THE EFFECT OF ASCORBIC ACID ON PATHOHISTOLOGICAL TUMOR CHARACTERISTICS AND PHENOTYPE CHARACTERISTICS OF LYMPHOCYTES DURING THE DEVELOPMENT OF EXPERIMENTAL MAMMARY CARCINOMA IN MICE

Zorica Pavlovic [1] and Voja Pavlovic [2]

TIn our previous study we demonstrated that high doses of ascorbic acid prolonged the survival of mice with experimental mammary carcinoma. In this work we studied, ussing the same model, pathohistological characteristics of the tumor and phenotypic changes of lymphocyte subsets in the spleen. Experiments were performed on CBA/H mice. The growh of experimental tumor was induced by injection of mammary adenocarcinoma cells intramuscularly at the femoral region of mice. The animals were divided into control group and three experimental groups (I, II and III). Mice from experimental groups were treated peroraly with 10 mg /, 100 mg / and 1000 mg / kg body mass (b.m.) of ascorbic acid, respectively, whereas control mice received physiological saline. Mice were sacrified after 7, 14 and 21 days from the beginning of the experiment. Total tumor mass and its pathohistological characteristics, spleen mass and cellularity as well as relative and total numbers of T cells, B cells and T cell subsets ($CD4^+$ and $CD8^+$) in the spleen, were analyzed. High doses of ascorbic acid decreased tumor mass, stimulated proliferation of fibroblasts and formation of capsula arround the tumor, induced tumor necrosis and increased the number of tumor infiltrating lymphocytes. Changes of lymphocyte subsets and their numbers varied depending on the applied dose of ascorbic acid and the time elapsed following tumor induction. The most prominent changes, manifested by an increase in the number of $CD4^+$ T cells were observed on the 14^{th} day in II experimental group. Our results suggest that the beneficial effect of ascorbic acid on experimental tumorogenesis in our model was the consequence of its influence on the tumor and on the immune system. *Acta Medica Medianae 2005; 44(2): 23–31.*

Key words: *mammary adenocarcinoma, mouse, ascorbic acid, histopathology, lymphocytes, phenotype*

Health Service, Nis[1]
Department of Physiology, Faculty of Medicine, Nis[2]

Correspondence to: Zorica Pavlovic
Health Service
18000 Nis, Serbia and Montenegro
Phone: 018/334-221

Introduction

The environmental factors are considered to have a very important role in the development of different types of carcinoma (1,2). Among them, the nutritive factors containing initiators and promoters of carcinogenesis or acting as genotoxic carcinogens are of special importance. On the other hand, some nutritive deficiencies may influence the appearance of malignant diseases (3,4). Therefore, Marshal et al. showed that the risk of oral carcinoma development increased along with the decrease of vitamin A and vitamin C (ascorbic acid) intake (5). These facts are supported by the results of Graham et al. claiming that vitamin C had a preventive role in the development of laryngeal carcinoma (6). Several epidemiological studies showed that the consumption of vitamin C within the food intake decreased the risk of gastrointestinal and other malignant tumors appearance (7,8). The study of Cameron and Pauling showed the beneficial effect of the application of high dosage of vitamin C on the patients' survival in the terminal stage of malignant disease (9). However, these results were not confirmed by Cregan et al. (10).

The results of several experimental studies concerning the relationship between vitamin C and malignant tumors have been published. Pauling et al. showed that spontaneous appearance of mammary carcinoma in mice corresponded with the decrease of ascorbic acid concentration in serum (11). Fraizer and McGinn published data claiming that the addition of vitamin C (1mg/ml) in drinking water led to the significant increase of the survival time of C_3H/HEJ mice after the implantation of mammary adenocarcinoma cells (12). However, Abul-Haij and Kelliher did not obtain the same results by applying a similar experimental model (13).

Our previous research using a model of experimental mammary adenocarcinoma in CBA/H mice showed that vitamin C (1000mg/body mass) prolonged the survival period of these animals in comparison to the control (14). In this work, we wanted to examine histopathological characteristics of tumor and some immunological parameters on the same model. This would in turn enable better perception of the possible mechanisms of the vitamin C effect on the development of experimental mammary carcinoma.

Materials and methods

Experimental animals

The experiments were carried out with female CBA/H mice, between 7 and 8 weeks of age, weighing 20 to 24 g. Until the onset of the experiment, the animals had lived under usual laboratorial conditions. They were fed with paletted food, while the water was given from feeding cup *ad libitum*. Vitamins of B group were added to the drinking water. 60 mice were used for the experiment.

Experimental tumor induction

Tumor induction in experimental animals was carried out by injecting $5x10^6$ mouse mammary adenocarcinoma living cells in femoral musculature. After that, the animals were randomly divided into one control and three experimental groups (I,II,III). The mice of the experimental groups were given ascorbic acid solution through gastric sonde in the overall volume of 0,5 ml during the period of 5 days in a single week. Experimental group I was given 10 mg of ascorbic acid/kg of body mass (b.m), experimental group II was given 100 mg/kg b.m., whereas experimental group III received 1000 mg/kg b.m. Ascorbic acid was dissolved in physiological solution. The mice of the control group were given the same volume of physiological solution.

