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Modulated release of IdUrd from poly (D,L-lactide-co-glycolide) microspheres by addition of poly (D,L-lactide) oligomers

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Abstract

This paper reports the release characteristics of a radiosensitizer, 5-iodo-2′-deoxyuridine (IdUrd), from poly (D,L-lactide-co-glycolide) 50: 50 (PLGA) microparticles obtained by a phase separation technique. Poly (D,L-lactide) oligomers (D,L-PLA) were incorporated into the PLGA matrix in order to accelerate the overall drug release rate and regulate the triphasic release profile exhibited by the standard PLGA microparticles. For D,L-PLA (800), the burst effect was large and the IdUrd release was complete between 28 and 35 days. These results were attributed to rapid pore formation on the periphery of the microspheres in the early stages of incubation, due to hydrosolubility of the smallest oligomers (D,L-PLA (800)). In the case of D,L-PLA (1,100), drug release occurred over a six week period, the standard time course of conventional radiation therapy. The period during which the radiosensitizer was incorporated in human brain tumor cell nuclei after its entrapment in biodegradable microspheres was determined by using an organotypical tissue culture. The presence of radiosensitizer in the DNA of tumor cell nuclei was detected by immunohistochemical labelling of tumor fragments. IdUrd release from standard microspheres (7 ± 0.5 weeks) was longer than from oligomer-containing batches. For D,L-PLA (800)-containing microspheres, the radiosensitizer was entirely released within 4.5 ± 0.5 weeks. The microspheres containing D,L-PLA (1,100) allowed an IdUrd release over a 5 to 6 week period. The ex vivo data were consistent with the in vitro findings in terms of release duration.

Keywords: IdUrd; Biodegradable microspheres; D,L-PLA oligomers; Phase separation; Tumor culture

1. Introduction

Failure to achieve an efficient treatment in most of the histological types of gliomas such as oligodendrogliomas remains a significant problem. The isolated malignant cell components of these tumors permeate functionally intact brain parenchyma and cannot be resected in important brain areas. In oligodendrogliomas, conventional radiotherapy allows a median survival from 3.5 to 11 years depending on the tumor grade [1].

One strategy to improve radiation efficacy is to increase the radiosensitivity of tumor cells by using thymidine analogs such as 5-iodo-2′-deoxyuridine (IdUrd). IdUrd is a powerful radiosensitizer [2] that

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competes with thymidine in the biosynthesis of DNA.

The mitotic activity of normal brain parenchyma is negligeable. Consequently, infiltrating gliomas such as oligodendrogliomas could represent an interesting target for IdUrd radiosensitization. The presence of radiosensitizer in the vicinity of malignant cells during radiotherapy might increase the lethal effects of ionizing radiations on the tumor cells that incorporate the radiosensitizer. This concept requires the use of localized delivery systems able to release therapeutic amounts of IdUrd into the tumor over the time course of a conventional radiation therapy (6 weeks). Stereotactic implantation of biodegradable microspheres in the tumor can meet these requirements [3].

Controlled release microspheres of IdUrd, made from biodegradable polyesters such as poly (D,L-lactide-co-glycolide) 50: 50 (PLGA) since they are biocompatible with the brain [4], were developed. A phase separation technique was used to entrap the active agent. In order to modulate drug release from PLGA microspheres, oligomeric polylactides (D,L-PLA) of different molecular weights and in variable amounts were used.

The specific aims of the study were threefold: to obtain an IdUrd in vitro release over 6 weeks; to verify the ability of the radiosensitizer to be incorporated in human brain tumor cell nuclei after its entrapment in biodegradable microspheres; to determine the period during which the IdUrd incorporation took place.

2. Materials and methods

2.1. Materials

5-Iodo-2′-deoxyuridine (≥99% pure) was obtained from Sigma-Aldrich Chimie (St Quentin Fallavier, France). PLGA was purchased from Boehringer Ingelheim (RG 506, B.I. Chimie, Paris, France). The gross compositions of the chains were 25% D-lactic units, 25% L-lactic units and 50% glycolic units. Weight average molecular weight (Mw) was 75,000 (I=1.56). Low molecular weight D,L-PLAs (LMW-PLA) containing 50% D-lactic units were supplied by URA CNRS 1465 (Montpellier, France, $\bar{M}w$=800, I=11) and Phusis (St Ismier, France, $\bar{M}w$=1,100, I=1.7). Methylene chloride, silicone oil (Rhodorsil® 300 cSt) and mono and disodium hydrogenophosphate were obtained from Prolabo (Paris, France). Heptane was purchased from Verbièse (Wasquehal, France), dimethyl sulfoxide (DMSO) from Carlo Erba (Val de Reuil, France) and tetrahydrofuran (THF) from Sigma-Aldrich Chimie (St Quentin Fallavier, France). Water used in the experiments was deionized by using a Millipore purification system (Milli RO plus 6, Millipore, St Quentin en Yvelines, France). IU4 and MIB1 antibodies were obtained from Tébu (Le Perray en Yvelines, France) and Immunotech (Marseille, France) respectively. Human brain tumor fragments were obtained from the Neurosurgery Department (Hôpital Ste Anne, Paris, France) and included oligodendrogliomas grades A/B, meningiomas and brain metastases of epidermoidal cancer and adenocarcinoma.

