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Chitosan microspheres in PLG films as devices for cytarabine release

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Abstract

Cytarabine was included in chitosan microspheres and several of these microspheres were embedded in a poly(lactide-co-glycolide) (PLG) film to constitute a comatrix system, to develop a prolonged release form. Chitosan microspheres, in the range of 92 ± 65 μm, having good spherical geometry and a smooth surface incorporating cytarabine, were prepared. The cytarabine amount included in chitosan microspheres was 43.7 μg of ara-C per milligram microsphere. The incorporation efficiency of the cytarabine in microspheres was 70.6%. Total cytarabine release from microspheres in vitro was detected at 48 h. Inclusion of cytarabine-loaded microspheres in poly(lactide-co-glycolide) film initiated a slower release of the drug and, in this way, the maximum of cytarabine released (80%) took place in vitro at 94.5 h. Comatrices, with 8.7 mg of cytarabine, signifying a dose of 34.5 μg/kg, were subcutaneously implanted in the back of rats. Maximum plasma cytarabine concentration was 18.5 ± 1.5 μg/ml, 48 h after the device implantation and the drug was detected in plasma for 13 days. The histological studies show a slow degradative process. After 6 months of implantation, most of the microspheres of the matrix seemed to be intact, the comatrix appeared surrounded by conjunctive tissue and small blood vessels and nerve packets were detected in the periphery of the implant.

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

1. Introduction

Biodegradable carriers have been extensively used in pharmaceutical design to obtain delivery systems that allow sustained drug release. The degradation products of these carriers must be non-toxic and metabolised by the organism (Jones et al., 1989).

Among the biodegradable devices used for drug controlled release, chitosan microspheres have been used to include different types of drugs. Chitosan, or β(1,4)2-amino-2-deoxy-D-glucose, is a hydrophilic biopolymer obtained industrially by hydrolysing the aminoacetyl groups of chitin —

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which is the main component of the shells of crab, shrimp and krill — by an alkaline treatment. Chitosan has attracted attention as a matrix for controlled release since it possesses reactive functionalities, is easily degraded by enzymes and the degradation products are non-toxic (Suheyla Kas, 1997). Chitosan is reported to find application as a drug in the treatment of hyperbilirubinaemia and hypercholesterolaemia (Nagyvary, 1982). Chitosan has been prepared and evaluated for its antitumour activity carrying several antineoplastic agents (Ouchi et al., 1989; Jameela and Jayakrishnan, 1995). Thus, chitosan appears to be a promising matrix for the controlled release of pharmaceutical agents.

The biological life of the carrier matrix is important from the point of view of determining the duration of chemical delivery that can be affected using a particular matrix. As part of an ongoing programme investigating the potential of natural as well as synthetic polymers as carriers for prolonged delivery of drugs, we examined the inclusion of cytarabine in chitosan microspheres and its release in vitro from them and also from a comatrix formed by these microspheres included in a poly(lactide-co-glycolide) (PLG) film. Besides the biodegradation of the comatrix implanted in the back of rats over a period of 6 months, the pharmacokinetic parameters of drug release were studied. Cytarabine is an agent commonly used in the treatment of acute myelo- and myelo-monocytic leukaemias (Thomas and Archimbaud, 1995) and it has been chosen as a model drug to be incorporated into chitosan microspheres, in order to study the in vitro release profiles of small molecules from the matrix and their in vivo biocompatibility.

2. Materials and methods

2.1. Materials

Chitosan (minimum 85% deacetylated) (Sigma-Aldrich Química, S.A., Madrid, Spain), di-potassium monohidrogen phosphate (K_2HPO_4) (Panreac, Barcelona, Spain), potassium di-hidrogen phosphate (KH_2PO_4) (Panreac), glutaraldehyde (25%) (Sigma-Aldrich Química), toluene (Panreac), petroleum ether (69–95°C) (Panreac), cottonseed oil (Sigma-Aldrich Química), methanol (Panreac), sodium hydrogen sulphite solution 40% w/v (Panreac), acetone (Panreac), lysozyme (50 000 U/mg solid) (Sigma-Aldrich Química), 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-Aldrich Química) and chloroform (Panreac).

