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Imaging and histological characterization of a human brain xenograft in pig: The first induced glioma model in a large animal

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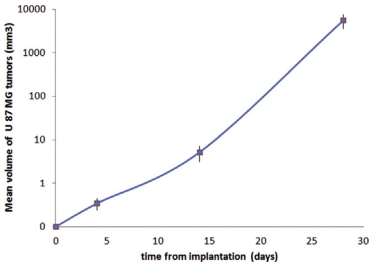
HIGHLIGHTS

- We have developed the first induced large animal glioma model.
- Ciclosporine was used for the immunomodulation.
- The time to develop a tumor is short and reproducible with U87 MG cells, allowing preclinical studies.
- Pig and human brains are macroscopically similar, providing realistic condition for convection enhanced-delivery protocol.

GRAPHICAL ABSTRACT



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ABSTRACT

The prognosis of glioblastoma remains poor despite significant improvement in cytoreductive surgery, external irradiation and new approach of systemic treatment as antiangiogenic therapy. One of the issues is the low concentration in the infiltrated parenchyma of therapeutic agent administered intravenously mainly due to the blood–brain barrier. An intracerebral injection is advocated to overpass this barrier, this kind of administration need a low flow and continuous injection. The development of sophisticated implanted devices for convection-enhanced delivery is a mandatory step to have a controlled released of a therapeutic agent in glioblastoma treatment. Before testing such a device in a clinical trial a serious preclinical studies are required, in order to test it in realistic conditions we have develop the first induced high grade glioma model in a non-rodent animal: the pig. 21 pigs have been implanted in the parietal lobe with human glioblastoma cell lineage under a chemical immunosuppression by ciclosporine. A MRI follow up was then realized. 15 pigs have been implanted with U87MG, 14 have presented a macroscopic significant tumor, with radiological and anatomapathological characteristics of high grade glioma. 6 pigs were implanted with G6, stem-like cells tumors of glioblastoma, 1 pig develops a macroscopic tumor. This is the first reproducible glioma model in a large animal described, it open the way to preclinical studies to test implanted devices in anatomic realistic conditions, without the ethical issues of a primate use.

1. Introduction

Glioblastoma multiform (GBM) is the most frequent primary malignant brain tumor (Wen et al., 2008; WHO, 1957). Despite

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decades of effort prognosis remains poor, with a median survival of 15 months (Brandes et al., 2008; Mirimanoff et al., 2006; Stupp et al., 2005). The current standard treatment for GBM consists in combining surgery with radio-chemotherapy (Deutsch et al., 1989; Laperriere et al., 2002; Perry et al., 2007; Walker et al., 1980) including temozolomide (Mirimanoff et al., 2006; Stupp et al., 2005). This combination therapy is necessary as, although the extent of the surgical resection is a major prognostic factor (Allahdini et al., 2010; Curran et al., 1992; Gorlia et al., 2008; Lamborn et al., 2004; Stummer et al., 2008), it remains non-curative because of the infiltrating properties of the tumor, resulting in seeding of distant tumor cells (Brem et al., 1972; D'Abaco and Kaye, 2007; Giese and Westphal, 1996; Tate et al., 2009; Zagzag et al., 2000). The therapeutic potential of radiotherapy alone is also limited due to the inherent radioresistance of glioblastoma cells (Bao et al., 2006; Hatanpaa et al., 2010; Shaifer et al., 2010). While, with chemotherapy, the major cause of failure is poor delivery of therapeutic agents across the blood–brain barrier (BBB) (Pardridge, 2007). This makes it necessary to use high plasma drug concentrations, which potentially expose patients to frequent and severe adverse effects. To circumvent the BBB, direct injection of chemotherapeutic agents into brain tissue is advocated (Bobo et al., 1994; Cokgor et al., 2000; Groothius et al., 1999; Lidar et al., 2004). This delivery technique provides higher intratumoral concentrations of the drug with lower plasma concentrations, theoretically providing increased antitumor efficacy with fewer side effects.

The main pitfalls with this delivery route are backflow and leakage (Buonerba et al.,2011; Chen et al., 1999; Sampson et al., 2007a,b), This makes it necessary to tightly control several parameters during infusion.

Backflow consists in fluid flowing along the outside of the catheter, causing reduced delivery to the tumor bed. This can result if the flow rate exceeds the diffusion properties within the tumor, thereby increasing local pressure; thus intratumoral infusion requires a slow infusion process. Infusion can be improved by creating a continuous positive pressure output based on interstitial convection; in this case, the bulkflow created by the volume of fluid injected enhances the delivery capacity. This concept is known as convection-enhanced delivery (CED) (Chen et al., 2004; Kalyanasundaram et al., 1997; Khan et al., 2005; Morrison et al., 1999; Patel et al., 2005).