The animals were sacrificed by ether anesthesia on the 7^{th},14^{th} and 21^{st} day after tumor induction. *There were 10 animals* in each group. After the tumor and spleen extraction, their mass was weighed. The tumor tissue was further treated for pathohystological analysis, whereas the spleen was used for immunological examinations.

Pathohystological analysis

Several cut-out parts were taken from each tumor and fixed in 10% buffered formalin; after dehydratation in alcohol and enlightening in xylol, they were embedded in paraffin. Paraffin blocks were cut on microtome (Richert-Jung) into 5-7 micrometers thick slices and mounted onto microscopic slides. After deparaffinizing process, the slides were stained according to Azan and Van-Gieson method. The pathohystological analysis was carried out by light microscopy. The results were descriptively presented and illustrated by the photographs.

Cytofluorimetric analysis

The spleen was macerated through the iron mesh soaked into Petryv's dishes with the solution of phosphate buffered saline (PBS) by pressing the plug of a syringe. Thus, we obtained cell suspension which was afterwards rinsed by centrifugation. The counting of cells was done in the standard manner using Turck's solution. Erythrocytes from the cells of the spleen were lysed with ammonium sulfate. After being counted once again, splenocytes were divided into test tubes for fluorescence, in concentration of $1x10^6$ cells per each test tube in the volume of 100 μL. Antibodies conjugated with fluorescein isotocianate (FITC) were added to cells. The following antibodies reactive with the mouse antigens were used: Thy1.2 (pan T cell marker); membrane immunoglobulin (Ig) (B-lymphocyte marker); CD4 and CD8. Antibodies were commercially obtained from Serotec, while CD4 was from Beckton-Dickinson. Antibodies were diluted in PBS with the addition of 2% of fetal calf serum, and 0,1% Na-azide, in concentration of 2,5 μg/ml. The control consisted of cell samples incubated without antibodies. After the 45-minute incubation at +4^{o}C the cells were washed in PBS and then fixed in 1% formalin and analyzed by flow cytometryeter (EPICS-CS, Coulter). The population of lymphocytes was identified on the basis of forward scater versus side scater characteristics. The level of specific fluorescence was determined on the basis of fluorescence profile in control samples that did not exceed 2%. The results were shown as percentages of positive cells for each marker. The total number of lymphocyte subpopulations was counted according to the relative values and the total number of lymphocytes.

Statistical Analysis

For the statistical data management, the Student's t test for small samples was used. Statistical significance between the examined groups was defined at the $p<0,05$ level.

Results

The effect of ascorbic acid on mass and histopathological characteristics of tumor during the development of experimental mammary carcinoma.

The first part of the results in this paper referred to the examination of growth dynamic and histopathological characteristics of tumor during the induction of experimental mammary carcinoma.

Table 1 shows that tumor growth in experimental groups of mice, measured by its total mass, was slower in relation to the control group. However, statistically significant difference in tumor mass was noticed only on the 21^{th} day between III experimental group (mice treated with ascorbic acid in dose of 1000 mg/kg b.m.) and the control group.

The results of pathohistological analysis of mammary carcinoma in control animals show that tumor tissues were predominately composed of malignant cells with high mitotic index, hyper-chromatic nuclei

Table 1. The effect of various doses of ascorbic acid on the dynamic of tumor mass changes in mice during the development of experimental mammary carcinoma

Animal group	Tumor mass (g)		
	7. day	14. day	21 day
Control	0.51 ± 0.01	4.25 ± 0.90	9.79 ± 1.03
Experim. I	0.43 ± 0.03	4.09 ± 0.87	8.87 ± 0.88
Experim. II	0.48 ± 0.04	2.97 ± 0.05	9.12 ± 0.99
Experim. III	0.43 ± 0.05	2.93 ± 0.07	8.37 ± 0.86*

Mean values were shown ±SD for 10 animals in each group
*=p<0.05 in comparison to the control.

and the disturbance of nucleo-cytoplasmatic ratio in favor of the nuclei. Tumor growth was followed by infiltration of surrounding musculature with gradual atrophy of muscle cells. Since the reactivity of connective tissue is lacking, the capsule was not formed even in the end of the examined period. Rare lymphoid cells were present in tumor. Hemorrhagic areas were noticed at some places (Figure 1).

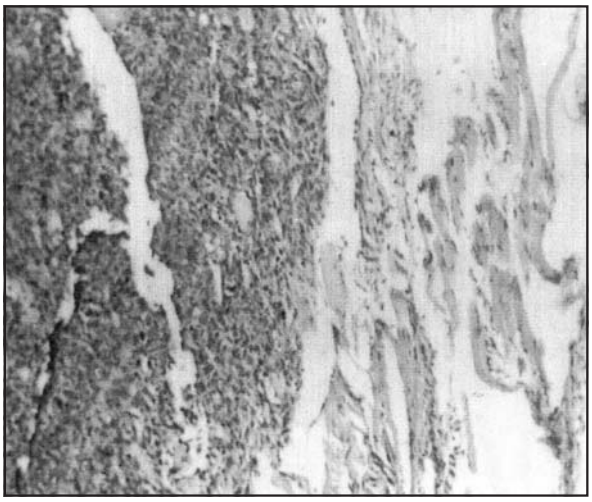

Figure 1. Strong tumor proliferation holds back the bundles of skeletal muscles and leads to their atrophy as well

