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Research paper

Cytarabine release from comatrices of albumin microspheres in a poly(lactide–co-glycolide) film: in vitro and in vivo studies

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Abstract

Cytarabine (ara-C) was included in albumin microspheres and these microspheres were immersed in a poly(lactide-co-glycolide) (PLGA) film to constitute a comatrix system to develop a prolonged form of release. Cytarabine-loaded albumin microspheres were synthesized by emulsion, and 25 or 50 mg of drug were included in the disperse phase. Thus, microspheres with 46 ± 4 μg drug/mg microspheres and 50 ± 5 μg drug/mg microspheres were obtained, which means a percentage of incorporation efficiency of 42 ± 4% and 25 ± 2%, respectively. These cytarabine-loaded microspheres were used to prepare PLGA-comatrices. Kinetic release studies indicated that total cytarabine release only takes place in the presence of protease, probably due to the fact that glutaraldehyde establishes covalent links with the amine side group of the drug and cross-links it with the protein matrix. A slower kinetic release of the drug was obtained from PLGA-comatrices, although only 80% of the included cytarabine was released on day 7. The comatrices were subcutaneously implanted in the back of rats and in both cases the ara-C administered dose was 36 mg of ara-C per kg of body weight. The drug was detected in plasma 10 days. The mean residence time (MRT) of the drug administered by these comatrices was 87–91 times larger when compared to the value obtained when the drug was administered in solution by intraperitoneal injection. The histological studies show that a degradative process of the comatrices takes place. The comatrices do not damage surrounding tissue; a normal regeneration of the implanted zone was observed.

Keywords: Albumin microspheres; Controlled release; Cytarabine; Histological studies; Pharmacokinetic; Poly(lactide-co-glycolide) film

1. Introduction

Subcutaneous implantable drug pellets using non-degradable polymers have been used for long-term, continuous drug administration. The procedure requires surgical implantation and removal of the drug-containing devices or polymeric matrices [1]. These facts have led to the research and development of novel, controllable, non-irritating, non-carcinogenic, biocompatible and bioabsorbable drug delivery systems for overcoming the drawbacks of non-degradable implantable pellets for prolonged continuous release [2]. Biodegradable implantable polymeric systems release the drug over a long period of time with simultaneous or subsequent degradation in the tissue of the polymer towards harmless constituents, thus avoiding removal once the therapy is complete.

Subcutaneous tissue is essentially a sheet of areolar tissue lying directly underneath the skin. It is rich in fat, but poor in nerve network and hemoperfusion. Therefore, the subcutaneous tissue is an ideal location for implantation and prolonged drug administration because of its easy access, slow drug absorption and low reactivity to the insertion of foreign materials.

Biodegradable devices must be made of materials whose degradation products can be metabolized by the organism in order to prevent their possible toxicity. In this way, biological materials, such as proteins or polysaccharides, have been used as support for controlling the release of several substances, often in the form of microspheres. Thus, teophyline has been released from bovine casein microspheres [3], doxorubicin [4] and 5-FU [5] from casein microspheres; mitoxantrone [6] and bupivacaine [7] from albumin microspheres, and albumin from chitosan beads [8] and from alginate–chitosan microcapsules [9].

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Furthermore, some synthetic polymers produce degradation products that can be metabolized by the organism and they can be used in the elaboration of these kinds of devices, such as lactic and glycolic acids and their copolymers [10–14].

On the other hand, among the antineoplastic drugs used in cancer therapy, cytarabine (ara-C) is used in the treatment of acute myelogenous leukemia [15,16] and non-Hodgkin lymphoma [17,18]. Different devices have been designed for the sustained release of this drug [19–23].

The aim of this paper was to obtain comatrices that allow a controlled release of cytarabine. Thus, cytarabine-loaded albumin microspheres were synthesized and were then included in poly(lactide-co-glycolide) to obtain a comatrix that caused a slower release of the drug. To evaluate the characteristics of these systems, in vitro kinetic studies of drug release were carried out in the presence of protease type VIII, as well as in vivo studies after subcutaneous implantation of the drug-loaded comatrices in rats. Cytarabine concentration in plasma and biodegradation of implanted comatrices were evaluated.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) fraction V [Merck, Darmastad, Germany], di-potassium monohydrogen phosphate (K_2HPO_4) [Panreac, Barcelona, Spain], potassium di-hydrogen phosphate (KH_2PO_4) [Panreac], glutaraldehyde (25%) [Sigma, St. Louis, MO, USA], dodecyl sulfate sodium salt ($C_{12}H_{25}NaO_4S$; SDS) [Merck], petroleum ether (69–95 °C) [Panreac], cottonseed oil [Sigma], isopropanol [Panreac], Tween 80 [Panreac], Protease type VIII [From Bacillus licheniformis, activity 7–15 U/mg solid; Sigma], monobasic sodium phosphate [Scharlau, Barcelona, Spain], dibasic sodium phosphate [Probus, Badalona, Spain], heparin [Analema, Vigo, Spain], diethyl ether anaesthetic [Panreac], poly(DL-lactide-co-glycolide) (50:50, M 40,000–75,000) [Sigma], chloroform [Panreac] were used.

