Original Article

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The Effect of Monosodium Glutamate on Neural Tube Development of Early Chicken Embryo: An *in vivo* Experimental Study

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Abstract

Aim: Although monosodium glutamate is widely used in the food industry, it has effects in terms of teratogenicity, especially during pregnancy and embryogenesis. Although monosodium glutamate is widely used in the food industry, it has effects in terms of teratogenicity, especially during pregnancy and embryogenesis. Our study analyses the effect on the development of the notochord by giving monosodium glutamate to chicken egg embryos.

Methods: The incubation and embryonic development follow-up of our study were held in the experimental animals laboratory of University of Health Sciences Turkey, Istanbul Bakirkoy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, and the preparation and microscopy of the samples were carried out in the Pathology Department of the Cerrahpasa Faculty of Medicine. 120 fertile, pathogen-free eggs were incubated at 75% humidity and 37.4±0.2 °C until embryos reached Hamburger and Hamilton stages 9-10 (30th hour). Eggs were divided into four groups. Group 1 consisted of uninjected eggs, group 2 consisted of eggs injected with saline (10 nL 0.9 NaCl), group 3 consisted of eggs injected with 15 mg/kg monosodium glutamate (MSG); and group 4 consisted of eggs injected at regular intervals.

Results: The correlation values between height, dilatation, autolysis, chest, and lumbar diameters of embryos in all groups were examined. The mean embryo length was 1.0017±0.36 cm. The mean chest diameter was 20.70±14.45 cm. The mean lumbar diameter was 10.31±14.34 cm. When the groups were compared in terms of embryo length, it was observed that the groups given MSG were significantly shorter than the control and SF groups (p=0.00).

Conclusion: The amount of MSG taken with food is important because it can affect the organs. Depending on the amount consumed, MSG may adversely affect notochord development.

Keywords: Embryo, monosodium glutamate, neural tube defect, teratogenicity

Introduction

Neural tube defects (NTDs) are a group of congenital anomalies affecting the meninges, vertebrae, muscles, and skin. They are the second most common congenital malformation after cardiac and vascular anomalies (1,2). Many children die from congenital abnormalities (1,3,4). The risk of NTD is between 1 and 10 per 1000 births worldwide (3,4). The period when the embryo is most sensitive to teratogenicity is the embryogenesis period between the 3rd and 8th weeks of pregnancy (1). Most spinal cord defects occur due to abnormal closure at the 3rd and 4th weeks of neural fold development (2). Anencephaly, a type of NTD, results in death soon after birth, while myelomeningocele or spina bifida can

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cause lifelong disability with high mortality, neurological, cognitive, urological, and gastrointestinal complications (2). Spina bifida is most commonly seen in the lumbosacral region (1).

Genetic factors are responsible for 30% of NTDs, while environmental and multifactorial causes account for 15% and 55%, respectively. Although more than 100 genes are thought to be involved in causing NTDs, only 20 of these have been identified. Studies on 20 other genes are ongoing (1).

Monosodium glutamate (MSG) is the sodium salt of non-essential glutamic acid and is a flavor enhancer used frequently in the food industry. It can cause conditions such as cardiotoxicity, hepatotoxicity, neurotoxicity, low-grade inflammation, metabolic disorder, premalignancy, asthma, Chinese restaurant syndrome, and personality changes (5-7). Although MSG is widely used in the food industry, it has effects in terms of teratogenicity, especially during pregnancy and embryogenesis.

Our study determines the effect of MSG injection on the development of chicken egg embryos regarding height, dilatation, and autolysis.

Materials and Methods

Compliance with Ethical Standards

The incubation and embryonic development follow-up of our study were held in the experimental animals laboratory of University of Health Sciences Turkey, Istanbul Bakirkoy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, and the preparation and microscopy of the samples were carried out in the Pathology Department of the Cerrahpasa Faculty of Medicine. It was reviewed and approved by the Ethics Committee of University of Health Sciences Turkey, Istanbul Bakirkoy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital (2015/46084). Animal care and all experiments comply with the Council of the European Communities Directive of November 24, 1986 (86/609/EEC) on the protection of animals for experimental use.

Study Design

Our study is an *in vivo* experimental animal study. The post-blastula periods of chicken eggs and mammalian embryos are similar. One hundred twenty fertile pathogen-free eggs (65±2 g) (Atabey, Gallus gallus, Poultry Research Institute, Ankara, Turkey), were incubated at 75% humidity and 37.4±0.2 °C until embryos reached Hamburger and Hamilton stages 9-10 (30th hour).

