

# ASCORBIC ACID MODULATES SPONTANEOUS THYMOCYTE APOPTOSIS

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The aim of the paper was to analyze the effect of various concentrations of ascorbic acid on spontaneous apoptosis of lymphocytes.

About 58% of all thymocytes are subjected to a spontaneous apoptosis, after 24-hour cultivation in the complete medium. The number of apoptotic thymocytes was much lower in the culture with different concentrations of ascorbic acid. The most dramatic effect was detected in the culture with the highest concentration of ascorbic acid (10000 µg/ml). In this culture, the number of apoptotic thymocytes was about 32%. These results, compared with the results of spontaneous apoptosis, show that the most significant inhibition of apoptosis was detected in the culture with 10000 µg/ml of ascorbic acid.

High concentration of ascorbic acid can inhibit spontaneous apoptosis of thymocytes. Such inhibition of T cell apoptosis, as the effector cells in immune system, can represent one of the major factors by which ascorbic acid influence the immune system. *Acta Medica Medianae* 2005;44(4): 21 – 23.

**Key words:** ascorbic acid, thymus, apoptosis

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## Introduction

Thymus is the central organ of the immune system. The proliferation, differentiation and T-lymphocytes selection processes take place in this organ (1). With humans, thymus reaches its maximum size during the 1<sup>st</sup> year after which its involution, as one of the examples of physiological involutions, starts (2). Thymocytes make the greatest thymus cell population. About 80% of thymocytes are located in the cortex and about 20% of thymocytes are located in the thymus medulla. All thymocytes are subjected to the process of positive or negative selection during the maturation process. This thymocytes selection is based on the thymocytes ability or inability to recognize antigenic peptide/own MHC molecule complex. The negative thymocytes selection represents acquired tolerance to own antigens. It is acquired by the thymocytes' apoptosis or by the anergy, that is the clone inactivation (1). Apoptosis or the programmed cell death is a physiological process necessary for the normal development and homeostasis of an organism. The process intensity is strictly controlled (3). Increased or decreased intensity of this process leads to many disorders.

Ascorbic acid is one of the basic nutritive factors, often connected with the immune system mo-

dulation. It has been proved earlier that the application of the ascorbic acid may increase T-cell proliferation and cytokines production. It also has antioxidant and anticancerogenic effect (4,5,6). All these effects point to the modulation of the nonspecific protection. The precise mechanisms for the ascorbic acid performance, aimed at the improvement of the specific protection, have not been fully examined. It is considered that one of the effects of the ascorbic acid is the modulation of T-cells survival, i.e. the modulation of the apoptosis intensity.

## Aims

The aim of the research is to examine the effects that different concentrations of ascorbic acid have on the intensity of the thymocyte spontaneous apoptosis.

## Material and Methods

Wistar rats of both sexes (male and female), 9-11 weeks old were used in the experiments. The animals were bred under the standard laboratory conditions in the vivarium of the Medical Research Institute, at the Faculty of Medicine in Nis.

The animals were sacrificed by ether inhalation, administering diethyl ether. Thymus was extracted by sterile instruments and put into Petri dish with RPMI/10% FCS (Sigma, USA). After the adjacent tissue had been removed, thymus was homogenized through sterile steel net. Thymus cell suspension was resuspended and rinsed in RPMI/10% FCS. The number of viable thymocytes was determined by counting in 0.1% Tripan blue solution.

