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Research paper

Anti-cancer drug diffusion within living rat brain tissue: an experimental study using [^{3}H](6)-5-fluorouracil-loaded PLGA microspheres

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Abstract

This study was performed (i) to monitor the diffusion of the anti-cancer drug 5-fluorouracil (5-FU) and (ii) to elucidate the fate of poly(lactide-co-glycolide) (PLGA) based microspheres within living rat brain tissue upon intracranial implantation. Drug-loaded microparticles were prepared using a solvent emulsion/extraction process and administered into healthy and C6 glioma-bearing Sprague–Dawley rats. The same surgical procedure was carried out with magnetite-loaded microspheres. To monitor 5-FU diffusion from the implantation site, tissue combustion was performed on animals implanted with tritiated drug microspheres. T_2-weighted nuclear magnetic resonance imaging was undertaken on animals implanted with magnetite-loaded microspheres to determine microsphere localization after deposit. Results show that an important microparticle backflow occurs in healthy rats, whereas the microspheres remain at the site of administration in C6 glioma-bearing rats. Drug diffusion is limited to the vicinity of the implantation site. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: 5-Fluorouracil; Drug delivery; Microsphere; Magnetite; Diffusion; C6 glioma; Tissue combustion

1. Introduction

Brain tumours have been for long an unsolved challenge to therapeutic gains. Although new drugs and techniques have contributed to improve patients' lives or even eradicate their suffering, research has failed to find a valuable way to improve the situation.

In the case of fast-spreading glioblastomas, the current treatment consists in surgically removing the tumourized area and submitting the patient to external radiotherapy. This leads to a median survival time of only 11 months [1]. Different strategies have been developed to overcome the blood–brain barrier (BBB) transport and to allow an increased amount of locally available drug to treat the tumour. Bakhshi and North [2] reviewed the role of drug infusions in the treatment of brain cancers, by the means of catheterization or implantable pumps. Although continuous infusions of chemotherapeutic agents give encouraging results, the implementation of such devices does not improve patients' welfare and life expectancy. The use of drug delivery systems represent an alternative way to reach the above-mentioned goal. Walter et al. [3] described a biodegradable implant, charged with paclitaxel, which significantly extended the survival time of 9L-gliosarcoma-bearing rats as compared to control animals. Carmustin was encapsulated within hybrid liposomes designed for intrathecal chemotherapy: in rats inoculated in the cisterna with C6 glioma cells, these liposomes prevented the subarachnoid dissemination of the malignant glioma [4]. Sampath and Brem [5] reviewed ongoing and planned clinical trials set up to evaluate safety and efficacy of a variety of encapsulated anti-tumour agents targeted against brain cancers.

Ongoing studies in our group aim at using poly(D,L-lactide-co-glycolide) (PLGA)-based microspheres to deliver drugs to the CNS [6–8]. In particular, 5-fluorouracil (5-FU)-loaded microspheres have been developed for stereotactic intracerebral implantation [9]. Preliminary experiments on C6 glioma-bearing rats showed promising results, the median survival time could be doubled [10]. Eight patients of a phase I–II pilot study, scored for high-grade glioma, underwent surgical removal before 5-FU microspheres were implanted in the resection bed. Eighteen months after the trial began, results were very encouraging as far as survival, welfare and future applications were fore-

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cast [11]. Compared with monolithic implants such as Gliadel [12], microspheres allow a higher adaptability of the treatment to the surgical act. Indeed, depending on how deep and how distant from each other microspheres are deposited, the area covered by the drug can be controlled. Nevertheless, to complete further clinical studies, some questions should be answered. The present work aims at working out some of them by examining microsphere fate and 5-FU-diffusion area from these particles in the brain. [^{3}H]5-FU microspheres were used to evaluate diffusion areas from the implantation site.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA 25/50, Resomer RG 506, molecular weight = 75 000; $I = 1.56$) and poly(-vinyl alcohol) (PVA Rhodoviol 4/125, 88% hydrolyzed) were respectively obtained from Boehringer Ingelheim (Germany) and Merck Eurolab (France), and [^{3}H]5-FU from NEN (1 mCi in 1 ml, 12.5 Ci/mmol, DuPont de Nemours, USA). 5-Fluorouracil (5-FU) was kindly donated by Roche (France).

Solvents and reagents were high-grade and used without further treatment (Merck Eurolab, France).

Fifteen female Sprague–Dawley rats, 160–180 g bodyweight (Elevages Dépré, France) were used in this study. They were kept in standard animal facilities, and given free access to water and food. Animal care was administered in strict accordance to the European Community guidelines.