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

2.2. Methods

2.2.1. Ara-C stability

The effect of temperature and lysozyme on drug degradation was studied. Thus, 25 mg of ara-C were dissolved in 100 ml phosphate buffer (0.1 M, pH 7.4). The dissolution was maintained at a constant stirring rate at 37°C for 25 days. At intervals, 100-μl samples were drawn from the solution in order to follow the change in cytarabine concentration. The removed volume was replaced with the phosphate buffer.

The concentration of cytarabine was determined by high-performance liquid chromatography (HPLC) (Spectra-Physics SP 8800 HPLC pump, SP 100 UV absorbance detector and SP 4400 computing integrator). The stationary phase was Spherisorb ODS, C_{18}, 5 μm (25 × 0.46 cm) (Teknokroma, Barcelona, Spain). The eluent was 0.005 M monobasic sodium phosphate and 0.005 M dibasic sodium phosphate in distilled water, with 5% v/v methanol (Quock and Sakai, 1985). The flow-rate was set at 1.2 ml/min and the detector wavelength was always 272 nm. The samples showed a single peak in the chromatogram (retention time 2.8 ± 0.4 min) which belonged to ara-C (Breithaupt and Schick, 1981; Quock and Sakai, 1985).

A similar experiment was carried out to determine the stability of cytarabine in the presence of lysozyme. The drug (25 mg) was dissolved in phosphate buffer (100 ml, 0.1 M, pH 7.4) with lysozyme (50 000 U/mg protein) (5 mg) and maintained at 37°C for 25 days. Aliquots of 100 μl were taken at intervals and cytarabine concentration was determined by HPLC.

2.2.2. Preparation of microspheres

The method is based on that of Jameela and Jayakrishnan (1995). A 4% solution of chitosan in 5% aqueous acetic acid containing 2% NaCl was prepared and 6 g of this solution was dispersed in a mixture of 60 ml cottonseed oil and 20 ml of petroleum ether in a 100 ml round-bottomed flask at room temperature. The dispersion was stirred using a stainless steel paddle stirrer at 2000 rev/min for 5 min and 1.6 ml of glutaraldehyde-saturated toluene (GST), prepared according to the method of Longo et al. (1982), was added. After 15 min, another 1.6 ml of GST was introduced. The stirring was continued for 1.5 h and then the hardened microspheres were washed several times with petroleum ether followed by methanol, a 5% solution of sodium bisulphite and finally, with acetone.

2.2.2.1. Cytarabine-loaded spheres. Cytarabine-containing chitosan microspheres were prepared by mixing 50 mg of ara-C with 6 g of chitosan solution, dispersing and cross-linking as before. The microspheres were washed several times with petroleum ether, once with a 5% solution of sodium bisulphite, once with methanol and finally with acetone and then dried.

The ara-C amount included in chitosan microspheres was determined by swelling of 80 mg ara-C-loaded microspheres in 57 ml phosphate buffer 0.1 M pH 7.4 at 37°C and vigorous stirring for 120 h.

2.2.3. Preparation of comatrix

A total of 400 mg poly(lactide-co-glycolide) (50:50, M 40 000–75 000) were dissolved in 5 ml 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. 21.85 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 evaporated completely. By this process, a comatrix system was developed by incorporating cytarabine loaded chitosan microspheres in poly(-lactide-co-glycolide) matrix. These co-matrices were dried at room temperature and maintained in a drier. Subsequently, the comatrix was divided in six parts of 1.2 × 3 cm (each included 3.64 mg of the drug), in order to proceed to in vitro studies.

In vivo experiments were performed by utilizing individual teflon moulds (1.5 × 1.8 cm). Two types of matrices with different amounts of PLGA were prepared. One with 75 mg of polymer (type A) and the other with 175 mg of PLGA (type B), dissolved in chloroform. In both cases, 200-mg drug loaded microspheres were included, as described above, thus the ara-C amount administered was 8.7 mg.

2.2.4. Cytarabine release from microspheres in vitro

A total of 80 mg of drug-loaded microspheres — i.e. 3.5 mg of cytarabine — were added to 7 ml phosphate buffer 0.1 M pH 7.4 inside a dialysis bag (Spectra/Por membrane MWCO: 6–8000), which was placed in a vessel containing 50 ml phosphate buffer at a constant temp- erature (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 lysozyme effect, a similar experiment was carried out. In this case, 5 mg of lysozyme was also added to the inside of the dialysis bag . Because of these experiments, the release kinetic of cytarabine in the presence of lysozyme was known.

All the experiments were carried out in triplicate.

