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Passing across the blood-brain barrier in glioblastoma multiforme (GBM)

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SUMMARY:

Introduction and purpose: Blood-brain barrier (BBB) consists of capillary endothelium, in which there are three types of intercellular junctions - adherent, tight and gap junctions. Efficient therapy involves delivering a therapeutic dose of drug into a specific site in the body, and maintaining this dose for adequate time afterwards. The aim of this study is to review current knowledge of new strategies in drug delivery to CNS and the effectiveness of these methods in glioblastoma multiforme (GBM) treatment. This review was performed using the PubMed database.

A brief description of the state of knowledge: Methods for delivering drugs to the brain are divided into invasive and non-invasive. Invasive methods involve temporary disrupting tight intercellular junctions of the vascular endothelial cells and delivering drugs intracerebrally or intraventricularly during neurosurgical procedures. In recent years, there has been a growing interest in the use of nanoparticles as drug carriers to the central nervous

system via blood-brain barrier. The usage of nanoparticles implies many advantages, such as non-invasive, low cost, good biodegradability, stability, ability to carry various types of agents, selectivity and ability to control drug release.
Conclusions: Limited options in treating brain located tumors, including glioblastoma multiforme, due to difficulties in drug penetration through the BBB engages scientists to search for new treatments. Crossing the BBB using invasive methods based on interruption of cell junctions show promising results, but they are associated with i.a. a high risk of uncontrolled influx of toxins to the CNS or ion-electrolyte imbalance, which may lead to neuronal dysfunction. Invasive methods can be effective only in tumors, while treatment of diseases such as Alzheimer's disease is impossible. Recent studies show that nanoparticles would be a great, non-invasive alternative, but they are difficult to use with relatively low permeability through undamaged BBB. In some studies using nanoparticles as nanocarriers (EDVDox) or SYMPHONY method (combining photothermal therapy with GNS and immunotherapy of checkpoints in a mouse model) against GBM shows positive results. More research is required to confirm the effectiveness and safety of these treatments.

Key words: the blood-brain barrier; oncology, treatment, nanoparticles

Description of the state of knowledge

1. **Blood-brain barrier (BBB).**

Blood-brain barrier (BBB) consists of capillary endothelium, in which there are three types of intercellular junctions - adherent, tight and gap junctions[1]. BBB function is to separate neurons from systemic circulation and thus provide a highly regulated central nervous system environment, which is crucial to synapses and neurons proper functioning. Blood vessels of the central nervous system have unique features, which enable highly strict ion, particles and cells transport regulation. This precise control of hemostasis protects brain tissue and if interrupted, pathological changes are likely to occur[2]. BBB disruption causes a flow of neurotoxic factors, cells and pathogens from systemic circulation to brain tissue, which could lead to immune reaction and inflammatory reaction development and thus neurons degradation[3].

The perfect mechanism to deliver therapeutic agents to the central nervous system should be easy to control, selective and not harmful to the blood-brain barrier. Moreover, drug carrier should be non-invasive, not toxic and biodegradable. Drug concentration should reach a therapeutic concentration in the site of action and remain for eligible time to induce the greatest effect[4].

2. **Blood-brain barrier in central nervous system neoplasms.**

According to American Cancer Society the number of new cases of cancer in 2040 will reach nearly 27,5 million, while in the end of 2020 over 23,8 thousand adults and 3,5 thousand children under the age of 15 will be diagnosed with primary brain tumors or

primary spinal cord tumors[5,7]. Primary brain tumors are 5-10% of all neoplasms and are challenging both in clinical oncology and research studies, as the 5-year survival rate for patients with CNS cancer is 36% and the 10-year survival rate is 31%[6,8].

The most common CNS tumors in adult patients are gliomas. They are classified based on histopathology image as astrocytic, oligodendroglial and ependymal tumors, or based on malignancy grade - II, III or IV grade. Depending on patients' age, 40 to 90% gliomas are malignant and because of that they are known as the most common lethal primary brain tumors. Gliomas annual morbidity is estimated at 0,5-2/100 thousand people. Men are diagnosed twice as often, usually in 5 or 6 decade of life. Among Polish population the incidence of gliomas is approximately 1300 cases per year, while malignant gliomas are about 600 cases[7].

