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Utilizing SPR as a Novel Technique to Measure Cell Aggregation for Ketamine Treated Brain Gliomas

N. Ya. Gridina

The State institution "A.P. Romodanov Institute of Neurosurgery NAMS of Ukraine", Kyiv, 04050, Kyiv, Ukraine
*Corresponding Author: gridinanina@ukr.net

Abstract This work is devoted to a new approach to use of the inhibitor of glutamate-ionotropic NMDA-receptor – ketamine in treatment of rat malignant gliomas. Optimal ketamine concentrations for inhibition of tumor-associated inflammation in gliomas was found through measurement of blood cells aggregation *in vitro* in patients with gliomas. For the first time, the method of surface plasmon resonance was used to determine the degree of blood cell aggregation as the II phase of inflammation. Experiments for measure of ketamine antitumor activity were performed using the high-invasive and malignant strain of the rat glioma 101.8. The correlation between antitumor activity and blood cells aggregation was found. Unlike highly toxic preparations administered during human gliomas chemotherapies, low doses of ketamine is nontoxic, efficacious and it does not produce side effects.

Keywords Rat Gliomas, Ketamine, NMDA-Receptors, Surface Plasmon Resonance, Blood Cell Aggregation

1. Introduction

Brain gliomas pose a particularly complicated problem in neurosurgical practice. On the average a life span with gliomas of the fourth degree ranges from 6 to 12 months. In recent years clinical investigations have shown that development of tumor-associated inflammation process stimulates growth and progression of malignant tumors, including gliomas [1-3]. In the latter case, glutamate acts on their progression mediately via a tumor microenvironment (TM) [4]. Long-term administration of drugs possessing anti-inflammatory action promotes a slowdown of the growth of gliomas and increases the life span of patients with these diseases [5]. However, the prolonged usage of the most of these drugs (aspirin, anti-inflammation drugs of nonsteroid and hormonal action) produces a side effect on a human organism.

The application of antagonists to NMDA-receptors may well become a new therapeutical approach to treatment of gliomas that provides protection of neurons from glioma-induced neuroexcitotoxicity and good permeability through the blood-brain barrier (BBB) [6]. Ketamine is able to produce the anti-inflammation effect at the level of the central nervous system by blocking expression of the nuclear complex NF-kβ, which is responsible for activation of gene transcription for the number of pro-inflammation cytokines, particularly TNF-α. Ketamine reliably slows down the induced by endotoxine expression of the transcription factor NF-kβ in cells of human glioma and in cells of an intact mouse brain [7].

It is worthwhile to clinically test this preparation as a neuroprotector since it possesses several positive properties simultaneously: block of NMDA-receptors, block of the synthesis of pro-inflammation cytokines, the great ability to penetrate through BBB [8].

The application of ketamine in clinical conditions requires new approaches to its use for treating gliomas. As is known, the aggregation of blood cells is a system factor in the inflammation process, including tumor-associated inflammation that can be studied with the method of surface plasmon resonance (SPR). For the first time, we have developed a technique enabling use of the SPR method for studying the processes of blood cell aggregation in the case of various inflammation and tumor deseases, including brain injuries and gliomas with various degrees of malignancy. There is no need to use electrolyte solutions in the contents of various buffers when using this technique. In consideration of the availability of ion channels and ionotropic receptors in blood cells, it makes this technique very attractive due to its objectivity and accessability [9 - 12].

The aim of this work was to develop new line of approach to the application of ketamine for producing the antitumoral effect in the case of re-inoculated rat brain glioma caused by the strain 101.8. This effect has been reached by selecting the optimum concentration of this preparation, which enables a reduction in the level of blood cell aggregation in patients with gliomas of various degrees of malignancy *in vitro*.

2. Materials and Methods

To attain the set aim, we chose two ways: observation

and treatment of patients with neurosurgical pathology as well as experiments on animals (rats) re-inoculated with brain gliomas. The investigations were performed with 312 patients separated by groups in dependence on the kind of neurosurgical pathology, namely: i) with trauma brain injuries of a medium degree of lesion – 73 patients; ii) with gliomas of various degrees of malignancy (II degree – 52 patients; III degree – 103 patients; IV – 84 patients). For comparison, we studied 52 samples of donor blood. Small amounts of blood were collected from a cubital vein of patients and donors on an empty stomach immediately after their arrival at the clinic; as an anticoagulant, we used heparin (0.1 ml per 10 ml of blood) that does not affect aggregation indices of blood cells.

