ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ВПЛИВУ СУПЕРНАТАНТУ НЕЙРОГЕННИХ КЛІТИН ЩУРА ТА ІМУНОМОДУЛЮЮЧОГО ПРЕПАРАТУ НА МОНОНУКЛЕАРИ ПЕРИФЕРИЧНОЇ КРОВІ ХВОРИХ З ГЛІОМАМИ ГОЛОВНОГО МОЗКУ in vitro

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Мета: *дослідити вплив супернатанту нейрогенних клітин (СНК) щура на мононуклеари периферичної крові (МНПК) хворих з гліомами головного мозку та порівняти його з дією препарату з встановленими імуномодулюючими властивостями (галавіт).*

Матеріали і методи. *Досліджували МНПК хворих з гліомами головного мозку (n=20): анапластичними астроцитомами (III ступінь злоякісності, n=9) і гліобластомами (IV ступінь злоякісності, n=11); та осіб групи порівняння (умовно здорові особи без онкологічного захворювання, n=20). СНК виготовляли з тканини мозку щура на 14-у добу гестації. Препарат СНК та галавіт (ЗАТ «ЦСМ «МЕДИКОР», РФ) у кількості 0,10 мг/мл додавали до суспензії свіжовиділених МНПК та інкубували протягом 24 год. До та після інкубації з препаратом у суспензії визначали кількість життєздатних клітин, апоптичних клітин (PI+, CD95+), експресію активаційних антигенів CD25, HLA-DR.*

Результати. *СНК не впливав цитотоксично або впливав несуттєво на МНПК хворих з гліомами. Галавіт проявляв достовірно більш виражену цитотоксичну дію на МНПК хворих з гліомами, ніж СНК. СНК і галавіт демонстрували тенденцію до проапоптотичного впливу на МНПК хворих з гліомами. СНК і галавіт суттєво не змінювали співвідношення диференційних та активаційних антигенів, експресованих імунокомпетентними клітинами хворих з гліомами.*

Висновок. *Порівняльне дослідження показало, що СНК щура в концентрації 0,10 мг/мл не впливає цитотоксично на МНПК хворих з гліомами, на відміну від імуномодулюючого препарату галавіт.*

Ключові слова: *мононуклеари периферичної крові, гліоми головного мозку, супернатант прогеніторних нейроклітин щура, галавіт, суспензійні культури.*

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ВЛИЯНИЯ СУПЕРНАТАНТА НЕЙРОГЕННЫХ КЛЕТОК КРЫСЫ И ИММУНОМОДУЛИРУЮЩЕГО ПРЕПАРАТА НА МОНОНУКЛЕАРЫ ПЕРИФЕРИЧЕСКОЙ КРОВИ БОЛЬНЫХ С ГЛИОМАМИ ГОЛОВНОГО МОЗГА in vitro

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Цель: *исследовать влияние СНК крысы на мононуклеары периферической крови (МНПК) больных с глиомами головного мозга и сравнить его с действием препарата с установленными иммуномодулирующими свойствами (галавит).*

Материалы и методы. *Исследовали МНПК больных с глиомами головного мозга (n=20): анапластическими астроцитомами (III степень злокачествен-*

ности, n=9) и глиобластомами (IV степень злокачественности, n=11); и лиц группы сравнения (условно здоровые лица без онкологического заболевания, n=20). СНК получали из ткани мозга крысы на 14-е сут гестации. Препарат СНК и галавит (ЗАО «ЦСМ «МЕДИКОР», РФ) в количестве 0,10 мг/мл добавляли к суспензии свежевыделенных МНПК и инкубировали в течение 24 ч. До и после инкубации с препаратом в суспензии определяли количество жизнеспособных клеток, апоптических клеток (PI+, CD95+), экспрессию активационных антигенов CD25, HLA-DR.

Результаты. СНК не влиял цитотоксически или влиял несущественно на МНПК больных с глиомами. Галавит проявлял достоверно более выраженное цитотоксическое действие на МНПК больных с глиомами, чем СНК. СНК и галавит демонстрировали тенденцию к проапоптотическому влиянию на МНПК больных с глиомами. СНК и галавит существенно не изменяли соотношение дифференцировочных и активационных антигенов, экспрессированных иммунокомпетентными клетками больных с глиомами.

