Original Article

Changes in the Correlation Between Peripheral Blood Cells and Membrane Charge in Brain Gliomas and Meningiomas

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Abstract

Mechanism of tumor progression in malignant gliomas and other tumors of the body were studied to identify the main pathogenetic link. Indicators of the charge of cell membranes, including the membranes of blood cells, can be considered in vein. These indicators are universal for any type of tumors, and not tissue-specific. Indicators of many significant processes in the body depend on the level of cellular charge, which emphasizes its dominant role. Interaction of reparative processes of inflammatory genesis with regenerative processes carried out by blood stem cell in relevant studies and in this study by mesenchymal blood stem cells. The interaction is under the control of the epidermal-mesenchymal transition depends on a large extent on the cellular charge of blood cell membranes. The paper presents the correlation features between the pools of cells of inflammatory and regenerative origin (leukocytes, lymphocytes, granulocytes, and monocytes), the charges of their membranes in gliomas of III grade of malignancy and meningiomas of I grade of malignancy studied *in vitro*. Membrane charges were determined indirectly, through the level of aggregation of blood cells, using the surface plasmon resonance method where the aggregation expressed in arbitrary SPR units. To detect a latent correlation, low concentrations of verapamil hydrochloride (10,000-fold dilution) were added to the blood samples before determining the level of cell aggregation and the samples were exposed to low-level laser radiation with an oscillation frequency of 1.2 Hz. Results indicate the great importance of maintaining a normal, rather than reduced, level of cell membrane charge.

Keywords: Gliomas, Meningiomas, Blood cells, Verapamil hydrochloride

Introduction

In previous studies [1, 2], a hypothesis was put forward about the relationship between the composition of blood cells and the charge of their membranes in patients with glioblastomas. This phenomenon is of considerable interest in connection with the transition of reparative processes in body tissues into regenerative processes, which is observed in malignant tumors of any genesis, characterized by necrotic cell death [3, 4]. A large number of works study the interaction between pools of blood cells without taking into account their charge characteristics [5-8].

The role of the chronic inflammatory process in the development and progression of malignant tumors is beyond doubt [9-12], while the significance of the transition of repair mechanisms to regeneration involving stem cells of mesenchymal origin from the bone marrow has not been extensively studied in case of brain gliomas.

In this work we utilized the effect of low concentrations of verapamil hydrochloride and electromagnetic oscillations of low-level laser radiation (LLLR) with a frequency of 1.2 Hz on the change in the level of aggregation of blood cells taken from patients with gliomas and meningiomas before surgical treatment and on day 7 after surgery under *in vitro* conditions. Laser radiation with an oscillation frequency of 1.2 Hz is used in medical institutions with a positive effect on the rehabilitation of patients [13].

Then we studied the correlation between changes in the level of aggregation of blood cells without exposure and under the

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influence of a 0.25% solution of verapamil hydrochloride and LLLR, and the cellular composition of blood in the same samples. The blood composition in each sample remained unchanged, and the charge of blood cell membranes and their correlation with the quantitative composition of blood cells were analyzed.

The work aimed to study the change in the correlation coefficient between the blood cells quantities and their level of aggregation, affected by the influence of verapamil hydrochloride diluted with water by a factor of 1:10.000 and LLLR with a frequency of 1.2 Hz in patients with grade III of gliomas and meningiomas with I grade of malignancy before and after surgery.

Materials and Methods

The object of the study was the peripheral blood cells taken from patients during the neurosurgical operations for the removal of tumors (in compliance with the norms of the Committee on Bioethics). A survey of 11 patients undergoing a course of complex treatment at the A. P. Romodanov Neurosurgery Institute of National Academy was performed to obtain the indicators of the degree of aggregation of blood cells before the start of therapeutic and surgical interventions, as well as on the 7th day after the operation. As a control, a similar blood sampling was performed in healthy donors (12 people) who underwent a clinical examination at the blood transfusion point. The study groups were formed subject to the condition of the absence of concomitant inflammatory diseases. For instance, the common clinical blood tests (ESR, the number of erythrocytes and other cellular elements of the blood) showed the absence of a pronounced inflammatory process in patients with gliomas of III grade of malignancy and meningiomas of I grade of malignancy. Patients accompanied by changes in the processes of blood cells aggregation (hypoproteinemia, autoimmune diseases, and diseases requiring the use of salicylates) were not included in the study groups.