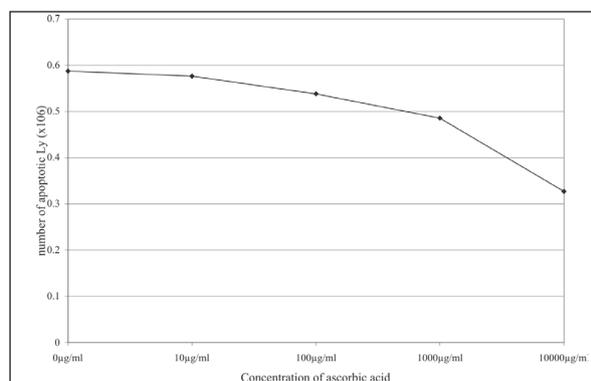
The obtained thymocytes were distributed in the cell-cultivating basal panels ( $1 \times 10^6$  thymocytes/200  $\mu$ l) with 96 places (Sarstedt, Newton, USA) and cultivated with different concentrations of ascorbic acid (10000, 1000, 100, 10  $\mu$ g/ml) (Galenika, Belgrade). The final volume of each pool was 200  $\mu$ l. The cells were cultivated for 24 hours in a thermometer at 37°C and 5% CO<sub>2</sub> (Assab, Sweden).

Türk's solution for leukocyte counting was used for the morphological detection of thymocyte apoptosis. This reagent enables the fixation and determination of the nucleus. In doing so, it makes possible a clear differentiation of the normal chromatin in viable cells, as well as the chromatin condensation and karyorrhexis of the apoptotic cells. All cultures were grown in triplicate. The obtained values are shown as mean values  $\pm$  SD.

Student's T-test was used for the comparison of the obtained results.

## Results

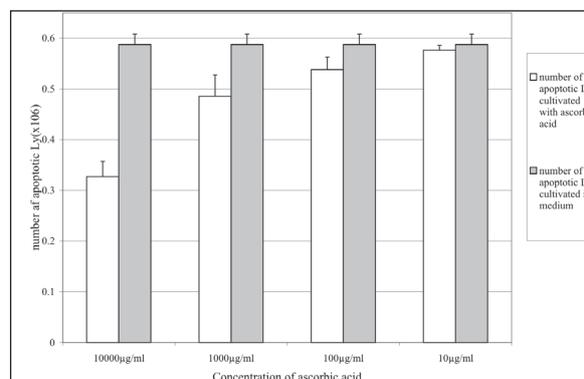
The first part of our research was related to the examination of the intensity of the spontaneous thymocyte apoptosis. Actually, these results indicate that more than a half of the isolated thymocytes (approximately 58%) was liable to the spontaneous apoptosis after 24-hour cultivation in the complete medium (Graph 1). However, cultivating of the isolated thymocytes with different concentrations of vitamin C demonstrate that the number of the apoptotic thymocytes decreases, proportionally to the increasing of the ascorbic acid concentration. The most dramatic effect was



Graph 1. Number of apoptotic Ly cultivated with ascorbic acid for 24h

detected in the cultures with the highest concentration of vitamin C (10000  $\mu$ g/ml), where the percentage of the apoptotic thymocytes decreased to approximately 32%. Also, the vitamin C concentration of 1000  $\mu$ g/ml reduced the number of apoptotic lymphocytes to 48%, while the remaining concentrations of the ascorbic acid, in a much lower degree, reduced the total number of the apoptotic cells (53-57%) in relation to the spontaneous thymocyte apoptosis. A comparison of the obtained data with the values of the spontaneous thymocyte apoptosis showed that statistically the most significant

apoptosis inhibition was verified in cultures with 10000  $\mu$ g/ml of vitamin C, as well as in other cultures (1000  $\mu$ g/ml  $p < 0.01$ ; 100  $\mu$ g/ml  $p < 0.05$ ), while in the culture with 10  $\mu$ g/ml of vitamin C, statistically significant apoptosis inhibition was not detected (Graph 2).



Graph 2. The effect of ascorbic acid on spontaneous thymocyte apoptosis

## Discussion

Ascorbic acid represents a significant nutritive antioxidant. Actually, the intensity of cell and tissue damage is significantly reduced by the oxidation of the ascorbic acid into ascorbat (by tying free oxygen to itself) (7). Nowadays, there are various data about the ascorbic acid effect on the immune system. Some authors deny its stimulatory effect (8, 9). The other group of authors emphasizes the protective and stimulatory effect on the immune system (7,10,11).