In mice treated with ascorbic acid, a slower infiltrative tumor growth inside the muscles was noticed. This process is more likely to be noticed in animals treated with vitamin C in dosages of 100 mg/kg t.m. and 1000 mg/kg b.m. Also, there is the presence of a strong lymphoplasmatic infiltration which is in direct correlation with the applied dosage of vitamin C. Proliferation of fibroblasts can be noticed inside the tumor, as well as in the capsule around it. The capsule was very discrete in I experimental group and developed and prominent in the III experimental group. Hemorrhagic and necrotic zones inside the tumor at the end of examined period were significantly noticeable in comparison to the control group. Necrosis was particularly characteristic for tumors in animals of the III experimental group in which proliferated collagen in tracts separate necrotic areas of carcinogenic tissue (Figure 2).

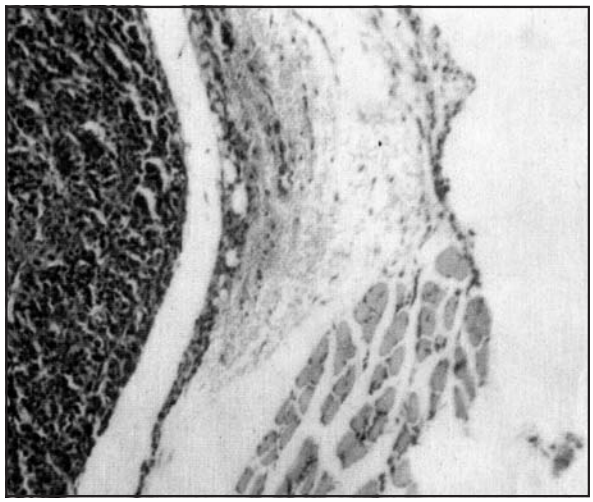

Figure 2. Compact, highly proliferative tumor mass surrounded by the area of hemorrhage and edematization of connective tissue

In mice treated with ascorbic acid (10 mg), a deceleration of infiltrative tumor growth progression inside the muscles was observed; there were a proliferation of connective tissue, a discrete formation of capsula infiltrate by lymphocytes in the first part of the experiment (Figure 3).

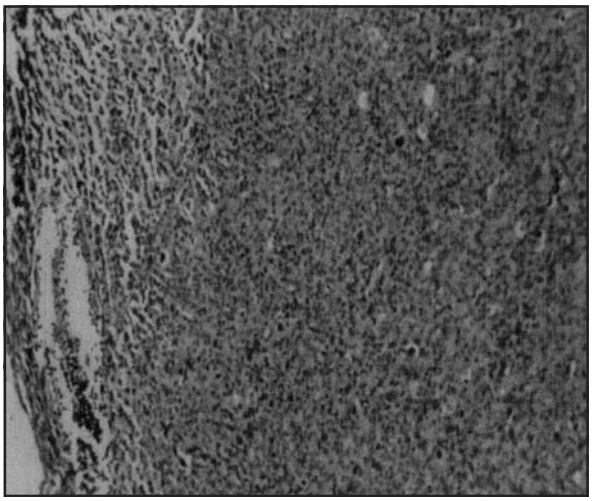

Figure 3. Wide tumor tissue zone with hardly perceivable capsula infiltrated by lymphocytes

In mice treated with 100 mg of ascorbic acid, on the pathohistological preparation obtained during the assay, some changes in tumor mass in the form of haemorrhagia, necrobiosis and necrosis were noticed. However, in the end of the assay, we noticed necrobiosis of cancer cells, their discomplexion and lymphocytic infiltration with both well-developed and wide girdle of fibro collagen capsula (Figure 4).

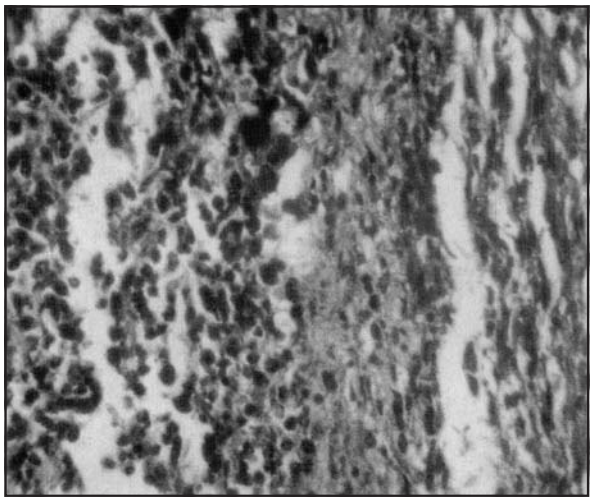

Figure 4. Subcapsular lymphocytic infiltration of a tumor capsula part which is well-developed and rich in both fibroblasts and collagen fibers

In mice treated with 1000 mg of ascorbic acid, during the first week of the assay, a lymphocytic infiltration occurs along with fibroblastic reaction, both inside the tumor and skeletal muscles (Figure 5).

