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Post-resection treatment of glioblastoma with an injectable nanomedicine-loaded photopolymerizable hydrogel induces long-term survival

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor. Despite available therapeutic options, the prognosis for patients with GBM remains very poor. We hypothesized that the intra-operative injection of a photopolymerizable hydrogel into the tumor resection cavity could sustain the release of the anti-cancer drug paclitaxel (PTX) encapsulated in poly (lactic-co-glycolic acid) (PLGA) nanoparticles and prevent GBM recurrence. The tumor was resected 13 days after implantation and a pre-gel solution composed of polyethylene glycol dimethacrylate (PEG-DMA) polymer, a photoinitiator and PTX-loaded PLGA nanoparticles (PTX PLGA-NPs) was injected into the tumor resection cavity. A solid gel filling the whole cavity was formed immediately by photopolymerization using a 400 nm light. PTX *in vitro* release study showed a burst release (11%) in the first 8 h and a sustained release of 29% over a week. *In vitro*, U87 MG cells were sensitive to PTX PLGA-NPs with IC_{50} level of approximately 0.010 μg/mL. The hydrogel was well-tolerated when implanted in the brain of healthy mice for 2 and 4 months. Administration of PTX PLGA-NPs-loaded hydrogel into the resection cavity of GBM orthotopic model lead to more than 50% long-term survival mice (150 days) compared to the control groups (mean survival time 52 days). This significant delay of recurrence is very promising for the post-resection treatment of GBM.

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive and lethal type of brain tumor. GBM exhibits a high proliferation rate, high tumor cell infiltration into adjacent brain tissue, resistance to conventional treatment and the ability to quickly develop recurrences, which are responsible for its poor prognosis (Milano et al., 2010). The current standard therapy includes surgical resection, balanced between the need to remove a maximum of the tumor and to limit any resulting impairments, followed by radiotherapy and oral alkylating chemotherapy with temozolomide (TMZ;Temodar®) (Davis, 2016; Wilson et al., 2014). Massive and thorough surgical resection of GBM is frequently not achievable because GBM frequently infiltrates the brain parenchyma beyond the main mass of the tumor, or the brainstem, the diencephalon and neocortical areas that control speech, motor function and sensation (Davis, 2016). If achievable, the macroscopically complete resection of the primary tumor is not curative since infiltrating tumor cells invariably remain in the surrounding brain parenchyma, leading to later tumor recurrences. As a consequence, approximately 70% of GBM patients experience local recurrence within one year of diagnosis (Stupp et al., 2005); the 2-year survival rate is only 27%, and the long-term survival rate is less than 5% (Ostrom et al., 2014; Stupp et al., 2009).

The blood brain barrier (BBB), which acts very effectively to protect the brain from harmful molecules, limits the entry of most systemically administered drugs to the brain (Bastiancich et al., 2016a). To overcome this issue, one of the promising strategies to limit or delay GBM recurrences is the direct administration into tumor resection cavity of a local drug delivery system, ensuring sustained release of cytotoxic drugs. Indeed, a local drug delivery system will ensure (*i*) a direct

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contact of the drug with tumor cells, (*ii*) a sustained drug release, (*iii*) a limited drug degradation and (*iv*) a reduction of off-target effects in healthy tissues (Bastiancich et al., 2016a). The rationale for this approach is that biocompatible materials can be introduced directly into the brain inside the surgical tumor resection cavity. Indeed, after surgical resection, patients have to wait a period of time before starting the radio/chemotherapy regimen because of the duration of the post-surgical wound healing process; thus, residual infiltrative cells will keep proliferating during this time (Patel et al., 2015). Consequently, in this time gap, a local drug delivery system could be administered before starting the conventional radio/chemotherapy regimen. In this approach, cytotoxic agents diffuse into the brain parenchyma to kill residual tumor cells around the resection cavity borders, which are responsible for recurrences. The only system applying this strategy approved by FDA is Gliadel®, a polymeric wafer loaded with carmustine. However, Gliadel® showed minimal and controversial advantages. Although a significant survival benefit for patients who received Gliadel® treatment was reported, the short sustained intracerebral release of most drug (1 week) and the local sides effects are involved in the major limitations of Gliadel® wafer (Bota et al., 2007; Engelhard, 2000; Perry et al., 2007).

Recently, we developed an innovative hydrogel uniquely comprised of photopolymerizable polyethylene glycol dimethacrylate (PEG-DMA) delivering TMZ for the local treatment of GBM (Fourniols et al., 2015). This injectable hydrogel presented mechanical properties compatible with brain implantation. In vivo, this system was well tolerated over one week in a healthy mouse brain and reduced tumor growth in a subcutaneous human U87 MG GBM model. However, O6-methylguanine-DNA methyltransferase (MGMT) is involved in the drug resistance mechanism of GBM, which limits the efficacy of TMZ (Kitange et al., 2009). To bypass this issue, other anti-cancer drugs were investigated for the treatment of GBM, such as lauroyl-gemcitabine ($GemC_{12}$) and doxorubicin (Bastiancich et al., 2016b; Fourniols et al., 2015; Qi et al., 2014; Vinchon-Petit et al., 2010). Among them, paclitaxel (PTX) could be an alternative as it is an efficient anti-tumor agent that inhibits cell proliferation and induces apoptosis (Gupta et al., 2003). Also, it kills GBM cells via a MGMT-independent mechanism (Shen et al., 2017; Xin et al., 2010; Zhan et al., 2010). However, the anti-glioma effect of PTX is very limited when administered systemically as it cannot cross the BBB and, in consequence, do not accumulate in the CNS at therapeutic doses (Chang et al., 2001). We hypothesized that PTX would be a promising drug for the local delivery before radio- and chemotherapy.

