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TOPICAL REVIEW

The role of ascorbic acid and monosodium glutamate in thymocyte apoptosis

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Abstract: The studies on experimental animals have confirmed toxic effect of monosodium glutamate in different organs, mainly manifested by increased oxidative stress and cytotoxicity, strongly correlated with numerous diseases. Continuous intake of this flavor enhancer in modern nutrition also resulted with toxic effects on human health, known as Chinese restaurant syndrome. The reference data about influence of monosodium glutamate on the cells of the immune system or primary immune organs and possible protective effects of specific antioxidants are still largely unknown. This review summarizes recently known facts about the role of monosodium glutamate in the cells of the immune system, especially in thymocytes. Also, in this review many new data on positive effects of ascorbic acid on immune system and the mechanisms of its protective influence on thymocytes are discussed *(Tab. 1, Ref. 52).* Full Text (Free, PDF) *www.bmj.sk.*
Key words: monosodium glutamate, vitamin C, thymus, apoptosis.

Monosodium glutamate (MSG) is a sodium salt of glutamic acid which is one of the most abundant amino acids. Although MSG occurs in many foods naturally, it is frequently added as a flavor enhancer, the fact of which makes MSG one of the most applied food additives in modern nutrition. High daily intake of MSG results in accumulation and rise of glutamic acid concentration in blood (1). Glutamic acid primarily serves as an important excitatory neurotransmitter in central nervous system but it also serves as an energy source for certain tissues and as previously reviewed as a substrate for glutathione synthesis (2). These effects of glutamic acid are carried out via multiple receptor types, namely ionotropic (iGluR) and metabotropic glutamate receptors (mGluR). iGluR are pharmacologically defined as NMDA, AMPA and kainate receptor, while mGluR consists of so far eight defined different receptors. It is well documented that excessive activation of glutamate receptors was associated with some neurodegenerative disorders such as Huntington`s and Alzheimer`s disease (3, 4). On the other hand, recent studies have showed expression of glutamate receptors in variuos cells,outside the central nervous system including those of the immune system (5, 6, 7). The latter fact indicates that the monosodium glutamate possibly has its role in immune cell signaling.

Vitamin C, also known as ascorbic acid, is synthesized by all animals except humans, monkeys, guinea pigs, bats, and several bird species (8). Vitamin C is a water-soluble nutrient essential for the biosynthesis of collagen, L-carnitine, and conversion of dopamine to norepinephrine (9). Furthermore, ascorbic acid plays an important role in thyroxine synthesis, amino acid metabolism and iron absorption (10). Antioxidant properties of vitamin C in extracellular and intracellular compartments are also well documented (11, 12, 13). Nowadays, it is believed that the reduction in antioxidant capacity is one of the major factors in several chronic diseases (14, 15, 16), in which the lover level of ascorbic acid is also detected (as reviewed in 17). On the other hand, cells of the immune system are very sensitive to changes in the antioxidant status, which usually ends with impaired immune function (18). The possibility of protective role of ascorbic acid in immune system is based on the fact that the concentration of vitamin C (1mmol/l) in immune and inflammatory cells is ten times higher than that in plasma (19). Such high levels of ascorbic acid probably reflect the level of potential oxidative stress within these cells. Having that in mind, the protective role of vitamin C in the treatment of autoimmune diseases, phagocytic disfunctions, immunosupressive disorders and lymphocyte toxicity (as reviewed in 20) is expected.

Monosodium glutamate and thymocytes

Toxic MSG effects were first reported in human adults as a Chinese restaurant syndrome. Thus, MSG has been reported to produce symptoms such as weakness, flushing, dizziness and headache beginning in ten minutes up to two hours after the MSG-containing meal (21). Other toxic effects of MSG such as damaged hypothalamic neurons, obesity, altered antioxidant status in several brain regions and different organs have also been documented so far (22, 23, 24, 25, 26). The mechanism of glutamate toxicity remains unknown but several studies have proposed an

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Tab. 1. Expression of various glutamate receptors in cells of the immune system.