Because of complexity in CNS neoplasms morphology, they are quite resistant to the treatment[6]. The presence of BBB makes the treatment even more challenging due to difficulties on drug delivery, which leads to frequent relapses after tumor removal surgery. Moreover, currently used agents are not specific for neoplasm tissue and are harmful for normal cells. As stated, targeted therapy is crucial for reducing relapsing frequency and treatment toxicity[9].

3. Challenges in crossing blood-brain barrier.

Efficient therapy involves delivering a therapeutic dose of drug into a specific site in the body, and maintaining this dose for adequate time afterwards. As BBB unables most of therapeutic agents administration to the brain tumor, both therapy, diagnosis and prevention of disease progression is difficult to accomplish[10]. Nearly 100% of macromolecular and 98% of micromolecular neurotherapeutics cannot pass through the BBB by passive diffusion due to the presence of tight gap junctions. Moreover, between epithelial cells there is high electric resistance (1500-2000 Ω cm 2), caused by the encapsulation of capillaries above pericytes and astrocytes. These features are significant limitations to introducing new agents to the central nervous system diseases, as the many of them showed efficacy *in vitro* and could not pass the BBB in the living organism models[12,13].

4. Strategies to cross the blood-brain barrier.

Strategies for delivering drugs to the brain are divided into invasive and non-invasive methods. Invasive methods involve temporary disrupting tight intercellular junctions of the vascular endothelial cells and delivering drugs intracerebrally or intraventricularly during neurosurgical procedures. On the other hand, non-invasive methods are based on molecular mechanisms and drug's chemical modifications[12,13]. Temporary disruption of proteins of the extracellular matrix is considered the most convenient way of delivering drugs to CNS. This method draws upon hyperosmotic solutions and the phenomenon of osmotic shock to constrict the endothelial cells and disturb cell-junctions' function. Another method that shows

therapeutic potential in chemotherapy is ultrasound-mediated drug delivery (USMD). In USMD, microbubbles with a diameter of 1-10 microns are used to mechanically break cell junctions and ultrasound waves, which are aimed at the area of a few millimeters, ensure precise delivery of chemotherapeutic agents[13]. Using USMD in animal models, drugs commonly used in chemotherapy, including: doxorubicin, carmustine, trastuzumab and temozolomide, have been successfully transported through BBB[14]. Carpentier et al. conducted a clinical trial evaluating effectiveness of pulsed ultrasounds in patients with recurrent glioblastoma multiforme before carboplatin treatment. For this purpose, the BBB was disrupted monthly using pulsed ultrasound with systemically injected microbubbles. The BBB was disrupted at sound pressure down to 1,1 MPa without any detectable adverse effects in radiological investigation and clinical examination. The study proved that sequential disrupting the BBB with a pulsed ultrasound in combination with systemic microbubbles injections, is safe and well tolerated and it could optimize the delivery of chemotherapy to the brain[15]. Despite promising results, invasive methods based on interruption of cell junctions cause the BBB integrity damage. They are associated with a high risk of an uncontrolled influx of toxins to the CNS, leakage of membrane proteins, release of cytokines and neurotransmitters, ion-electrolyte imbalance, which may lead to neuronal dysfunction[12]. The most direct methods of drugs supplying to the CNS tumor are intracerebral and intraventricular injections as well as biodegradable plates impregnated with chemotherapeutic agents implanted into post-resection cavities. Both methods draw upon the phenomenon of drug diffusion into the nervous tissue. However, this technology has significant limitations caused by the irruptive decrease of diffusion depending on the distance travelled by the drug into nervous tissue. It requires high accuracy in gauging the injection or implantation site towards maximally targeting the drug to the tumor[13]. It is necessary to enter high drug's concentrations to create an appropriate concentration gradient, which is fundamental to distribute active ingredients over a considerable distance through nervous tissue. Unfortunately, such high concentration of chemotherapeutic agents might be toxic to undamaged nervous tissue and lead to significant side effects[16]. To improve the delivery of drugs into the nervous tissue the convection-enhanced delivery (CED) has been developed[17]. In CED, implanted intracranial catheters administer the drug at precisely controlled infusion rate[16]. The employment of these invasive methods is limited only to the treatment of localized pathological processes of CNS, e.g. tumors. It is not possible to use these methods in case of widespread demyelinating diseases such as Alzheimer's disease or multiple sclerosis. Moreover, they are associated with a high risk of infections, mechanical injuries, vascular thrombosis or cerebral hernia[13].

