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Anti-Angiogenic and Oxidant Effects of Monosodium Glutamate at Different Concentrations in Chorioallantoic Membrane Model

Farklı Konsantrasyonlardaki Monosodyum Glutamatın Koryoallantoik Membran Modelinde Anti-Anjiyojenik ve Oksidan Etkileri

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Öz

Monosodyum glutamat (MSG), lezzet artırıcı bir gıda katkı maddesidir. İşlenmiş gıda tüketimindeki yükselişe bağlı olarak MSG maruziyeti her geçen gün artmaktadır. MSG maruziyeti çeşitli doku ve organlara zarar vermektedir. Bu çalışmanın amacı, MSG'nin anjiyogenez ve oksidan-antioksidan dengesi üzerindeki etkilerini araştırmaktır. Üç farklı konsantrasyonda MSG (10^{-4} M, 10^{-5} M ve 10^{-6} M), kontrol ve bevasizumab (10^{-6} M) hazırlanıp, embriyoların korioallantoik membranına (CAM) yerleştirildi. Deneyden önce ve sonra tavuk yumurtalarından sıvı alındı. Yumurta kabuğu üzerinde açılan pencereden anjiyogenez araştırıldı. Kontrol ve 10^{-6} M MSG grubunda (ortalama skor: 0.3) anjiyogenez normal bulundu. Anti-anjiyojenik etkilerin, 10^{-5} M MSG grubunda (ortalama puan: 0.5) ve 10^{-4} M MSG grubunda (ortalama puan: 0.7) orta düzeyde ve bevasizumab grubunda (ortalama puan: 1.1) güçlü olduğu tespit edildi. Sonuçlarımıza göre MSG daha yüksek dozlarda antianjiyogenik özellikler göstermektedir. Araştırmamızın sonuçlarına göre MSG'nin CAM modelinde anjiyogenezi doza bağımlı bir şekilde inhibe ettiği ve oksidan-antioksidan dengesini bozarak oksidatif hasarda artışa neden olabileceği görülmektedir. MSG'nin CAM modelinde anjiyogenez ve oksidan-antioksidan denge üzerindeki etkilerine ilişkin literatürde daha önce yapılmış bir çalışma tespit edilemediği için araştırma sonuçlarımızın literatürdeki önemli bir eksikliği gidereceğini düşünmekteyiz.

Anahtar Kelimeler: Anjiyogenez, Koryoallantoik Membran Modeli, Monosodyum Glutamat, Oksidatif Stres

Abstract

Monosodium glutamate (MSG) is a flavor-enhancing food additive. MSG exposure is rising day by day because of the high commercial food consumption. MSG exposure causes damage to various tissues and organs. The aim of this study is to investigate the effects of MSG on angiogenesis and oxidant-antioxidant balance. Three different concentrations of MSG (10^{-4} M, 10^{-5} M, and 10^{-6} M), control, and the bevacizumab (10^{-6} M) were prepared and placed on the chorioallantoic membrane (CAM) of the embryos. Albumen was taken from the embryos before and after the experiment. Angiogenesis was investigated through the window that was opened on the eggshell. Angiogenesis was found to be normal in the control and 10^{-6} M MSG group (average score: 0.3). Anti-angiogenic effects were moderate in the 10^{-5} M MSG group (average score: 0.5) and in the 10^{-4} M MSG group (average score: 0.7), and strong in the bevacizumab group (average score: 1.1). According to our results, MSG shows anti-angiogenic properties in higher doses. MSG increased oxidative stress. According to the results of our research, it is seen that MSG inhibits angiogenesis in a dose-dependent manner in the CAM model and may cause an increase in oxidative damage by disrupting the oxidant-antioxidant balance. Since no previous study has been found in the literature regarding the effects of MSG on angiogenesis and oxidant-antioxidant balance in the CAM model, we think our results will fill an important gap in the literature.

