Cukurova Medical Journal

Araştırma Makalesi / Research Article

Vitamin C-E and Monosodium Glutamate-Induced Ovarian Toxicity

Vitamin C-E ve Monosodyum Glutamata Bağlı Ovaryan Toksisite

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CukurovaMedicalJournal 2014; 39 (3):517-524.

ABSTRACT

Purpose: Our study aimed to investigate the combinatorial effect of vitamin C and E on oxidative stress, the number of folicles, and hormonal level in female rats orally exposed to monosodium glutamate (MSG).

Materials and Methods: Female, twelve weeks old Wistar rats ingested with of MSG at dose 140 mg/200 gram body weight (bw) with or without combination of vitamin C and E. Twenty five rats were divided into five group (n = 5 each), control group, MSG-treatment group, MSG-treatment + 0.2 mg/g bw vitamin C + 0.04 IU/g bw vitamin E (MSG + CE_1), MSG-treatment + 0.4 mg/g bw vitamin C + 0.04 IU/g bw vitamin E (MSG + CE_2), and MSG-treatment + 0.6 mg/g bw vitamin C + 0.04 IU/g bw vitamin E (MSG + CE_1). Analysis of malondialdehyde (MDA) level was done colorimetrically. Analysis of the number of ovarian follicles was done histopathologically with hematoxylin eosin staining. Analysis of 17β-estradiol and follicle stimulating hormone (FSH) levels were done by ELISA technique.

Results: There were significantly ($P < 0.05$) increased ovarium MDA levels and atresia follicle number in groups exposed to MSG compared to non-exposure group. The level of FSH, 17β-estradiol, the number of primary, secondary, de Graaf follicles were significantly lower in MSG-treatment group compared to control group ($P < 0.05$). The administration of combined vitamin C and E (second and third dose) significantly ($P < 0.05$) decreased the MDA levels and the number of atresia follicle compared to the MSG-exposed groups, to reach level in control group ($P > 0.05$). MSG + CE_2 and MSG + CE_3 significantly increased FSH level, number of primary follicles, compared to MSG-treatment group ($P < 0.05$), to reach similar level in control group ($P > 0.05$). All dose of combined vitamin C and E significantly increased 17β-estradiol level and the number of secondary and de Graaf follicles compared to MSG-treatment group ($P < 0.05$), to reach significantly higher level compared to control group ($P < 0.05$).

Conclusion: The present data suggest that combination of vitamin C and E as in this study inhibited ovarium toxicity caused by MSG treatment.

Keywords: glutamate; antioxidant; folliculogenesis; hormone; lipid peroxidation

ÖZET

Amaç: Bu çalışmada oral monosodyumglutamat (MSG) verilmiş dişi sıçanlara, C ve E vitaminleri kombine olarak uygulandığında meydana gelen tesire bağlı olarak oksidatif stres durumu, folikül sayısı ve hormon düzeyi gibi değişkenlerin nasıl etkilendiklerini test etmeyi amaçladık.

Materyal ve Metot: 12 haftalık dişi Wistar sıçanlarından bir kısmına sadece MSG (140mg/200 g) verildi, ikinci kısmına ise MSG ile C ve E vitaminleri kombine olarak verildi. Yirmibeş sıçan toplamda 5 gruba ayrıldı (her grup için n=5). Bu

gruplardan ilk ikisi kontrol grubu ve sadece MSG verilen grup olarak belirlendiler. Son üç gruba ise MSG'ye ilave olarak farklı dozlarda C ve E vitaminleri kombine olarak verildi. Değişik C ve E dozlarına göre oluşturulan bu gruplar aşağıdaki gibidirler; üçüncü gruba MSG'ye ilave olarak 0.2 mg/g bw(vücut ağırlığı) oranında C vitamini ve 0.04 IU/g bw oranında E vitamini (MSG + CE_1) verildi. Dördüncü gruba, MSG'ye ilave olarak 0.4 mg/g bw oranında C vitamini ve 0.04 IU/g bw oranında E vitamini (MSG + CE_2) verildi. Beşinci gruba ise , MSG'ye ilave olarak 0.6 mg/g bw oranında C vitamini ve 0.04 IU/g bw E vitamini (MSG + CE_3) verildi. Malondialdehit (MDA) analizi kolorimetrik olarak yapıldı. Folikül sayısı, hematoksileneozin boyama yöntemi kullanılmak suretiyle hispatolojik olarak belirlendi. 17β-estradiol ve folikül stimüle edici hormon (FSH) düzeyleri, eliza tekniği kullanılarak belirlendi.