Leakage during cerebral infusion is mainly induced by sulci or ependymal surfaces close to the site of infusion. An important, and largely underestimated, bias in animal studies is that rodents' brains do not contain sulci. This minimizes leakage and improves local delivery during infusion in animal experiments (Chen et al., 1999, 2004; Kalyanasundaram et al., 1997; Khan et al., 2005; Morrison et al., 1999; Patel et al., 2005; Sampson et al., 2007a,b), which could explain why the encouraging results from studies on small animals cannot be reproduced in humans, resulting in the systematic failure of phase 3 clinical studies (Buonerba et al., 2011).

A large animal model is therefore needed to test CED protocols in pre-clinical studies for several reasons. Firstly, this model would make it possible to analyze intratumoral infusions in relevant anatomic conditions, where leakage phenomena can also occur. Secondly, the volume required for human applications and parameters that must be controlled during intratumoral infusion (flow rate, pressure level), makes it necessary to design an implanted device. Any such device must be implanted and tested in a large animal before being used in humans.

As yet, no induced model of a malignant glioma has been developed in large animals, as discussed by some authors (Lopez et al., 2008). A spontaneous dog glioma (brachycephalic breeds) exists, but unfortunately, it is quite a rare and constitutes a non-reproducible model (Foster et al., 1988; Heidner et al., 1991; Snyder et al., 2006). One of the issues is the lack of non-rodent athymic animals. An athymic strain is mandatory if a human glioblastoma cell lineage is to be implanted, to avoid rejection of the implanted cells, which would be seen as a xenograft. Although the BBB confers a privileged immune status on the brain, rejection is an issue in this context, as previously seen with xenogeneic transplanted neurons (Michel-Monigadon et al., 2010).

This article describes a pig model of human glioma, induced by implantation of human glioblastoma cell lines.

2. Materials and methods

2.1. Ethical considerations

All operative procedures and animal care were performed in line with the French Government's guidelines (decree 87-848, 1987) and with the Laboratory Animal Care and Use Committee.

2.2. Animals

Animals were white large Land-race three-month-old female pigs obtained from Fournand stockbreeding (Les Halles, France). The animals were transferred to the Claude Bourgelat Institute (Marcy l'Etoile, France) and were thoroughly examined by a veterinary physician to detect any latent infection before the immunosuppressive treatment began. Any focal infection was a criterion for exclusion from the study.

2.3. Cells

Two different cell lines were tested: U87 MG and G6. U87 MG was chosen as it is widely use in pre-clinical studies, it is reproducible and readily available. G6 cells are tumor stem cells, which can theoretically reproduce the cell heterogeneity of glioblastoma.

2.3.1. U87 MG

Cells were obtained from the American Type Culture Collection, (Manassas, VA, USA). They were cultured as an adherent monolayer, in DMEM supplemented with 10% fetal bovine serum, 2 mmol/l L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 40 μg/ml gentamycin.

Prior to implantation, cells were dispersed with a 0.05% solution of trypsin/EDTA and adjusted to a final concentration of 10×10^6 cells/20 μl in DMEM.

2.3.2. G6

Cells were obtained from a primary culture of glioblastoma as previously described (Platet et al., 2007). Cells were grown and maintained as floating neurospheres under 3% O_2 in a MCO-5M multi-gas incubator (Sanyo), in a medium consisting of DMEM/glucose 4,5 g/l, DMEM/glucose 1 g/l/F12 (1:1:1) containing 100 units/ml penicillin, 100 μg/ml streptomycin, 40 μg/ml gentamycin, 20 ng/ml EGF, 20 ng/ml bFGF, 2 μg/ml heparin, 0.5 units N_2 and 0.5 units B-27 supplements. Cells were centrifuged before implantation and resuspended at a final concentration of 10×10^6 cells/20 μl in DMEM.

2.4. Surgical procedure

The whole surgical procedure was performed at the Claude Bourgelat Institute. Animals were pre-medicated with an intramuscular injection of atropine sulfate (0.04 mg/kg) and azaperon (1 mg/kg). Twenty to thirty minutes later, anesthesia was administered by intravenous injection of tiletamine-zolazepam (5 mg/kg). Once unconscious, orotracheal intubation was performed and sedation was maintained by a continuous inhalation of isoflurane 2%.