Distilled and deionized water [Milli-Q, MA, USA] was used. Cytarabine (ara-C), molecular weight of 248, was supplied by Upjohn FarmoquÍmica, S.A. (Madrid, Spain) as a crystalline powder with a purity of 99.7%.

2.2. Preparation of microspheres

The method is based on that of Lee et al. [24]. The disperse phase was 200 mg bovine serum albumin (BSA) dissolved in 400 μl phosphate buffer 1 mM pH 7.5, containing 0.1 wt.% sodium dodecyl sulfate (SDS). Either 25 or 50 mg cytarabine dissolved in 500 μl of distilled water was added to the protein solution in order to obtain microspheres with different amounts of cytarabine, which were called type A and type B microspheres, respectively. The continuous phase (7 ml) was a mixture of cottonseed oil and petroleum ether (6:4 v/v). The two phases were mixed in a vortex at a constant stirring rate for 5 min to obtain a water-in-oil emulsion. Crosslinking of the spheres was carried out by adding 100 μl of glutaraldehyde (25 %v) and stirring the dispersion for 20 min at room temperature. The resulting albumin microspheres were washed in petroleum ether (3 ×), isopropanol (2 ×) and phosphate buffer containing 0.5% Tween 80 (2 ×). Microspheres were dried over anhydrous $CaSO_4$ at room temperature for 72 h.

The amount of ara-C included in the microspheres was determined in the presence of protease type VIII: 80 mg of cytarabine-loaded microspheres and 5 mg of protease were placed in phosphate buffer 1 mM, pH 7.5 at 37 °C and were stirred vigorously. The amount of the drug was evaluated by HPLC (Spectra-Physics SP8800 HPLC pump, SP 100 ultraviolet detector and SP 4400 computing integrator). The stationary phase was Spherisorb ODS, C_{18}, 5 μm (25 cm × 0.46 cm) (Teknokroma, Barcelona, Spain). The mobile phase was 0.005 M monobasic sodium phosphate in distilled water containing 5% v/v methanol [25,26]. The flow rate was set at 1.2 ml min^{-1} and the wavelength detector was 272 nm. Cytarabine standards of 0.1–100 μg ml^{-1} were used for external standardization and linear curves with a correlation coefficient of 0.999 were calculated from the area under the peak measurements. These areas were computed by the integrator. The chromatogram of the samples contained a single peak (retention time 2.8 ± 0.4 min) belonging to cytarabine.

2.3. Preparation of comatrices

A total of 400 mg poly(lactide-co-glycolide) (50:50, M = 40,000–75,000) was dissolved in 5 ml of chloroform; 2.5 ml of the solution was cast on a Teflon mould (7 × 3 cm). The film was allowed to set at room temperature until dry. Then, 500 mg of drug-loaded microspheres (i.e. 23 ± 2 mg of cytarabine) was dispersed on the film. Finally, 2.5 ml of the poly(lactide-co-glycolide) solution was added to the microspheres, and the chloroform was completely evaporated. These poly(lactide–co-glycolide)-comatrices were dried at room temperature over anhydrous $CaSO_4$ until reaching a constant weight. Subsequently, the comatrix was divided into six 1.2 × 3 cm pieces (each included 3.8 ± 0.3 mg of the drug), in order to proceed to the in vitro studies.

In vivo experiments were performed by utilizing individual Teflon moulds (1.5 × 1.8 cm). Both type A and type B poly(lactide–co-glycolide) comatrices were developed by incorporating type A and type B cytarabine-loaded albumin microspheres, respectively. The amount of microspheres used was that in order to obtain an ara-C load of 9 mg, which was about 200 mg of microspheres. The polymer amount was 51 mg, dissolved in chloroform.

2.4. In vitro cytarabine release

A total of 80 mg of drug-loaded microspheres (type A or type B) were added to 7 ml phosphate buffer 0.1 M pH 7.4 contained in a dialysis bag (Spectra/Por membrane MWCO: 6-8000), which was placed in a vessel containing 50 ml phosphate buffer at a constant temperature (37 °C) and stirring rate. At intervals, 100-μl samples were drawn from the solution in order to follow the change in ara-C concentration by HPLC. The removed volume from the vessel was replaced with phosphate buffer. In this way, release of cytarabine by simple swelling of microspheres was studied. In order to study cytarabine release from microspheres as a consequence of the protease effect, a similar experiment was carried out. In this case, 5 mg of protease was also added to the dialysis bag. Because of these experiments, the kinetic release of cytarabine in the presence of protease was determined. All the experiments were carried out in triplicate.

In order to study cytarabine release from poly(lactide–co-glycolide) comatrices, the comatrices (7 × 3 cm) were divided into small pieces (1.2 × 3 cm), each with a known amount of drug-loaded microspheres (drug content: 3.8 ± 0.3 mg ara-C/piece). A comatrix piece was placed inside a dialysis bag with 10 ml of phosphate buffer. This bag was submerged in a vessel with 90 ml of buffer at constant temperature (37 °C) and stirring rate. At different intervals, 100-μl samples were drawn from the solution to determine ara-C concentration by HPLC. This volume was replaced with phosphate buffer to maintain the vessel volume. A similar experiment was carried out in the presence of protease: 10 mg of protease type VIII was placed inside the dialysis bag with the device. The conditions of the experiment were maintained. All the experiments were carried out in triplicate.