Bulgular: MSG verilmiş tüm gruplar kontrol grubu ile karşılaştırıldıklarında, MDA düzeylerinin ve atrezik folikül sayısının istatistiksel olarak önemli oranda artmış olduğu tespit edilmiştir ($P < 0.05$). Sadece MSG verilen grup ile kontrol grubu karşılaştırıldıklarında; sadece MSG verilmiş grupta, FSH ve 17β-estradiol hormon düzeyleri ile primer, sekonder ve graff folikülleri sayılarının anlamlı olarak düşmüş oldukları tespit edildi ($P < 0.05$). Sadece MSG verilmiş grup ile karşılaştırıldığında, C ve E vitaminlerinin kombine olarak verilmesinin atrezikfolikül sayısı ve MDA düzeylerini anlamlı olarak düşürdüğü tespit edilmiştir. MSG'ya ilave olarak C ve E vitaminlerinin kombine olarak verildiği durumlarda MDA düzeyleri ve atrezik folikül sayısı kontrol grubu düzeyine düşmüştür ($P < 0.05$). Buna göre MSG + CE_2 ve MSG + CE_3 grupları ile sadece MSG verilmiş grup karşılaştırıldıklarında, MSG + CE_2 ve MSG + CE_3 gruplarında FSH düzeyleri ile primer folikül sayısının anlamlı olarak artmış olduğu (kontrol grubu düzeyine ulaşmış olduğu) tespit edilmiştir($P > 0.05$). Sadece MSG verilmiş grup ile karşılaştırıldıklarında; C ve E vitaminlerinin tüm dozlarının, 17β-estradiol düzeyi ile sekonder ve graff folikül sayılarını anlamlı olarak arttırdıkları tespit edilmiştir ($P < 0.05$). C ve E vitaminlerinin verildiği gruplarda, 17β-estradiol düzeyi ile sekonder ve graffolikül sayıları, kontrol grubunun üzerindeki düzeylere ulaşmıştır ($P < 0.05$).

Sonuç: Bulgularımız C ve E vitamin kombinesinin MSG'ya maruz kalmaya bağlı olarak gelişen ovaryum toksisitesini inhibe ettiği sonucunu ortaya koymuştur.

Anahtar Kelimeler: glutamat; antioksidan; folikülogenez; hormon; lipid peroksidasyonu

INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of glutamic acid[1]. MSG is used as a flavor upgrader all over the world, mainly inChinese food. 'ChineseRestaurant Syndrome' and the production of lesions in the hypothalamus of newborn mice and monkeys are reported toxic effects of MSG[2].Previous studies have shown that MSG affect male fertility by causing a significant oligozoospermia and increases abnormal sperm morphology in a dose-dependent fashion in male Wistar rats[3]. It has been implicated in male infertility by causing testicular hemorrhage, degeneration and alteration of sperm cell population and morphology[4].

Female infertility can be caused by reduction the capacity of the ovary. The capacity may include the quantity and quality of oocytes. Several factors that may serve as predictors include age, ovarian volume, number of antral follicles and hormonal markers such as estradiol. A woman with a reduced capacity of ovaries has a high rate of stimulation failure and a high rate of pregnancy failure[5].Folliculogenesis in the ovary is affected by xenobioticswhich may cause loss of oogonia, oocytes, somatic cells and even a significant decline in the number of follicles[6]. Every substance that induces oxidative stress, an imbalance between prooxidant and antioxidants, can cause some of reproductive diseases such as infertility[7].Previous studies revealed that subcutaneous administration of MSG (4 and 8 mg/g body weight 6 days) to normal adult male mice caused a significant increase in erythrocyte lipid peroxidation[8]. In order to diminished oxidative stress in MSG treatment, previous study have been applied an pharmacological treatment, including quercetin, COX-2 inhibitor, and vitamin C[9-11].