The C6 glioma brain tumour line used in this study was kindly provided by INSERM U298 (Angers, France). Cells were grown on F12 medium enriched with 10% foetal bovine serum and 1% anti-microbial condensed solution (penicillin/streptomycin/amphotericin B) in a humidified atmosphere of 5% CO_2, at 37 °C. Once in an exponential growth phase, cells were detached with 0.25% trypsin, centrifuged at $1000 \times g$ for 10 min and resuspended in DMEM (Dulbecco's modified Eagle medium) to a final concentration of 4×10^4 cells per 10 μl. All cell culture devices and solutions were obtained from Gibco (France).

For implantation purposes, microspheres were suspended in a sodium carboxymethylcellulose (Na-CMC) solution containing: 3.76% (w/w) mannitol, 0.94% (w/w) polysorbate 80 and 1.18% (w/w) sodium carboxymethylcellulose (all supplied by Cooper, France).

Animals were anaesthetized by a mixture of acepromazine (4 mg/kg) and ketamine (80 mg/kg). Stereotactic injections were performed using a stereotactic frame (Stoelting, USA).

2.2. Microsphere preparation

2.2.1. [^{3}H]5-FU crystallization

Microsphere preparation requires the use of 5-FU crystals [9]. Yet [^{3}H]5-FU is only commercially obtained as a solution in ethanol/water (7:3). We set up a process to obtain crystals: [^{3}H]5-FU solution was poured, under stirring, into a beaker containing 200 mg 5-FU dissolved in 27 ml methanol then added to 200 ml heptane placed in a reactor, kept under an argon flow (0.5 ml/min) for 50 h at 35 °C. Crystals appeared when solvents evaporated; 5-FU is insoluble in heptane. Then, [^{3}H]5-FU crystals were collected and vacuum-dried from heptane at 20 °C.

2.2.2. [^{3}H]5-FU encapsulation

Microspheres were prepared by the solvent evaporation/extraction method, as previously reported [9]. Briefly, [^{3}H]5-FU crystals were suspended in a PLGA/methylene chloride/acetone solution and poured into a PVA aqueous solution under mechanical stirring. The emulsion was added to water, still under mechanical stirring, allowing solvent extraction to take place.

2.2.3. Magnetite-loaded microspheres

Magnetite particles (0.2 ± 0.1 μm) were prepared as previously described [13] and were subsequently encapsulated as an aqueous suspension by the double emulsion process [8]. Briefly, the aqueous suspension containing the magnetite particles was emulsified in a PLGA/methylene chloride/acetone solution then added to a PVA aqueous solution under mechanical stirring. The resulting emulsion was added to water, still under mechanical stirring.

2.3. Microsphere characterization

2.3.1. Encapsulation rate

Scintillation counting (β counting apparatus type 1214, EG&G Instruments, France) and spectrophotometric assay (266 nm, Uvikon 922, Kontron Instruments, Italy) allowed to assess 5-FU content after dissolution of 5-FU microspheres in dimethyl sulphoxide.

2.3.2. Size distribution

Crystal and microsphere size distributions were evaluated by laser diffractometry (Mastersizer S, Malvern Instruments, UK).

2.3.3. In vitro kinetics studies

Samples of about 40 mg of microspheres were placed into dialysis bags (6000–8000 mol. wt. Co; Fisher Bioblock, France), then placed into glass vials containing 300 ml 0.13 M phosphate buffer (pH 7.4) in a USP dissolution apparatus (100 rpm, 37 °C; AT7, Sotax, Basel, Switzerland) and protected from light. Medium was sampled over time and drug concentrations were determined by β radioactivity counting and spectrophotometry assay (266 nm).

2.4. Cell and microsphere implantation

The 15 animals were divided into two groups, one bearing C6 glioma ($n = 5$), the other not ($n = 10$). To inoculate the

tumour, anaesthetized animals were placed in the stereotactic head frame and a burr hole was drilled (anterior 0 mm, lateral 3 mm, depth −7 mm according to bregma). The cell suspension (40 000 C6 cells, 10 μl) was inoculated over a 10-min period. The scalp was closed with surgical thread. Tumours were allowed 12 days to develop before they admitted a mean volume of 20 μl [14]. On day 12, microsphere suspensions were injected into all rats at the same coordinates, at the rate of 1 μ/min, using a 23-gauge needle. Microsphere concentrations were adjusted so the delivered 5-FU amount was 0.40 mg per animal, corresponding to 2.88 μCi ($n = 13$, four C6-bearing rats and nine healthy rats), i.e. 61.5 mg of microspheres per animal. Magnetite microsphere concentration was 62 mg per animal ($n = 2$, one C6-bearing and one healthy).