Eggs were divided into four groups. The first group consisted of uninjected eggs; the second group of eggs were injected with physiological saline (10 nL of 0.9 NaCl); the third group of eggs were injected with a normal dose of MSG (15 mg/kg); and the fourth group of eggs were injected with a neurotoxic dose of 60 mg/kg. After MSG was dissolved in water, the calculated doses were injected into the blastoderm with a 24-gauge syringe. The eggs covered with sterile drapes were opened 72 hours later, their shells were opened, and the embryos were removed microscopically. All the eggs were normal and were classified according to whether there was a defect or not. Embryogenic, vascular, and developmental conditions were observed.

All embryos were fixed with a 10% formalin solution. After 24 hours, they were examined macroscopically for their craniocaudal lengths. Also, measurements were made from the thorax and waist levels of the embryos. Additionally, if the embryo was longer or shorter, it was sampled more or less. Then, all samples underwent routine pathology procedures, and slides were prepared and stained with hematoxylin and eosin (H&E) (Figure 1). All slides were examined by microscope, and all neural tubes at each level were measured anterior-posteriorly and right-left laterally via an ocular micrometer (Figure 2).

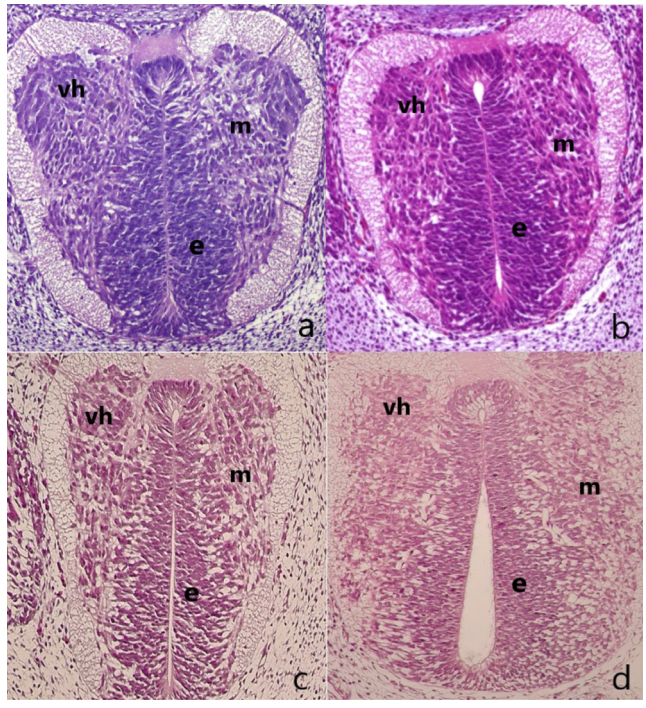

Figure 1. a) Normal neural tube of a normal chicken embryo in the control group (H&E, x 100), **b)** The neural tube of a chicken embryo in the group given physiological saline is seen (H&E, x100), **c)** Dilatation is seen in the chick embryo given MSG 15 mg/g (H&E, x100), **d)** Serious dilatation is seen in the chick embryo given a toxic dose of MSG 60 mg/g (H&E, x100)
e: Ependymal cells, m: Mantle region, vh: Ventral horn, H&E: Hematoxylin and eosin, MSG: Monosodium glutamate

Statistical Analysis

The SPSS v20.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis. The chi-square test was used to evaluate the differences between groups in terms of categorical variables. P-values <0.05 were considered statistically significant.

Results

The height, dilatation, and autolysis of all embryos were examined, as were the thoracic and lumbar diameters. The mean embryo length was 1.0017±0.36 cm. The mean thoracic diameter was 20.70±14.45 cm. The mean lumbar diameter was 10.31±14.34 cm (Table 1).

There was significantly less dilatation in the control and saline-treated groups than in the MSG-administered groups (p=0.000). Dilatation was significantly higher in high-dose MSG groups and saline (SF) groups (p=0.032). When the groups were compared in terms of embryo length, it was observed that the groups given MSG were statistically shorter than the control and SF groups (p=0.00). When the control group and SF group were compared, there was a decrease in embryo height in the SF group (p=0.048).

When the SF group was compared with the control group, it was found that there was a statistically significant enlargement in the thoracic diameter (p=0.00). However, while the thoracic diameter increased in the SF group, there was a decrease in the lumbar diameter. Thoracic diameters in the high-dose MSG group were found to be statistically larger than in the normal-dose group (p=0.004).

There was a statistically significant relationship between embryo length and lumbar diameter (p=0.000). As the embryo length decreased, the lumbar diameters decreased. As the embryonic height decreased, there was a statistically significant increase in autolysis (p=0.039) and dilatation (p=0.002).

