the filter holder was clamped off and incubation was continued for 30 min at room temperature. After incubation, the samples were allowed to drip to dryness. The lysed samples were washed with 5 ml of 0.02 M EDTA solution (pH 10.0). After final washing, each filter was carefully removed and placed in a scintillation vial to which 0.4 ml of HCl was added. The vial was heated for an hour at 70°C. After cooling, 0.5 ml of 2N NaOH was added. The vials were placed in a shaker bath at 25°C and were shaken vigorously for 1 hr. Finally, 0.4 ml of aliquots was taken and 7 ml of Aquasol containing 0.7% acetic acid were then added to each vial for radioactivity counting. For DNA assay, 0.5 ml of aliquots was taken and DNA concentration was measured by the diphenylamine procedure of Burton (1956).

Statistical Analysis

Mean values obtained for control and treated cultures were compared by the Student's t-test. Significance was set up at $p < 0.05$.

RESULTS

To assess the cytotoxicity of MSG on hepatocytes culture, cells were exposed to various doses of MSG for 24 hr and the viability was measured using Tryphan Blue exclusion test. As shown in Table 1, MSG did not show any cytotoxic effects up to 0.5% level. At 1.0% MSG, the viability of hepatocytes was slightly decreased to 90%.

Table 1. Cytotoxic effects of MSG on primary cultures of rat hepatocytes.

Treatment	Viability (% of control)
MSG 0.05% (w/v)	100
0.1	100
0.5	100
1.0	90

Table 2 shows the effects of MSG on lactic dehydrogenase release in primary cultures of rat hepatocytes. In this experiments, hepatocytes were exposed to MSG for 4 hr. Similar to the results of Tryphane Blue exclusion test (Table 1), LDH release was similar to control cultures up to 0.5% MSG. At 1.0% level, MSG induced LDH release higher than controls.

Table 2. Effects of MSG on lactic dehydrogenase release in primary cultures of rat hepatocytes[a].

Treatment	% Release of LDH
Control	6.96 ± 0.85
MSG 0.05% (w/v)	6.94 ± 0.65
0.1	7.38 ± 1.44
0.5	8.48 ± 0.84
1.0	11.41 ± 1.73*

a. Hepatocytes were exposed to MSG for 4 hr. Values shown are the mean ± SE of triplicate determinations. An asterisk indicates values significantly different from the control ($p < 0.05$).

Fig. 1. shows the effect of MSG on DNA single-strand breaks as measured by the alkaline elution technique. At 0.1, 0.5 and 1.0% level of MSG, the fraction of DNA remained on filter was similar to untreated control cultures. Meanwhile, MMS, the positive control, resulted in the increase of DNA single-strand breaks.

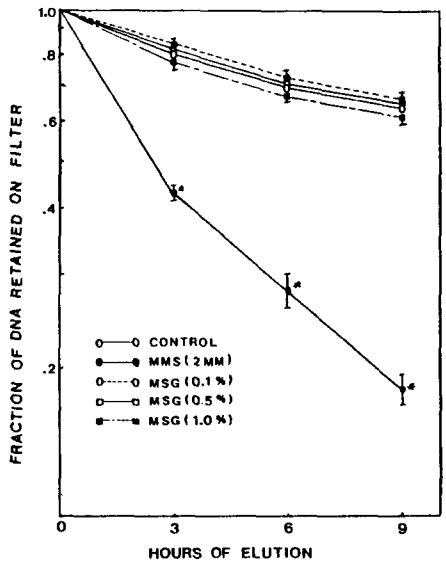

Fig. 1. Effects of MSG on DNA single-strand breaks in primary rat hepatocyte cultures. Hepatocytes were treated with MSG for 24 hr or MMS for 1 hr. All values represent the mean ± SE of triplicate determinations. An asterisk indicates values significantly different from the control ($p < 0.05$).

Table 3 shows the results of unscheduled DNA synthesis. Similar to the DNA single-strand breaks assay, MSG did not induced unscheduled DNA synthesis in hepatocytes cultures up to 1% level while aflatoxin B_1 (AFB_1) increased unscheduled DNA synthesis significantly.

Table 3. Effects of MSG on unscheduled DNA synthesis in primary cultures of rat hepatocytes[a].

Treatment	dpm/μg DNA
Control	494 ± 91
AFB_1 (10^{-6} M)	2,550 ± 640*
MSG 0.05% (w/v)	547 ± 148
0.1	557 ± 25
0.5	481 ± 92
1.0	550 ± 69

a. Hepatocytes were exposed to MSG for 24 hr. Values shown are the mean ± SE of triplicate determinations. An asterisk indicates values significantly different from the control ($p < 0.05$).