In this work, was studied the ketamine-hydrochlorid (57,6 mg/ml, Ukraine, Kiev, firm "FARMAK") influence for various dilutions with de-ionized water in conditions in vitro on the degree of blood cell aggregation in patients with neurosurgical pathology. Also studied was the role of the concentration dilutions for the above preparation on the degree of blood cell aggregation as well as its dependence on the kind of neurosurgical pathology.

After centrifuging at the speed 3000 rev/min, blood plasma was removed, and blood cells with the volume 200 µl were placed into test tubes preliminary filled with 20 µl of ketamine diluted with de-ionized water by 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} times. As a reference sample, we used blood added with 20 µl of de-ionized water (control - without addition of pharmacological medication). The position of the SPR peak for this sample served as a reference characteristic. The total amount of blood samples investigated using the above technique was 636. The dispensatory preparation was 2 ml of 5% solution of ketamine-hydrochloride with some pharmacological additives (benzetonium-chloride, natrium-chloride), therefore the preparation concentration was represented not in moles but in degrees of water dilution.

Wistar line rats (70 animals in the age of 4 to 6 weeks) were re-inoculated with one million of glioma cells (strain 101.8) into the right crown region. Since 8 days after glioma re-inoculation, ketamine diluted by 10^{-2}, 10^{-3} and 10^{-4} times was daily introduced into peritoneum in the dose 50 µl. In the same manner, the reference animals were introduced with the natrium- chloride solution in the identical volume. The strains of glioma 101.8 were received from the Institute of Human Morphology, Russian Academy of Sciences, and are the highly malignant and invasive analog of human glioblastoma. The life span of animals after glioma re-inoculation is approximately 18 to 21 days, tumor is well vascularized, re-inoculation of the brain and death-rate of animals are equal to 100%.

As an indicative characteristic, we chose the shift of the SPR peak position measured in angular degrees. The "Plasmon" spectrometer is sensitive only to a thin layer with the thickness 200 to 300 nm, which is adjoined to gold. Therefore, the SPR characteristic depends not only on the amount of blood cells in a sample deposited onto gold but on the total area of these cell membranes that directly interact with plasmons. Before experiment glass chip with gold film was many times washed by distilled water. Soon after that 200 ml blood cells was introduced by micro pump into measure chamber, so blood cells contacted a gold surface. As it known, in the case of blood cell aggregation the ratio of the surface inherent to cell particles to their volume is reduced (rouleau creation). Thereof, the total amount of cells, as compared to the reference one, can be higher, while the contact area with the gold film is lower.

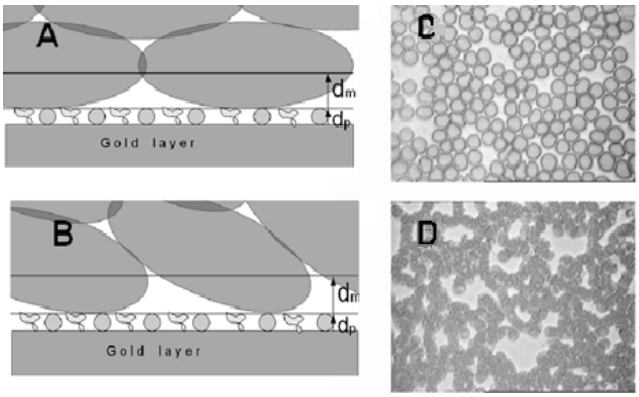


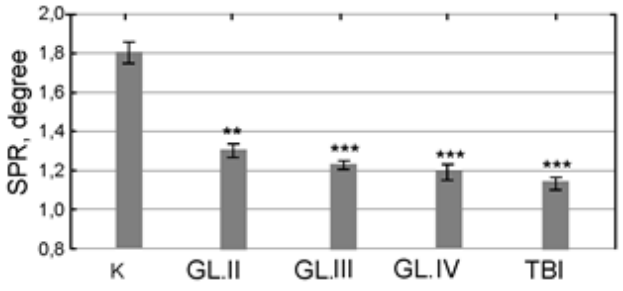
Figure 1. Blood cells topology on glass (enlargement x 200): A - C – donor cells; B - D – patient cells with glioma high malignancy degree. A-B schematic designation of blood cell membranes position on glass.

As seen from Fig. 1, the area of glass occupied by the blood cells of patients with gliomas of the high degree of malignancy (B-D) has more blank spaces, given the equal amount of blood with the previous preparation of donor blood cells (A-C), which was put on glass.

