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Research Article

The Mechanism of Tumor Progression Inhibition by Verapamil-Hydrochloride in the Long-Term Period after Complex Therapy in Patients with Glioblastomas

N YA Gridina[1]*, O YA Glavatsky[1], AN Morozov[1], YU V Ushenin[2], LA Kot[1], NG Draguntsova[1], AD Belousova[1], OI Korolyova[1] and IV Boltina[3]

[1]*The State Institution, "Romodanov Neurosurgery Institute, National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine*

[2]*Institute of Semiconductor Physics, V. E. Lashkaryova, Kyiv, Ukraine*

[3]*Institute of Ecohygiene and Toxicology, L. I Medvedya, Kyiv, Ukraine*

***Corresponding Author:** N YA Gridina, The State Institution "Romodanov Neurosurgery Institute, National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine.

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Abstract

The paper presents preliminary results of verapamil- hydrochloride use in the treatment of glioblastomas after radiation and chemotherapy, including courses of treatment with temozolomide and lomustine. After the end of the courses with lomustine treatment, followed by continuous treatment with verapamil - hydrochloride at low concentrations, the result of the patient's life expectancy for a period of 35 months was presented as a case-report after surgical removal of glioblastoma. Relapse after surgery occurred 29 months later. Patients treated with temozolomide and verapamil-hydrochloride showed a life expectancy result that did not differ from the literature data. It has been hypothesized about the reasons for the increase in life expectancy when using lomustine with verapamil-hydrochloride. The latter at low concentrations does not have a direct cytotoxic effect on glioblastoma cells, but it helps to inhibit the growth of their recurrence. It was shown that an increase in the degree of malignancy of gliomas coincides with an increase in the manifestations of tumor-associated inflammation, as well as an increase in the number of chromosomal aberrations and aneuploidy in peripheral blood lymphocytes. Studies carried out on blood culture have shown that verapamil- hydrochloride helps to stabilize the blood cell genome. After the course of treatment with lomustine, which has a more prolonged cytotoxic effect on blood cells than temozolomide, verapamil manages to stabilize their genome to a greater extent due to an increase in the level of transmembrane potential, which ultimately leads to a decrease in inflammation and prevents the early appearance of glioblastoma relapses.

Keywords: *Glioblastoma Cells; Temozolomide; Lomustine; Verapamil-Hydrochloride; Low Concentration*

Introduction

The achievements of modern oncology in the treatment of many types of malignant tumors are obvious and beyond doubt. However, there are some types of tumors, the treatment of which is still ineffective. These include malignant brain gliomas, in which the mortality rate reaches 100%, with a short life span after diagnosis [1-3]. The immediate cause of mortality in patients in the long-term postoperative period is the appearance of glioma relapses, which have a highly malignant phenotype compared to the primary tumor in the preoperative period. Recurrences of malignant gliomas in most cases are chemoresistant and radioresistant, which requires new developments in the tactics of treating this type of tumor.

What is the reason for the appearance of relapses after the conducted courses of rather aggressive chemotherapy? Unfortunately, it is in the activation of the inflammatory process as a result of the appearance of necrosis of tumor and healthy tissues after treatment of patients. The presence of tumor-associated inflammation accompanying the growth and progression of malignant gliomas is recognized by many authors [4-8]. We hope that over time, scientists will come to the conclusion that the mechanism of malignant tumors should be considered not only at the molecular-cellular level. This is only a subjective idea of the process, which objectively is a complex of interaction of several systemic processes that perform vital functions in the body of a healthy person.

Activation of inflammation mechanisms by necrotic tissues [9], which are found in the tissues of malignant gliomas, especially glioblastomas, leads to a long-term decrease in the transmembrane potential of peripheral blood cells, which in our studies was studied using the Plasmon biosensor, recording the level of blood cell aggregation in stage II inflammation [10]. These changes in the body, as shown in our previous studies, led to the transition of repair mechanisms to mechanisms of tumor tissue regeneration. Almost all cellular elements of the blood are involved in the mechanisms of regeneration. Recently, some of them have been identified with the potential of embryonic stem cells [11-15]. However, in the presence of a malignant tumor in the body, the processes of repair - regeneration acquire pathological characteristics. A decrease in the transmembrane potential contributes to the blocking of stage III inflammation, leading to the phenomenon of "non-healing wound" [16], as a result of which stem cells and blood cells are exposed to a large number of damaging inflammatory factors. These factors can be not only cytotoxic, but also genotoxic, damaging both the cellular genome and the entire cell.