Keywords: Angiogenesis, Chorioallantoic Membrane Model, Monosodium Glutamate, Oxidative Stress

Introduction

Monosodium glutamate (MSG), known with the international code E621, is a flavor-enhancing food additive frequently added to processed foods and ready meals as a flavor enhancer. Among the food and ready meals where MSG can be added as a flavor-enhancing food additive, mainly hamburgers, chips, salami, sausage, crackers, French fries, fish, grills, ready-made meatballs, and raw meatballs, instant soups, broth tablets, chicken broth tablets, canned food, frozen appetizers, sausage can be counted (1,2). The effects of MSG, which is widely used worldwide, on human health are being investigated as an essential question. Various experimental studies have shown that MSG exposure can cause damage to various tissues and organs, especially the brain and nerve tissue (3,4). However, the mechanism by which the harmful effects of MSG occur has not been fully elucidated. It is thought that MSG can cause these harmful effects by increasing oxidative stress and inhibiting angiogenesis that causes cell and tissue damage and delaying the healing of the damage (5-7).

The chick embryo chorioallantoic membrane (CAM) is an extra-embryonic membrane that mediates gas transfer between the embryo and the air. The mesodermal layers of the allantois and chorion fuse and form the CAM. It has an extensive vascularization which is easy to access. Therefore, the CAM is commonly used to investigate angiogenesis, especially to evaluate the efficacy and mechanisms of molecules for angiogenic and anti-angiogenic effects (8,9).

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In this study, it was aimed to show the changes in oxidative stress and angiogenesis inhibition, which may be possible mechanisms in the damage caused by MSG exposure, on an experimental CAM model.

Material and Method

The CAM model does not require ethics committee approval, and the document stating this was nevertheless obtained from Akdeniz University Animal Ethics Committee.

Ross 308 genus fertilized chickens' eggs were used. Embryos were placed at 37.5°C and 60% relative humidity. 6 days after the incubation 5 mL of albumen was aspirated with a syringe and used to evaluate oxidative stress markers, and the eggshell was removed on the opposite side. Normal embryo development was evaluated (Figure 1A) through the aperture, and malformed or dead embryos were excluded. Embryos were divided into five groups, 10 for each group. Each group consisted of ten chorioallantoic membrane models, which were applied as given below, respectively. Negative Control; Contains only agar. MSG 10^{-4} M; Agar containing 10^{-4} M monosodium glutamate (MSG). MSG 10^{-5}; Agar containing 10^{-5} M MSG. MSG 10^{-6} M; Agar containing 10^{-6} M MSG. Bevacizumab; Agar containing 10^{-6} M Bevacizumab. Doses were determined based on previous studies (10). Pellets were placed on the CAM. The aperture was covered with stretch film, and embryos were put into an incubator for 2 days. On the eighth day of incubation, the vascular densities were evaluated. After that, 5 ml of albumen was taken to assess oxidative stress markers.

Angiogenesis Scoring

Vascular development and the effects of the pellets on capillary density were evaluated and photographed and then scored as described. If the score was 0, then there is no effect, this means normal embryo formation and no change with respect to surrounding capillaries. If the score was 0.5, then there is a weak effect and this means no capillary-free areas and decreased capillary density but not more than the pellet. If the score was 1, then there is a moderate effect and this means the small capillary-free area or capillary density decreased in a specific area, and effects are not bigger than twice the pellet size. If the score was 2, then there is a strong effect, and this means a capillary-free area around the pellet at least twice the pellet size. Ten embryos were used for each group, and pellets just containing agar were used as a negative control group. A developed mean scoring system was used to evaluate the active ingredients used on the CAM. The following formula was used for the determination of the average score:

Average score= [number of embryos (score 2) X 2+ number of embryos (score 1)]/ total number of embryos (score 0, 1, 2).

According to the average score, the obtained values were expressed as follows: Normal development or no anti-angiogenic activity: score < 0.5, Mild or moderate anti-angiogenic property: score 0.5-1, and Marked or powerful anti-angiogenic property: score>1 (10).