Вывод. *Сравнительное исследование показало, что СНК крысы в концентрации 0,10 мг/мл не влияет цитотоксически на МНПК больных с глиомами, в отличие от иммуномодулирующего препарата галавит.*

Ключевые слова: *мононуклеары периферической крови, глиомы головного мозга, супернатант прогениторных нейроклеток крысы, галавит, суспензионные культуры.*

Treatment of patients with malignant brain gliomas remains one of the unsolved and urgent problems of modern neurooncology. One of the directions that are actively being developed to optimize the treatment of these patients is the search of drugs aimed at stimulating of antitumor immunity and inhibition of tumor proliferation. It is known that changes at all levels of immunity are in patients with brain gliomas [3]: relative and absolute lymphopenia, low content of CD4+, CD8+, CD16+ lymphocytes, HLA-DR-cells, inhibition of the lymphocytes proliferative ability, reduced secretion of effector cytokines. Along with the decrease in the number and function of CD4+, CD8+ cells, the number of T-regulatory CD4+CD25+FoxP3+ cells, producing immune depressing mediators, increases.

Topical approach in the treatment of brain gliomas is to use neural progenitor cells (NPC) and their products that are known to be able to exhibit anticancer properties [8,9,15]. In previous studies we have established antitumor activity of rat progenitor neurogenic cells supernatanat (RPNS) in dissociated cultures of human gliomas [2] and when administered to rats with experimental glioma 101.8A [4]. Also, the immunomodulatory potential of NPC is investigated [13].

The aim of this work was to study the influence of RPNS on the peripheral blood mononuclear cells (PBMC) of patients with brain gliomas and to compare it with influence of the preparation with established immunomodulatory properties. As such preparation drug halavit (5-amino-

1,2,3,4-tetrahidroftalazyn 1,4-diyenosodium salt) was chosen – antiinflammatory and immunomodulatory drug used in general oncology [1,10]; as in previous in vitro studies its direct cytodestructive and antiproliferative effects on cells of malignant gliomas were demonstrated [5].

Materials and methods of researches. PBMC of patients with brain gliomas (n=20) and persons of comparison group (conditionally healthy individuals without cancer, n=20) were studied. As a result of histological verification of the diagnosis in patients the 9 anaplastic astrocytomas (III degree of malignancy (d.m.)) and 11 glioblastomas (IV d.m.) were diagnosed according to the International histological classification of tumors of the central nervous system [16].

PBMC were isolated by centrifugation in fikoll-verohrafin gradient (d=1,077) at 1500 rev/min for 30 min, then washed twice in buffered saline pH 7,2-7,4. Cell viability was determined by the permeability of the plasma membrane for 0.2% trypan blue ("Merch", Germany) [6].

RPNS was received from rat brain tissue on 14th day of gestation [2].

RPNS and halavit («MEDICOR», Russia) in concentration 0.10 mg/ml was added to fresh-isolated PBMC suspensions and incubated for 24 h. The choice of this concentration was based on previous reports, which were found the cytotoxic effect of RPNS and halavit on brain tumors samples in concentration 0,02-0,10 mg/ml [2,5]. Before and after incubation with preparations cell suspensions were analized for number of viable cells, apoptotic cells (PI+, CD95+), level of activation antigen expression (CD25+, HLA-DR+).

RPNS and halavit cytotoxic effects were evaluated by cytotoxic index (CI):

$$CI = \frac{VCi - VCi_{+P}}{VCi} \times 100\%,$$

where VCi – number of viable cells in the initial suspension;

VCi_{+P} – number of viable cells in the suspension after incubation with preparation (RPNS or halavit).

Apoptotic PI+cells were determined by cytofluorymetryc method using propidium iodide (PI) (0.05 mg/ml) according to the recommendations [6].