Due to lipid peroxidation in MSG exposure therefore need antioxidant which act in lipid compartment. The vitamin E is an antioxidant in the lipid compartment to protect against lipid peroxidation[12]. Other biological functions of the tocopherol are changing gene expression, modulation of cell signaling and proliferation[13]. Besides, the vitamin Ealso found in significant amounts in ovaries and follicular fluid[14].In order to neutralize the prooxidant coming from vitamin Ereaction its need second another antioxidant.Vitamin C able to scavenges reactive oxygen species and may, thereby, prevent oxidative damage to the important biological macromolecules, such as DNA, proteins, and lipids[15].

This study aimed to investigate the combinatorial effect of vitamin C and E onoxidative stress, the number of folicles, and hormonal level in female rats orally exposed to monosodium glutamate (MSG).

MATERIAL and METHODS

Animal

Female, twelve weeks old,Wistar rats ingested with of MSG at dose 140mg/200 gram body weight (bw) with or without combination of vitamin C and E. MSG dose in seven until ten fold higher than previous studies[16,17].The duration of MSG treatment is subchronic (42 days)[18]. Twenty five rats were divided into five group (n = 5 each), control group, MSG-treatment group, MSG-treatment + 0.2 mg/g bw vitamin C + 0.04 IU/g bw vitamin E (MSG + CE_1), MSG-treatment + 0.4 mg/g bw vitamin C + 0.04 IU/g bw vitamin E (MSG + CE_2), and MSG-treatment + 0.6 mg/g bw vitamin C + 0.04 IU/g bw vitamin E (MSG + CE_1). The investigation was approved by the Ethical Committee for Animal Research of the Faculty of Medicine,University of Brawijaya and conforms to the Guide for the Care and Use of Laboratory Animals. Cycle determination was doneat day 43 to determined the estrus cycle. The research was done in the Pharmacological, Biomedical, Pathology Laboratories, Medical Faculty of Brawijaya University, Malang, East Java, Indonesia.

MSG, Vitamin C and E Treatment

The powder of MSG was dissolved withaquades 1 cc.Vitamin Cwas dissolved withaquades0.5 cc and Vitamin Ewas dissolved withsesame oil0.5 cc. All these compounds were orally treatment by gavage into rats at 10 p.m every day for 42 days after MSG-treatment.

Determination Estrous Cycle

The estrous cycle was determined to know the execution time of experimental animals. Cotton buds, cover glass, glass objects, Giemsa and the microscope were prepared for vaginal swap. Put cotton buds soaked with 0.9% physiological saline into the vaginal opening and rotate 360^o to obtain vaginal discharge, and then put the vaginal discharge on glass objects, dried and then soaked in methanol 9% for 10 minutes. It was then stained with methylebne blue for 30 minutes. After stained with Giemsa, it was then washed in running water and dried, then observe using a microscope with a magnification 100 times. Results of a vaginal swap for phase determination of white rats were based on the presence of and quantity of vaginal epithelial cells[19].

MalondialdehydeLevel

Malondialdehyde level from left ovaries was analyzed using TBA assay kit (R & D system Inc, USA, Catalog number bKGE013).

The Number of Follicles

The numbers of primary, secondary, tertiary and de Graaf follicles were calculated from the right ovary cut transversely and then preparation was made and stained histologically with HE and the follicles were calculated using Dotslide Olympus Camera XC 10. The entire cross-section was analyzed and further identified with magnification of 400 X.

Dissection

Before the rats were killed, the vaginal swap was done to determine the estrous cycle in rats; rats that were on proestrus phase would be killed

and if rats were not the proestrus phase, wait until the proestrus phase.

Measurement of FSH Level

FSH in serum was measured immunoenzymaticallyusing an ELISA method (Abnova Rat FSH Elisa Kit, Catalog Number 2535). All procedure was done according kit instruction.

Measurement of 17β-Estradiol Level

Level of 17β-Estradiol in serum was measured immunoenzymaticallyusing an ELISA method (Cusabio China, Catalog series CSB-E05110r). All procedure was done according kit instruction.

Ethics

This research has been approved by research ethics committee Faculty of Medicine University of Brawijaya, Malang, Indonesia

Statistical Analysis

Data are presented as mean ± SD and differences between groups were analyzed using 1-way ANOVA with SPSS 17.0 statistical package. Post Hoc test was used if the ANOVA was significant. $P < 0.05$ was considered statistically significant.

