Ketotifen-Loaded Microspheres Prepared by Spray-Drying Poly(D,L-Lactide) and Poly(D,L-Lactide-*co*-Glycolide) Polymers: Characterization and *In Vivo* Evaluation

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ABSTRACT: Ketotifen (KT) was encapsulated into poly(D,L-lactide) (PLA) and poly(D,L-lactide-*co*-glycolide) (PLGA 50/50) by spray-drying to investigate the use of biodegradable drug-loaded microspheres as delivery systems in the intraperitoneal cavity. Ketotifen stability was evaluated by HPLC, and degradation was not observed. Drug entrapment efficiency was $74 \pm 7\%$ (82 ± 8 μg KT/mg for PLA) and $81 \pm 6\%$ (90 ± 7 μg KT/mg for PLGA 50/50). PLA microspheres released ketotifen (57% of encapsulated KT) in 350 h at two release rates (221 μg/h, 15 min to 2 h; 1.13 μg/h, 5–350 h). A quicker release of ketotifen took place from PLGA 50/50 microspheres (67.4% of encapsulated KT) in 50 h (322 μg/h, 15 min to 2 h; 16.18 μg/h, 5–50 h). After intraperitoneal administration (10 mg KT/kg b.w.), microsphere aggregations were detected in adipose tissue. Ketotifen concentration was determined in plasma by HPLC. The drug released from PLA and PLGA 50/50 microspheres was detected at 384 and 336 h, respectively. Noncompartmental analysis was performed to determine pharmacokinetic parameters. The inclusion of ketotifen in PLGA and PLA microspheres resulted in significant changes in the plasma disposition of the drug. Overall, these ketotifen-loaded microspheres yielded an intraperitoneal drug release that may be suitable for use as delivery systems in the treatment of inflammatory response in portal hypertension. J Pharm Sci 97:3153–3169, 2008

Keywords: ketotifen; poly(D,L-lactide); poly(D,L-lactide-co-glycolide); microspheres; spray-drying; *in vitro–in vivo* correlation

INTRODUCTION

Allergic symptoms can be treated with first-generation antihistamines, lipid-soluble drugs that block peripheral H1 receptors. Among them, ketotifen fumarate (KT), 4-(methyl-4-piperidylidene)-4H-benzo[4,5]cyclophepta[1,2-b]thiophen-10(9H)-one hydrogen fumarate, has been widely used as an antiallergic and antianaphylactic agent in adults and children.[1] It is available orally and as eye drops for the treatment of allergic symptoms.[2] Since ketotifen is a mast cell stabilizer, it can prevent local tissue damage and multiorgan dysfunction due to vasoactive and pro-inflammatory mediators derived from these cells, particularly histamine, after intestinal ischemia/reperfusion.[3] At clinically relevant drug concentrations, ketotifen also induces primary necrosis

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in human eosinophils. Ketotifen and other histamine H1-receptor antagonists freely cross the blood–brain barrier (BBB) and can affect the central nervous system (CNS); therapeutic doses can lead to drowsiness and a worsening of epileptic symptoms, and overdoses may cause fatal seizures. Ketotifen has also been investigated in multidrug resistance in human breast cancer cells and doxorubicin toxicity in mice.[4] It has been demonstrated that this drug reverses multidrug resistance due to P-glycoprotein overexpression and provides cardioprotection against doxorubicin. There is not much published pharmacokinetic data on ketotifen,[1,5–7] most of which is in humans receiving an oral route of administration. The dosage of ketotifen administered to adult patients with bronchial asthma and allergic responses is 1 mg orally taken twice daily.[1] Doses of 2, 10 and 50 mg/kg body weight have been orally administered to rats for 2 weeks to study their effect on fertility and pregnancy.[8] To study the epileptogenic activity of ketotifen, doses of 20, 30 and 40 mg/kg body weight were intraperitoneally administered to amygdala-kindled and sham rats.[9] Pharmacokinetic studies of ketotifen after intravenous, intranasal and rectal administration in rabbits have been carried out with doses of 1 mg/kg body weight, and 5 mg/kg body weight for oral administration.[7]

The diverse administration routes of ketotifen and the possible advantages of loading the drug in drug delivery systems have led to designing different approaches in modulating ketotifen delivery. Thus, for topical and transdermal administration ketotifen has been included in deformable liposomes and ethosomes,[10] in pressure sensitive adhesives (PSA) matrices[11] and in polyisobutylene patches.[12] Due to its use in the treatment of bronchial asthma, particularly of an allergic origin, dry powder inhalation formulations of liposomally entrapped drug have been prepared[10] for direct ketotifen delivery in the respiratory tract. Since ketotifen is used in the treatment of allergic eye disease, the drug has been loaded in contact lenses.[13]

Microparticles represent drug delivery systems suitable for diverse administration routes. Over the years, a variety of natural and synthetic polymers have been explored for the preparation of microparticles, of which poly(lactic acid) and its copolymers with poly(glycolic acid) have been extensively investigated because of their biocompatibility and biodegradability.[14,15] We have carried out degradation studies of microspheres prepared by spray-drying poly(D,L-lactic acid) (PLA) and poly(D,L-lactide-*co*-glycolide) (PLGA) polymers.[16] Significant differences in decreasing average molecular weight were observed between PLA and PLGA 50/50 microspheres from the beginning of the degradation process; the rate constant of the first stage of degradation was larger for PLGA 50/50 ($k = 14.3 \times 10^{-3}$ day^{-1}) than for PLA microspheres ($k = 2.1 \times 10^{-3}$ day^{-1}).[16] Thus, this study focuses on the preparation of ketotifen-loaded biodegradable microspheres based on PLA and PLGA 50/50 polymers, their morphology and size characterization as well as drug release experiments. In this study *in vivo* evaluation of the systems has been carried out by intraperitoneal administration in rats. This administration route has been chosen due to the possible use of these polymeric systems in the treatment of the inflammation observed in portal hypertension, a clinical syndrome that is frequently studied using partial portal vein-ligated (PVL) rats.[17] Thus, as a prior and necessary step, ketotifen-loaded PLA and PLGA microspheres were administered to non-operated rats to evaluate plasma levels of the drug.

EXPERIMENTAL

Materials

PLA (Sigma–Aldrich, Barcelona, Spain), PLGA [lactide:glycolide 50:50] (Sigma–Aldrich), dichloromethane (Panreac, Barcelona, Spain), acetonitrile (Panreac), chloroform (Panreac), potassium monohydrogen phosphate (K_2HPO_4) (Panreac), potassium dihydrogen phosphate (KH_2PO_4) (Panreac), heparin (Analema, Vigo, Spain), Tween 80 (Panreac) were used as received. Milli-Q® water (Millipore, Madrid, Spain) was used. Ketotifen hydrogen fumarate was kindly supplied by Novartis (Madrid, Spain).

Methods

Preparation of Microspheres

Preparation of microspheres was carried out by a spray-drying process (Mini Spray-dryer B-191, Büchi, Flawil, Switzerland). To obtain microspheres without drug, PLA or PLGA 50/50 was dissolved in dichloromethane (2 wt%).[18–21] Microspheres with KT were prepared from 1.8 wt%

of polymer and 0.2 wt% of ketotifen dissolved in dichloromethane. The polymeric solutions (100 mL) were maintained under constant stirring (900 rpm) and sprayed through the nozzle of the spray-dryer. Assay conditions were: inlet air temperature 63–66°C, outlet air temperature 51–53°C, spray flow 5 mL/min, and compressed spray air flow (represented as the volume of the air input) 700 L/h. Microspheres were collected from the spray-dryer cyclone separator, and were then placed in a vacuum oven (Bioblock Scientifics, Illkirch, Strasbourg) for 24 h at 100 mBar of pressure and at 37°C. Microspheres were stored in a dessicator under vacuum conditions.