Heparinized venous blood samples taken from patients with gliomas and meningiomas before the start of treatment and on the 7th day after the operation, were separated by centrifugation (1500 rpm for 10 minutes) into blood cells and plasma. The cell fraction was used to determine the surface plasmon resonance (SPR) angle shift in degrees, which reflects the degree of aggregation of blood cells. The study of the initial processes of blood cell aggregation has become possible with the opening of a new direction in biosensorics, using the SPR effect [14, 15], which does not require a radioactive or fluorescent label. The SPR phenomenon consists of the resonant excitation of surface plasmons at the dielectric-metal (gold or silver) interface at a certain angle of incidence of a TM-polarized electromagnetic (laser) wave on this interface. The value of the resonant angle of incidence is sensitive to the refractive index of the external medium, which is employed in this sensor. The unit of measurement is considered to be the SPR shift in degrees, which depends not only on the number of cells deposited on a glass plate coated with a thin layer of gold, but also on the total area of the membranes of these cells directly interacting with the plasmon. A plasmon is a cloud of free electrons generated by the action of a laser beam passing through a glass prism on a thin layer of gold. As is known, during agglutination or aggregation of blood cells, the ratio of the surface of cell particles to their volume decreases, while the total number of cells, compared to the control, may be greater, and the area of contact with the gold plate may be smaller, which leads to a decrease in the shift in the SPR readings, measured in degrees. The study of SPR parameters with blood cells in healthy individuals and patients with neurosurgical pathology was carried out using a PLASMON SPR spectrometer (Biosuplar) with an optical excitation source (GaAs laser, λ=650 nm), developed at the Institute of Semiconductor Physics of National Academy of Sciences of Ukraine. Glass plates (n=1.612) with a layer of gold (45-50 nm) deposited onto an intermediate adhesive layer of chromium (~ 5 nm) were fixed on a retroreflecting glass prism (n = 1.612) of the spectrometer using an immersion liquid (polyphenyl ether, n = 1.612). In the course of work with blood cells, plates with a deposited layer of gold were reused after repeated washing with ion-free water. The interaction of the gold layer with the charge of blood cell membranes leads to a shift in the plasmon resonance curve (SPR shift in degrees), which was measured and processed by the device with the BSS55 software package, with the results displayed in graphical form on a computer monitor [14].

Before the studies, the instrument micro cuvettes were washed with ion-free water. The supply of the cell fraction, with a volume of about 400 μl, was carried out using an electric pump at a rate of 100 μl per minute. At the end of the study, blood cells were washed off the substrate with a large volume of water, while monitoring the return of the original curve on the display screen to its original position.

To reduce the level of aggregation of blood cells, a technique was developed under *in vitro* conditions using a blocker of NMDA-dependent Ca^{2+} ion channels, e. g. verapamil, which can change the indicators of transmembrane potential on the membranes of blood cells, mediated in our studies by their level of aggregation.

It is known that under physiological conditions, NMDA receptors are activated by millimolar concentrations of glutamate, which is present in the synaptic cleft for several milliseconds. During pathological impulses, receptors are activated by micromolar concentrations, but for a much longer time [16]. We took the same pattern as a basis concerning the effects of milli- and micromolar concentrations of verapamil hydrochloride to select its optimal concentrations to change the level of blood cell aggregation.

In the control blood samples, an equal volume of ion-free water was used for dilution instead of verapamil hydrochloride.

Blood samples were irradiated with a laser generating electromagnetic radiation with a pulse frequency of 1.2 Hz in Petri dishes for 5 minutes each. Then, in these samples, the level of aggregation of blood cells was determined individually, measured in SPR angular units.

The number of blood cells was determined in the same blood samples using an automatic hematological analyzer Mindray-3000.

Monocytes were detected in the pool of Mid cells of the peripheral bloodstream.

Statistical studies were performed using the "Statistics-10v" package.

Method for Determining the Correlation Coefficient

Spearman's rank correlation coefficient is a nonparametric method that is used to statistically study the relationship between phenomena. In this case, the actual degree of parallelism between the two quantitative series of the studied features is determined and an assessment of the tightness of the established relationship is given using a quantitatively expressed coefficient.