RESULTS

The exposure of MSG to rat ovarium affected the MDA levels, as shown in Table. 1. There were significantly ($P< 0.05$) increased ovariumMDA levels in groups exposed to MSG compared to non-exposure group. MSG + CE_2 and MSG + CE_3 significantly ($P< 0.05$) decreased the MDA levels compared to the MSG-exposed groups, to reach level incontrol group ($P> 0.05$).

Table 2 shows the FSH and 17β-estradiollevels in all groups. The level of FSH and 17β-estradiol were significantly lower in MSG-treatment group compared to control group ($P< 0.05$). Second and third dose of combined vitamin C and E significantly increased FSH level compared to MSG-treatment group ($P< 0.05$), to reach similar level in control group ($P> 0.05$). All dose of combined vitamin C and E significantly increased 17β-estradiol level compared to MSG-treatment group ($P< 0.05$), to reach significantly higher level compared to control group ($P< 0.05$).

Table 3 shows the number of follicles in all groups. Treatment to MSGcould significantly reduce the number of primary, secondary, de Graaf follicles compared to the control ($P <0.05$), and significantly increasedatresia follicles compared to the control ($P< 0.05$). MSG + CE_2 and MSG + CE_3significanltyincreased the number of primary follicles compared to MSG-treatment group ($P< 0.05$), reaching the number in the control group ($P> 0.05$). All the groups administered with vitamin C and E, the number of secondaryand de Graaffollicles was significantly higher than MSG-treatment group ($P < 0.05$), reaching the number in the control group. Beside, MSG + CE_2 and MSG + CE_3 also significantly decrease the number of atresia follicle than that MSG-treatment group ($P <0.05$).

Table 1. Level of ovaries malondialdehyde in MSG-treatment groups and control female rats

		Treatment groups			
Level	Control	MSG	MSG + CE_1	MSG + CE_2	MSG + CE_3
MDA	0.87 ± 0.16	1.28 ± 0.13^a	1.13 ± 0.25	1.04 ± 0.13^b	0.92 ± 0.15^b

Note: values are presented as mean ± SD; $^a p<0.05$; in comparison with control (non exposure)l group; $^b p<0.05$; in comparison with MSG-treatment groups; MSG: monosodium glutamate; MDA: malondialdehyde.

Table 2. TheFSH and 17β-estradiollevels in MSG-treatment groups and control female rats

		Treatment groups			
Level (pg/ml)	Control	MSG	MSG + CE1	MSG + CE2	MSG + CE3
FSH	34.71 ± 6.19	22.92 ± 5.2[a]	28.36 ± 2.79	32.54 ± 4.22[b]	36.08 ± 5.61[abcd]
17β-estradiol	41.38 ± 17.98	22.20 ± 8.09	48.95 ± 21.69[ab]	72.89 ± 15.68[ab]	117.48 ± 13.39[abcd]

Note: values are presented as mean ± SD; [a]p<0.05; in comparison with control (non exposure)l group; [b]p<0.05; in comparison with MSG-treatment groups; [c]p<0.05; in comparison with MSG-treatment + vitamin C (0.2 mg/g bw) + vitamin E (0.04 IU/g bw); [d]p<0.05; in comparison with MSG-treatment + vitamin C (0.4 mg/g bw) + vitamin E (0.04 IU/g bw); MSG: monosodium glutamate; FSH: follicle stimulating hormone; pg/mL: picogram/ml.

Table 3. Thenumber of follicles in MSG-treatment groups and control female rats

		Treatment groups			
Follicle (number)	Control	MSG	MSG + CE_1	MSG + CE_2	MSG + CE_3
Primary	1.40 ± 0.54	0.40 ± 0.54[a]	0.60 ± 0.54[a]	1.20 ± 0.44[b]	1.80 ± 0.44[bc]
Secondary	3.80 ± 0.44	2.60 ± 0.54[a]	3.40 ± 0.54[b]	4.00 ± 0.70[b]	4.60 ± 0.54[abc]
De Graaf	3.40 ± 0.54	2.60 ± 0.54[a]	3.60 ± 0.54[b]	4.00 ± 0.70[b]	4.80 ± 0.44[abcd]
Atresia	3.80 ± 0.44	4.60 ± 0.54[a]	4.00 ± 0.70	3.40 ± 0.54[b]	3.20 ± 0.44[bc]

Note: values are presented as mean ± SD; [a]p<0.05; in comparison with control (non exposure)l group; [b]p<0.05; in comparison with MSG-treatment groups; [c]p<0.05; in comparison with MSG-treatment + vitamin C (0.2 mg/g bw) + vitamin E (0.04 IU/g bw); [d]p<0.05; in comparison with MSG-treatment + vitamin C (0.4 mg/g bw) + vitamin E (0.04 IU/g bw); MSG: monosodium glutamate.

