THE STUDYING OF BUTYRIC ACID INFLUENCE ON EPSTEIN–BARR VIRUS ACTIVATION IN LYMPHOID AND EPITHELIAL CANCER CELLS

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Abstract: Symbiotic microbiota system functions in homeostasis maintenance of organism are performed through production of multiple microbial low-molecular-weight compounds. Short-chain fatty acids (SCFAs) have a special and multifunctional role among similar compounds. The most important SCFA is a butyric acid which provides barrier and metabolic functions mainly in large intestine. Hyperacetylasion of histones due to histone deacetylase (HDAC) inhibition is one of the key mechanisms, by dint of which the butyric acid influences biologically the large intestine atypical cells. Butyric acid influence at the molecular level is studied insufficiently, in particular, even taking into account all positive effects, there is a danger of reactivation of latent infections which are in cells in their latent form and which may enter acute lytic phase when the transcriptional apparatus is activated. This article presents the study results of butyric acid mechanisms of influence on replication process of Epstein–Barr virus in cancer cells of different origin. It is demonstrated that under the butyric acid influence in epithelial and lymphoid cells transition from latent virus phase to lytic one takes place by means of BZLF–1 and BRLF–1 genes activation. Besides, the butyric acid inhibiting effect on epithelial nasopharynx cancer cells migration *in vitro* is demonstrated. Based on the obtained data, conclusions were made concerning practicability of butyric acid studying for further use as a functional product in the fight against cancer.

Keywords: Epstein–Barr virus, short-chain fatty acids, viral genes, virus reactivation, migration

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INTRODUCTION

It is generally recognized that gut microbiota has an enormous metabolic potential and plays an essential role in human health maintenance. Symbiotic microbiota system functions in homeostasis maintenance of organism are performed through production of multiple microbial low-molecular-weight compounds which are effectors, cofactors and/or signaling molecules which regulate speed and intensity of various physiological functions course, metabolic and behavioral reactions [1, 2]. Short-chain fatty acids (SCFAs) have a special and miscellaneous (multifunctional) role among similar compounds.

SCFAs are carboxylic acids which has less than 6 carbon atoms in their compound. In recent decades there has been an increasing academic interest in studying of the SCFA physiological effects within the norm, their role in different gastrointestinal tract (GI tract) diseases pathophysiology, as well as in design and appearance of drugs (metabiotics) based on large intestine microbiota metabolites [3, 4].

Nowadays, a large number of data are accumulated in scientific literature which show conclusively the most important SCFA functional role in regulation of various physico-biochemical processes at the GI tract level, as well as at the entire organism in whole. The most important SCFA is a butyric acid which provides barrier and metabolic functions mainly in large intestine [5, 6].

Butyrate plays an important role in normal colonocytes phenotype preservation by means of cell apoptosis regulation and intensification of DNA reparation processes [7, 8]. Thus, it was demonstrated that butyrate influences clearly gut cells aging *in vitro*, as well as *in vivo*, which is implemented through regulation of their proliferation and differentiation processes and normalization of cells correlation which are at proliferation or apoptosis stage [9, 10].

Hyperacetylasion of histones due to histone deacetylase (HDAC) inhibition is one of the key mechanisms, by dint of which the butyric acid influences biologically the large intestine atypical cells. This butyric acid quality compensates for imbalance in acetylation of histones which may lead to transcription dysregulation and inactivation of genes, controlling the number of cell division cycles, cell differentiation and apoptosis processes, as well as cancer pathological process [11, 12].

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Thus, regular regeneration of lower section mucous layer in the presence of butyrate allows to get rid of functionally dead coloncytes without dysfunction of the neighboring healthy cells and without occurrence of local inflammatory reactions. This is the most important mechanism of colonic mucosa tissue homeostasis maintenance because large intestine epithelium has a difference in the high speed of cell regeneration in comparison with other body tissues (a average coloncyte lifespan is 3 days), and an adequate energetic and plastic large intestine epithelium provision and proliferation and differentiation processes regulation are critical under these conditions [13, 14].

If we consider butyric acid as a therapeutic substance, its influence at the molecular level is studied insufficiently. In particular, even taking into account all positive effects, there is a danger of latent infections reactivation which are in cells in their latent form and which may enter acute lytic phase upon transcriptional apparatus activation [15].

