CELLS OF BUCCAL EPITHELIUM CHARGE STATE IN CHILDREN WITH LESIONS OF THE ORAL MUCOSA AT THE BACKGROUND OF ACUTE LYMPHOBLASTIC LEUKEMIA IN THE DYNAMICS OF THE TREATMENT

Ilona Kovach, *Doctor of Medical Sciences,*
Julia Khotimskaya, *Candidate of Medical Sciences,*
Yana Lavreniuk, *Candidate of Medical Sciences,*
Kristina Buniatian,
SE «Dnepropetrovsk Medical Academy of Ministry of Health Care of Ukraine»

Annotation. *To study the charge state of buccal epithelial cells (BEC) in children with lesions of the mouth mucous membrane at the background of acute lymphoblastic leukemia in the dynamics of the treatment. The charge state of BEC in 126 children with lesions of the oral mucosa at the background of acute lymphoblastic leukemia (ALBL) in different periods of its course in the dynamics of treatment has been studied. All the children were divided into two groups – the basic one (n=91) and the one of comparison (n= 35). Depending on acute lympho-blastic leukemia clinical stage all the children under examination were divided into three sub-groups (the acute phase, remission, relapse). At the first acute period and at the relapse of ALBL the treatment-and-prophylactic complex including kvertsetin and polyphenol containing mucosal gel applications, the treatment of oral cavity mucosa with antimycotic agents, antiseptic with a mixture of alkaloid bisulfate with sanguinarine with chelerythrine and keratoplastic agent, probi-otic preparation INSIDE were used. At the period of remission polyphenol containing gel, kera-toplastic remedy and probiotic were administered. For the individual hygiene of mouth cavity alcohol-free lysozyme dentifrice water with anti-inflammatory effect was used.*

The children under study had a reduced level of BEC functional activity in comparison with norm in both groups of the patients. During treatment, there was an increase in the number of BEC moving nuclei and nuclei and plasmolemma amplitudes in the main group of children.

Key words: *children, oral mucosa, acute lymphoblastic leukemia, the charge state of buc-cal epithelial cell.*

Introduction. The last years are marked by a significant raise of interest to the functions of the mucosal epithelium (epithelium of the mucous mem-branes). This is due to the recognition of its coordinating position in the reac-tions connecting the mechanisms of congenital (nonspecific) and specific im-munity, initiation and stabilization of inflammatory processes [1-5]. It turned out that mucosal epitheliocytes have a significant effector potential in inflam-mation and immunity reactions, realizing it in response to the stimulating effect of exogenous (microorganisms, allergens, pollutants) and endogenous (cyto-kines, etc.) nature [4, 5]. Due to this, mucosal epitheliocytes acquire the ability to enter into cooperation with "professional" inducers and effectors of inflam-mation and immunity, such as neutrophils, eosinophils, mast cells, dendritic cells, macrophages, T - and B - lymphocytes. This turns them into active par-ticipants in cascading and network interactions that determine the development and regulation of inflammatory and

immune processes. It is not coincidentally that mucosal epitheliocytes are referred to as "inflammatory cells" [4, 5] more often, and the pathology associated with inflammation of the mucous mem-branes is called "epithelial cell disease". This is due to the fact that, being under the sights of exogenous and endogenous stimuli, mucosal epitheliocytes are able to change their functional status, joining in the formation of vicious circles that support chronic pathology in the mucosal membranes system.

As a part of the mucosal system, the buccal epithelium (BE) retains the elements of its active position in relationships with stimuli emanating from the external and internal environment. This makes it possible to use it to study the physiology and reactivity of mucous membranes, including as an indicator of local and general disorders of the oral mucosa.

The objective: to study the charge state of buccal epithelial cells (BEC) in children with lesions of the mouth mucous membrane at the background of acute lymphoblastic leukemia in the dynamics of the treatment.

Materials and methods. 126 children aged 2 - 18 years old with acute lymphoblastic leukemia (ALBL) participated in clinical trials. They had the fol-lowing dental pathology: generalized chronic catarrhal gingivitis (GCCG), ero-sive-ulcerative and oral candidiasis. The children underwent clinical examina-tions were divided into 2 groups – the basic and comparative. Dental plaque was removed and oral cavity sanation was performed in them. Hygienic care was systematically repeated and controlled.