2.5. Animals: cytarabine administration

Male Wistar rats weighing 252 ± 10 g were obtained from the Animalario of the Universidad Complutense de Madrid (Spain), which operates according to the requirements related to animal experimentation regulations (RD 223/1988; OM 13/X/1989). The animals were kept on a 12-h light, 12-h dark schedule and were fed standard rat food and water ad libitum.

The animals were divided into two groups. One group (12 animals) consisted of rats implanted with comatrices with drug (one piece 1.5 × 1.8 cm). The animals were anesthetized with diethyl ether and a single incision 1–2 cm long was made on their backs; blunt-scissor dissection was then used to create lateral implant sites by tunneling immediately beneath the skin in a lateral direction. The implants were then inserted a distance away from the incision point and subsequently they were sutured. Half of the animals in this group were implanted with type A comatrices and the other half with type B comatrices, both of them containing 9 mg of the drug, which corresponds to a dose of 36 mg/kg. The second group of animals (six rats) received a single daily intraperitoneal injection of cytarabine (0.9 mg of ara-C in 1 ml saline solution) for 10 days. Thus, the total amount of ara-C was also 9 mg.

2.6. Plasma cytarabine determination

At predetermined times after the implantation of the drug-loaded comatrix, the animals were anesthetized with diethyl ether. Blood (1 ml) was collected by puncturing the jugular vein and then placed in polypropylene tubes containing 75 units of heparin (15 μl). The heparinized blood was centrifuged at 12,000 × *g* for 10 min in a Sigma 202 M centrifuge, immediately after collection so as to obtain plasma, which was stored at −20 °C.

The concentration of ara-C was measured by HPLC after the addition of trichloroacetic acid (2 M, 5 μl) to plasma (100 μl) to precipitate plasma protein. This treatment of the samples did not affect ara-C levels.

Blood samples were taken from rats implanted with comatrices 6 and 24 h after the implant and then at 24-h intervals. For rats that received ara-C by intraperitoneal administration, four samples were taken daily: 30 min, 1 h, 2 h and 4 h after drug injection.

The animals were killed with diethyl ether 1, 4 and 6 months after the comatrix implantation, and then an incision was made in their backs to remove the implanted comatrix.

2.7. Pharmacokinetic parameters

Non-compartmental methods can be used to determine certain pharmacokinetic parameters without deciding on a particular compartmental model. The basic calculations are based on the area under the plasma concentration versus times curve (AUC) (zero moment) and the first moment curve (AUCM). The AUC can be calculated by the trapezoidal rule. The AUCM is the area under the concentration times time versus time curve, and it can be also calculated by the trapezoidal rule. From the AUC and AUCM values, the mean residence time (MRT) can be calculated (MRT = AUCM/AUC). It can be related to the average elimination rate constant as 1/MRT.

2.8. Scanning electron microscopy

The surface morphology of the microspheres and poly(lactide–co-glycolide)-comatrices was studied by scanning electron microscopy (Jeol JSM-6400 Electron Microscope). The size distribution of microspheres was determined by direct measurement in SEM photographs. The microspheres were dispersed in acetone, fixed on a rigid support and coated with gold. The comatrices were fixed with glue on a rigid support and coated with gold.

For the in vivo studies, the morphology of the comatrices after 1, 4 and 6 months of implantation was studied. The comatrices were dehydrated, fixed with glue on a rigid support and shadowed with gold.

2.9. Histological studies

The implants removed after 4 and 6 months of implantation were fixed with formol (10% v/v). They were immersed in paraffin. Sections (10 μm) were obtained with a paraffin microtome (Minot type). Samples were dyed using the alcian blue hemalum picro-indigo method [27].

2.10. Statistical analysis

Results are expressed as mean ± S.D. of six rats per group. Data analysis of the pharmacokinetic parameters was performed by unpaired Student's *t*-test. A value of $P < 0.05$ was considered significant.

3. Results and discussion

3.1. In vitro studies

Different biodegradable compounds have been used to design drug delivery systems. Among these compounds, proteins have been utilized to prepare microspheres [5], in which a wide variety of bioactive substances have been included in order to obtain sustained drug delivery devices [6,28,29].

Type A cytarabine-loaded albumin microspheres were spherical in overall shape, their average size was 53 ± 34 μm: 50% between 60 and 84 μm, 25% between 40 and 60 μm, and 25% between 40 and 23 μm; and the surface morphology was non-porous. Type B cytarabine-loaded albumin microspheres were larger, their average size was 76 ± 35 μm: 15% between 140 and 100 μm, 14% between 95 and 80 μm, 43% between 70 and 80 μm, and 28% between 55 and 45 μm; their surface was less smooth and they stuck to each other.

The amount of cytarabine included in albumin microspheres was 46 ± 4 μg of drug/mg of microspheres (type A) or 50 ± 5 μg of drug/mg of microspheres (type B), depending on the composition of the disperse phase. That means a percentage of incorporation efficiency of 42 ± 4% for type A microspheres and 25 ± 2% for type B microspheres. Two factors could contribute to these low percentages; one of them is the high solubility of cytarabine in aqueous medium (148 mg/ml), which causes some of the drug to be eliminated in the washing process after synthesis. The other one is the interference of the drug in the cross-linking process, since the amine group of cytarabine can react with glutaraldehyde making the formation of albumin microspheres difficult. The amount of cytarabine in the disperse phase had an influence on the characteristics of albumin microspheres, and the larger the amount of cytarabine included in microspheres, the larger their average size.