Particle Size and Appearance

The size and appearance as well as the size distribution of PLA- and PLGA50/50-based microspheres and KT-loaded microspheres were characterized by scanning electron microscopy (Jeol JSM-6400 Electron Microscope, resolution 36 mm from Centro de Microscopía Electrónica Luis Brü, UCM). The samples were fixed to an adhesive sheet on a rigid support and coated with gold for their later visualization. Micrographs (Fig. 1) were recorded in randomly selected particle populations. For each of the SEM micrographs 250 particles were measured using micrograph enlargements. The counted particles were used to check the convergence of the polydispersity index. The combined diameters were used to calculate the number-average diameter D_n and the weight-average diameter D_w using the following equations:[22]

$$D_n = \frac{\sum N_i D_i}{\sum N_i}$$

$$D_w = \frac{\sum N_i D_i^4}{\sum N_i D_i^3}$$

$$U = \frac{D_w}{D_n}$$

N_i is the number of particles measured, and D_i the diameter of the measured particle. By using D_n and D_w the polydispersity index U was calculated. The particle distribution is considered to be monodisperse when the polydispersity index is between 1.0 and 1.1.[23]

Ketotifen Stability in Different Drug Release Media

Stability of ketotifen in different drug release media, phosphate buffer 1 mM pH 7.4 and pH 2 as well as chloroform was studied by HPLC. A ketotifen solution was prepared (60 μg/mL) in either phosphate buffer 1 mM pH 7.4 or pH 2 and was maintained at 37°C and at a constant stirring rate (200 rpm) for 20 days. At intervals, 100 μL samples were withdrawn from the solution in order to determine ketotifen concentration by HPLC (Spectra-Physics SP8800 HPLC pump, SP 100 UV absorbance detector and SP 4400 computing integrator). Furthermore, ketotifen stability in chloroform was determined; a ketotifen solution (60 μg/mL) in chloroform was maintained for 2 h at room temperature and then aliquots of 100 μL were evaporated under N_2 stream to obtain a residue. The residue was reconstituted with mobile phase and ketotifen concentration was determined by HPLC. The HPLC method is based on that developed by Yagi et al.[7] The stationary phase was Spherisorb ODS2, C_{18}, 5 μm (25×0.46 cm; Waters). The mobile phase was KH_2PO_4 0.1 M, pH 4 with 40% (v/v) of acetonitrile. The flow rate was set at 1 mL/min and the detector wavelength was 297 nm. The calibration curve was obtained from KT solutions between 0.1 and 100 μg/mL and a good linear correlation ($r^2 = 0.99$) was obtained. The validation of the HPLC method was demonstrated by the precision, calculating the coefficient of variation (CV).[24] The CV for intra-run and inter-run of KT was calculated from concentrations of 100, 1 and 0.1 μg/mL. The ketotifen retention time was 6.0 ± 0.2 min. Stability of ketotifen in the aforementioned solvent media was also studied by UV/V spectra (188–600 nm) (UNICAM 8700 Spectrophotometer).

Estimation of Drug Content

PLA and PLGA 50/50 as well as ketotifen are soluble in chloroform. UV/V spectra (188–600 nm) of the polymer solutions and the drug in chloroform were carried out. UV/V spectrum of ketotifen shows a maximum absorbance at 297 nm; however, the polymer solutions did not absorb at this wavelength. Thus, to determine the amount of ketotifen (KT) included in the polymeric microspheres, 10 mg of the drug-loaded microspheres was dissolved in 1 mL of chloroform. The amount of ketotifen was determined by UV/V spectroscopy at 297 nm using a microcell (50 μL). The absorbance of the samples was interpolated in a calibration curve, which was obtained from ketotifen solutions in the range of 0.1–100 μg/mL KT. It was

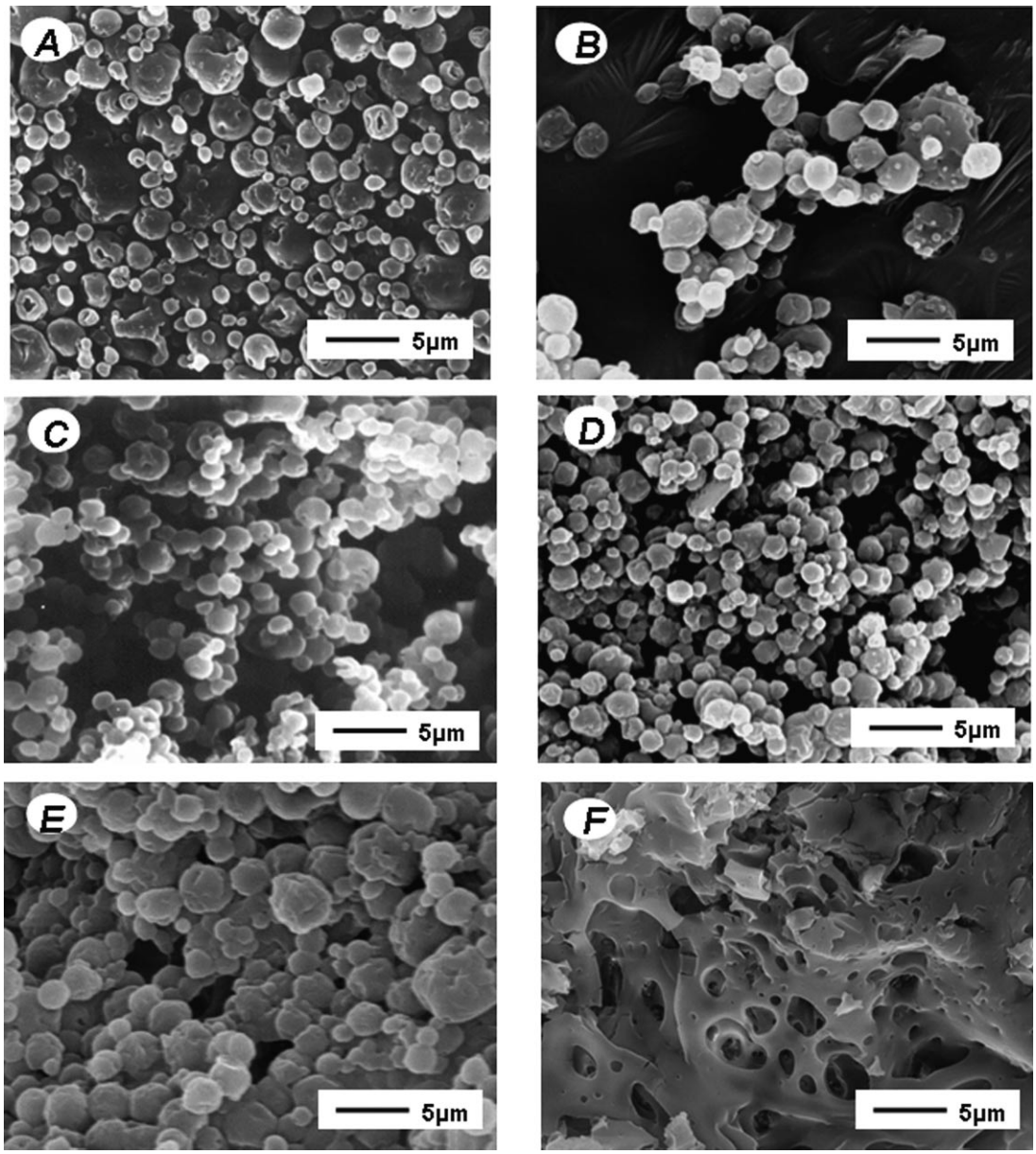

Figure 1. Scanning electron micrographs (SEM) of PLA microspheres (A), ketotifen-loaded PLA microspheres (B), PLGA 50/50 microspheres (C) and ketotifen-loaded PLGA 50/50 microspheres (D). SEM of ketotifen-loaded PLA microspheres (E) and ketotifen-loaded PLGA 50/50 (F) microspheres after 350 h in the release medium (phosphate buffer 1 mM, pH 7.4 at 37°C).

previously verified that the absorbance of KT was not modified when the polymers and the drug were dissolved in freshly prepared solutions. The experiment was carried out in triplicate. The amount of drug entrapped per weight of microspheres was calculated [Drug loading = (weight of ketotifen in microspheres/microsphere sample weight)]. The percentage of entrapment efficiency was expressed by relating the actual drug entrapment to the theoretical drug entrapment.[25]

In Vitro Drug Release Studies

For drug release studies, 30 mg of KT-loaded microspheres was added to 50 mL of phosphate buffer (1 mM; pH 7.4), which was placed in a vessel covered with Parafilm® at 37°C and at a

constant shake (200 rpm) in an orbital incubator (Ecotron® Inforts AG CH-4103). At intervals, 100 μL samples were withdrawn from the solution in order to follow the change in ketotifen concentration by UV/V spectroscopy at 297 nm. In order to confirm the stability of ketotifen released from PLA and PLGA microspheres, the concentration of the drug in the release medium was also evaluated by HPLC. The sampling time points were: a sample every 15 min during the first hour; a sample every 20 min during the second hour; a sample every 30 min during the following 2 h; a sample every hour during the following 3–4 h and then one or two samples a day for 13 days. The volume removed from the vessel was replaced with phosphate buffer. The concentration of the pharmaceutical formulation of ophthalmic solution of ketotifen (ZADITOR™; Novartis Ophthalmics) is 0.25 mg/mL. Solubility of ketotifen in phosphate buffer pH 7 at 26°C is 10.75 mg/mL.[26] In drug release experiments ketotifen concentration was always very much lower than its solubility in phosphate buffer; thus, sink conditions were maintained. The experiments were carried out in triplicate.

