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Research Article

Peculiarities of the Spectrum of Chromosome Aberrations in the Peripheral Blood Lymphocytes in Cases of Brain Gliomas and their Correction with Verapamil and Ketamine

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Abstract: This work is devoted to question, is the genetic anomalies in glioma cellular genome is asubject to reasons or consequences of the malignant process? For answer on this question new approach was used, such as comparison of the genetic abnormalities data between brain gliomas and cerebral brain injury. This two pathologies have different view of inflammations, and it influence on the genome can investigate by cytogenetic methods. Making into account the connection between membrane and cell nuclear, pharmacology medications verapamil and ketamine was used for possible stabilization of nuclear chromosomes. Thus, a disorder of cellular genome in cases of inflammatory processes of neoplastic and non neoplastic genesis largely is defined with charge properties of cellular membranes and the influence of channel antagonists (verapamil and ketamine) on cellular membranes promotes the normalization of charge properties of membranes and stabilization of cellular genome. This result is evidence about such fact, that more of the genetic aberrations are not reasons, but only consequences of malignant process and it is very important result for utilization in gene therapy.
Keywords: Braingliomas, Genetic aberrations, Tumor-association inflammation, Cytogenetic methods, Membrane-nuclear interactions, Verapamil, Ketamine, and Stabilization of nuclear chromosomes.

INTRODUCTION

Nowadays it is commonly believed that genetic anomalies in the cells of the malignant tumors serve as a dominant factor of their pathogenesis. The information that allows attacking the problem critically is presented in this investigation. The role of genetic anomalies in cases of tumor growth consider as a result of genome destruction of a chaotic nature.

It's known that gliomas keep a widely varying level of genetic anomalies that increases during the process of their progression. The maximum number of such anomalies is observed in cases of malignant gliomas of IV degree of malignancy (d.), and the minimum number is fixed in cases of gliomas of II d. [1]. Nevertheless, the intents to correct the genome of the malignant glioma by methods of gene therapy turned to be dull. This brings up a question: the genetic anomalies in cellular genome of gliomas are subject to reasons or consequences of the malignant process. There is no clear response for these questions yet. As a rule, the genomic aberrations, that also include gene expression level changes, are compared with similar indices of sound organism cells. Taking into account existence of tumor-association inflammation (TAI) that accompanies the growth and progression of malignant tumors [2], it's required to make a comparison of genomic aberrations with similar indices of genomes of inflammatory genesis cells, thus, it turns to be more careful method of problem solution concerning cause-effect relations in the tumor growth pathogenesis.

Among the cells of inflammatory process the peripheral blood lymphocytes are the most convenient object for conduction of investigations of cellular genome, because during the process of culture with phytohemagglutinin (PHA) they turn into lymphoblasts with a big nucleus that helps to explore chromosome aberrations in both cases: strictly inflammatory processes and TAI processes, and also allows to examine their comparative characteristics.

The discovery of connection between cellular genome activity and cellular membranes state permits us to suggest a possibility of cellular genome stabilization via normalization of cellular membranes

charge. Among other issues, the peripheral blood cells are the most convenient object for search of medications that stabilize cellular genome, because there is a strong correlation between changes of properties of blood cells membranes and cellular membranes of vitals [4-6].

The goal of the experiments was to conduct a comparative test of chromosomes aberrations number in peripheral blood lymphocytes among healthy people, among patients who suffered from trauma brain injury, and also among the ones who suffered from cerebral gliomas, depending on the rate of their malignance and on possibility of stabilization of blood cells genome in cases of gliomas with the help of verapamil and ketamine medications taken into certain concentrations under in vitro conditions.

MATERIALS AND METHODS

97 patients, suffering from different degree of malignancy brain gliomas had been examined before the surgeries were performed. Moreover, than 25 patients suffering from trauma brain injuries (TBI) of medium severity had been examined. The similar blood sampling was held among sound donors (25 persons).