To assess the tightness of interrelation, the Chaddock scale **(Table 1)** can be used [17]:

Table 1. The Chaddock scale

Absolute value r_{xy}	The tightness (strength) of the correlation
less than 0.3	weak
from 0.3 before to 0.5	moderate
from 0.5 before 0.7	noticeable
from 0.7 before 0.9	high
more than 0.9	very high

The statistical significance of the obtained coefficient is assessed using the Student's t-test. If the calculated value of the t-test is less than the tabular value for a given number of degrees of freedom, the statistical significance of the observed relationship is absent. Otherwise, the correlation is considered statistically significant.

Results and Discussion

We studied the change in correlation coefficient between cellular charge under the influence of low concentrations of verapamil hydrochloride and LLLR with an oscillation frequency of 1.2 Hz and cellular content in 12 blood samples from 6 patients (47±2.9 years old) with gliomas of III grade of malignancy and 5 patients with meningiomas of I grade of malignancy before and after treatment. The control group for patients with gliomas consisted of 12 practically healthy individuals (48±5.5 years).

Table 2. Correlation coefficients between the number of peripheral blood cells and indicators of their aggregation in 12 practically healthy individuals.

	SPR Cells number	WBC	Lym	Mid	Gran
		6,109±1,242	2,2634±0,560	0,854±0,521	3,3090±0,979
Blood +H2O	1,634±0,424	-0,25968	-0,23207	-0,18887	0,18182
Blood+Ver. in 1/10000 dilution	1,545±0,304	-0,27335	-0,18101	0,11432	0,06364
Blood+H_2O+LLLR	1,670±0,609	-0,25968	0,1346	-0,17396	-0,1
Blood+Ver. In 1/10000 dilution +LLLR	1,613±0,570	-0,31435	0,2135	-0,47218	-0,26364

Table 3. Correlation coefficients between the number of peripheral blood cells and indicators of their aggregation in 6 patients with grade III glioma before surgery.

	SPR Cells number	WBC	Lym	Mid	Gran
		12,95±1,529	2,616±0,825	1,383±0,421	8,95±1,089
Blood +H_2O	1,032±0,104	-0.65714	-0.31429	0.14494	-0.31429
Blood+Ver. in 1/10000 dilution	1,078±0,124	-0.88571	-0.31429	-0.05798	-0.65714
Blood+H_2O+LLLR	0,967±0,156	-0.48571	-0.08571	-0.20292	-0.14286
Blood+Ver. In 1/10000 dilution +LLLR	0,976±0,233	-0.2	0.6	-0.63775	-0.42857

Table 4. Correlation coefficients between the number of peripheral blood cells and indicators of their aggregation in 6 patients with grade III glioma after surgery (7 days).

	SPR Cells number	WBC	Lym	Mid	Gran
		14,66±3,550	3,383±0,571	3,4833±0,257	7,8±2,003
Blood +H2O	1,249±0,3553	0.82857	-0.63775	0.78269	-0.08571
Blood+Ver. in 1/10000 dilution	1,325±0,4551	0.71429	-0.37685	0.66674	-0.25714
Blood+H2O+LLLR	1,346±0,5601	0.88571	-0.60876	0.92763	-0.14286
Blood+Ver. In 1/10000 dilution +LLLR	1,292±0,4001	0.82857	-0.43483	0.81168	-0.2

Table 5. Correlation coefficients between the number of peripheral blood cells and indicators of their aggregation in 5 patients with meningioma I grade of malignancy before surgery.

	SPR Cells number	WBC	Lym	Mid	Gran
		12,95±1,529	2,616±0,825	1,383±0,421	8,95±1,089
Blood +H2O	1.386± 0.5364	0.7	0.2	0.2	0.6
Blood+Ver. in 1/10000 dilution	1.621± 0.5426	0.6	-0.1	0.9*	0.8
Blood+H2O+LLLR	1.397± 0.4099	0.5	-0.2	0.7	0.6
Blood+Ver. In 1/10000 dilution +LLLR	1.668± 0.5484	0.6	-0.1	0.9*	0.8

In the group of healthy individuals, no significant correlation was found between SPR readings and blood cells. On the 7th day after the operation, the number of cells, except for granulocytes, increases: 2.5 times the number of monocytes, 1.3 - lymphocytes, 1.1 times - leukocytes, and the number of granulocytes decreases by 1.1 times **(Table 2)**.

With grade III glioma before surgery, a significant negative correlation was observed between leukocytes and SPR readings in blood samples with the addition of verapamil hydrochloride diluted by a factor of 10.000 **(Table 3)**.