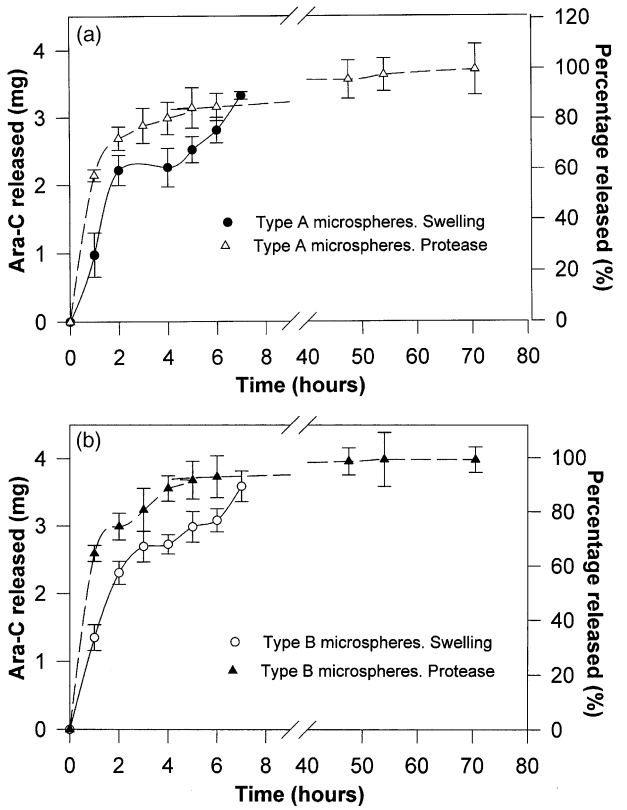

Fig. 1. Cytarabine (ara-C) released from type A (A) and type B (B) albumin microspheres as a function of time by swelling and in the presence of protease type VIII.

The release kinetics of the drug from type A and type B microspheres by swelling showed a hyperbolic profile (Fig. 1). The maximum drug release from type A microspheres had taken place in the first 7 h, and a burst effect was observed in the first hour, when 29% of the drug was released. In the case of type B microspheres, maximum drug release had been observed in the first 7 h, and 37% of that amount was released in the first hour.

In the presence of protease type VIII a quicker drug release from both types of microspheres was observed. Thus, 57% and 65% of the total released drug had been released in the first hour from type A and type B microspheres (Fig. 1), respectively. With regard to the total amount of cytarabine released from albumin microspheres, it was larger in the presence of protease from both types of microspheres. Thus, for type A microspheres the maximum amount of cytarabine in the release medium was 3.32 ± 0.06 mg in the absence of protease, whereas in the presence of the enzyme it was 3.71 ± 0.38 mg. A similar situation was observed for type B microspheres; in the absence of protease in the release medium the maximum cytarabine amount was 3.59 ± 0.23 mg, and in the presence of protease it was 3.99 ± 0.40 mg. Therefore,

the amount of released cytarabine was larger in the presence of protease type VIII. Latha and co-workers (Latha et al., 1994b) have described that drugs with amine side groups could establish covalent links with the protein matrix by cross-linking with glutaraldehyde. In this way, only degradation of the protein microspheres allowed the complete release of the drug. When protease was in the release medium, holes in the surface of the microspheres were observed at 24 h, and total hydrolysis of microspheres was detected at day 8.

The preparation of poly(lactide–co-glycolide) comatrices by inclusion of the drug-loaded microspheres in a polymer film was carried out with type A microspheres. When the release rate of cytarabine from the comatrix during the first stage of the process was determined, its value was almost the same in the presence and in the absence of protease (0.96 ± 0.05 mg/h and 1.0 ± 0.08 mg/h, respectively; $P > 0.5$). Thus the polymer film protected drug-loaded microspheres from the hydrolytic action of the enzyme. This protection effect was more evident when the release rate of the drug from microspheres was considered, thus the cytarabine release rate from microspheres type A was 1.35 ± 0.06 mg/h and 1.11 ± 0.08 mg/h ($P < 0.05$) in the presence and in the absence of the enzyme, respectively. In fact, the release rate of cytarabine from the comatrix in the first stage of the process was almost the same as that from the microspheres in the absence of the enzyme in the solvent medium. The polymer film protects microspheres and only 70% of the drug included in the albumin microspheres had been released in the first 27 h in the absence of protease (Fig. 2). When the enzyme was present in the release medium, about 80% of cytarabine included in the comatrices had been released by day 7, however, total hydrolysis of microspheres of the comatrices had taken place by day 20. Thus, these comatrices increased the time period at which cytarabine release took place.

3.2. In vivo studies

On the basis of theoretical considerations, the total dose of drug required to achieve a sustained optimum level in vivo in a particular organ over a period of time can be reduced both by administering it as a controlled release formulation and by directing the drug to the organ in question. Thus, by using the appropriate technology, it may be possible to maintain higher drug concentration gradients over a specific area for a longer period of time than by bolus injection.