2.5. [^{3}H]5-FU diffusion studies

One animal of each group (with or without C6 glioma) was killed at 24, 48, 96 and 168 h post-implantation of [^{3}H]5-FU-loaded microspheres by being frozen into isopentane at about −70 °C under deep halothane-induced anaesthesia. They were kept at −18 °C before further treatment.

Heads were fixed horizontally on the freezing microtome plate and sliced in a perpendicular way to both the sagittal axe of the animal and the course of the needle used for microsphere injection, so horizontal sections of the brains were obtained. Two-hundred-micrometre thick consecutive slices were collected in paper devices and burnt in an Oxidizer apparatus (type 307, Packard, France). β radioactivity was then gathered and evaluated by β-counting.

2.6. NMRI assessments

Nuclear magnetic resonance imaging (NMRI) was performed with a Bruker Avance DRX 300 (Germany) apparatus equipped with a vertical superwide-bore magnet of 7T. Quantitative T_2-weighted NMR images of the brain were obtained using a multi-spin echo sequence (TR = 2500 ms, TE = 15, 30, 45, 60, 75, 90, 105 and 120 ms; FOV = 3 × 3 cm; matrix 128 × 128; nine contiguous slices of 1 mm, two acquisitions).

3. Results

3.1. In vitro studies

The tritiated 5-FU crystals had a mean diameter of 28.4 ± 12.7 μm. [^{3}H]5-FU-loaded microspheres were developed which showed similar in vitro drug release profiles as the microspheres used in the clinical trials conducted in Angers Hospital Centre [11]. The prepared microparticles had a mean diameter of 35 ± 14 μm and a 5-FU loading of 13 ± 1%. Radioactivity level was 937 μCi/g of microspheres. The release profile was characterized by an initial drug release of 22% within the first 24 h, followed by a slower constant release rate for a 3-week period (Fig. 1). Both radioactivity evaluation and spectrophotometry gave similar results.

One of the potential pitfalls with tritiated compounds comes from the exchange of this molecule with water protons. This point was checked by counting samples before and after lyophilization in order to assess this exchange. The calculated prior/after lyophilization ratio ρ equaled $\rho = 1.01$.

3.2. In vivo studies

During surgery, the [^{3}H]5-FU microsphere suspension flowed back importantly around the trepanation hole in healthy brain tissues whereas this almost did not occur during implantation in C6 glioma-bearing rats. To quantify this phenomenon, the apparent microsphere backflow occurring during surgery was retrieved on cotton tips and consequently counted after combustion. To minimize this backflow, different suspension volumes, ranging from 20 to 50 μl ($n = 3$), and microsphere concentrations, 0.15 and 0.24 mg of microspheres per μl of Na-CMC ($n = 2$), were tested in the healthy group. The best results were obtained for 20 μl of a suspension containing 0.15 mg microspheres per μl. Nevertheless, despite this optimization, this backflow represented for the subsequent eight animals treated, approximately 30–60% in healthy tissues ($n = 4$) whereas it was 0–5% in tumour tissues ($n = 4$) in terms of microsphere quantity.

The localization of implanted microspheres in the brain was confirmed using magnetite-loaded PLGA-microspheres. Magnetite is a superparamagnetic compound that significantly reduces transverse relaxation time. Therefore, on T_2-weighted nuclear magnetic resonance images, magnetite-loaded microspheres appeared as a dark spot and were located at the exact coordinates where they had been deposited in the tumour-bearing brain. In the healthy rat, microspheres were observed along the needle track, quite homogeneously spread. The measured diameter of the spot in the tumour-bearing rat was about 2.5 mm (Fig. 2)

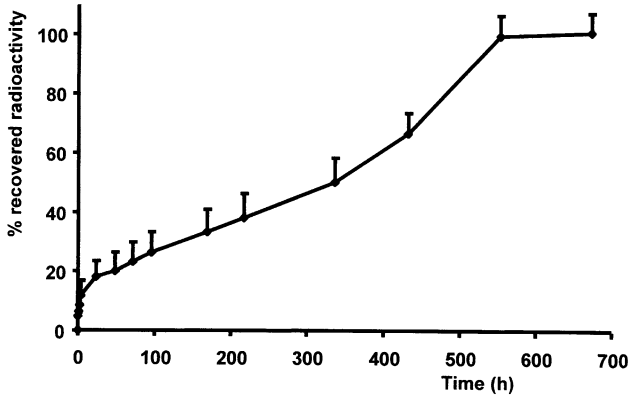

Fig. 1. [^{3}H]5-FU release profile in 130 mM sodium phosphate buffer, pH 7.4, 37 °C.

