EVALUATION OF THE EFFICACY OF SURGICAL RESECTION OF MALIGNANT GLIOMAS USING 5-AMINO-LEVULINIC ACID

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EVALUATION OF THE EFFICACY OF SURGICAL RESECTION OF MALIGNANT GLIOMAS USING 5-AMINO-LEVULINIC ACID (Abstract): Aim: To evaluate the efficacy of a modern therapeutic procedure, surgical resection of malignant gliomas using 5-Amino-Levulinic Acid (5-ALA) compared with tumor resection associated with radiotherapy and/or antineoplastic medication. Material and methods: The investigation was performed on 127 patients diagnosed with malignant glioma, aged 20 to 80 years, admitted to the Neurosurgical Department of the Iasi “Prof. Dr. N. Oblo” Hospital between 2009-2015. Of these patients, 17 underwent surgical resection and 5-ALA (Gliolan®) treatment. 5-ALA was administered orally, 3 hours before the induction of general anesthesia, under the strict supervision of an anesthetist. The study protocol was approved by the Hospital Ethics Committee and informed consent was obtained from all patients enrolled in this study. A Kaplan-Meier analysis was used for the evaluation of therapeutic efficiency and assessment of the surviving subjects for a certain period of time after treatment. Results: Survival rate was significantly higher in the Gliolan® group compared to the group treated by classic methods at 6 months, 1 year, but especially after 2 years of treatment (0.65 vs 0.48). Conclusions: 5-ALA is a relevant intraoperative marker for direct visualization of the tumor and infiltrative tissue, providing the basis for a feasible future in the therapeutic management of glioblastoma and promising an increase in the survival rate compared to classic surgical method. Keywords: MALIGNANT, GLIOMA, 5-ALA.

In adults, malignant brain tumors account for approximately 2% of all neoplasms, their incidence increasing with age and declining in elderly patients aged over 75 (1, 2). Despite their relative rarity, primary central nervous system tumors are an important source of morbidity and mortality and still represent a major health problem (3). Researches on the etiology and the causes that contribute to the occurrence of gliomas are underway, but the relative rarity of the disease and the rapid death of the patients diagnosed with aggressive subtypes hamper these processes (4). Therefore, to date no causal factors are known and it is only assumed that exposure to therapeutic doses of ionizing radiation for the treatment of cancer results in an increased risk of glioma, as well as of meningioma, and nerve sheath tumors (5, 6). Different hereditary disorders or genetic mutations are also known to be involved in
the etiopathogenesis of gliomas (7).

In the literature, a link between cerebral trauma and brain tumors as a risk factor has been suggested. However, so far there is no conclusive evidence to support this assumption (3). On the other hand, it is considered that exposure to insecticides, pesticides, petrochemicals, organic solvents, lead compounds and nitro-derivatives increases the possibility of developing glioblastoma (8). Malignant brain gliomas are locally invasive tumors that inevitably lead to death within 1-2 years after diagnosis. These tumors remain associated with poor prognosis despite the maximum surgical treatment, completed with radiotherapy and chemotherapy. The degree of tumor resection is accepted as important for optimal surgical treatment, and recent studies provide evidence for a maximal cytological reduction in the management of newly diagnosed malignant gliomas (9, 10).

Patients with brain tumors may have different symptoms and clinical signs depending on their location, growth rate and presence of intracranial hypertension. The symptomatology associated with brain tumors is mainly determined by the tumor mass, adjacent edema, or normal brain tissue infiltration and destruction (11). Tumors positioned in functional areas of the cortex or along the ventricular system, with a high rate of growth, are clinically manifest shortly after onset, unlike those located in nonspecific areas, with late-onset manifestations (12). Among the most common symptoms are: headache, convulsions, sensory disturbances, motor or language deficits, confusion, personality changes, memory loss and visual deficits (11, 13).

