Expert Opinion

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Drug delivery into the brain using poly(lactide-co-glycolide) microspheres

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Among the strategies developed for drug delivery into the CNS, locally controlled drug release by the way of an implantable polymeric device has been developed in recent years. The first polymeric devices developed were macroscopic implants needing open surgery for implantation. Over the last few years, poly(lactide-co-glycolide) microspheres have been shown to be safe and promising for drug delivery into the brain. Poly(lactide-co-glycolide) is biodegradable and biocompatible with brain tissue. Due to their size, these microspheres can be easily implanted by stereotaxy in discrete, precise and functional areas of the brain without causing damage to the surrounding tissue. Brain tumour treatments have been developed using this approach and clinical trials have been performed. Potential applications in neurodegenerative diseases have also been explored, particularly neurotrophic factor delivery and cell therapy.

Keywords: brain tumour, cell therapy, chemotherapy, drug delivery, microspheres, neurotrophic factors, PLGA, polymer, stereotaxy

Expert Opin. Drug Deliv. (2005) 2(2):363-376

1. Introduction

Drug delivery to the brain remains a pluridisciplinar and challenging area of research. The application of numerous new and potent drugs is limited by their weak passage through the blood–brain barrier. In addition, the brain is not made of the repetition of the same functional unit, as in the liver or the kidney, but is a complex assemblage of different anatomo-functional structures. This complexity explains the need to target precisely one area to avoid neurological side effects. For example, cerebroventricular infusion of glial-cell-derived neutrophic factor (GDNF) in the aim to rescue dopamine-mediated mesencephalic neurons and to cure Parkinson's disease, lead to loss of appetite, intermittent hallucinations, depression and inappropriate sexual conduct due to the action of GDNFs on other populations of dopamine-mediated neurons [1].

While waiting for the design of a 'magic bullet' that is able to cross the blood–brain barrier and reach the target cell in the desired brain area, strategies based on polymeric devices have been developed. The principle of incorporating a drug into an implantable polymeric carrier for controlled release was first mentioned in the 1960s by Folkman and Long who demonstrated in dogs that a silicone rubber device implanted into the myocardium could release digoxine [2]. The first application of a controlled-release polymeric system in the brain was aimed at improving labelling of perivascular meningeal projections from cat trigeminal ganglia [3]. Since these studies, different research groups have developed implantable polymeric devices permitting the controlled and localised release of neuroactive substances directly into the brain. The first polymeric devices developed were macroscopic implants also called monolithic devices. Drugs are incorporated into polymers by triturating a dry powdered drug with a similarly treated polymer and compressing weighed aliquots of the mixture in a press (trituration method), or by dissolving both the polymer and the drug in a solvent, evaporating the solvent and punching out the resulting material (solution method). These macroscopic

implants are shaped as a nail, disc, wafer or pipe. The first clinical applications for the local treatment of brain tumours were reported by Japanese neurosurgeons using Silastic® (Dow Corning Corporation) implants loaded with 5-fluorouracil (5-FU) and polymethylmethacrylic acid needle-shaped systems releasing mitomycin, adriamycin, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-1-nitrosourea (ACNU) or 5-FU [4-6]. However, the real breakthrough was the development and FDA approval of biodegradable polyanhydrides wafers releasing 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (Gliadel®; Guilford Pharmaceuticals) for the treatment of glial brain tumours [7,8].

The limitation of these implants is their size, of several centimetres, which does not allow a real intratumoural or intraparenchymal implantation, neither by a stereotaxic nor endoscopic administration. Indeed, progress in neurosurgery now enables neurosurgeons to hit any target in the brain, either deep or as small as 1 mm, using a stereotaxic frame, or frameless trough computer-assisted neurosurgery (neuronavigation) [9]. These guided techniques allow the implantation of electrodes, catheters and the realisation of biopsies or any type of injection. The morbidity of stereotaxic procedures is low (mortality < 1%). Neuroendoscopy, which is gaining momentum due to the miniaturisation of endoscopic probes, also allows multiple biopsies or injections in the ventricles, cistern or skull base structures such as the pituitary gland [10]. Due to their size (1 – 1000 µm), microparticles in suspension can be easily implanted by these techniques in discrete, precise and functional areas of the brain, using needles as thin as 21 gauge, without causing damage to the surrounding tissue [11] (**Figure 1**). This implantation avoids the inconvenient insertion of large implants by open surgery and can be repeated if required.

Microparticles can be prepared by various methods [12,13]: i) physicochemical processes such as simple or complex coacervation, or emulsion solvent extraction–evaporation; ii) chemical processes such as interfacial polycondensation; and iii) mechanical processes including spray coating, spray drying and spray congealing [102]. The aforementioned microencapsulation processes can either lead to the production of microspheres or microcapsules. The microspheres are matrix systems in which the drug is dispersed throughout the polymer, whereas the microcapsules can be viewed as reservoir systems, in which each capsule is delimited by an individual polymeric membrane. Mechanisms of drug release are different depending on the microparticle structure and the polymer type. The drug either diffuses through the polymeric barrier or in the pores filled with water. If the polymer is biodegradable, a combination of diffusion and degradation phenomena regulates the release kinetics.

2. Poly(lactide-co-glycolide) microspheres

For surgical use, the polymer employed should be totally biocompatible and biodegradable in the brain tissue. Natural macromolecules (human or bovine serum albumin, gelatin, collagen, alginate, chitosan and so on) have been tested, but special attention should be given to the purity and homogeneity of samples, and to the risk of disease transmission (as the spongiform bovine encephalopathy).

Many synthetic polymers have been investigated, but attention has been focused on aliphatic polyesters including poly(α-hydroxyacids) (PLGA) [14-17]. These polymers are constituted of lactic and/or glycolic acid units. When the two types of monomeric units are associated along the same chain, a copolymer is generated: PLGA. Its degradation, which is caused by the cleavage of the ester bonds by hydrolysis, is then independent of any enzymatic process and requires the presence of water alone. PLGA is totally biodegradable and is finally metabolised into CO_2 and H_2O (**Figure 2**). The physicochemical and degradation properties of PLGA depend on many parameters including the molar ratio of the two monomers in the polymer backbone, the molecular weight polymer and the polydispersity index. The biodegradation rate of the PLGA copolymers may vary from < 1 month to a period of a few years, depending on the polymer composition. It can, therefore, be modified and adapted to suit clinical purposes. The PLGA systems can be easily sterilised by γ-irradiation despite alteration of the polymer properties, thus allowing their surgical use [18,19].