There was a statistically significant relationship between autolysis and thoracic diameter (p=0.01). As the thoracic diameter increased, autolysis increased (Table 2). There was a statistically significant relationship between thoracic diameter and embryo length (p=0.044).

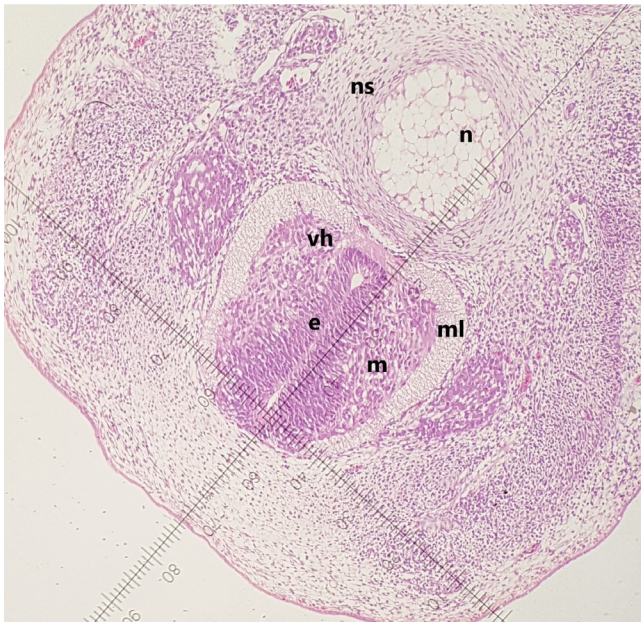

Figure 2. All embryos' neural tubes are measured via ocular micrometer by microscope.
n: Notochord, ns: Notochordal sheath, e: Ependymal cells, m: Mantle region, vh: Ventral horn, ml: Marginal layer

Discussion

Neural tube defect is a complex congenital malformation of the nervous system. Spina bifida, myelomeningocele, meningocele, anencephaly, encephalocele, and other types are frequently seen (4). Anencephaly and spina bifida are the most common types (7). The risk of NTD is 1.67±1000; the risk of spina bifida is 1.13±1000, the risk of anencephaly is 0.25±1000; and the risk of encephalocele is 0.15±1000 (4). When terminated patients are added, the rate becomes 2.55±10004 for the risk of NTD (4). The rate of incidence in the USA has decreased to 1±1500 cases after folic acid use. Multiple gene mutations are associated with NTD. The live birth rate of important structural anomalies is 3% (1).

Neurulation is complete on the twenty-eighth day (1). Here, the spinal cord and brain develop. On the 28th day, when neurulation is complete and the neural tube is closed, most mothers are not aware of the pregnancy. Morphological activities regulated by genes in the closure of NT must perform functions in a coordinated manner. Cell death, neural migration, neuroepithelial development, cytoskeletal microfilament contraction, and bending sites in neural tube development are all in a certain order (7).

Neural tube defect is a disease caused by complex interactions between environmental factors, epigenetic infections, and genetic susceptibility (2). Folic acid deficiency is the highest known risk factor for NTD. Maternal diabetes, obesity (body mass index >30), hyperthermia, hypervitaminosis A, maternal age over 40 and under 19, socioeconomic status, lack of education, nutritional deficiency, heavy metal work during pregnancy, air pollution due to coal, smoking, alcohol, caffeine, and medical drug intake (valproic acid, thalidomide, and serotonin reuptake inhibitors), measles infection, and phenylketonuria are among the other causes of NTD (1-4).

Glutamate is the most abundant amino acid in plasma. It has many biological functions and is a non-

Table 1. Embryo height and thoracic and lumbar diameters			
Groups	**Embryo height (cm) (p=0.000)**	**Thoracic diameters (1/1000 cm) (p=0.131)**	**Lumbar diameters (1/1000 cm) (p=0.000)**
Group 1	1.22±0.41	12.0±15.29	15.40±15.16
Group 2	1.006±0.40	26.93±10.89	13.66±15.17
Group 3	0.94±0.23	25.4±13.18	10.20±14.70
Group 4	0.84±0.25	18.46±13.57	2.00±7.61