One of the most widespread viruses is the Epstein-Barr virus (EBV). According to the data of most researchers, today approximately 80–90% of population are infected with EBV. EBV was firstly extracted from Burkitt's lymphoma cells 35 years ago. It soon became known that the virus can cause mononucleosis and nasopharyngeal carcinoma. Nowadays, it is established that EBV is associated with a number of cancer, mainly lymphoproliferative and autoimmune diseases (classic rheumatic diseases, vasculitis, nonspecific ulcerative colitis etc.) [16, 17].

There are two types of reproduction in cells, infected with this virus: lytic, that is leading to death, lysis of the host cell, and latent, when the number of viral copies are small and the cell is not destroyed. EBV can for a long time exist in B-lymphocytes and epitheliocytes of nasopharyngeal area and salivary glands. In acute or active infection lytic viral replication dominates [18, 19]. EBV proteins, synthesized at the early lytic infection stage, called early antigen complex, are expressed prior to DNA synthesis beginning, some of these proteins are associated with DNA replication. But the products of the late infection lytic stage are expressed after the DNA synthesis beginning and contain structural proteins, included in the virion compound, in their compound. The latter pass through the cytoplasmic membrane, becoming mature viral particles which can embed in not yet infected cells [20, 21].

The lytic EBV reproduction starts with viral genes expression: BZLF–1 and BRLF–1 which activate the early viral genes. In a state of latent reproduction only a small number of genes, responsible for the viral genome replication, remain in an active state. Some of the active genes influence different molecular and cell cascades, in particular, the signal activity through the transcription factor NF-kB which is of particular importance for latency maintenance. A state of latency is reversible, mechanisms, causing viral genome transcription reactivation, are studied insufficiently [22, 23].

The choice of the target gene was determined by the necessity to capture the moment between transitions from latent stage to lytic. Thus, at the lytic phase beginning the virus starts to produce a cascade of new proteins for the assembly of capsids and formation of active viral particles in cell. The proteins of immediate reaction BZLF1 (ZTA) and BRLF1 (RTA) play a substantial role in this process. EBV genome epigenetic reaction is the main regulation mechanism in determining different types of EBV infection in tumors, associated with it [24].

EBV produces up to 20 micro RNA in latently infected cells. They are located in two clusters: 14 micro RNA are located in the introns of the BARTA viral gene. BARTA micro RNA are expressed at a high level in latently infected epithelial cells, and at lower, though detected, levels - in B-cells. In other words, the BARTA gene expression is an indicator for the fact that the cells under studying really contain viral particles [25, 26].

However, despite the seeming negativity of the virus lytic replication phenomenon, this quality can be used for good when one deals with cancer cells, because viruses are able not only to destroy cancer cells physically, tearing them apart, but also to deliver genes into cells, increasing their sensitivity to usual chemotherapeutic drugs. Tumor-specific viral particles can be marked either with fluorescent dyes, or with radioisotopes. When they enter the body, they connect with tumor cells, making them detec Table [27, 28].

The objective of the article was to study the mechanisms of the butyric acid influence on virus replication processes in cancer cells of different origin. BZLF–1 and BRLF–1 genes were taken for studying which are activated in case of acute lytic infection, starting the process of viral particles collection. Primers to BARTA gene were used to prove the existence of the viral genome in cell, from this gene the virus RNA are transcribed at all kinds of phases.

In addition, in this article issues, concerning cancer cells migration in culture *in vitro*, were studied because metastasis is one of the main problems when treating cancer. Metastasis is secondary growth foci of any malignant neoplasms. The basic cancer diseases result in formation of such foci in regional and local lymph nodes, lungs, liver, backbone etc. Cancer cells migration from the primary localization to the remote area is a difficult biological process which includes changes at the molecular, cell and physical level [29]. Based on the obtained data, one can make a conclusion concerning practicability of butyric acid studying for further use as a functional product in the fight against cancer.