At the end of the experiment, in animals of experimental group, there is subcapsular direction, that is, towards the tumor. Moreover, there were areas of progressive proliferation of fibroblast, the appearance of collagen in the capsular part (Figure 6) as well as its condensation (Figure 7).

The influence of ascorbic acid on mass and spleen cellularity in mice during the development of experimental mammary carcinoma.

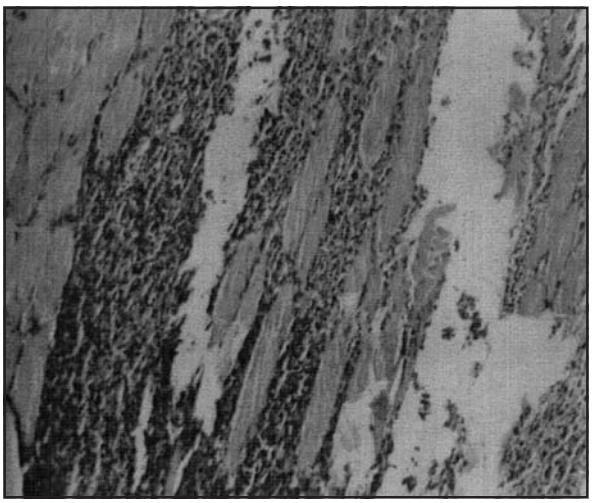

Figure 5. Tumor mass seizes and stratifies skeletal muscle, shows the signs of necrobiosis, fibroblasts proliferation and lymphocytic infiltration

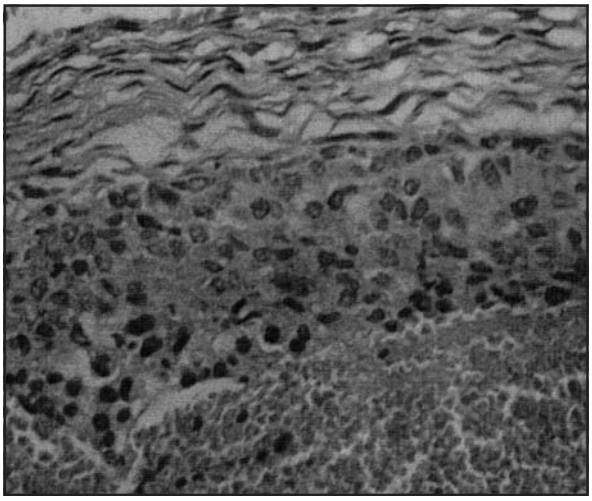

Figure 6. Above the hemorrhagic- necrotic area, there is a relatively vital tumor tissue; above the tissue, there is a wide fibroblast capsula with the domination of collagen fibers bundles

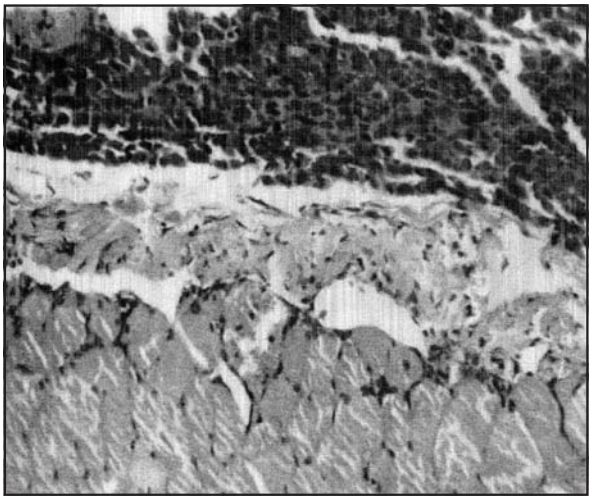

Figure 7. Strong connective tissue interposes between the tumor and muscular tissue

Our further research checked the kind of changes taking place in spleen of animals during the development of experimental mammary carcinoma. We chose spleen having in mind that it comprises the largest peripheral lymphoid organ in mice, and which is in turn the most significant source of lymphocytes for both *ex vivo* and *in vitro* research.

The results presented in Table 2 show that in the control group of animals, the tumor growth was followed by the increase in mass and spleen cellularity. In the experimental group of mice, the increase of cellularity and mass of spleen was less in relation to the control group. At the end of the examined period (21^{th}day) the greatest decrease of spleen cellularity (around 35%), in relation to the control group, was noticed in animals of the III experimental group.

The influence of ascorbic acid on phenotype characteristics of spleen lymphocytes in mice during the development of experimental mammary carcinoma.

The results of research of relative and total values of T and B lymphocyte numbers as well as $CD4^+$ and $CD8^+$ T subpopulations of spleen lymphocytes of

Table 2. The effect of various dose of ascorbic acid on the dynamic of mass and spleen cellularity changes in mice during the development experimental mammary carcinoma

Animal group		Spleen mass (mg) (A)		
		Cellularity (x 10^7) (B)		
		7. day	14. day	21. day
Control	A B	148 ± 10 8.4 ± 0.7	342 ± 57 14.0 ± 1.4	488 ± 70 26.4 ± 4.5
Experim. I	A B	138 ± 15 8.7 ± 1.0	296 ± 38 11.6 ± 2.1*	408 ± 12* 22.5 ± 5.6
Experim. II	A B	122 ± 12** 7.6 ± 0.2*	248 ± 43* 12.9 ± 1.7	436 ± 85 22.3 ± 4.3
Experim. III	A B	110 ± 21** 7.0 ± 0.2*	262 ± 49** 11.5 ± 1.6*	384 ± 63* 17.7 ± 3.8*