The cytarabine-loaded albumin microspheres of the comatrices included 46 ± 4 μg of drug/mg of microspheres (type A) or 50 ± 5 μg of drug/mg of microspheres (type B). The amount of microspheres included in the comatrices allowed obtaining 9 mg of cytarabine in the implanted sample. This amount of drug administered to rats with a body weight of 252 ± 3 g represented a dose of 36 mg/kg. Specific doses of ara-C in rats have not been described. Doses from 10 to 225 mg/kg of weight have been assayed in mice [30,31].

The plasma concentration of ara-C released from type A comatrices is shown in Fig. 3. The maximum amount released, which produced a cytarabine plasma concentration of 16.5 ± 7 μg/ml, took place within the first 6 h. From this time, plasma drug concentration gradually decreased and the drug was not detected at day 12.

From type B comatrices, the drug release was slower and ara-C plasma concentration increased as a function of implantation time of the sample. The maximum plasmatic concentration of ara-C was 15.6 ± 0.8 μg/ml, and it was reached 48 h after implantation. From this time, plasma drug concentration gradually decreased and drug was not detected at day 12 (Fig. 3).

The influence of the method of administration on ara-C plasma levels was studied by comparing drug

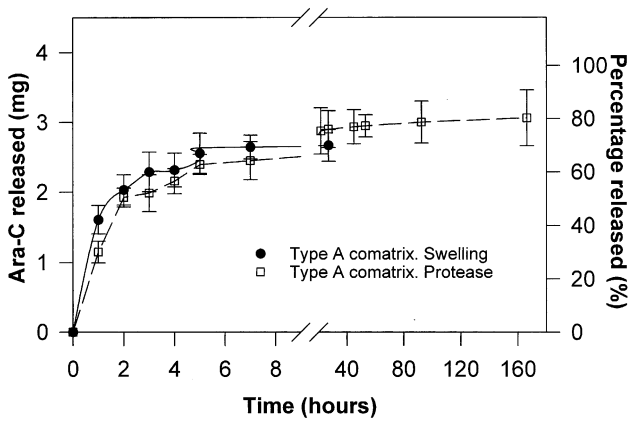

Fig. 2. Cytarabine (ara-C) released from type A comatrices as a function of time by swelling and in the presence of protease type VIII.

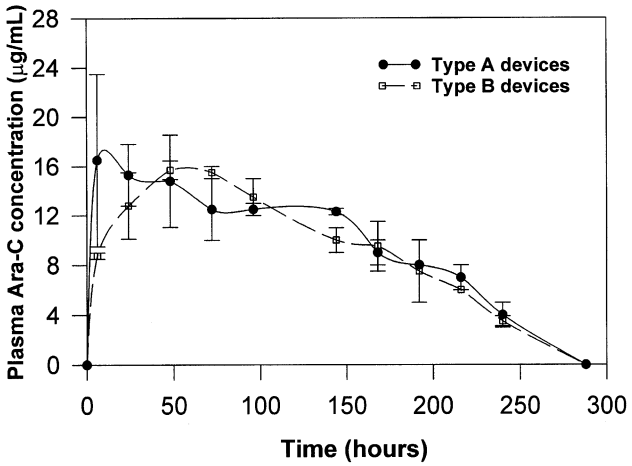

Fig. 3. Plot of cytarabine (ara-C) plasma concentration as a function of time for drug released from type A and type B cytarabine-loaded comatrices.

Table 1
Pharmacokinetic parameters of cytarabine (ara-C) after intraperitoneal injection (0.9 mg ara-C/injection daily for 10 days) and subcutaneous implantation of cytarabine-loaded comatrices (9 mg ara-C/comatrix)

	AUC (μg h/ml)	AUCM (μg h^2/ml)	MTR (h)	1/MTR (h^{-1})
Type A comatrix	3430 ± 505*	266,834 ± 4090*	77.79 ± 8.10*	0.013 ± 0.003*
Type B comatrix	3264 ± 410*	265,993 ± 2542*	81.49 ± 6.20*	0.012 ± 0.002*
Injection	4.10 ± 0.80	3.65 ± 0.21	0.89 ± 0.26	1.12 ± 0.16

AUC, area under the plasma concentration versus times curve. AUCM, area under the concentration times time versus time curve. MTR, mean residence time (MTR = AUCM/AUC); *significant difference with regard to the injection group, $P < 0.005$.

administration in the form of subcutaneously implanted comatrices to intraperitoneal injection form. Intraperitoneal injection is a parenteral via of drug administration, usually used in animal experimentation due to its very simple use and fast absorption of the drug into the blood, essentially by the porta vein [32]. In both cases the ara-C dose was the same. Since the release of ara-C from the comatrices took place in about 10 days, daily intraperitoneal administration of ara-C was performed over a 10-day period. Thus, by intraperitoneal administration the ara-C plasmatic concentrations were 3.62 ± 0.54 μg/ml, 1.63 ± 0.48 μg/ml and 0.69 ± 0.14 μg/ml, 30 min, 1 h and 2 h after the injection, respectively, and the drug was not detected in plasma at 4 h.