Currently, treatment of malignant gliomas involves both surgical resection and multimodal treatment (radiotherapy, chemotherapy), considering that the best treatment option is to remove as much tumor volume as possible, while maintaining morbidity at a minimum level (14). There is increasing evidence that a maximum surgical cytoreduction (more than 98% of the initial tumor mass) is an important prognostic factor for patient survival, but this is not easy to achieve due to the infiltrative nature of these tumors (15). Despite the indisputable advances in imaging-guided surgical technique (neuronavigation), targeted radiotherapy and new chemotherapies (e.g. temozolamide, carmustine), the prognosis of patients diagnosed with malignant gliomas is currently gloomy, so it is imperative to develop new antitumor strategies (16, 17, 18). Remarkable discoveries in molecular biology and biotechnology, and particularly a better understanding of tumor biology (angiogenesis, apoptosis, immune response) open major possibilities for improving the prognosis of these patients. Biotech and especially biologic progress will open new attractive therapeutic targets to decrease the proliferation process of malignant brain tumors (14).

In the present study we aimed at evaluating the efficacy of a modern therapeutic procedure, surgical resection of malignant gliomas using 5-Amino-Levulinic Acid (5-ALA).

**MATERIAL AND METHODS**

We conducted a study of 127 patients diagnosed with malignant glioma, aged 20 to 80 years, admitted to Neurosurgical clinics (I, II, III) of the Iasi "Prof. Dr. N. Oblu" Hospital, between 2009 and 2015. Of these patients, 17 underwent surgical resection and 5-ALA (Gliolan®) treatment. 5-ALA was administered orally, 3 hours before the induction of general anesthesia,
Evaluation of the efficacy of surgical resection of malignant gliomas using 5-amino-levulinic acid

under the strict supervision of the anesthetist. It is considered to be a relevant biomarker for the gross total resection of glioblastomas which has received approval for use in Europe since 2008 (19). The experimental protocol has been approved by the Hospital Ethics Committee, in accordance with the international regulations regarding the clinical trials and use of medical data, and the informed consent was obtained from all the enrolled patients. Inclusion criteria were: age over 18 years, diagnosis of malignant glioma confirmed by imagining methods. Exclusion criteria were: refusal of subjects or their family to be included in the clinical study, history of allergy to any of the 5-ALA compounds, porphyria, chronic liver disease, mental illness, non-cooperating patients. The following data were collected from all patients: socio-demographic data, clinical manifestations, biochemical parameters, tumor imaging and histopathologic appearance, therapeutic method used, efficacy of treatment over time, survival rate, relapses.

Evaluating the therapeutic efficiency and assessing the surviving subjects for a certain period of time after treatment was performed using the Kaplan-Meier analysis (20). This method (21) estimates data regarding the survival of patients with various diseases under a specific treatment, at different times during the investigation (especially when not all subjects continue the study). It also provides the comparative analysis of the efficacy of different applied therapeutic methods. In clinical trials, the effect of a therapeutic procedure is measured by counting the number of subjects surviving or being rescued at a specified time after applying the treatment. This type of analysis involves calculating the probability of occurrence of an event at a set time point (20).

The probability of survival at a given time point in the study is calculated using the following formula: (number of subjects living at the beginning of the study – number of deceased subjects) / number of subjects living at the beginning of the study (20).

Data were expressed as +/- standard deviation and significance was tested using un-paired t-test and Fisher’s method. P-values below 0.05 were considered statistically significant.

RESULTS

Kaplan-Meier’s analysis revealed that patients with glioma who underwent surgery associated with Gliolan® treatment had a higher survival rate at 6 months, 1 year and 2 years, respectively, compared to the group without Gliolan®, treated using classic procedures (tumor resection associated with radiotherapy and/or antineoplastic chemotherapy). The survival rate was significantly higher in the Gliolan® group (0.65), compared with the group treated by classic methods (0.48), especially after 2 years of treatment (fig. 1).