Glutamate receptor	Cells	Reference data
mGluR 1, 2, 3, 5	Mouse thymocytes, thymic stromal cell line	50
mGluR 2, 3, 4, 5	Rat thymocytes	34
iGluR	Human lymphocytes	33, 51
mGluR and iGluR	Rodent lymphocytes	52

apoptotic pathway (27, 28, 29, 30, 31). The studies carried out in the past decade were focused on the relation of glutamate receptors and cells of immune system. Numerous findings indicated a significant expression of glutamate receptors on immune cells (summarized in Table 1). However, despite the intensive studies, the precise function of these receptors in white blood cells remains unknown. The possible relationship between glutamate concentrations and lymphocyte reactivity has been documented earlier (32) as well as the inhibitory effect on lymphocyte proliferation. The latter effect is probably mediated by mGluR5 activation (33, 34). It increases the intracellular calcium level and via several reactions, it leads to programmed cell death. Recent findings demonstrate that MSG inhibits the in vivo (28) and in vitro (35) proliferation of thymocytes while the inhibition depends on dose and time. Furthermore, these studies demonstrated that the inhibited thymocyte proliferation was due to a decrease in cell viability, while thymocytes die via apoptotic mechanism under both in vitro (29) and in vivo conditions (28, 30, 31). High MSG-induced cytotoxicity was also documented in liver, kidney and brain (26), the fact of which indicates that MSG plays a possible role in inducing immune disorders as well as various chronic diseases. One of the mechanisms involved in MSG-induced thymocyte apoptosis was the down-regulation of Bcl-2 protein expression, while the level of Bax protein has not been changed (28, 29, 30). This fact suggests an important role of Bcl-2/Bax ratio, rather than that off Bax level alone in MSG-induced apoptosis of rat thymocytes. The importance of Bcl-2/Bax ratio in controlling the lymphocyte apoptosis has been confirmed earlier (27, 36). Bax homodimer is a potent regulator of cell death signal and the anti-apoptotic effect of Bcl-2 protein is due to its association with Bax. The latter association results in the reduction of free Bax pool for homodimerization. On the other hand, MSG-induced apoptosis and altered level of Bcl-2 protein in thymocytes are also related with oxidative stress. Namely, the treatment of animals with MSG resulted with an increase in oxidative stress within the kidneys, liver, brain and thymus (26, 30) and presented the possible mechanism of cell toxicity. MSG-induced oxidative stress in rat thymus showed a time-dependent manner and close relation with the rate of cells apoptosis . The most significant apoptosis and reduction in Bcl-2/Bax level in thymocytes are seen when the extensive oxidative stress occurs (30). The excessive generation of oxygen reactive species (ROS) in cells is known to damage DNA, lipids and proteins. Lipid peroxidation in cellular membranes damages the polyunsaturated fatty acids especially in lymphoid cells, and sensitizes T cells to apoptosis by decreasing the expression of Bcl-2 protein (37).