2.2.5. Cytarabine release from the comatrix in vitro

The comatrix (7 × 3 cm) was divided into small pieces (1.2 × 3 cm), each with a known amount of drug-loaded microspheres (drug content was 3.64 mg of cytarabine). A comatrix piece was placed inside a dialysis bag with 10 ml phosphate buffer 0.1 M pH 7.4. This bag was submerged in a vessel with 90-ml buffer at constant temperature (37°C) and stirring rate. At 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 constant the vessel volume.

All the experiments were carried out in triplicate.

2.2.6. Animals: cytarabine administration

Male Wistar rats, weighing 252 ± 3 g, obtained from the Animal Department of the Universidad Complutense of Madrid, Spain, were used. The animals were kept on a 12:12 h light:dark cycle and were fed standard rat food and water ad libitum.

The animals were divided into two groups. The first group consisted of rats implanted with the comatrix (one piece 1.8 × 1.5 cm) with the drug. The animals were anaesthetized with diethyl ether and a single incision, 1–2 cm long, was made on their backs; blunt-scissor dissection was then used to create a lateral implant site by tunnelling immediately beneath the skin in a lateral direction. The implants were then inserted a distance from the incision and sutured. The amount of cytarabine of the comatrix piece was 8.7 mg, signifying a dose of 34.5 μg/kg. The second group of animals was intraperitoneally administered a daily single injection of saline solution (1 ml) containing 0.9 mg of ara-C for 10 days. This drug amount was a tenth of that administered by comatrix, in order to administer the total amount of drug in a similar period of time as that obtained through comatrix-administration. 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 (Benet and Sheiner, 1986). This way of administration establishes a comparison between the drug plasma levels and its pharmacokinetic parameters with regard to drug administration using a sustained drug release device.

2.2.7. Plasma cytarabine determination

At predetermined times after implantation of the drug-loaded comatrix, animals were anaesthetized with diethyl ether. Blood was obtained by puncture of the jugular vein. Samples of blood (1 ml) were collected in polypropylene tubes containing 75 U 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 and the plasma obtained was stored at 253 K.

The concentration of ara-C was measured by HPLC. Plasma was chromatographed with a previous addition of trichloroacetic acid (TCA) 2 M. In this way, 5 μl of TCA were added to 100 μl of plasma in order to obtain the precipitation of the plasmatic proteins. This treatment of the samples does not modify ara-C.

Blood samples were taken from rats implanted at 24 h intervals. For rats which received ara-C by intraperitoneal administration, two samples were taken daily, 30 min and 1 h after drug injection.

Animals were sacrificed with diethyl ether and an incision was made on their backs to remove the implanted comatrix. The surrounding tissue of the remaining polymer matrices was removed and then each matrix was divided in two pieces. One was dried and used for scanning electron microscopy; the other was placed in 10% formalin solution for histology.

2.2.8. Pharmacokinetic parameters

Plasma cytarabine data were analyzed using a non-compartment model. The elimination constant (K_e) and the corresponding half-life ($t_{1/2} = \ln 2/K_e$) were derived from the terminal slope of the semilogarithmic plots of plasma concentration versus time. The areas under curve (AUC) were determined by trapezoidal method. The total body clearance (TBC) was derived from dose/AUC and the volume of distribution (V_d) from TBC/K_e.

2.2.9. Scanning electron microscopy (SEM)

The surface morphology of the microspheres and comatrices was studied by scanning electron microscopy (Jeol JSM-6400 Electron Microscope). The microspheres were dispersed in acetone, fixed on a rigid support and coated with gold. The comatrix was fixed with glue on a rigid support and coated with gold.

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

2.2.10. Histological studies

Implants extracted after 4 and 6 months of implantation were fixed with formol (10% v/v), and immersed in paraffin. Cuts (10 μm) were carried out with a paraffin microtome (Minot type). Samples were dyed using the Alcian blue hemalum picro-indigo method (Humason, 1979).

2.2.11. Statistical analysis

Results are expressed as mean ± S.E.M. of five 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.