From plots of cumulative ketotifen released versus time, two release rates were calculated. The first release rate was determined between 15 min and 2 h; a straight line was obtained by using a least square fit and the release rate was determined from the slope of this line. The second release rate was determined between 5 and 350 h for KT-loaded PLA microspheres, and from 5 to 50 h for KT-loaded PLGA microspheres by using the same procedure.

In Vivo Ketotifen Administration

Male Wistar rats, weighing 227 ± 8 g, were obtained from the Animalario of the Universidad Complutense de Madrid (Spain) (DC 86/609/CEE; OM 13/X/1989, RD 1201/2005). Guidelines contained in the NIH publication on the principles of laboratory animal care, 85–23 revised in 1985, were followed throughout. Experiments were approved by the Animal Care Committee of Universidad Complutense de Madrid. The animals were housed in cages under environmentally controlled conditions of light (12:12 h light:dark cycle), temperature (22 ± 2°C) and were fed standard rat food and water *ad libitum*. Just before injection, the dissolution solvent was put under ultraviolet light (Ecogen Lamp, Viber Lourmat, Intensity 7 mW/cm^2) at 254 nm for 4 s because of the germicidal action of this wavelength.

Different groups of animals were established. Before the drug release experiments were carried out, a group of animals (three rats) was administered the vehicle used for microsphere administration; thus, 1 mL of saline solution (0.9% NaCl) containing 0.06 wt% of Tween-80 was intraperitonealy injected for 1 min. This solution was biocompatible. The animals that were administered the drug were divided into three groups. GROUP 1: Animals injected with KT-loaded microspheres. The microspheres were dispersed in 1 mL of saline solution (0.9% NaCl) containing 0.06 wt% of Tween-80. The animals were anaesthetized with halothane (Burtons Series 5 T.C.V., Kent, United Kingdom) and the microsphere dispersion was then intraperitonealy injected in the rat for 1 min using a sterile syringe with a 1.2×40 mm nozzle (Microlance 3). Group 1A (six rats): 27 mg of PLA microspheres, whose KT content was 2.21 mg. Group 1B (six rats): 26 mg of PLGA 50/50 microspheres, whose KT content was 2.34 mg. GROUP 2 (six rats): Animals intraperitonealy injected with 1 mL of an aqueous solution of KT of 2 mg/mL for 1 min. Furthermore, a control group of animals was intraperitonealy injected with PLA (three rats) or PLGA 50/50 (three rats) microspheres without drug. The microspheres were dispersed in 1 mL of saline solution (0.9% NaCl) containing 0.06 wt% of Tween-80.

Determination of Ketotifen in Plasma

At predetermined times after the injection of KT-loaded microspheres and KT solution, animals were anaesthetized with halothane. Blood (0.2 mL) was collected by puncturing the jugular vein in heparinized (15 units = 3 μL) polypropylene tubes. In animals administered KT-loaded microspheres, blood samples were taken 6, 24 and 30 h after the injection and at 24-h intervals thereafter. In animals administered a KT solution, blood samples were taken 20, 40 and 84 min and then every hour up to 4.4 h and every 2 h up to 8.4 h after the injection. The heparinized blood was centrifuged at 11000*g* for 10 min in a Sigma 202 M centrifuge immediately after collection so as to obtain plasma. Plasma samples were then stored at −20°C.

Ketotifen was precipitated from plasma samples by acetonitrile according to a modification of the method proposed by Yagi et al.:[7] 250 μL

of acetonitrile was added to 100 μL of plasma. After vigorous shaking for 5 min and centrifugation (3500*g*, 10 min), the supernatant was evaporated with N_2 to remove the organic solvent, and the sample was then placed in a vacuum oven (Bioblock Scientifics) for 12 h at 300 mBar of pressure and at 30°C, to obtain a residue. The residue was reconstituted with 12.5 μL of an aqueous solution with 50 v% of acetonitrile, and ketotifen concentration in the sample was determined by the HPLC system described above. For calibration, drug-free plasma pooled with known amounts of KT, to obtain a KT concentration between 0.1 and 100 μg/mL, was used after undergoing the same extraction procedure. KT standards were run for external standardization and a linear curve with a correlation coefficient of 0.989 was generated from the area under the peak measurements. The validity of the method was investigated by the determination of precision of the assay based on the reported guidelines.[24] Five replicates of control samples at each concentration of 100, 5 and 0.1 μg/mL were used to determine the inter- and intra-run validity. The precision was demonstrated by the CV values. The ketotifen retention time was 6.3 ± 0.2 min.

Pharmacokinetic Parameters

Noncompartmental analysis was performed. The terminal elimination rate constant (K_e) was estimated from the log-linear portion of the plasma concentration-time courses. The absorption rate constant (K_a) was estimated from the log plasma concentration-time course; the elimination slope was back-extrapolated to the ordinate, and using the intercept, the differences between the actual blood level points during the absorptive phase and the concentration on the back-extrapolated line at the same time were plotted as a function of time. By combining these different points, a straight line was obtained by using a least square fit, and the absorption rate constant was determined from the slope of this line.[27] The area under the curve (AUC) of plasma concentration-time was estimated from the plasma concentrations at different time points using the linear trapezoidal rule with extrapolation to infinity. The area under the concentration times time versus time curve (AUMC) was also calculated by the trapezoidal rule. From the AUC and AUMC values, the mean residence time (MRT) was calculated (MRT = AUMC/AUC).[28] Apparent total body clearance (Cl) and volume of distribution at steady-state (V_{ss}) were also estimated. Data analysis of the pharmacokinetic parameters was performed by using unpaired Student's *t*-test. A value of $p < 0.05$ was considered significant.

In Vivo/In Vitro Correlation (IVIVC)

In order to establish level B IVIVC,[29] the mean *in vitro* dissolution time (MDT) was compared to the MRT *in vivo*.[30] The MDT was calculated using the following equation:

$$\mathrm{MDT} = \frac{\mathrm{ABC}_{\text{in vitro}}}{M_\infty}$$

where $\mathrm{ABC}_{\text{in vitro}}$ is the area between the release curve and its asymptote, calculated by the trapezoidal rule from time zero to the last measured time point, and M_∞ is the total amount of released drug at this time point.

Histological Studies

Animals were sacrificed in a CO_2 atmosphere 17 days after the intraperitoneal injection of the microspheres. An incision was made on the peritoneal region of the rat to examine tissues. Tissues where the presence of microspheres was detected were removed. A piece of the removed tissue, fixed with formol (10%, v/v), was immersed in paraffin. Cuts (10 μm) were carried out with a paraffin microtome (Minot type). Samples were dyed using the hematoxilin-eosin method.[31]

Statistical Analysis

Statistical comparisons were performed with unpaired Student's *t*-test. A value of $p < 0.05$ was considered significant.

RESULTS

Preparation of ketotifen-loaded microspheres was carried out using spray-drying technology.[21] Polymer concentration and solvent as well as assay conditions were chosen based on previously published data on preparation and optimization of PLA and PLGA microspheres by spray drying.[18–21] The polymers and the drug were dissolved in dichloromethane, which allowed obtaining outlet temperatures between 50 and 53°C. The temperature of microspheres obtained by the spray-drying technique used were about 15–20°C lower than the outlet temperature;[32] thus, it is very probable

that the temperature of ketotifen in the experiment was only around 30–35°C.

