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Commentary

Aiming for the brain: a new thermogel-based drug delivery platform

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Abstract

Glioblastoma Multiforme (GBM) is one of the most lethal human cancer types, with a 5-year survival rate of approximately 5%. A key reason for this is usually considered its poor accessibility to systemically administered drugs that only limitedly overcome the Blood Brain Barrier, ultimately causing the likely dismal appearance of recurrences. Here, we comment on our successful use, in GBM preclinical models, of novel thermogel based drug-delivery platforms for loco-regional treatment of tumor recurrences after primary surgery. The innovation as well as the pitfalls of our processes are outlined and discussed with an eye towards potential advancements in the realm of personalized medicine applications.

Keywords: Glioblastoma, Tumor recurrences, Surgery, Locoregional therapy, Thermogel, Preclinical models, Nanotechnology, Drug repurposing

Abbreviations: 3R: Refinement, Reduction, Replacement; BBB: Blood Brain Barrier; CED: Convection Enhanced Delivery; FDA: Food and Drug Administration; FUS: Focus Ultrasounds; GBM: Glioblastoma Multiforme; GSC: Glioblastoma Stem Cell; ID50%: Inhibitory Dose 50%; IVIS: In Vivo Imaging System; PLC: PolyCaprolactone; SiO_2: Silicon dioxide, silica; THG: Thermogel; THG@PCL-TMZ: Thermogel containing polycaprolactone particles loaded with temozolomide; THG@SiO_2-TMZ: Thermogel containing mesopourus silica particles loaded with temozolomide; TMZ: Temozolomide

Introduction

The design of safe drug delivery systems that locally allow efficacious concentration of curative molecules has become the latest frontier in nanomedicine. It usually relies on the entrapment of drugs into micro/nano carriers guaranteeing for the controlled release of the drugs while protecting them from degradation and fast clearance. Often, this is achieved in combination with biocompatible gel matrices that allow semi/controlled prolonged availability of drugs [1]. This becomes a priority when the disease is poorly "accessible to drugs", like it often occurs in cancer, frequently doomed by tumor heterogeneity and by inaccessibility of the lesion site. A notable example of "hard-to-reach" tumors is Glioblastoma Multiforme (GBM), one of the most lethal human cancer types, with a 5-year survival rate of approximately 5% [2].

Despite the recent advances in pharmacology and nanotechnology, an effective drug delivery strategy to locally target the intracranial tumor is still lacking. Indeed, glioblastoma is grimly characterized by a poor success of cures, with surgical removal followed by radiotherapy and temozolomide (TMZ) chemotherapy often representing the first line of intervention [3]. However, the current chemotherapeutic standard of care, represented by several cycles of TMZ, is poorly effective, limited by its only partial (20%) ability to overcome the Blood Brain Barrier (BBB) that confine and tightly regulate exchanges between the circulating therapies and tumor cells [4,5].

Recently, low frequency localized ultrasounds (FUS) [6], Convection Enhanced Delivery (CED) [7], and the use of osmotic forces [8] have been all proposed to improve the success of systemically delivered chemotherapeutics. However, to date, the only FDA-approved strategy for a local treatment in glioblastoma is represented by the implant of polymeric matrix enriched in carmustine (Gliadel), yet important side effects, mainly attributed to the stiffness of the scaffolds causing damages to adjacent healthy tissues and the use of carmustine as active molecule, have strongly limited its use [9] after a conservative resection of the tumor mass.

Notably, the lack of effective treatments able to completely eradicate the primary tumor often results in recurrences and the growth of cells frequently resistant to treatment. In our work presented in "Loco-regional treatment with temozolomide-loaded thermogels prevents glioblastoma recurrences in orthotopic human xenograft models" [10], we designed and tested a tunable and adaptable drug delivery platform to convey treatments to fight intracranial tumor growth and to prevent its inevitable recurrences in a preclinical model of human GBM. We chose innovative chitosan-based thermogels (THG), whose gelation is induced by environmental temperature changes, that constitutes the matrix for hosting mesoporous SiO_2 (silica) particles loaded with TMZ or TMZ-sprayed poly-caprolactone (PCL) microparticles, ultimately obtaining TMZ-enriched formulations, namely THG@SiO_2-TMZ or THG@PCL-TMZ. The rational was to create an amenable injectable gel-based fluid to fill the cavity created into the tissue by the resection of the primary tumor in intracranial GBM bearing mice.

In developing the optimal formulation, we first needed to tackle some critical challenges concerning the loading of the TMZ into the silica mesoporous silica particles and into the spray-drying the PCL particles and the suitability of the new formulations to achieve effective releasing of TMZ for the *in vivo* therapy. The choice of testing silica nanoparticles might appear quite challenging as these nano-vectors have been rarely used for harnessing delivery into the brain [11] while PCL is commonly used as a delivery platform [12]. Given the distinct features of the two systems, we also verified *in vivo* safety and biocompatibility for each component. While PCL is considered biocompatible and is approved by FDA [13], on the other hand, silica nanoparticles have been safely used as peripheral drug delivery [14] but have been reported to cause an inflammatory response and cytotoxicity in an *in vitro* model of microglia [15]. In our case, we confirmed the non- cytotoxic effect *in vitro* for SiO_2 nanoparticles alone or within the matrix. Further test confirmed the non-toxicity of our formulations applied to the healthy brain tissue.

