

# Effect of Antioxidants and Membrane-Stabilizing Drugs on Paraneoplastic Disorders of Erythrocytes and Microcirculation

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## ABSTRACT

Has been investigated the intensity of local hemocirculation, functional state of adrenoreactive structures of mesenteric blood vessel walls and erythrocytes' deformability (ED) in mice and rats during experimental malignant tumor growth (Ehrlich carcinoma, Walker carcinoma, Sarcoma C-45) before and after treatment with antioxidants and membrane-stabilizing drugs, with the use of  $H^+$  clearance, bio-microscopy, pharmacological analysis using the vasoactive agents, and filtration-photometry methods. The received data were analyzed statistically by means of Student's criterion. Has been stated that during the process of experimental malignant tumor growth the local hemocirculation and erythrocytes' deformability are decreased, the functional state of adrenoreactive structures of mesenteric blood vessels is impaired. At the early stage of cancer growth the malfunctioning of  $\alpha$ -receptors is expressed; at the late stage of malignant growth both, functional state of  $\alpha$ - and  $\beta$ -receptors are impaired. Administration of antioxidants and membrane-stabilizing drugs markedly improved the above-mentioned disturbances. Revealed improvements after treatment with antioxidants indicates the leading role of peroxidative processes in pathogenesis of paraneoplastic disorders of erythrocytes and microcirculation.

**KEYWORDS:** cancer, paraneoplasia, erythrocyte, deformability, adrenoreceptors, alpha-tocopherol, proxanol P-268, plaferon LB

It is generally recognized that cancer growth is accompanied by paraneoplastic processes. Investigation of disorders of "intact" organs (organs that are not directly affected or involved in the malignant process) is of great value, since such "innocent" processes in most cases are the first messengers of grave and cruel disease. Moreover, the paraneoplastic disorders are manifested long before the malignant process reveals itself; they aggravate the course of disease and often play the leading role in lethal outcomes [1,2].

Among various paraneoplastic processes, erythrocytes' and microcirculatory disorders are of especial importance, since the mentioned structures are very labile, fragile [3], and immediately reflect any deviations occurred in organism, therefore it's of great diagnostic value. On the other hand they provide organism with oxygen and nutrients. Rises problem - Cancer growth, microcirculation, hemorheology, erythrocytes and oxidative processes. The components, involved in the above-mentioned problem are closely interrelated. Intensity of microcirculation depends on blood micro rheology (fluidity), which in turn depends on erythrocytes functional state. On the other hand erythrocytes' membrane and adrenoreactive structures that adjust blood vessels' luminal size are under influence of oxidative processes. The paraneoplastic disorders of all of these interrelated processes are responsible for developed generalized hypoxia characteristic for malignant tumor growth.

Thus, detection of paraneoplastic disorders and their correction by all available means and methods is of paramount importance in modern oncology. It is expected that early diagnostics and correction of paraneoplastic disorders along with surgical removal of cancer tissue would increase the treatment efficiency.

The present work was aimed to study microcirculation, local blood flow, adrenergic structures of blood vessels, and erythrocytes' deformability during malignant tumor growth in experimental animals and on the bases of revealed paraneoplastic disorders, using various antioxidants and membrane stabilizing drugs, find the ways for their correction.

## MATERIAL AND METHODS

Experiments have been carried out on mice of mixed population (body weight 25-30 g) and on pubertal white rats (body weight 220-250 g). Days of investigation were: 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> after implantation of Ehrlich carcinoma; 5<sup>th</sup> and 12<sup>th</sup> - after implantation of carcinoma Walker and 30<sup>th</sup> and 40<sup>th</sup> - after implantation of Sarcoma C-45.

Intra-peritoneal injections of  $\alpha$ -tocopherol (1,2 ml/100g a day, beginning from the next day after cancer implantation), proxanol P-268 (40 mg/100g a day, beginning from the next day after cancer implantation) and plaferon-LB (10 mc.g/100g a day, during 10 days before experiment) were used as membrane stabilizing and antioxidant agents and urethan - as anesthetics (1,3 g/kg - for mice; 1,4 g/kg - for rats).

Experimental animals were divided into three major groups: Norm (healthy animals. 10 mice and 10 rats), Control (untreated animals. 30 mice and 40 rats) and Experimental (animals treated with  $\alpha$ -tocopherol - 30 mice, proxanol P-268 - 20 rats and plaferon LB - 20 rats).