PBMC expression of antigens CD25 (IL-2 receptor □-chain (IL-2RA)), CD-95 (FAS-receptor) and HLA-DR (class II histocompatibility antigen) was determined by indirect immunofluorescence method using monoclonal antibodies («Sorbent», Russia). Cell suspensions were analyzed before and after incubation with preparations by flow immunocytofluorymeter FACSCalibur (USA) as recommended by [6].

Statistical analysis of data was performed using the statistical software package "Statistica 6.0", the reliability difference was evaluated using Student t-test.

Results and discussion. Incubation with RPNS slightly affected the number of viable PBMC of patients with gliomas. RPNS did not have cytotoxic effect on 55.6% of PBMC samples of patients with gliomas of III d.m., on 11.1% samples effected with CI_{25}, on 22.2% – had CI_{50}. The RPNS cytotoxic effect on PBMC of patients with glioblastomas on average was also low: the preparation had no effect on 54.5% of PBMC samples, on 9.1% – effected with CI_{25}.

Average values of halavit cytotoxicity in PBMC suspension cultures of patients with glioblastomas were higher than those in PBMC cultures of patients with anaplastic astrocytomas (fig.1); halavit affected on 71.4% of the studied PBMC samples of patients with anaplastic astrocytomas and on all samples of the PBMC of patients with glioblastomas.

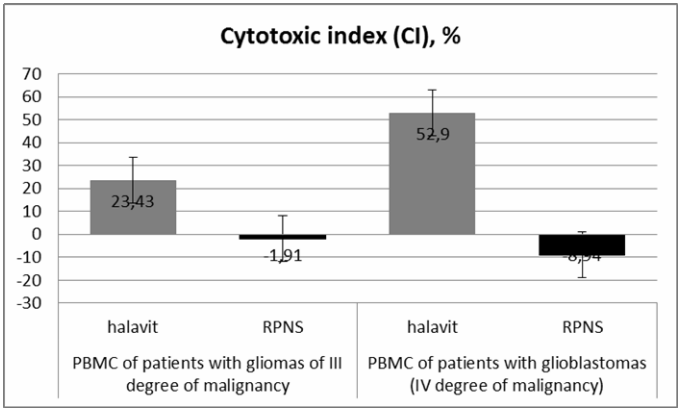

Figure 1. **Cytotoxic effect of halavit and RPNS on the PBMC of patients with gliomas (%).**

Difference was found in cytotoxic impact of halavit and RPNS in concentration 0.10 mg/ml on the PBMC of patients with glioblastomas (fig.1): mean CI of RPNS was significantly lower than CI of halavit ($p<0,026$). Ratio of studied PBMC samples of patients with gliomas of III and IV d.m., on which halavit had cytotoxic effect, significantly exceeded the proportion of samples on which RPNS affected similarly.

The number of PI+cells in PBMC suspension cultures of patients with gliomas characterized by large scale fluctuations of individual parameters. The initial PBMC suspensions of patients with glioblastomas contained fewer

amounts of CD95+ and PI+cells than PBMC suspensions of patients with anaplastic astrocytomas of III d.m. (fig.2). Under conditions of RPNS influence in PBMC suspension cultures of patients with glioblastomas the number of CD95+cells slightly increased (by an average of 2%); number of apoptotic PI+cells increased by an average of 5% in PBMC suspension cultures of patients with gliomas with III and IV d.m.

It is known that the expression of CD95+ only demonstrates the commitment to apoptosis, and PI dye detects the hypodyploid cells in terminal stages of apoptosis and necrosis, when they have lost some DNA [6]. So RPNS in concentration 0.10 mg/ml showed a tendency to proapoptotic effects on PBMC of patients with gliomas, increasing the number of CD95+PBMC carrying the FAS-receptor which expression reflects the commitment to apoptosis (in suspension cultures of patients with glioblastomas) and PI+PBMC on the end-stage of apoptosis (in patients with gliomas of III and IV d.m.).

Under the influence of halavit the number of PI+cells slightly increased (an average of 3%) in PBMC suspension cultures of patients with gliomas of III d.m., and CD95+cells (an average of 3%) – in patients with glioblastomas, but significant differences of these parameters were not found (fig.2).

