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Genotoxicity of monosodium glutamate

Nazmiye Ataseven [a], Deniz Yüzbaşıoğlu [a, *], Ayten Çelebi Keskin [b], Fatma Ünal [a]

[a] Gazi University, Faculty of Science, Department of Biology, Genetic Toxicology Laboratory, 06500, Teknikokullar, Ankara,Turkey
[b] Kırıkkale University, Faculty of Engineering, Department of Bioengineering, 71450, Kırıkkale, Turkey

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ABSTRACT

Monosodium glutamate (MSG) is one of the most widely used flavor enhancers throughout the world. The aim of this study is to investigate the genotoxic potential of MSG by using chromosome aberrations (CAs), sister-chromatid exchanges (SCEs), cytokinesis-blocked micronucleus (CBMN), and random amplified polymorphic DNA-polimerase chain reaction (RAPD-PCR) in cultured human lymphocytes and alkaline comet assays in isolated human lymphocytes, which were incubated with six concentrations (250, 500, 1000, 2000, 4000 and 8000 μg/mL) of MSG. The result of this study indicated that MSG significantly and dose dependently increased the frequencies of CAs, SCE and MN in all treatments and times, compared with control. However, the replication (RI) and nuclear division indices (NDI) were not affected. In this paper, *in vitro* genotoxic effects of the MSG was also investigated on human peripheral lymphocytes by analysing the RAPD-PCR with arbitrary 10-mer primers. The changes occurring in RAPD profiles after MSG treatment include increase or decrease in band intensity and gain or loss of bands. In the comet assay, this additive caused DNA damage at all concentrations in isolated human lymphocytes after 1-h *in vitro* exposure. Our results demonstrate that MSG is genotoxic to the human peripheral blood lymphocytes *in vitro*.

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1. Introduction

Nowadays people have to suffer the mutagenic and carcinogenic effects of many genotoxic agents in daily life and working environments due to changing life styles and introduction of novelties. For instance, use of chemical substances such as drugs, food additives, pesticides, and nanomaterials in all areas of life and work environments as well as nature increases fastly. So, finding out the negative effects of these chemical substances on human genome has gained much importance. Monosodium glutamate (MSG) (E621), the sodium salt of L-Glutamic acid being constituent of proteins, is a well known and widely used flavour enhancer worldwide. It is used to enhance the natural flavors of poultry, meats, snacks, seafood, soups and stews (Adeyemo and Farinmade, 2013; Fuke and Shimizu, 1993). MSG, a prototypical umami ligand, is frequently added in Asian cuisine for improving the taste of food (Li et al., 2013). The umami taste is the fifth taste that is unique (Ninomiya, 2002). Umami substances are present in abundance in various foods including vegetables (tomato, potato, cabbage, mushroom, carrot, soybean and green tea), seafood (fish, kelp, seaweed, oyster, prawn, crab, sea urchin, clam and scallop), meat and cheese and contribute greatly to the characteristic taste of them. There are four basic tastes, namely, sweet, sour, salty and bitter (Appaiah, 2010).

The MSG sensitivity, also known as Chinese restaurant syndrome (CRS), includes symptoms of numbness, weakness, and heart palpitations (Appaiah, 2010; Kwok, 1968). Since the detection of MSG sensitivity, a range of studies have claimed the association between MSG and several health outcomes including asthma, diabetes, obesity, and allergic rhinitis (Shi et al., 2013; Williams and Woessner, 2009). Over the past 40 years, anecdotal reports and small uncontrolled studies alleging a variety of MSG-induced reactions have been published. These reports have raised concerns regarding the safety of dietary consumption of MSG (Williams and Woessner, 2009). There is considerable evidence to suggest that consumption of MSG places humans at risk and the greatest risk is for children. It is reported that MSG may be linked with brain tumors and neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease

Abbreviations: CAs, chromosome aberrations; SCEs, sister chromatid exchanges; MN, micronuclei; MI, mitotic index; RI, replication index; NDI, nuclear division index; SCGE, single cell gel electrophoresis; BN, binucleate; MMC, mitomycin-C; RAPD-PCR, random amplified polymorphic DNA-polimerase chain reaction.

* Corresponding author. Tel.: +90 312 202 1205; fax: +90 312 212 2279.
E-mail address: deniz@gazi.edu.tr (D. Yüzbaşıoğlu).

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(Appaiah, 2010; Plaitakis and Shashidharan, 2000). Studies show that rats who had been fed with MSG since birth could not escape mazes or discriminate between stimuli as good as non-MSG-fed rats. The implications for children are that MSG could seriously affect their cognitive skills and cause learning difficulties (Appaiah, 2010; Leber, 2008).