The local hemocirculation was studied using the method of polarography ( $H^+$  clearance). The blood flow was defined by the formula  $f = 100 \times K \cdot 0,693/T_{1/2}$  ml/min/100g; where  $T_{1/2}$  - is the biological half live of  $H^+$  in tissue (defined by semi-algorithmic scale);  $K$  - is the partial coefficient of gas distribution in tissue-blood. For reliable electrical contact, indifferent chlorine-silver electrode with square 10 cm<sup>2</sup> was placed in high density KCl solution. In the same solution was placed the experimental mouse-tail. Recording of local hemocirculation in skeletal muscle was performed by insertion of platinum electrode with active surface 0,15 - 0,3 mm<sup>2</sup> in *m. biceps femoris* at 1-2 mm depth. Active and indifferent electrodes were connected to polarographic scheme and polarogram owing to millivoltmeter (pH-450) was registered on the paper of potentiometer KCP-4.

Erythrocytes' deformability was defined by means of filtration - computed photometry method [4], which



Norm 2,86±0,12 0	5 <sup>th</sup> day after Walker carcinoma implantation	12 <sup>th</sup> day after Walker carcinoma implantation
Animals with Walker carcinoma (Control; untreated animals)	4,62±0,09 1	5,06±0,11 2
Animals with Walker carcinoma + $\alpha$ -tocopherol	3,63±0,11 3	2,90± 0,09 4
Animals with Walker carcinoma + proxanol P-268	4,24±0,16 5	3,56±0,17 6

$P_{0,1}<0,001$   $P_{0,2}<0,001$   $P_{3,1}<0,001$   $P_{4,2}<0,001$   $P_{5,1}>0,05$   $P_{6,2}<0,001$   $P_{3,4}<0,001$   $P_{6,5}<0,01$   $P_{0,3}<0,001$   $P_{0,4}>0,2$   $P_{0,5}<0,001$   $P_{0,6}<0,005$   $P_{1,2}<0,01$

**Tab.3** Time of erythrocytes' passage through the filter (deformability) in case of Walker carcinoma growth (control), in norm and after treatment with  $\alpha$ -tocopherol and proxanol P-268.

by 9,4% ( $P<0,05$ ) and on the 21<sup>st</sup> day - by 27,7% ( $P<0,001$ ).

In case of treated with  $\alpha$ -tocopherol animals ED is significantly increased by 25,0% only on the 7<sup>th</sup> day of cancer growth ( $P<0,001$ ) compared to control. The data obtained on the 14<sup>th</sup> day revealed increase tendency in ED by 8,1% ( $P>0,05$ ). Although, the results of ED on the 14<sup>th</sup> day compared to control are not statistically significant, they are still increased. Noteworthy that there is not significant difference between the results of ED in norm and results obtained on the 14<sup>th</sup> day ( $P>0,1$ ). We should take into consideration the fact, that erythrocytes' deformability in control group on the 14<sup>th</sup> day after cancer implantation is relatively improved and increased (at the expense of increased number of immature erythrocytes as a result of compensatory reaction developed after hemolysis) compared to ED detected on the 7<sup>th</sup> day; this reduces the difference in ED between control and treated animals. That's why, despite increase in ED, we could not observe the significant improvement of ED in treated animals on the 14<sup>th</sup> day of cancer growth.

On the 21<sup>st</sup> day of cancer growth  $\alpha$ -tocopherol could not improve ED significantly and increase in ED was not statistically significant ( $P>0,2$ ).

In case of proxanol P-268, on the 7<sup>th</sup> day of implantation ED is increased by 19,3% ( $P<0,001$ ), on the 14<sup>th</sup> day - by 3,5% ( $P>0,1$ ) and on the 21<sup>st</sup> day - by 10,6% ( $P<0,005$ ) compared to control.

The results of investigations obtained in case of Walker carcinoma and Sarcoma C-45 growth are summarized in Tab.2 and Tab.3.

Investigation of adrenoceptors of mesenteric blood vessels revealed that on the 7<sup>th</sup> day of Ehrlich carcinoma implantation after adrenalin application diameter of arterioles dilated by 20,4%, on the 14<sup>th</sup> day - by 3,2 ( $P<0,001$ ) and on the 21<sup>st</sup> day arterioles constricted by - 2,8% however, it was not statistically significant ( $P>0,1$ ).

On the dihydroergotamine background adrenalin instillation caused arterioles' dilation by 14,4% ( $P<0,01$ ), 2,7% and 1,8% ( $P>0,1$ ) correspondingly.

On the obzidane background, arterioles' dilatation was not statistically significant (1,7%, 7,9% and 0,9%,  $P>0,1$ ).

In treated with  $\alpha$ -tocopherol animals, on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days of cancer growth adrenalin instillation caused vasoconstriction by 19,8%, 17,4% and 9,1% respectively ( $P<0,001$ ) and in case of proxanol P-268 microvessels were constricted by 17,1%, 19,4% and 10,8% ( $P<0,001$ ) correspondingly.