Analysis of the activation antigens expression showed (fig.2) that the initial PBMC suspensions of patients with gliomas of III d.m. contained slightly increased amounts of CD25+cells (mean 2.5%). Under the conditions of RPNS influence these rate tended to increase in PBMC of patients with glioblastomas (an average of 3% compared to PBMC of relatively healthy individuals). Thus, the increasing of viability and number of cells under the influence of RPNS in PBMC suspension cultures of patients with glioblastomas correlated with the tendency to increasing of the number of CD25+cells bearing the IL-2 receptor α-chain (IL-2RA).

It should be noted that the increase of the number of CD25+cells under the influence of RPNS on PBMC of patients with glioblastomas may have ambiguous interpretation. On the one hand, the expression of IL-2RA indicates the activated state of cells, their ability to proliferate in response to the stimulus of IL-2 and restore the pool of immunocompetent cells in patients with glioblastomas, which immune system is characterized by lymphopenia [3]. On the other hand, IL-2RA is one of the

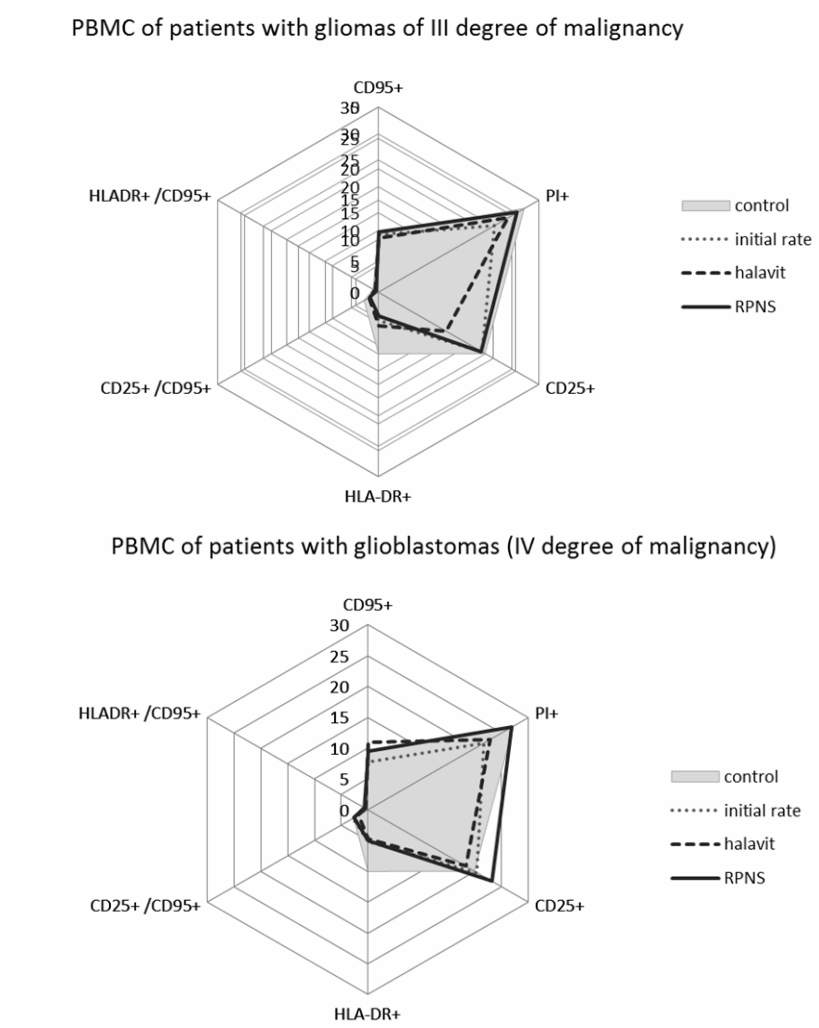

Figure 2. **The ratio of differential and activation antigens expressed by immunocompetent cells of patients with gliomas (%).**

8 genes with immune function associated with glioblastoma [14]. IL-2RA expressed on the surface of T-regulatory CD4+FoxP3+cells that form the immunosuppressive microenvironment of glioblastoma and is the dominant mechanism for avoiding of gliomas from immune response; number of

T-regulatory CD4+CD25+FoxP3+cells increased in tumors and peripheral blood of patients with glioblastomas [14].