SEM studies indicated that PLA and PLGA 50/50 microspheres were small in size and their surface was smooth and slightly porous. Some hollows or deformations, which are common characteristics of microspheres obtained by this spray-drying process,[33] were observed in PLA microspheres (Fig. 1A and B). This appearance is mainly determined by the solvent evaporation process.[20] The solvent evaporation rate of dichloromethane is quick, and this can allow achieving more spherical microspheres than those obtained using solvents with a higher boiling point.[20] Significant differences in appearance between ketotifen-loaded microspheres and unloaded-microspheres were not observed (Fig. 1C and D). The size distribution of microspheres without drug was similar to that of ketotifen-loaded microspheres, and most of the particles were the same size in both groups (Fig. 2). Microspheres without drug reach average diameters of 1.2 μm (PLA) and 0.9 μm (PLGA 50/50), whereas the sizes were 1.2 μm (PLA) and 0.8 μm (PLGA50/50) for ketotifen-loaded microspheres. All the types of particles are considered polydisperse, with a polydispersity index over 1.1[23] (Tab. 1). The presence of the drug in the feed mixture did not influence the size of the ketotifen-loaded microspheres.

Stability of ketotifen was determined by HPLC. The precision of the HPLC assay was demonstrated by the coefficients of variation. The coefficients of variation for intra-run of KT at 0.1, 1 and 100 μg/mL were 8.9%, 6.1% and 0.5%, respectively, and 7.0%, 7.3% and 1.0% for inter-run at the same concentrations. Figure 3 shows chromatograms of ketotifen in phosphate buffer

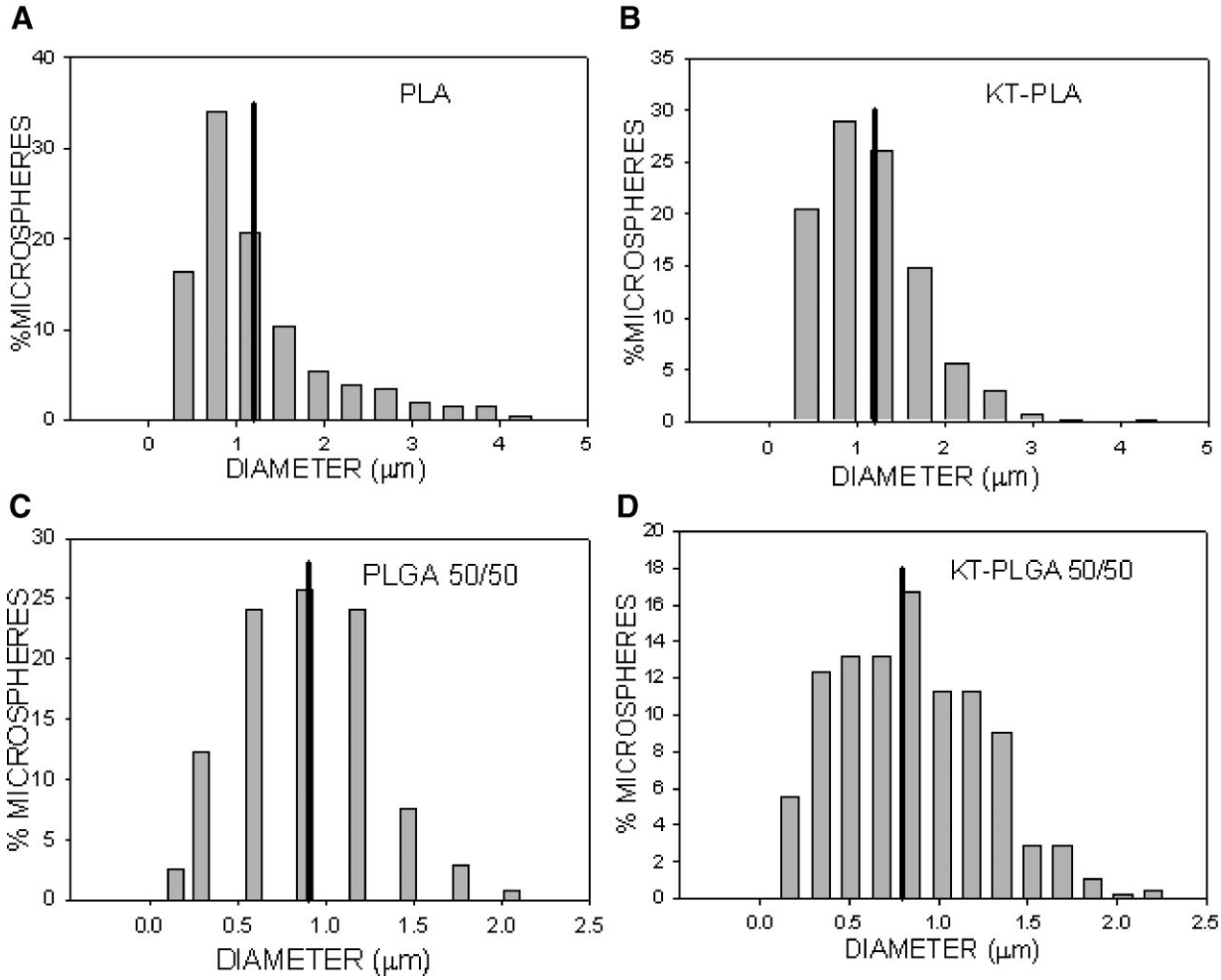

Figure 2. Size distribution of PLA (A), ketotifen-loaded PLA (B), PLGA 50/50 (C) and ketotifen-loaded PLGA 50/50 (D) microspheres. Average diameter: solid line.

Table 1. Particle Size and Polydispersity Index (U) of Microspheres

Sample	D_n (μm)	U
PLA microspheres	1.2	2.3
Ketotifen-loaded PLA microspheres	1.2	1.6
PLGA 50/50 microspheres	0.9	1.4
Ketotifen-loaded PLGA 50/50 microspheres	0.8	1.7

D_n, number-average diameter.

1 mM pH 7.4 (Fig. 3A) and pH 2 (Fig. 3B). The peak of KT was observed in the chromatograms at 6.0 ± 0.2 min, retention time of the drug standards. Differences as a function of the incubation time were not observed. Ketotifen was also stable after dissolution in chloroform, evaporation under N_2 and then reconstitution with mobile phase. Thus, the drug was stable in the assay conditions. The drug stability in the above-mentioned solvent media was also observed by UV/V spectra, which showed two characteristic peaks at 190 and 297 nm.

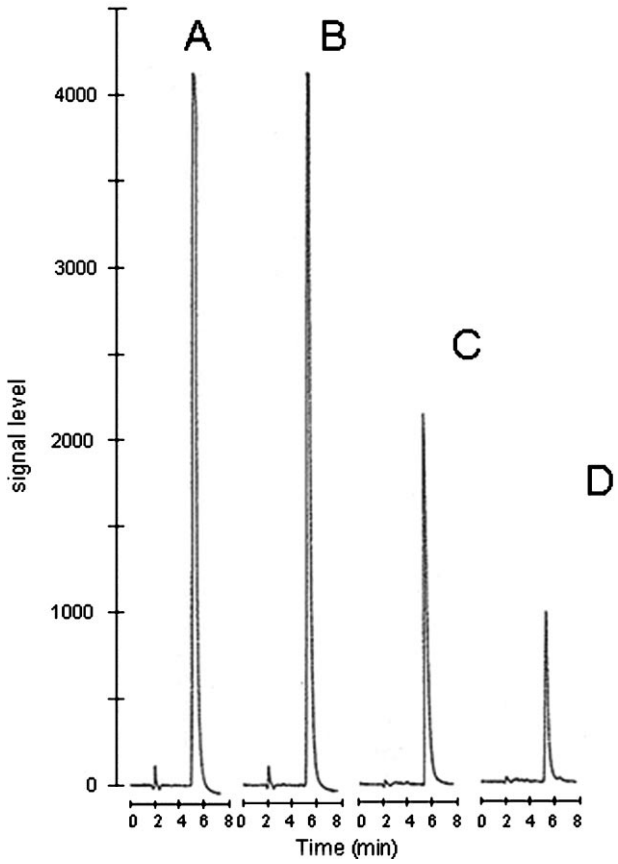

Figure 3. Chromatograms of ketotifen (KT) in phosphate buffer 1 mM, pH 7.4 (A) and pH 2 (B) at 37°C, and chromatograms of KT released from PLA (C) and PLGA 50/50 (D) microspheres after 250 h of drug release in phosphate buffer 1 mM, pH 7.4 at 37°C.

The percentage of entrapment efficiency of ketotifen was $74 \pm 7\%$ for PLA microspheres and $81 \pm 6\%$ for PLGA 50/50 microspheres. Drug-loaded microspheres included between 82 ± 8 and 90 ± 7 μg KT/mg microspheres for PLA and PLGA 50/50 microspheres, respectively.