5. Nanoparticles.

In recent years, there has been a growing interest in the use of nanoparticles as drug carriers to the central nervous system via blood-brain barrier. The usage of nanoparticles implies many advantages, such as non-invasive, low cost, good biodegradability, stability, ability to carry various types of agents, selectivity and ability to control drug release. Nanoparticles approved for drug transmission through BBB include polymers, for example poly(lactic-co-glycolic acid) (PLCA) and poly(lactic acid) (PLA), liposomes and non-organic

nanoparticles, such as AuNP, zinc oxide nanoparticles and carbon quantum dots[12]. Depending on therapeutic or diagnostic method, the drug can be dissolved, entrapped, adsorbed, encapsulated or covalently attached to the nanoparticle and give nano-sized conjugates of drug and its vehicle[10]. Coating nanoparticles with surface acting agents, such as polysorbate 80 and poloxamer 80, enables their transport into endothelial cells. After entering the vessel nanoparticles absorb apolipoprotein E or A-I, which binds to receptors on the endothelial cells surface and induces endocytosis. In the next 15-30 minutes nanoparticles are delivered to brain cells due to transcytosis[18]. Moreover, attaching certain ligands, such as specific antibodies, to the nanoparticles, allows agents to enter to the particular site with overexpressing proteins through the mechanism of molecular recognition. It is also possible to manipulate drug release from nanoparticles, depending on pH and temperature of the environment[19]. However, there are some difficulties, such as not yet well-understood physiological response to the nanoparticles, their deposition in the body and inducing inflammatory, oxidative and cytotoxic reactions. Other obstacles refer to the complicated production process, which can lead to instability of the nanoparticle over prolonged storage, as it is sensitive to changes in temperature and pressure[20].

6. Nanotechnology on glioma therapy.

Gliomas are difficult to treat. Therapy includes surgery resection and radiotherapy and chemotherapy afterwards. Pharmacology treatment is based on temozolomide, doxorubicin and monoclonal antibodies, however new methods are required. Nanotechnology provides new ways for therapy development. Using nanoparticles as drug carrier facilitate drug delivery and are promising novel agents in CNS tumors treatment[21,22,23].

First attempt to use nanoparticles in glioblastoma multiforme treatment was carried out by Whittle et al. They used nanoparticles as doxorubicin vehicle (EDVDDox) to target epithelial growing factor receptor (EGFR), which demonstrate overexpression in 40-50% GBM cases. Results of the study showed that nanoparticles were well tolerated in patients with relapsed GBM and did not demonstrate serious adverse effects[24]. Other researchers also studied the usage of nanotechnology in cancer therapies and it resulted in novel methods, such as Synergistic Immuno-Photothermal Nanotherapy (SYMPHONY) created by Yang Liu et al. This method is based on using plasmonic gold nanostars (GNS) as photothermal inducers and inhibiting checkpoints by immunotherapy. In this study researchers implanted glioblastoma cells into mouses to induce tumor growth. Afterwards, mouses were randomized and treated with different methods - photothermal therapy, GNS and immunotherapy anti-PD separately and in various combinations. Size of the tumor was monitored and SYMPHONY group demonstrated the best results. Moreover, after SYMPHONY therapy mice were implanted with cancer cells again, but they reject it due to gained immune memory. This study showed efficacy of using complex therapy with nanoparticles and protection from relapses in animal model [25].

7. Clinical and preclinical trials.

The study by White et al. focused on establishing the maximum tolerated concentration of CED-administered carboplatin in the peritumor area. The study enrolled 18 patients with relapsed or progressive glioblastoma multiforme who were assigned to 6 cohorts with ascending carboplatin levels (starting with 0.03 mg/ml carboplatin in the first group and ending with 0.18 mg/ml in the sixth group)[26].

Ding et al. conducted a preclinical study on 40 rats to assess the safety of glioblastoma therapy with MR1-1 infusion (recombinant immunotoxin, targeted at EGFRvIII antigen, specific for GBM tumor) and Gd-DTPA (gadolinium conjugated with diethylenetriaminepentaacetic acid) using the CED method. For this purpose, all animals were implanted with intracerebral cannulae and randomly divided into 4 groups receiving different concentrations of MR1-1 and Gd-DTPA. The rats were clinically monitored for 6 weeks. Then a histological examination was performed to assess the CNS toxicity of the used method. All rats survived the entire study without clinical or histological toxicity ascribed to drugs. Co-infusion of MR1-1 with Gd-DTPA by the CED was considered safe for long-term preclinical treatment. There was no evidence of neurological toxicity or characteristic symptoms of neurological deficits such as head tilt, hemiparesis, or ataxic gait in any of the rats[27].