Encapsulation of a bioactive molecule in nanoparticles (NPs) is one of the strategies widely applied to solubilize lipophilic drugs, to protect them from degradation and to obtain a sustained delivery. Poly (lactic-co-glycolic acid) (PLGA) is one the most successful polymers developed to formulate polymeric NPs for the delivery of anti-cancer drugs. PLGA has attracted considerable attention due to the following favorable attributes: (*i*) biodegradability and biocompatibility, (*ii*) FDA/EMA approval for parenteral administration, (*iii*) possibility of sustained drug release and (*iv*) efficient encapsulation of poorly soluble drugs (Danhier et al., 2012). Additionally, PTX-loaded PLGA-NPs have been previously developed with good encapsulation and loading efficiencies and significant regrowth delays of various *in vivo* tumor models (Amoozgar et al., 2014; Cui et al., 2013; Danhier et al., 2009b; Luo et al., 2016).

The aim of the present study was to develop an injectable photopolymerizable hydrogel capable of sustaining the release of PTX over a period to fill the gap between surgical resection and the conventional radio/chemotherapy regimen (Fig. 1). The hydrogel consists of a PEG-DMA polymer, the Lucirin-TPO® photoinitiator and PTX PLGA-NPs. PLGA NPs are incorporated to slow down the PTX release from the hydrogel. The pre-gel solution is injected into the resection cavity right after surgery and then photopolymerized under blue light (400 nm) irradiation. The PTX release as well as the cytotoxic effect of PTX PLGA-NPs on U87 MG cells were studied, the mid- and long-term tolerability in healthy mouse brain and anti-tumor efficacy after local injection in the resection cavity in an orthotopic U87 MG model were investigated *in vivo*.

2. Materials and methods

2.1. Hydrogel formulation

2.1.1. Formulation of PTX PLGA-NPs

PTX PLGA-NPs were formulated using a modified emulsion-evaporation method as previously reported (Danhier et al., 2009a). Briefly, PLGA (Resomer® RG 502, Mw = 7000–17,000 g/mol, 56 mg, Sigma-Aldrich, USA), PLGA-PEG (M_W = 4600–10040 g/mol, 12 mg), PCL-PEG (M_W = 5000–13,100 g/mol, 12 mg) (synthesized as previously described (Schleich et al., 2013)) and PTX (6 mg, Chemieliva, China) were dissolved in dichloromethane. This organic solution was then added to an aqueous solution containing 3% (w/v) PVA (M_W = 30–70 kDa, Sigma-Aldrich, USA), emulsified using a vortex for 2 min, and then sonicated (4 × 30 s, 50 W). The mixture was rotary evaporated for 1 h to remove the organic solvent. To remove the non-encapsulated drug, the suspension was filtered (1.2 µm), washed using centrifugation (15,000 rpm, 45 min) and suspended in water.

2.1.2. Physico-chemical characterization of PTX PLGA-NPs

The average particle size and polydispersity index of the PTX PLGA-NPs were measured by dynamic light scattering and zeta (ς) potential measurements performed by laser Doppler velocimetry using a Zetasizer NanoZS (Malvern Instruments, UK) (n = 3).

2.1.3. Quantitative determinations of the PTX in PTX PLGA-NPs

PTX was quantified by high-performance liquid chromatography (HPLC) using a Shimadzu Prominence system (Shimadzu, Japan). The separation was conducted using a BDS Hypersil C18 (Thermo Scientific, USA) (100 × 4.6 mm; particle size 3 µm) column with a mobile phase comprising acetonitrile (VWR Chemicals, France) and water at a ratio of 45:55 (v/v). The detection wavelength was set to 227 nm, and the flow rate was maintained at 1.2 mL/min. Under these conditions, the retention time of PTX was approximately 6.5 min. A calibration curve was obtained by diluting PTX in acetonitrile at concentrations between 1 and 100 µg/mL (correlation coefficient of R^2 = 0.9993). The limit of quantification was 0.84 µg/mL, and the limit of detection was 0.28 µg/mL. The total quantity of PTX loaded in the PTX PLGA-NPs was evaluated by the dissolution of an amount of nanoparticles in acetonitrile (dilution ratio 1:100) and quantification by HPLC. Encapsulation efficiency was calculated by final drug amount in nanoparticles divided by the total drug added, while the drug loading is calculated by final drug amount in nanoparticles divided by the initial amount of polymers as previously described (Danhier et al., 2009a).

2.1.4. Preparation of PTX PLGA-NPs-loaded hydrogel

PTX PLGA-NPs were diluted in water to reach 3.5 mg/mL of PTX and mixed with PEG-DMA (average Mw = 550 g/mol) (Sigma-Aldrich, USA) at a 75:25 v/v ratio. Next, 0.5% of Lucirin-TPO® (BASF) was added as the photoinitiator. Then, the pre-gel solution was irradiated at 750 mW/cm^2 for 15 s with a blue light (Lumencor, USA). The hydrogel formation was re-optimized with different concentrations of PEG-DMA and photoinitiator (Supplementary data, Fig. S1). Unloaded and blank PLGA-NPs-loaded PEG-DMA hydrogels were also prepared as controls by adding water or blank PLGA-NPs instead of the PTX PLGA-NPs in the pre-gel solution. The stability of PTX under blue light exposure as well as that of PTX in the PTX PLGA-NPs-loaded PEG-DMA hydrogel was assessed by HPLC by measuring the recovery of the total amount of PTX, the PTX peak integrity and retention time (Supplementary data, Fig. S2).