Summary

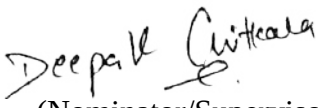
Actively targeted nano-carrier system for the co-delivery Temozolomide and an autophagy modulator for the treatment of glioblastoma multiforme

Glioblastoma (GBM) is the most prevalent and deadly primary malignant brain tumor in adults, accounting for 16% of all brain and central nervous system tumors. Conventional treatment primarily involves surgical resection, radiotherapy, and adjuvant temozolomide (TMZ) chemotherapy. TMZ is a second-generation imidazotetrazine DNA alkylating agent marketed as Temodal® in the form of capsules and injectables. TMZ being a potent molecule, it exhibits limitations, including development of resistance, short half-life, pH-dependent hydrolysis, and rapid clearance, resulting in <1% of the dose reaching the brain. Additionally, the studies have shown the direct involvement of the PI3K/Akt/mTOR/p53/autophagy pathway in the survival and progression of GBM. Still, the delivery of the molecule (autophagy inducer, rapamycin) to the target site of action is quite challenging, mainly due to drug-related (logp >5, high mol. wt) and physiological barriers (tissue binding affinity, plasma binding, blood-to-plasma ratio, and blood-brain barrier (BBB)). Therefore, co-delivering autophagy inducer rapamycin and TMZ could help achieve multidimensional GBM treatment; however, the process is still problematic to achieve. Herein, we propose to design and synthesize a carrier system that can co-deliver hard-to-deliver molecules rapamycin and TMZ to the targeted site of action, *viz.,* transversing the BBB.

In the current research, we prepared a cRGD peptide-functionalized polymer-polymer hybrid system using polycarbonate TMZ polymer conjugate and polyester mPEG-PLA polymer coloaded with rapamycin against GBM. Initially, a series of TMZ-polymer conjugates were prepared and screened, wherein TMZ was conjugated to polymer with a payload of ~30% w/w of TMZ. Further, the hybrid TMZ nanoconjugates (hybrid TMZ NCs) were prepared with PEG-PLA polymer, exhibiting an average particle size of ~100 nm, improved stability half-life from 1.8 h to 194 h, and colloidal stability for over 7 days. However, the BBB plays a vital role in separating the brain microenvironment from blood-related infection, demonstrating a problematic process to deliver small molecules through it. To permeate across the BBB, we have functionalized the hybrid TMZ NCs with the cell-penetrating cRGD peptide motif and coloaded hybrid TMZ NCs with rapamycin for potential synergism with TMZ, acting *via.* multiple pathways for killing tumor cells multi-dimensionally (*viz.* PI3K/Akt/mTOR/p53/autophagy). The coloaded hybrid TMZ NCs (non-targeted and cRGD

targeted) were assessed for *in vitro* and *in vivo* efficacy in the C6-induced syngeneic orthotropic glioma model, demonstrating marked improvement in brain physiology, survival rate, tumor burden, and better histopathological parameters with reduced toxicity and improved biocompatibility.

Overall, the study confirms the cRGD peptide functionalization and coloaded with autophagy modulator rapamycin with TMZ has demonstrated substantial improvement in the delivery-associated limitations (loading capacity, stability half-life, permeation across BBB, hematological and organ toxicity), *in vitro* and *in vivo* performance compared to free drug. Thereby opening multiple avenues with the co-delivery of hydrophilic (TMZ) and hydrophobic (rapamycin) molecules for the betterment of glioblastoma.



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