Abstract

Glioblastoma multiforme (GBM) is one of the most common brain tumors in adults. Its current first-line treatment is a surgical resection with systemic chemotherapy and radiotherapy. Despite extensive research, it remains challenging to treat and to cure. Photodynamic therapy (PDT), a local and minimally invasive procedure for cancer treatment, is promising in the context of specificity in comparison to traditional forms of anticancer therapies. The purpose of the study was to verify the feasibility of PDT with AGuIX nanoparticles functionalized to act as a photosensitizer (tetraphenyloporphyrin) targeted for tumor-associated blood vessels (DKPPR peptide homing for neuropilin-1 overexpressed in tumor vascular endothelial cells) against GBM and its vasculature.

The photodynamic effect of proposed treatment was verified in *in vitro* cell culture studies using immortalized macrovascular endothelial cells (ECRF-24 cell line) and human glioblastoma cells (U87-MG cell line). The therapeutic effect of PDT with functionalized AGuIX was examined in various *in vivo* models, i.e., human glioblastoma U87-MG xenografts grafted to chicken chorioallantoic membrane (CAM) model and mouse ectotopic tumor model, where U87-MG tumors were grown in Balb/c nude mouse limb. Vascular were investigated by USG with Doppler and EPR oximetry. Additionally, the orthotopic tumor model of human U87-MG tumors in Balb/c nude mice and the murine GL-261 model implanted in the brains of C57BL/6J mice using a stereotactic device was optimized. Its feasibility to test AGuIX-mediated PDT was possible with MRI imaging. AguIX solution in various concertations was added to cell culture medium for *in vitro* studies or injected i.v. for *in vivo* models and then cells/tumors were irradiated with various light doses (λ_{em}=650 nm).

In vitro, immortalized macrovascular endothelial cells and GBM cells were shown to accumulate AGuIX nanoparticles containing TPP photosensitizer coupled with vascular-targeting peptide. The compound was not toxic *in vitro* in the concentration range tested. Metabolic activity decreased with increasing drug and light doses, with no differences between the target peptide and the scrambled peptide. PDT was effective in inhibiting tumor growth in CAM, while in the ectopic mouse model, tumors regrew after therapy. GBM tumors that grow in mice responded differently to AGuIX photodynamic therapy. The response was linked to the initial state of functionality of the vascular system (% of blood vessels and pO_2) during therapy. In PDT-respondent tumors, the percentage of functional vasculature in tumor volume decreased immediately after therapy. Two orthotopic models: human U87 cells in nude mice and murine GL-261 C57BL/6J mice were optimized. Interstitial pilot PDT experiments showed poor tumor response and high inflammation. Interstitial PDT of tumors in the mouse brain is feasible, providing tumor monitoring of the tumor by magnetic resonance imaging is performed before or during PDT.

I showed the moderate effectiveness of PDT with AGuIX functionalized nanoparticles and outlined some vascular and oxygen-related explanations of the partial response to photodynamic therapy in biological models of GBM. Obtained results and optimized models give ground for further analysis.

Małgorzata Elas

Patrycja Nowak-Sliwinska