Biochemical Analysis

Total antioxidant status (TAS) and total oxidant status (TOS) levels were measured colorimetrically using a commercial kit (Mega Tip Inc. Gaziantep, Turkey) (6). The oxidative stress index (OSI) was calculated with the TOS/TAS formula (7). The Erel method in the literature formed the basis of oxidant and antioxidant measurements (6,7).

Statistical Analysis

The angiogenesis scores were compared with an average score system described in previous studies. The raw values were presented as the mean ± standard deviation (SD). Oxidative stress markers were compared by the one-way analysis of variance (ANOVA) test. For post-hoc comparing, the groups, Tukey and Duncan's tests were used. A p of less than 0.05 was determined as statistically significant.

Results

The number of embryos were shown according to the degree of anti-angiogenesis on the CAM (Table 1). In the control (drug-free pellet) group, normal vessel development formation was detected on all CAMs (Figure 1B). Although a weak anti-angiogenic effect was detected on some CAMs in the 10^{-6} M MSG group, vascular development was normal in general (Figure 1C), and MSG did not show any significant anti-angiogenic effect according to the mean score (Table 1). A moderate anti-angiogenic effect on 5 CAMs (50%) was detected in the 10^{-5} M MSG group (Figure 1D), while a moderate anti-angiogenic effect on 3 (30%) CAMs in the 10^{-4} M MSG group and a strong anti-angiogenic effect on 2 (20%) CAMs in the 10^{-4} M MSG group (Figure 1E) anti-angiogenic effect was detected. When evaluated according to the mean score, angiogenesis was found to be mild to moderately affected in the 10^{-5} M MSG group (average score: 0.5) and in the 10^{-4} M MSG group (average score: 0.7) (Table 1). In the positive control group, the mean score was 1.1, and as expected, bevacizumab strongly suppressed angiogenesis (Figure 1F). ANOVA analyses of TAS, TOS, and OSI values in MSG-exposed groups were given in Figures 2, 3, and 4.

Table 1. Anti-angiogenic scoring of MSG and Bevacizumab at different doses

Score	0	0.5	1	2	Average score**
Control group	**10**	**0**	**0**	**0**	0
***MSG 10^{-4}M n(10)**	**3**	**2**	**3**	**2**	**0.7**
MSG 10^{-5}M n(10)	**2**	**3**	**5**	**0**	**0.5**
MSG 10^{-6}M n(10)	**4**	**3**	**3**	**0**	**0.3**
Bevacizumab 10^{-6} M n(10)	**0**	**2**	**5**	**3**	**1.1**

*MSG: Monosodium glutamate. ** Score < 0.5: Normal development or no anti-angiogenic activity. Score 0.5-1: Mild or moderate anti-angiogenic property. Score>1: Marked or powerful anti-angiogenic property.