Experimental studies and the long history of the clinical use of these types of copolymers, particularly as surgical sutures, have demonstrated their excellent histocompatibility [20-22]. PLGA microspheres are currently used in the clinic as subdermal implants for the controlled release of bromocriptine or luteinising hormone releasing hormone (LHRH) analogues. The total biocompatibility of PLGA with brain tissue has been demonstrated [23-28]. In brain tissue, a vacuole degradation starts in the middle of the 50:50 PLGA microspheres, which lose their spherical shape after 1 month. This phenomenon is due to the autocatalytic effect of the carboxylic groups of oligomers formed in the matrix during the degradation process. At the surface of the microsphere, these hydrosoluble low molecular weight degradation products are easily eluted. However, in the matrix they are imprisoned and accelerate the degradation process. The microspheres brake in several fragments and only a few fragments are observed after 2 months. The brain tissue reaction observed is a nonspecific astrocytic reaction with hypertrophia and overexpression of glial fibrillary acidic protein (GFAP), which begins 3 – 7 days after implantation, before decreasing between weeks 2 and 4, to persist locally as a scar along the injection tract. A macrophagic-microglial reaction occurs in parallel, starting the first day after implantation and decreasing dramatically after 1 month, although degraded microspheres persist in the area. Some microspheres and fragments are engulfed by macrophages and foreign-body giant cells. No T lymphocyte reaction is elicited. There is no neuronal toxicity and some healthy neuronal somas or axons can be observed by optical or electronic

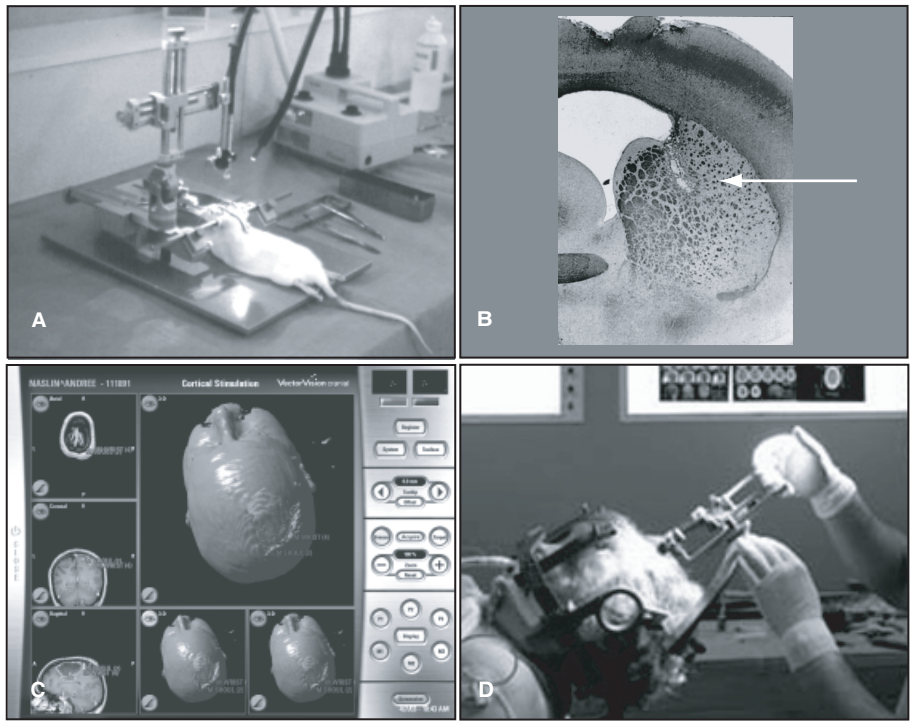

Figure 1. (A) Brain implantation of microspheres in an anaesthetised rat using a stereotaxic frame; (B) microspheres implanted in the striatum of a rat brain (arrow); (C) programming of stereotaxic intratumoural implantation of microspheres on the neuronavigation software; (D) stereotaxic implantation of 5-fluorouracil-releasing microspheres.

microscopy, in contact with microspheres. This lack of neurotoxicity, despite a local decrease of the pH during the degradation process, could be explained by the potent buffer capacity of the brain tissue, due to the astrocytic functions.

In total, > 100 different molecules have been incorporated in PLGA microspheres since their first description. Formulations of drug-releasing PLGA microspheres are well characterised for low molecular weight organic molecules. However, for high molecular weight molecules, such as peptides and proteins, the conformation modifications during the encapsulation process still pose some problems. Therefore, drug-releasing microspheres are easier to formulate for peptides than for proteins, which are frequently modified and become inactive.

3. Applications in neuro-oncology

Malignant gliomas represent 13 – 22% of brain tumours and, regardless of the method of treatment, the median survival is < 1 year [29]. Despite surgical treatment, external beam radiation therapy and systemic chemotherapy, these tumours tend to recur within centimetres of their original location [30-31]. In an attempt to decrease the local recurrence, recent efforts have focused on designing polymer devices, such as PLGA microspheres, that allow local delivery of chemotherapeutic agents after intratumoural implantation.

After the original report of modest efficacy of intravenous BCNU therapy for malignant glioma > 20 years ago, BCNU became the mainstay of single-agent chemotherapy for brain tumours, and the standard against which other chemotherapeutic regimens have been judged. Unfortunately, the systemic use of BCNU is associated with considerable toxicity including myelosuppression. In addition, when given intravenously, BCNU has a short half-life both in plasma and within the brain. BCNU was the first cytotoxic drug for which local therapy by polymeric devices was developed [7,8]. BNCU-releasing PLGA microspheres were formulated and evaluated in a rat glioma model, thus allowing an enhanced survival [32-36].

Platinum analogues, which have shown modest activity against glioma when given intravenously have also been tested [34-39]. Carboplatin-releasing PLGA microspheres were evaluated on a rat glioma model, showing an increased survival of animals, despite a limited diffusion of the carboplatin of 0.5 mm from the injection site.

