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Local implantation of doxorubicin drug eluting beads in rat glioma

S. Vinchon-Petit[a,b,*], D. Jarnet[b], S. Michalak[c], A. Lewis[d], J.-P. Benoit[a], P. Menei[a,e]

[a] INSERM, U646, Université d'Angers, Angers F-49100, France
[b] Centre Paul Papin, Angers F-49100, France
[c] Anatomopathology, CHU d'Angers, F-49933 Angers Cedex 9, France
[d] Biocompatibles UK, Ltd., Farnham, Surrey, UK
[e] Department of Neurosurgery, CHU d'Angers, F-49933 Angers Cedex 9, France

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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

* Corresponding author at: Department of Radiation Therapy, Centre Paul Papin, 49933 Angers Cedex 09, France. Tel.: +33 241 35 29 16; fax: +33 241 35 27 35.
E-mail addresses: s.vinchon-petit@unimedia.fr, skaya2@free.fr (S. Vinchon-Petit).

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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

and (5) a chemotherapy plus radiotherapy group, receiving one injection of 1 mg/ml CM-BC1 followed by WBI for a total dose of 18 Gy. Groups 4 and 5 received 4 μg of doxorubicin. Radiotherapy was administered on days 8, 11 and 14 to animals in groups 3 and 5.

2.6. Animal observation

Rats were examined daily and staged for activity and well-being according to a classification developed in our animal facilities (data not published). Rats too weak to feed and to stand (corresponding to stage 2) were sacrificed. The day of euthanasia was recorded and used in the survival analysis. Rats were weighed weekly.

2.7. Statistics

Survival was calculated from the day of the tumor implantation and presented as median and mean ± SE (standard error). The Student's *t*-test comparing combination treatment groups was performed. SPSS® software was used for that purpose and tests were considered as significant with p values < 0.05. Any rat surviving longer than 120 days was regarded as a 'long survivor'. The Kaplan–Meier method was used to plot animal survival.

3. Results

3.1. Biocompatibility and safety of bead implantation in the brain

The rat brains were evaluated for brain tissue reactions as a result of the implantation. For the "unloaded control beads" group, there was no unexpected mortality. Clinical staging and weight curves were normal for all animals until sacrifice. There was no issue with wound healing following the procedure. For the nine rats analyzed, the pack of beads was localized in the striatum. It is important to note that in all the slides, some beads were displaced from their implantation site during the slicing, with sometimes a tearing of the surrounding tissue, probably due to the difference in consistency between brain tissues and beads. Their location was still perfectly visible as a round and well limited hole in the tissue. No beads were in the subarachnoidian space. The beads appeared round, and stained similar to basophile material (H&E staining), striated by the effect of the microtome knife. This is a sign of a hard material (these soft, hydrated beads become dehydrated during the tissue fixing process). There are no signs of deformation, degradation or vacuolization of the beads over time (Fig. 1A). The striking point is the total lack of a foreign body reaction around the beads on H&E staining (Fig. 1B). Immunohistochemistry showed a very weak, non-specific, astrocytic reaction due to the implantation. This lack of strong astrocytic reaction supports the supposition that the biocompatibility of the material in the brain is excellent. There was neither lymphocytic infiltration, nor demyelinisation. Neuronal markers showed healthy neurons and neuronal fibers in close proximity to the beads.

For the "1 mg/ml CM-BC1" group, rats received 4 μg of doxorubicin per dose of beads. All rats presented a good neurological and general state before sacrifice. Animals were sacrificed at day 8, month 3 and month 6. There was no issue with wound healing in any of these rats. There was no evidence of necrosis or haemorrhage on any of the macroscopic examinations. It is important to note that for all the sections, as for the control beads, some beads were displaced from their implantation site during the slicing, again, with some tearing of the surrounding tissue on occasion, due to brain tissue and bead differences. Their localization was still perfectly visible as a round and well limited hole in the tissue. The beads (or their localization) were exactly in the predefined target in the middle of the striatum for most animals. They were localized slightly more

