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Local implantation of doxorubicin drug eluting beads in rat glioma

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ABSTRACT

We evaluated the safety and the efficacy of doxorubicin drug eluting beads "CM-BC1" when used locally in a 9L glioma model. Twenty microlitres of 1 mg/ml CM-BC1 (4 μg/rat), 10 mg/ml CM-BC1 (40 μg/rat) or unloaded beads were injected into the brain of 27 rats which was analyzed on day 8, month 3 or month 6. Then, after tumor implantation, rats were treated locally: (1) control group; (2) a group receiving 20 μl of unloaded beads, (3) a group "3 × 6 Gy whole-brain irradiation" (WBI), (4) a group receiving 20 μl of 1 mg/ml CM-BC1 and (5) a group receiving 20 μl of 1 mg/ml CM-BC1 followed by a WBI. Both the unloaded beads and the lower dose of 1 mg/ml CM-BC1 were well tolerated with no early deaths in opposite to 10 mg/ml CM-BC1. Medians of survival for the "1 mg/ml CM-BC1" group and the combination group are respectively 28.9 and 64.4 days. These results were significant compared to the "unloaded beads" group. The rat's survival was not significantly improved in comparison with the radiotherapy group. This preliminary evidence suggests that 1 mg/ml CM-BC1 could be interesting for recurrent high-grade gliomas. Further work is necessary to improve this seducing tool.

1. Introduction

Malignant gliomas are the most common types of primary central nervous system tumors and have a growing incidence of 5–8/100 000 (Bauchet et al., 2007; Stupp et al., 2005). Regardless of methods of treatment, most of these tumors recur locally. In an attempt to decrease these local recurrences, recent efforts have focused on designing polymer devices that deliver antitumor drugs into the resection surgical cavity. Macroscopic nonbiodegradable devices and more recently biodegradable wafers have been used for local chemotherapy of brain tumors in humans (Brem et al., 1995; Kubo et al., 1986; Oda et al., 1982; Valtonen et al., 1997; Westphal et al., 2003, 2006). Efficacy of local chemotherapy with BCNU-wafers has been previously demonstrated in patients with recurrent glioblastoma and more recently in primary malignant glioma (Brem et al., 1995; Westphal et al., 2003, 2006). But their size (several centimetres) does not allow a real intra-tumoral or intra-parenchymal implantation, neither a stereotactic administration. Moreover their incidence on survival is poor. Many other drug delivery devices have been developed and evaluated in animal models (Bartoli et al., 1990; Krauze et al., 2007; Kubo et al., 1986; Lesniak et al., 2005; Menei et al., 1996; Mu and Feng, 2003; Rousseau et al., 2009; Vauthier et al., 2003). Nevertheless, very few have come to clinical trial (Menei et al., 2004, 2005a; Sapin et al., 2006). One potential strategy is to test microparticles in suspension. Due to their size (1–1000 μm), they can be implanted easily in discrete, precise and functional areas of the brain, using needles as narrow as 21 gauge, without causing damage to the surrounding tissue (Menei et al., 2005b).

One such device that is currently the subject of widespread clinical investigations is a drug delivery embolisation system produced from a biocompatible polyvinyl alcohol (PVA) hydrogel known as DC Bead®, or LC Bead® in the USA (Biocompatibles, UK Ltd.). This device is indicated for the treatment of hypervascular tumors (Aliberti et al., 2006, 2008; de Baere et al., 2008; Eyol et al., 2008; Fiorentini et al., 2007; Forster et al., in press; Gonzalez et al., 2008; Keese et al., 2009; Lencioni et al., 2008; Lewis et al., 2006a,b, 2007; Malagari et al., 2008; Poon et al., 2007; Tang et al., 2008) and is being used to treat both primary and metastatic liver cancer. Outside of its use as a drug delivery embolisation system, DC Bead has also been shown in preclinical models to have the potential for treatment of other tumors such as pancreatic cancer (Forster et al., in press) and peritoneal carcinomatosis (Keese et al., 2009), by local direct injection of the microspheres. DC Bead microspheres studied in this

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paper are produced from a biocompatible polyvinyl alcohol (PVA) hydrogel that has been modified with sulphonate groups for the controlled loading and delivery of chemotherapeutic agents (Lewis, 2009). Beads are prepared by the reverse suspension polymerisation of the PVA macromer with 2-acrylamido-2-methylpropane sulfonate (AMPS) by free radical polymerisation. The sulphonate groups of the AMPS component interact reversibly with cationically charged drugs such as doxorubicin (Lewis et al., 2007). The drug loading and release characteristics have been reported elsewhere (Lewis et al., 2007; Gonzalez et al., 2008). In brief the presence of these sulfonate groups permits the efficient loading, sequestering and release of drugs such as doxorubicin, thereby allowing the delivery of a significant dose of chemotherapy into the surrounding tissue. Polymers that contain fewer sulfonate groups, or none at all, may be able to load small amounts of drug, however any drug that is loaded is rapidly lost in solution (Lewis et al., 2006a).

DC Bead has been shown to deliver drug over a protracted period of time (both in vitro and in vivo) (Gonzalez et al., 2008; Hong et al., 2006; Lewis et al., 2006a). Therefore, we predict that sustained local delivery of doxorubicin from the beads in patients with glioma may provide sufficient levels of chemotherapy to reduce tumor volume. Because doxorubicin is known to be efficient against brain tumors, using doxorubicin-loaded DC Beads could be a new strategy to reduce glioma volume (Stan et al., 1999). The purpose of this project was to study the long term biocompatibility and toxicity of doxorubicin-loaded DC Beads in the rat brain. We also evaluated the efficiency of doxorubicin-loaded beads on a rat glioma model, alone or associated to a fractionated radiation therapy.