Stability of ketotifen released from KT-loaded PLA- and PLGA- microspheres was confirmed by HPLC. Figure 3C and D shows chromatograms of ketotifen released from KT-loaded microspheres after 250 h. Degradation of the drug was not observed. The cumulative release of ketotifen did not reach 100% (Fig. 4). A burst effect was observed in both types of microspheres, 9% (7.4 ± 3.0 μg/mg microspheres) and 10% (8.6 ± 3.4 μg/mg microspheres) of the loaded KT was released from PLA and PLGA 50/50 microspheres, respectively, during the first 15 min. A quick release of the most external ketotifen from PLA microspheres was observed; it took place at a rate of 221 μg/h [KT (μg) = 322 + 221 time (h), $r^2 = 0.98$] from 15 min to 2 h, and then a slower rate of 1.13 μg/h [KT (μg) = 1048 + 1.13 time (h), $r^2 = 0.90$] from 5 to 350 h was determined. The release rate of ketotifen from PLGA 50/50 microspheres was 322 μg/h [KT (μg) = 114 + 322 time (h), $r^2 = 0.98$] from 15 min to 2 h, and 16.18 μg/h [KT (μg) = 1044 + 16.18 time (h), $r^2 = 0.96$] from 5 to 50 h. Thus, the release of ketotifen took place at two rates from both types of microspheres; however, the different composition of the polymer of the microspheres determined the value of those rates, which were higher in PLGA 50/50 microspheres. The maximum release of ketotifen from PLA microspheres was 57% of the loaded drug at 350 h (Fig. 4A). The release of the drug from PLGA 50/50 microspheres reached the maximum (67.4% of the loaded drug) at 50 h (Fig. 4B); from this point a decrease in drug concentration in the release medium was observed. In fact, ketotifen-loaded PLA microspheres maintained their morphology after 350 h of incubation in phosphate buffer (Fig. 1E). However, at that time the degradation of ketotifen-loaded PLGA 50/50 microspheres was evident (Fig. 1F). Thus, the release of ketotifen from these microspheres must be related to their degradation characteristics.

In this study 10 mg KT/kg body weight was the dose used to evaluate the intraperitoneal administration of ketotifen-loaded PLA and PLGA 50/50 microspheres as well as a solution of the drug. The

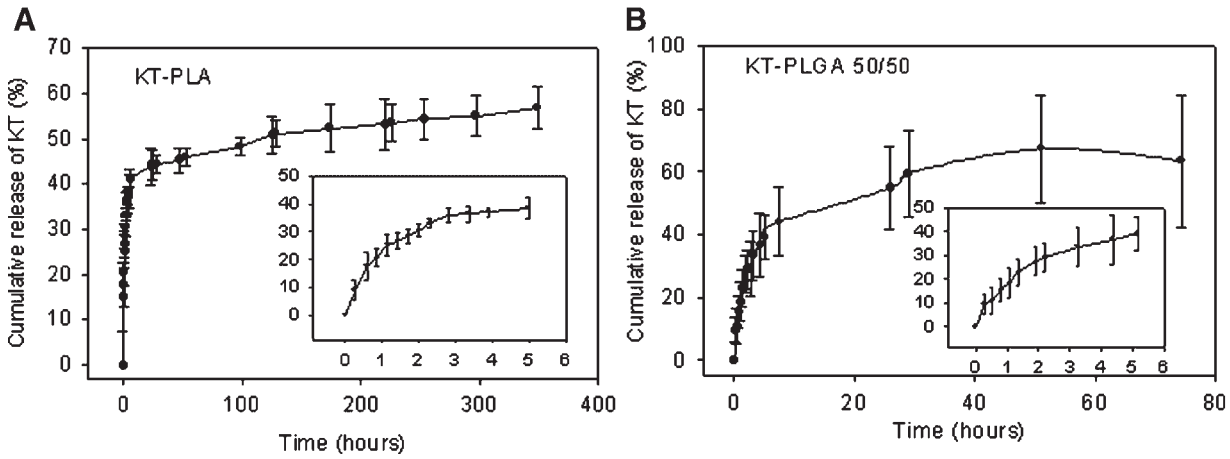

Figure 4. *In vitro* release kinetic of ketotifen (KT) from ketotifen-loaded PLA (A) and ketotifen-loaded PLGA 50/50 (B) microspheres. Inset: KT release during the first hours. Phosphate buffer (1 mM, pH 7.4) at 37°C. Each point shows average values ± standard deviation ($n = 3$).

coefficients of variation of HPLC assay for intra-run of ketotifen at 0.1, 5 and 100 μg/mL were 5.0%, 6.1% and 1.8%, respectively. The coefficients of variation for inter-run at the same concentrations were 8.0%, 5.2% and 12%. After administration of ketotifen-loaded PLA microspheres (Fig. 5A), the drug started to be detected in plasma at 30 h, and the maximum drug concentration (571 ng/mL) was reached at 169 h (Tab. 2); then, the drug concentration began to decrease up to hour 384. The ketotifen concentration in plasma decreased at 120 h and then increased again to reach the maximum; thus, the presence of the drug in plasma during the first 120 h can be due to the initial quick release of ketotifen observed *in vitro*. Plasma levels of ketotifen after drug-loaded PLGA 50/50 microspheres also showed two peaks (Fig. 5B); the maximum of the first peak (177 ng/mL) took place at 24.4 h and the maximum of the second peak (102 ng/mL) was detected at 169 h (Tab. 2), where there was a decrease in plasma concentration of the drug up until hour 336. The quicker release of ketotifen from PLGA 50/50 microspheres observed *in vitro* (Fig. 4B) caused a lower drug plasma concentration when microspheres were administered *in vivo*. The drug is probably protected in the microsphere, and ketotifen is metabolized when it

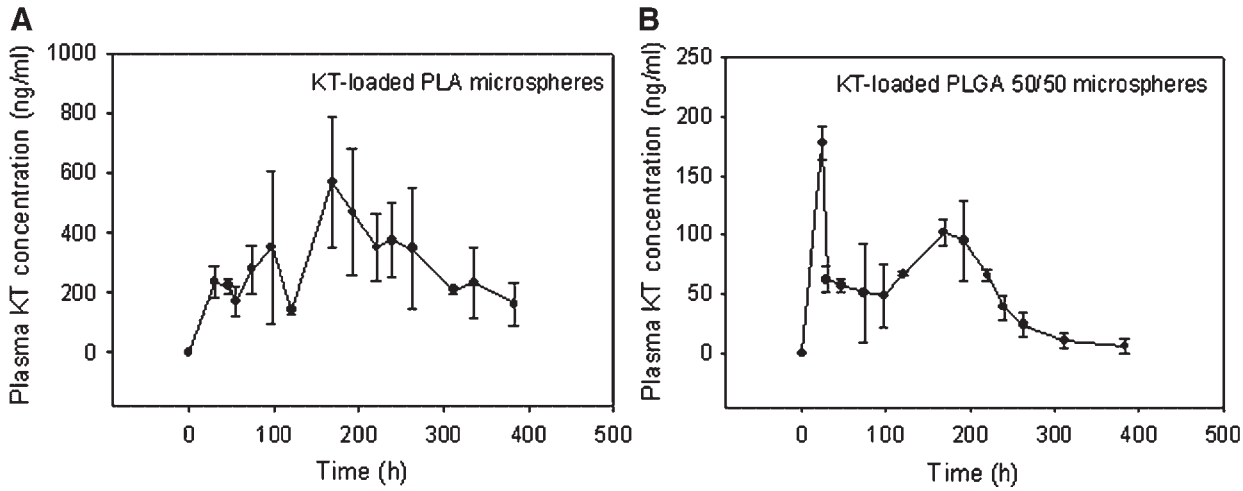

Figure 5. Plasma concentration of ketotifen after intraperitoneal injection of ketotifen-loaded PLA microspheres (A) and ketotifen-loaded PLGA 50/50 microspheres (B). Each point shows average values ± standard deviation ($n = 6$).