2.2. Size-exclusion chromatography (SEC) measurements

2.2.1. PLGA and LMW-PLA analyses

Weight average molecular weights were determined in THF by size-exclusion chromatography (SEC) equipped with a 510 pump, and a 410 differential refractometer detector (Waters, St Quentin en Yvelines, France) as referred to polystyrene standards. PLGA was analysed with a high molecular weight mixed column (5 μm, 60 cm) and LMW-PLA with two low molecular weight columns connected in series, at a flow rate of 1ml/min (10 μm, 60 and 30 cm, PLGel, Interchim, Montluçon, France).

2.2.2. LMW-PLA content determination in microparticles

Blank and IdUrd-loaded microparticles (30 mg) of different types were dissolved in THF. Samples were analysed by using the high molecular weight column. D,L-PLA (800 and 1,100) contents of the microparticles were determined by the oligomer surface peak/total surface peak ratio expressed in percentage and referred to calibration curves established with both types of oligomers. As an example, Fig. 1 represents

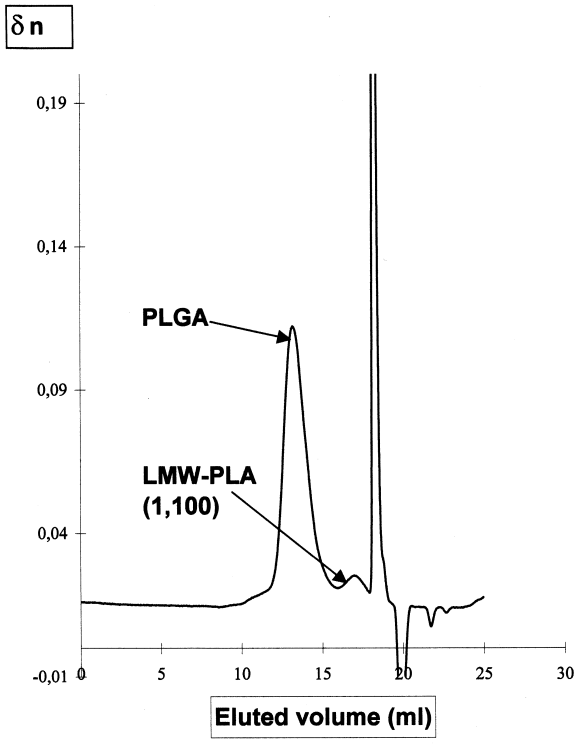

Fig. 1. SEC chromatogram of PLGA/LMW-PLA (1,100) 92/8 microspheres loaded with 20% IdUrd.

a SEC chromatogram of PLGA/LMW-PLA (1,100) 92/8 microspheres loaded with 20% IdUrd.

2.3. Determination of the stability windows

The 'stability window' originally defined by Ruiz et al. [5] corresponds to the experimental conditions that yield a stable dispersion of polymer coacervate droplets in the dispersing phase following the addition of silicone oil. It can be determined by the establishment of ternary diagrams [6–8]. In the phase diagram, each point corresponds to a defined weight percentage of methylene chloride, silicone oil and PLGA.

Different polymer and oligomer concentrations in methylene chloride (1 to 10% w/w) were used to define the stability window. Silicone oil was progressively added to the organic phase and the samples were observed by an optical microscope (Olympus BH2, Osi, Paris, France) equipped with a video camera (Cohu) and a computerized image analyser (Microvision Instruments, Evry, France) after 2 minutes of magnetic stirrer agitation following each addition.

2.4. Microsphere preparation

PLGA 50:50 (200 to 250 mg) and LMW-PLA (0 to 50 mg) were dissolved in methylene chloride. Polymer and oligomer concentration was fixed at 1.3% (w/w). Initial polymer/oligomer mass ratios of 100/0, 90/10 or 80/20 were used. Milled IdUrd crystals (70 to 100 mg) (18 ± 3 μm), were dispersed in the organic phase by sonication (3 minutes). A phase separation inducer (silicone oil) (8 g) was added to the mixture stirred (magnetically) at room temperature for 2 minutes in order to precipitate the polymer and the oligomer around the drug particles. The resulting dispersion (semi-formed microparticles or coacervates) was poured into 400 ml of heptane (hardening agent) stirred at 600 rpm (Heidolph RGH500, Prolabo, Paris, France). After 30 minute agitation, the solidified microparticles were filtered on a 0.45 μm filter (HV type, Millipore SA, Maurepas, France) and washed with heptane (50 ml). The resulting microspheres were dried under reduced pressure for 60 h at 30–35°C. They were stored at +6°C in a dessicator shielded from light.

2.5. Size distribution analysis

Microparticle average size was determined by using a Coulter® Multisizer (Coultronics, Margency, France) after dispersion of microparticles in a conducting liquid (Isoton® II, Coultronics, Margency, France). Additional size analyses were made by optical microscopic evaluation of 10 fields that contained approximately 50 particles each.

2.6. IdUrd content determination

Microspheres (8 mg) were dissolved in DMSO (50 ml). IdUrd content in microparticles was assayed spectrophotometrically at 287 nm (Uvikon 922,

Kontron Instruments, St Quentin en Yvelines, France).

2.7. Degradation studies

For degradation studies, the formulation parameters were slightly modified in order to produce bigger microspheres allowing easier AFM surface scanning. The concentration of polymer and oligomer in the organic phase was adjusted to 5% (w/w). Blank microspheres (65±5 μm) were obtained. They contained LMW-PLA (800 and 1,100 Da) according to an initial PLGA/D,L-PLA mass ratio of 90/10 incorporation. _Three dialysis bags (Spectra/Por® Membrane, Mw 6,000–8,000 Da, Bioblock, Illkirch, France) containing 40 mg microspheres and 4 ml phosphate buffer were incubated in a large volume of phosphate buffer (200 ml) pH 7.4, at 37°C under 100 rpm agitation. After 24, 96 and 336 h, one dialysis bag was removed. The 40 mg microspheres were filtered, washed with 200 ml deionized water, freeze-dried (RP2V Serail, SGD, Argenteuil, France) and analysed.