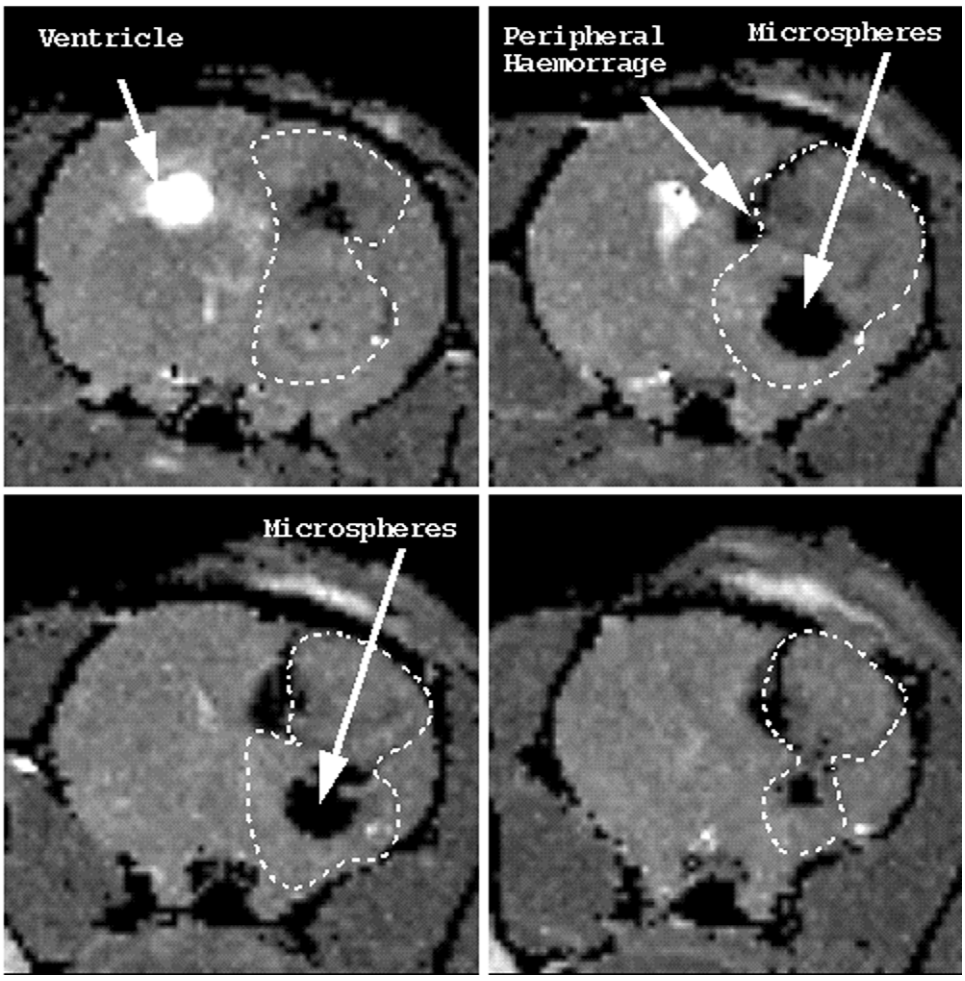

Fig. 2. One-millimetre thick contiguous NMR T_2-weighted images of a C6 glioma-bearing rat brain, 16 days post cell inoculation (4 days after magnetite microparticle implantation). Tumour was manually delimited by the dashed line. The striatal hypointense spot seen on two consecutive images correspond to the magnetite nanoparticles.

whereas it was 5.5–6 mm in the case of the healthy rat brain (Fig. 3). These hyposignals were due to the magnetite entrapped in microsphere matrices. In vitro release evaluation of magnetite microspheres showed no release of magnetite for more than 14 weeks (Fig. 4).

Microsphere localization was also confirmed by radioactivity. Radioactivity diffusion in brains was established by sample combustion of contiguous 200-μm thick slices, taking into account the apparent backflow. Radioactivity gathered on cotton tips during surgery was deduced from the total activity implanted to determine the activity really deposited in the brains, from which percentages were calculated (Fig. 5). After stereotactic injection of [^{3}H]5-FU-loaded microspheres in healthy rat brains, radioactivity was recovered along the needle track (Fig. 5A) whereas, after injection in tumour-bearing rat brains, radioactivity was mainly located at depth 7 ± 1.5 mm, i.e. the site of microsphere implantation and spreading (Fig. 5B). Similar distribution patterns were observed throughout the whole experimental time.

