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VERAPAMIL - HYDROCHLORIDE AT LOW CONCENTRATIONS INHIBITS THE GROWTH OF RELAPSE GLIOBLASTOMA

N. Ya. Gridina*[1], A. N. Morozov[1], V. D. Rozumenko[1], A. A. Shmeleva[1] and Yu. V. Ushenin[2]

[1]Institute of Neurosurgery n. A. P. Romodanova, Kiev, Ukraine.
[2]Institute of Semiconductor Physics n. V. E. Lashkaryova, Kiev, Ukraine.

***Corresponding Author: N. Ya. Gridina**

Institute of Neurosurgery n. A. P. Romodanova, Kiev, Ukraine.

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ABSTRACT

The life expectancy of glioblastoma patients was investigated in the distant postoperative period. These patients were treated with such concentrations of verapamil – hydrochloride, that minimize the level of blood cells aggregation in II stage of the inflammatory process. 11 patients received treatment (I group) and 32 patients did not received treatment (II group) with calcium blocker using verapamil – hydrochloride examples in pills. For the objectification of tumor association inflammation (TAI), that present's in the patient's body, the definition of blood cells aggregation was examined on the "Plasmon" sensor. Peripheral blood cells were collected from patients to determine the indicators of blood cells aggregation with the addition of various aqueous of verapamil – hydrochloride (from 1: 10 to 1: 100,000 times). Patients took drugs at the lowest level of blood cells aggregation doses. Drug dose was performed by decreasing its concentration by a factor of 10, 000. From the 43 patients, admitted to the clinic, only 3 patients did not undergo chemotherapy at will. After the surgical removal of glioblastoma and the postoperative irradiation course, they only took verapamil – hydrochloride at low concentration daily. In the I group of 8 patients, undergoing combined treatment courses and taking verapamil - hydrochloride, the average life expectancy was 18.6 ± 1.82 months and 8.47 ± 1.02 months in the group of 32 patients (Cox's F - test p = 0,00108). Three patients without chemotherapy in the postoperative period received daily treatment with verapamil – hydrochloride without interruption for life. All 3 patients continue to live for 19, 25 and 29 months, respectively. The paper presents for the first time the results of highly malignant glioblastoma treatment with low concentrations of the NMDA-dependent calcium blocker verapamil – hydrochloride. The results indicate a high antitumor activity of the drug, both when used together with traditional methods of treatment or separately.

KEYWORDS: Glioblastoma, tumor-associated inflammation, verapamil-hydrochloride, low concentrations, life expectancy.

INTRODUCTION

Brain gliomas are one of the most malignant human tumors with an unfavorable prognosis and a short period of the life in the postoperative period, averaging about 9 months.[1-3] Breach of relationship between the mechanisms of reparation (the predominance of the inflammatory process) and regeneration (replacement of the defect with stem cells) can lead to the development of a tumor. Tumor growth begins when the transition of repair to regeneration is incomplete and the process of stem cell development is adversely affected by cells and factors of inflammatory genesis over a fairly long time. In the body, there are mechanisms of protection against such effects on stem cells as apoptosis, which dominates during embryonic development, and the epithelial-mesenchymal transition (EMT).[4-5]

The study of the inflammatory process that accompanies the growth of malignant gliomas, called tumor-associated inflammation (TAI), becomes relevant.[6-9] In our previous studies, a relationship was found between the increased inflammatory component and the growth of the degree of glioma malignancy.[10] Therefore, the use of methods for inhibiting TAI in the postoperative period acquires considerable interest in order to prevent possible continued gliomas growth. Most anti-inflammatory drugs have a number of side effects and are not recommended for long-term use in patients with malignant tumors. Suppression of TAI can be accomplished by reducing the activity of ionotropic receptors, such as NMDA – receptors.[11-12]

The structure of NMDA - receptors includes calcium channels. Calcium ions are essential in the development of the inflammatory process in many pathological processes, including tumors. The action of ketamine (selecting blocker of NMDA-receptors) was more effective than verapamil, but long-term use of ketamine

in the clinic can be dangerous. This indicates a link between the mechanisms of blocking calcium channels by verapamil and a decrease in the activity of ionotropic NMDA - receptors, due to the structural feature of the NMDA- receptor containing the built-in calcium channel. The inhibition of TAI using NMDA-dependent calcium blocker verapamil can help to slow the growth of glioblastomas, without causing toxic effects on the body with prolonged use.