Table 2. Pharmacokinetic Parameters of Ketotifen (KT) after Intraperitoneal Injection of the Drug-Loaded Microspheres and a Solution of the Drug

	KT Formulation		
Parameter	KT-Loaded PLGA 50/50 Microspheres	KT-Loaded PLA Microspheres	KT Solution
K_a (h^{-1})	0.0199 ± 0.0070^a	0.0125 ± 0.0038^a	2.29 ± 0.34
K_e (h^{-1})	0.0194 ± 0.0085^{ab}	0.0058 ± 0.0003^a	1.23 ± 0.02
AUC (μg h/mL)	20 ± 6^{ab}	142 ± 61^a	51 ± 16
AUMC (μg h^2/mL)	641 ± 238^{ab}	10044 ± 4771^a	51 ± 17
C_{max} (ng/mL)			
1st peak	177 ± 14	102 ± 11	$39.4 \times 10^3 \pm 11.7 \times 10^3$
2nd peak	352 ± 258	571 ± 217	
T_{max} (h)			
1st peak	24.4	169	2.4
2nd peak	97.4	169	
MRT (h)	31 ± 2^{ab}	71 ± 4^a	1.00 ± 0.02
Cl (L/h kg)	0.53 ± 0.13^{ab}	0.081 ± 0.031^a	0.21 ± 0.06
V_{ss} (L/kg)	16.43 ± 3.3^{ab}	5.75 ± 1.79^a	0.21 ± 0.04

KT dose: 10 mg KT/Kg body weight.
[a]Significant difference with respect to KT solution group ($p < 0.05$).
[b]Significant difference with respect to KT-PLA microsphere group ($p < 0.05$).

is released; the slower release of ketotifen from PLA microspheres seems to cause longer and higher levels of the drug. When ketotifen was intraperitoneally administered at the same dose in solution (Fig. 6) the maximum drug concentration (39.4 μg/mL) was observed at 2.4 h, and the drug was not detected starting at 4.4 h.

The inclusion of ketotifen in PLGA and PLA microspheres resulted in significant changes in the plasma disposition of the drug. The pharmacokinetic parameters are summarized in Table 2. The absorption constant (K_a) was slower and C_{max} was lower when ketotifen was released from both types of microspheres. The steady-state volume of distribution (V_{ss}) of ketotifen was 78- and 27-fold larger when the drug was administered by KT-loaded PLGA and PLA microspheres, respectively, than the corresponding value of the drug administered in the form of a solution. The elimination rate constant (K_e) was 63- and 212-fold lower when the drug was administered by KT-loaded PLGA and PLA microspheres, respectively. Total body clearance (Cl) depended on AUC values, which were 2.5-fold lower for KT released from PLGA microspheres and 2.7-fold larger when the drug was released from PLA microspheres in comparison with the administration of ketotifen in the form of a solution. The value of the MRT of ketotifen in plasma was between 30 and 70 times larger when ketotifen was administered by drug-loaded PLA or PLGA 50/50 microspheres.

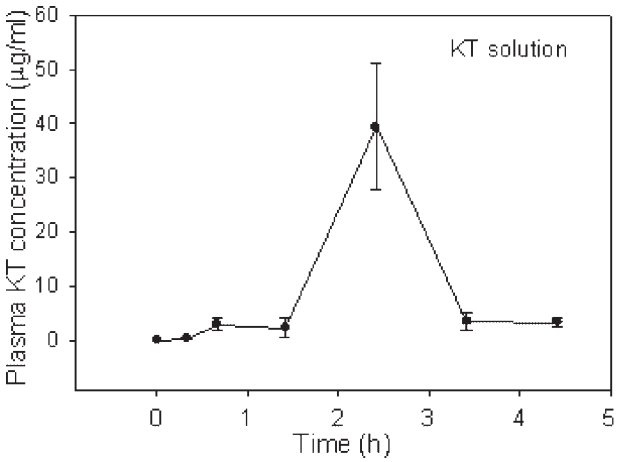

Figure 6. Plasma concentration of ketotifen after intraperitoneal injection of a KT solution (dose 10 mg KT/kg body weight). Each point shows average values ± standard deviation ($n = 6$).

Although at least three different formulations are necessary to establish an *in vitro*/*in vivo* level B correlation, MDT values were calculated from in vitro ketotifen release from microspheres: 307 ± 6 h for KT-loaded PLA microspheres; 63.0 ± 0.7 h for KT-loaded PLGA 50/50 microspheres. The linear relationship for *in vitro* MDT and *in vivo* MRT was: MRT = 20.93 + 0.16 MDT. Furthermore, a linear relationship was established between the absorption constant (K_a) and the *in vitro* release rates of the drug (Fig. 7); thus, *the in vitro* release rates of KT from KT-loaded PLGA

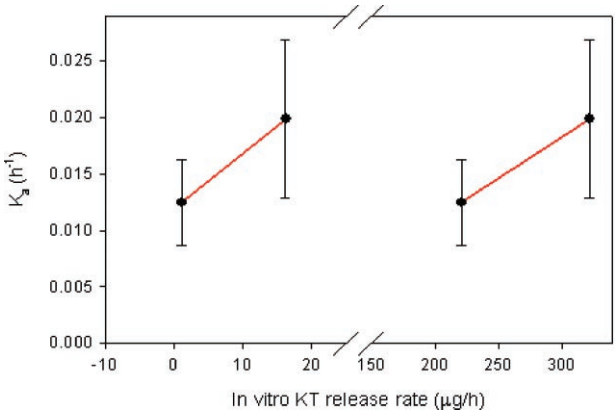

Figure 7. Relationship between the absorption constant (K_a) of ketotifen in plasma and *in vitro* release rates of ketotifen from ketotifen-loaded PLA and PLGA 50/50 microspheres. Each point shows average values ± standard deviation ($n = 6$).

microspheres were larger, and the absorption constant of the drug was also larger than the corresponding values of the drug released from KT-loaded PLA microspheres.

To examine the peritoneal region, animals were sacrificed 17 days after the injection, when ketotifen was not detected in plasma. From a macroscopic point of view, microspheres were not observed in the liver, kidney, pancreas, intestines or tegument. However, unloaded and ketotifen-loaded microspheres were detected in adipose tissue. In Figure 8 an example of ketotifen-loaded PLGA 50/50 microspheres included in adipose tissue is shown. Connective tissue surrounding

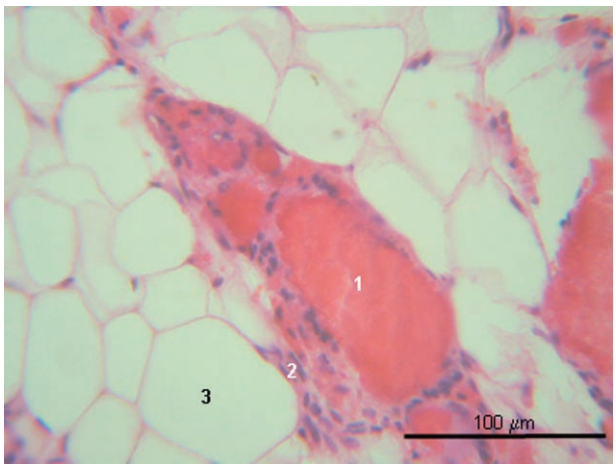

Figure 8. Photomicrograph of microspheres and the surrounding tissue after 17 days of intraperitoneal injection of ketotifen-loaded PLGA 50/50 microspheres. Microspheres (1), connective tissue (2), adipose tissue (3).

groups of microspheres is observed. This fact can be considered in accordance with the normal body reaction to a biocompatible material, which consists of walling it off in an avascular, collagenous bag.

DISCUSSION

Antihistaminic drugs used in the treatment of asthma have been included in drug delivery systems[34,35] to improve their pharmacological efficiency. Ketotifen[36] is one of these types of drug. Furthermore, this drug has been used in the treatment of some inflammatory intestinal diseases caused by parasite infections,[37] allergy to milk proteins[38] or eosinophil gastroenteritis.[39] From an experimental point of view, ketotifen can be an interesting drug in decreasing the inflammatory response caused in the endothelium of rats used in experimental models of prehepatic portal hypertension.[17] In this case, ketotifen administration in the intraperitoneal cavity using sustained drug delivery systems could be useful in improving the antiinflammatory effect of the drug.