OBJECTS AND METHODS OF STUDY

The subject matters of the study in this article were cell lines of different origin. The epithelial cell lines C666–1 and CNE2 were derived from undifferentiated nasopharyngeal carcinoma of South China origin. Growth regime is the attached monolayer. Cells are relatively big in diameter (12–15 μm), they have an irregular nucleus shift and nearly vast cytoplasm. C666–1 grow in clusters, CNE2 grow in even monolayer. Cells have an elongated shape, capable of taking an amoeboid

appearance while moving. C666–1 cells are Epstein-Barr virus carriers in the long-run regime. The cell line CNE2 is the carrier of the artificially embedded plasmid of the viral membrane protein LMP2A, responsible for the primary cancer cells migration mechanisms which arise from Epstein-Barr virus, contained in them. It allows to use this cell line for studying the EBV-induced migration processes *in vitro* [30].

Raji is immortilized limphoid cells which also produce the Epstein-Barr virus. This cell line originates from the B-lymphocytes of the 11–year-old Nigerian male patient from Burkitt's lymphoma. Cells are relatively big in diameter (5–8 μm); they have an irregular nucleus shift and nearly vast cytoplasm with free ribosomes which have a tendency for grouping. Generally, cells grow in clusters in suspension. Some cells have an elongated pear-shaped form, others have a round shape and are multinuclear.

AGS is a cell line which originates from the cells of adenocarcinoma of the stomach of the 54–year-old woman who was living in the Caucasus without the prior treatment for cancer. It is the hyperploid human cell line. The modal chromosome number is 49 in 60% of cells. For the experiment the cell line was artificially infected with EBV. Growth regime is the attached monolayer. The cells are relatively big in diameter (10–15 μm) and have an irregular nucleus shift.

Cell cultivation and reagent treatment

Cell culture cultivation took place under the conditions of CO2-incubator in the atmosphere of 5% CO2, 100% humidity and at a temperature of 37°C. For lymphoid cells the medium RPMI 1640 Media was used with addition of Defined Fetal Bovine Serum (FBS) to a concentration of 10%, L-Glutamine to a concentration of 5% and Penicillin-Streptomycin 100X Solution to a one-fold volume. The IMDM epithelial cell medium was prepared in the same manner except FBS, concentration of which was 15%. All the used reagents for cell cultivation were produced by the companies HyClone™, Thermo Scientific™.

C666-1 and CNE2 epithelial culture cell growth was conducted in 20 ml of the IMDM medium with a concentration of $0.8x10^6$/ml. Raji lymphoid culture cell growth was conducted in 20 ml of the RPMI medium with a concentration of $0.3x10^6$/ml.

The working acid solution preparation was conducted by means of dilution with the IMDM and RPMI medium to a concentration of 10 mM in case of butyric (Merck Kebo, Germany) and hydrochloric (Sigma-Aldrich, USA) acids. 12–O–Tetradecanoylphorbol–13–acetate (Sigma-Aldrich, USA) (TPA) was used at a concentration of 0.002% and was also diluted in mediums for cell line growth. Epithelial cells were transplanted on the 12-hole Tablet in the amount of $1x10^6$/hole. In the experiment TPA was used as a positive control, being a widely known lytic virus replication inducer. When C666–1 was used, after seeding one waited 24 hours for the adhesive cell attachment. Then the medium was replaced by a new one with the acid solution in the volume of 1 ml/ hole. When treating the Raji lines acid treatment was conducted right after the seeding in the amount of 2 ml of the medium/hole. After 24 hours the cells were collected for total RNA separation.

Total RNA separation

To separate RNA the method according to the RNeasy Mini Kit Protocol (Qiagen, USA) was used with the use of this set of reagents. RNA quality and quantity was checked on the device NanoDrop 2000c UV-Vis Spectrophotometer. Then the total RNA pharmaceutical was treated with DNase I RNase-free (Thermo scientific, Sweden) at a concentration of 1U/mkL to remove the genomic DNA admixture.

Reverse transcription polymerase chain reaction

As a tool for execution of this item of study the kit RevertAid First Strand cDNA Synthesis Kit (Thermo scientific, EU) was used, the reaction was conducted according to the company protocol with the use of random hexamer primers. In RT-PCR reaction 1 μg of total RNA was selected for each sample. Test tubes with the ready mixture were placed in thermal cycler "PTC–200 peltier thermal cycler" with primer annealing regime of 25°C during 5 minutes, elongation of 42°C during 60 minutes and reaction inhibition of 70°C during 5 minutes.