With grade III glioma on the 7th day after the operation, a significant positive correlation was found between leukocytes in control blood samples and after exposure to LLLR and verapamil hydrochloride in combination with LLLR. A positive correlation is observed between monocytes (Mid) and SPR readings in the blood samples exposed to low concentrations of verapamil hydrochloride (1:10,000) and in combination with LLLR **(Table 4)**.

Table 6. Correlation coefficients between the number of peripheral blood cells and indicators of their aggregation in 5 patients with meningioma I grade of malignancy after surgery.

	SPR Cells number	WBC	Lym	Mid	Gran
		12,95±1,529	2,616±0,825	1,383±0,421	8,95±1,089
Blood +H2O	1.428± 0.5324	-0.82	-0.9*	0.1	-0.7
Blood+Ver. in 1/10000 dilution	1.380± 0.5168	-0.82	-0.9*	-0.1	-0.7
Blood+H2O+LLLR	1.368± 0.7506	-0.82	-0.9*	0.1	-0.7
Blood+Ver. In 1/10000 dilution +LLLR	1.437± 0.69411	-0.667	-1	0.3	-0.5

In blood samples taken from patients with meningiomas before surgery, a significant correlation was found between blood cells and SPR values obtained with the addition of verapamil hydrochloride, as well as with the combined action of verapamil and radiation. After surgery, the samples showed a significant correlation between the number of lymphocytes and SPR indicators apart from the joint impact of verapamil and LLLR **(Table 5)**.

The research results show that there are differences in the correlation between the indicated indicators in gliomas and meningiomas **(Tables 4 and 6)**. Meningiomas are benign tumors of the meninges, while gliomas have malignant growth characteristics.

It is known that the cellular pool of monocytes contains mesenchymal stem cells, which are involved in the processes

of regeneration of damaged body tissues [18, 19]. With tumor progression, stem cells contribute to the growth of tumor tissue. In a healthy organism, the functioning of mesenchymal stem cells is controlled by the epithelial-mesenchymal transition (EMT) [3, 4]. Reparative processes in the body are carried out mainly in stage III of the inflammatory process. When inflammation passes into the chronic stage and repair is not fully carried out, EMT promotes the activation of the functions of mesenchymal stem cells (MSCs) for the implementation of regenerative processes in the lesion. During the tumor development process, MSCs carry a damaged genome [20], which does not allow full regeneration. As a result of the pathological functioning of MSCs, uncontrolled growth of tumor tissue occurs without signs of cytoarchitectonics.

The results of this work indicate that with gliomas III grade of malignancy there is a significant positive correlation of the blood cells aggregation indicators to a large extent with monocytes (Mid - cells) and leukocytes, and to a lesser extent with lymphocytes and granulocytes. The number of monocytes increases after surgery the most compared to other cell fractions. This is explained by the fact that in the tumor focus, along with the regeneration process, which mainly involves monocytes and leukocytes, the processes of repair of inflammatory genesis with the participation of lymphocytes, granulocytes, and other blood cells of inflammatory genesis are also involved. Factors of secondary alteration (stage I of inflammation) prevent the passage of the normal process of regeneration and disruption of the cytoarchitectonics of the regenerated tissue, turning it into a tumor conglomerate.

In meningiomas, a significant correlation with SPR indicators is inherent to a greater extent in monocytes and lymphocytes. Probably, cells with regenerative potentials, such as monocytes and lymphocytes, differ in the mechanisms of regeneration in benign and malignant tumors.

A significant correlation was observed mainly in blood samples after the addition of verapamil hydrochloride at a dilution of 1:10.000 and after exposure to blood with LLLR *in vitro*. Such treatments of the blood lead to a change in the charge on the cell membranes, which is reflected in the indicators of the level of aggregation of blood cells, measured in SPR units.

Conclusion

Obtained data indicate that by acting on the blood with chemical or physical factors, it is possible to change the correlation coefficient between the charge of the membranes and the number of blood cells. Consequently, the regulation of the cellular composition of peripheral blood in the body may depend on the indicators of aggregation of blood cells, which contributes to the passage of inflammation and subsequent restoration of damaged tissues within the framework of physiological repair or in tumors as an example of pathological regeneration. Probably, the mechanisms of regeneration in benign and malignant tumors differ and are implemented by different cell pools depending on the charge of cell membranes.

These studies can be used as new approaches to adjuvant chemotherapy to develop methods for treating patients with malignant brain tumors using pharmacological drugs of the calcium channel blockers group, using low concentrations of verapamil hydrochloride as an example.

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