Peripheral blood lymphocytes of the patients were cultivated by Hungerdorf method during 52 hours [7]. Metaphase chromosome plates were received and evenly stained chromosomes for karyotyping were explored. All structural aberrations of chromatic and chromosomal karyotypes, and numerical aberrations (aneu- and polyploidy cells) were taken into consideration. The division of aneuploid cells into hypo- (the number of chromosomes in the metaphase was less than 44) and hyperploid ones (the number of chromosomes in the metaphase exceeded 48).

The impact was realized in vitro with 0.25 % verapamil-chloride (verapamil) solution (in concentration of 1:1000) and ketamine-chloride (ketamine) solution (50 mg/ml) in concentration of 1:1000. Dilution of channel antagonist medications in 10.000 times contributed more to optimal blood cells aggregation decrease among the patients who suffered from brain gliomas and demonstrated statistically valid effect in case of tumor growth inhibition [8]. Into the PRMI culture media of 2 ml volume were introduced 20 micro liters (mcl) of verapamil and ketamine solutions in concentration balance of 1:1000, at that, the final concentration of medications – 1:10.000 was achieved. All test solutions were prepared immediately before cultivation, during 10 minutes 20 mcl of verapamil and ketamine solutions were cultivated separately with 200 mcl of blood cells, then 2 ml of PRMI media were added and cultivated at $+37^0$C during 3 days. Statistical data analysis was conducted applying Students't-test.

RESULTS AND DISCUSSION

It was demonstrated that the percent of chromosomes aberration among patients who suffered from TBI was higher than among healthy people. In previous experiments a relationship between blood cells aggregation rate and number of chromosome aberrations in nucleuses of peripheral lymphocytes was determined [9]. In cases of high malignant gliomas of IV d. a maximum amount of chromosomes aberrations in the nucleuses of the peripheral lymphocytes was detected and increased to the maximum blood cells aggregation level. Thus, modification of transmembrane potential of leucocytes membranes in case of inflammatory process may contribute to chromosomes aberrations number increase in blood lymphocytes. In cases of gliomas the number of aberrations increases in a greater degree when high malignant gliomas (III and IV d.), in comparison with the low malignant ones (II d.). In addition to the above TAI promotes the decrease of transmembrane potential of blood cells in cases of high malignant gliomas more than in cases of low malignant ones. The frequency of aneuploid cells determination among healthy people and among patients who suffered from TBI composed correspondingly: (7, 8±0, 5) % and (9, 2±0, 3) %. The similar indices among patients, who suffered from gliomas of the III d. and IV d. was improved to a greater extent than among patients who suffered from gliomas of II d. (Table 1).

Table 1*:* The frequency of aberrations and aneuploidy among patienswhith trauma brain injuries and gliomas

Data	Gliomas II d.	Gliomas III-IV d.	TBI	Health
Media group data of the metaphase frequency with aberration, %	3,4±0,4* * p≤ 0,01.	4,5±0,3* * p≤ 0,01.	2,7±0,2* * p≤ 0,01.	1,7±0,2

In such a manner, there is a tendency for increase of frequency of detection of chromosomes aberrations and aneuploidy in peripheral blood lymphocytes in cases of inflammatory processes of nonneoplastic (TBI) and tumor genesis (glioma). Moreover, a connection with glioma malignancy was disclosed.

The spectrum of chromosomes aberrations consists of aberrations of chromatic and chromosomal types (Table 2).

When the analysis of the frequency of basic types of chromosomes aberrations is carried out – the spectrum will move towards chromatic aberrations. It should be appreciated that among glioma patients this

percent is significantly higher: 90 % - in cases of gliomas of II d., 82, 9 % - in cases of gliomas of III d. and IV d., while in case of TBI – 71, 1 %, and among healthy person – 63, 8 %. The percent of exchanges was higher among glioma patients: 8, 0%; 13,8%; in comparison with 5,1 % in case of TBI; and 3,4 % - among healthy persons.