These studies on BCNU and carboplatin-releasing microspheres demonstrated that local and sustained delivery is superior to an equipotent bolus dose following tumour resection, and that direct injection of the microspheres in the cavity walls of the debulked tumour provide superior effect over deposit in the surgical cavity. Interestingly, in deep non-operated tumours, injection of microspheres into the tissue surrounding

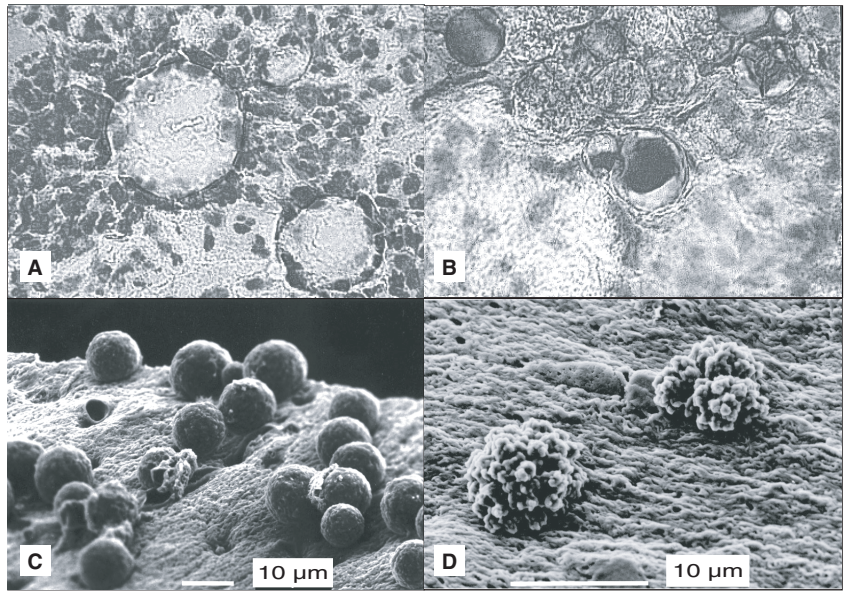

Figure 2. Poly(D-L lactide-co-glycolide) microspheres implanted in the rat striatum, optical microscopy, toluidine blue X400. (A) At 24 h; **(B)** after 3 weeks, some microspheres are vacuolised, allowing the penetration of the dye; scanning electron microscopy **(C)** after 7 days, **(D)** after 4 weeks; note the shrunken aspect. **(A)** and **(B)** reprinted from MENEI P, DANIEL V, MONTERO-MENEI C, BROUILLARD M, POUPLARD-BARTHELAIX A, BENOIT JP: Biodegradation and brain tissue reaction to poly(D-L lactide-co-glycolide) microspheres. *Biomaterials* (1993) 14:470-478, with permission from Elsevier. **(C)** and **(D)** reprinted from VEZIERS J, LESOURD M, JOLLIVET C, MONTERO-MENEI C, BENOIT JP, MENEI P: Analysis of brain biocompatibility of drug-releasing biodegradable microspheres by scanning and transmission electron microscopy. *J. Neurosurg.* (2001) **95**:489-494.

the growing tumour may provide a superior effect over implantation into the tumour itself [35].

Antimetabolite drugs, such as carboplatin, act on the synthesis of puric and pyrimidic bases. Through their action they are all potential radiosensitisers, and thus promising because of the systematic use of external radiotherapy in the treatment of malignant brain tumours. The neurotoxicity of platinum analogues has nevertheless limited their development. Other antimetabolites, such as 5-iodo-2'-deoxyuridine, have been microencapsulated in PLGA [40-43]. Among these drugs, 5-FU was revealed as the most promising during the preclinical studies. This hydrophilic and antimetabolic drug was chosen because it does not efficiently cross the blood–brain barrier, its anticancer activity may be improved by sustained administration and it is a powerful radiosensitiser [44]. As 5-FU is a pyrimidine base, which is only taken up into cells that are in S-phase, and as its cytotoxicity is potentiated by radiation, a specific action against the malignant glial cells that infiltrate intact parenchyma is expected. 5-FU-loaded PLGA microspheres have been prepared by a solvent evaporation process and characterised [45,101]. A sustained delivery for ≥ 3 weeks, a diffusion of several millimetres and a lack of toxicity has been demonstrated in the murine brain [46-48]. Implanted by stereotaxy, these 5-FU-releasing microspheres improved the survival of C6 glioma-bearing rats [46]. Studies in another murine model, F98 glioma, have shown 5-FU-releasing microspheres and radiotherapy combination to be more effective than 5-FU-releasing microspheres alone [49,50]. Based on these results, we proceeded with an evaluation on newly diagnosed malignant glioma in two clinical situations, resected tumours and deep nonoperable tumours.

3.1 Peroperative implantation of 5-fluorouracil-releasing microspheres after tumour resection

The rationale of this approach is to prevent the preferential recurrence of glioblastoma from the brain parenchyma near the resection site (**Figure 3A**). The PLGA 5-FU-loaded microspheres used in these clinical trials had a 48 ± 20 µm mean diameter, a drug content of 23 ± 3.5% and were radiosterilised at 25 kGy (**Figure 3B**). *In vitro* kinetics studies, performed for each batch before implantation, showed a burst effect in the first 24 h followed by a sustained release of 5-FU for 20 days. For clinical use, the microspheres were supplied as a lyophilised powder in single-dose vials to be reconstituted in the operating room with a sterile aqueous solution (**Figure 3C**).

A Phase I pilot study, including eight patients with newly diagnosed glioblastoma was conducted first [51]. The inclusion criteria were as follows:

- aged 18 – 68 years
- clinical and radiological features suggesting a supratentorial glioblastoma
- a Karnofsky performance scale score of ≥ 60
- the possibility of performing a macroscopically complete tumour resection according to investigator opinion