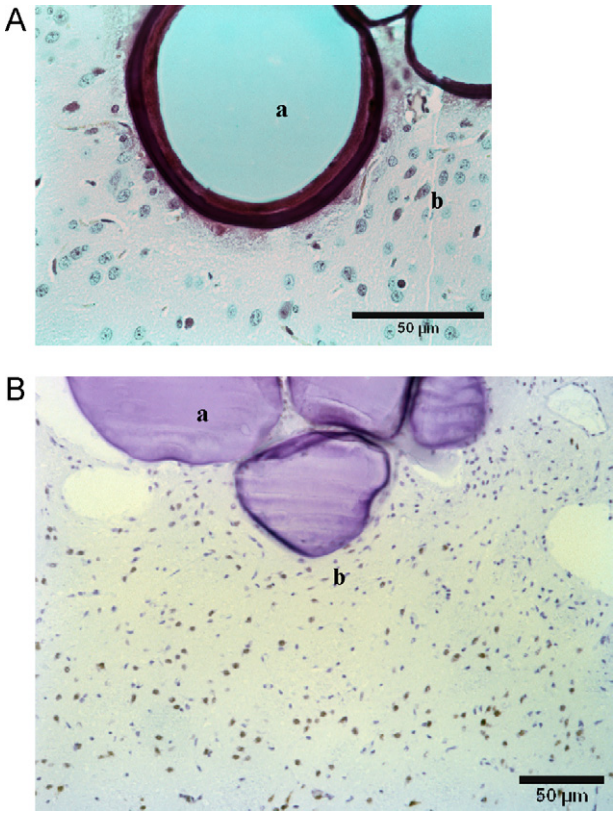

Fig. 1. (A) Unloaded beads in rat right striatum on day 8 (H&E staining 40×). (a) Unloaded beads. (b) Respected neuronal nucleus. (B) Unloaded beads in rat right striatum on month 6 (NeuN staining 2.5×). (a) Unloaded beads. (b) Respected neuronal nucleus.

medially in the striatum for one animal, opening the lateral ventricle, without hydrocephaly. In one animal, one bead was observed on the cortical point of injection. Except for this one, there were no beads in the subarachnoidian space in any of the observed slides. The beads appeared exactly as the unloaded control beads: round, stained similarly to basophile material (H&E staining) and striated by the effect of the microtome knife. On H&E staining, there was a moderate cell reaction with a moderate inflammatory reaction in two animals (one at month 3, one at month 6). There was no cell loss and no anomaly of brain architecture in any of the observed animals (Fig. 2).

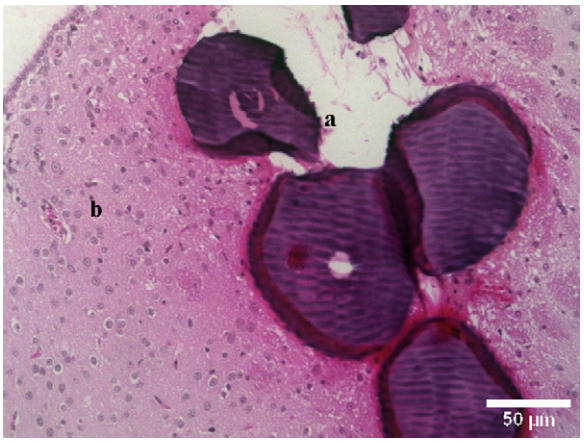

Fig. 2. 1 mg/ml CM-BC1 in rat right striatum on month 6 (H&E staining 20×). (a) 1 mg/ml CM-BC1; (b) expected architecture.

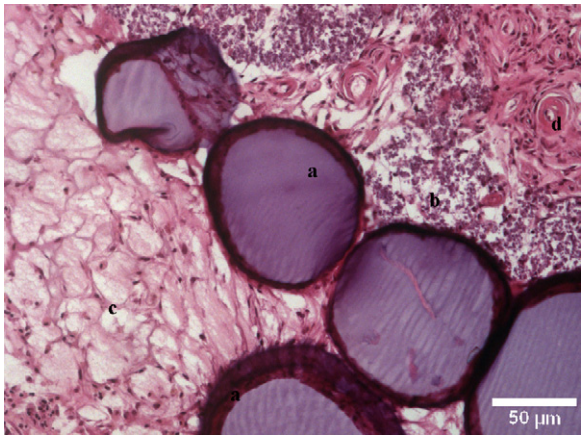

Fig. 3. 10 mg/ml CM-BC1 in rat right striatum on day 21 (HES 20×). (a) 10 mg/ml CM-BC1; (b) necrosis; (c) vacuolisation of brain tissue; (d) neoangiogenesis (with abnormal vessels).