PCR with the use of DNA was conducted in the solution, prepared on the basis of ten-fold buffer solution for Taq DNA Polymerase, Recombinant (Thermo scientific, Sweden) with primer concentration of 0.8 mM and magnesium ion concentration of 2 mM. All other components were taken according to the protocol to the PCR conduction kit. Test tubes with the ready mixture were placed in thermal cycler "PTC-200 peltier thermal cycler" with denaturation regime of 95°C during 20 seconds, primer annealing regime of 58°C during 15 seconds and elongation of 72°C during 20 seconds with the total number of cycles 36.

The chosen primers were selected with the use of NCBI database of the Blast programme and ordered from the company Invitrogen™ Life Technologies and are represented in Table 1.

Table 1. Primer parameters for PCR

Target gene name	Molecular mass of expected product	Nucleotide sequence of selected primers
BZLF1	255–bp	FW GGGAGAAGCACCTCAACCTG RV TTGCTTAAACTTGGCCCGGC
BRLF1	443–bp	FW CAAACAGACGCAGATGAGGC RV GCGGTGCCTATGGTGGCAGG
BARTA	232–bp	FW AGAGACCAGGCTGCTAAACA RV AACCAGCTTTCCTTTCCGAG
GAPDH	303–bp	FW GATGACCTTGCCCACAGCCT RV ATCTCTGCCCCCTCTGCTGA

For quantitative balancing of the results the primers for Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used. GAPDH is one of the most important glycolytic enzymes because it conducts glycolitic oxidoreduction reaction, which results in high-energy compound formation – 1,3-diphosphoglycerat - and NADH. Is expressed in cells constantly, due to this it marks that the PCR reaction was successful, and also is a mathematical control, from the amount of which the results of the target gene expression are counted.

The product detection was conducted in 1% agarose gel (Bionordika, Sweden) with addition of ethidium bromide (Sigma-Aldrich, USA) in one-fold buffer solution TAE. For this purpose, 15 mkL of thermal cycling reaction products were used, mixed with 5 mkL of the weight substance. Markers were used on 1 kb (Fermentas, USA). The reaction was conducted at a voltage of 11 V/cm. The results were detected in gel-documentation ChemiDoc™ XRS+ with the use of the application Image J.

Cell migration studying

The present study included the measurement of cells mobility by dint of the method of experimental wound or scratch healing, which were made in the monolayer. For this method adhesive cell cultures are needed which form the level monolayer on the Tablet surface. In our case the cell line CNE2 was used. The cell culture in the amount of 1 mln was placed on a standard 6-hole Tablet. After 24 hours a scratch was made and the components under studying were added. After 10 hours cells were dyed with an aqueous solution of crystal violet and the results were detected in the application Image Lab™ Software 170–82657.

Western blot analysis

The collected cells were lysed in buffer solution RIPA, which contain THAM base of 50 mM, 150 mM NaCl, 0.1% of SDS, 0.5% of deoxycholic acid, 1% of Triton X100, 5% of protease inhibitors. The buffer solution was used in the amount of 100 mkL per one sample, then the samples were centrifuged at 13000 rpm and supernatant was selected. Then the samples were analyzed by dint of electrophoretic method in 4-10% of polyacrylamide gel at a voltage of 140 V in electrophoretic cell Mini-PROTEAN® Tetra Vertical Electrophoresis Cell (Bio-Rad, USA). 15 µg of protein of each sample were applied to track, the concentration of which was measured in advance according to the Bradford method, as well as 2 mkL of 200 kDa marker (Fermentas, USA). Then the electrophoresis products were transferred by dint of dry transfer to nitrocellulose membrane at a voltage of 17 V during 40 minutes.