Mean values were shown ±SD for 10 animals in each group
*=p<0.05; **= p<0.01 in relation to the corresponding control group

mice during the development of mammary carcinoma were presented in Tables 3 and 4. In mice belonging to the control group, the percentage of T lymphocytes (Thy 1.2^+ cells) was progressively becoming lower during the examined period of time. However, due to the increased spleen cellularity, their absolute number was not significantly changed. The relative values of B lymphocytes increased in the period of 7 -14 days and then decreased from the 14^{th} – 21^{st} day. Similar dynamics of change was noticed in the overall number of B lymphocytes. Relative values of $CD4^+$ and $CD8^+$ T lymphocytes were decreased from 7-14-day period, while absolute values gradually increased during the examined period. (Table 3,4).

Table 3. The effect of various doses of ascorbic acid on the dynamic of relative value changes of lymphocyte subpopulation in spleen of mice during the development of experimental mammary carcinoma

Animal group subpopulations	Lymphocyte	%		
		7. day	14. day	21 day
Control	Thy 1.2	29.8±4.1	18.7±3.2	14.9±2.1
	B Ly	51.4±6.6	61.6±2.2	41.8±3.6
	$CD4^+$	20.3±3.2	12.5±2.4	15.5±3.2
	$CD8^+$	5.8±0.8	3.7±0.9	3.6±0.5
Experim. I	Thy 1.2	26.6±2.4	23.4±2.6*	15.3±2.7
	B Ly	54.4±3.2	63.7±6.9	44.8±6.8
	$CD4^+$	20.2±1.2	16.6±2.3*	13.2±1.9
	$CD8^+$	5.6±0.5	4.9±0.4*	4.1±0.8
Experim. II	Thy 1.2	27.5±2.2	23.1±1.5*	15.1±1.8
	B Ly	51.6±2.9	64.1±1.9	48.8±9.5
	$CD4^+$	19.6±1.6	16.8±1.7*	17.4±3.3
	$CD8^+$	5.7±0.9	4.7±0.3*	3.9±0.6
Experim. III	Thy 1.2	24.4±2.7	18.1±2.6	16.4±1.1
	B Ly	56.2±3.8	64.1±3.0	49.2±6.0*
	$CD4^+$	19.4±1.6	15.8±1.2*	18.6±2.3
	$CD8^+$	5.5±1.1	4.0±0.9	4.6±1.2

Mean values were shown ±SD for 5 animals in each group
*=p<0.05 in relation to the corresponding control group

Table 4. The effect of various doses of ascorbic acid on the dynamic of absolute values change of lymphocyte subpopulation in spleen of mice during the development experimental mammary carcinoma

Animal group subpopulations	Lymphocyte	(x 10^6)		
		7. day	14. day	21 day
Control	Thy 1.2	1.6±0.2	1.7±0.2	1.9±0.5
	B Ly	2.8±0.4	5.6±0.4	5.3±0.7
	$CD4^+$	1.1±0.1	1.1±0.2	2.0±0.6
	$CD8^+$	0.3±0.1	0.3±0.1	0.4±1
Experim. I	Thy 1.2	1.4±0.1	1.8±0.3	1.7±0.5
	B Ly	2.9±0.5	4.9±1.1	4.9±1.0
	$CD4^+$	1.1±0.1	1.2±0.2	1.5±0.5
	$CD8^+$	0.3±0.1	0.4±0.1	0.5±0.1
Experim. II	Thy 1.2	1.3±0.2	2.0±0.3	1.6±0.2
	B Ly	2.5±0.1	5.6±0.8	5.0±1.0
	$CD4^+$	0.9±0.1	1.5±0.2*	1.8±0.3
	$CD8^+$	0.3±0.1	0.4±0.1	0.4±0.1
Experim. III	Thy 1.2	1.1±0.2**	1.4±0.3	1.4±0.3
	B Ly	2.7±0.6	4.9±0.9	4.1±1.0
	$CD4^+$	0.9±0.2	1.1±0.2	1.6±0.5
	$CD8^+$	0.2±0.01*	0.3±0.1	0.4±0.1

Mean values were shown ±SD for 10 animals in each group
*=$p<0.05$; **= $p<0.01$ in relation to the corresponding control group

In mice treated with ascorbic acid (group I and II) statistically more significant relative values of T lymphocytes were found on the 14th day compared to the control group. However, the values of the total number of T lymphocytes did not significantly change, except on the seventh day when fewer numbers of T lymphocytes were recorded in the III experimental group.

When relative values of B lymphocytes were analyzed, it could be noticed that a statistically significant increase of these cells occurred only in III experimental group, on the 21st day ($p < 0,05$) in relation to the control group. No significant differences were found in the total number of B lymphocytes during the whole examined period between the experimental groups and the control group of mice.