The surface morphology of the microspheres before and during the degradation studies was determined by using SEM and AFM measurements. The SEM studies were performed by mounting the microspheres onto metal stubs using double sided adhesive tape, vacuum-coated with a carbon film (10 nm thick) with a MED 020 (Bal-Tec, Balzers, Lichtenstein) and SEM evaluations were made with a JSM 6310F (JEOL, Paris, France) at an accelerating voltage of 5 kV. The AFM analysis was carried out in the contact mode, with an Autoprobe® CP (Park Scientific Instrument, Geneva, Switzerland) fitted with a 2 μm cantilever and monocristalline silicium tip.

2.8. In vitro release studies

IdUrd released at 37°C from microspheres into phosphate buffer 0.13M, pH 7.4, was assayed spectrophotometrically at 287 nm.

A USP dissolution apparatus shaken at 100 rpm (Sotax AT7, Paris, France) was used to obtain the release data. A dialysis bag containing 80 mg microparticles and 7 ml phosphate buffer was placed into 400 ml release medium.

2.9. Ex vivo release studies

2.9.1. Tumor tissue culture

Tumor cultures were performed as follows: after tumor fragment selection, tumor tissues were trimmed to approximately 1 mm^3. Sterile gelatine sponge (Gelfoam®, Upjohn, Kalamazoo, MI, USA) pieces were deposited in Petri dishes containing 3 ml of an aseptic RPMI medium 1640 (Sigma-Aldrich, St Quentin Fallavier, France). The tumor fragments were placed onto Gelfoam ®. The Petri dishes were kept in an oven under 5% CO_2 atmosphere at 37°C during the study.

2.9.2. IdUrd release from microspheres

After 50 Gy γ-irradiation at a 2 Gy/min flow (662 Kev) with a ^{137}Ce source (irradiator IBL 637, Cis bio-International, Gif sur Yvette, France), 20–23% IdUrd-loaded microparticles (1 mg) were placed in a filter-type bottom basket, allowing free drug diffusion. The basket was placed within the medium in a Petri dish. IdUrd could thus diffuse in the RPMI and reach the tumor cells. The microspheres were maintained in contact with the tumor tissue implants for 96 h before they were analysed. In order to assess the IdUrd release period from microspheres, the basket was transferred every 96 h to a new tumor tissue culture set. From week 5, incubation times were shortened to every 2 days in order to precisely determine the end of drug release. Experiments were performed for PLGA and PLGA/LMW-PLA microsphere batches during periods varying from 5 to 10 weeks.

2.9.3. IdUrd incorporation in tumor tissues

After each incubation time with microspheres, tumor tissue samples were formalin-fixed and paraffin-embedded. Adjacent 4 μm sections were used for routine staining (Hemalum-phloxine) and for immunohistochemistry studies according to the method of Hsu et al. [9]. IU4 antibody was used for the detection of IdUrd incorporation within tumor cell nuclei. In addition, by using MIB1 antibody, immunolabelling of 4 μm adjacent paraffin section was performed in order to control the presence of proliferating cells within the tumor tissue culture.

3. Results and discussion

3.1. Microsphere preparation

Phase diagrams were obtained for blank microparticles made from initial PLGA/LMW-PLAs (1,100) and (800) mass ratios of 100/0 and 90/10 as shown in Fig. 2. The presence of oligomers in the organic phase slightly shifted the stability window towards lower silicone oil values (15 to 32%) in the phase diagram as compared to limits obtained for the standard PLGA microsphere stability window (20 to 38%). These results confirmed the findings from Ruiz et al. [6] concerning the influence of overall copolymer hydrophobicity, affected here by the presence of oligomers in the organic phase, on the amount of silicone oil necessary to induce formation of stable coacervate droplets [10]. Increasing the oligomer percentage reduces the silicone oil needed to induce phase separation. Phase separation conditions yielding 35±5 μm (SD on microsphere mean size) blank microspheres were selected. These conditions correspond to percentages of PLGA/LMW-PLA, silicone oil and dichloromethane of 1%, 29% and 70% respectively in the phase diagram. These experimental conditions were used to prepare standard and oligomer-containing microspheres loaded with IdUrd. Microsphere diameter of 45±5 μm (SD on microsphere mean size) were obtained with drug loadings varying from 20 to 28%. The obtained microsphere size met the surgical and technical requirements [11]. Encapsulation yields reached 90±5%. Consequently, the phase separation technique was found suitable for the entrapment of IdUrd crystals.

3.2. In vitro release studies

The goal of the present study was to develop a 6 week controlled release formulation of IdUrd by using PLGA. The IdUrd release profile from standard PLGA microspheres obtained by the phase separation process was triphasic as shown in Fig. 3.