The nitrocellulose membrane with proteins was incubated the whole night at a temperature of +4ºC in primary antibodies EBV ZEBRA Antibody (BZ1): sc-53904 (Santa Cruz Biotechnology, USA), diluted in blocking buffer solution in the ratio of 1 to 100 which has in its compound 5% milk, diluted with PBS with addition of tween 20 to a concentration of 0.1%. Then the excessive antibodies were washed in the wash buffer solution which is a PBS with addition of tween 20 to a concentration of 0.1% during 30 minutes. Then the membrane was incubated with secondary antibodies Goat Anti-Mouse IgG (H+L)-HRP (BioRad, USA) and diluted in the blocking buffer solution in the ratio of 1 to 1000 at a room temperature during one hour, after that the membrane was washed in the same manner and detected with the use of the system Gel Doc™ XR+ Imager (Bio-Rad, USA). Then the membrane was incubated with antibodies β-Actin Antibody (C4): sc-47778 (Santa Cruz Biotechnology, USA) in dilution in the blocking buffer solution in the ratio of 1 to 2000 at a room temperature during 2 hours.

RESULTS AND DISCUSSION

RT-PCR is a method of thermal cycling of ribonucleic acid (RNA) specific fragment. A single-stranded RNA molecule is converted into the complementary DNA (cDNA) in the reverse transcription reaction and then already the single-stranded DNA molecule is amplificated with the use of traditional PCR. For this purpose the reverse transcriptase (RT) enzyme is used. After the thermal cycling cycles millions of copies of the necessary sequence are formed. In the experiment RT-PCR is used as an indicator of the target gene expression.

Fig. 1 shows the results of thermal cycling of fragments in the Raji cells system.

In comparison with control cells, Raji cells, treated with butyric acid, demonstrated the significant increase in BZLF1, BRLF1 lytic genes expression in relation to GAPDH gene and BARTA gene (Fig. 2). Moreover, this pattern is not associated with stress, experienced by the cells in connection with the foreign components presence, because the this pattern is not linked to a pH decrease (Table 2). One more proof of this fact is that in cells, treated with hydrochlorhydric acid, we did not observe the induction of the early lytic genes expression.

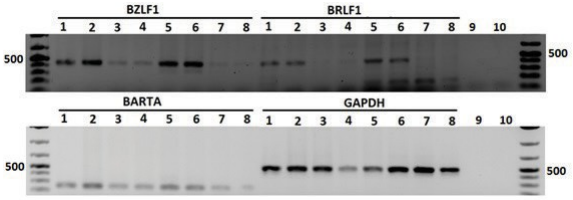

Fig. 1. Thermal cycling products of Epstein-Barr virus early lytic genes in the Raji cell line: 1–2 – 10 mM butyric acid, 3–4 – 10 mM hydrochlorhydric acid, 5–6 – TPA, 7–8 - control, 9–10 – PCR-control.

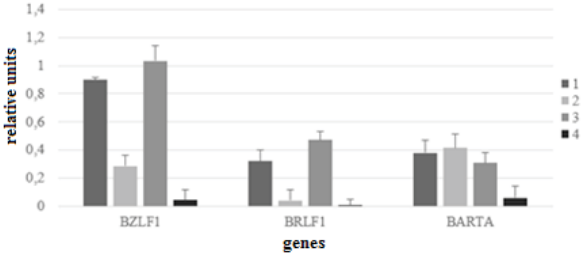

Fig. 2. Results of Epstein-Barr virus early lytic genes thermal cycling in relation to GAPDH in the Raji cell line: 1 – 10 mM butyric acid, 2 – 10 mM hydrochlorhydric acid, 3 – TPA, 4 – control.

Similar pattern was in epithelial cells (Fig. 3–6) where butyric acid was an active inducer of the lytic gene cascade activation start. Thus, 10 mM butyric acid inducecc the lytic replication in cancer cells by means of «silent» genetic sites activation.

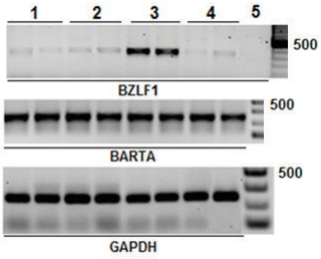

Fig. 3. Thermal cycling products of Epstein-Barr virus early lytic genes in the cell line C666–1: 1 – control, 2 – 10 mM hydrochlorhydric acid, 3 – 10 mM butyric acid, 4 – TPA, 5 – PCR-control.