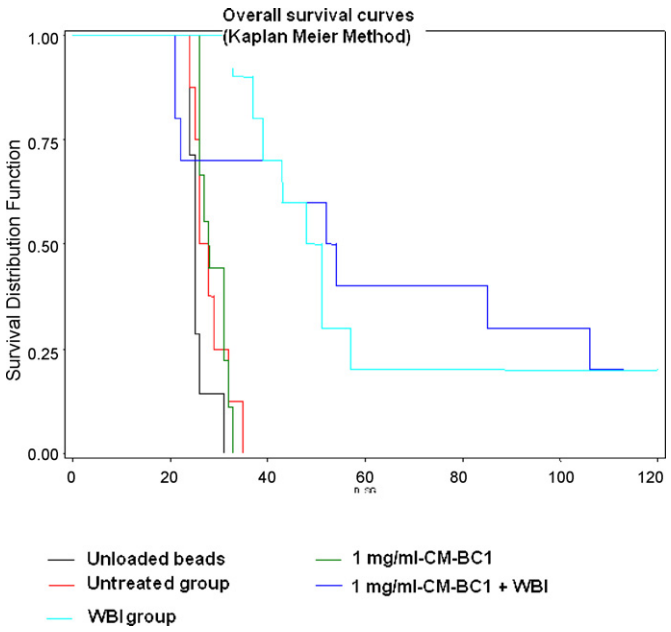

Fig. 4. Survival curves were obtained by Kaplan–Meier method.

For the "10 mg/ml CM-BC1" group, rats received 40 μg of doxorubicin per dose of beads. On day 5, neurological condition of two rats started to deteriorate. Those two rats died spontaneously: one on day 9 and one on day 21 (animals were found dead in the morning and histological examination was not possible). Seven rats were too weak to feed and to stand (stage 2) and were sacrificed 21 days after the bead implantation according to the protocol. The 10th rat was still alive at 3 months and was sacrificed according to the protocol. There was no issue with wound healing. Moderate oedema was evident in seven rats, in addition to significant necrosis with haemorrhage at the level of the injection site and into the adjacent cortex. For the eight rats analyzed, beads were observed in the middle of the striatum in six animals. No beads were visible in two animals, one because slicing problems and another probably due to unsuccessful bead implantation. As for the group 1, some beads were displaced from the implantation site during the slicing, with sometimes a tearing of the surrounding tissue, probably due to the difference in consistency between brain tissues and beads. Their localization was still perfectly visible as a round and well limited hole in the tissue. For each rat assessed, microscopic examination showed significant oedema, necrosis (sometimes calcified, with calcifications confirmed by a Kossa staining), and haemorrhage around the 10 mg/ml CM-BC1. This necrosis was surrounded by a marked cell reaction (macrophages, with some T lymphocytes), confirmed by Perls reaction, and neoangiogenesis, indicated by proliferation of abnormal microvessels with an enlarged wall. The brain tissue around this cell reaction was vacuolated (Fig. 3). For all the animals, GFAP IHC staining showed a lack of astrocytes in contact with the beads and an astrocytic reaction in the surrounding area. Neurofilament, NeuN and synaptophysin immunostaining showed the disappearance of neurons and axons in a large area around the implantation site, indicating massive neuronal death. For rat 10 which was still alive and sacrificed at month 3, macroscopic and microscopic examinations showed the lack of beads, probably due to a technical problem during the implantation procedure. The differential toxicity profile of 1 vs. 10 mg/ml beads following direct implantation into the rat brain indicates the local release of the doxorubicin was evident at the higher dose.

3.2. Combined effects of chemotherapy and radiotherapy

After the biocompatibility study, rats were randomised in only 5 experimental groups. In the efficacy study, the lower doxorubicin dose was selected due to the toxicity issues. All non-treated rats died before d35, with a median and mean survival of 27 and 28.1 days (Fig. 4 and Table 2). Similar survival was observed in animals that received unloaded control beads, with all animals dying by day 31 ($p = 0.16$ versus control). Animals receiving WBI had an extended survival compared to groups 1 and 2, with a median survival of 49.5 days. There were two long term survivors in this group.

