Improved Activity of a New Angiotensin Receptor Antagonist by an Injectable Spray-Dried Polymer Microsphere Preparation

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Received January 23, 1997; accepted April 8, 1997

Purpose. To characterize and evaluate *in vitro* and *in vivo* the release mechanisms involved in spray-dried biodegradable microspheres having differents Poly(D,L-lactide) blend formulations and containing an antihypertensive drug (L-158,809).

Methods. Microspheres and blended polymers were characterized by DSC, SEM, confocal laser microspcopy and size analysis. In vitro release studies were evaluated by using microspheres made from various blends of high and low molecular weight polymer. In vivo studies were evaluated by L-158,809 antagonist AT1 function versus the shift of the normal dose-response curve of blood pressure induced by Angiotensine II.

Results. The average yield of L-158,809 microspheres (10% (w/w)) was 95% of the theoritical loading. The average diameter of the microspheres was from 1 to 3 micrometers. In all release experiments, a significant burst effect (<15%) was observed followed by a near zero-order release kinetics. In vivo studies with two different formulations show a strong shift of angiotensin II dose-response curve.

Conclusions. The release kinetics and photomicrographs suggest that the system is best described as a multi-parameter controlled released system in which the drug is molecularly dispersed. In vivo results demonstrating the controlled release of L-158,809.

KEY WORDS: microspheres; spray-drying; polymer blend; polylactic acid; L-158-809.

INTRODUCTION

During the last decade, the use of biodegradable polymers has become very popular in drug delivery systems (1). Homopolymers of lactic acid, glycolic acid, ϵ-caprolactone and their copolymers have been shown to be biocompatible and biodegradable. As a consequence many implantable controlled release systems have been developed (2). Microspheres have been prepared by various methods (e.g., solvent evaporation (3–5), coacervation (6–7) and spray-drying (8–12)). The solvent evaporation method requires the use of a surfactant such as polyvinyl alcohol (PVA) which has been reported to be carcinogenic (13–14). Intravenous injections require small microspheres (diameter less than 5 mm) (15), which are more readily obtained by spray-drying than by other methods. The spray-drying method is very versatile as it can be applied to suspensions for hydrophilic drugs in a polymer solution as well as to hydrophobic drugs dissolved in the polymer solutions. The objective of this work was to improve the release of a non-peptide antagonist (L-158,809) of angiotensin II receptor (AT1). The characteristics and activity of that drug have been described previously (16–20). One of the properties that guided the design of this controlled release dosage form, was its low hydrosolubility. L-158,809 was encapsulated by spray-drying in an amorphous and biodegradable polymer, poly(D,L-lactide) (PLA) from a polymer solution in chloroform. PLA is degraded by auto-catalytic hydrolysis of ester bonds. It has been shown that body's esterases do not degrade the polymer (21–22). The half-life of PLA degradation is up to seven months (MW 100,000) *in vivo* (23) and less depending on the molecular weight of the polymer. The control of the drug release was accomplished through the blending of homopolymers with different molecular weights (24). Native PLA microspheres have a strong tendency to aggregate. To insure good dispersion, polyethyleneglycol 400 distearate (PEG) was added to the polymer blend. Release studies were conducted in phosphate buffer (pH = 7.4). In all release experiments, a significant burst effect (15%) was observed, followed by near constant release kinetics. The release kinetics and photomicrographs suggest that the system is best described as a multi-parameter controlled release microsphere system in which the drug is molecularly dispersed. Characterization of microspheres was done using scanning electron microscopy (SEM), confocal microscopy and differential scanning calorimetry (DSC). In vivo studies were done using Sprague-Dawley rats and the results showed a significant shift of angiotensin II dose-response curve demonstrating the controlled release of L-158,809.