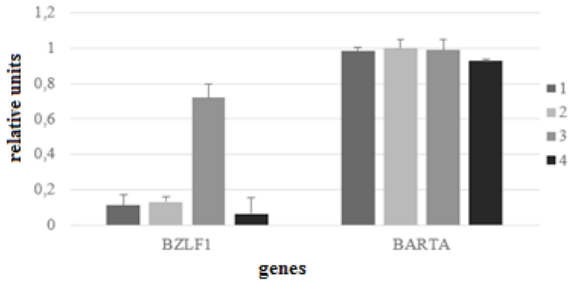

Fig. 4. Results of Epstein-Barr virus early lytic genes thermal cycling in relation to GAPDH in the cell line C666–1: 1 – control, 2 – 10 mM hydrochlorhydric acid, 3 – 10 mM butyric acid, 4 – TPA.

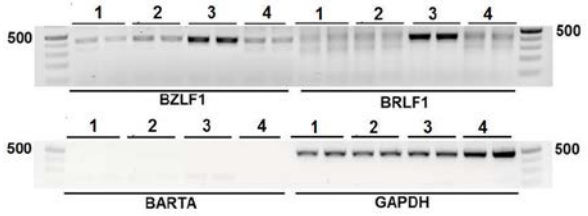

Fig. 5. Thermal cycling products of Epstein-Barr virus early lytic genes in the AGS cell line: 1 – control, 2 – 10 mM hydrochlorhydric acid, 3 – 10 mM butyric acid, 4 – TPA.

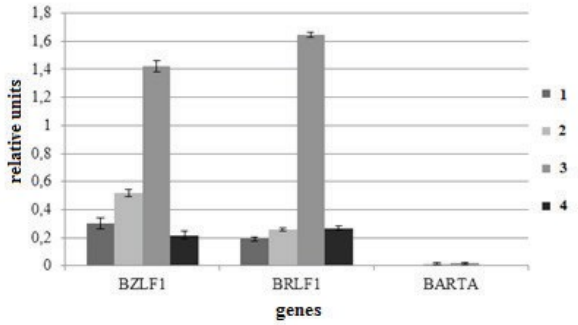

Fig. 6. Results of Epstein-Barr virus early lytic genes thermal cycling in relation to GAPDH in the AGS cell line: 1 – control, 2 – 10 mM hydrochlorhydric acid, 2 – 10 mM butyric acid, 4 – TPA.

According to the set of experiments butyric acid initiates at the level of RNA the cascade of the EBV virus lytic infection in cells. In addition, the obtained results were proved at the protein level by dint of Western blot analysis in the cell lines under studying (Fig. 7).

The obtained results testify to the absence of positive signals in control cells in comparison with cells, treated with butyric acid. These results attest to the ones, obtained during the RNA analysis, and if earlier we were dealing with weak confirmation signals of early lytic genes activity in cell, then the present results testify to the start of infection lytic stage course which leads to the collection of viral particles, causing cell death.

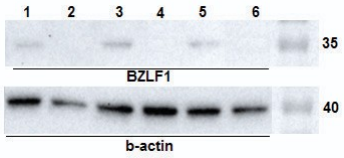

Fig. 7. 1 – Raji, treated with 10 mM butyric acid, 2 – Raji, treated with 10 mM hydrochlorhydric acid, 3 – C666–1, treated with 10 mM butyric acid, 4 – C666–1, treated with 10 mM hydrochlorhydric acid, 5 – AGS, treated with 10 mM butyric acid, 6 – AGS, treated with 10 mM hydrochlorhydric acid.

Lytic autonomous infection causes cell death. Multiplying in cell, the virus induces synthesis of virus-specific proteins, capable to one extent or another of suppressing cell metabolism. During the reproduction cycle viral components are accumulated in cell which have a damaging effect on cell structure. In the lysosome damage process the contents are freed and cell autolysis is conducted [31].

Lytic autonomous infection can be activated by several reasons, one of which is medium conditions deterioration, wherein the immediate viral particles collection begins [32]. To decrease the probability of influence of this factor on the study results, we measured pH in mediums with the components under studying. The obtained results are represented in Table 2.