Close analysis of the radioactivity profiles showed that the diffusion within the rat brain is very limited (Fig. 5A,B). Underneath the injection site (−7 mm), radioactivity level was important over a 1.5-mm distance, which, according to the NMR images, corresponds to the natural spreading of the microsphere suspension after implantation. Then it dropped to very small amounts, which are nevertheless significant (ten times noise level at the minimum for 3 mm). There is no difference of profile whatever time of sacrifice is considered but intensity is greater for animals killed at 96 and 168 h. These results, supported by magnetite microsphere localization, demonstrate that radioactivity recovered underneath 8.5 mm is due to ^{3}H diffusion alone: tritium is detected 3 mm beneath the site of microsphere deposit (i.e. from 8.5 mm under bregma), similarly in the two groups, as a maximum.

4. Discussion

Among many parameters, drug release kinetics from microspheres depend on crystal size distribution and encap-

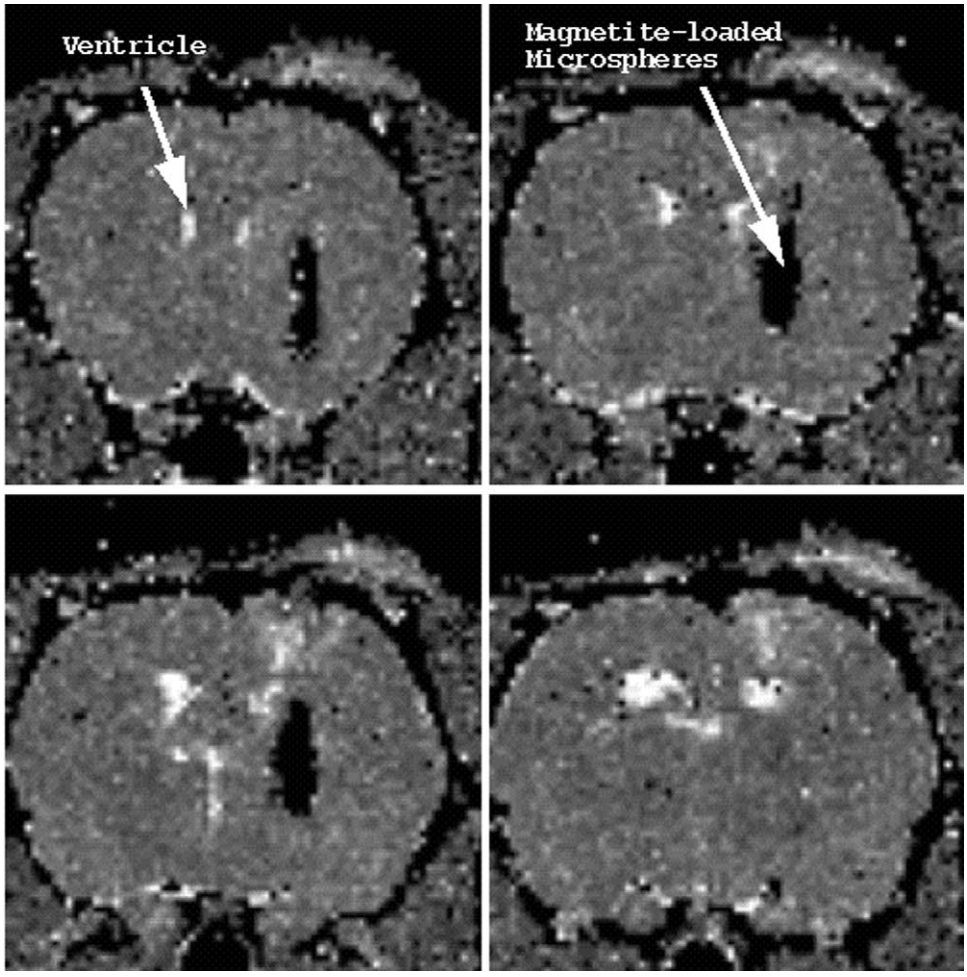

Fig. 3. One-millimetre thick contiguous NMR T_2-weighted images of a healthy rat brain, 4 days after magnetite microparticle implantation. Spots due to magnetite are seen on three consecutive slices.

sulation rates [15]. To achieve release profiles that would match the requirements we wished (i.e. a burst effect less than 40% at 24 h and microsphere sizes compatible with stereotactic injection), we investigated these factors. Usually, a milling step can be performed to obtain an adequate size distribution [7] but, with a radiolabelled compound, this step is difficult to consider. Consequently, the current experimental conditions were set up to obtain crystals to be directly encapsulated. Moreover, in our case, it was necessary that radioactivity was homogeneously spread into the initial 5-FU powder. A concomitant crystallization of the radiolabelled and cold 5-FU led to an homogeneous mixture. The obtained crystals presented a larger mean diameter than usually used but allowed to prepare appropriate microspheres by keeping the encapsulation rate low (13%).