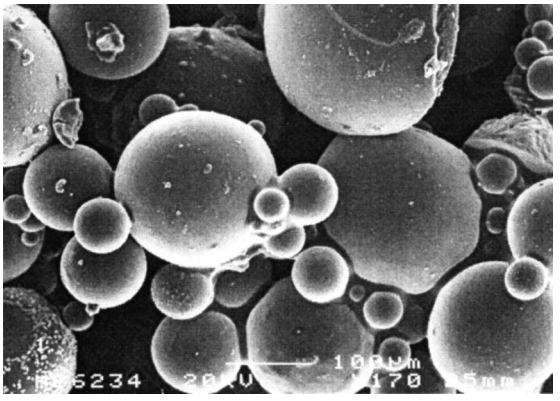

Fig. 1. Scanning electron micrograph of cytarabine loaded chitosan microspheres in the size range of 92 ± 65 μm.

3. Results and discussion

Chitosan, which is insoluble in water, can be solubilized in dilute acetic acid due to the formation of the acetate salt. The viscosity of a 4% solution in 5% acetic acid was found to be the optimum for dispersing into droplets even with high drug loading (> 70%) (Austen and Sennet, 1986). Because a slow and uniform crosslinking of the droplets, particularly on the surface, was felt desirable to generate spheres of good sphericity, glutaraldehyde saturated toluene was chosen instead of an aqueous solution to induce the crosslinking.

The stability of cytarabine in phosphate buffer (pH 7.4) at 37°C in the absence and presence of lysozyme has been studied for 600 h. The initial concentration of the solution, 0.25 mg/ml of ara-C, was maintained constant for the studied period of time. No degradation of the drug as a function of time was observed.

The surface morphology of dried cytarabine-loaded chitosan microspheres, using SEM, is shown in Fig. 1. The microspheres were ≈92 ± 65 μm in size and spherical in overall shape. The surface morphology of the chitosan microspheres also revealed them to be non-porous. Drug loading did not cause any change in the shape, size or surface morphology of the microspheres. Chitosan microspheres loaded with mitoxantrone were synthesized by Jameela and Jayakrishnan (1995) using paraffin liquid/petroleum ether containing sorbitan sesquiolate as continuos phase, reaching an average size of 120 μm.

The amount of cytarabine included in chitosan microspheres was 43.7 μg ara-C per milligram of microspheres. Ara-C release from microspheres was studied by simple swelling and as a consequence of the lysozyme effect. The release kinetic of cytarabine from chitosan microspheres by swelling shows a hyperbolic profile (Fig. 2). At the first stages of the process, a large amount of cytarabine is released — 36% of the drug is released during the first hour. Total release of cytarabine was obtained at 48 h. No degradation of microspheres during the incubation was visualized by SEM.

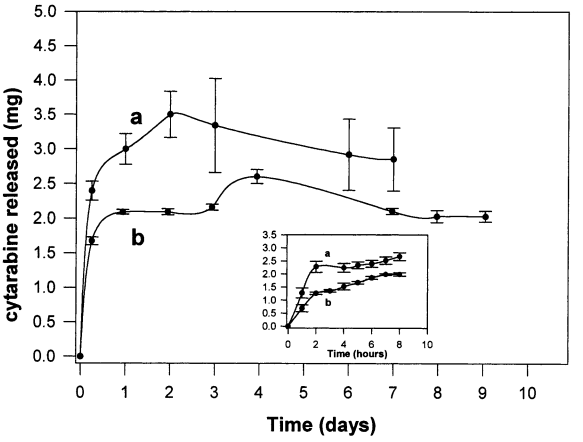

Fig. 2. Cytarabine released from (a) chitosan microspheres and (b) the comatrix, in phosphate buffer, 0.1 M, pH 7.4 at 37°C, by swelling as a function of time.

The incorporation efficiency of the drug was 70.6%. Cytarabine is soluble to the extent of 148 mg/ml in water. During the cross-linking and hardening process, water is exuded from the microspheres along with the dissolved drug and this appears to be responsible for the percentage of incorporation efficiency. The inclusion of aspirin, griseofulvin and theophylline in chitosan microspheres (Tanoo et al., 1992), shows incorporation efficiency of 77.3, 73.8 and 78%, respectively.