Different studies have evaluated the effect of various processing parameters on the preparation of PLA and PLGA using spray-drying technology. Thus, the effect of the solvent on PLA microspheres was studied by Bain et al.,[18] and they concluded that microspheres prepared by dichloromethane were technologically superior. Furthermore, the lowest solvent residue was obtained when dichloromethane was used in microsphere preparation, and its amount significantly decreased as a function of *in vacuo* storage time.[18] Dichloromethane achieved more spherical PLGA and PLA microspheres due to its rapid solvent evaporation rate.[20] Although dichloromethane is a class 2 solvent according to the ICH classification, and its administration should be limited (PDE 6 mg/day; concentration limit 600 ppm), it is considered one of the halogen solvents that causes the least toxicity.[40] Studies on particle size and distribution, and morphology of PLA and PLGA microspheres prepared by spray drying based on polymer concentration and solvent used were carried out by Wang and Wang.[20] Their results showed that the viscosity of the liquid exerts a significant influence on the average size of atomized droplets. They demonstrated that the viscosities of the polymer solutions were several times the viscosity of dichloromethane solvent,

and increased as the percentage of lactic acid increased in the copolymer; furthermore, a higher polymer concentration produces more particles with a larger particle size.[20] Low PLA concentration (<1.5%, w/v) tended to form poor spherical particles; in addition, high PLA concentration (>3%, w/v) produced fibrin products.[19] Regarding the drug load of microspheres, studies carried out with chlorambucil (25–75%, w/w of polymer) indicated that higher drug loading decreased the encapsulation efficiency of the drug in PLA microspheres.[19] PLGA microspheres were loaded with paclitaxel and the drug load was kept at 1% (w/w) of polymer.[20] Thus, the parameters selected for the preparation of ketotifen-loaded PLA and PLGA microspheres were chosen considering the above mentioned data as well as our previous studies on 5-fluorouracil-loaded PLA and PLGA microspheres[21] and bupivacaine-loaded poly(ε-caprolactone) microspheres.[41] Unloaded and KT-loaded PLA and PLGA microspheres were small in size and polydisperse, and their average diameter and appearance were similar to those obtained in equivalent experimental designs.[18,20]

Microspheres prepared by spray-drying technology allow obtaining high percentages of drug entrapment efficiency when the drug and the polymer can be dissolved in a solvent or in miscible solvents.[41] Thus, the high entrapment efficiency obtained for ketotifen in PLA and PLGA 50/50 microspheres was similar to that reported for the encapsulation of drugs that were soluble in the same solvent as polymers using the spray-drying technique.[41] The entrapment of ketotifen in liposomes[42] used to obtain a dry powder inhalation formulation took place with a maximum efficiency of $97.92 \pm 0.54\%$ starting from 200 μg of ketotifen per 200 mg of formulation. Lower entrapment efficiency of ketotifen was obtained for deformable liposomes and ethosomes, $74.51 \pm 0.86\%$ and $43.98 \pm 0.96\%$, respectively, prepared for topical delivery.[10] In the preparation of liposomes and ethosomes with ketotifen, drug and lipids were dissolved in the same solvent. However, in order to remove the unentrapped ketotifen from liposomal dispersion dialysis[42] and ultracentrifugacion[10] were used. These procedures can contribute to decreasing the percentage of entrapped drug. This is a significant difference regarding the spray-drying procedure used in this case, since ketotifen and each one of the polymers were dissolved in dichloromethane, and this dissolution was used to obtain drug-loaded microspheres. Furthermore, ketotifen has been included in PSA matrices (maximum drug content 313.1 ± 19.7 μmol/cm^3) for skin permeation[11] as well as in silicone-containing and p-HEMA hydrogel contact lens materials (maximum drug uptake 227 ± 9 μg/lens for Vifilcon, a p-HEMA based contact lens with methacrylic acid).[13] The high ketotifen content of silicone-typed PSA was due to the casting method used in their preparation, in which the amount of ketotifen depended on its solubility in the organic solvent used.[42] On the other hand, the uptake of ketotifen in the contact lens depended on the concentration of the drug solution used (222 μg/mL). The uptake increased with high water content materials, p-HEMA containing materials and with ionic materials.[13] Thus, the amount of ketotifen loaded was small (approximately 8 μg of ketotifen per mg of lens).

Drug release experiments indicated that the most external ketotifen was released at the beginning of the process. This burst effect observed during the first 15 min of ketotifen release is usual in drug release from microparticulate systems due to the quick diffusion of the drug from the external part of the microspheres.[20] The burst effect is not an advantage or disadvantage of the formulation; it depends on the type of drug entrapped and also on the type of application of the microspheres.[43,44] Since ketotifen is an antihistaminic drug, a quick release of the drug could be an advantage in the control of histamine delivery from cells during the first stage of the treatment. Then, a slow release of the drug allows maintaining the antihistaminic effect, where the microspheres probably act as a reservoir to prevent enzymatic metabolism of the drug.

Furthermore, a significant interaction between ketotifen and the polymers seems to exist, since total release of the loaded drug from microspheres did not take place. In previous studies we have observed that degradation of PLA microspheres was slow;[16] mass loss was not observed during the 150 days of incubation in phosphate buffer and the average molecular weight of the microspheres decreased only 12% during the first 2 months. Thus, the slow hydrolysis of backbone ester groups due to the hydrophobic character of PLA microspheres makes the release of the most internal ketotifen difficult. KT-loaded PLA microspheres maintained their morphology after 350 h of drug release (Fig. 1E), which was not observed in KT-loaded PLGA 50/50 microspheres at the same time (Fig. 1F). Two considerations must be taken into account in the release kinetics

of the drug from PLGA 50/50 microspheres. On the one hand, the percentage of ketotifen released was larger than that from PLA microspheres in a shorter period of time. This fact is very likely related with its quicker degradation and with the larger hydrophilic nature of the polymer, which facilitates the uptake of aqueous solution. The hydrolytic effect of the aqueous medium on polymeric chains allows the formation of larger pores and channels inside the microspheres, which makes drug release more favourable.[45] A decrease of 94% in molecular weight during the first 2 months of incubation in phosphate buffer has been observed in the degradation process of PLGA 50/50 microspheres.[16] Decrease in molecular weight of PLGA 50/50 microspheres took place in two phases; the length of the first one was the first 22 days.[16] The second consideration in the *in vitro* kinetic release of ketotifen was the decrease in the drug concentration observed starting at 50 h of incubation (data not shown), which led to a KT concentration of 28% of the loaded drug at 350 h. The link breaks that took place in PLGA 50/50 microspheres very likely causes the release of small polymer chains as well as glycolic and lactic acid, which probably interact with the drug in the solvent medium. Ketotifen has a carbonyl group. The carbonyl group is polar because of the greater electronegativity of oxygen, and the presence of oxygen with its nonbonding electron pairs turns aldehydes and ketones into hydrogen-bond acceptors. Thus, hydrogen bonds may be established between the carbonyl group of ketotifen and the hydroxyl of the carboxylic group of the polymer chains. The degradation of the PLGA 50/50 microspheres increases the amount of small polymer chains and as a consequence the amount of carboxylic groups to form hydrogen bonds. Furthermore, ketotifen is a weak base ($pK_a = 8.5$),[11] which is ionized at acid pH ($pH < pK_a$). Thus, the protoned nitrogen of the drug may also establish interactions with the carboxylic group of the polymer chains. This way, ketotifen released from PLGA 50/50 microspheres may interact with the carboxylic groups generated by degradation of the polymer and be removed from the release medium, with the drug concentration decreasing. Another possibility that could explain the decrease in ketotifen concentration after 50 h of release from PLGA 50/50 microspheres is the degradation of the drug. However, chromatograms of ketotifen in solution at pH 2 and pH 7.4 and of the drug released from PLGA 50/50 microspheres (Fig. 3) showed a peak at the retention time of ketotifen standards. Thus, degradation does not seem to be the best option to explain the decrease in drug concentration.

The pathophysiology of prehepatic portal hypertension has been studied using the experimental model of partial PVL rat. One of the characteristics of PVL rats is an increased infiltration of the intestinal mucosa and submucosa by mast cells, whose inflammatory mediators could produce vasodilatation and angiogenesis.[46] Thus, the administration of ketotifen in the intraperitoneal cavity could exert its antihistaminic activity, decreasing the level of inflammatory mediators.