The initial step revealing a marked drug release was followed by a phase where the release rate was lowered considerably, probably due to a lack of porosity inside the matrix. The observed burst effect was due to the presence of uncoated drug crystals present at the microsphere surface, as revealed by SEM studies (unshown results). It must be noted that the dissolution of these IdUrd crystals does not create enough pores at the microsphere surface to allow the diffusion of drug located in the underlying layers. After 2 weeks (336 h), the IdUrd release rate increased again to reach a fairly constant value. This change was attributed to a noticeable PLGA degradation associated with progressive pore formation throughout the matrix. This pattern was observed

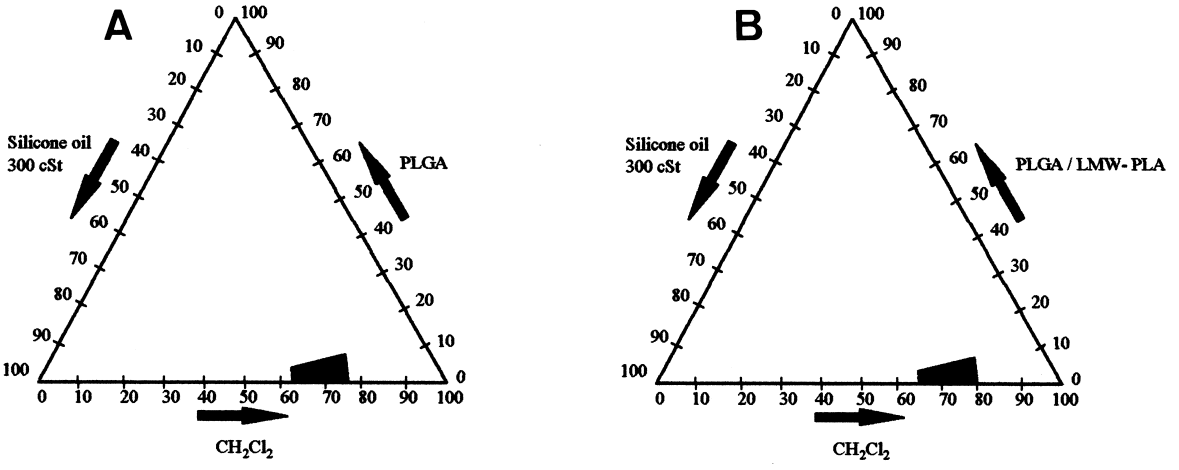

Fig. 2. Phase diagrams for the coacervation of (A) PLGA and (B) PLGA/LMW-PLA (1,100) or (800) blends with initial mass ratio of 90/10.

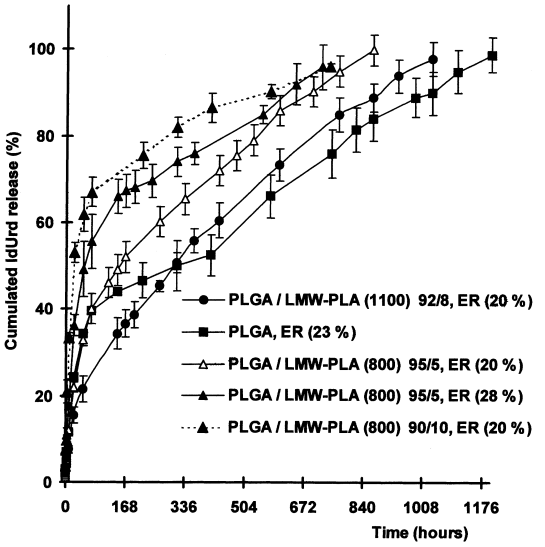

Fig. 3. IdUrd release from PLGA/LMW-PLA microparticles obtained by a phase separation technique (ER: drug encapsulation ratio).

with biodegradable triptorelin-loaded microspheres [10]. In addition, only 85% of IdUrd was recovered after 6 weeks (1008 h). Consequently, in order to accelerate and to regulate overall release rate, LMW-poly (D,L-lactide) was incorporated into the PLGA microparticles.

Oligomer incorporation in the microspheres, as determined by size-exclusion chromatography was 80±5% and 50±20% for the D,L-PLA (1100) and (800) respectively (Table 1). The incorporation was lowered and irregular when the oligomer chain length decreased. It is postulated that a fraction of the smallest chains did not coacervate and stayed in the solvent during microparticle formation.

Fig. 3 illustrates the cumulative amounts of IdUrd released from the different microsphere batches. Overall duration of IdUrd release depended on the amount and chain length of oligomers in the particles.

Incorporation of LMW-PLA clearly enhanced IdUrd overall release rate.

For D,L-PLA (800), a biphasic release profile was observed. A burst effect, that was a function of drug loading and amount of oligomer in the microparticles was followed by a slower release phase. For the same encapsulation ratio, the burst effect was enhanced with increasing amount of oligomer in microspheres. It was generally more pronounced than that displayed by the standard PLGA microspheres and was attributed mainly to rapid pore formation on periphery of the microspheres, due to hydrosolubility of the oligomers. The D,L-PLA chains were very polydispersed (I=11). So, a non negligeable fraction of oligomers was constituted by chains lower than 10 lactic units. It is worth it reminding that 1-to-7-lactic-unit chains were water soluble as evidenced by capillary electrophoresis assay [12]. An other characteristic of these kinetic patterns was that IdUrd release was complete between 4 and 5 weeks (840 h). This was too rapid with respect to the goal of the final application.