Almost any treatment regimen for malignant tumors is aimed at destroying cells and tissues, with a side effect of necrosis of non-tumor tissues of the patient's body. Consequently, after the death of tumor cells and a short period of remission, processes begin that provide the appearance of gliomas relapses.

It is considered the most effective treatment of malignant brain gliomas with temozolomide in combination with radiation therapy [17-22], which is called the "gold standard" today. In the postoperative period, the life expectancy of patients receiving classical treatment regimens with lomustine, procarbazine, vincristine and some other chemotherapeutic drugs increases slightly. It is known from the literature that the median time to progression of glioblastomas in patients after irradiation was 5 months, with lomustine - 7 months, with temozolomide - 13 months [22]. These results clearly require the development of innovative approaches to treatment with these drugs, as well as the search for new drugs with a pathogenetic mechanism of action.

We have previously shown that verapamil - hydrochloride at low concentrations helps to reduce the level of blood cell aggregation, which is stage II of the inflammatory process, suppressing the spread of inflammation in the body [23], inhibiting the growth of malignant brain gliomas and their relapses. In high concentrations, verapamil directly inhibits the growth of malignant tumors, but this is not acceptable for its long-term use.

In our studies, temozolomide and lomustine, as representatives of the drugs of the imidazotetrazine group and the alkylating action from the nitrosourea group, were used in the treatment of patients with glioblastomas.

After receiving courses of radiation therapy and chemotherapy with temozolomide and lomustine, the patients were prescribed a course of therapy with low concentrations of verapamil - hydrochloride.

We analyzed the data on life expectancy of patients treated with temozolomide and lomustine in combination with radiation therapy (RT) and a subsequent course of verapamil- hydrochloride. In the control group, patients simultaneously underwent similar courses. This group included patients who, for various reasons, from the very beginning were unable to carry out long-term therapy with verapamil-hydrochloride. The toxic and genotoxic effects of temozolomide and lomustine on blood cells are well known, therefore, it was of interest to study the effect of verapamil on blood cells, in particular, on the level of chromosomal aberrations of lymphocytes, as well as to what extent this effect can contribute to inhibition of the growth of glioblastoma recurrences.

Aim of the Study

The aim was to comparatively analyze the effectiveness of treatment of patients with verapamil - hydrochloride, carried out after receiving courses of radiation therapy in combination with temozolomide and lomustine, and also to try to explain the differences in the mechanism of their antitumor action.

Materials and Methods

The work investigated the life expectancy of 11 patients with glioblastomas who received, along with temozolomide and other chemotherapy regimens, verapamil-hydrochloride, the use of which at very low concentrations was justified in previous works [23]. The control group consisted of 37 patients who did not take verapamil - hydrochloride.

The study of blood cells aggregation level was continued using the Plasmon biosensor in order to select the optimal concentrations of verapamil - hydrochloride prescribed for permanent treatment of patients after courses of RT and CT (chemotherapy) [24].

The presence of glioblastomas relapses was investigated using imaging methods of NMR tomography.

To determine the number of chromosomal aberrations in lymphocytes and aneuploid cells in 32 patients with gliomas and 8 health individuals, the blast transformation reaction of lymphocytes was modified by adding all 10 µl solutions to the culture medium RPMI *in vitro*, including phytohemagglutinin (PHA), antibiotics, dilutions of verapamil-hydrochloride in 10,000 times.

Statistical treatment of findings was realized by "Statistics - 10v" package.

Results and Discussion

Treatment of patients with verapamil- hydrochloride after LHT courses showed group differences between patients with verapamil (group I-Verapamil) and group II without verapamil (control), which amounted to a difference of 14 months (Table 1). Individual fluctuations were within 12 - 36 months. (I group) and 2 - 28 months (control) (Figure 1). It is worth noting that in the group of patients treated with verapamil - hydrochloride, all patients lived for more than 12 months.