In order to examine the enzymatic degradation of this material, 80 mg of the ara-C loaded microspheres were incubated in 57 ml of phosphate buffer containing 5 mg of lysozyme. The release kinetic of the drug was the same as that for simple swelling. Microspheres were not degraded by lysozyme, neither were pores observed on their surface, as it was visualized by SEM. Even after 20 days, no degradation of the material appeared when the spheres were examined. Although Hirano et al. (1989) have reported that partially N-acetylated derivatives of chitosan, having a degree of substitution of 0.4–0.8 for N-acetyl, are digested several times faster than N-acetylchitosan (degree of substitution 1.0 for N-acetyl) by lysozyme and Pangburn et al. (1982) have reported that glutaraldehyde cross-linked hydrogels of 31% deacetylated chitin are also degraded by lysozyme, but at a slower rate compared to uncross-linked material, the glutaraldehyde crosslinked chitosan microspheres, prepared from 74% deacetylated chitin, were not found to be degraded to a significant extent in vivo in 6 months. Thus, the ara-C loaded-chitosan microspheres, obtained from 85% deacetylated chitosan and crosslinked with glutaraldehyde, are highly stable.

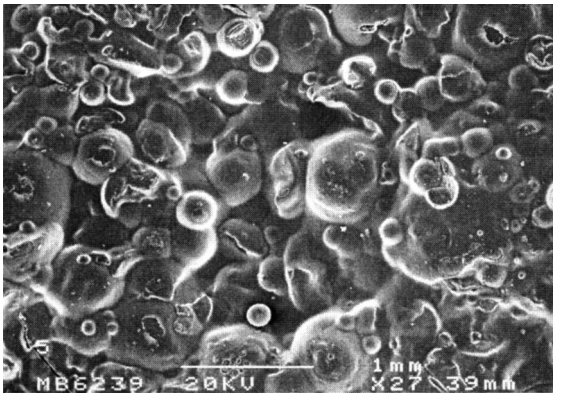

Fig. 3. Scanning electron micrograph of cytarabine-loaded chitosan microspheres included in poly(lactide-co-glycolide).

The SEM microphotography of the comatrix (Fig. 3) shows chitosan ara-C-loaded microspheres included on the poly(lactide-co-glycolide) film and the external polymeric surface is smooth. The in vitro release of cytarabine from the co-matrix showed a slower rate compared with that from microspheres. A 22.4% ara-C release was observed after the first hour. Maximum release of ara-C took place after 4 days (94.5 h) (Fig. 2), so drug delivery was slower than from microspheres. Only 80% of the ara-C included in the microspheres is released from the comatrix, thus an amount of the drug is maintained in the comatrix, probably in the microspheres more coated for the PLG film. The release kinetic shows an initial burst followed by slow behaviour (Fig. 2). This study was carried out by swelling because no degradation of microspheres by lysozyme was shown.

The cytarabine-loaded chitosan microspheres of the comatrix included 43.7 μg of drug per milligram of microspheres, which means 8.7 mg of cytarabine in the implanted comatrix. This amount of drug administered to rats with 252 ± 3

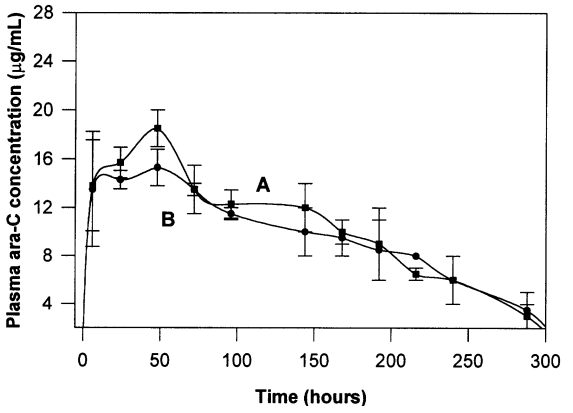

Fig. 4. Plot of cytarabine plasma concentration as a function of time for drugs released from the cytarabine-loaded comatrices type A (■) and B (●).

g of body weight means a dose of 34.5 mg/kg (135.9 μmol/kg). Doses of ara-C in rats have not been described. In this way, Allen et al. (1992) have assayed total doses between 10 and 100 mg/kg weight in mice of 18–30 g by intravenous (i.v.) administration. Riva et al. (1985) have used doses between 10 and 100 mg/kg weight in mice of 20 g by i.v. or i.p. injections. Also, Onishi et al. (1991) have studied, by i.p. injection, total doses of ara-C between 37 and 150 mg/kg weight in mice of 20 g. Higher doses, between 156 and 225 mg/kg weight and by i.p. injection have been used by Agur et al. (1992) in 6–10 week-old mice. Our group, in previous studies of ara-C release has employed doses of 201–230 mg/kg by subcutaneously implanted hydrogels (Blanco et al., 1998; Gómez et al., 1998).