The intracellular concentration of cytarabine depends on the administered dose [33]. From an ara-C plasmatic concentration of approximately 10–15 μmol/l (2.4–3.7 μg/ml) the transporting of ara-C inside the cell is independent of its concentration [34], allowing intracellular ara-C concentrations to be larger than the K_m of the deoxycitidine kinase for ara-C (K_m = 1.5 μmol/l), and as a result ara-CTP formation is maximum. In this way, by intraperitoneal injection, plasma ara-C concentration was below 2.4 μg/ml 1 h after its administration, and therefore ara-CTP formation was not optimal [33], the effectiveness of ara-C decreasing. When ara-C was administered by subcutaneously implanted comatrices the drug plasma concentration was above 10 μmol/l (2.4 μg/ml) for about 240 h, thus the intracellular ara-CTP formation would be optimal for a long period of time.

The pharmacokinetic parameters of ara-C after both intraperitoneal injection (0.9 mg ara-C/injection) and subcutaneous implantation of drug-loaded comatrices (9 mg ara-C/comatrix) are shown in Table 1. Administration of cytarabine as comatrices of drug-loaded albumin microspheres included in a poly(lactide–co-glycolide) film seems to result in improved therapeutic results. Not only was the area under the plasma concentration–time curve (AUC) larger, but the mean residence time (MRT) of the drug was also 87–91 times larger.

In order to evaluate the biodegradation of the comatrices and their biocompatibility, the animals implanted with type A and type B cytarabine-loaded comatrices were killed at 1, 4 and 6 months, and the comatrices and surrounding tissue were removed.

The scanning electron microscopy (SEM) of a transversal section of the type A comatrix removed 1 month after implantation (Fig. 4a) indicated that the degradation process had begun. The poly(lactide–co-glycolide) film showed discontinuous zones and only some microspheres were detected. A similar degradation effect was observed in type B comatrices 1 month after subcutaneous

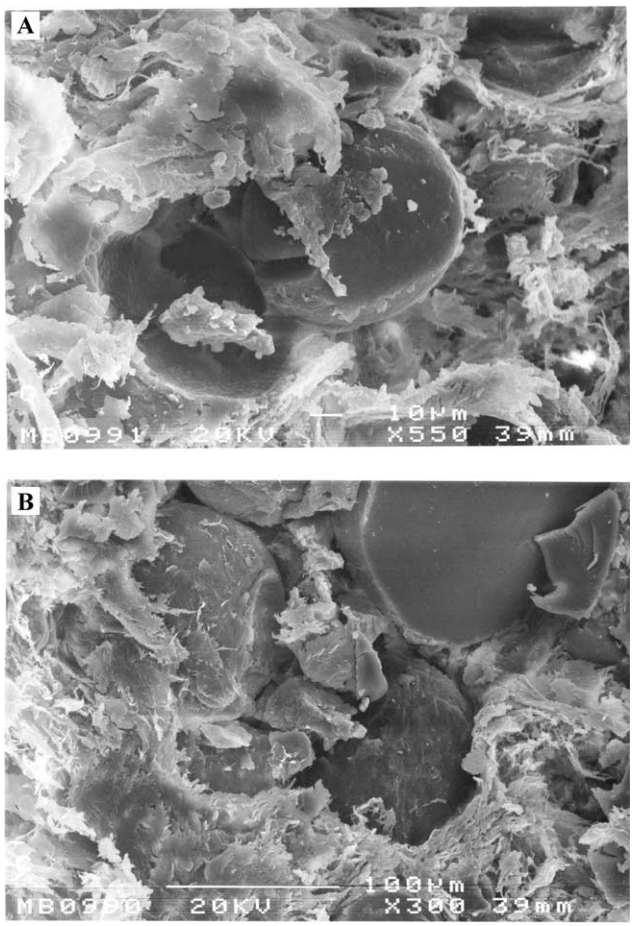

Fig. 4. Scanning electron micrograph of type A cytarabine-loaded comatrix (A) and type B cytarabine-loaded comatrix (B) after 1 month of implantation in the back of rats.