When LMW-PLA (1,100) was incorporated in the PLGA matrix, the IdUrd release profile was not modified, still presenting two phases. The burst effect was decreased with respect to LMW-PLA(800)-containing microspheres. As there were

Table 1
Incorporation yields of the different types of LMW-PLAs in the PLGA matrix

Microsphere type	Initial oligomer/polymer mass percentage (%)	Experimental oligomer/polymer mass percentage (%)	Oligomer incorporation yield (%)
PLGA/LMW-PLA (800)			
ER=24±4%	10 (n=3)	5±2	
ER=0%	10 (n=1)	5	50±20
	20 (n=1)	10	
PLGA/LMW-PLA (1,100)			
ER=20±2%	10 (n=3)	8±0.5	80±0.5
ER=0%	10 (n=2)	8	

p ER: drug encapsulation ratio.

no crystals on the microsphere surface (unshown results), the origin of the burst effect was attributed to pore formation by dissolution of the smallest oligomers, in contradiction to the origin of the burst effect for the standard microspheres. The remaining 80% encapsulated drug released over a 40 day period (960 h) according to a pseudo-zero order law. It is believed that a fraction of the oligomer molecules were not solubilized following the microsphere immersion in the aqueous medium. They probably remained in the matrix and autocatalysed the PLGA chain hydrolysis. The beginning of the degradation phenomenon occurred sooner than in the case of the standard PLGA particles.

These biocompatible oligomers which did not chemically modify the matrix were preferred to classical plasticizers and pore-forming agents currently used to accelerate drug release from biodegradable microparticles [13–17]. This strategy was reported in order to promote the drug release from poly (D,L-lactide) microparticles obtained by an emulsion solvent evaporation process [18–20]. Thus, testing the ability of very LMW-PLA (Mw <2,000 Da) to be incorporated into the PLGA matrix during phase separation represented a major challenge, the small D,L-lactic-unit chain coacervation being uncertain. Indeed, they were expected to stay in the supernatant after the silicone oil was added. It is well known that high molecular weight PLGA chains first coacervate following the addition of silicone oil, the thermodynamic incompatibility of the two polymers increasing with their molecular weights [6,8].

In conclusion, the differences observed on IdUrd release profiles were explained by the presence of small D,L-lactic unit chains which were unexpectedly incorporated in the PLGA matrix. In order to support this finding, surface morphology studies were performed.

3.3. Degradation studies

AFM and SEM studies were only performed on blank microspheres in order to understand the sole effect of the entrapped oligomers on the microsphere degradation. Firstly, surface morphology changes concerning freshly-made microspheres were noted as a function of the type of batch. Secondly, surface analyses were carried out on microspheres submitted to increasing incubation times in phosphate buffer at 37°C.

Before immersion, PLGA and PLGA/LMW-PLA microparticles presented similar surfaces. Spherical particles with a non porous surface were observed with SEM. A homogeneous background with a mean roughness of 5 nm appeared in AFM. Dispersed aggregates of about 200 to 400 nm diameter and 40 nm high were also present on the surface certainly corresponding to coacervate residues as shown in Fig. 4.

However, after a 96 h immersion, a noticeable change of the surface morphology occurred with the oligomer-containing microspheres. Indeed, a porous and granulous structure appeared as revealed by SEM (Fig. 5). The porosity indicated leakage of the oligomers located at the microsphere surface. It meant that the smallest chains coacervated last, which is in agreement with the findings of Ruiz et al. [6]. In addition, the porosity was much higher when the oligomer chain length decreased. These results explain the rapid IdUrd release from loaded PLGA/LMW-PLA (800) microparticles observed in vitro.

After a 336 h immersion, pores with a 500 nm diameter appeared on the surface, irrespectively of the oligomer chain length, as revealed by both methods (Fig. 6). Conversely, the surface of the standard blank microspheres did not change over 2 weeks. A porousless surface was observed. The low porosity of the surface after a 2 week incubation was consistent with the slow IdUrd release phase following the burst, observed in vitro with the standard microspheres.

In conclusion, this study confirmed the presence of very LMW-PLA in the PLGA matrix. These oligomers caused a change of the surface morphology in the early stages of microparticle immersion in phosphate buffer.

3.4. Ex vivo studies

This study consisted in determining the periods of time during which the IdUrd release from the microspheres was associated with its incorporation into tumor cell nuclei. This was a main issue because of the clinical application of the microspheres.

Firstly, in the case of standard PLGA microspheres, a high number of cell nuclei originating