The results obtained in our study indicate that the thymocyte cultivation *in vitro* conditions leads to apoptosis of more than a half of the cultivated thymocytes (approximately 58%). Such a high degree of apoptosis is probably the consequence of the thymocyte isolation. Actually, a sudden isolation of the nonstimulated thymocytes leads to an abrupt halt of the effect of the thymic microenvironment on thymocytes, as well as to the halt of the effect of many cytokines within thymus itself. All the aforementioned factors can significantly increase a spontaneous apoptosis in the thymocyte culture. Similar results were obtained in Perandones and the associates' research (12).

Our further research showed that high ascorbic acid doses significantly reduced the apoptosis intensity. The greatest apoptosis inhibition was detected in the cultures with 10000  $\mu$ g/ml ( $p < 0.001$ ). The lowest apoptosis inhibition was detected in the cultures with 100  $\mu$ g/ml ( $p < 0.05$ ). A significant inhibition was not detected in the cultures with 10  $\mu$ g/ml. According to the obtained results, we can conclude that high concentrations of ascorbic acid significantly inhibit a spontaneous thymocyte apoptosis. The spontaneous apoptosis inhibition is directly proportional to the ascorbic acid concentration. Campbell and the associates' earlier researches (7) also indicate the protective effect of the ascorbic acid on the spontaneous splenocytes' apoptosis. Similar results were issued out by Vissers et al. as-

sociates (13). Their results indicate antiapoptotic effect of the ascorbic acid on the activated as well as nonactivated human neutrophils. One of the reasons of such a phenomenon may be that many different ways of apoptosis induction make one common way in which the ascorbic acid manifests its antiapoptotic effect (7). Total reduction of the apoptotic cell number, resulting in the number increase of the T-cell pool, can be one of the mechanisms through which the ascorbic acid has the stimulatory effect on the immune system (13).

## Conclusion

Apoptosis represents a strictly controlled process. Disturbances in its intensity lead to serious disorders. Ascorbic acid has a very significant effect in the intensity modulation of the spontaneous thymocyte apoptosis. Such apoptosis inhibition of the T-cells, as effectory cells of the immune system, can represent one of the basic factors through which the ascorbic acid has stimulatory effect on the immune system.

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## ASKORBINSKA KISELINA MODULIŠE SPONTANU APOPTOZU TIMOCITA

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Tokom procesa maturacije, svi timociti podležu procesu pozitivne ili negativne selekcije. Negativna selekcija timocita dovodi do stvaranja tolerancije na sopstvene antigene. Danas se smatra da jedan od načina delovanja askorbinske kiseline jeste modulacija preživljavanja T ćelija, tj. modulacija intenziteta apoptoze.

U radu je ispitan efekat različitih koncentracija askorbinske kiseline na intenzitet spontane apoptoze timocita.

Rezultati pokazuju da oko 58% izolovanih timocita podleže spontanoj apoptozi, nakon 24 sata kultivacije u kompletnom medijumu. Kultivacija izolovanih timocita sa različitim koncentracijama vitamina C, pokazuje da se broj apoptotičnih timocita smanjuje, srazmerno povećanju koncentracije askorbinske kiseline. Najdramatičniji efekat je detektovan u kulturama sa najvećom koncentracijom vitamina C (10000 µg/ml), gde se procenat apoptotičnih timocita smanjuje na oko 32%. Upoređivanje dobijenih podataka sa vrednostima spontane apoptoze timocita, pokazuje da je statistički najznačajnija inhibicija apoptoze verifikovana u kulturama sa 10000 µg/ml vitamina C.

Visoke koncentracije askorbinske kiseline značajno inhibiraju proces spontane apoptoze timocita. Ovakva inhibicija apoptoze T ćelija, kao efektornih ćelija imunološkog sistema, može predstavljati jedan od osnovnih faktora preko kojih askorbinska kiselina stimulatивно utiče na imuni sistem. *Acta Medica Medianae* 2005;44(4): 21 – 23.

**Ključne reči:** askorbinska kiselina, timus, apoptoza