Although the therapeutic potential of drug-loaded microspheres is established in animal and human brain tumours [10,16,17], information on their fate after implantation had to be supplemented.

The required administration way of microspheres, i.e. the stereotactic implantation, involves a viscous isotonic medium. Na-CMC aqueous solution addresses this point. Nevertheless, healthy brain tissues are considered as viscoelastic [18]. They show great difficulties to 'absorb' the injected suspended medium, which results in an unavoidable backflow. This phenomenon has been quantified by Morrison et al. [19]. They showed that backflow intensity is inversely proportional to viscosity and shear modulus and proportional to inflow rate, tissue resistance and needle radius. In our work, despite adaptations of implantation conditions, results show an important backflow in the healthy group whereas the event remains negligible in the tumour-bearing group. Some explanation could arise from the aforementioned work, as tumourized and healthy tissues do not present the same features. The C6 tumour is a fast-growing, disorganized, infiltrating tumour [20]. Hence, tissue resistance must decrease and shear modulus increase in brain tumour tissues. Globally, the backflow phenomenon must be reduced compared to healthy tissues. This could explain the suspension retention at the site of injection in the tumour for C6 glioma-bearing brains. For therapeutic purposes in clinics, microspheres are presently implanted in the tumour removal bed, i.e. in healthy tissues including

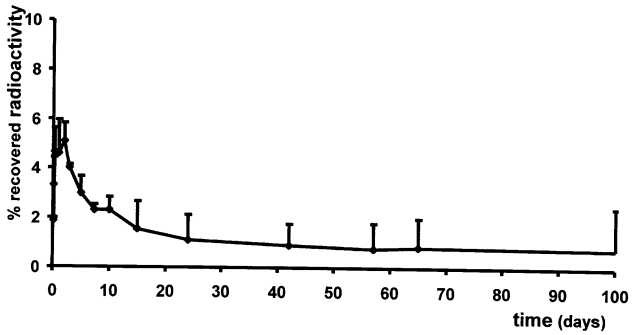

Fig. 4. In vitro magnetite release profile from microspheres, in 130 mM sodium phosphate buffer, pH 7.4, 37 °C. The initial rise in radioactivity recover was due to microspheres being removed as the same time as supernatant, so this value was deducted afterward. This graph allows to establish that magnetite release is negligible for a very long period of time (>25 weeks).