Patients were selected from the general group who received temozolomide or lomustine along with RT, followed by a course of verapamil - hydrochloride. The research results are presented in table 1 and figure 1.

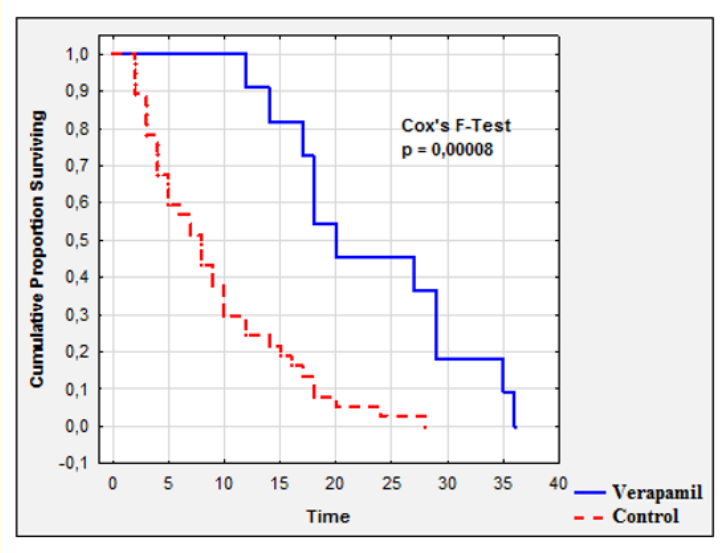

Figure 1: *Surviving data for the I (11 patients) and II (37 patients) groups of patients are significant by the Cox's F-test. The significance level is 0,00008.*

Group	Number of patients	Mean (months)	Standard Deviation (months)	Median life expected (months)
Patients, treated without verapamil-hydrochloride	37	9,11	1,5	7,5
Patients, treated with verapamil-hydrochloride	11	23,17	2,52	19

Table 1: *The life expectancy of patients treated with verapamil-hydrochloride during the process of combined treatment of glioblastomas in the late postoperative period.*

Patients who received additional treatments other than temozolomide, lomustine, and verapamil -hydrochloride were not included in the groups. The indicators of the life expectancy of these patients are presented in table 1. It was necessary to analyze how the combination of temozolomide or lomustine courses followed by constant treatment with verapamil affected the life expectancy of patients.

In the I group of patients with glioblastomas (11 people), after standard treatment, they took verapamil - hydrochloride at low concentrations for life. The data of life expectancy of patients who received courses of treatment with temozolomide or lomustine followed by a course of treatment with verapamil- hydrochloride were, respectively: temozolomide + verapamil hydrochloride - 17.7 months; lomustine + verapamil hydrochloride - 21.6 months. Consequently, lomustine in combination with verapamil - hydrochloride contributed to a significant increase in the life expectancy of patients with glioblastomas in comparison with the literature [22]. At the same time, therapy with verapamil- hydrochloride after a course of RT and temozolomide had almost no effect on the increase in the life expectancy of patients and coincided with the data obtained by other researchers [25].

Let us consider an individual case of treatment of patient Sh. (55 years old), who, after courses of RT and lomustine followed by a course of verapamil- hydrochloride in low concentrations (dilution of the drug in 10,000 times), lived 35 months after the first operation to remove glioblastoma. The patient was found to have intermediate sensitivity to temozolomide (MGMT - deficient glioblastoma), in connection with which he was prescribed adjuvant therapy with lomustine at a dosage of 200 mg 1 time for 4 weeks, after which the patient received daily verapamil - hydrochloride without interruption. The relapse-free period was 29 months, which was objectively confirmed by examination using NMR tomography. The patient's life expectancy was 35 months, which is currently a difficult to achieve result in the treatment of glioblastomas [22].