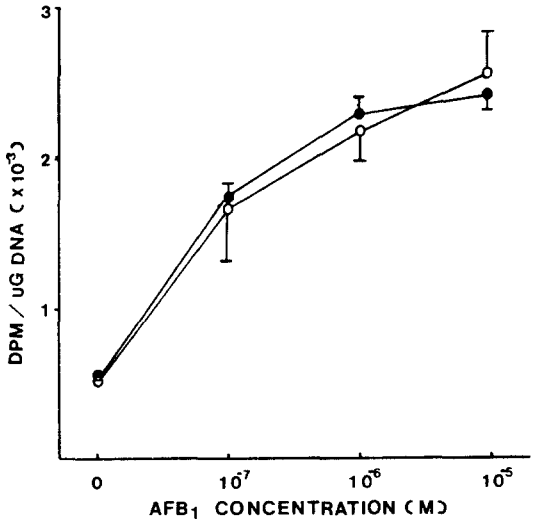

Fig. 2. Effects of MSG on Aflatoxin B_1-induced unscheduled DNA synthesis in primary rat hepatocytes cultures. Hepatocytes cultures were treated with aflatoxin B_1 alone (—○—) or aflatoxin B_1 + 1.0% MSG (—●—) for 24 hr. All values represent the mean ± SE of triplicate determinations.

Since MSG itself did not induce any DNA single-strand breaks and unscheduled DNA synthesis, we investigated possible synergistic effects of MSG to aflatoxin B_1. Fig. 2 shows the results of unscheduled DNA synthesis. In this experiment, hepatocytes were treated with 1.0% MSG along with aflatoxin B_1. Aflatoxin B_1 induced a dose-related increase of UDS up to 10^{-5} M. Meanwhile, MSG did not increased aflatoxin B_1-induced UDS.

DISCUSSION

The measurement of chemically induced DNA repair as unscheduled DNA synthesis has been shown to be an excellent predictor of genotoxic and carcinogenic potential (Williams, 1982). In UDS, the non-S-phase uptake of ^{3}H-thymidine incorporated into DNA is measured. In hepatocyte cultures, the percentage of replicating cell is very small. Nevertheless, it is enough to create a level of ^{3}H-thymidine incorporation into DNA to interfere with measurement of UDS. Therefore, hydroxyurea was used to prevent G_1 cells from entering S phase.

The alkaline elution assay for observation of DNA damage is based on the molecular-weight-dependent retention of the molecule on 2 μm pore size polyvinyl chloride filters. The sensitivity of the measurement is of the order of one DNA lesion per 10^7 nucleotides (Kohn *et al.* 1981). In order to inhibit the semiconservative DNA replication and excision repair, ara-C together with hydroxyurea were added to the culture during treatment with MSG.

Although the safety of MSG has been extensively studied through long term animal studies including teratogenicity, very few informations are available for the direct cytotoxicity on mammalian cell cultures. U.S. Food and Drug Administration reported that cells (kangaroo-rats cell line) were exposed continuously for 72 hr at 0.1% MSG without showing any toxic effects (1969). The results in the present study indicate that MSG does not have any cytotoxic effect on hepatocyte cultures when exposed for 24 hr up to 0.5%. At this concentration MSG also did not induce unscheduled DNA synthesis or DNA single-strand breaks. At 1.0% level, MSG showed slight cytotoxicity on liver cell. However, it did not induce the increased DNA damage at this concentration and had no synergistic effect on aflatoxin B_1-induced mutagenicity.

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일차배양 간세포에서 Monosodium Glutamate에 의한 돌연변이 유발성의 검증

김동현 · 양규환

한국과학기술원 생물공학과

조미료로 널리 사용되고 있는 monosodium glutamate (MSG)의 세포독성 및 돌연변이 유발성을 일차 배양 간세포에서 조사하였다. MSG를 세포 배양액에 첨가하여 24시간 동안 간세포에 처리한 결과 0.5% 농도까지는 간세포에 아무런 독성을 나타내지 않았으며 비주기성 DNA합성이나 DNA 단사 절단도 유발시키지 않았다. 1% MSG에서는 간세포의 생존율이 약간 저하되었으나 이 농도에서도 돌연변이 유발성이 전혀 없는 것으로 판명되었으며, aflatoxin B_1과 동시 처리시에도 aflatoxin B_1에 의한 DNA손상을 증가 또는 감소시키지 않았다.