Considering previous studies,[1,7–9] the dose of KT intraperitonealy administered to rats in this study was 10 mg KT/Kg body weight. When ketotifen was administered by drug-loaded microspheres, they were in the adipose tissue. Thus, ketotifen was detected in plasma 336 and 384 h when it was administered by drug-loaded PLGA 50/50 and PLA microspheres, respectively (Fig. 5). The values of the pharmacokinetic parameters of ketotifen (Tab. 2) showed the effect of the different polymer formulations of the drug. Except when the drug is administered intravenously in the form of a solution, the drug has to be released from the dosage form and then be absorbed into systemic circulation by passing through various membranes.[27] A drug given in different dosage forms will yield varying amounts of drug absorbed and, hence, differences in onset, intensity and duration of the pharmacologic or clinical effect.[27] Thus, the administration of the KT-loaded PLA microspheres produced a maximum drug plasma concentration between 112 and 69 times lower than the maximum drug plasma concentration obtained when the KT solution was administered; regarding KT-loaded PLGA 50/50 microspheres, the maximum drug plasma concentration was between 386 and 222 times lower. The polymer composition of KT-loaded microspheres influenced their *in vitro* drug release characteristics (Fig. 4), and it determined not only the maximum plasma KT concentration but also the time the drug was present in plasma. Significant differences in MRT and AUC, pharmacokinetic parameters reflecting the residence time and amount of free KT, respectively, in systemic circulation, between the i.p. administration of the drug by KT-loaded microspheres and KT solution, as well as between different types of microspheres intraperitonealy administered were observed. Thus, the

relative bioavailability,[27] determined from AUC values, was very different for both types of microspheres (Tab. 2). Whereas it was 39% for KT-loaded PLGA 50/50 microspheres, the relative bioavailability of the drug was 278% when KT-loaded PLA microspheres were administered. On the other hand, the higher MRT values of KT suggest a more prolonged action when KT is formulated with PLA or PLGA 50/50. In general, polymer drug delivery systems exert protection on the loaded drug against its degradation or metabolism; thus, the drug absorption from this type of systems after i.p. administration depends on the release of the drug from the polymer system, its permeability through tissue barriers, and its dissolution under physiological conditions. Studies carried out with rabbits[7] have shown that the intravenous administration of KT in solution at a dose of 1 mg/kg body weight produced an AUC value of 0.514 ± 0.048 μg h/mL, and the AUC value was 0.338 ± 0.069 μg h/mL when the drug in solution was administered intranasally. Thus, these AUC values are about 10 times lower than those obtained in our experiments when KT solution was intraperitonealy administered to rats using a dose of 10 mg/kg body weight. Transdermal delivery studies with ketotifen patches of polyisobutylene and fatty acids[12] containing 10 mg of ketotifen were carried out in rabbits (2.5–3.5 kg); the maximum concentration of ketotifen (0.0552 μg/mL) was obtained at 6 h, the drug was detected in plasma for 72 h and the AUC was 2.56 μg h/mL. The bioavailability of the ketotifen patch, based on AUC data, indicated that about 0.6 mg of KT was absorbed into systemic circulation.

On the other hand, ketotifen was absorbed rapidly after the i.p. administration of the drug solution to rats ($K_a = 2.29 \pm 0.34$ h^{-1}) (Tab. 2). A similar value of absorption constant has been determined for ketotifen after rectal administration in rabbits ($K_a = 2.45 \pm 0.47$ h^{-1}).[7] The administration of KT-loaded microspheres significantly decreased the absorption constant of ketotifen and, as a consequence, the C_{max}. Thus, C_{max} was proportional to the rate and quantity of absorbed drug. Furthermore, since t_{max} is related to the absorption rate, it was longer when the drug was released from the microspheres. In a similar way, the value of the elimination constant of ketotifen administered in the form of a solution to rats (Tab. 1) was similar to the value determined for the intravenous and oral administration to rabbits (1.25 ± 0.16 h^{-1} and 1.27 ± 0.10 h^{-1}, respectively).[7] The administration of the drug by KT-loaded microspheres caused a significant decrease in the elimination constant; the lowest value was for the KT released from PLA microspheres, which was in accordance with the slower *in vitro* release of KT from PLA microspheres. The total body clearance of ketotifen administered in the form of a solution to rats was 10 times lower than the value determined in rabbits after intravenous administration (2.04 ± 0.23 L/h kg);[7] this was in accordance with the corresponding AUC value, which was 10 times lower for rats in comparison with the values obtained in rabbits, as has been indicated above. The slow release of the drug from KT-loaded PLA microspheres caused a low clearance, 2.6 times lower than the clearance of the drug administered in the form of a solution, and a slow elimination ($K_e = 0.0058 \pm 0.0003$ h^{-1}). However, the release of ketotifen from PLGA caused a very small plasma concentration of the drug, which allowed a quick clearance in spite of the slow elimination.

The steady-state volume of distribution (V_{ss}) of ketotifen when it was administered by intraperitoneal injection to rats in the form of a solution (Tab. 2) suggests that the distribution of the drug was mainly into extra-cellular fluids. In rats, the mean circulating blood volume is 64 mL/kg,[47] and the mean extra-cellular fluid volume is 200 mL/kg,[48] a value very similar to V_{ss} value determined for the intraperitoneal administration of the drug in solution ($V_{ss} = 0.21 \pm 0.04$ L/kg). The intraperitoneal administration of ketotifen by KT-loaded PLA and PLGA microspheres significantly increased the steady-state volume of distribution of the drug, and it suggests that these microspheres facilitate the distribution of the drug into tissues, which may be related to the hydrophobic characteristics of the polymers and the interaction of the microspheres with peritoneal cavity tissues. The binding of a drug to polymers may modify its distribution; thus, conjugation of methylprednisolone with dextran-70 drastically altered the distribution of the steroid by converting methylprednisolone from a large volume of distribution drug (2290 mL/kg) to a prodrug with a small volume of distribution (102 mL/kg), probably because the hydrophilic carrier decreased the lipophilicity of the drug.[49]

From *in vitro* KT release studies and *in vitro* degradation of PLA and PLGA microspheres,[16] it can be deduced that KT release from PLA and PLGA microspheres occurs mainly by dissolution of the drug inside the microspheres, and then by

the diffusion of the drug from them, even though a combination of drug diffusion and microsphere erosion processes also takes place. A level B IVIVC can be established not only when drug dissolution is the rate-limiting step for the *in vivo* ADME,[30] but also when drug release occurs by a combination of diffusion and erosion.[50] In this study with KT-loaded PLA and PLGA microspheres, a relationship has been established between mean *in vitro* dissolution time (MDT) and *in vivo* MRT. Although only two KT formulations have been studied, which questions the possibility of predicting MRT values of KT from *in vitro* experiments, a clear relationship exits between both parameters. The largest AUC value of KT when it was released from PLA microspheres corresponded with the longer release observed *in vitro*, whereas the lower AUC of the drug was obtained when KT was released from PLGA 50/50. Another relationship has also been observed between the *in vitro* release rates of ketotifen from the microspheres and the *in vivo* absorption constant (Fig. 7). A faster absorption of the drug was observed when the drug was quickly released from PLGA 50/50 microspheres, and this took place at each one of the two release rates. On the contrary, the slower release of the drug from PLA microspheres caused a lower absorption constant. Thus, a correlation seems to exist between the release rate and the absorption rate of the drug.

The presence of unloaded or KT-loaded PLA and PLGA 50/50 microspheres was observed in the adipose tissue after i.p. injection (Fig. 8). The implantation of biocompatible and biodegradable microspheres induces the activation of humoral and cellular mechanisms to produce inflammatory and healing responses of the material.[16] The acute and chronic inflammatory responses are of short duration, 1–2 weeks, regardless of polymer composition of the biodegradable microspheres. Polymorphonuclear leukocytes, monocytes and lymphocytes are the cell types associated with this event. However, the presence of lymphocytes or mast cells in the adipose tissue close to the injected microspheres was not observed. Groups of microspheres surrounded by very active connective tissue were observed, with the large amount of nuclei around the polymer indicating the activity of this tissue. The formation of the fibrous capsule is one of the local events following the implantation of microspheres. On the other hand, microspheres were not observed in internal organs. Thus, the formation of fibrous capsule in metabolically essential tissues did not take place, and possible undesired consequences were not detected.

In conclusion, the preparation of ketotifen-loaded PLA and PLGA 50/50 microspheres by spray-drying technology allows obtaining a high percentage of entrapped efficiency. A quick release of the drug takes place during the first 2 h and then a slower release rate is observed. The intraperitoneal administration of ketotifen-loaded microspheres allows detecting the drug in plasma between 336 and 384 h, and the MRT of the drug increases between 30 and 70 times with respect to the i.p. administration of ketotifen in solution at the same dose. The concentration and the presence of KT in plasma can be modulated depending on the composition of the polymer. These systems may be used to improve the treatment of the inflammation observed in portal hypertension, a clinical syndrome that is frequently studied using partial portal vein-ligated rats.

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