Oral cavity hygiene was administered with the alcohol-free anti-inflammatory tooth wash "Lizomukoid", which was developed by the De-partment of Biotechnology of the Institute for Stomatology and Maxillofacial Surgery of the Ukrainian Academy of Medical Sciences (head of the depart-ment – Doctor of Biology, Professor Levitsky A. P). The children of the main group, in addition to oral hygiene, got the developed treatment-and-prophylactic complex (TPC). At its administration the period of the diseases's course was taken into account. The first variant of the local treatment was applied in the acute period and relapse of the disease and included: application of mucosal gels "Vinogradny" and "Kvertulin" on the oral mucosa twice a day in turn for 2 weeks, the application of probiotic "Biovestin" in the appropriate age-related dosage (1 month), treatment of the affected areas of the mucosa with an antimycotic solution for the mouth of Candid (20 drops thrice a day for 10 - 14 days), oral cavity mucosa rinsing with an antimicrobial Sangviritrin 3 times a day for 10-14 days in dilution of 40- 50 drops on 200 ml of water. To accelerate the processes of epithelialization, the appliqués of the antioxidant vitamins and provitamins Catomas were used twice a day in 30 minutes after meal during 14 days, starting from the third week of treatment.

The second variant of the local treatment included: oral hygiene, the use of anti-inflammatory tooth wash Lizomukoid, mucosal gel Vinogradny, probi-otic Biovestin which were used at the remission of the disease.

Variants of oral cavity mucosa treatment at acute lymphoblast leukemia by the groups of children are presented in Table 1.

Table 1

Variants of oral cavity mucosa treatment at ALBL by the groups of children

groups		Variants of treatment	N of patients
basic	I	Oral cavity hygiene+"Lysomucoid" + muco-sal gel "Grape" + "Quertulin" + probiotic "Biovestin" + "Candide" + "Sangviritrin" + "Katomas"	71
	II	Oral cavity hygiene +"Lysomucoid" + mu-cosal gel "Vine" + "Biovestin"	20
comparison		Oral cavity hygiene + "Lysomucoid" rinsing	35
Total			126

I - acute period + relapse; II - remission

The electrophoretic mobility of BEC's nuclei was determined by the method of Shahkbazov (1986) in the modification of Denga O. V. (1997). The method consists in assessing the level of general and local nonspecific resistance of the body, in particular, the oral cavity, through the complex of BEC measured charge parameters: the percentage of mobile nuclei and cellular plasmalemas, their amplitudes and shift rate, the ratio of these amplitudes [6].

Due to this technique, it is possible to objectively assess the state of cellular metabolism, and consequently, the general functional activity of the cells. The BECs were taken with a slight scraping on an empty stomach, after rinsing the oral cavity. Samples were prepared according to the technique [6]. The per-centage of BEC mobile nuclei and plasmols was estimated with biological mi-croscope with a 480 magnification for 100 intact cells in each sample. Ampli-tudes of nuclei and plasmolemmas displacement was estimated with an eye-glass ruler.

Results of the study and their discussion. The decreased level of BECs func-tional activity has been revealed in all the groups of the children under study in comparison with the norm. These changes took place both at acute period, re-lapse and remission of ALBL. A low percentage of BEC motile nuclei and small amplitude of their displacement prove it. At the same time, the ampli-tude of displacement of plasmolemmas is even more reduced, and accordingly, the ratio Apl / An equals 1.03. In children of the main group with ulcerative or candidal stomatitis in the oral cavity, these indices turned out to be the lowest, which, in our opinion, testifies to the stress reaction of the body and the insta-bility of the adaptive processes in the oral cavity in the first acute period and during the relapse of the main blood diseases. Thus, the amplitude of the nuclei was 1.22±0.07 μm, the amplitude of the plasmolemmas was 1.26 ± 0.07 μm, and the number of mobile nuclei was 22.05±1.11 %.

We have developed systems of treatment and prevention for the children of both groups which initiate nuclear-cytoplasmic ratio in cells, increase metabol-ic processes. The latter is evidenced by rising immediately after the correction percentage of motile BEC nuclei and displacement of their amplitude (Table. 2).

Analysis of BEC charge state changes digital values in children with ALBL in the

first acute phase and during relapse within 6 months after initiation of the researches showed increase of BEC motile nuclei at 24.6 % in the main group of the children after the application of TPC developed, with the use of kvertsetin-containing mucosal gel, and its combination with gel containing a large amount of polyphenols, flavonols, anthocyanins, and catechins, mixture of sanguinarine and chelerythrine bisulfate alkaloids with probiotic and anti - mycotic drugs.