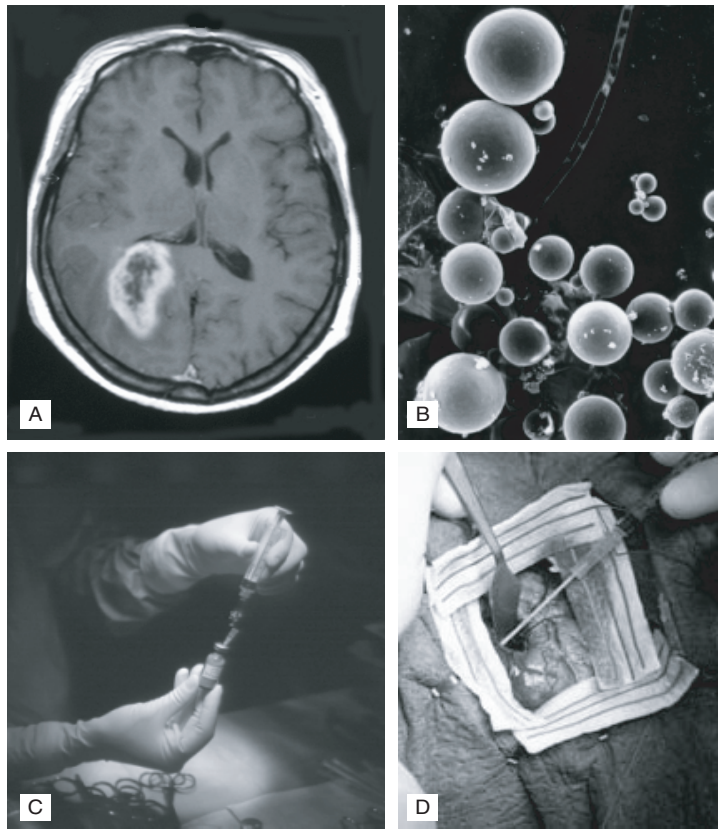

Figure 3. (A) Glioblastoma on magnetic resonance imaging before surgery; (B) scanning electronic microscopy of 5-fluorouracil-releasing microspheres; (C) preparation of microspheres suspension in the operative room (D) implantation of 5-fluorouracil-releasing microspheres, after resection of the glioma. (C) Reproduced with permission from MENEI P, JADAUD E, FAISANT N *et al*.: Stereotaxic implantation of 5-FU-releasing microspheres in malignant glioma. *Cancer* (2004) 100:405-410. Cancer © copyright 2004 American Cancer Society.
5-FU: 5-Fluorouracil.

After the diagnosis of a glioblastoma was confirmed by intraoperative histological examination, microspheres were implanted all around the walls of the surgical resection cavity, every cm^2, to a depth of 2 cm. A total volume of 1.5 – 2.5 ml (proportional to the dose) of microsphere suspension was injected using 100 µl per injection site (**Figure 3D**). Two groups with increasing doses of 5-FU (70 and 132 mg) were sequentially studied. External beam radiotherapy was initiated between the second and seventh day after surgery (total dose of 59.4 Gy in 33 fractions).

Tolerance was good except for one patient, who experienced a recurrent brain swelling with the second dose. The chemistry and cellular composition of cerebrospinal fluid (CSF) were normal from day 3 to day 30 and no abnormal wound healing, infection or systemic side effects were observed. The 5-FU release profiles in CSF showed a peak level at day 10 and day 20 for the first and second doses, respectively. Significant concentrations of 5-FU were still present in the CSF of patients 1 month after implantation. This longer release profile *in vivo* than *in vitro* was previously described in animal studies and could be explained by a slower hydrolysis of the PLGA in the brain parenchyma than in the saline. This sustained release, *in vivo*, for > 1 month during the radiotherapy allowed an optimal radiosensitisation effect. The peak of the release profile in CSF was interpreted as the result of the 5-FU redistribution from the extracellular space, to ventricular and subarachnoidian CSF. In contrast, passage of 5-FU in systemic circulation was slight and transitory, explaining the lack of systemic complication and healing abnormality.

The median survival time for all the patients was 98 weeks. In the group treated with the first dose, two patients died of local recurrence at 61 and 125 weeks and one died at 114 weeks of lung metastases of the glioblastoma. In the group treated with the second dose, one patient died 31 weeks after surgery of a local recurrence, two patients died at 59 and 82 weeks of a recurrence distant to the original tumour, and outside of the irradiated volume. The other patients of this group presented an unusual long survival. One died 4 years after the diagnosis of cerebellar recurrence. The last patient is

still in remission 7 years after the treatment. She developed a normal pressure hydrocephalus a few years after the treatment, necessitating a shunt, but she is in good neurological condition. The overall median survival time (98 weeks), the recurrences distant to the original tumour site and the long survival of two patients were in accordance with a local control of the tumour.

These encouraging results lead the authors to perform a randomised Phase II multi-centre study [52]. Objectives were to assess efficacy (local progression-free survival), safety and overall survival. This trial involved 12 french departments of neurosurgery and 90 patients were included. The inclusion criteria were the same as in the Phase I study except for histology, which included glioblastoma and high grade oligoastrocytomas. Two arms were randomised, one with surgical resection and conventional radiotherapy starting within 7 days following surgery (arm B), another with surgery followed by implantation of microspheres (5-FU 132 mg) and radiotherapy in the 7 days (arm A). A total 95 patients were enrolled. Of these 95 patients, 77 were treated according to the study treatment plan, and all were evaluable in intention to treat (ITT) for efficacy and safety. A total of 18 of the 95 enrolled patients were excluded from the protocol due to an absence of peroperative confirmation of a high-grade glioma (e.g., metastasis and lymphoma). Performance status, tumour size, number of glioblastoma, median time between randomisation and surgery, and median follow-up were not significantly different between the two treatment arms. As expected, the radiological review did not always confirm the peroperative estimation of complete resection. More patients underwent second-line therapy (surgery and/or chemotherapy) in arm B (61%) than in arm A (44%). Of the 77 ITT patients, 78% had progressed at the cut-off date: 74% in arm A and 82% in arm B. All relapses that occurred in patients with incomplete resection occurred locally, whereas 16% of relapses in patients who had a complete resection were only distant. A total of eight patients (10%) had distant progression only (arm A: five; arm B: three). Time to tumour progression was not significantly different for the two treatment arms (arm A: 6.5 months; arm B: 6.3 months). Median overall survival was almost 2 months longer in arm A (15.2 months) than in arm B (13.5 months). Moreover, a 2.9 month difference in overall survival was also observed in favour of patients with complete resection in arm A over arm B. These differences, however, do not reach statistical significance.