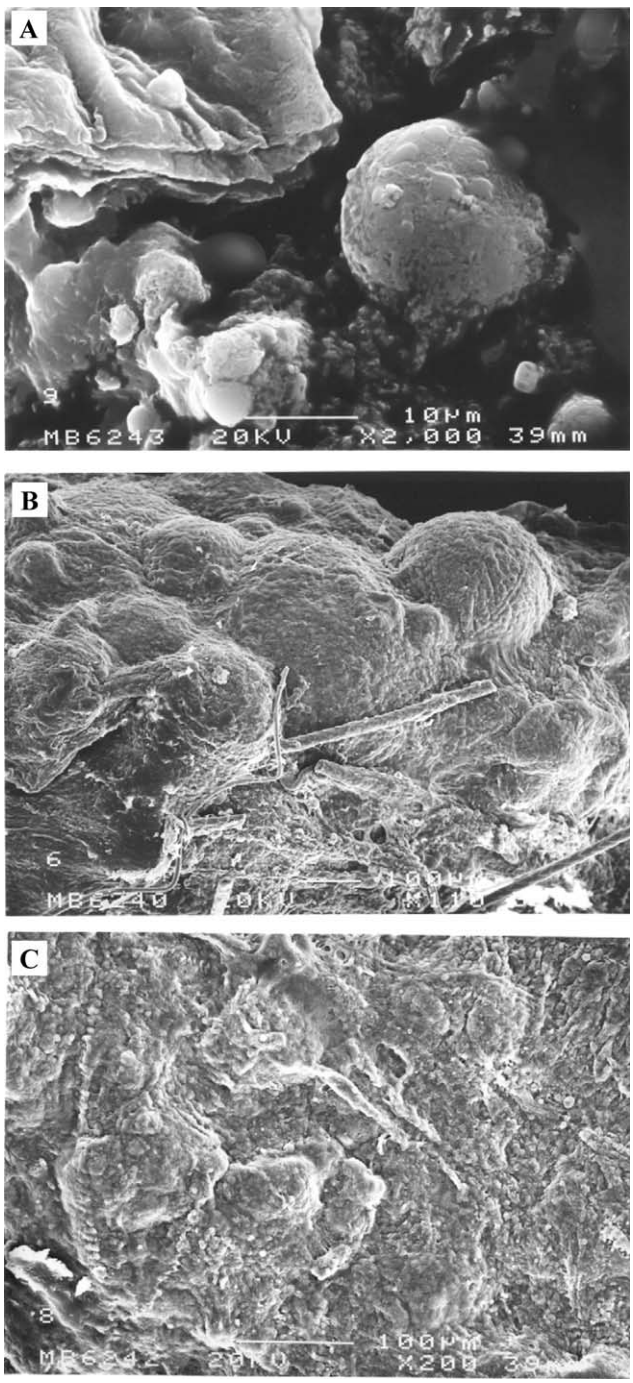

Fig. 5. Scanning electron micrograph of type A cytarabine-loaded comatrix after 4 months of implantation (A), external surface of type B cytarabine-loaded comatrix after 4 months of implantation (B), and external surface of type A cytarabine-loaded comatrix after 6 months of implantation in the back of rats (C).

implantation (Fig. 4b). After 4 months of implantation (Fig. 5a) the number of microspheres decreased in a significant way and it was difficult to observe them in the comatrix remains by SEM. Furthermore, the external surface of the comatrices indicated the degradation of the polymer film (Fig. 5b), which had a wrinkled aspect, which was quite different of the smoothness of the non-implanted comatrix (results not shown). The degradation process of the comatrices increased with the time of implantation, and at 6 months (Fig. 5c) the surface comatrix was more wrinkled, and protuberances due to microspheres were not observed.

Willmott and collegues [35] have studied the biodegradation of albumin and casein microspheres embolized in liver, lung and kidney. The SEM study shows an erosion process, which is characterized by the progressive loss of sphericity and surface smoothness. On the other hand, the polymer degradation of polyesters based on lactic and glycolic acid is mainly based on the bioerosion due to water [36]. Furthermore, the degradation products are finally metabolized to carbon dioxide and water or excreted via the kidneys. Thus, the comatrices of cytarabine-loaded albumin microspheres enclosed in a poly(lactide–co-glycolide) film were slowly degraded. The polymer film, whose degradation is very slow, protected albumin microspheres against enzyme degradation improving their half-life and allowing a sustained cytarabine release.

When a polymeric implant is introduced in the body some mechanisms are activated to start immunologic reactions, usually as inflammation around the implant zone [37]. The concentration of active physiological substances, physiological medium pH, oxygen concentration, the number and kind of metal ions and the anion composition of the body are altered. Macrophages and other kinds of cells invade the implant zone. A collagen layer that covers the polymer surface, and whose thickness depends on the implant biological compatibility, is generated [38]. The most common biodegradative agents in the body are water, salts, pH of the physiological medium and enzymes.

The histological studies of type A comatrices after 4 months of implantation (Fig. 6a) showed microspheres included in conjunctive tissue. The microspheres were in different phases of disintegration. Mulinucleate foreign body giant cells inside partially empty microspheres were observed. Type B comatrices at 4 months of implantation (Fig. 6b) showed a similar situation, although the degradation stage looks larger, and more microsphere remains were observed.

The type A comatrices removed at 6 months (Fig. 7a) indicated a greater biodegradation stage. The microsphere remains were surrounded by conjunctive tissue, and blood vessels, as well as nerve packets in the periphery of the implant (Fig. 7b), were observed. The histological study of the type B comatrices at 6 months (Fig. 7c) was similar to that of type A, although more blood vessels and muscular fibers were observed. Therefore, the type B comatrices seem to be more easily biodegraded, probably due to the fact that cytarabine-loaded microspheres obtained from 50 mg of drug in the disperse phase were less stable, which means that their crosslinking was less