Along with this, there was a tendency to an increase in the amplitude of displacement both BECs nuclei and plasmolemma and, hence, their ratio in the main group children. The increase of plasmolemma charge further led to opti-mization of the displacement of both plasmolemma amplitudes and nuclei ratio that is typical for the normal physiological state of adaptation reactions, start-ing from the cellular level. Thus, in children of the main group in 6 months af-ter the beginning of the research, the displacement amplitudes of plasmolem-mas and nuclei increased by 39 % and 23 %, respectively.

At the same time, in this group children, at the end of the study, the BEC charge state was almost normal (Table 2).

Table 2

Dynamics of changes in the charge state of BEC in children with ALBL in the first acute period and in the period of relapse, (M ± m)

Period of oservation		Initial data	In 6 months	In a year
Indicators of the charge state of BEC	Motile nuclei, %	comparison	22.08±1.13	23.17±1.16 p>0.05	22.73±1.15 p>0.05
		main	22.05±1.11 p>0.05	27.52±1.38 p>0.05 p1<0.05	28.16±1.41 P<0.05 P1<0.05
	Nuclei's amplitude	comparison	1.22±0.07	1.24±0.07 p>0.05	1.23±0.07 p>0.05
		main	1.21±0.06 p>0.05	1.49±0.08 P<0.05 p>0.05	1.48±0.08 P<0.05 P1<0.05
	Plasmolemma amplitude	comparison	1.35±0.07 p>0.05	1.31±0.07 p>0.05	1.29±0.07 p>0.05
		main		11.88±0.10 P<0.05 P1>0.05	1.65±0.09 P<0.05 P1<0.05
	Apl/An	comparison	1.03	1.06	1.05
		main	1.03	1.26	1.11

Notes: p - an indicator of reliability of differences in comparison with the initial data; p1 - the indicator of reliability of differences in comparison with the com-parison group

In the comparison group, in 6 months after lysozyme rinsing agent use, the electrophoretic indices of the BEC improved somewhat, however, did not reach normal values in somatically healthy children. Subsequently, a decrease in the electrophoretic

activity of the cells was observed and their indices practi-cally approached the initial values (p> 0.05).

In a year of observations, the numerical values of the percent of motile BEC nuclei in the main group children continued to increase and were 27.7% higher than the baseline data at the beginning of TPC use. There was also a tendency towards an increase in amplitudes displacement of both BEC nuclei and plasmolemma, and, therefore, their ratio in the children under study. The displacement amplitudes of the nuclei and plasmolemmas increased and at the end of the observation were 22.3 % and 22.2 % larger than the original values, and Apl / An equals 11.1

However, in the comparison group, where only the lysozyme rinsing agent was used, the electrophoretic activity of the cells was reduced, and their indices practically approached the initial values (p> 0.05).

The digital values of BEC functional activity were reduced in children with GCCG and stomatitis against the background of ALBL at the period of remission. This was proved by a low percentage of motile BEC nuclei and small amplitude of their displacement. In this case, the amplitude of displace-ment of the plasmolemmas, and accordingly, the ratio Apl / An was even low-ered to a greater extent and composed 1.02. Noteworthy is the fact that the main group children who had either ulcer or thrush in the mouth had the low-est indexes. This, in our opinion, may indicate instability of adaptive processes in the oral cavity during ALBL remission. Thus, the amplitude of the nuclei was 1.23 ± 0.06 μm, the amplitude of the plasmolemmas was 1.26±0.07 μm, and the number of mobile nuclei was 23.09 ± 1.16 %.

Use of the TPC developed in both groups of children initiated nuclear-cytoplasmic ratio in cells that results in increase of metabolic processes in BEC and the increase of motile nuclei their displacement amplitude immediate-ly after the correction evidences this (Table 3).

In 6 months after the beginning of the charge state of BEC studies in children with ALBL in remission we have found the increase of motile nuclei of BEC at 25.2 % in the main group children after the use of TPC developed. The complex involved the use of kvertsetin-containing mucosal gel, as well as its combination with gel containing a large number of polyphenols, flavonols, anthocyanins and catechins, a mixture of sanguinarin and chelerythrine bisul-fate alkaloids with probiotic and antimycotic agents.