Neurological adverse events were more frequent in arm A than in arm B. This increased occurrence was associated with relatively stereotyped adverse events, occurring within the first 2 weeks of the beginning of radiation therapy, with a peak between days 10 – 15. They consisted of the exacerbation of pre-existing symptoms, sometimes associated with radiological features of brain oedema and rapidly resolving on high-dose steroids. Once this safety issue was identified, the protocol was amended to homogenise the dose of steroids across centres. The incidence of neurological adverse events decreased after this amendment and the difference between the two arms became less apparent. No biological abnormalities were noted during the study. No scar healing issue was reported. The implantation of microspheres in the wall of the tumour cavity was not associated with haemorrhagic complications.

The absence of a statistically significant effect in the present Phase II study may be related to its weak power and/or methodological issues, which were not anticipated at this exploratory stage of development. One of these methodological issues was the randomisation based on a diagnostic assumption by the investigator before histological confirmation. As a consequence, 18 of the 95 randomised patients were excluded from the protocol due to an absence of confirmation of a malignant glioma diagnosis. This high rate of exclusion was expected due to the study design with randomisation prior to confirmation of the histological diagnosis. A second methodological issue was the open status of the study. A comparator arm with placebo microspheres was initially planned, but due to the lack of enough data concerning the safety of multiple implantations of microspheres in the resection cavity, this option was refused by the ethics committee. More patients underwent chemotherapy and/or second surgery in arm B than in arm A. This difference, which can be favoured by the open status of the study, may have influenced the overall survival. Taken together with an acceptable safety profile, the efficacy results could justify a Phase III study, appropriately powered to show the expected difference in survival.

3.2 Stereotaxic implantation of 5-fluorouracil-releasing microspheres for deep and nonoperable malignant glioma

Due to the good tolerance during the previous studies and the possibility of injecting the microspheres suspension through a thin needle, a Phase I study was designed concerning deep and no resectable malignant glioma [53,105]. The inclusion criteria were:

- aged 18 – 70 years
- a Karnofsky performance scale score of ≥ 60
- malignant glioma (grade III or IV) confirmed by biopsy

A total of 10 patients with newly diagnosed, nonoperable, malignant gliomas were included in this trial and one dose of 5-FU was studied (132 mg). After histological confirmation, 2.5 ml of a PLGA 5-FU-loaded microspheres suspension were implanted by stereotaxy (with a stereotaxic frame or by neuronavigation) in the tumour, in one or several trajectories, with one to seven deposits per trajectory (**Figure 1C** and **D**). External beam radiation (59.4 Gy) was started before the seventh day postsurgery. Patients were followed by clinical examination, computerised axial tomography (CT) scan, magnetic resonance imaging and 5-FU assays in blood and CSF.

This study confirmed that stereotaxic intratumoural implantation of biodegradable drug-releasing microspheres is

feasible in human beings. This treatment can be applied in one stereotaxic procedure, immediately after the diagnosis of brain tumour is confirmed. The treatment can also be applied during a second surgery, if the diagnosis cannot be done on frozen sections.

The number of trajectories and microsphere deposits was adapted to the size, shape and necrotic/cystic components of the tumour. If one injection should be adapted for a tumour cyst, multiple depots were considered necessary for a solid tumour. 5-FU diffuses from the microspheres into the brain tissue for a distance of 3 mm [47]. It is then possible to programme the injection sites in a way that the spherical volume of 5-FU diffusion, from each microsphere depot, matches the tumour volume, as a drug dosimetry.

The 5-FU dose and injected volume was the same whatever the size of the tumour, and was determined from previous studies with peroperative microsphere injection. No acute intracranial hypertension was observed despite the intratumoural injection of 2.5 ml of microsphere suspension, sometime in large tumours. This fact can be explained by the fast resorption of the liquid vehicle. Nevertheless, the four patients who received a single trajectory (with one to five deposits) experienced a worsening of their neurological symptoms. For an equal volume of microsphere suspension, the tolerance is better when the volume is distributed in several trajectories.

5-FU assays in the CSF showed some traces of 5-FU in 50% of the day 10 samples, and in one sample in five at day 20. In the blood, traces of 5-FU were detected in some patients at day 10 and day 20. Compared with 5-FU kinetic study realised after peroperative implantation of microspheres in the wall of a tumour resection cavity [51], the concentration of 5-FU in the CSF and the systemic circulation were in the same range (0 – 25 ng/ml). There was no parallelism between the 5-FU concentrations and the neurological side effects observed. These very low blood concentrations of 5-FU explain the lack of haematological complications or healing problems. By comparison, 5-FU blood concentrations can go up to 2000 ng/ml during a standard 8 h continuous intravenous infusion and decrease to 0 ng/ml 1 h after the end of the perfusion.

The median overall survival was 40 weeks with two long survivors (71 and 89 weeks), which is encouraging. These two patients presented a small size or a cystic tumour. It can be hypothesised that these cases could represent the best indications and/or that the dose must be adapted to the tumour volume.

3.3 Future developments

Other drugs are currently being tested and interesting results have been obtained with other radiosensitive components such as taxane derivatives. A different group of promising molecules is represented by the antitumoural proteins such as tumoural necrosis factor and the cytokines for immunotherapy. Indeed, PLGA microspheres can be successfully used to deliver proteins such as growth factors and cytokines for several months in brain tissue, and preclinical studies are undertaken concerning intratumoural immunotherapy [54].

4. Applications to neurodegenerative diseases

The neurodegenerative diseases present a slow and irreversible evolution and are characterised by a progressive loss of one or several neural cell populations. Among them, Parkinson's disease is one of the most frequent and certainly the most studied. Several clinical trials including the delivery of neurotrophic factors have been performed; nevertheless, a curative therapy is still lacking.

4.1 Transmitter replacement

In some neurodegenerative diseases, there is a striking depletion of one or more neurotransmitters, due to neuronal death. Restoration of neurotransmission by implanted microspheres releasing appropriate pharmacological agents or neurotransmitters has been one of the first strategies evaluated.

In Parkinson's disease, the main neurochemical characteristic is a marked degeneration of nigrostriatal dopamine-mediated neurons, which provide the dopamine-mediated striatal innervation. Dopamine itself cannot be taken orally because it does not cross the blood–brain barrier and the current medication used, L-Dopa, can cause serious adverse reactions and its effectiveness decreases with time. In an attempt to deliver dopamine directly in the striatum, PLGA microspheres have been developed and studied. It is possible to attain functionally significant amounts of dopamine for a prolonged period of time by implantation of PLGA microspheres into the rat striatum [55,56]. Dopamine or noradrenaline loaded PLGA microspheres both allow a 30 – 50% reduction of rotation in unilateral 6OH-dopamine-lesioned rat for $\leq$ 8 weeks [57,58]. However, as these neurodegenerative disease are chronic and as neurotransmitter supplementation is required for several years, sustained delivery by implanted microspheres could not be a reasonable therapeutic option.