Plasma concentration of ara-C from both comatrices A and B, is shown in Fig. 4. The drug is slowly released from the comatrix and its plasma concentration increases as a function of implantation time, up to 48 h. From this time, plasma drug concentration gradually decreases and the drug was not detected after 13 days.

The intracellular concentration of ara-C depends on the administered dose (Capizzi et al., 1991). From ara-C plasmatic concentrations ≈ 10–15 μmol/l (2.4–3.7 μg/ml) transport of ara-C inside the cell is independent of its concentration (White and Capizzi, 1991). The plasma concentrations of ara-C from comatrices type A were between 74.60 μmol/l (18.5 μg/ml) at 48 h and 12.10 μmol/ml (3 μg/ml) at 12 days of implantation. From comatrices type B, the plasma concentrations of ara-C were between 61.69 μmol/l (15.3 μg/ml) at 48 h and 14.11 μmol/ml (3.5 μg/ml) at 12 days of implantation. These concentrations are higher than 10 μmol/l, thus the amount of intracellular ara-C (60% of ara-C extracellular) is larger than K_m of the deoxycitidine kinase for ara-C ($K_m = 1.5$ μmol/l) and ara-CTP formation can be considered.

The total amount of drug administered by the comatrices was given by i.p. injection, distributed in ten doses of 0.9 mg/day, dissolved in 1 ml of saline solution, in order to establish an approximation with the plasma concentration of ara-C released from the comatrices. Thus, by i.p. administration, the ara-C plasmatic concentrations were 13.5 μmol/l (3.35 μg/ml) and 6.6 μmol/l (1.63 μg/ml) 30 min and 1 h after the injection, respectively. In this way, by i.p. injection, plasma ara-C concentration is smaller than 10 μmol/l, 1 h after its administration and therefore, ara-CTP formation could not be maximum, the effectiveness of ara-C decreasing.

Table 1
Pharmacokinetic parameters of cytarabine after intraperitoneal injection (0.9 mg ara-C per injection) and subcutaneous implantation of ara-C-loaded comatrix (8.7 mg ara-C per comatrix)[a]

	Comatrix A	Comatrix B	Injection
K_e (h^{-1})	$5.4\ 10^{-3} \pm 0.6\ 10^{-4}$*	$4.5\ 10^{-3} \pm 0.3\ 10^{-4}$*	0.29 ± 0.06
$t_{1/2}$ (h)	130.1 ± 15.4*	154.3 ± 11.7*	2.4 ± 0.5
AUC (mg/h per mililitre)	244.3 ± 51.8*	225.3 ± 36.2*	3.2 ± 1.0
V_d (L)	6.9 ± 1.5*	8.8 ± 1.5*	1.2 ± 0.4

[a] K_e, elimination constant; $t_{1/2}$, elimination half-life; AUC, total area under the blood level-time curve; V_d, volume of distribution.
* Significant difference with regard to the intraperitoneal injection group, $P < 0.005$.

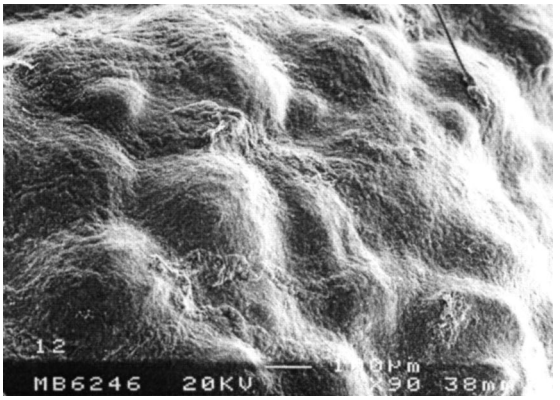

Fig. 5. Scanning electron micrograph of cytarabine-loaded comatrix after 6 months of implantation in the back of rats.

Pharmacokinetic data are shown in Table 1. The time at which the maximum cytarabine concentration occurs is longer when comatrix is used opposite to the i.p. administration, and the half-life the drug and the total area under the blood level-time curve (AUC) improve 62 and 72 times, respectively, for comatrices with 75 and 175 mg of poly(lactide-co-glycolide). No significant differences between both types of comatrices were determined.