3. Results and Discussion

Studying the shifts of SPR curves for blood cells enabled to find statistically significant differences between the group of healthy persons and patients with gliomas of the II ($p \leqslant 0.01$) to IV($p \leqslant 0.001$) degrees of malignancy (Fig. 2). The decrease in the shift value of SPR curves for blood cells is indicative of enhancement of the aggregation level for these cells and, indirectly, of lowering the electric charge on cell membranes. It is typical for initial stages of the inflammation process.

It should be not that lowering the shift value in peripheral blood cells in the case of gliomas of the II to IV degrees of malignancy is gradual, and the minimum values are typical for patients with the glioma IV degree of malignancy. However, in comparison with corresponding values for patients with trauma brain injury, they remain higher, which can indicate the availability of a subacute inflammation process or microinflammation inherent to malignant gliomas.



Note: ** $p \le 0.01$;
*** $p \le 0.001$.

Figure 2. Characteristics of blood cell aggregation (i.e. values of the angular shift of the SPR peak position) typical for patients with various degrees of malignancy. K – blood samples without additions of preparations; GL.II, GL.III and GL.IV – blood samples for the cases of gliomas of the II, III, IV degrees of malignancy, respectively; TBI – the case of trauma brain injury.

Ketamine in all the dilutions did not influence the aggregation characteristics of donor blood cells (Fig. 3).

In the group of patients with a trauma brain injury, ketamine in all the dilutions, except 10^{-4}, lowered blood cell aggregation. For the dilutions 10^{-1}, 10^{-2} and 10^{-3}, it took place with the confidence $p \leqslant 0.05$, and for the dilution 10^{-5} – with the confidence $p \leqslant 0.01$ (Fig. 4).

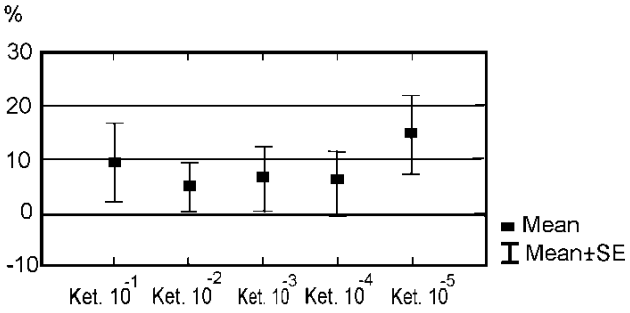


Figure 3. Influence of ketamine dilutions on donor blood cells aggregation data. Note: 0 – blood cells with 20 mkl of water for a control. All dates take in relation with control (%)

In the group of patients with gliomas of the II degree of malignancy, ketamine in the dilution 10^{-1} provided enhancement of the blood cell aggregation degree, and in the dilution 10^{-5} it reliably ($p \le 0.05$) lowered the aggregation degree (Fig. 5).

In the group of patients with gliomas of the III degree of malignancy, ketamine in all the dilutions, except 10^{-4}, 10^{-1} reliably lowered the blood cell aggregation degree. For the dilutions 10^{-2}, 10^{-3} and 10^{-5}, it provided lowering with the confidence $p \leqslant 0.01$, and for the dilution – with the confidence $p \leqslant 0.05$ (Fig. 6).

As to the patients with gliomas of the IV degree of malignancy, ketamine for all the dilutions, except 10^{-1}, reliably lowered the blood cell aggregation degree for the dilution values 10^{-2} ($p \leqslant 0.01$), and 10^{-3}, 10^{-4} and 10^{-5} dilutions with the confidence $p \leqslant 0.001$ (Fig. 7).

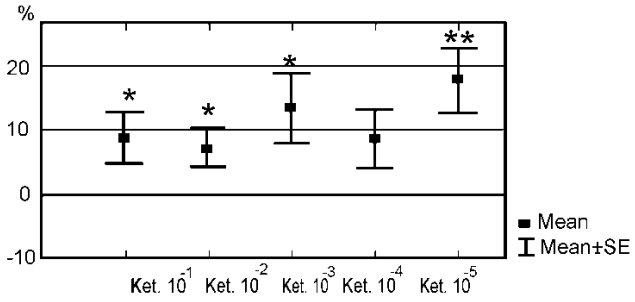


Figure 4. Influence of ketamine dilutions on blood cells aggregation data of patients with TBI. Note: * $p \le 0.05$; ** $p \le 0.01$.