Relative $CD4^+$T values of lymphocytes in animals of all experimental groups were statistically higher on the 14th day in relation to the control group, whereas the relative values of $CD8^+$T lymphocytes, during the same examined period, in animals of I and II experimental groups were higher in comparison with the mice of the control group. The absolute values of $CD4^+$T lymphocytes in animals belonging to II experimental group were statistically higher on the 14th day when compared to the control one, while absolute values of $CD8^+$T lymphocytes in animals of III experimental group were statistically lower on the 7th day from tumor induction, in relation to the control.

Discussion

This experimental work was initiated by the up-to-date knowledge about the importance of vitamin C for numerous physiological processes in organism including immunological, metabolic and anti-oxidative processes, as well as its lower concentration in patients with malignant diseases, and the controversial literary data concerning anti-tumor effect of high doses of vitamin C (9,10,12,13,15,16,17,18). According to our previous results, ascorbic acid prolongs the survival of mice with experimental mammary adenocarcinoma (14). In this paper, we wished to examine, using the same model, histopathological characteristics of tumor and some of immunological parameters for better understanding of the previously defined phenomenon.

Our results showed that vitamin C in a dose-dependant manner decreased the tumor mass, and that the highest dose (1000 mg/kg b.m.) had the highest effect. The decrease of the tumor mass may be the consequence of the direct effect of vitamin C on the proliferation of malignant cells or, its indirect effect on the stimulation of fibroblast proliferation and tumor encapsulation. Even though direct inhibitory effect of high dose of vitamin C on malignant cells proliferation was shown in some *in vitro* examinations, more detailed mechanisms have not been studied further (4,19). Tu-

mor cells release an enzyme hyaluronidase, which breaks the hyaluronic acid in intracellular matrix of the surrounding tissues, thus weakening these tissues and permitting their infiltration by malignant cells. It changes physio-chemical features of the tissue including viscosity and adhesiveness. Thus, proliferation is alleviated, as well as metastasis of malignant cells. Ascorbic acid is necessary for synthesis of one glycoprotein called physiological hyaluronidase inhibitor (PHI). It blocks the depolymerization process of glycosaminoglycan and depolymerization of matrix (20,21,22). During the tumor growth, due to the increase utilization of ascorbate, the reservoirs of vitamin C are wasted, particularly in the central zones of the tumor (23). Therefore, the concentration of PHI is reduced, which in turns provides necessary conditions for the faster growth of malignant cells. Therefore, it is logical to consider that application of vitamin C will have protective effect by the activation of the synthesis of PHI.

The change in histopathological structures of mammary adenocarcinoma which was presented in this paper can also be explained by the effect of vitamin C on collagen synthesis. Namely, the precursor of collagen (pro-collagen) which is synthesized in fibroblasts is rich both in proline and in lysine. Ascorbic acid which enables hydroxylation of proline and lysine into hydroxyl-proline and hydroxyl-lysine is necessary for the conversion of pro-collagen into collagen. Thus, maturation of collagen is stimulated as well as further formation of collagen fibers, which are parts of connective tissue (22,24,25). Besides hampering its growth, tumor encapsulation blocks the transportation of nutritive substances into the tumor tissue. This is the reason for the appearance of necrosis and hemorrhage which were present in tumors of animals treated with the high dose of vitamin C in our study.

Malignant tumors stimulate immunological system of a host (26). This is also confirmed by our results based on the following phenotypic features and the number of lymphocytes in the spleen of the control animals. At the beginning, the growth in the number of B lymphocytes was greater in relation to the growth in the number of T lymphocytes. Later (during the second and the third week), relative values of all subpopulations of lymphocytes were decreasing, whereas their absolute values did not increase despite the expectation, having in mind a progressive increase of the total spleen cellularity. That may be the consequence of inhibitory effect of already developed tumor on the immune system. Numerous clinical or experimental studies, which have been conducted so far, showed that tumor had immunosuppressive effects on activation, proliferation, and effector functions of both specific and nonspecific immunity components (26,27,28). One of the immunosuppressive mechanisms could be explained as the deficiency of vitamin C during the period of tumor growing, considering its already proven effect on immune system (4,15).

In animals treated with vitamin C, various changes were noticed in phenotype features and in the number of lymphocytes in spleen. They depended on the dose of vitamin C as well as the examined period. Even if the changes were noticed in animals having been given the smallest dose of vitamin C, they were more visible in II and III experimental group. Generally speaking, it can be said that in the first part of examination (up to the period of two weeks) the number of T lymphocytes statistically increased compared to the control, mostly on the number of CD4T cell subset. This increase was the most obvious in II experimental group. It is well-known that CD4T lymphocytes are the most significant cells for the onset of T cell immune response, activation of specific cytotoxicity mediated by NK cells, macrophage activation, as well as for activation and regulation of humoral immunity to protein antigens (29). All these components of the immune system are involved in antitumor immunity. In some other experimental tumor models it has been shown that the ascorbic acid treatment brought to the increase in the number of CD4T lymphocytes (30,31). Vitamin C exerts also the stimulating effect on the proliferation of lymphocytes in culture in the presence of mitogens (20,21,30,32,33), and the phenomenon is in correlation with the increase in vitamin C concentration in activated lymphocytes (20,21,30,32,33,). Since ascorbic acid is important for lymphocytes proliferation, lower concentrations of this vitamin during the period of advanced tumor growth may be also significant for the suppression of immunological reactivity.