Poly(DL-lactic acid) was implanted subcutaneously under controlled in vivo conditions by Ali et al. (1994), the external surface of the virgin poly(DL-lactic acid) is relatively smooth. An increased porosity as well as exposure of internal structure due to severe cracks appeared on the external surface of the poly(DL-lactic acid).

In order to determine the biological degradation and the biocompatibility of these comatrix, some of the animals implanted with the cytarabine-loaded comatrix were sacrificed at 4 and 6 months. The comatrix, that was included in a thin fibrous capsule, was removed. The SEM of the comatrices, 4 months after implantation, indicates that the microspheres included in the poly(lactide-co-glycolide) are not degraded and the film of polymer appears quite different to that observed in the initial comatrix (Fig. 3). Thus, hydrolytic degradation of the polymer in vivo contribute to ara-C delivery. Six months after implantation, the appearance of the polymer of the comatrix surfaces is more wrinkled and fibrous (Fig. 5), which indicates a degradative process.

Histological studies, using optical microscopy, show that no significant differences between A and B comatrices exist. Besides, implants removed after 4 and 6 months of implantation were quite similar. In general, implants are constituted by a dense mass of conjunctive tissue, in which microspheres are included (Fig. 6a). Most of microspheres are not dissolved, even at 6 months, although those more degraded contain multinucleate foreign body giant cells (Fig. 6b). In regions situated between microspheres, collagen fibres, macrophages and small blood vessels are observed (Fig. 6c).

The presence of macrophages and giant cells is related to the tissular biodegradation of polymers, originating changes of the physical properties of the material: loss of mechanical resistance, plasticity of the polymer or fragmentation. Degradation of implants by cells is essentially caused and regulated by macrophage action. Macrophages secrete enzymes that digest the material and produce fragmentation. At the same time, these enzymes induce fibroblasts to synthesize collagen to form a fibrous capsule around the implant. When a particle is so large that it cannot be encompassed by a macrophage acting alone, giant cells appears. These cells form when macrophages coalesce to produce a phagocyte large enough to deal with large particles or to attempt to deal with rough surfaces. The presence of giant cells at an interface sometime after implantation can indicate a persistent stimulus (Wilson, 1997).

Thus, histological studies indicate the implant has undergone a degradative process that can be considered a normal response of the organism. The implant is being biodegraded, although this process is very slow in this case.

This comatrix of ara-C loaded-chitosan microspheres included in a poly(lactide-co-glycolide) film is biodegradable and biocompatible, and injurious effects are not detected in the animals after 6 months of implantation. The administration of ara-C by implants of comatrix, from which the drug is released, results in, from a therapeutic viewpoint, suitable levels of ara-C in plasma for a definite period of time, despite the short average half-life on this drug. The implanta-

tion of these slow-release devices containing ara-C would decrease, at least in part, the secondary effects produced by the high doses of this drug used usually. The plasmatic ara-C concentrations obtained when it is released from the comatrix are in the range 1–50 μmol/l, plasma concentrations obtained when therapy with intermediate and high doses of ara-C are used. Probably a quicker degradation of the implant would be desirable, so experiments are on course to improve this aspect