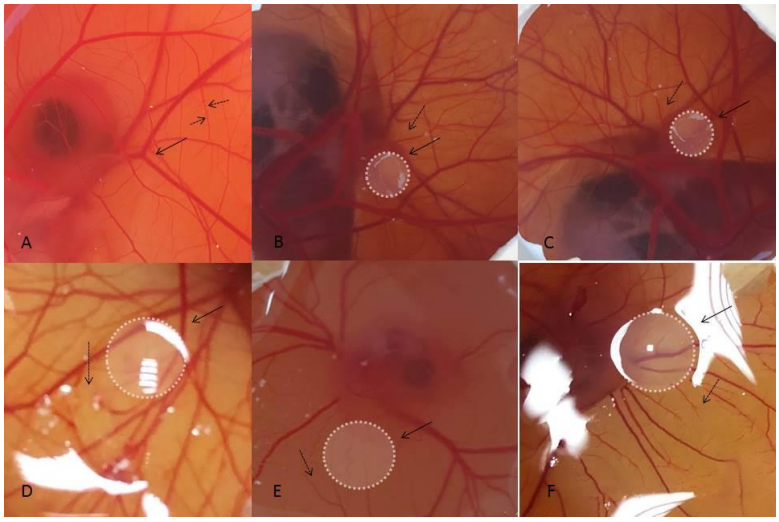

Figure 1. **A:** Normal development of chick embryo (black arrow shows the normal main vessel formation) with normal capillary vessel (dashed arrows) formation. **B:** Drug-free pellet with a non-disrupted main vessel (black arrow) and normal capillary formation (dashed arrow). **C:** Unaffected main vessel (black arrow) and capillary (dashed arrow) formation on pellet-applied CAM treated with 10^{-6} M MSG concentration. **D:** Unaffected main vessel (black arrow) growth with disrupted capillary (dashed arrow) formation on pellet-applied CAM treated with 10^{-5} M MSG concentration (weak anti-angiogenic effect). **E:** Marked decreased vascularity (black arrow) with decreased capillary density on pellet-applied CAM treated with 10^{-4} M MSG concentration (marked anti-angiogenic effect). **F:** Marked disruption on the main vessel (black arrow) formation with a marked decrement of capillary density (dashed arrow) on pellet-applied CAM treated with 10^{-6} M bevacizumab concentration (marked anti-angiogenic effect).

Discussion

MSG can be added as a flavor-enhancing food additive, mainly to hamburgers, chips, salami, sausage, crackers, French fries, fish, grills, etc. It is widely used around the world and exposure is rising day by day. MSG exposure causes damage to various organs and tissues (1,2). There is not enough research on MSG's anti-angiogenic and oxidant-antioxidant balance in the CAM model.

According to our results, MSG shows anti-angiogenic properties in higher doses. These findings are among the first results presenting the anti-angiogenic effects of MSG in different doses. Moreover, our results show that MSG experimentally increased oxidative stress in each dose.

There are conflicting results about the effects of MSG on angiogenesis, especially in studies on enteropathy, it has been claimed that MSG has beneficial effects on ulcerated or damaged tissues. It has been explained that these curative effects increase mucus secretion in various ways and stimulate angiogenesis by increasing vascular endothelial growth factor (VEGF) (11,12). On the contrary, in another study investigating the effects of MSG on the endometrium, it was suggested that MSG has a toxic effect on the endometrium by suppressing VEGF release (13). In another study investigating the effects of MSG on mouse mesenchymal stem cells, it was determined that MSG did not cause cytotoxicity. In this study, which experimentally investigated the toxic effects, the effects of MSG on leptin stimulating angiogenesis were investigated and it was claimed that no effect was observed at the doses used in the experiment (14). In a study that investigates neonatal excitotoxicity, the findings support VEGF-mediated signaling taking the role in excitotoxicity triggered by neonatal MSG treatment. Investigators found fast incremental immunoreactivity levels to VEGF-A protein in both the cerebral motor cortex and hippocampus following the administration of MSG (15). We did not evaluate VEGF levels, but we

identified the direct effects of MSG on capillaries in the CAM model. Depending on our findings, MSG shows different effects according to dosage. There was an anti-angiogenetic effect at high doses, while there was no effect at low doses. The contradictory results presented in different studies in the literature may be due to this dose-related effect.

If the increase in oxidative stress cannot be balanced by the antioxidant system in the cell, then oxidative damage occurs. Various harmful effects such as degradation of DNA structure, lipid peroxidation, deterioration and destruction of protein structure, destruction of membranes as a result of damage to proteins and lipids of membrane structures, and deterioration of enzyme structures occur in the cell with the effect of oxidative damage (16-20). Nowadays, researchers have gained importance to show the role of free radicals in human diseases. The harmful effects of free radicals have been shown to play a role in the formation mechanisms of diseases such as cancer, heart diseases, diabetes mellitus, and many vascular pathologies (21-27).

According to the results of our research, it is seen that MSG inhibits angiogenesis in a dose-dependent manner in the CAM model and may cause an increase in oxidative damage by disrupting the oxidant-antioxidant balance. There is not enough data in the literature on the subject. We think that will make an essential contribution to the literature with the data obtained from this study. If the oxidant and angiogenesis inhibitory effects of MSG are demonstrated by new studies, various suggestions, such as limiting the use of MSG or adding antioxidants to products using MSG may come to the fore.