Table 2. Group 1; Control (un-injected eggs), Group 2; physiological saline, Group 3; injected with a normal dose of MSG (15 mg/kg), Group 4; (60 mg/kg) injected high dosage, undeveloped, autolysis and dilatation observation numbers			
Total 120 eggs	**Undeveloped egg at oven**	**After 72 hours living blastoderm's* autolysis (p=0.008)**	**Dilatation (p=0.000)**
Group 1 (n=30)	2	0	0
Group 2 (n=30)	4	0	0
Group 3 (n=30)	0	2	12
Group 4 (n=30)	4	4	8
*Undeveloped egg at oven at hour 30 and autolysis=Developed blastoderm after 72 hours were autolytic MSG: Monosodium glutamate			

essential amino acid (8). It acts as a neurotransmitter through glutamate receptors to produce physiological and pathological effects. It is also involved in cellular proliferation, spermatogenesis, and immune functions. In the glutamate transamination reaction, it participates in energy production by converting -α-ketoglutarate. It takes part in the metabolism of proteins, carbohydrates, and lipids (9). Excessive glutamine intake also increases its conversion to other amino acids and ATP production (10). In addition, the risk of damage to the central nervous system, ischemia, and seizures increases with an increase in glutamate intake beyond 30 mg/kg/day (5,6).

Monosodium glutamate is a white, crystalline substance fermented from sugar cane and beet. It is described as umami, unlike the other four senses, because it has both an umami and salty taste. It does not change during cooking but can be dried in an acidic environment at high temperatures. Due to these features, it offers good stability (11). Monosodium glutamate can be present in both an ionic and solid state and decomposes in an aqueous solution (12). Monosodium glutamate is used as an additive in the food industry. It is widely used as a flavor enhancer because of its ability to strengthen the taste of food (5). Monosodium glutamate is found naturally in meat, cheese, and seafood. There are several reasons why it is so widely used in the food sector; no need for special permits, low cost, easy transport in powdered form, and ease of purchase. They can be found in convenience foods, salad dressings, ketchup, mayonnaise, and preserves (6). In industrial countries such as Europe, daily MSG intake is around 0.3-1.0 grams (5-15 mg/kg) in humans (5,6). The effect of MSG is more pronounced in certain regions of the brain. By affecting the hypothalamus, it disrupts adipose tissue homeostasis and may cause weight gain. It has also been stated that it may cause obesity (13). High glutamate intake may have deleterious effects on multiple organs and systems (5). In neurotoxic doses (60 mg/kg), interactions with the hypothalamus, hippocampus, amygdala, cerebrum, and cerebellum cause personality changes, aggression, loss of muscle strength, a decrease in locomotor activity, and an increase in NO (5,6). Since there is no study in the literature showing the effect of MSG on the notochord, our study is the first on this subject.

In our study, the embryo lengths of the control group and the groups given SF were longer than those of the groups given MSG. Additionally, chest diameter increased, cell migration slowed down, and midline fusion was delayed in the MSG group compared to the control group. When MSG-administered groups were compared with each other, it was observed that embryos given a toxically high dose of MSG were shorter in length and had more autolysis. In another experimental study, MSG was examined in Wistar Albino rats, and they found a decrease in fetal weight, crown vertebral lengths, and placental weight (14). In 19-day rat fetuses, they found no condrification and latency on ossification of the cervical vertebrae and absence of the caudal vertebrae (14). Another study showed degenerative and apoptotic changes in motoneurons and neuroglia in MSG-given rats (15), whereas our study was found compatible with the literatüre (14,15).

The blood-brain barrier restricts the passage of glutamine to the brain (16). In a study, blood-brain barrier permeability increased in newborn male rats administered a toxic dose of MSG, and it was stated that increased MSG consumption was related to an increase in vascular endothelial growth factor type-2 receptor levels. It has

been reported that the increase in the vascular endothelial growth factor type-2 receptor causes an increase in the vascular permeability of the blood-brain barrier (17). In another study, it was stated that since the blood-brain barrier is not well developed in the newborn period, it is more affected by the increase in the amount of glutamine (18). An increase in glutamine levels causes neuronal overstimulation. This may cause apoptosis and necrosis (19). In our study, growth retardation on the notochord increased with an increase in MSG dose. Although MSG does not cross the placenta, it is found in high doses in the fetal circulation (20,21). Additionally, it is difficult to determine the daily intake of MSG in processed foods since the number of additives is unknown. As it is not possible to calculate the amount of MSG consumption, care should be taken when consuming foods containing MSG, especially during pregnancy. Our study showed that MSG can cause neural tube developmental abnormalities in chicken embryos.

Study Limitations

There are some limitations to our study. Although there is abnormal embryonic development in the spinal cord after MSG ingestion, it is not enough to say that MSG has a teratogenic effect. More high-quality research is needed to establish if MSG consumption has a teratogenic effect.

Conclusion

Care should be taken when consuming foods containing MSG due to the negative side effects associated with excessive intake. Avoiding excessive consumption is most important during pregnancy, when MSG ingestion increases the risk of notochord development defects.

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