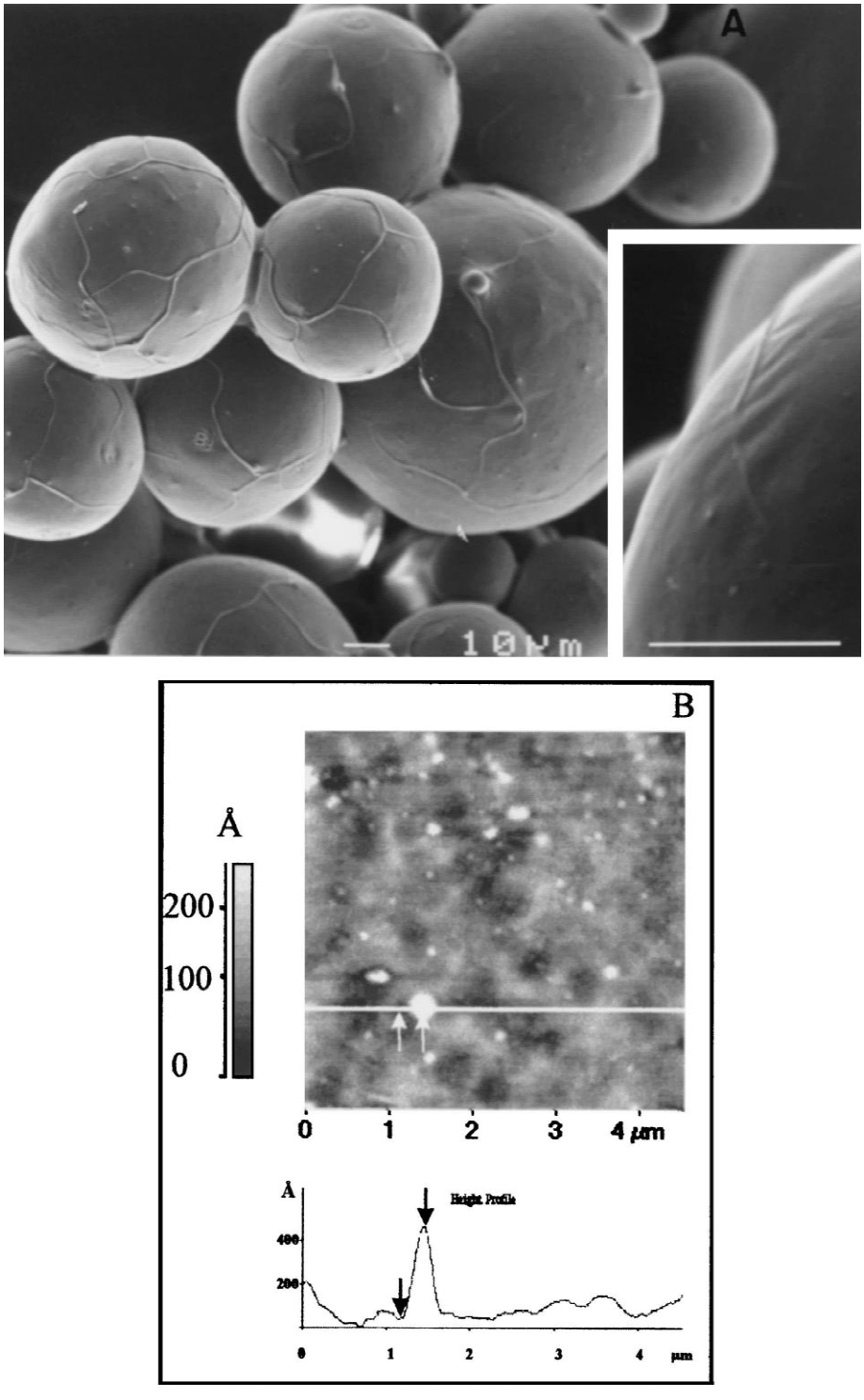

Fig. 4. SEM aspect (A) and AFM surface analysis (B) of PLGA/LMW-PLA (1,100) microparticles before immersion in phosphate buffer. The height measurement (40 nm) was taken between two points (indicated by arrows) 300 nm apart on the X scan line (in white).

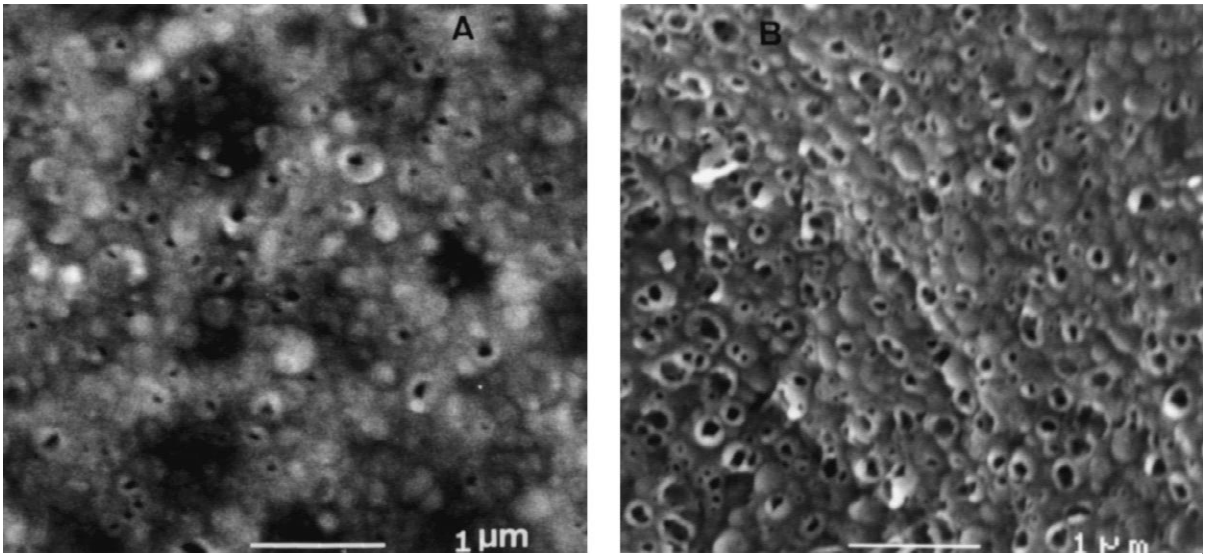

Fig. 5. SEM analyses of surface of (A) PLGA/LMW-PLA (1,100) and (B) PLGA/LMW-PLA (800) microparticles after a 96 hour immersion.

from new tumor tissue fragments were IU4-labelled until day 45 to day 52, depending on the batches. A typical IU4-immunostaining of oligodendroglioma cells is shown in Fig. 7B as compared to a standard hemalum-phloxine staining revealing the morphological appearance of the tumor fragment (Fig. 7A). The subsequent IU4-immunolabellings were negative despite the presence of MIB1-labelled dividing cells in the tumor fragment (Table 2). This result indicated that the IdUrd content was entirely ex vivo released from standard PLGA microspheres within 7 ± 0.5 weeks.