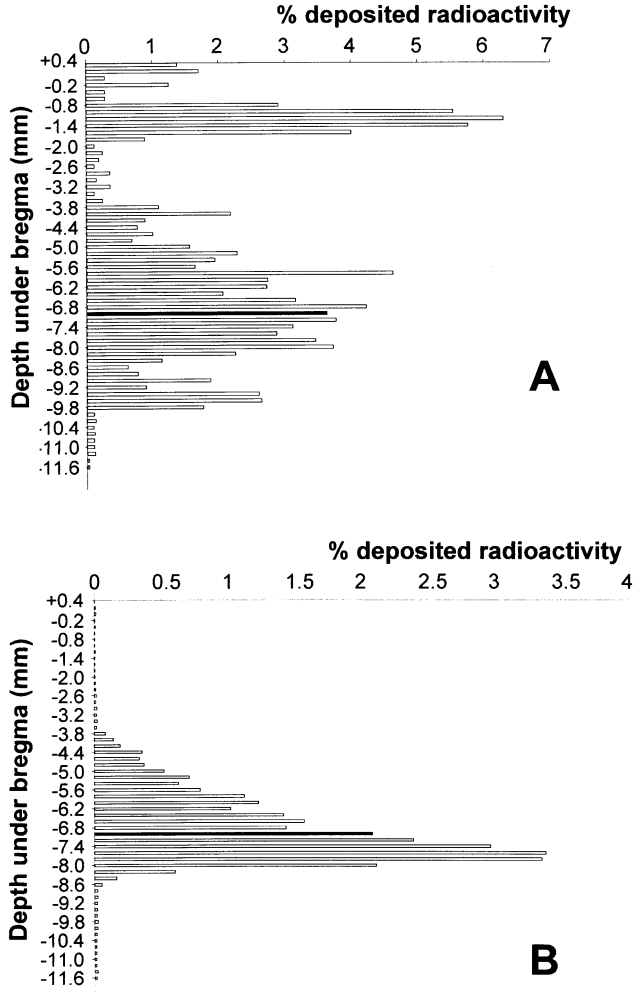

Fig. 5. Radioactivity distribution in brains as a function of depth (in mm) under the bregma in a healthy (A) and a tumour-bearing (B) rat 168 h after [^{3}H]5-FU-loaded microsphere implantation. Similar patterns were observed for the other rats of each group and subsequently are not shown. Black lines point at the *z*-coordinate (depth) used for implantation purposes (7 mm under bregma).

disseminated tumour cells [11]. The observed backflow is being countered by maintaining microspheres in touch with the surgical resection bed, with the help of a draining gel (Surgicel, cellulose oxide). Future developments of formulation and surgical techniques should lead to treatment of patients whose gliomas are currently too deep or complex to undertake surgery. Microspheres seem particularly suitable for direct implantation in the tumour because they spontaneously remain in situ in tumourized areas. They offer a new means to prolong survival in these patients.

Our results show a limited distribution of 5-FU in the brain. A maximum of 3 mm was noted if microspheres are considered to remain where they are deposited. This is in good agreement with previous findings by Kassis et al. [21]. These authors established that, in a human brain invaded with a glioma, [^{123}I]iododeoxyuridine (IUdR) diffused only in the vicinity of the injection site, in the tumour volume, with diffusion distances in accordance with our conclusions. IUdR and 5-FU belong to the same group of antimetabolites and are both quite hydrophilic (5-FU: 12 mg/ml; IUdR: 2 mg/ml). This characteristic could partially explain the poor distribution in a 'lipophilic' tissue. From this statement, we could deduce that a more lipophilic molecule would diffuse better. But Fung et al. [22] noted short diffusion distances (<3 mm) for bis-chloronitrosourea (BCNU) encapsulated in a monolithic implant in rat brains. Wang et al. [23] established that BCNU presents a very short penetration distance because it gets drained out of the system before being able to diffuse to any appreciable distance; this was correlated with its transvascular permeability. Nevertheless, 5-FU exhibits a poorer transvascular permeability than BCNU. Thus, even if its hydrophilic characteristics prevents the molecule from diffusing extensively, it is more likely to stay a longer period of time in the tumour vicinity, with rising concentrations. Hence, its local efficacy may increase.

The short diffusion distance supports the choice of a multi-point administration during surgery. It is essential that the whole area of the tumour location be covered with 5-FU. With the microsphere form, it is possible to multiply very close injection sites. This guarantees a local, sustained delivery of the drug, with minimal damage to surrounding healthy tissues. Microspheres permit a wider and deeper delivery of the drug to the tumour site, and even to otherwise inaccessible areas of the brain. 5-FU-loaded microspheres should authorize a great benefit in terms of survival in glioma-bearing patients, with negligible side effects and an important improvement in their welfare.

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References

[1] P.L. Kornblith, M. Walker, Chemotherapy for malignant gliomas, J. Neurosurg. 68 (1998) 1–17.

[2] S. Bakhshi, R. North, Implantable pumps for drug delivery to the brain, J. Neurooncol. 26 (1995) 133–139.

[3] K. Walter, M. Cahan, A. Gur, B. Tyler, J. Hilton, O. Colvin, P. Burger, A. Domb, H. Brem, Interstitial taxol delivered from a biodegradable polymer implant against experimental malignant gliomas, Cancer Res. 54 (1994) 2207–2212.

[4] I. Kitamura, M. Kochi, Y. Matsumoto, R. Ueoka, J. Kuratsu, Y. Ushio, Intrathecal chemotherapy with 1,3-bis(2-chloroethyl)-1-nitrosourea encapsulated into hybrid liposomes for meningeal gliomatosis: an experimental study, Cancer Res. 56 (1996) 3986–3992.

[5] P. Sampath, H. Brem, Implantable slow-release chemotherapeutic polymers for the treatment of malignant brain tumors, Cancer Control J. 5 (1998) 130–137.

[6] P. Menei, V. Daniel, C. Montero-Menei, M. Brouillard, A. Pouplard-Barthelaix, J.P. Benoit, Biodegradation and brain tissue reaction to poly(D,L-lactide-co-glycolide) microspheres, Biomaterials 14 (1993) 470–478.