MATERIALS AND METHODS

Materials

D,L-PLA synthesis was performed as described previously (25) with slight modification. Briefly, D,L-dilactide (Aldrich, Montreal) was synthesized by a ring opening method using tetraphenyltin as a catalyst under dry and high vacuum conditions. The crude reaction product was purified by precipitation of PLA in water after dissolution in acetone. PLA was then dried under vacuum over phosphorous pentoxide for several days. L-158,809 (M.W 409.518) was obtained from Merck-Frosst (Kirkland, Canada). PEG 400 distearate was purchased from Aldrich (Montreal) and poloxamer 188 (Pluronic F-68) from BASF (Parsipanny, N. J.). Except for PLA, all reagents were used without further purification.

Characterization of PLA

Molecular weight determinations of PLA samples were performed by GPC using a Waters 717 Autosampler, a Waters 600E System Controller and a Waters 410 Differential Refractometer. The columns used were Waters Ultrastyragel 10^3 Å and 10^4 Å and calibrated with polystyrene standards (Polysciences, Warrington, PA.) . Chloroform was used as mobile phase at a rate of 1 ml/min.

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0724-8741/97/0700-0887$12.50/0

Preparation of Microspheres

Microspheres consisted of L-158,809, PEG 400 distearate and different ratios of PLA 82,000 and 10,000. The following ratios of PLA 82,000/10,000 were used: 100%/0%, 90%/10%, 80%/20%, 70%/30%, 60%/40 and 50%/50%. Microspheres were prepared according to the following procedure: 1% (w/v) of a chloroform solution containing 0.8% of PLA, 0.1% of L-158,809 and 0.1% of PEG 400 distearate, was prepared. The solution was spray-dried with a Büchi Mini Spray Dryer-Model 190 (Büchi, Flawil, Switzerland) using a 0.5 mm nozzle. The process parameters were set as follows: inlet air temperature, 40–42°C; aspirator control, 10; pump control 10 (245 ml/h); air flow, 500 Nl/h.

Particle Morphology

Morphological characterization of the microspheres was performed using scanning electron microscopy (SEM) and confocal microscopy. For the SEM characterizations, the microspheres were attached to the specimen holder by a double-coated adhesive tape. The specimens were coated for 3 min. with a layer of gold.

Particle Size Distribution

The mean particle size of the samples was determined using photon correlation spectroscopy (N4 Plus, Coulter Electronics Inc., Hialeah, FL). Microspheres were diluted in phosphate buffer pH 7.4 to give a particle count rate between 5×10^4 and 1×10^6 counts per sec.

Mean particle diameter was calculated in size distribution processor mode (SDP) using the following experimental conditions: fluid refractive index 1.33; temperature 20°C; viscosity 0.93 centipoise; angle of measurement 90.0°; sample time 10.5 μs, and sample run time 90 sec.

L-158,809 Encapsulation Efficiency

10 mg of microspheres were dissolved in 50 ml of tetrahydrofuran (THF) and analyzed using a U.V. spectrophotometer (HP 8452A Diode Array Spectrophotometer) at 284 nm.

In Vitro L-158,809 Studies

Drug release from various blends of PLA microspheres was studied at 37°C using a thermostated shaker bath. The release studies were performed in 250 ml phosphate buffer (pH 7.4) containing 0.05% Pluronic F-68 as a wetting agent to further minimize aggregation. 10 ml samples were removed and centrifuged for 5 min at 3000 rpm. The supernatants were analyzed using a U.V. spectrophotometer (HP 8452A Diode Array Spectrophotometer) at 286 nm and returned to the medium.

In Vivo Studies

All animal experimentation followed the guidelines from the Canadian Council for Animal Care (CCAP). The presence of L-158,809 in the circulation was evaluated by its antagonist AT2 function versus the shift of the normal dose-response curve of blood pressure induced by Angiotensine II. Batches made of 30% and 40% PLA 10,000 were chosen because of their different *in vitro* half-time release characteristics. Male Sprague-Dawley rats (300–350g) received an intraperitoneal injection of encapsulated L-158,809 microspheres (n = 3), free L-158,809 (n = 3) or saline solution (n = 8). The dose of L-158,809 used was 7 mg/kg. After a specified time interval (17 or 85 hours), animals were anesthetized by an IM injection of a ketamine-xylozine solution (87 mg/kg and 13 mg/kg respectively). Systolic and diastolic blood pressure of unconscious rats were measured directly with a catheter in the right carotid artery using a Grass polygraph with 23X1 Statham pressure transducer. Bolus injection of Angiotensin II (0.1 ml) were administered intravenously through the left vein and flushed with an additional 0.1ml of saline solution.