AIMS

The aim was to investigate the life expectancy of patients with glioblastomas in the distant postoperative period who were treated with such concentrations of verapamil - hydrochloride, which minimize the level of blood cells aggregation in the II stage of the inflammatory process.

MATERIALS AND METHODS

43 patients were divided into 2 groups, that received (group I) or who did not receive treatment (group II) with a calcium blocker using the example of verapamil - hydrochloride in pills ("Pharmak"). For the objectification of the presence of TAI in the patient's body, the definition of aggregation of blood cells was examined on the instrument "Plasmon" sensor.[13] Under physiological conditions, NMDA - receptors are known to be activated by millimolar concentrations of glutamate, which is present in the synaptic cleft for several milliseconds. In pathological impulses, receptors are activated by micromolar concentrations, but for a significantly longer time.[14] We took the same pattern as a basis for the effects of millimolar and micromolar concentrations of verapamil - hydrochloride on the level of blood cells aggregation, given the location of the calcium channel in the structure of the NMDA- receptors of lymphocytes and whose function is NMDA-dependent.[14-15] Selection of optimal concentrations of verapamil - hydrochloride was carried out with the aim of treating patients with glioblastomas in the postoperative period.

Peripheral blood cells were collected from patients to determine the indicators of aggregation of blood cells with the addition of various aqueous dilutions of verapamil - hydrochloride (from 1:10 to 1: 100,000 times). This made it possible to indirectly determine the level of blocking of the NMDA-dependent calcium blocker verapamil on the membranes of blood cells, including leukocytes and lymphocytes.[15] Patients took drugs at a dosage at which the level of aggregation of blood cells was the lowest. Dosing of the drug was performed by reducing its concentration by a factor of 10,000.

From the 43 patients, admitted to the clinic, only 3 patients did not undergo chemotherapy. After the surgical removal of glioblastoma and the postoperative irradiation course, they only took verapamil - hydrochloride at low concentrations daily. Histological studies of the bioptic material were carried out according to standard methods, the sections were stained with hematoxylin-eosin and pikrofuksin.

RESULTS AND DISCUSSION

In I group of patients, undergoing combined treatment courses and taking verapamil, the average life expectancy was 18.6 ± 1.82 months. In II group of patients, undergoing combined treatment courses but without taking verapamil, the average life expectancy was 8.47 ± 1.02 months. Three patients without chemotherapy in the postoperative period received daily treatment with verapamil - hydrochloride without interruption. All 3 patients continue to live for 19, 25 and 29 months, respectively (table 1, fig. 1).

The effectiveness of such an approach to the treatment of glioblastoma can be compared with the data of other authors, in which with early diagnosis of primary glioblastoma and new medical tactics, the life expectancy of patients averaged only 15.3 months in postoperative period.[16]

The total number of patients who lived more than one year after surgery and did not take verapamil was 29%. The number of patients taking verapamil, along with chemotherapy and radiation, who lived more than a year after surgery, was 100%.

Table 1: The life expectancy of patients receiving a course of treatment with verapamil - hydrochloride with a combined treatment of glioblastomas in the late postoperative period.

Group	Number of patients	Mean (months)	Standard deviation (months)	Median life expected (months)
Patients, treated without verapamil - hydrochloride	32	8,47	1,02	7
Patients, treated with verapamil – hydrochloride	8	18,63	1,82	18