The reason for these results should be considered in the mechanism of action of these chemotherapy drugs on tumor cells. The cardinal mechanism of influence on the integrity of the structures of the cell nucleus is that the TP53 tumor suppressor gene encodes a transcriptional activator that controls fundamental cellular responses to various stress signals, including DNA damage. TP53 mutations and loss of heterozygosity are often observed in glioblastomas multiforme [17].

Suppression of the growth of glioblastomas by temozolomide lies in the violation of the structural integrity of DNA molecules as an alkylating agent that methylates DNA at the N7 position of guanine, O3 adenine and O6 guanine. The drug damages DNA indirectly through exposure to O6-methylguanine [25]. It should be noted that temozolomide also has an immunosuppressive effect, which is observed already during the first treatment cycles, with a maximum between 21 and 28 days.

The mechanism of action of lomustine is the alkylation of DNA and RNA with damage to their matrices. With prolonged use, a long-term effect is possible - the development of bone marrow dysplasia with damage to the genomes of peripheral blood cells, including lymphocytes.

The literature contains data on the study of chromosomal aberrations in induced pluripotent cells [26]. Considering the fact that temozolomide and lomustine disrupt the structure of DNA and RNA of glioblastoma cells and bone marrow cells, it can be assumed that the

positive effect of verapamil-hydrochloride on the effectiveness of additional treatment in patients with glioblastoma can be attributed to the difference in the mechanisms of its action on the blood cell genome. The toxic effect of temozolomide on bone marrow cells in time occurs faster than lomustine. Probably, verapamil-hydrochloride after the courses of RT and CT manages to protect part of the bone marrow and peripheral blood cells from genotoxic damage with lomustine, but not with temozolomide, which had a positive effect on increasing the life expectancy of patient Sh. This assumption can be confirmed or denied in future experiments with artificial blood that does not contain cellular elements.

In connection with the above assumption, it is of interest to study the effect of verapamil - hydrochloride on the level of chromosome aberrations in peripheral blood lymphocytes in cerebral gliomas with various degrees of malignancy prior to RT and CT.

The results of cytogenetic studies of peripheral blood lymphocytes showed that the average group frequency of aneuploid cells in the group of patients with malignant gliomas (grade III-IV) was significantly higher (22.0 ± 0.9%) compared with similar indicators in patients with benign gliomas (II grade of malignancy) (14.41 ± 0.9%), and in healthy individuals (7.8 ± 0.5%) (Figure 2).

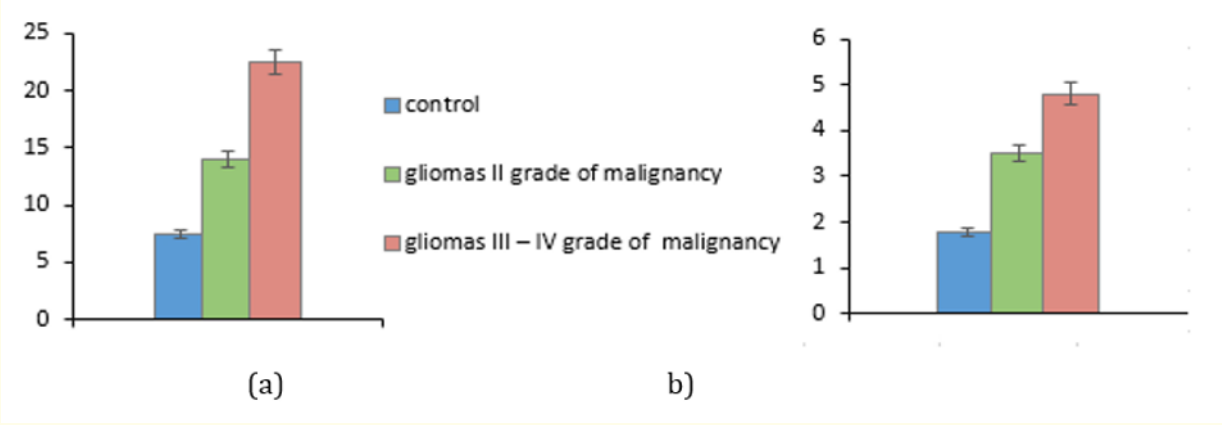

Figure 2: *a) The number of aneuploid cells and b) the frequency of registration of chromosomal aberrations in peripheral blood lymphocytes in patients with gliomas of various degrees of malignancy.*

In our previous studies, it was shown that with an increase in the glioma degree of malignancy, the level of aggregation of blood cells increases, included lymphocytes, indicating and increased levels of inflammation. Therefore, a decrease in the level of transmembrane potential leads to an increase in the level of chromosome aberrations and the number of aneuploid cells in the peripheral cells blood of patients with glioblastomas.

