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Research article

Novel model of orthotopic U-87 MG glioblastoma resection in athymic nude mice

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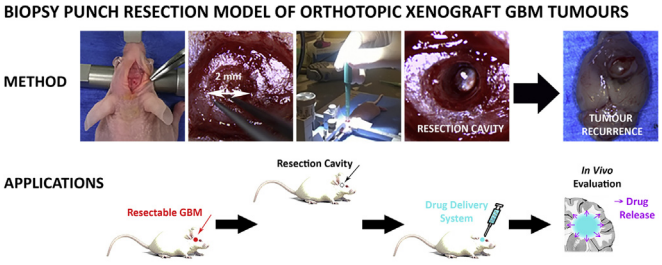
HIGHLIGHTS

- Biopsy punch resection of U-87 MG tumour is effective, affordable and reproducible.
- Biopsy punch cavity suitable for drug delivery system evaluation.
- Clinically relevant model for studying GBM treatments.

GRAPHICAL ABSTRACT



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ABSTRACT

In vitro and *in vivo* models of experimental glioma are useful tools to gain a better understanding of glioblastoma (GBM) and to investigate novel treatment strategies. However, the majority of preclinical models focus on treating solid intracranial tumours, despite surgical resection being the mainstay in the standard care of patients with GBM today. The lack of resection and recurrence models therefore has undermined efforts in finding a treatment for this disease. Here we present a novel orthotopic tumour resection and recurrence model that has potential for the investigation of local delivery strategies in the treatment of GBM. The model presented is simple to achieve through the use of a biopsy punch, is reproducible, does not require specific or expensive equipment, and results in a resection cavity suitable for local drug delivery systems, such as the implantation or injection of hydrogels. We show that tumour resection is well tolerated, does not induce deleterious neurological deficits, and significantly prolongs survival of mice bearing U-87 MG GBM tumours. In addition, the resulting cavity could accommodate adequate amounts of hydrogels for local delivery of chemotherapeutic agents to eliminate residual tumour cells that can induce tumour recurrence.

1. Introduction

Glioblastoma (GBM) is the most common and aggressive malignant tumour of the central nervous system in adults. These tumours show a high proliferation rate with diffuse infiltration of adjacent brain tissue (Bernardi et al., 2009). Conventional therapeutic procedures, aiming at increasing patient life expectancy, focus on maximal surgical resection combined with adjuvant radiotherapy

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and/or chemotherapy by oral delivery of Temozolomide (TMZ) (Stupp et al., 2005). However, tumour recurrences due to residual infiltrative cells at the resection margin are inevitable, leading to a median survival of about 14 months, with a 5 year-life expectancy of less than 10% (Lefranc et al., 2006). In consequence, there is a much unmet medical need that necessitates solving. Innovative drug delivery systems aiming at delivering drugs to the tumour site present a promising approach in treating this disease (Bastiancich et al., 2016). The local drug delivery of cytotoxic agents, using injectable systems in the tumour resection cavity with sustained drug release characteristics, aims at preventing the growth of cancer cells that cannot be resected during surgery.

Local delivery seems very promising for the treatment of GBM for a number of reasons. One is that it allows for bypassing the blood brain barrier through direct administration of a drug into the brain. Another is that sustained drug release, reaching therapeutic concentrations at the tumour site without involving other organs, can be obtained (Bastiancich et al., 2016). The rationale for the use of local delivery strategies in GBM treatment has been highlighted by approval of Gliadel® by the FDA. However, due to some conflicting results being obtained with the use of Gliadel®, and limitations in current treatment options available, novel avenues of treating GBM through local drug delivery strategies need to be investigated (Ashby et al., 2016; Bock et al., 2010).

To evaluate the efficacy of these drug delivery systems on GBM recurrence, a clinically relevant tumour resection model is needed. Despite many preclinical studies, most *in vivo* GBM models do not mimic the clinical scenario of surgical debulking and instead focus on treating solid intact intracranial tumours. Therefore, in light of the central role of tumour resection in clinical therapy, development of rodent models of GBM resection and recurrence are a necessity (Kauer et al., 2011), and indeed, several models do currently exist. Akbar and colleagues were the first to perform an intracranial resection in a rat model of C6–green fluorescent protein (GFP) intracranial glioma model. Through the use of a fluorescent dissecting microscope, they were able to detect the tumour and subsequently guide a suction tip that allowed for the precise microsurgical resection of the tumour (Akbar et al., 2009). This method was also reproduced in nude rats by Denbo and colleagues (Denbo et al., 2011), while Kauer and colleagues further modified it to develop an efficient GBM subtotal resection model in nude mice (Kauer et al., 2011). A simplified technique, using mere aspiration for 5 s to remove a GBM tumour in rats, has also been reported, although it was found less effective in completely or efficiently resecting the tumour tissue, with no difference in survival observed between resected and control animals (Ozeki et al., 2012). Nevertheless, the drawback of these techniques is the need of specific or expensive equipment that are not always available.