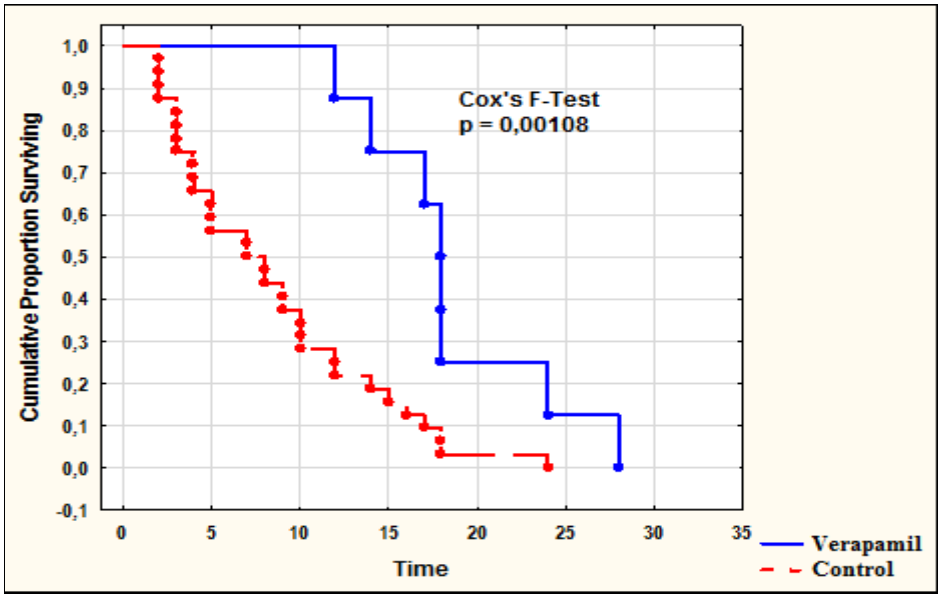
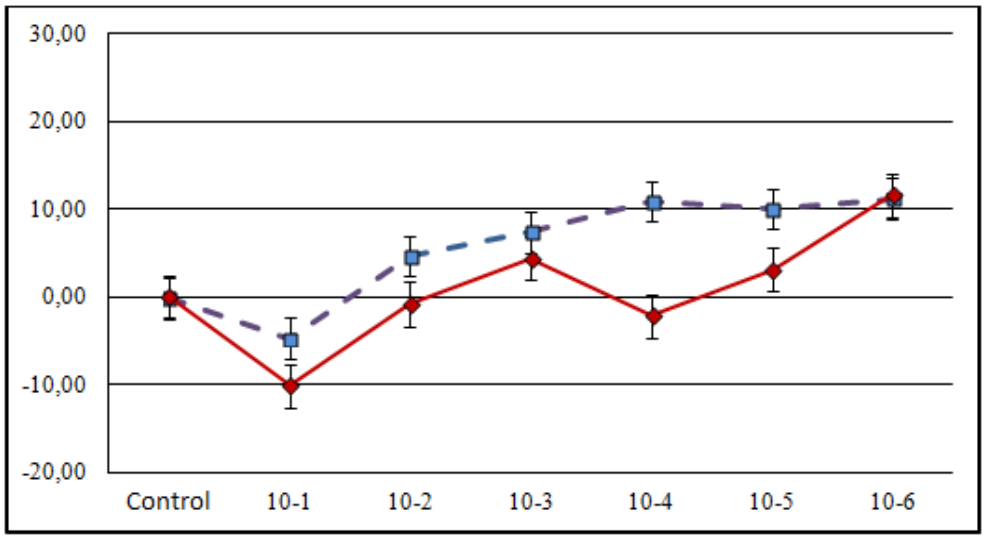


Fig. 1: I (8 patients) and II (32 patients) groups of patients, who received and did not receive verapamil treatment, are significant by the Cox's F- test. The significance level is 0.00108.

Studies of the level of aggregation of blood cells were expressed in terms of SPR, which quantitatively reflected the level of aggregation of the blood cells of patients, since nanoscale intercellular distances (of the order of 200-300 nm) in the flow cell between blood cells located on a glass plate covered with a thin layer were determined with high accuracy gold**.**

Baseline indicators of SPR before treatment with verapamil - hydrochloride in both groups of patients differ slightly, which indicates the adequacy of the selection of groups. In order to select the optimal concentration of verapamil - hydrochloride for treating patients, aqueous dilutions of verapamil - hydrochloride (from 1:10 to 1: 100.000 times) were added to the blood *in vitro* beforehand. Dilutions of verapamil tenfold led to an increase in the level of aggregation, and large dilutions of verapamil (10,000 times), on the contrary, contributed to a decrease in the aggregation of blood cells. The concentration of verapamil – hydrochloride, that most decreases the level of blood cells aggregation, was then used in the patients treatment in the postoperative period. Patients took a solution of the drug 3 times a day, constantly, without interruption, for life. Indicators of SPR in patients of groups I and II are presented in Fig. 2.