Vitamin C and MSG-induced thymocyte apoptosis

There is a long-standing debate about the implication of ascorbic acid in boosting the immune system. Vitamin C has been found to be a stimulant in leukocyte functions, especially the neutrophil and monocyte movement (38). Furthermore, vitamin C was shown to improve the human immune response such as antimicrobicidal natural killer cell activities, lymphocyte proliferation and chemotaxis (39-44), indicating the important role of this vitamin in regulating the immune response. MSG-induced thymocyte apoptosis was successfully ameliorated with high doses of ascorbic acid in vivo. The protective role of vitamin C was mainly obtained by up-regulating the Bcl-2 expression in thymocytes with the resulting change in Bcl-2/Bax protein ratio possibly playing a more significant role in MSG-induced thymocyte apoptosis than the Bax expression itself (45). The antiapoptotic roles of vitamin C in apoptosis induced by dexamethasone and hydrogen peroxide as well as in spontaneous thymocyte apoptosis have been confirmed by earlier studies (40, 46, 47). These findings confirm the earlier investigations showing that ascorbic acid prevents hepatic, renal and brain cytotoxicity after MSG treatment by reducing the intensity of oxidative stress (26). Ascorbic acid as a potent antioxidant is involved in antioxidant defense of cells and participates in free-radical scavenging. The imbalance between antioxidant and prooxidant systems is critical for immune cell functions. The MSG-induced oxidative stress in thymocytes seems to be one of the major inducers of cell toxicity, which later results with imbalance of expression of pro-apoptotic and anti-apoptotic proteins. Under these circumstances, the ascorbic acid may prove its antioxidant properties and anti-apoptotic role. Campbell et al. showed that vitamin C could modulate the immune system by inhibiting the T-cell apoptosis signaling pathways (40), among them the oxidant sensitive transcription factor Nf-kB, which is also included in T-cell apoptosis signaling pathways (48). These findings are confirmed by a study showing that in different cell types, vitamin C prevents apoptosis by upregulating the Bcl-2 protein expression level while the latter process results in a change in Bcl-2/Bax protein ratio (49). Intensive oxidative stress sensitizes T cells to apoptosis by decreasing the expression of Bcl-2 protein. By increasing the expression of Bcl-2 protein and enhancing the Bcl-2/Bax protein ratio, the protective role of ascorbic acid may allow the thymocytes to cope better with the effect of oxidative stress in MSG-induced thymocytes toxicity. By detoxifying the ROS, antioxidants may therefore reverse the oxidative stress-induced decline in Bcl-2 and prevent the cell death (37).

By enabling a continuous intake of high amounts of MSG, modern nutrition can increase the oxidative stress and result in cytotoxicty in many organs, especially in thymus. Potent anti-

oxidant nutrients such as vitamin C may significantly restore the MSG-induced oxidative stress and apoptosis in thymus and simultaneously prevent the secondary immunological consequences.

References

1. Garattini S. Glutamic acis, twenty years later. J Nutr 2000; 130 (4S Suppl): 901S - 909S.

2. Freeman M. Reconsidering the effects of monosodium glutamate: A literature review. J Am Acad Nurse Pract 2006; 10: 482 – 486.

3. Ariano MA, Wagle N, Grissell AE. Neuronal vurnerability in mouse models of Huntington`s disease: membrane channel protein changes. J Neurosci Res 2005; 80: 634 – 645.

4. Tsai VW, Scott HL, Lewis RJ, Dodd PR. The role of group I metabotropic glutamate receptors in neuronal excitotoxicity in Alzheimer`s disease. Neurotox Res 2005; 7: 125 – 141.

5. Gill SS, Pulido OM. Glutamate receptors in peripheral tissues: current knowledge, future research, and implications for toxicology. Toxicol Pathol 2001; 29: 208 – 223.

6. Skerry TM, Genever PG. Glutamate signalling in nonneuronal tissues. Trends Pharmacol Sci 2001; 22: 174 – 181.

7. Hinoi E, Takarada T, Ueshima T, TsuchihashiI Y, Yoneda Y. Glutamate signaling in peripheral tissues. Eur J Biochem 2004; 271: 1 – 13.

8. Linster CL, Van Schaftingen E. Vitamin C: Biosynthesis, recyclingand degradation in mammals. FEBS J 2007; 274: 1 – 22.

9. Rebouche CJ. Ascorbic – acid and carnitine biosynthesis. Am J Clin Nutr 1991; 54: S1147 – S1152.

10. Deruelle F, Baron B. Vitamin C: is supplementation necessary for optimal health? J Altern Complement Med 2008; 14: 1291 – 1298.

11. Dasgupta A, Zdunek T. In vitro lipid peroxidation of human serum catalyzed by cupric ion: antioxidant rather than prooxidant role of ascorbate. Life Sci 1992; 50: 875 – 882.

12. Berger TM, Polidori MC, Dabbagh A et al. Antioxidant activity of vitamin C in iron - overloaded human plasma. J Biol Chem 1997; 272: 15656 – 15660.

13. Krajcovicova - Kudlackova M, Babinska K, Valachovicova M, Paukova V, Dusinska M, Blazicek P. Vitamin C protective plasma value. Bratisl Lek Listy 2007; 108: 265 – 268.