DISCUSSION

In the present study, we observed a significant increase in MDA levels in rat ovarium exposed to MSG. The ovariumMDA is a decomposition product of peroxidized polyunsaturated fatty acids that as marker for detection of ROS reactivity toward lipid peroxidation[20,21].This altered lipid profile accompanied with elevation in reactive oxygen species formation and reduction of antioxidant activities, including SOD and GSH-Px[9,22].

Previous studies show that oral ingestion of MSG at dose level 4 mg/g body weight and above increased the oxidative stress in cardiac tissue[23]. The administration of second and third dose of combined vitamin C and E significantly (P< 0.05) decreased the MDA levels compared to the MSG-exposed groups, to reach level in control groups. This finding indicated that combined vitamin E and C able to diminished ovarium lipid peroxidation caused by orally MSG.The competition by glutamate for the cystine:glutamate antiporter induces an imbalance in the homeostasis ofcystine, the precursor of glutathione (GSH). Therefore, the administration of vitamin E and C may give rise to an ability to maintain intracellular GSH levels[24].

The level of FSH and 17β-estradiol were significantly lower in MSG-treatment group compared to control group (P< 0.05). The ability of monosodium glutamate to damage nerve cells of the hypothalamus is a pointer to the fact that it may alter the neural control of reproductive hormone secretion via the hypothalamic–pituitary–gonadal regulatory axis. In this study, such alterations in FSH and 17β-estradiol secretion may adversely affect the reproductive capacity of the affected animals[16,25]. MSG + CE_2 and MSG + CE_3 significantly increased FSH level compared to MSG-treatment group (P< 0.05), to reach similar level in control group (P> 0.05). All dose of MSG + CEsignificantly increased 17β-estradiol level

compared to MSG-treatment group (P< 0.05), to reach significantly higher level compared to control group (P< 0.05).

Treatment to MSGcould significantly reduce the number of primary, secondary, de Graaf follicles compared to the control (P <0.05), and significantly increasedatresia follicles compared to the control (P< 0.05). This finding indicated that orally MSG is very toxic to all type follicles. Previous studies show that the injection of monosodium glutamate (4 mg/g b.w.) to rats resulted in a decrease in the number of Graafian follicles [26]. MSG + CE_2 and MSG + CE_3significanltyincreased the number of primary follicles compared to MSG-treatment group (P< 0.05), reaching the number in the control group (P> 0.05). For all the groups administered with vitamin C and E, the number of secondaryand de Graaffollicles was significantly higher than MSG-treatment group (P < 0.05), reaching the number in the control group. Beside, second and third doses combined vitamin E and C also significantly decrease the number of atresia follicle than that MSG-treatment group (P <0.05). The current study showed that a decrease in 17β-estradiol level as a result of administration of MSG was also accompanied by increased levels of MDA and atresia follicel. Previous studies shows that oxidative damage due to a decrease in estradiol causes apoptosis and follicular atresia[27,28]. Lipid peroxidation in the plasma membrane of luteal cells and may be associated with loss of gonadotropin receptors and decreased cAMP formation thereby reducing steroidogenic ability of the corpus luteum during involution[29].

In conclusion, the present data suggesting that combined vitamin C and E able to inhibit ovariumtoxicity caused by MSG treatment via reducing oxidative stress, prevent decrease the number of follicles, and hormone (FSH and 17β-estradiol) level.

Acknowledgment

The author acknowledged to all technician in Laboratory of Pharmacology, Pathology and Biomedical Science for helping this study.

Declaration of interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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Geliş tarihi/Received on: 04.02.2014
Kabul tarihi/Accepted on:03.03.2014