[7] A. Gèze, M.C. Venier-Julienne, P. Saulnier, P. Varlet, C. Daumas-Duport, P. Devauchelle, J.P. Benoit, Modulated release of IdUrd from poly(D,L-lactide-co-glycolide) microspheres by addition of poly(D,L-lactide) oligomers, J. Controlled Release 58 (1998) 311–322.

[8] J.M. Péan, M.C. Venier-Julienne, F. Boury, P. Menei, B. Denizot, J.P. Benoit, NGF release from poly(D,L-lactide-co-glycolide) microspheres. Effects of some formulation parameters on encapsulated NGF stability, J. Controlled Release 56 (1998) 175–187.

[9] M. Boisdron-Celle, P. Menei, J.P. Benoit, Preparation and characterization of 5-fluorouracil-loaded microparticles as biodegradable anticancer drug carriers, J. Pharm. Pharmacol. 47 (1995) 108–114.

[10] P. Menei, M. Boisdron-Celle, A. Croué, G. Guy, J.P. Benoit, Effect of stereotactic implantation of biodegradable 5-fluorouracil-loaded microspheres in healthy and C6 glioma-bearing rats, Neurosurgery 39 (1996) 117–123.

[11] P. Menei, M.C. Venier, E. Gamelin, J. Saint-André, G. Hayek, E. Jadaud, P. Mercier, G. Guy, J.P. Benoit, Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of glioblastoma, Cancer 86 (1999) 325–330.

[12] W. Dang, T. Daviau, P. Ying, Y. Zhao, D. Nowotnik, C. Clow, B. Tyler, H. Brem, Effects of GLIADEL wafer initial molecular weight on the erosion of wafer and release of BCNU, J. Controlled Release 42 (1996) 83–92.

[13] L. Babes, B. Denizot, G. Tanguy, J.J. Le Jeune, P. Jallet, Synthesis of iron oxide nanoparticles used as MRI contrast agents: a parametric study, J. Colloid Interface Sci. 212 (1999) 474–482.

[14] L. Lemaire, F. Franconi, J.P. Saint-André, V.G. Roullin, P. Jallet, J.J. Le Jeune, High-field quantitative transverse relaxation time, magnetization transfer and apparent water diffusion in experimental rat brain tumour, NMR Biomed. 13 (2000) 116–123.

[15] L. Martini, J. Collett, D. Attwood, The release of 5-fluorouracil from microspheres of poly(e-caprolactone-co-ethylene oxide), Drug Dev. Ind. Pharm. 26 (2000) 7–12.

[16] M. Glaize Ewend, J. Williams, K. Tabassi, B. Tyler, K. Babel, R. Anderson, M. Pinn, D. Brat, H. Brem, Local delivery of chemotherapy and concurrent external beam radiotherapy prolongs survival in metastatic brain tumor models, Cancer Res. 56 (1996) 5217–5223.

[17] R. Gutman, G. Peacock, D. Lu, Targeted drug delivery for brain cancer treatment, J. Controlled Release 65 (2000) 31–41.

[18] L. Bilston, Z. Liu, N. Phan-Tien, Linear viscoelastic properties of bovine brain tissue in shear, Biorheology 34 (1997) 377–385.

[19] P. Morrison, M. Chen, R. Chadwick, R. Lonser, E. Oldfield, Focal delivery during direct infusion to brain: role of flow rate, catheter diameter, and tissue mechanics, Am. J. Physiol. 277 (1999) R1218–R1229.

[20] J. Mokry, S. Nemecek, J. Adler, Inoculation of C6 glioma cell suspension into the brain of adult rats: morphological study, Sb. Ved. Pr. Lek. Fak. Karlovy Univerzity Hradci Kralove 35 (1992) 293–305.

[21] A. Kassis, S. Tumeh, P. Wen, J. Baranowska-Kortylewicz, A. Van der Abbeele, R. Zimmerman, P. Carvalho, B. Garada, W. DeSisto, N. Bailey, F. Castronovo, G. Mariani, P. Black, S. Adelstein, Intratumoral administration of 5-[^{123}I]iodo-2′-deoxyuridine in a patient with a brain tumor, J. Nucl. Med. 37 (1996) 19S–22S.

[22] L. Fung, M. Shin, B. Tyler, H. Brem, W. Saltzman, Chemotherapeutic drugs released from polymers: distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea in the rat brain, Pharm. Res. 13 (1996) 671–682.

[23] C. Wang, J. Li, C. Teo, T. Lee, The delivery of BCNU to brain tumors, J. Controlled Release 61 (1999) 21–41.