Statistical data analysis revealed that all 17 patients who were treated with Gliolan® survived 6 months after surgery. Long-term monitoring of the subjects included in the study showed that: at the 6-month follow-up the disease recurred in 2 of the subjects; 15 of the patients survived 1 year after therapy (1 patient with relapse); 11 of the patients treated with Gliolan® survived at 2 years (in 9 of them the disease recurred) (fig. 2, 3). Statistical data processing revealed that all 110 glioma patients treated by classic methods (radiotherapy and/or antineoplastic chemotherapy) survived 3 months after surgical intervention (fig. 4, 5).
Fig. 1. Survival rate of patients with glioma according to the administered treatment

Fig. 2. Assessing the efficacy of Gliolan® therapy by survival rate in time

Fig. 3. The presence of relapses in patients treated with Gliolan®

Fig. 4. Assessing the efficacy of classic therapy (without Gliolan®) by survival rate at 6 months, 1 year and 2 years after treatment
Evaluation of the efficacy of surgical resection of malignant gliomas using 5-amino-levulinic acid

The long-term assessment of the subjects included in the study showed that: one month after surgery the disease recurred in 2 patients; at the 3-month follow-up, it was found that the disease relapsed in 7 of the investigated patients; 65 patients survived 6 months after therapy (5 with relapses); 56 patients survived 1 year after therapy (in 18 the disease recurred); 32 patients survived at 2 years (but 26 presented disease relapse).

DISCUSSION
Over the past six decades, a variety of innovative techniques have been developed to help neurosurgeons and to increase the effectiveness of malignant brain tumor resections. Among these methods that have contributed to both improved and increased life expectancy, we mention: intraoperative electro-physiological monitoring, neuro-navigations techniques, intraoperative ultrasound and magnetic resonance, and also fluorescein-guided cytoreductive surgery, involving the use of 5-aminolevulinic acid (9, 10).

Since its approval by the US Food and Drug Administration (FDA) in 2007, numerous studies have reported the use of 5-aminolevulinic acid in various brain tumors such as: meningioma, metastases or gliomas (19, 20). 5-Aminolevulinic acid is a natural compound produced in hemoglobin metabolism pathways, and its exogenous administration allows it to penetrate the blood-brain barrier and to be captured in neoplastic cells from both tumor and infiltration zones. By photodynamic action it induces apoptosis and tumor cell death, thus allowing maximum resection (23).

The steps taken to approve the use of this substance began after several clinical trials conducted in the neurosurgery departments of various university clinics which showed the benefits of maximal resection, with an improvement of the survival rate. In 2001, Lacroix M. (24) demonstrated an increase in the 5-month survival interval in patients with glioblastoma after maximum resection and treatment with Gliolan®. Four years later, Van den Bentet MJ (25) substantiated an increase in survival rate of 18.3 months in patients treated with Gliolan® compared with 13.5 months in those treated by the classical method, result which supports the efficacy of this substance.

CONCLUSIONS
In our clinical investigation we proved that intraoperative use of the navigation system has been of real help taking into account the changes that may occur, reach-
ing an accuracy of less than 1 cm, without altering the actual location of the tumor. This study showed that the survival rate was significantly higher in the Gliolan® group compared to the group treated by classic procedures, particularly 2 years after tumor resection. Based on the observations that 5-ALA is an important intraoperative marker for direct visualization of the tumor and infiltrative tissue, we can support the idea that it provides the basis for a feasible future in glioblastoma therapeutic management, promising an increase in survival rate compared to classic surgical method, by achieving a maximum resection.

REFERENCES

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**NEWS**

**SYNTHESIS OF HIERARCHICAL CuO NANOSTRUCTURES: BIOCOMPATIBLE ANTIBACTERIAL AGENTS FOR GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA**

Copper Oxide is one of the important materials among other antimicrobial agents such as silver, zinc oxide, or titanium dioxide. CuO nanostructures have stimulated great interest in the field of environmental remediation because of their important characteristics which include: high stability, structural properties, antimicrobial activity. The novelty of this study is the biocompatibility determination of nanostructured CuO. The findings of the present work proved that the prepared CuO nanostructures are highly stable in aqueous solution and biocompatible in blood. They also exhibited pronounced bactericidal activity towards both Gram positive and Gram negative bacteria (Sonia S, Jayasudha R, Jayram ND, et al. Synthesis of hierarchical CuO nanostructures: Biocompatible antibacterial agents for Gram-positive and Gram-negative bacteria. *Current Applied Physics* 2016; 16: 914-921).

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