Secondly, the ex vivo release studies concerning the microspheres containing the different types of oligomers were carried out during 4 to 7 weeks. The radiosensitizer released over one month in case of 800 Da oligomers. Indeed, a positive IU4 labelling was obtained until days 29 to 33, according to experiments. After this time, a positive MIB1 and negative IU4 labellings were observed. In the case of 1,100 Da LMW-PLA-containing microspheres, IdUrd was released in between 5 and 6 weeks. Indeed, tumor cells nuclei were IU4-labelled for 34 to 40 day periods. Afterwards, despite the presence of dividing cells in tumor tissue, IU4 labelling was negative, indicating the end of drug release.

The results of the ex vivo study which is a quite original approach allowing to determine the IdUrd release periods from the different batches, were consistent with the in vitro release duration data.

The type of tissue culture used in the study which consisted in maintaining ex vivo human tumor tissues on gelatine sponge stands, although different from the in vivo conditions, was quite interesting. The tissue architecture, the heterogeneity and contacts of cellular populations were maintained. This would not be the case with a monolayer cell culture method. Moreover, as it would be the case in vivo, the radiosensitizer needed to diffuse within the tissue fragments to reach the cell nuclei.

The use of human brain tumor fragments made the ex vivo experiments dependent on neurosurgical recruitment. Consequently, different histological types of brain tumors, involving variable proliferation coefficients, were used in the studies, even in a same experiment (Table 2). However, this tumor heterogeneity used in the protocol did not introduce any bias in the study results. Indeed, our objective only consisted in controlling the presence or absence of IdUrd in tumor cell nuclei but the percentage of tumor cells that incorporate IdUrd, which is highly dependent on tumor proliferation characteristics, was not considered in here.

Finally, the degree of thymidine replacement by

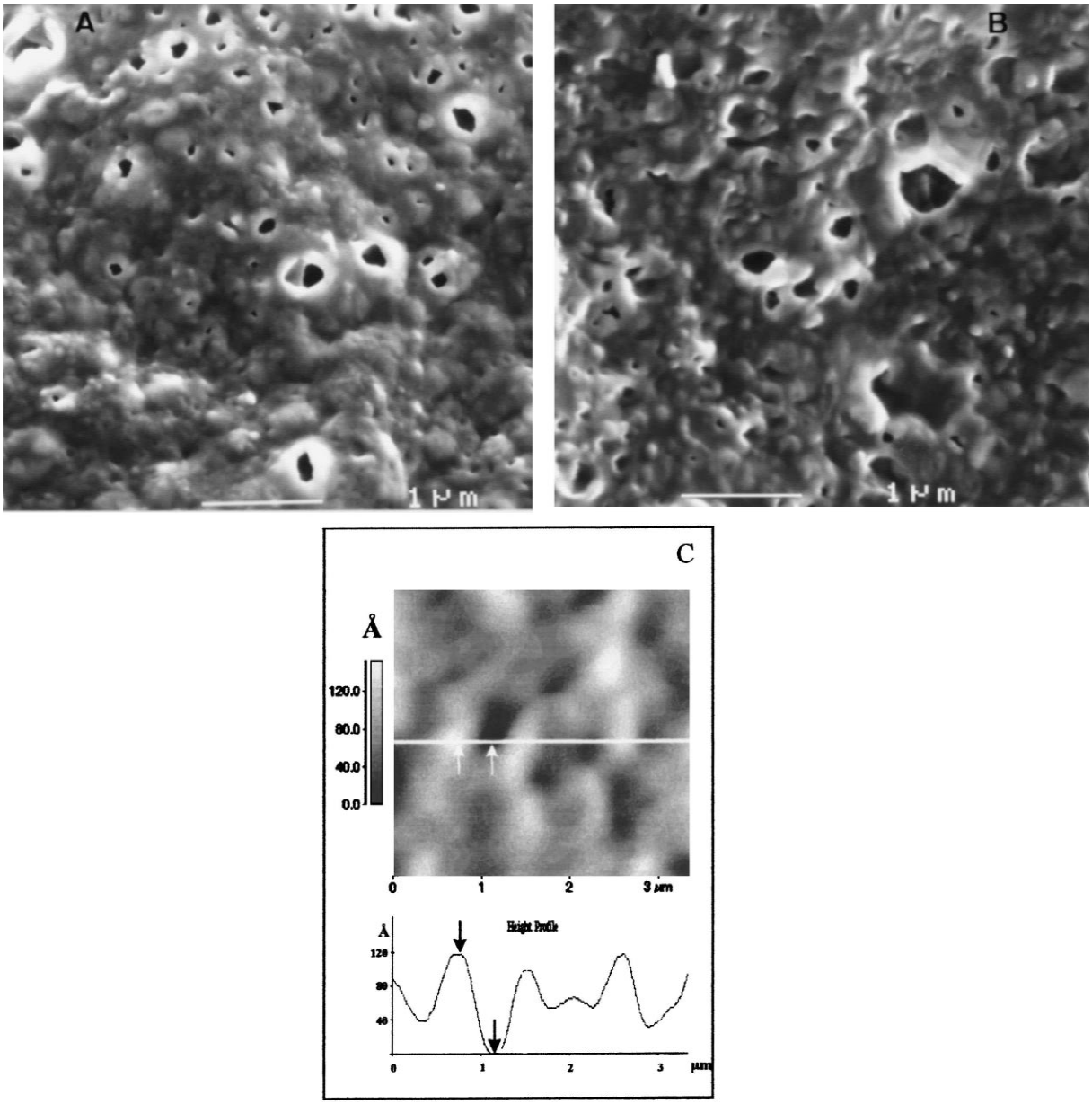

Fig. 6. SEM analyses of surface of (A) PLGA/LMW-PLA (1100) and (B) PLGA/LMW-PLA (800) microparticles and AFM analysis of surface of (C) PLGA/LMW-PLA (800) after a 2 week immersion. The height measurement (12 nm) was taken between two points (indicated by arrows) 300 nm apart on the X scan line (in white).