4.2 Neuroprotection and neural regeneration by neurotrophic molecules

Neurotrophic factors are proteins, which have a profound influence on developmental events such as naturally occurring cell death, differentiation and process outgrowth. It has been shown using different animal models that they can protect the neurons from several aggressions and prevent the neural degeneration [59]. Based on these studies, it has been suggested that tropic factors could be used for treating neurodegenerative diseases. A sustained and controlled delivery of these neurotrophic proteins in a definite region of the brain is necessary for this therapeutic approach and still remains a challenging area of research. Until now, long-term administration of growth factors has been limited to intraventricular infusions using cannulae or pumps. This route of administration

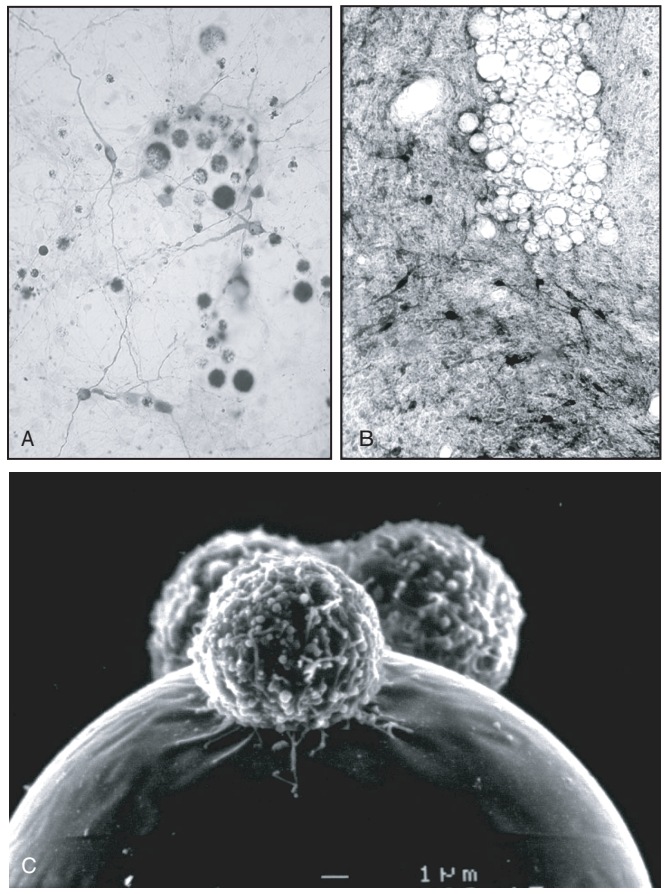

Figure 4. (A) GDNF-releasing microspheres implanted with fetal dopamine-mediated neurons in the rat brain; (B) NGF-releasing microspheres implanted in the septal area of a rat brain, in contact with healthy cholinergic neurons; (C) scanning electronic microscopy of a PAM carrying PC12 cells.

GDNF: Glial cell line derived neutrophic factor; NGF: Nerve growth factor; PAM: Pharmacologically active microcarrier.

requires repeated injections or refilling of pump reservoirs. In addition, efficient intraparenchymatous concentrations of a neurotrophic factor are difficult to sustain using current pump technology due to the degradation of the drug in solution and the low penetration of proteins from ventricular CSF. Moreover, due to the ventricular distribution, other cells than those targeted can respond to the factor, causing undesirable side effects [1]. Preliminary results of a 6-month open-label trial in which GDNF was infused directly into the striatum were promising [60]. However, the continuation of the study, in a double-blind, placebo-controlled trial was disappointing and failed to prevent functional decline (Amgen press release, 2004). One explanation for the lack of efficacy could be that GDNF did not penetrate far enough beyond the tip of the infusion catheter.

Recent efforts have concentrated on the transplantation of genetically modified cells. Direct gene transfer to the brain has also been evaluated. These strategies allow a prolonged and intraparenchymatous delivery of the protein, but the safety of the method and the long-term expression of the transgene still need to be determined. Moreover, the delivered doses, which depend on cell survival and the stability of the transgene, could be difficult to control in a precise manner.

4.2.1 Nerve growth factor-releasing microspheres

In Alzheimer's disease, the deficit in memory and learning are well correlated with the degeneration of basal forebrain cholinergic neurons, which give the cortical and hippocampal cholinergic innervation. Nerve growth factor (NGF) has been shown to protect these neurons in various conditions of stress and degeneration. This protein was the first neurotrophic factor to be identifed and is very well characterised. NGF-releasing microspheres have thus been developed. NGF-releasing PLGA microspheres were prepared and the NGF release kinetics were characterised *in vitro* using radiolabelled NGF, immunoenzymatic assays and PC12 cells bioassay, and then *in vivo* after implantation in the intact rat striatum [61-66]. *In vitro* studies showed that these microspheres allowed a sustained release of bioactive NGF for ≥ 1 month. NGF-releasing microspheres were thus implanted in the rat brain, near the septal cholinergic neurons, axotomised by an unilateral transection of the fornix-fimbria [67] (**Figure 4B**). The hippocampal cholinergic

denervation by surgical resection of the fornix–fimbria produces spatial memory deficit in rats and has been proposed as a model of Alzheimer's disease. In the non-treated animals, the percentage of economised surviving neurons, when compared with the controlateral intact side, was 31 ± 2% and 27 ± 1% at 2 and 6 weeks, respectively. Unloaded microspheres caused no protective nor neurotoxic effects (40 ± 9 and 39 ± 6% of surviving cholinergic neurons at 2 and 6 weeks, respectively). In contrast, NGF-loaded microspheres showed a significant effect on the survival of economised cholinergic neurons at 2 and 6 weeks after implantation (66 ± 9 and 61 ± 5% when compared with the controlateral intact side, respectively). These results showed that PLGA microparticles present no neurotoxicity and release sufficient amounts of bioactive NGF to significantly limit the lesion-induced disappearance of cholinergic neurons in the septum for ≥ 6 weeks. Co-encapsulated NGF and ganglioside GM1, in PLGA microspheres have the same type of effect on a similar model [68].