14. Tak PP, Zvaifler NJ, Green DR, Firestein GS. Rheumatoid arthritis and p53: how oxidative stress might alter the course of inflammatory diseases. Immunol Today 2000; 21: 78 – 82.

15. Lefer DJ, Granger DN. Oxidative stress and cardiac disease. Am J Med 2000; 109: 315 – 323.

16. Ginter E. Chronic vitamin C deficiency increases the risk of cardiovascular diseases. Bratisl Lek Listy 2007; 108: 417 – 421.

17. McGregor G, Biesalski H. Rationale and impact of vitamin C in clinical nutrition. Curr Opin Clin Nutr Metab Care 2006; 9: 697 – 703.

18. Knight JA. Review: free radicals, antioxidants and the immune system. Ann Clin Lab Sci 2000; 30: 145 – 158.

19. Moser U. Uptake of ascorbic acid by leukocytes. Ann N Y Acad Sci 1987; 498: 200–215.

20. Kodama M, Kodama T. Vitamin C and the genesis of autoimmune disease and allergy. In Vivo 1995; 9: 231 – 238.

21. Schaumberg HH, Byck R, Gerst R, Mashman JH. Monosodium l - glutamate; its pharmacology and role in the Chinese restaurant syndrome. Science, 1969; 163: 826 – 828.

22. Park CH, Choi SH, Piao Y et al. Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. Toxicol Lett 2000; 115: 117 – 125.

23. Mozes S, Sefcíkova Z, Lenhardt L, Racek L. Obesity and changes of alkaline phosphatase activity in the small intestine of 40- and 80-day old rats subjected to early postnatal overfeeding or monosodium glutamate. Physiol Res 2004; 53: 177 – 186.

24. Singh K, Ahluwalia P. Studies on the effect of monosodium glutamate [MSG] administration on some antioxidant enzymes in the arterial tissue of adult male mice. J Nutr Sci Vitaminol (Tokio) 2003; 49: 145 – 148.

25. Cekic S, Filipovic M, Pavlovic V, Ciric M, Nesic M, Jovic Z, Brankovic S. Histopathologic changes at the hypothalamic, adrenal and thymic nucleus arcuatus in rats treated with monosodium glutamate. Acta medica medianae, 2005; 44: 35 – 42.

26. Farombi EO, Onyema OO. Monosodium glutamate-induced damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and quercetin. Hum Exp Tox 2006; 25: 251 – 259.

27. Schelman WR, Andres RD, Sipe KJ, Kang E, Weyhenmeyer JA. Glutamate mediates cell death and increases the Bax to Bcl - 2 ratio in a differentiated neuronal cell line. Brain Res Mol Brain Res 2004; 128: 160 – 169.

28. Pavlovic V, Cekic S, Sokolovic D, Djindjic B. Modulatory effect of monosodium glutamate on rat thymocyte proliferation and apoptosis. Bratisl Lek Listy 2006; 107: 185 – 191.

29. Pavlovic V, Cekic S, Kocic G, Sokolovic D, Zivkovic V. Effect of monosodium glutamate on apoptosis and Bcl - 2/Bax protein level in rat thymocyte culture. Physiol Res 2007; 56: 619 – 626.

30. Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Jevtovic - Stoimenov T, Cekic S, Velickovic D. Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus. Mol Cell Biochem 2007; 303: 161 – 166.

31. Pavlovic V, Cekic S. The effect of monosodium glutamate on the apoptosis of rat thymocytes and Bcl - 2 expression. Arch Med Sci 2006; 2: 28 – 31.

32. Droge W, Eck HP, Betzler M, Schlag P, Drings P, Ebert W. Plasma glutamate concentration and lymphocyte activity . J Can Res Clin Onc 1988; 114: 124 – 128.

33. Lombardi G, Dianzani C, Miglio G, Canonico PL, Fantozzi R. Characterization of ionotropic glutamate receptors in human lymphocytes. Briti J Pharmacol 2001; 133: 936 – 944.

34. Rezzani R, Corsetti G, Rodella L, Angoscini P, Lonati C, Bianchi R. Cyclosporine-A treatment inhibits the expression of metabotropic glutamate receptors in rat thymus. Acta Histochem 2003; 105: 81 – 87.