The aim of TMZ encapsulation was to decrease its diffusion rate in the hydrogel, allowing for a prolonged locoregional release of the drug rather than a burst release that would be observed if free TMZ was simply dissolved in the thermogel matrix. Yet, encapsulation of the drug inside the microcarriers did not appreciably slow down TMZ degradation. Certainly, in accordance with previous reports [16], the discriminant to success of our nano-assisted delivery was the ability to locally concentrate a much higher amount of TMZ (approximate 1.75 mg/kg), than what is achievable by the systemic delivery. It is noteworthy that each of the gel-based formulations was effective as single anticancer treatment, significantly reducing the occurrence of tumor recurrences and preserving the animal general wellbeing from unfavorable side effects.

Importantly, for our experiments, we used U87-MG-Red-FLuc cells of human origin. The sensitivity of the human U87MG glioblastoma model to TMZ, expressed as the effective dose that kills 50% of the cells (ID50%) *in vitro*, has been reported to vary, ranging from 0.1 and 1 mM [17], after 72 hours exposure. To fully characterize our model for further experiments, we verified *in vivo* that U87-MG- Red-FLuc cells coherently respond to TMZ treatment, showing a IC50% of 0.735 mM [10]. However, the value of IC50% in the high range indicates a low sensitivity that might be due to the transfection and stabilization procedures used to express luciferase protein that, while endowing the model with useful live imaging features, might have modified the reactivity of the original cells to chemotherapeutics.

This also emphasizes the importance of using the best representative cells model to draw experimental conclusions. Xenograft models based on established cell lines such as human U87MG cells often offer limited patient tumor representation because injection of such cell lines into the brains of mice fail to develop diffuse infiltration into surrounding healthy tissue and microvascular proliferation, that represent the defining morphological features of GBM. Like in our case, to obtain reliable models, researchers often face the choice of using animal-derived over human-derived tumorigenic cells and, accordingly, to choose a suitable animal model. For GBM as well as for other tumors, any of the animal models holds features that could be suitable for a specific scientific quest [18-20]. Using human U87-MG-Red-FLuc cells, we were able to translate the characteristics of the formulations tested *in vitro*, directly into the *in vivo* immune-compromised mice. Despite the limitation intrinsically expressed using a quite robust animal model such as athymic mice and the lack of a complete immune-competent system to recapitulate all the features of glioblastoma, the U87MG model was suitable to sustain repeated surgical sessions with no signs of discomfort or deterioration of well-being rather that caused by the development of the disease in untreated animals. It was, therefore, possible to determine the longitudinal development and remission of the intracranial glioblastoma in each animal treated for two weeks using an extremely low number of animals, thanks to the use of IVIS live imaging, in accordance with the 3R principles. Our goal was, in fact, to validate the two gel-based formulations as an efficient platform for drug delivery regardless the drug and the specificity of the treatment. However, the use of patients' derived cells to establish recurrences' murine models will represent the natural progression towards an approach of personalized medicine in GBM [20-22]. In fact, evidence suggests that the Glioblastoma Stem Cells (GSC) subpopulations of GBM are very important to maintain tumor heterogeneity, therefore becoming the most accepted standard for studying GBM biology *in vitro* and *in vivo* [23], but their use must be sustained by a systematic clinical characterization of the tumors from which the cell lines derive to allow to correlate the findings obtained with these cells to patient's parameters.

Nevertheless, to completely prevent the onset of recurrences, we strongly believe that we will necessarily need using drugs more efficacious than TMZ, possibly targeted drugs inhibiting specific GBM drivers/oncogenes. In this context, we also trust that a more efficient cure will be possible when existing anticancer drugs will be re-evaluated with extensive GBM drug repurposing efforts [24] independently of their ability to pass the BBB. Indeed, a wide range of anti-cancer drugs have been found to kill GBM cells *in vitro*,

possibly even more efficiently than TMZ. Still, most of them are often doomed to fail in clinical trials because of their inability to cross the BBB. For these reasons, our effort is now concentrated on developing an interchangeable local delivery system, in the form of thermogels, able to release the most innovative and effective drugs directly into the brain.

Conclusions

Once approved, loco-regional gel-based therapies are expected to provide a safe and effective tool to reduce systemic cycles of chemotherapies and improve survival of patients, ultimately improving quality of life and facilitating their compliance to therapies.

Author Contribution

MC and LG wrote the manuscript.

Conflicts of Interest

The authors declare no competing interests.

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