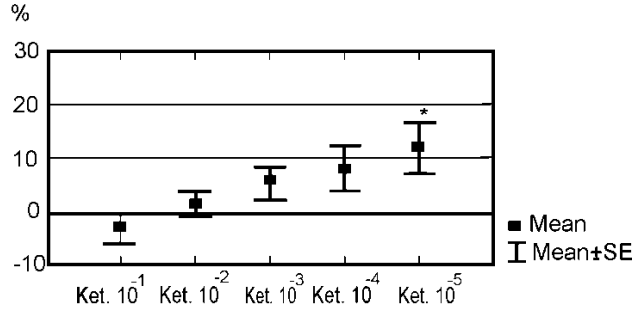
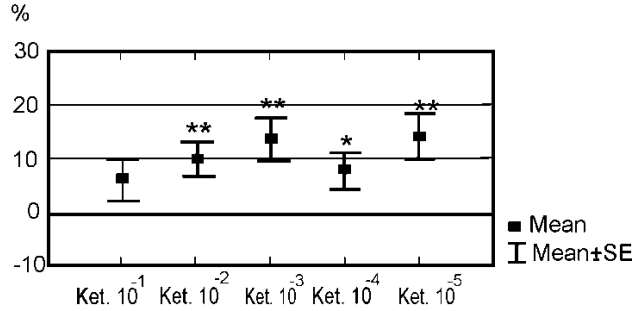


Figure 5. Influence of ketamine dilutions on blood cells aggregation data of patients with gliomas II degree of malignancy. Note: * $p \le 0.05$.



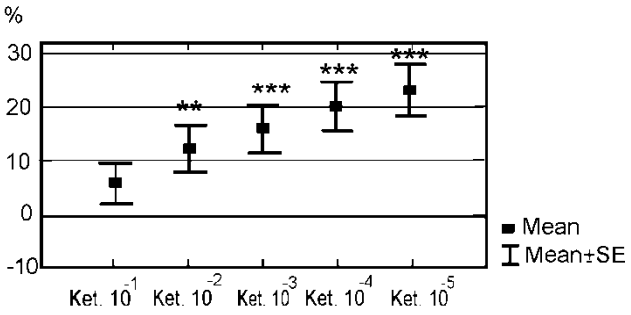
Note: * $p \le 0.05$; ** $p \le 0.01$.

Figure 6. Influence of ketamine dilutions on blood cells aggregation data of patients with gliomas III degree of malignancy.

The above results enabled us to conclude that small-dose concentrations of ketamine are more efficient in lowering the blood cell aggregation degree in the case of gliomas with a high degree of malignancy than in the case of benign gliomas (Fig. 7, 5).

The concept of using the channel blockers is based on their ability to link with some part inside the channel only in conditions of NMDA receptor activation, i.e, when the channel is open. This mechanism inherent to action of channel blockers provides a basis to assume that they will be especially efficient in conditions of pathological

activation of NMDA receptor complex [13]. The enhanced activity of NMDA receptor complex can be a consequence of either excess stimulation of NMDA-receptors or stable changes at the receptor or post-receptor levels. This hypothesis of the enhanced efficiency of channel blockers in the conditions of pathology is based on the convenience that in these conditions NMDA-receptors are activated by higher glutamate concentrations than those in physiological conditions.



Note: ** $p \le 0.01$; *** $p \le 0.001$.

Figure 7. Influence of ketamine dilutions on blood cells aggregation data of patients with gliomas IV degree of malignancy.

However, in real physiological conditions NMDA-receptors are activated with millimolar glutamate concentrations that are available only for several milliseconds [14]. At the same time, even in conditions of acute ischemia of nervous tissue NMDA-receptors are activated by low micromolar concentrations of glutamate but for considerably longer time periods.

If the affinity to the channel part was the only parameter determining pharmacological activity of channel blockers, then the effects of these substances would be more pronounced in physiological conditions than in the state of pathology. However, they are able to leave the binding place in conditions of strong depolarization [15]. Contrary to other receptors, the NMDA ones are simultaneously susceptible to ligands and to changes in the membrane potential (they are voltage-dependent). It served as a base for the assumption that channel blockers are the most efficient in conditions of durable stimulation of NMDA-receptors with low glutamate concentrations, which probably is one of the key mechanisms in pathogenesis of many neurodegenerative diseases [15, 16].

The obtained data concerning the influence of various dilutions of ketamine investigated in vitro showed that low concentrations of the preparation reliably lower the blood cell aggregation degree, which can be taken as a basis for further investigations devoted to development of the methods providing anti-inflammation therapy in the cases of tumors arising in the central nervous system. Ketamine does not influence the blood cell aggregation degree in healthy patients. At the same time, when the degree of glioma malignancy is increasing, maximum ketamine dilutions (low concentrations) conduce to a change in the blood cell aggregation degree more efficaciously.