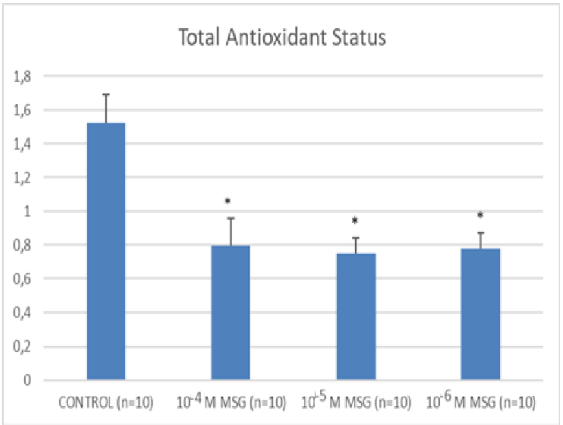

Figure 2. Significant difference compared to the control group. All the experimental groups differ significantly from the control group ($p<0.001$). The 10^{-4} M MSG, 10^{-5} M MSG, and 10^{-6} M MSG groups did not differ significantly ($p>0.05$).

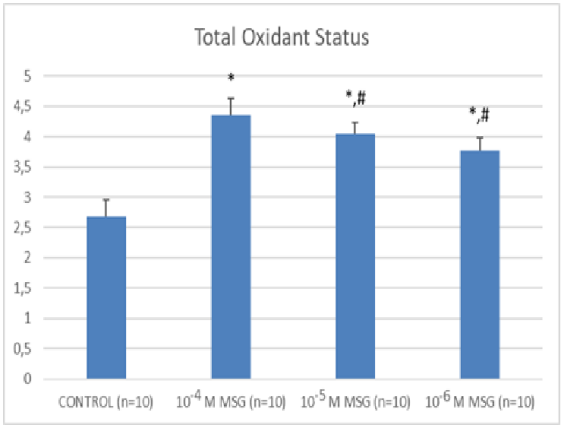

Figure 3. Significant difference compared to the control group. # = Significant difference compared to the 10^{-4} M MSG group. All the experimental groups differ significantly from the control group ($p<0.001$). When the 10^{-4} M MSG group was compared with the 10^{-5} M MSG and 10^{-6} M MSG groups, there were significant differences between both groups (0.03, <0.001, respectively). 10^{-5} M MSG and 10^{-6} M MSG groups did not differ significantly ($p>0.05$).

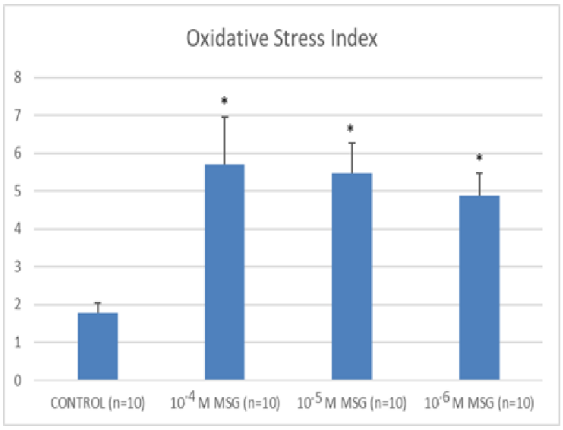

Figure 4. Significant difference compared to the control group. All the experimental groups differ significantly from the control group ($p<0.001$). The 10^{-4} M MSG, 10^{-5} M MSG, and 10^{-6} M MSG groups did not differ significantly ($p>0.05$).

In conclusion, according to the detailed results of our research, it has been shown that MSG causes an increase in TOS and OSI values, a decrease in TAS, an increase in oxidative damage, and inhibits angiogenesis in a dose-dependent manner. Limiting the use of MSG may be an important measure to protect the health of living organisms.

Ethics Committee Approval: Ethics committee approval was obtained from ASM Hospital Ethics Committee (ASM-EK-21/160) for the study.

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