Animals in group 4 (1 mg/ml CM-BC1) all died by day 33, with a median survival of 28 days. The survival was not significantly different from untreated animals. When 1 mg/ml CM-BC1 was combined with WBI (group 5), there was a significant increase in median survival (53 days) compared to unloaded beads or CM-BC1 alone. In this group there were two long term survivors. The association gives the best median of survival compared to the "WBI" group with 64.4 days versus 59.9 days. Unfortunately, this difference is not significant. We noted that in the combined group, there were early deaths. Some rats died before the untreated animal and 30% of the animals died before the first rats died in "WBI" group. Sur-

Table 2
Descriptive and statistical data from the survival study depending on groups of treatment.

Groups (n)	Range (days)	Median of survival (days)	Mean time of survival (days) ± SE	Mean ILT (%)	Long term survivors
Group «control» ($n = 8$)	24–35	27	28.1 ± 1.33	–	0
Group «unloaded beads» ($n = 7$)	24–31	25	25.7 ± 0.92	–	0
Group «WBI» ($n = 10$)	33–120	49.5	59.9 ± 2.85	133	2
Group «1 mg/ml CM-BC1» ($n = 10$)	26–33	28	28.9 ± 0.95	12.4	0
Group «1 mg/ml CM-BC1 + WBI» (n =v10)	21–120	53	64.4 ± 11.62	133.1	2

Group 1, group "control"; group 2, "unloaded beads"; group 3, "WBI (whole brain irradiation)"; group 4, "1 mg/ml CM-BC1"; and group 5, "1 mg/ml CM-BC1 + WBI" of 18 Gy (3 × 6 Gy).
Increase in survival time (ILT) is calculated in comparison to the "unloaded beads" group (%). SE means "standard error".

vival curves for all groups were obtained by Kaplan–Meier method, including long survivor animals (Fig. 4).

4. Discussion

DC Bead microspheres have been studied in particular as a treatment option for hepatocellular carcinoma (Aliberti et al., 2008; de Baere et al., 2008; Eyol et al., 2008; Gonzalez et al., 2008; Lencioni et al., 2008; Lewis et al., 2006a,b; Malagari et al., 2008; Poon et al., 2007). The incorporation of doxorubicin into these small PVA beads was performed to enhance the cytotoxic effect through local administration. Preclinical testing of this product in embolization models has demonstrated the reproducibility and reliability of the device, together with reduced systemic drug exposure and sustained local drug delivery (Hong et al., 2006; Lewis et al., 2006a). Studies have shown that doxorubicin was still being released from the beads at 90 days post implantation (Lewis et al., 2006a,b; Namur et al., 2010). Therefore DC Bead presents advantages over other techniques designed for direct administration to the tumor bed, such as wafer implants, conjugated nanoparticles, liposome encapsulated drug, or intratumoral infusion with Ommaya reservoir. There have been already more than 10,000 procedures carried out with this doxorubicin bead combination in the treatment of liver tumors, demonstrating their safety, and also a phase II randomised control trial which shows lower side effects and benefits for advanced patients (Lammer et al., 2010). There have been a number of pharmacokinetic studies which demonstrate the release of doxorubicin into the bloodstream is very low compared to the traditional treatments and also preclinical work which demonstrates the release of drug occurs over many weeks (Namur et al., 2010). Characterisation of the drug loading beads has been extensively studied in order to understand the kinetics of drug loading and release. Data from in vitro studies show drug uptake is dependent on the dose of drug and the size of the beads, with loading efficiency of >99% being achieved at doses up to 40 mg dox/ml hydrated beads. The presence of drug in the bead results in displacement of water, and consequently a decrease in the average diameter of the beads and a decrease in compressibility. Doxorubicin is present throughout the structure of the bead; however fluorescent microscopy revealed the drug to be concentrated in the outer regions of the bead. Release of the drug measured in vitro showed slow release of the doxorubicin from the bead, with half-lives over 1500 h predicted for across the bead size range, although elution rates were faster with smaller beads. A strong linear relationship was found between these in vitro data and pharmacokinetic data obtained from patients treated with DC Bead loaded with doxorubicin in transarterial chemoembolisation procedures (Gonzalez et al., 2008). The sustained local release of the drug has been confirmed in vivo models showing limited systemic drug levels, presence of drug in the beads and surrounding tissue at 90 days post-implantation (Lewis et al., 2006a; Namur et al., 2010). Again, smaller beads were shown to result in higher peak plasma levels and increased tissue necrosis, indicative of their faster release profile. These data could have been interesting for a sustained release during all the radiation therapy time for a concomitant effect. If other drug delivery devices have been developed and evaluated in animal models, very few are supported by so encouraging data. In our study, 10 mg/ml CM-BC1 (total dose 40 μg of doxorubicin per rat) was highly clinically toxic. The lower dose of 1 mg/ml CM-BC1 (total dose of 4 μg of doxorubicin per rat) was well tolerated with no early deaths. However, little clinical effect was seen of the CM-BC1 alone, indicating that a therapeutic effect was not achieved at this dose. Nonetheless, the amount of doxorubicin loaded into the beads can be modified and as the feasibility of this approach has been demonstrated, intermediate doses of doxorubicin could be tested. In one publication describing local therapy in a rat glioma model, doxorubicin was loaded in polyanhydride polymers (PCPP-SA). PCPP-SA were prepared using the mix-melt method (Lesniak et al., 2005). It was found that the doses of 1 mg, 700 μg and 500 μg of doxorubicin were toxic, whereas 300 μg was well tolerated. The type of toxicity observed was the same as in our study, namely necrosis and oedema. It is important to note that this polyanhydride polymer allows a slow release of the drug: 1% loaded polymer released 6.5% of drug over 200 h, and 10% loaded polymer released 21% of drug over 200 h. This release, slower than that obtained with the DC-beads, could explain the differences in term of tolerance over different dose ranges. While the in vitro potency of doxorubicin is remarkable and its current indications in treating peripheral tumors have proven efficacious, doxorubicin is highly toxic and presents a very narrow therapeutic window.