In the cases of inflammation processes and malignant gliomas, ketamine influences the processes of cell aggregation in the most efficient manner, probably due to the dependence of NMDA-receptor activity on changes in the membrane potential. The latter, as said above, is tightly related with the degree of blood cell aggregation. Lowering the ketamine concentration has a more pronounced effect on fall of blood cell aggregation characteristics.

Fig. 8 shows the data on the increase of a life span of animals under the action of ketamine in various concentrations. 10^{-3}-fold dilution of the initial ketamine concentration conduced to an increase in the life span of rat with glioma 101.8 ($p \le 0.05$). And 10^{-4} - fold dilution of the ketamine solution provided the maximum increase in the life span ($p \le 0.01$). As far as the efficacy is concerned, the usage of highly toxic preparations to treat a case of glioma 101.8 has slightly differed from the above data on the application of ketamine. It is important to note that unlike chemotherapies, 10^{-4} - fold dilution of ketamine is nontoxic and it does not produce side effects.

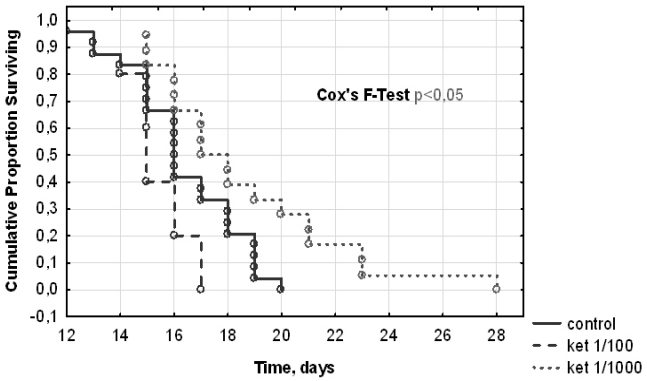


Figure 8. Low-dose (dilution 10^{-3}) and high-dose (dilution 10^{-2}) actions of ketamine on the life span of rats with re-inoculated glioma 101.8.

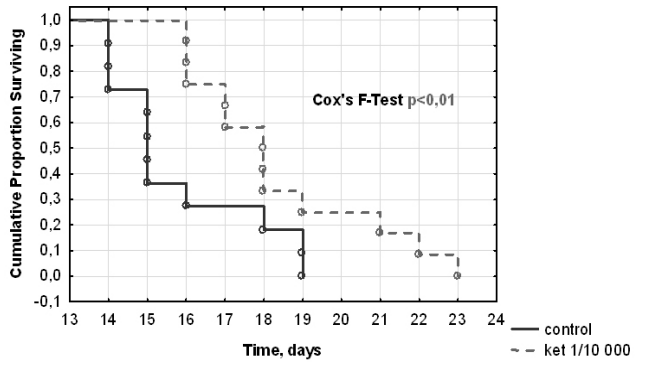


Figure 9. Low-dose (dilution 10^{-4}) actions of ketamine on the life span of rats with re-inoculated glioma 101.8.

As seen from Fig. 9, the higher concentration of ketamine does not promoted any increase in the life span of experimental animals. At the same time, the lower ketamine concentration promoted the increase of the animal life span in its maximum by 5 days longer than at higher ketamine

concentrations. These data are in accordance with the pathophysiological mechanism providing inhibition of ionotropic NMDA-receptor by ketamine [14].

The obtained data confirm that the antitumoral effect of ketamine correlates well with the degree of lowering blood cell aggregation.

Our investigations showed that blood cell aggregation, being the most important mechanism in development of the inflammation process, is also a fundamental system characteristic influencing the progression of malignant gliomas.

The antitumoral effect of ketamine depends on its influence on the blood cell aggregation degree. The lower is this factor, the higher antitumoral action of this preparation. For the first time, we have developed the principally new approach taking into account the mechanism of ketamine action on NMDA-receptors allowing pathogenetic application of inhibitors of ionotropic receptors with the aim to suppress the glioma growth.

4. Conclusion

It is experimentally found for the first time that ketamine in small-dose concentrations lowers the degree of blood cell aggregation in neurosurgical patients.

Developed in our work are new approaches to using the pharmacological preparation ketamine to obtain the optimal antitumoral effect in the case of brain gliomas. These approaches take into account the influence of ketamine small doses on NMDA-receptors, which provides lowering the degree of blood cell aggregation and prevents development of inflammation related with a tumor, which promotes the increase of the life span of animals with re-inoculated rat glioma 101.8.

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