A study by Prados et al. assessed the effect of RMP-7, a bradykinin analog that temporarily increases BBB permeability, on carboplatin glioma therapy. This randomized study included 122 patients with glioblastoma multiforme or recurrent anaplastic glioblastoma, who were randomized to receive RMP-7 plus carboplatin (n=62) and carboplatin plus placebo (n=60). The median TTP was 9.7 weeks in the RMP-7 and carboplatin groups and 8.0 weeks in the placebo group, and the median survival was 26.9 weeks and 19.9 weeks, respectively. There was no difference in time between groups to worsening neuropsychological assessments, functional independence, or quality of life ratings. The use of RMP-7 had no effect on the pharmacokinetics and toxicity of carboplatin. The study showed that RMP-7, at this specific dose and schedule, did not significantly improve the efficacy of carboplatin, and higher doses of RMP-7 may be required to increase carboplatin delivery to the tumor[28].

Shen et al. used specially designed nanoparticles angiopep with high transcytosis capacity and parenchymal accumulation to improve the penetration of nanoparticles into the brain. The study tested the effectiveness of nanoparticles, labeled with ^{125}I angiopep-PEG-PLA micelles, by administering them intravenously to mice. Then the accumulation in the brain was checked, it showed high accumulation in the brain lasting up to 24 hours. These results showed that the angiopep modified PEG-PLA micelle is a promising brain-targeted nanocarrier for lipophilic drugs[29].

Conclusions:

Limited options in treating brain located tumors, including glioblastoma multiforme, due to penetration difficulties of substances through the BBB engages scientists to search for new treatments.

Crossing the BBB using invasive methods based on interruption of cell junctions shows promising results, but they are associated with i.a. a high risk of uncontrolled influx of

toxins to the CNS or ion-electrolyte imbalance, which may lead to neuronal dysfunction. Invasive methods can be effective only in tumors, and treatment of diseases such as Alzheimer's disease is impossible. Research shows that nanoparticles would be a great, non-invasive alternative, but they are difficult in usage with relatively low permeability through undamaged BBB. In some studies, using nanoparticles as nanocarriers (EDVDox) or SYMPHONY method (combining photothermal therapy with GNS and immunotherapy of checkpoints in a mouse model) against GBM shows positive results. More research is required to confirm the effectiveness and safety of these GBM's treatments.

References:

[1]Zhou Y, Peng Z, Seven ES, Leblanc RM. Crossing the blood-brain barrier with nanoparticles. *J Control Release*. 2018;270:290-303.

[2]Daneman R, Prat A. The blood-brain barrier. *Cold Spring Harb Perspect Biol*. 2015;7(1):a020412. Published 2015 Jan 5.

[3]Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol.* 2018 Mar;14(3):133-150.

[4]Jain K.K. Nanobiotechnology-based strategies for crossing the blood–brain barrier. Nanomedicine. 2012;7:1225–1233.

[5]Zottel A, Videtič Paska A, Jovčevska I. Nanotechnology Meets Oncology: Nanomaterials in Brain Cancer Research, Diagnosis and Therapy. *Materials (Basel).* 2019 May 15;12(10):1588.

[6]Lim M., Xia Y., Bettegowda C., Weller M. Current state of immunotherapy for glioblastoma. Nat. Reviews. Clin. Oncol. 2018;15:422–442.

[7]http://www.neurosciencereview.eu/index.php?option=com_content&view=article&id=68 &Itemid=54 [online accessed: 15.09.2020].

[8]https://www.cancer.net/cancer-types/brain-tumor/statistics [online accessed:15.09.2020]

[9]Patel D, Wairkar S, Mayur Yc. Current Developments in Targeted Drug Delivery Systems for Glioma. *Curr Pharm Des*. 2020;10.2174/1381612826666200424161929.

[10]Li X, Tsibouklis J, Weng T, et al. Nano carriers for drug transport across the blood-brain barrier. *J Drug Target*. 2017;25(1):17-28.