The other purpose of this study was to assess the anti-tumor activity, in terms of survival benefit, of administration of drug-eluting beads in rats bearing a cerebrally implanted syngeneic glioblastoma 9L. The potential added effect of radiotherapy was also evaluated. In terms of survival, the control groups (untreated and unloaded beads) median survival was as expected for this animal model in the laboratory (27 and 25 days, respectively), with a small standard error of the mean confirming the quality and reproducibility of the model. Although the 9L gliosarcoma is not a perfect model of human glioma because it is less invasive, we used it because of its ability to create very similar sized tumors. CM-BC1 (4 μg/rat) in combination with radiotherapy significantly increased the median survival, compared to the chemotherapy alone group and there were two long survivors for this group. However it was not significantly different, in comparison with the radiotherapy alone group. The major finding of this work is that doxorubicin beads, administered in combination with external beam irradiation, resulted in a significant enhancement in median survival time compared to the chemotherapy group ($p = 0.02$). The association gives the best median of survival, even compared to the "WBI" group with 64.4 days versus 59.9 days even though this result is not significant.

This work gives interesting preliminary results because the behaviour of beads in rat brain was unknown until now. In this study, doxorubicin-loaded beads were mixed with a solution of ultra-pure high molecular weight alginate. Alginate, a viscous polymer derived from brown algae, is an established compound in many biomedical and nutritional applications. Due to alginate's biocompatibility and simple gelation with divalent cations such as Ca^{2+}, it is widely used for cell immobilization and encapsulation. Mixing the alginate with the beads prior to intracerebral implantation was performed in order to minimize displacement of the beads. As we observed in biocompatibility studies, we have little back flow with this technique since only a few beads can be observed in the subarachnoid space in a few animals. A pilot study by Brinker et al reported on the investigation of DC Bead with doxorubicin or irinotecan in a B4TCa rat glioma model (Blates et al., 2010). While both drugs were efficacious in treating the tumor, doxorubicin was shown to be far more toxic than irinotecan and the method of microsphere implantation was also not readily translatable into the clinic.

Our work demonstrates that DC Beads are well tolerated in rat brain and can be easily used in patients when combined with the alginate carrier. Further work is now necessary to improve this concept for better preclinical results. We have the opportunity to change the concentration of the loaded therapeutic agent or to increase their volume of distribution for example. Others drugs could also be an option because DC Beads can be loaded with other interesting therapeutic agents, such as irinotecan, topotecan and mitoxantrone (Lewis, 2009). They have shown the same safety and efficiency in patients for hepatic tumors and irinotecan can be efficient against glioma cells (Buie and Valgus, 2008).

5. Conclusion

This preclinical evidence suggests that 1 mg/ml CM-BC1 may be easily used to treat high-grade malignant brain tumors. Our data represent the first demonstration of the compatibility between these compounds and rat brain, and potentially indicate a therapeutic option for this class of tumors which often present problems of accessibility and therapeutic resistance.

Conflict of interest

None declared.

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