SPR data



Concentrations of diluted verapamil – hydrochloride; Control – without diluted).

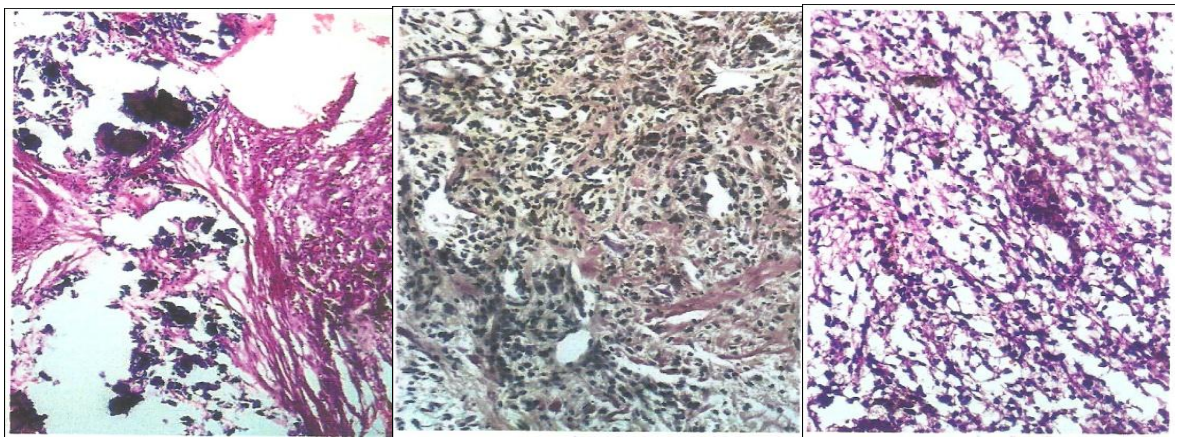
Fig. 2: The ratio of indicators of blood cells aggregation of under the action of verapamil dilutions in patients with glioblastomas, treated and not treated with verapamil (line with a stroke). A comparison was made of SPR indices in relation to the indices of blood dilution with water (20 µl of water and 200 µl of blood, with no hemolysis in any of these dilutions).

As can be seen from Figure 2, the decrease in the SPR curve under the action of verapamil when diluted with water is 10,000 times the same as postoperative remission in patients with glioblastoma, as evidenced by visualization methods performed. An increase in the SPR curve was observed in patients with recurrent glioblastoma.

The dependence of the level of blood cells aggregation of on the presence of a tumor in the brain is well expressed when verapamil is added to the blood at a dilution of 10,000 times (concentration 10^{-4}), which leads to a decrease in SPR. This is characteristic of benign or conditionally benign tumors, such as meningiomas or grade II gliomas, which were shown in our previous studies.[17,18]

Preliminary experiments to determine the level of aggregation of blood cells in patients with glioblastomas under the action of verapamil and ketamine, which is a selective blocker of NMDA - receptors, as well as in the treatment of experimental rats with a transplantable glioma of strain 101.8 (human glioblastoma analog) showed coincidence of data on the effect of verapamil and ketamine both on the level of patient cell aggregation in glioblastoma and on the life expectancy of experimental animals.[17]

The action of ketamine was more effective than verapamil, but long-term use of ketamine in the clinic can be dangerous. This indicates a link between the mechanisms of blocking calcium channels by verapamil and a decrease in the activity of ionotropic NMDA - receptors, due to the structural feature of the NMDA-receptor containing the built-in calcium channel. Movalis, the non - steroidal anti-inflammatory pharmacological drug, is a selective blocker of COX-2 (cyclooxygenase-2) and at a dilution of 10,000 times did not have an antitumor effect on rat glioma 101.8. Consequently, the effective inhibition of growth of glioblastoma obtained in the work was achieved using the mechanisms of blocking the NMDA- receptor and the calcium channel on the membranes of glioblastoma cells and peripheral blood cells of patients, which leads to a change in the microenvironment of malignant brain tumors and inhibition of their growth.



a) b) c)

Figure 3: Morphological studies of the glioblastoma tissue from patient B. a) areas of glioblastoma calcification and lysis. Staining by hematoxylin-eosin (x 200); b) areas of fibro-sclerotic transformation. Staining by pikrofuksin (x 200); c) areas of thin tissue of glioblastoma. Staining by hematoxylin-eosin (x 200). On histological specimens, massive foci of calcium deposits with tissue lysis sites were found (a-b). In certain areas, glioblastoma tissue was subjected to fibro-sclerotic transformation along with areas of loosening of tumor tissue (3c).