Table 2: Chromosome aberration spectrum in peripheral blood lymphocytes with TBI and gliomapatiens

Diagnosis	All aberrations	Chromatid aberrations						Chromosomal aberrations					
		Total		Single fragments		Exchanges		Total		Conjugated fragments		Anular and dicentric chromosomes	
		т	%	т	%	т	%	т	%	т	%	т	%
The patients group with glial tumors.													
Gliomas II d.	50	45	90,0	41	82	4	8,0	5	10,0	4	8,0	1	2,0
Gliomas III - IУ d.	94	78	82,9	65	69,1	13	13,8	16	16,9	9	9,5	7	7,4
The patients group with TBI and health.													
TBI	138	98	71,1	91	66,0	7	5,1	60	28,9	34	24,6	6	4,3
Health	58	37	63,8	35	60,4	2	3,4	21	36,2	21	36,2	0	0

Among aberrations of chromosomal type the conjugated fragments constitute the majority. Their number is three times less than in the group of glioma patients (8, 0% and 9, 5% comparative to 24, 6 and 36, 2 % in context of TBI and among healthy people. For anular and dicentric chromosomes happen to be 2, 0%; 7,4% in the group of glioma patients, 4, 3 % - in the groups of TBI patients and 0 % - among people of control set.

It means that decrease of indices of chromatid aberrations chromosomes number mainly is detected in cases of gliomas of II d. And increase of conjugated fragments is typical for TBI patients and healthy people.

A positive correlative relationship between the grade of malignancy and some indices of chromosomes aberration was detected. A very remote unreliable correlative relationship between the number of aberrations of chromosomal type and the grade of glioma malignancy was determined. In addition, a reliable direct positive correlative relationship of mean power between the grade of glioma malignancy and frequency of aberrations of chromatic type (coefficient + 0, 70 at 0, 06 mis-action) was fixed, and number of aberrations between chromatic type (correlative coefficient +0, 69 at 0, 06 mis-action), all the above mentioned is not in contradiction with literature data [10-12]. Taking into account the relationship between cellular membranes and nucleuses of cells, decrease of transmembrane potential in cases of TBI leads to the appearance of chromosomal aberrations with the same grade of probability as in cases of malignant gliomas [9]. If we compare the number of chromosomal aberrations in case of inflammatory process in consequence of TBI and in cases of TAI due to the fact of gliomas growth, we can note that in case of TBI chromosomal aberrations also do exist, thought their number is reduced, that fact can be explained by quick regeneration of lymphocytes population. These results speak for the relationship between state of blood cells membranes and organs and number of chromosomal aberrations. Is there an analogy between modifications of blood cells transmembrame potential and organs cells, in particular, brain? The previous investigations gave an affirmative answer for that question.

The analysis was conducted to explore a cellular density of tissues of low malignant and high malignant gliomas applying stained morphological preparations.

The level of cellular density depends on transmembrane potential quite as much as the level of blood cells aggregation. It was found that cellular density was increasing in comparison with targets at adjacent tissues of brain, at that a high density of cells was noted in cases of high malignant gliomas in comparison with low malignant ones.

Therefore, the total score of chromosomal aberrations doesn't serve as specific marker of tumor growth and depends on the charge state of cellular membranes in cases of all types of pathology. At the same time, the indices of aneuploidy are typical for gliomas growth, because in cases of TBI the indices are unreliably raised in relation to targets among healthy people. Aneuploidy is characterized by chromosomes number that is not a multiple of haploid set, and appears as a result of disturbances of segregation of chromosomes in the context of mitosis or meiosis. Aneuploidy is typical for malignant cells, in particular for cells of solid tumors [13-17].

A more intense increase of indices of number of chromatid aberrations is noted in cases of low malignant gliomas of II d., and increase of conjugated fragments is typical for the TBI patients and for healthy people. Thus, it's unlikely that increase of conjugated fragments is closely related to gliomas proliferation.

There are some anomalies of peripheral lymphocytes cells genome, the lymphocytes that take part in inflammatory process among TBI patients and in the context of various grades of brain gliomas malignancy. It is fair to assume that such alterations of genome can be related to transmembrane potential. The effect of some medications that modify transmembrane potential of blood cells membranes, supposedly, can affect the grade of peripheral blood leukocytes genome stabilization.