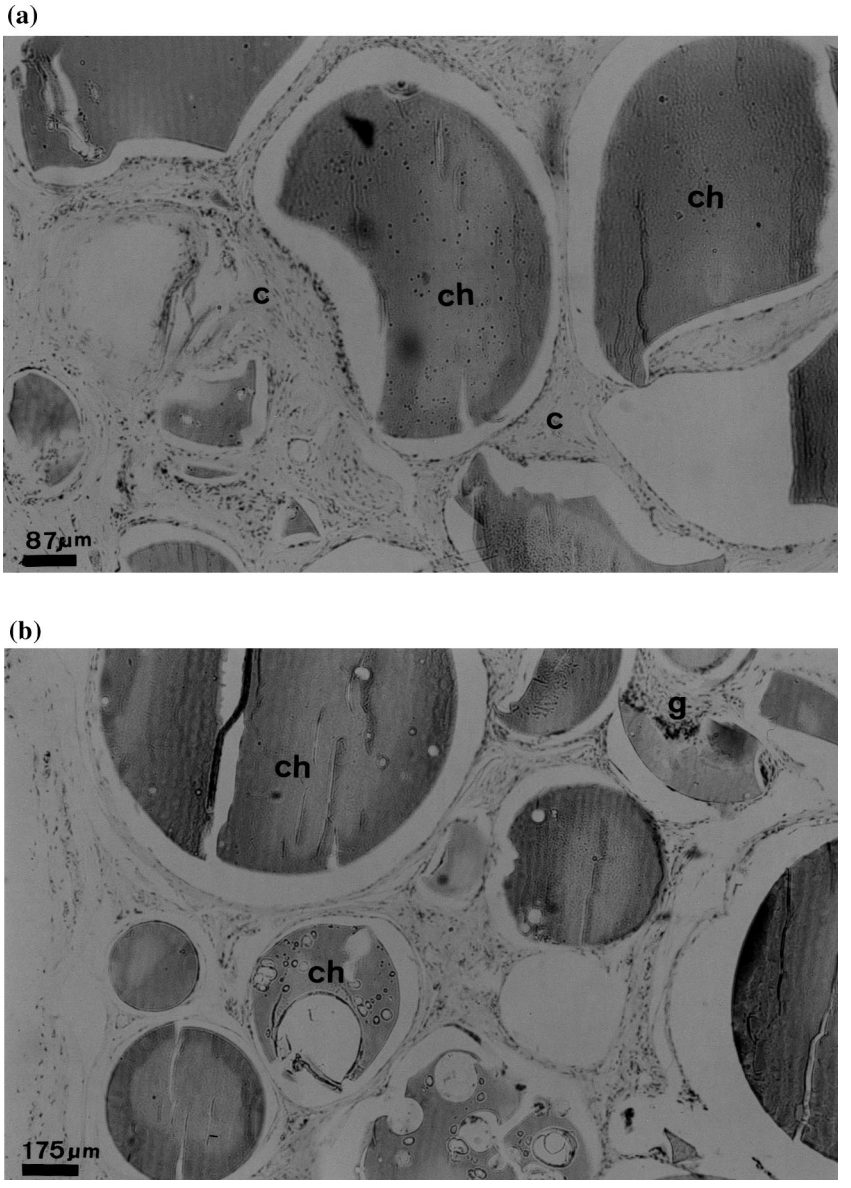

Fig. 6. Photomicrographs of the comatrix type B and the surrounding tissue: (a) after 4 months of implantation in the backs of rats (original magnification × 115; 1 cm = 87 μm), (b) after 6 months of implantation (original magnification × 57; 1 cm = 175 μm), (c) Detail of a 6 months implanted comatrix (original magnification × 141; 1 cm = 71 μm). Chitosan microspheres (ch), conjunctive tissue-connective fibres (c), multinucleate foreign body giant cell (g), macrophages (m) and small blood vessels (v) are observed.

(c)

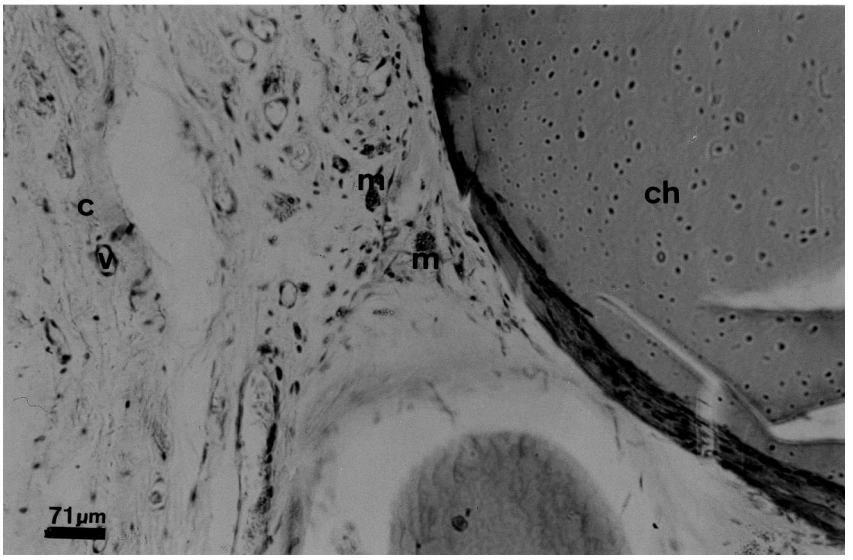

Fig. 6. (*Continued*)

without decreasing the ara-C amount included in microspheres or changing the period of time of the drug release.

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