On the background of obzidane, in case of  $\alpha$ -tocopherol, adrenalin influence revealed vasoconstriction by 21,2%, 19,2% and 9,3% ( $P<0,001$ ) and in case of P-268 arterioles were constricted by 16,9%, 19,5% and 14,4% ( $P<0,001$ ) correspondingly.

On the background of dihydroergotamine, in case of  $\alpha$ -tocopherol, adrenalin influence revealed vasodilatation by 18,7%, 17,3% and 11,6% ( $P<0,005$ ), and in case of P-268 - by 18,1%, 15,8% and 13,5% ( $P<0,001$ ) respectively.

Unlike  $\alpha$ -tocopherol and proxanol P-268, plaferon LB did not reveal significant improvement in functioning of mesenteric arterioles' adrenoceptors.

The results of investigations of mesenteric arterioles in case of sarcoma C-45 growth are presented in Tab.4,5,6.

## DISCUSSION

As the results of investigation have shown in the process of experimental malignant tumor growth erythrocytes' deformability are significantly reduced. Erythrocytes' membrane disturbances could be explained by decreased antioxidant protection of organism which in turn supports activation of lipid peroxidation leading to destructive processes [5]. In our previous studies we have stated activation of peroxidative processes and its effect on biomembranes in case of malignant growth [6].

During cancer growth the functioning of mesenteric arterioles' adrenergic structures is impaired as well. As it was stated, at the early stage of cancer growth adrenalin application revealed abnormal reaction of mesenteric arterioles - instead of constriction, arterioles dilated. The mentioned reaction indicates malfunctioning of arteriolar  $\alpha$ -adrenoceptors. This is confirmed by the fact that preliminary blockade of  $\alpha$ -adrenoceptors did not change reaction of arterioles on adrenalin application. Moreover, the preliminary blockade of  $\beta$ -receptors had no effect on adrenalin application. Noteworthy that at the early stage of malignant growth, the  $\beta$ -receptors are still intact - on the background of  $\alpha$ -adrenoceptors'

blockade or without it, arterioles dilate after adrenalin application and preliminary blockade of  $\beta$ -receptors does not reveal arterioles' reaction on adrenalin application.

The above-mentioned disturbances of arterioles' adrenergic structures could be considered as a result of activated lipid peroxidation, increased concentration of nitric oxide (NO) and catecholamines (especially of epinephrine) in blood of experimental animals.

At the late stage of cancer growth arterioles practically had no reaction on adrenalin application, neither on the background of  $\alpha$ - and  $\beta$ -receptors' blockade, nor without it. This fact clearly indicates that at the late period of cancer growth the function of both,  $\alpha$ - and  $\beta$ -receptors are exhausted.

Disorders of local hemocirculation expressed during experimental malignant growth could be related to the above-mentioned erythrocytes' and adrenergic structures' disturbances. Dilatation of arterioles, decreased linear speed of blood flow and reduced erythrocytes' deformability supporting the process of aggregation, in cooperation to factor "Plasma" probably play an important role in paraneoplastic disorders of microhemocirculation.

In an attempt to restore paraneoplastic disturbances of erythrocytes and microcirculation the antioxidants and membrane-stabilizing drugs were used.  $\alpha$ -tocopherol is a quite powerful antioxidant; proxanol P-268 is the nonionic surface active agent, membrane-stabilizing drug that is able to change rheologic features of the blood; plaferon LB reveals antioxidant and membrane-stabilizing effects.

The results of our investigations have shown that administration of  $\alpha$ -tocopherol in common positively effects on erythrocytes' deformability, however long-term use of  $\alpha$ -tocopherol reveals opposite reaction and worsens erythrocytes' deformability. There are suggestions that in mice  $\alpha$ -tocopherol injections are positively tolerated during 10 to 12 days, however long-term use has negative effect, because in such case  $\alpha$ -tocopherol serves as a substance for oxidation, which in turn exaggerates the process of lipid peroxidation [7,8].

Proxanol P-268 has the similar, positive effect on ED and it is better tolerated than  $\alpha$ -tocopherol. In case of plaferon LB, effect is better when treatment is started earlier and at the early stage of disease.

The function of adrenoactive structures of mesenteric arterioles in cases of  $\alpha$ -tocopherol and proxanol P-268 were more or less improved. Plaferon LB has not had the effect on adrenoceptors.

After treatment, the local hemocirculation was improved in all cases that is probably the result of improved deformability of erythrocytes however, plaferon LB revealed the less effect. Probably, along with erythrocytes' deformability we have to take into consideration the state of adrenoceptors, erythrocytes' aggregability, and factor "plasma". According to literature proxanol P-268 is the surface-active agent, reducing adhesiveness and aggregability of erythrocytes, that's why it had the better effect nearly in all cases of malignant growth when we observed the changes in paraneoplastic disorders of erythrocytes and microcirculation.