IdUrd in the DNA of malignant cell nuclei, was not tackled in this work. However, it could be considered, using a flow cytometry technique, with the same type of tissue culture.

4. Conclusions

The successful incorporation of LMW-PLA into a biodegradable matrix using the phase separation

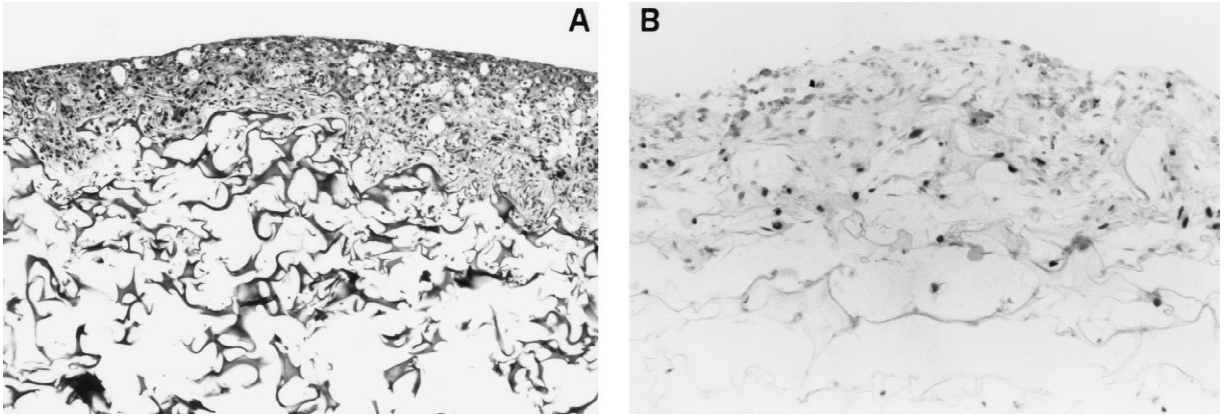

Fig. 7. A and B: Paraffin section of an oligodendroglioma maintained in tissue culture during 52 days. (A) Hemalum-phloxine staining showing the tumor implant on top of gelatine sponge and (B) IU4-immunostaining: tumor cell nuclei that incorporated IdUrd are seen in black (optical microscopy, ×200).

process yielded IdUrd-loaded microspheres, releasing in vitro the drug over a 6 week period, corresponding to the time of a conventional radiotherapy course. The drug release duration was also evaluated with brain human tumor tissue fragments maintained ex vivo in contact with the microspheres. The results obtained by this original method were consistent with the in vitro data, in terms of IdUrd release duration.

It will be interesting to follow up this study by first quantifying the IdUrd incorporation into the DNA of infiltrating gliomas such as oligodendro-

Table 2
Ex vivo protocols for release studies – IU4 and MIB1 immunolabelling results

Microsphere type	Tumor nature	Positive IU4 labelling (IdUrd)	Positive MIB1 labelling (dividing cells)
PLGA/LMW-PLA (800)	$OligoB_1$ d0-d31;		
95/5	$OligoB_2$ d33-d36 (n=2)	until d33	until d36
ER=20%			
(2 different batches)	OligoA d0-d47 (n=2)	until d33	until d40
	$OligoB_3$ d0-d42 (n=1)	until d29	until d40
PLGA / LMW- PLA (1,100)	OligoA d0-d47 (n=2)	until d38	until d40
92 / 8	$BMEC_1$ d0-d52 (n=1)	until d34	until d50
ER=20%			
(3 different batches)	Meningioma d0-d26;		
	$BMEC_2$ d28-d48 (n=1)	until d35	until d50
	$OligoB_3$ d0-d45 (n=1)	until d40	until d45
PLGA/LMW-PLA	$OligoB_1$ d0-d52;		
100/0	$OligoB_2$ d55-d64 (n=1)	until d52	until d64
ER=20-23%			
(2 different batches)	$BMEC_1$ d0-d52 (n=1)	until d45	until d48
	Meningioma d0-d26;		
	$BMEC_2$ d28-d48 (n=1)	until d48	until d52
	BMA d0-d50 (n=1)	until d52	until d55

ER: drug encapsulation ratio. OligoA: grade A oligodendroglioma. OligoB: grade B oligodendroglioma (according to Daumas-Duport's grading [1]). BMEC: brain metastases of epidermoidal cancer. BMA: brain metastases of adenocarcinoma. d: day.

gliomas. It is worth it reminding that the efficiency of radiotherapy is highly dependent on the degree of thymidine replacement by its halogenated analog in DNA. The determination of the concentration and the incubation time necessary for an optimal degree of replacement are two other important issues. Finally, the effects of ionizing radiations on brain tumor cells that incorporate the radiosensitizer will have also to be considered.

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