2. Materials and methods

2.1. Drug-eluting bead resuspension preparation

Unloaded beads (DC Bead) and doxorubicin loaded drug eluting beads (CM-BC1) were provided by Biocompatibles UK Ltd., Surrey, UK. A bead size range of 100–300 μm beads was used for these studies. All manipulations were made in a special room dedicated to cytotoxics handling under a fume extraction hood.

Two loading levels in the CM-BC1 were studied: 1 mg/ml and 10 mg/ml CM-BC1 provided lyophilized and sterile.

Beads were rehydrated in the vial. Five minutes after the addition of 1 ml of sterile water, the mixture was decanted in a BD Falcon™ 10 ml Conical Tube. The vial was rinsed with 1 ml of sterile water and this was added to the other 1 ml in the BD Falcon™ 10 ml Conical Tube. Finally, the bead suspension was mixed with 3 ml 0.6% alginate solution, to reach a final volume of 5 ml. The alginate used for suspension and viscosity increase was ultra-pure Phycomer E01 (CellMed AG, Alzenau, Germany) which is mannuronic acid rich and has a molecular weight of approximately 800,000. For the intracranial injection, we had the solution was drawn into a Kendall Monoject syringe with a BD Hypodermic 0.6× 25 mm 23G-needle.

2.2. DC Beads brain biocompatibility and toxicity

DC Beads were analyzed for their biocompatibility in brain parenchyma before therapeutic studies. After intraperitoneal anesthesia by xylazine (Rompun, Bayer, Puteaux, France) (10 mg/kg) and ketamine (Clorkétam, Vétoquinol, Lure, France) (50 mg/kg), rats were placed in a small-animal stereotactic frame (Kopf Instruments, Phymep, France). After shaving and skin disinfection, sagittal incision of 2 cm was made to expose the skull, followed by a burr hole 0.5 mm anterior and 3 mm lateral from the bregma using a small drill. Twenty microlitres of beads resuspension were injected manually with a rate of 0.5 μl/min, 5 mm deep in right striatum of 27 female Wistar rats (according to the Paxinos atlas) with a Kendall Monoject syringe and a BD Hypodermic 0.6× 25 mm 23G-needle. After a final wait of 5 min, the needle was removed and the wound was sutured.

Table 1
Immunohistochemistry stains.

Antibodies anti-	Company	Clone	Dilution	Staining
CD3	Dako	Poly	100	T lymphocytes
GFAP[a]	Dako	Mono 6F2	900	Astrocytes
NeuN	Zymed	Mono A60	100	Neuronal bodies
Neurofilament	Monosan	Mono 2F11	120	Axons
Synaptophysine	Biogenex	Mono Snp88	200	Neurons and their axons

[a] GFAP, glial fibrillary acidic protein.

Groups were as follows: (1) a unloaded beads group (control group) (n = 9); (2) a 1 mg/ml CM-BC1 group (n = 9); (3) a 10 mg/ml CM-BC1 group (n = 9). Rats were examined daily. Three animals were scheduled to be sacrificed (atmosphere saturated with CO_2) at each time point, on day 8, month 3, and month 6. The brain was surgically removed, fixed, dehydrated and paraffin embedded. Standard staining (hematoxylin and eosin (H&E)), Perls coloration (for siderophages staining), Lugol Blue staining (for myelin) and Kossa staining (for microcalcifications) were performed. The immunohistochemistry (IHC) stains used are shown in Table 1. The histology was reported in a descriptive manner, in particular to evaluate the successful implantation of the beads, the location/distribution of the beads in the brain and the brain tissue reaction.

2.3. Tumor cell line

Rat 9L-glioma cells (European Collection of Concealment Culture, n° 94110705, Salisbury, U.K.) were cultivated in the "DMEM" medium ("Dulbecco's Modified Eagle's Medium", Biowhittaker, Verviers, Belgium) added with 10% of foetal calf serum (FBS, Biowhittaker) and of a mixture of antibiotics: penicillin (100 UI/ml), streptomycin (0.1 mg/ml) and amphotericin B (25 μg/ml) (ABS, Sigma, Saint Quentin Fallavier, France). Cells were maintained in a balanced wet atmosphere (37 °C and CO_2 5%). Cells were ready to be used to induce a brain tumor, after trypsinisation (trypsin/EDTA (Sigma)) and resuspension in "EMEM" ("Eagle's Minimum Essential Medium", Biowhittaker).

2.4. Animals and intracranial tumor implantation

Female Fischer-F344 rats were obtained from Charles River Laboratories France (L'Arbresle, France). Ten-weeks-old, they weighted 150–200 g. They were housed in groups of 4 in cages in conformity with the standards of the directives of the Union European and dealt with by the animal facilities of the Faculty of Medicine of Angers, establishment approved according to the law.

Ten microlitres of 10^3 9L-cells suspension were implanted by stereotactic conditions with a 10 μl-26G Hamilton syringe (Harvard Apparatus, Ulis, France) into the right striatal region of the rat brain as describe above for beads implantation.

2.5. Therapeutic protocol

On day 6 after tumor cells implantation, rats were assigned into 5 experimental groups and 20 μl of beads suspension were injected according to the same coordinates as the 9L cells. Groups were as follows: (1) control group (n = 8); (2) a group receiving one injection of unloaded beads (n = 7), (3) a group only irradiated by a whole-brain irradiation (WBI) with a total dose of 18 Gy, (4) a chemotherapy group, receiving one injection of 1 mg/ml CM-BC1