35. Pavlovic V, Cekic S. The effect of monosodium glutamate on rat thymocyte proliferation and Bcl-2/Bax protein expression. Arch Med Sci 2006 2: 247 – 251.

36. Almawi WY, Melemedjian OK, Jaoude MM. On the link between Bcl-2 family proteins and glucocorticoid - induced apoptosis. J Leukoc Biol 2004; 76: 7 – 14.

37. Hildeman DA, Mitchell T, Aronow B, Wojciechowski S, Kappler J, Marrack P. Control of Bcl - 2 expression by reactive oxygen species. Proc Natl Acad Sci 2003; 100: 15035 – 15040.

38. Anderson R, Oosthuizen R, Maritz R, Theron A, Van Rensburg AJ. The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. Am J Clin Nutr 1980; 33: 71 – 76.

39. Heuser G, Vojdani A. Enhancement of natural killer cell activity and T and B cell function by buffered vitamin C in patients exposed to toxic chemicals: the role of protein kinase-C. Immunopharmacol. Immunotoxicol 1997; 19: 291 – 312.

40. Campbell JC, Cole M, Bunditrutavorn B, Vella AT. Ascorbic Acid Is a Potent Inhibitor of Various Forms of T Cell Apoptosis. Cell Immunol 1999; 194: 1 – 5.

41. Wolf G. Uptake of ascorbic acid by human neutrophils. Nutr Rev 1993; 51: 337–- 338.

42. Pavlovic V, Cekic S, Rankovic G, Stojiljkovic N. Antioxidant and pro-oxidant effect of ascorbic acid. Acta Med Medianae 2005; 44: 65 – 68.

43. Pavlovic Z, Pavlovic V. The effect of ascorbic acid on pathohistological tumor characteristics and phenotype characteristics of lymphocytes during the development of experimental mammary carcinoma in mice. Acta Med Medianae 2005; 44: 23 – 31.

44. Pavlovic Z, Pavlovic V, Pavlovic Z. The effect of ascorbic acid on development of experimental mammary carcinoma in mice. Acta Med Medianae 2004; 43: 5 – 9.

45. Pavlovic V, Pavlovic D, Kocic G, Sokolovic D, Sarac M, Jovic Z. Ascorbic acid modulates monosodium glutamate induced cytotoxicity in rat thymus. Bratisl Lek Listy 2009; 110 (4): 206–209.

46. Maellaro E, Bello B, Comporti M. Protection by Ascorbate against Apoptosis of Thymocytes: Implications of Ascorbate-induced Nonlethal Oxidative Stress and Poly (ADP-ribosyl)ation. Exp Cell Res 1996; 226: 105–113.

47. Pavlovic V, Cekic S, Bojanic V, Stojiljkovic N, Rankovic G. Ascorbic acid modulates spontaneous thymocyte apoptosis. Acta Med Medianae 2005; 44: 21–- 23.

48. Haertel C, Strunk T, Bucsky P, Schultz C. Effects of vitamin C on intracytoplasmic cytokine production in human whole blood monocytes and lymphocytes. Cytokine 2004; 27: 101 – 106.

49. Saitoh Y, Ouchida R, Kayasuga A, Miwa N. Anti - Apoptotic Defense of bcl-2 Gene Against Hydroperoxide-induced Cytotoxicity Together With Suppressed Lipid Peroxidation, Enhanced Ascorbate Uptake, and Upregulated Bcl-2 Protein. J Cell Biochem 2003; 89: 321 – 334.

50. Storto M, de Grazia U, Battaglia G et al. Expression of metabotropic glutamate receptors in murine thymocytes and thymic stromal cells. J Neuroimmunol 2000; 109: 112 – 120.

51. Lombardi G, Miglio G, Dianzani C et al. Glutamate modulation of human lymphocyte growth: in vitro studies. Biochem Biophys Res Commun 2004; 318: 496 – 502.

52. Boldyrev AA, Kazey VI, Leinsoo TA et al. Rodent lymphocytes express functionally active glutamate receptors. Biochem Biophys Res Commun 2004; 324: 133 – 139.

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