As it known, verapamil and ketamine act as channel antagonists and have an effect on NMDA – receptors of membranes of leukocytes, gliomas cells and other cells of the organisms [18], distorting in such event the level of transmembrane potential. Verapamil blocks calcium channels in the structure of NMDA - receptors, and ketamine entirely blocks the activity of ionotropic NMDA – receptor. The results that speak for stabilizing effect of verapamil and ketamine on genome of lymphocytes that are cultivated with the addition of these medications were obtained (Table 3). The research results demonstrated that while verapamil impacting on blood cells during the process of cultivation the frequency of lymphocytes chromosomal aberrations was unreliably decreased comparative to targets. Under the effect of ketamine an unreliable increase of indices of chromosomal aberrations frequency was fixed. Under the effect of verapamil and ketamine a reliable decrease of uneuploidy cells frequency among patients is observed, this fact may witness about stabilizing effect of channel antagonists at the level of cellular genome that has been mediated through cellular membrane. The impact of verapamil under in vitro conditions leads to reliable decrease of number of polyploidy cells, an increased amount of which serves as extreme form of chromosomes misbalance, and leads to the break of ontogeny at an early stage among people [19]. We also know about antitumor effect of verapamil among cell cultures [20].

Table 3: The total cytogenetic data in the patients groups with modification by verapamil and ketamine in vitro

Data		Groups		
		Control group	With verapamil and ketamine in vitro	
			Verapamil	Ketamine
Aberration frequency, %		7,87 ± 0,49	6,48 ± 0,61	8,47 ± 1,15
The cells number, %	Aneuplody	16,13 ± 0,67	11,86 ± 1,33*	12,91 ± 0,83*
	Polyploidy	0,70 ± 0,15	0,24 ± 0,12*	0,26 ± 0,19
	Multy aberrance	1,73 ± 0,24	1,15 ± 0,26	1,84 ± 0,49
Exchange types, %		0,53 ± 0,13	0*	0,26 ± 0,19
DAC (damage of aberrance cell)		2,00 ± 0,25	1,90 ± 0,33	2,18 ± 0,60

* - $p \leq 0,05$

Ketamine leads to unreliable decrease of these indices. Aberrations of exchange types are absent in case if verapamil is added in the cell culture. The minimum number of multiaberrant cells and damage of aberrant cell (DAC) are also observed while impact of verapamil on blood cells.

CONCLUSION

For the first time were performed comparative tests between indices of chromosomal aberrations in lymphocytes of peripheral blood during the inflammatory process in terms of TBI and in terms of TAI in case of various malignancy grades gliomas of using of cultivation of peripheral blood leukocytes models in the context of above mentioned pathologies. The similar modifications detected in the genome of lymphocytes in case of TBI showed dependence of chromosomal aberrations number on charge state of cellular membranes that was mediated by the level of blood cells aggregation. As is well-known the II stage of the inflammatory process is characterized by the level of blood cells aggregation. The particularities of TAI in comparison with inflammation of non neoplastic origin were disclosed. Thus, aneuploidy is more typical for lymphocytes in case of TAI and cerebral gliomasthan in case of inflammation among patients that suffer from TBI. The indices of number and frequency of chromatid aberrations reliably correlate with the grade of malignance of cerebral gliomas. The impact on charge properties of membranes with the help of channel antagonists verapamil and ketamine, this leads to stabilization of number of aberrations in blood lymphocytes nucleuses, does confirm the existence of relationship between functional status of membranes of organism cells and the level of stability of cellular genome. The dependence of glioma malignancy grade on the level of transmembrane potential, probably, is of strictly quantitative nature, and not of the tumor associated one. Consequently, giving an answer for the question, we can say that the largest number of blood cells chromosomes aberrations of malignant gliomas turn to be a consequence of tumor process, not a reason.

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