In the control variant (without verapamil - hydrochloride) (Table 2), we found a statistically significant difference between the frequency of chromosome aberrations in patients with gliomas II grade of malignancy and gliomas III-IV grade of malignancy, which was observed in previous studies. Verapamil significantly reduces the frequency of chromosome aberrations in patients with gliomas III-IV grade of malignancy. It should be noted that in patients with gliomas II grade of malignancy the frequency of chromosome aberrations is less common, although the data are not reliable. Therefore, the lowest frequency of chromosome aberrations corresponds to the group that was modified with verapamil- hydrochloride.

Data	Glioma patients	
	II degree of malignancy	IV degree of malignancy
Without verapamil	6,24 ± 0,73	8,80 ± 0,65*
With verapamil	5,90 ± 0,95	6,83 ± 0,75$^{\acute{a}}$

Table 2: *Frequency of chromosome aberrations (%) of lymphocytes under the action of verapamil hydrochloride in vitro in patients with gliomas.*

*Note: * - p ≤ 0.05 in comparison with the data on benign tumors; ά - p ≤ 0.05 compared with data without the addition of verapamil.*

A statistically significant decrease in the number of aneuploid cells is observed in patients with gliomas III - IV grade of malignancy with the action of verapamil hydrochloride (Table 3). Considering the mechanism of suppression of the activity of ionotropic NMDA receptors and blocking of calcium channels on the membranes of blood cells by verapamil - hydrochloride, it can be assumed that ionotropic channels and calcium blockers are involved in the instability of the cell genome.

Data	Glioma patients	
	II degree of malignancy	IV degree of malignancy
Without verapamil	13,30 ± 1,03	17,75 ± 0,87*
With verapamil	11,15 ± 1,27	12,17 ± 2,16$^{\acute{a}}$

Table 3: *The number of aneuploid cells (%) under the action of verapamil hydrochloride in vitro in patients with gliomas.*

*Note: * - p ≤ 0.05 in comparison with the data on benign tumors; ά - p ≤ 0.05 compared with data without the addition of verapamil*

Conclusion

The hypothesis put forward in the course of the work on the mechanism of increasing the life expectancy of patients receiving courses of treatment with lomustine followed by treatment with verapamil - hydrochloride receives experimental confirmation of the stabilizing effect of verapamil - hydrochloride on the lymphocyte genome. In the literature, there is evidence of an increase in the effectiveness of the action of some CT drugs by verapamil [27]. But at the same time significantly higher concentrations of verapamil were involved, in addition, verapamil in low concentrations has a significant increase in life expectancy mediated through inhibition of tumor-associated inflammation, noted in patients who did not undergo chemotherapy courses at will.

In a comparative analysis of the effectiveness of treatment of patients with verapamil - hydrochloride, carried out after the course of radiation therapy in combination with temozolomide and lomustine, according to preliminary data, it was found that the combination of RT regimens with CT lomustine followed by verapamil can be used to select the most effective combination of treatment regimens for patients with glioblastomas. However, this assumption should be confirmed on a larger sample.

Activation of tumor-associated inflammation and the presence of chromosomal aberrations in blood cells involved in reparative-regenerative processes are one of the reasons for the appearance of recurrence of malignant gliomas in the long-term postoperative period, that is accelerated by the use of cytotoxic and genotoxic chemotherapeutic agents and courses of radiation therapy. They lead to remission only for a short period of time, and then compensate for this by rapid tumor growth with the presence of resistance mechanisms to RT and CT. Therefore, some scientists predict that from 2025 cytotoxic chemotherapy drugs will gradually be replaced by pathogenetic drugs that do not cause side effects.

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