At the same time, there was a tendency to increase the displacement am-plitudes of BEC both nuclei and plasmolemma, and, consequently, their ratio in the children of the main group. An increase in the charge of plasmolemmas subsequently led to an optimization of the ratio of displacement amplitudes, of both plasmolemmas and nuclei, which is typical for the normal physiological state of adaptation reactions, beginning from the cellular level. Thus, in chil-dren of the main group in 6 months after the beginning of the study, the dis-placement amplitudes of plasmolemmas and nuclei increased by 55.6 % and 28.5 %, respectively.

Table 3

Dynamics of BEC charge state in children with ALBL in remission, (M ± m)

	Periods of observation		Initial data	In 6 months	In a year
Indicators of BEC charge state	Motile nuclei, %	Comparison	23.11±1.18	23.97±1.21 p> 0.05	23.97±1.21 p> 0.05
		Basic	23.09±1.16 P1>0.05	28.91±1.45 P<0.05 P1<0.05	29.85±1.51 P<0.05 P1<0.05
	of nuclei, mcm	Comparison	1.24±0.06	1.27±0.07 P>0.05	1.25±0.07 p>0.05
		Main	1.23±0.06 P1>0.05	1.58±0.08 P<0.05 P<0.05	1.57±0.08 P<0.05 P<0.05
	Plasmolemma amplitude, mcm	Comparison	1.27±0.07	1.34±0.07 p>0.05	1.33±0.07 p<0.05
		Main	1.26±0.07 P1>0.05	1.96±0.10 P<0.05 P1<0.05	1.82±0.10 P<0.05 P1<0.05
	Apl/An	Comparison	1.02	1.06	1.06
		Main	1.02	1.24	1.16

Notes: p - an indicator of reliability of differences in comparison with the initial data; p1 - the indicator of reliability of differences in comparison with the com-parison group

However, in the comparison group in 6 months after the application of the lysozyme-containing rinse, the electrophoretic indices of BEC improved somewhat, but did not reach normal values in somatically healthy children. Later, the electrophoretic activity of the cells decreased, and their indices did not differ significantly from the initial values (p> 0.05).

At the same time, after a year of observations, the numerical values of the percentage of motile BEC nuclei in the children of the main group contin-ued to increase and were 29.3 % higher than the baseline data at the beginning of the TPC. In addition, a tendency was established to increase the displace-ment amplitudes of BEC both nuclei and plasmolemma and amplitude of their displacement increased, and at the end of the observation they were 27.6 % and 44.4 % higher than the initial values.

However, in the comparison group, where only the lysozyme rinsing agent was used, the electrophoretic activity of the cells was reduced, and their indices practically were equal to the initial values (p> 0.05).

Conclusions. Thus, based on the results of the studies made, we may suggest that the use of the TPC developed consisting of kvertsetin – containing mucosal gel, and its combination with gel containing a large amount of poly-phenols, flavonols, anthocyanins, and catechins, and mixtures of sanguinarine and chelerythrine bisulfate alkaloids with probiotic and antimycotic agents re-sults in normalization of energy processes in BEC, stabilization of the nuclear and membrane potentials in them and is

an indicator of adaptive and function-al reactions normalization, starting at the cellular level. This ultimately leads to increase of general and local nonspecific resistance in ALBL children in all groups. In this case, the most pronounced reaction to correction by the method proposed was established in the children with remission of the main disease.

References:

1. Rastogi D., Rather A.G., Prince A. Host-bacterial interactions in the initiations of inflammation. Paediatr Respir Rev 2001; 2: 245–252.

2. Svanborg C., Godaly G., Hedlund M. Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. Curr Opin Microbiol 1999; 2: 99–105.

3. Godaly G., Bergsten G., Hang L., Fiscer H., Frendeus B., Lundstedt A.-C., Samuelsson M., Samuelsson P., Svanborg C. Neutrophil re-cruitment, chemokine receptors, and resistance to mucosal infection. J. Leukoc Biol 2001; 69: 899–906.

4. Kagnoff M.F., Eckmann L. Epithelial cells as sensors for microbial infection. J Clin Ivest 1997; l00: 6–10.

5. Stadnyk A.W. Cytokine production by epithelial cells. FASEB J 1994; 8: 1041–1047.

6. Den'ga O.V. Method of evaluation of surface charge of plasma membranes of buccal epithelium cells in children./ Visnyk stomatologii'. 1997;3:449–451.