At the same time, under the influence of halavit the number of CD25+cells decreased in patients with gliomas of III and IV d.m. (average 7.5% and 3% respectively). This is clearly explained by the known ability of this drug to inhibit hyperactivated cells [1].

Percentage of PBMC of patients with malignant brain gliomas expressing class II histocompatibility antigen HLADR was lower by an average of 5% less than in the PBMC of control group. These data confirm the known literature data that the loss of ability to express major histocompatibility complex molecules is one of the pathogenetic links of malignant gliomas [11, 12].

Under the conditions of both RPNS and halavit influence, the number of HLADR+cells in PBMC suspension cultures of patients with gliomas were not significantly changed.

Activation index CD25+/CD95+, reflecting the option "formation/ elimination" and indicating the predominance of one of the ways of development: readiness to FAS-mediated apoptosis or readiness of lymphocytes to proliferate and differentiate, changed slightly in PBMC of patients with gliomas under the influence by RPNS and halavit compared with the PBMC of control group (fig.2). Initial index HLADR+/CD95+, reflecting the option "mature/elimination" was significantly reduced in PBMC of patients with gliomas of III and IV d.m. compared to PBMC of control group ($p<0,0006$, $p<0.016$ respectively), demonstrating the prevalence of readiness of lymphocytes to FAS-mediated apoptosis on the readiness to acquiring the late differentiation antigen, and may lead to a shortage of main populations of immune cells. In addition, the index HLADR+/CD95+ in PBMC of patients with gliomas of III d.m. was significantly lower than that of patients with glioblastomas ($p<0,042$). After 24 h of incubation with both RPNS and halavit index HLADR+/CD95+ in PBMC of patients with gliomas remained relatively lower than respective indicator of conditional control ($p<0,0012$).

So humoral factors of fetal rat brain neurogenic cells, as well as halavit, did not significantly change the ratio of differential and activation antigens expressed by immunocompetent cells of patients with gliomas.

Some differences in the impact of the investigated preparations on PBMC of patients with anaplastic astrocytomas and glioblastomas, apparently are explained by the degree of molecular-genetic changes in cells of tumor-carriers patients, depending on the histobiological type of glioma and level of malignant transformation, the status of apoptosis signaling pathways etc.

Unlike previously established cytotoxic effects of RPNS on brain gliomas in experiment in vitro and in vivo [2,4], RPNS showed no significant cytotoxic effects on immune cells of patients with gliomas. We believe that

further research in this direction will conclude on RPNS possible administration as the drug for therapeutic purposes in patients with gliomas.

Conclusions

1. RPNS in concentrarion 0.10 mg/ml did not have the cytotoxic effect or had only slightly cytotoxic effect on PBMC of patients with gliomas. Halavit in concentrarion 0.10 mg/ml showed significantly more pronounced cytotoxic effect on PBMC of patients with gliomas than RPNS.
2. RPNS as well as halavit showed a tendency to proapoptotic effects on PBMC of patients with gliomas.
3. RPNS as well as halavit did not significantly change the ratio of differential and activation antigens expressed by immunocompetent cells of patients with gliomas.

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ОСОБЛИВОСТІ ПЕРЕБІГУ МНОЖИННОЇ МІЄЛОМИ У ХВОРИХ З ЦИТОГЕНЕТИЧНИМИ АНОМАЛІЯМИ В УМОВАХ СУЧАСНОЇ ТЕРАПІЇ

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Резюме. *В роботі представлено аналіз ефективності лікування хворих на множинну мієлому з несприятливими хромосомними аберраціями та у пацієнтів з нормальним каріотипом. На основі даних літератури і власного клінічного досвіду обґрунтовано необхідність цитогенетичного та молекулярно-генетичного (FISH) обстеження хворих для визначення у них оптимальної лікувальної тактики.*

Ключові слова: *множинна мієлома, хромосомні аберації, лікувальна тактика.*