NGF can also protect other neuronal populations from excitotoxicity. Excitotoxicity has been implicated in acute neurological lesions (trauma, ischaemia) and neurodegenerative diseases such as Huntington's disease. In this regard, it has been shown that NGF-releasing microspheres, implanted into the rat striatum 7 days prior to infusing quinolinic acid, reduce the lesion size by 40% when compared with the control groups [68]. A marked neuronal sparing was noted, principally concerning the cholinergic interneurons but also neuropeptide Y/somatostatin interneurons and GABA-mediated striatofuge neurons. This neuroprotection was confirmed by an autoradiographic study of D2 dopamine receptors (D2R) [69]. Tolerance of microspheres was demonstrated by *in vitro* autoradiography with the marker of gliosis, [^{3}H]-PK 11195 [70]. In this study, implanted microspheres in the intact striatum still contained NGF after 2.5 months and they were totally degraded after 3 months.

It has also been shown that NGF-releasing microspheres implanted in the rat striatum after an injection of 6-hydroxy-dopamine (6-OHDA), which is a neurotoxin destroying the dopamine-mediated innervation, induced the apparition of dopamine-mediated neurons [71].

4.2.2 *Glial cell-derived neutrophic factor-releasing microspheres*

The recent identification of neurotrophic factors, such as the GDNF, acting on mesencephalic dopamine-mediated neurons, offers the possibility to protect or stimulate axonal regeneration of these cells, which are affected in Parkinson's disease. Nevertheless, a safe and efficient GDNF delivery system that may be used in clinical trials is still lacking. For this reason, GDNF-loaded microspheres have been developed, which could release bioactive GDNF for ≥ 2 months and may be implanted by stereotaxy in the brain; they thus offer an alternative strategy in the treatment of Parkinson's disease [72]. A water-in-oil-in-water (w/o/w) extraction–evaporation technique was chosen to prepare protein-loaded microspheres. In order to assess the *in vitro* release profile of the GDNF-loaded microspheres, a preliminary study was performed to select an appropriate buffer for GDNF stabilisation, using experimental designs. Released GDNF was measured by both enzyme-linked immunosorbant assay (ELISA) and radioactivity using [^{125}I]-GDNF. The GDNF-loaded microsphere release profile was assessed in a low continuous flow system, and showed a sustained release for > 56 days of biologically active GDNF at clinically relevant doses. The *in vitro* release profile was characterised by a typical biphasic pattern: a marked initial GDNF release (17% within 24 h) followed by a continuous and sustained release (28.4% at day 49). A bioassay with fetal rat dopamine-mediated neurons confirmed the bioactivity of the released protein. Moreover, these GDNF-releasing microspheres are capable of delivering the neurotrophic factor for ≥ 2 months *in vivo*.

These GDNF-releasing microspheres were first evaluated in an acute lesion of the nigrostrial pathway. Intrastriatal injections of 6-OHDA, a selective excitotoxic agent for dopamine-mediated neurons, and GDNF-releasing microspheres were performed simultaneously [72]. The effects of GDNF released from the implanted microspheres were assessed by classical behavioural evaluation such as amphetamine-induced rotating behaviour and histologically by tyrosine hydroxylase (TH) immunoreactivity. In addition, quantitative autoradiography using *N*-(3-ioprop-2E-enyl)-2-β-(4-methylphenyl)nortropane (PE2I), a dopamine transporter (DAT) radiotracer, which is also suitable for single photon emission tomographic imaging in humans, was performed. During the first 3 weeks after lesion and implantation, the amphetamine-induced rotating behaviour of GDNF-treated rats was improved compared with controls and an increase in TH expression (26%) was measured in the striatum 6 weeks after lesion. In accordance with these results, an increase in striatal PE21-labelled DAT density was obtained (+ 17%) after 3 and 6 weeks of treatment.

GDNF-releasing microspheres were then evaluated on a model of chronic degeneration of the dopamine-mediated nigrostriatal pathway, which is more representative of the disease [74,75]. Two doses of 6-OHDA were injected into the rat striatum in order to induce a partial progressive and retrograde lesion of the nigrostriatal system. In this case, microsphere implantations were performed 2 weeks after the lesion, at the same coordinates. At this time period, the striatum is widely denervated, and only a few fibres persist in its rostral part and medially, along the ventricular wall. Each rat received two implantations of 1.5 mg of microspheres in a volume of 10 µl. For GDNF-releasing microspheres, this amount corresponded to a total dose of GDNF 3.75 µg per rat. Animals were examined 8 weeks after implantation of the microspheres.

The rats implanted with GDNF-loaded microspheres presented a dramatic amelioration of the amphetamine-induced rotation behaviour. The sensorimotor orientation test,

performed 8 weeks after the treatment, highlights this amelioration. This functional recovery observed in the treated animals was accompanied by an increase of dopamine-mediated innervation in the striatum due to the sprouting of the fibres spared by the lesion. Numerous TH positives fibres were observed that were clearly directed towards the microsphere-containing GDNF or were running between them. In some regions, their density was sufficient to reconstitute the striosome-matrix dopamine-mediated innervation of the normal striatum. Transmission electronic microscopy showed that this reinnervation was accompanied by the development of TH-immunoreactive (TH-ir) synapses in the vicinity of microspheres.

A second experiment was performed, to evaluate the fate of this dopamine-mediated reinnervation, in the long term, after the complete degradation of the microspheres. In the present study, the animals were followed for 32 weeks after implantation of the microspheres. The results demonstrated that restorative effect obtained with 2 months of GDNF-delivery persists after complete degradation of the microspheres, for ≥ 24 weeks after the end of delivery.