To provide a clinically relevant model for studying GBM treatments, we developed a novel approach for resection of U-87 MG mouse intracranial GBM in a validated and reproducible manner, using a biopsy punch. The advantages that our resection technique provides include simplicity, reproducibility, and the lack of necessity for any specific or expensive equipment.

2. Materials and methods

2.1. Animals

All experiments were conducted on six week old, female, specific opportunistic pathogen-free (SPOF) NMRI nude mice (Janvier, France) in accordance with Belgian national regulation guidelines as well as with EU Directive 2010/63/EU. All experiments were approved by the ethical committee of the Université catholique de Louvain (2014/UCL/MD/004). Mice were maintained on standard laboratory food and water *ad libitum*, with a 12 h artificial light/dark cycle.

2.2. Cell culture

U-87 MG glioma cells (ATTC, USA) were cultured in Eagle's Minimum Essential Medium (EMEM; ATTC, USA), supplemented with 10% foetal bovine serum (Gibco, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, USA). Cells were cultured as monolayers in 75 cm^2 culture flasks (Sigma, USA) and maintained at 37 °C/5% CO_2.

2.3. Orthotopic U-87 MG human glioblastoma tumour model

For the intracranial glioma model, animals were anesthetised by intraperitoneal injection of ketamine/xylazine (100 and 13 mg/kg, respectively) and positioned in a stereotactic frame. Once immobile, an incision 5 mm long was made along the midline. A burr hole was drilled into the skull at the right frontal lobe, 0.5 mm posterior and 2.1 mm lateral to the bregma using a high-speed drill (Dremel Inc., USA). A 5 μL Hamilton syringe fitted with a 26 gauge needle was used to inject 2.5 μL of complete culture medium containing 3×10^4 U-87 MG glioma cells at the junction between the cortex and striatum at a depth of 2.5–3.0 mm from the outer border of the cranium over a five minute period. After injection, the needle was kept in place for 5 min before slowly being extracted to prevent a vacuum and cell build-up into the needle track. The wound was then sutured and the animals were allowed to awaken under an infrared heating lamp (Danhier et al., 2015). No post-surgery analgesics were administered following the procedure. Animals awoke and were active between 1 and 2 h following surgery and did not display any signs of distress. The presence, volume and localisation of tumours was determined by Magnetic Resonance Imaging (MRI) between day 9 and 12 post inoculation of the U-87 MG cells. Animals were killed when they presented ≥20% body weight loss or 10% body weight loss plus clinical signs of distress (paralysis, arched back, lack of movement).

2.4. Magnetic resonance imaging

MRI was performed using a 11.7 T Bruker Biospec MRI system (Bruker, Germany) equipped with a 1 H quadrature transmit/receive surface cryoprobe after anesthetising animals with 1% isoflurane mixed with air (2.5% for induction, 1% for maintenance). Respiration was continuously monitored while animal core temperature was maintained throughout the experiment by hot water circulation in the cradle. Tumour volume was assessed using rapid acquisition with relaxation enhancement (RARE) sequence (TR = 2500 ms; effective echo time (TE_{eff}) = 30 ms; RARE factor = 8; FOV = 2 × 2 cm; matrix 256 × 256; twenty-five contiguous slices of 0.3 mm, $N_{average}$ = 4). Volumes were calculated from manually drawn region of interest (ROI).

2.5. Biopsy punch resection of tumour mass

On the 13th day post-inoculation of the tumour, mice were randomly assigned into control (no resection, no treatment) or resection (resection, no treatment) groups (n = 11 in each group). For intracranial glioma resection, animals were anaesthetised with ketamine/xylazine as described above before being immobilised in a stereotactic frame. A 7 mm incision was made in the midline along the previous surgical scar. The periosteum was removed revealing the bregma and previous burr hole. A high-speed drill was used to thin the skull area centred around the burr hole, after which fine tip tweezers (Dumont, Switzerland) were used to obtain a 2.1 diameter circular cranial window exposing the brain. A biopsy punch