Table 2. pH measurement in mediums with the components under studying

SCFA	Raji		C666–1		AGS	
	0 h	24 h	0 h	24 h	0 h	24 h
Medium	8.0	7.78	7.8	7.5	7.8	7.6
Hydrochlorhydric acid 10 mM	6.5	7.2	6.0	7.4	6.0	7.5
Butyric acid 10 mM	6.5	7.6	6.1	7.7	6.0	7.5
TPA	8.0	8.0	7.8	7.7	7.8	7.3

In contrast to epithelial cells, blood cells in vivo have a more consistent life medium with the result that medium pH should be varied moderately, estimating the experiment effect life Optimum for normal lymphoid cells is 7.35–7.40 [33], but for cancer Raji cells it is lower and is 6.5–7.0 [34]. It is due to the peculiarity of tumor processes development in organism, wherein glycolysis exchange is shifted towards increase in lactic acid production [35].

Acidity in nasopharynx is 5.5–6.5 and this makes C666-1 cells resistant to acidic medium [36]. AGS cell line is even more resistant because the normal acidity in gastric antrum varies widely between 1.3–7.4 pH [37]. Consequently, medium acidification factor during experiments does not influence the viral infection induction.

We studied butyric acid influence on cell migration on the basis of CNE2 cell line. It was impossible to use C666–1 line because this cell culture does not form the level monolayer and grows in clusters, and it complicates significantly the interpretation of the obtained results. The results are represented in Fig. 8, which demonstrates the difference in cell migration