[12]Zhou Y, Peng Z, Seven ES, Leblanc RM. Crossing the blood-brain barrier with nanoparticles. *J Control Release*. 2018;270:290-303.

[13]Tam VH, Sosa C, Liu R, Yao N, Priestley RD. Nanomedicine as a non-invasive strategy for drug delivery across the blood brain barrier. *Int J Pharm*. 2016;515(1-2):331-342.

[14]Azad TD, Pan J, Connolly ID, Remington A, Wilson CM, Grant GA. Therapeutic strategies to improve drug delivery across the blood-brain barrier. *Neurosurg Focus*. 2015;38(3):E9.

[15]Carpentier A, Canney M, Vignot A, et al. Clinical trial of blood-brain barrier disruption by pulsed ultrasound. *Sci Transl Med*. 2016;8(343):343re2.

[16]White E, Bienemann A, Taylor H, Hopkins K, Cameron A, Gill S. A phase I trial of carboplatin administered by convection-enhanced delivery to patients with recurrent/progressive glioblastoma multiforme. *Contemp Clin Trials*. 2012;33(2):320-331.

[17]Kunwar S, Chang S, Westphal M, et al. Phase III randomized trial of CED of IL13-PE38QQR vs Gliadel wafers for recurrent glioblastoma. *Neuro Oncol*. 2010;12(8):871-881.
[18]Tosi G, Duskey JT, Kreuter J. Nanoparticles as carriers for drug delivery of macromolecules across the blood-brain barrier. *Expert Opin Drug Deliv*. 2020;17(1):23-32.
[19]Tsou YH, Zhang XQ, Zhu H, Syed S, Xu X. Drug Delivery to the Brain across the Blood-Brain Barrier Using Nanomaterials [published correction appears in Small. 2018 Jun;14(25):e1801588]. *Small*. 2017;13(43):10.1002/smll.201701921.
[20]Awasthi R, Roseblade A, Hansbro PM, Rathbone MJ, Dua K, Bebawy M. Nanoparticles in Cancer Treatment: Opportunities and Obstacles. *Current Drug Targets*, 2018;19:1696-1709.
[21]Bush NA, Chang SM, Berger MS. Current and future strategies for treatment of glioma. *Neurosurg Rev*. 2017;40(1):1-14.
[22]Patel D, Wairkar S, Mayur Yc. Current Developments in Targeted Drug Delivery Systems for Glioma [published online ahead of print, 2020 Apr 24]. *Curr Pharm Des*. 2020;10.2174/1381612826666200424161929.
[23]Xiong L, Wang F, Qi Xie X. Advanced treatment in high-grade gliomas. *J BUON*. 2019;24(2):424-430.
[24]Whittle JR, Lickliter JD, Gan HK, et al. First in human nanotechnology doxorubicin delivery system to target epidermal growth factor receptors in recurrent glioblastoma. *J Clin Neurosci*. 2015;22(12):1889-1894.
[25]Liu Y, Chongsathidkiet P, Crawford BM, Odion R, Dechant CA, Kemeny HR, Cui X, Maccarini PF, Lascola CD, Fecci PE, Vo-Dinh T. Plasmonic gold nanostar-mediated photothermal immunotherapy for brain tumor ablation and immunologic memory. *Immunotherapy.* 2019 Oct;11(15):1293-1302.
[26]White E, Bienemann A, Taylor H, Hopkins K, Cameron A, Gill S. A phase I trial of carboplatin administered by convection-enhanced delivery to patients with recurrent/progressive glioblastoma multiforme. *Contemp Clin Trials*. 2012;33(2):320-331.
[27]Ding D, Kanaly CW, Bigner DD, et al. Convection-enhanced delivery of free gadolinium with the recombinant immunotoxin MR1-1 [published correction appears in J Neurooncol. 2010 May;98(1):9. Dosage error in article text]. *J Neurooncol*. 2010;98(1):1-7.
[28]Prados MD, Schold SC Jr, Fine HA, et al. A randomized, double-blind, placebo-controlled, phase 2 study of RMP-7 in combination with carboplatin administered intravenously for the treatment of recurrent malignant glioma. *Neuro Oncol*. 2003;5(2):96-103.
[29]Shen J, Zhan C, Xie C, et al. Poly(ethylene glycol)-block-poly(D,L-lactide acid) micelles anchored with angiopep-2 for brain-targeting delivery. *J Drug Target*. 2011;19(3):197-203.