	30 <sup>th</sup> day after sarcoma C-45 implantation		40 <sup>th</sup> day after sarcoma C-45 implantation	
	+Adrenalin		+Adrenalin	
Animals with sarcoma C-45 (Control; untreated animals)	22,8±0,8	20,1±0,7 P<0,05	21,7±0,7	23,9±0,7 P<0,05
Animals with Sarcoma C-45 + plaferon LB	23,5±0,5	20,6±0,5 P<0,001	21,5±0,6	23,6±0,6 P<0,05

**Tab.4** Diameter of mesenteric arterioles (mcm) in case of sarcoma C-45 growth, after treatment with plaferon LB, before and after application of adrenalin.

	30 <sup>th</sup> day after sarcoma C-45 implantation		40 <sup>th</sup> day after sarcoma C-45 implantation	
	+ Obzidane +Adrenalin		+ Obzidane +Adrenalin	
Animals with sarcoma C-45 (Control; untreated animals)	23,5±0,5	19,6±0,5 P<0,001	22,8±0,7	22,4±0,7 P>0,2
Animals with sarcoma C-45 + plaferon LB	23,1±0,4	18,8±0,4 P<0,001	22,7±0,6	22,1±0,5 P>0,05

**Tab.5** Diameter of mesenteric arterioles (mcm) in case of sarcoma C-45 growth, after treatment with plaferon LB, on the obzidane background, before and after application of adrenalin.

	30 <sup>th</sup> day after sarcoma C-45 implantation		40 <sup>th</sup> day after sarcoma C-45 implantation	
		+DHE +Adrenalin		+DHE +Adrenalin
<b>Animals with sarcoma C-45</b> (Control; untreated animals)	20,0±0,5	23,5±0,6 P<0,001	21,6±0,5	24,8±0,6 P<0,005
<b>Animals with sarcoma C-45 + plaferon LB</b>	20,6±0,4	24,9±0,7 P<0,001	22,4±0,4	25,9±0,4 P<0,001

**Tab.6** Diameter of mesenteric arterioles (mcm) in case of sarcoma C-45 growth, after treatment with plaferon LB, on the dihydroergotamine (DHE) background, before and after application of adrenalin.

## CONCLUSION

Thus, it could be concluded that in case of experimental malignant tumor growth erythrocytes deformability, local hemocirculation and function of adrenoreactive structures of mesenteric blood vessels are significantly reduced; At the early stage of cancer growth the malfunctioning of  $\alpha$ -receptors is expressed; At the late stage of malignant growth both, functional state of  $\alpha$ - and  $\beta$ -receptors are

impaired;  $\alpha$ -tocopherol and proxanol P-268 reveal positive effect on paraneoplastic disorders of erythrocytes and microcirculation; plaferon LB positively effects on erythrocytes deformability and local hemocirculation; Revealed improvements after treatment with antioxidants and membrane - stabilizing drugs indicates the leading role of activated peroxidative processes in pathogenesis of paraneoplastic disorders of erythrocytes and microcirculation.

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## Эффект антиоксидантных и мембраностабилизирующих средств на паранеопластические нарушения эритроцитов и микрогемодиализации

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## РЕЗЮМЕ

Изучены интенсивность локального кровотока (ИЛК) в скелетной мышце, функциональное состояние структур стен сосудов брыжейки тонкой кишки и деформабельность мембран эритроцитов мышей и крыс в процессе роста карциномы Эрлиха, Уокера и саркомы C-45 до и после применения  $\alpha$ -токоферола, проксанола П-268 и плаферона ЛБ. ИЛК изучалась методом полярографии по водороду, функциональное состояние адренореактивных структур микрососудов изучалось с помощью фармакологического анализа с использованием вазоактивных средств а деформабельность мембран эритроцитов - с помощью фильтрационно-фотометрического метода. В эксперименте установлено, что в процессе роста опухоли уменьшается ИЛК, деформабельность мембран эритроцитов и чувствительность адренореактивных структур микрососудов, в частности: на ранних стадиях роста опухоли снижается чувствительность  $\alpha$ -, а на более поздних этапах и  $\beta$ -адренореактивных структур стенки микрососудов. Использование отмеченных антиоксидантов и мембраностабилизирующих препаратов значительно улучшает эти нарушения, что указывает на ведущую роль пероксидационных процессов в патогенезе паранеопластических нарушений эритроцитов и микрогемодиализации.

**КЛЮЧЕВЫЕ СЛОВА:** злокачественная опухоль, эритроцит, деформабельность, адренорецепторы, альфа-токоферол, проксанол П-268, плаферон-ЛБ