One paradoxical finding in this paper is the decrease of spleen cellularity as well as further limitation in the increase of the number of lymphocytes in the third week of the experiment carried out on animals treated by vitamin C, although the opposite results would be expected. These results may be explained by the fact that despite vitamin C application along with the advance of tumor growth, its immunosuppressive effects surpass beneficial effects of vitamin C. However, they can be explained by the increased migration of lymphocytes from spleen into the tumor tissue, which is in accordance with our histopathological findings. Namely, we showed that with progressive tumor growth and the increase in vitamin C dosage, the number of tumor lymphocytes increased as well. Tumor infiltrating lymphocytes whose T cell receptors are specific for tumor antigens, possess antitumor effects both *in vitro* and *in vivo* (26,34). Up to now, there have been no experimental confirmations whether vitamin C has a direct or indirect effect on the migration of tumor infiltrating lymphocytes, whether therapeutic doses of vitamin C lead to their activation *in vivo*, and whether these cells are involved in the process of tumor necrosis. Therefore, our preliminary results are a good starting point - basis for further more complex examinations of this rather intriguing phenomenon.

According to our results, it can be concluded that vitamin C modulates both growth and histopathological characteristics of experimental mammary carcinoma in mice by stimulating the synthesis of collagen, proliferation of connective tissue and tumor encapsulation, increasing infiltration of lymphocytes in the tumor tissue and activating the immune system.

References

1. Higginson J, Muir CS. Environmental carcinogenes: Misconceptions and limitations to cancer control. J Natl Cancer Inst 1979; 63:1291–98.
2. Ross LP. Future possibilities in the prevention of breast cancer Fat and fiber breast cancer research. Breast Cancer Res 2000; 2: 268–76.
3. Berg JW. Can nutrition explain the pattern of international epidemiology of hormone-dependent cancers? Cancer Res 1975; 35: 3345–50.
4. Elliot M JR, Chithan K, Theoharis CT. The effects of plant flavonoids on Mammallian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000; 52: 673–751.
5. Marshal L, Graham S, Mettlin C, Shedd G, Swanson M. Diet in the epidemiology of oral cancer. Nutr Cancer 1982; 3:145–9.
6. Graham S, Mettlin C, Marshall J, Priore R, Rzepka T, Shedd D. Dietary factors in the epidemiology of cancer in larynx. Am J Epidemiol 1981; 113: 675–80.
7. Zaridze DG, Muir CS, McMichael AJ. Diet and cancer: Value of different types of epidemiological studies. Nutr Cancer 1985; 7: 155–66.
8. Vojdani A, Bazargan N, Vojdani E, Wright J. New evidence for antioxidant properties of vitamin C. Cancer Detect Prev 2000; 24(6): 508–23.
9. Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: prolongation of survival times in terminal human cancer. Proc Natl Acad Sci 1976; 73: 3685–9.
10. Creagan ET, Moertal CG, O'Fallon JR, Schutt AJ, O'Connell MJ, Rubin J, et al. Failure of highÐdose vitamin C therapy to benefit patients with advanced cancer control trial. New Engl J Med 1979; 301: 687–90.
11. Pauling L, Nixon JC, Stitt F, Marcuson R, Dunham WB, Barth R, et al. Effect of dietary ascorbic acid on the incidence of spontaneous mammary tumor in RIII mice. Proc Natl Acad Sci 1985; 82: 5185–9.
12. Frazier T, McGinn M. The influence of magnesium, calcium, and vitamin C on tumor growth in mice with breast cancer. J Surg Res 1979; 27: 318–20.
13. Abul-Haij Y, Kelliher M. Failure of ascorbic acid to inhibit growth of transplantable and dimethy lbenzanthracenc induced rat mammary tumors. Cancer Lett 1982; 17: 67–73.
14. Pavlović Z. Preventivno i terapijsko svojstvo askorbinske kiseline pri eksperimentalnoj indukciji malignih procesa. Niš: Doktorska disertacija; 1993.
15. Field CJ, Johnson IR, Schley PD. Nutrients and their role in host resistance to infection. L Leukoc Biol 2002; 71(1): 16–32.
16. Long CL, Maull KI, Krishnan RS, Laws HL, Geiger JW, Borghesi L et al. Ascorbic acid dynamics in the seriously ill and injured. J Surg Res 2003; 109(2): 144–8.
17. Karl-Johan M, Rodica L Max P, Tomas O, Fumiko I, Bengt G, Jan-Erik, et al. A short-term dietary supplementation of high doses of vitamin E increases T helper 1 cytokine production in patients with advanced colorectal cancer. Clinical Cacncer Research 2002; 1772–8.
18. Bogdansky O, Wroblewsky F, Markardt B. Concentrations of ascorbic acid in plasma and white cells of patients with cancer and non-cancerous chronic diseases. Cancer Res 1951; 11: 678–84.
19. Futakuchi M, Hirose M, Miki T, Tanaka H, Ozaki M, Shirai T. Inhibition of DMBA-initiated rat mammary tumour development by 1-0-hexy l-2,3,5-trimethy lhydroquinone, phenylethyl isothiocyanaate, and new synthetic ascorbic acid derivates. Eur J Cancer Prev 1998; 7(2): 153–9.
20. Anderson R, Osthusen R, Maritz R, Theron A, van Rensburg AL. The effects of increasing weekly doses of ascorbate on certain cellualar and humoral immune functions in normal volunters. Am J Clin Nutr 1980; 33: 71–82.
21. Eftychis H, Anderson R. Prevention of induction of suppression activity in human mononuclear leucocytes by ascorbate and cysteine. Int J Vitam Nutr Res 1983; 53: 398–407.
22. Barnes MJ, Kodicek E. Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. Vitam Horm 1972; 30: 1–19.
23. Chinoy NJ. Histochemical studies on ascorbic acid in human cancerous tissue and its significance. J Anim Morphol Physiol 1972; 19: 238-46.
24. Caygill C, Clucas IJ. The role of ascorbic acid in collagen biosynthesis. J Klin Chem Klin Biochem 1971; 1: 63–75.
25. Levene CI, Bates CI. Ascorbic acid collagen synthesis in cultured fibroblasts. Ann New York Acad Sci 1975; 258: 288–96.
26. Sogn JA. Tumor immunology: the glass is half full. Immunity 1998; 10:281–7.
27. Cheever MA, Disis ML, Bernhard H, Gralow JR, Hand SL, Huseby ES, et al. Imunity to oncogenic proteins. Immunological Reviews 1995; 145: 33–60.
28. Henderson RA, Finn OJ. Human tumor antigens are ready to fly. Advances in Immunology 1996; 62: 217:56.
29. Oxenius A, Zinkernagel RM, Hengartner H. $CD4^+$ T cell induction and effector functions: a comparison of immunity against soluble antigens and viral infections. Advances in Immunology 1998; 70: 313–67.
30. Siegel B, and Morton JI. Vitamin C, interferon and the immune response. Int J Vitam Nutr Res Suppl 1977; 16: 245–58.
31. Fraser R, Pavlović S, Kurahara C, Murata A, Petrson N, Taylor K et al. The effect of variations in vitamin C intake on cellular immune response of guinea pigs. Am J Clin Nutr 1980; 33: 839–47.
32. Delafuente JC, and Panush RS. Modulation of certain immunologic responses by vitamin C. Int J Vitam Nutr Res 1980; 50: 44–52.
33. De la Fuente M, Victor VM. Ascorbic acid and N-acetylcysteine improve in vitro the function of lymphocytes from mice with endotoxin-induced oxidative stress. Free Radic Res 2001; 35(1): 73–84.
34. Coulie PG. Human tumor antigens recognized by T cells: new perspectives for anti-cancer vaccines? Molecular Medicine Today 1997; 3: 261–8.