The capacity of the microspheres to release active GDNF was confirmed on a genetic model of Parkinson's disease, the Weaver mice, which present a spontaneous degeneration of the dopamine-mediated fibres in the dorsal part of the striatum. Implantation of GDNF-releasing microspheres was followed by a sprouting of these fibres toward these microspheres (R Raisman, data not published). A pilot study was performed on a primate model of Parkinson's disease (S Palfi, data not published). Two macaques intoxicated by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were treated by intrastriatal implantation of GDNF-microspheres: 20.8 mg of microspheres containing 5 µg of GDNF, distributed in five implantation sites (one in the caudate, four in the putamen). One animal died of a subdural haematoma; another showed a transitory recovery of its symptoms. This experiment led to the conclusion that despite an important volume of microspheres, the dose of GDNF used was insufficient. For the future, a design of microparticles containing more GDNF and allowing a controlled release for 6 – 12 months is needed. If this strategy proves to be efficient, the risk/benefit ratio could justify a recurrent stereotaxic implantation in patients if the clinical improvement appears superior to the bleeding risk of a stereotaxic procedure. Nevertheless, the therapeutic use of neurotrophic factor delivery by PLGA microspheres seems to be limited for chronic neurodegenerative diseases. On the other hand, for a short delivery, such as is needed in neural acute injury or cell therapy, implantable biodegradable microspheres are very promising.

5. Cell therapy

Cell therapy is commonly used in bone marrow reconstitution and is promising for the treatment of several diseases including neurodegenerative disorders such as Huntington's and Parkinson's disease. In these latter cases, clinical trials with fetal dopamine-mediated cells reveal that the functional benefit is limited by the low survival rate and poor integration of transplanted cells. Indeed, ~ 90% of the cells die in the first 2 weeks, requiring multiple donors for each patient to assure a sufficient number of surviving cells. Progress in neurotransplantation, as in cell therapy in general, thus requires an improvement in the preparation and the delivery of the cell product in order to increase the survival rate and cell integration in the host environment. Associating nerve cell transplantation and neurotrophic factor releasing-microspheres is an interesting strategy to overcome this problem.

The first attempts were performed by injecting the cells and the neurotrophic factor at the same time (**Figure 4A**). A more sophisticated approach is to use the microspheres as microcarriers that may convey the cells on their surface while delivering an active molecule. For this purpose, a new tool has been developed: the pharmacologically active microcarriers (PAM) (**Figure 4C**) [104]. PAM are biocompatible and biodegradable microparticles coated with cell adhesion or extracellular matrix molecules, conveying cells on their surface, and presenting a controlled delivery of a growth factor. Thus, the combined effect of the growth factor and the coating may influence the transported cells by promoting their survival and differentiation and favouring their integration in the host tissue after their complete degradation. Furthermore, the released factor may also influence the microenvironnement.

To evaluate this strategy, NGF-releasing PAM conveying PC12 cells were transplanted into a rat model of Parkinson's disease [76]. Indeed, when PC12 cells, which express TH, are exposed to NGF, they stop cell division, extend long neurites, become excitable and after depolarisation they can release large amounts of dopamine, the missing neurotransmitter in Parkinson's disease. Moreover, transplantation of PC12 cells, which are used as a source of dopamine, have been extensively studied in animal models with encapsulated biomaterials. After brain implantation of NGF-releasing or unloaded PAM conveying PC12 cells, or PC12 cells alone, cell survival, differentiation and apoptosis, as well as behaviour of the treated rats were studied. It was observed that the NGF-releasing PAM coated with two synthetic peptides (poly-D-lysine and fibronectin-like) induced PC12 cell differentiation and reduced cell death and proliferation. Moreover, the animals receiving this implant presented an improved amphetamine-induced rotational behaviour.

These PAM represent a very interesting tool for cell therapy as the cells may be cultured and sorted in the same support before implantation. Furthermore, in the tissue, they can induce cell differentiation and limit cell death and proliferation, which is essential when stem cells are employed. Depending on the molecule released, PAM may also modify the microenvironment (favour angiogenesis, local immunodepression or any interaction with the host cells) and thus favour the integration of grafted cells in the host tissue. In this regard, a recent article reported that the implantation of neural stem cells seeded on polymer scaffolds augmented the

constitutive reparative response in the injured brain [77]. It should be noted that PAM may be employed for different cell therapies as they can be produced in advance, stored as a freeze-dried powder and the cell adhesion protocol may be easily adapted to many cell types.

6. Conclusions

If drug-releasing PLGA microspheres could be viewed as old vectors, compared with the new systems developed by molecular biology, they have an important potential, especially in neurosurgery when used in combination with stereotaxic and computer-assisted surgery approaches. Other interesting approaches have not been included in this review, such as injection of PLGA microspheres in the CSF, for the treatment of pain [78,79] or spasticity [80,81].

7. Expert opinion

Our group has an experience of several years on microparticle engineering and drug delivery in the brain. During our preclinical and clinical studies, some positive and negative points of PLGA microspheres have emerged.

7.1 Positive points

PLGA microspheres allow a controlled and tailored dose and time/duration delivery, with the complete disappearance of the vector after its degradation.

The microparticle enable easy delivery of multiple compounds by simply mixing particles loaded with different drugs.

Compared with other approaches such as gene transfer or grafting of genetically modified cells, these systems are safe, without potential problems of immunotolerance, viral security and carcinogenic hazard.

In comparison with monolithic polymeric devices, such as wafers, microspheres can really be implanted in the brain tissue, allowing an intraparenchymal delivery; moreover, their size allows multiple and spatially distributed implantations.

7.2 Negative points

Implanted microspheres constitute a passive vector, the drug is released around the particles, which stay in place until degradation. The area of diffusion could be limited, especially if the released drug is hydrophilic or has a high molecular weight. This problem could be solved by multiple and scattered implantation sites.

This strategy allows a drug to be released for a period of only several months. This fact could be a limitation for the treatment of neurodegenerative disease, for example. However, it may represent an advantage as it is finite in time.

The apparent simplicity of the concept of microencapsulation should not mask the fact that microparticle engineering is a high-tech field. Even if several hundred molecules have been encapsulated in PLGA microspheres, obtaining a reproducible and well-characterised formulation (in terms of encapsulation ratio, kinetics release, activity of the released molecule) takes time and effort. The difficulties are increased if a scaling and good manufacturing practice production are needed (for a clinical trial) or if the encapsulated molecule is a protein.

It is interesting to point out that if numerous animal experiments with brain implantation of drug-releasing microspheres have been published, to our knowledge, there were only three reported clinical trials [51-53]. However, in the meantime, while awaiting more sophisticated vectors, this approach is the most appropriate for the delivery of neuroactive molecules that do not cross the blood–brain barrier.

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