In the patient, who did not take chemotherapy courses, upon re-operation in the case of continued tumor growth (relapse at the 12-th month) histopathological changes were observed while taking verapamil, which corresponded to the symptoms of medical pathomorphosis (Fig. 3). Calcium deposits indicate that low concentrations of verapamil prevent calcium from entering the cells due to the blockage of calcium channels.

All known methods of treatment of malignant tumors are aimed at cytoreduction and suppression of the tumor growth. Inflammation, contributing to an increase in cell mass with glioblastoma growth, is a protective-compensatory reaction that cannot be completely suppressed. To inhibit TAI in patients, it is necessary to act not on the third stage of inflammation with the mechanisms of proliferation, but on the first stage, on alteration, and to reduce the activity of calcium blockers and ionotropic NMDA - receptors. The latter activate inflammation and in the mechanisms of growth and destruction of brain cells during the tumor process play an important role, which can be indirectly determined by indicators of the level of aggregation of blood cells.[17,18] This is achieved by reducing the appearance of necrotic tissues by suppressing the activity of NMDA-dependent calcium channels by verapamil. Thereby, conditions are created in the tumor microenvironment to hinder the reproduction of glioblastoma cells. The use of verapamil

in traditional doses does not provide an antitumor effect, which was noted in animal experiments.[17]

The effectiveness of such an approach to the treatment of glioblastomas can be compared with the data of other authors, in whom, with early diagnosis of primary glioblastomas and new medical tactics, the life expectancy of patients averaged only 15.3 months.[16]

The question of the adverse effect of calcium antagonists on the occurrence of cancer has been discussed. However, a critical analysis of data on the treatment of hypertension with these drugs allowed WHO experts to conclude that such information was unreasonable.[19–20] In addition, it was shown that with the introduction of certain carcinogens (7,12-dimethylbenzantracene) into animals, verapamil suppressed the development of tumors in rats.[21] Other researchers have shown the absence of tumor-stimulating activity of verapamil during prolonged exposure in rats.[22] As you know, the drug is used widely throughout the world for the treatment of cardiac arrhythmias, without showing pronounced adverse reactions. It was shown that verapamil did not increase the risk of complications in patients after myocardial infarction.[19]

Verapamil improve the effectiveness of chemotherapy in patients.[23] The paper presents data on the treatment of 3 patients with glioblastomas only by verapamil, after irradiation courses, but without chemotherapy, which certainly indicates the presence of antitumor activity in this drug.

Traditional methods of glioblastoma treatment should be combined with techniques of low concentrations of verapamil - hydrochloride in the late postoperative period, since the activation of TAI after chemotherapy and radiotherapy contributes to the growth of tumor residues in the brain. The results obtained can be used to decide the question of the individual tactics of patient treating.

CONCLUSION

The paper presents for the first time the results of treatment of highly malignant glioblastomas with the low concentrations of the dependent calcium blocker verapamil - hydrochloride. The results indicate a high antitumor activity of the drug, both when used together with traditional methods of treatment or separately. The treatment method is pathogenetic in contrast to the empirical one, which is mainly used by modern chemotherapy. With low concentrations of verapamil hydrochloride, NMDA - receptor activity is reduced and calcium channels are blocked. This, in turn, leads to changes in the transmembrane potential on the blood cells membranes, which contribute to the suppression of the tumor-associated inflammation. TAI determines the tumor microenvironment, which promotes tumor growth and recurrence of glioblastoma in the postoperative period, in particular. Suppression of TAI helps prevent recurrence and, therefore, prolongs the life expectancy of patients with glioblastoma. The presented method can be used after courses of traditional postoperative chemotherapy throughout the patient's life, since this method, unlike other anti-inflammatory drugs, is not toxic to the body.

Our results can be useful for treatment not only patients with brain malignant tumors, but for another kind of malignant tumors, also subject to an individual approach to treatment.

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