UTICAJ ASKORBINSKE KISELINE NA PATOHISTOLOŠKE KARAKTERISTIKE TUMORA I FENOTIPSKE KARAKTERISTIKE LIMFOCITA ZA VREME RAZVOJA EKSPERIMENTALNOG MAMARNOG KARCINOMA U MIŠEVA

Zorica Pavlović i Voja Pavlović

U prethodnoj studiji prikazali smo da visoke doze askorbinske kiseline prolongiraju preživljavanje miševa sa eksperimentalnim mamarnim karcinomon. Koristeći isti model, studirali smo u ovom radu patohistološke karakteristike u tumoru i fenotipske promene u subsetovima limfocita slezine. Za eksperimente smo koristili CBA/H miševe. Rast eksperimentalnog tumora bio je indukovan injekcijom ćelija mamarnog adenokarcinoma intramuskularnom inokulacijom u femoralnu regiju miševa. Životinje su bile podelje na kontrolnu grupu i tri eksperimentalne podgrupe (I, II, III). Miševi eksperimentalnih grupa bili su tretirani peroralno sa 10 mg/,100 mg/ i 1000 mg na kilogram telesne mase askorbinske kiseline u odnosu na kontrolnu grupu miševa koja je primala fiziološki rastvor. Miševi su žrtvovani 7. 14. i 21. dana od početka eksperimenta. Određivana je ukupna tumorska masa i njena patohistološka karakteristika, masa slezine i celularnost kao i relativni i ukupan broj T, B ćelija i T ćelijske subpopulacije ($CD4^+$ i $CD8^+$) u slezini. Velike doze askorbinske kiseline smanjuju tumorsku masu, stimulišu proliferaciju fibroblasta i formiranje kapsule oko tumora, indukuju tumorsku nekrozu kao i porast broja tumorom infiltriranih limfocita. Promene u subpopulaciji limfocita kao i njihov broj varira zavisno od primenjene doze askorbinske kiseline i vremena trajanja tumorske indukcije. Najznačajnije promene, manifestovane u porastu broja $CD4^+$T ćelija, dobijene su 1,4. dana u drugoj eksperimentalnoj grupi. Rezultati sugerišu da je povoljan efekat askorbinske kiseline na eksperimentalnu tumoro-genezu u našem modelu posledica uticaja tumora na imuni sistem. *Acta Medica Medianae 2005;44(2): 23–31.*

Ključne reči: *mamarni adenokarcinom, miševi, askorbinska kiselina, histopatologija, limfociti, fenotip*