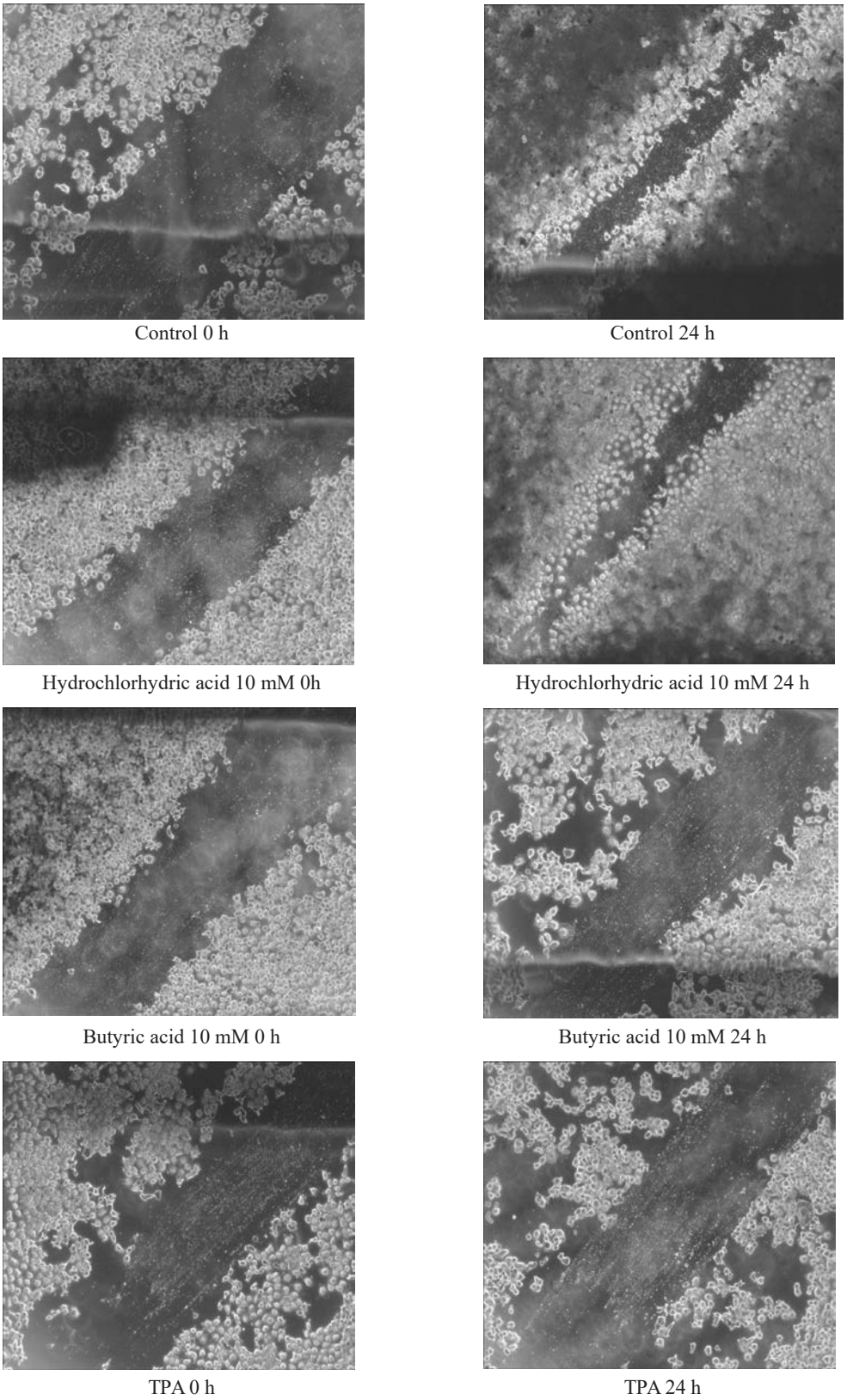

Fig. 8. Photos of dyed CNE2 cells under the microscope.

after 24 hours in control cells and cells, treated with hydrochlorhydric acid. In cell cultures, treated with butyric acid and TPA, "scratch healing" was not observed visually.

After the application measurement of the scratch square (Fig. 9), the obtained data testified to the fact that butyric acid and TPA reduce the cell ability to migrate nearly twice in comparison with hydrochlorhydric acid and the control.

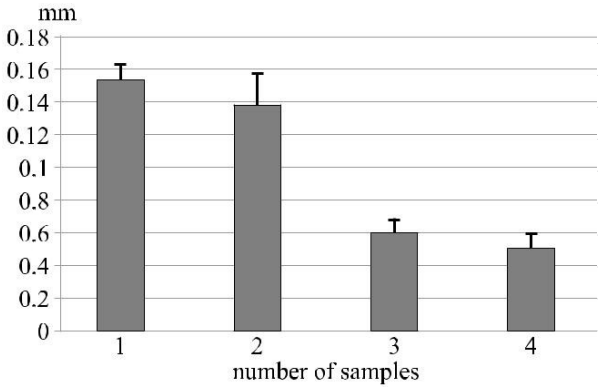

Fig. 9. Cell migration measurement (scratch size): 1 – 10 mM butyric acid, 2 – TPA, 3 – 10 mM hydrochlorhydric acid, 4 – control.

The molecular metastasis mechanisms are being studied. Other issues should be noted: during multiplication cells produce specific growth factors which stimulate capillary and vascular networks formation around neoplasm cells, an this, in turn, allows tumor to get all nutrients, necessary for existence [38]. Frequently, time, required for first metastatic cells manifestation, is determined by the neoplasm type.

CONCLUSIONS

Analyzing RNA, extracted from the samples, in comparison with control, cells, treated with butyric acid, had a significant more than triple increase in BZLF1 and BRLF1 lytic genes expression in relation to GAPDH gene and BARTA gene. Moreover, this effect was not observed in case of hydrochlorhydric acid, which indicates the effect of precisely butyric acid as the histoneacetylase inhibitor. This conclusion was proved at the protein level by dint of Western blot analysis and the results appeared to be similar in all cell lines. Thus, we should consider the idea of butyric acid use in cancer diseases fight with caution because, despite the fact that butyric acid produces virus lytic replication in cells, destroying them, on the other hand, a large number of viral particles are freed in this process which can infect healthy cells, causing inflammation and various complications.

Lytic autonomous infection can be activated by several reasons, one of which is medium conditions deterioration, wherein the immediate viral particles collection begins. To decrease the probability of influence of this factor on the study results, we measured pH in mediums with the components under studying. The range of measured values was from 6.0 to 8.0 and is nearly optimal for growth and cell lines development under studying. Consequently, medium acidification factor did not influence the viral infection induction.

It was demonstrated that butyric acid inhibits cell migration *in vitro*. The obtained data testify to the fact that butyric acid and TPAreduce the cell ability to migrate nearly twice in comparison with hydrochlorhydric acid and the control. This quality can be used for fight against the main cancer problem - metastasis.

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