Test methods searching genotoxic effects are applied for the purpose of establishing mutagenic/carcinogenic potentials of chemicals to be introduced or introduced to use in different areas. The level of genetic damage and the effect of mutagens on human health can be determined by a range of test systems in which bacteria, various mammalian cells, *Drosophila* or plants are used. One of the most widely used tests is human lymphocyte cultures (Doak et al., 2012; Soltani et al., 2009). Setting genotoxic potentials of food additives that increasingly become a part of life in recent years is quite crucial in terms of human health and life quality. No study has been found in literature on genotoxicity of MSG on human peripheral lymphocytes using CAs, SCEs, MN, RAPD-PCR and single-cell gel electrophoresis (SCGE)/comet tests. Moreover, previous investigations revealed that MSG showed conflicting results. The US FDA has confirmed that the use of MSG is safe for the general population and it has classified MSG as GRAS. The safety aspects were reviewed by the American Medical Association (AMA), the Joint FAO/WHO (1988) Expert Committee on Food Additives (JECFA), and the Scientific Committee for Food of the European Union (SCF, 1991); and the conclusion was similar to JECFA. JECFA concluded that the total dietary intake of glutamates arising from their use at levels necessary to achieve the desired technological effect and from their acceptable background in foods do not represent a hazard to health. MSG has been allocated an ADI not specified by JECFA, which indicates that no toxicological concerns arise when used as a food additive in accordance with good manufacturing practices (GMPs). For that reason, the establishment of an acceptable daily intake (ADI) was not deemed necessary (Appaiah, 2010). Contrary to this finding, various studies have reported that MSG had toxic (Abass and El-Haleem, 2011; AL-Mosaibih, 2013; Bojanić et al., 2009; Ebaid and Tag, 2013; Farombi and Onyema, 2006; Moreno et al., 2005; Nayanatara et al., 2008; Nwaopara et al., 2004, 2007, 2008; Ortiz et al., 2006; Pavlovic et al., 2007; Vinodini et al., 2008) and genotoxic effects (Adeyemo and Farinmade, 2013; Farombi and Onyema, 2006; Ismail, 2012; Khatab and Elhaddad (2015); Prasath et al., 2013; Renjana et al., 2013; Türkoğlu, 2013), in some test system.

In this paper, genotoxic effects of MSG on human lymphocytes *in vitro* were examined through CAs, SCEs, MN, RAPD-PCR and comet assay because, despite its extensive use, there is any study carried out before by using these tests. These test systems were chosen as they are routinely performed to identify mutagenicity, clastogenicity, and carcinogenicity potential of chemical agents.

The purpose of *in vitro* genotoxicity tests is to determine whether a compound, product and environmental factor induce damage in the genetic material of cells or organisms or may cause cancer by different mechanisms. Therefore, these tests are used as the biological markers and can be controlled development of organisms to genotoxic substances with the information obtained from the tests (Liou et al., 2002). Especially, the increase of CA and MN frequencies in human lymphocytes is among the most often used biomarkers of cancer risk in human (Ginzkey et al., 2014; Hagmar et al., 1998; Huerta et al., 2014). An increase of SCE frequency can display of permanent DNA damage (Cardoso et al., 2001; Sebastià et al., 2014). The cytokinesis-blocked micronucleus test is used to determine acentric fragments or whole chromosomes which are unable to migrate with the rest of the chromosomes during the anaphase of cell division and observed as small nuclei in interphase cells. Hence, the appearance of micronuclei indicate to clastogenic and/or aneugenic effects of chemical substances (Giri et al., 2002). RAPD analysis was also carried out for determination of DNA damage and mutation in response to genotoxic agents that directly and indirectly effects at the level of DNA sequence and structure. These DNA damage can be in the form of single- and double-strand breaks, rearrangements, loss of excision repair, cross linking, point mutations and structural and numerical chromosomal aberrations (Atienzar and Jha, 2006). The comet assay is a sensitive method for determining breaks in DNA (Collins, 2014). Furthermore it is generally necessary to use more than one test system to obtain a full assesment of the genotoxic and mutagenic profile of chemical substances because there is currently no single validated test that can provide knowledge on gene mutations, clastogenicity, and aneuploidy (Formigli et al., 2002).

2. Materials and methods

2.1. Chemicals

The test substance, MSG (CAS No.: 142-47-2), was purchased from Sigma. The chemical structure, molecular formula, and molecular weight of MSG is shown in Fig. 1. This chemical was dissolved in distilled water. The other chemicals cytochalasin-B (Cas. No:14930-96-2), mitomycin C (Cas. No: 50-07-7), bromodeoxyuridine (Cas. No: 59-14-3), NaCl (Cas. No: 7647-14-5), colchicine (Cas. No: 64-86-8), CTAB (Cas. No: 57-09-0) were obtained from Sigma. DMSO (Cas. No: 67-68-5), NaOH (Cas. No: 1310-73-2), Tris (Cas. No: 77-86-1), EDTA (Cas. No: 6381-92-6), Triton X-100 (Cas. No: 9002-93-1), Low Melting Agarose (Cas. No: 9012-36-6), Normal Melting Agarose (Cas. No: 9012-36-6), EtBr (Cas. No: 1239-45-8), H_2O_2 (Cas. No: 7722-84-1) were obtained from Applichem.

2.2. Lymphocyte cultures and isolations

This study has complied with the principles of the local ethics committee. The study was carried out on human peripheral blood samples taken from three healthy (two female and one male; RAPD-PCR analysis of two healthy volunteers), non-smoking volunteers, aged 23–26 years, not exposed to any drug therapy for at least three weeks prior and with no history of chromosome fragility or recent viral infection. The experiments were conducted using the same blood samples, divided into two parts: CAs, SCEs, MN, and RAPD-PCR were measured in whole blood, whereas the alkaline comet assay was used to evaluate DNA damaging effect of MSG on isolated human lymphocytes.

2.3. Dose selection

To detect the concentrations of MSG in our tests, LD_{50} values in rodents (Table 1), earlier study reports, and Turkish Food Codex (almost all foodstuff: 10 g/kg and varieties of spices and seasonings: no maximum level is specified for the additive in question) were analyzed and then a preliminary study was carried out to determine the best concentration range compatible with a good cell-proliferating activity, and then resulting in a sufficient number of metaphases for Mitotic index.

2.4. Chromosomal aberrations and sister chromatid exchange assay

For the CA and SCE assays, whole blood was added to chromosome medium supplemented with 10 μg/mL bromodeoxyuridine. The cultures were incubated in the dark at 37 °C for 72 h. The cells were treated with 250, 500, 1000, 2000, 4000 and 8000 μg/mL concentrations of MSG for 24 h (MSG was added 48 h after initiating the culture) and 48 h (MSG was added 24 h after initiating the

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