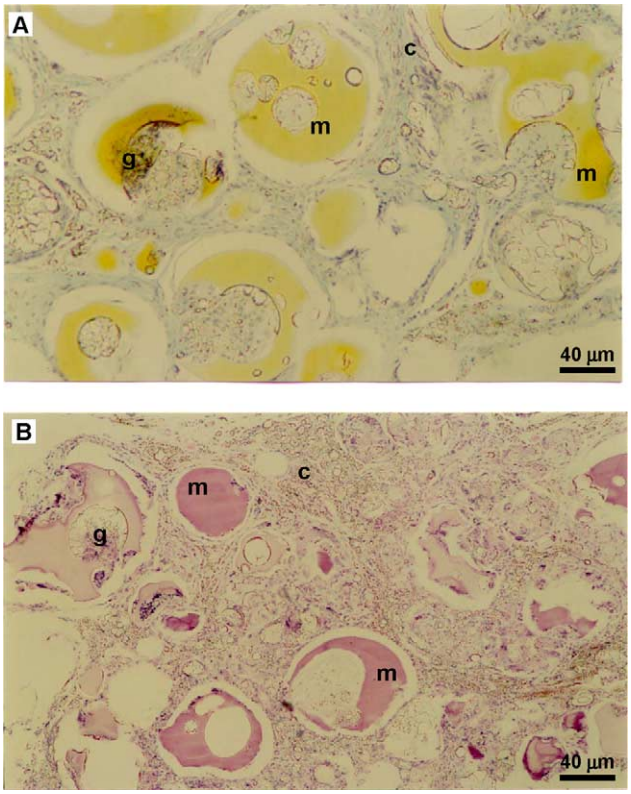

Fig. 6. Photomicrographs of type A cytarabine-loaded comatrix (A) and type B cytarabine-loaded comatrix (B) after 4 months of implantation. Albumin microspheres (m), connective tissue (c); multinucleate foreign body giant cells (g). [Magnification 252 ×].

efficient than that obtained from 25 mg of drug in the disperse phase.

The exposed results show that both types of comatrices have undergone a degradative process with a positive response of the receptor organism. The implants seem to have been totally accepted. In summary, the comatrices of cytarabine-loaded albumin microspheres included in a poly(lactide–co-glycolide) film were biodegradable and injurious effects were not detected in the animals after 6 months of implantation. The administration of cytarabine by comatrix implants, from which the drug is released, results, from a therapeutic viewpoint, in suitable levels of ara-C in plasma for a long period of time. The implantation of these slow-release comatrices containing ara-C would decrease, at least in part, the side effects produced by the high doses of this drug usually used. The plasmatic ara-C concentrations obtained when it was released from the comatrices were in the range of 1–50 μmol/l, so they were quite similar to those obtained when intermediate and high doses of ara-C are used in therapy. A quicker degradation of the implant would probably be desirable, so experiments are underway to improve this aspect without decreasing the ara-C amount included in the microspheres or changing the period of time of drug release.

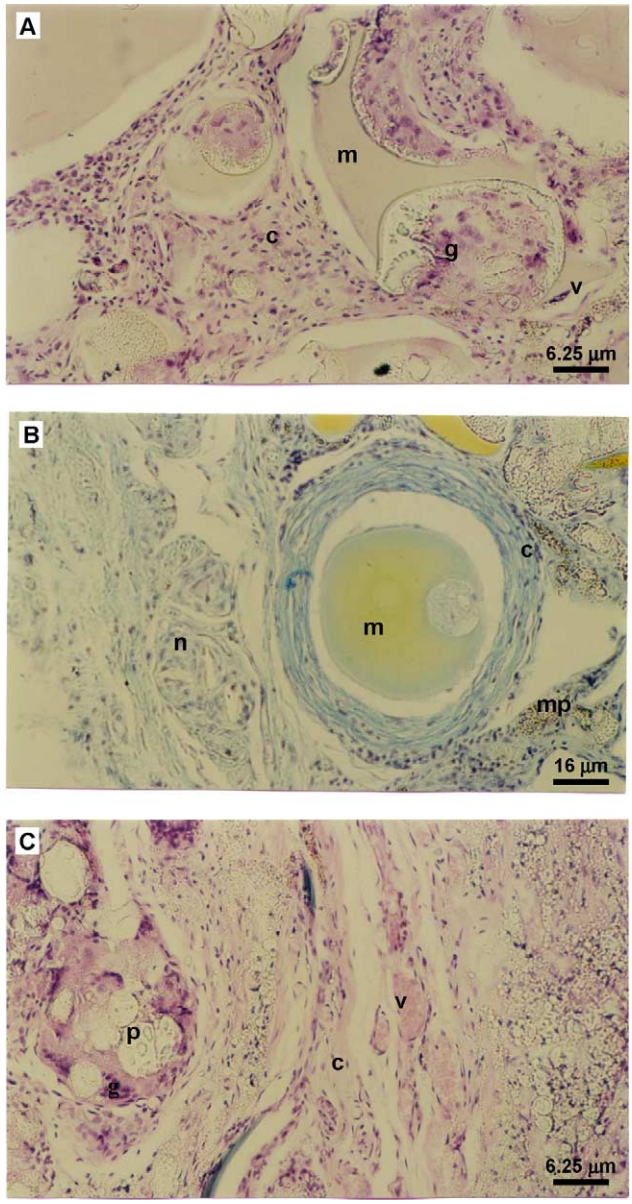

Fig. 7. Photomicrograph of type A cytarabine-loaded comatrix [Magnification 1600 ×] (A); and one microsphere surrounded by conjunctive tissue [Magnification 640 ×] (B) after 6 months of implantation. Photomicrograph of type B cytarabine-loaded comatrix after 6 months of implantation [Magnification 1600 ×] (C). Blood vessels (v); macrophages (mp); nerve packets (n), polymeric material (p).

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