Table I. Effect of Polymer Blend on Tg

PLA (10,000/82,000)	Mean Tg in °C	Start/End in °C
0/100	52.8	49.6/56.3
10/90	32.0	24.0/39.0
20/80	30.0	24.0/37.0
30/70	34.0	26.0/42.0
40/60	32.0	23.0/40.0
100/0	31.5	26.6/36.5

RESULTS AND DISCUSSION

Polymer blends of high molecular weight and low molecular weight PLA were characterized by DSC. Table Ishows that the addition of 10% of low molecular weight PLA in the blend decreases the Tg from 52.8°C to 32°C. There was no significant change in the Tg with further increase in the amount of the low molecular weight LA. The skewing of the onset and ending value of the Tg with the various blends could be related to the increase in polydispersity of the polymer blend. The addition of 0.1% of PEG 400 distearate to PLA 82,000 decreases the Tg from 52.8°C down to 39.3°C. This suggests that the Tg of the microspheres is affected by PEG 400 distearate and confirms that it is molecularly dispersed.

L-158,809 Encapsulation Efficiency

The L-158,809 content of microspheres was 9.93 ± 0,18 (w/w). This shows a very good efficiency of 98.2 ± 1.11. This demonstrates that the loading is not affected by initial polymer composition.

Particle Morphology

In Figure 1, scanning electron photomicrographs of the microspheres show very spherical particles with a smooth surface. No visible macropore can be noted using this method. The composition of the microspheres does not affect their morphology and their size. The mean diameter of microspheres made with 100% PLA 82,000 was 988.2 nm ± 113.7 nm, Figure 2.

Figure 3 demonstrates that the microspheres made from low molecular weight (10,000) PLA coalesced into strings resembling pearl necklaces during the release studies.

Confocal laser photomicrographs, Figure 4, indicate that L-158,809 is uniformly and molecularly dispersed within the

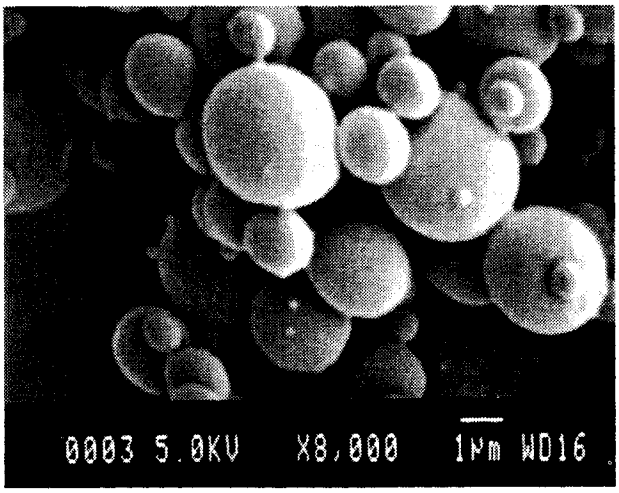

Fig. 1. SEM microphotograph of PLA microspheres before release studies.

microspheres. Lack of a melting point for L-158,809 in the polymer by DSC confirms this. The confocal laser photomicrograph does not support the existence of hollow spheres obtained by the spray-drying process as described previously (26) but it would not reveal the existence of a porous internal structure.

The addition of PEG 400 distearate was chosen in order to improve the microsphere dispersion as this technique was used successfully in liposome formulation. The hypothesis is that PEG 400 distearate has a amphiphilic structure as shown in Figure 5a. The effect of PEG 400 distearate as a dispersant during the release study could be explained by the tendency of PEG to form a hydrophilic head at the surface. Figure 5b,c, shows a scheme of a possible PEG 400 distearate molecule positioning at the surface in microspheres made by spray-drying. Using X-ray Photoelectron Spectroscopy, it has been shown that the PEG portion of a block copolymer is positioned at the surface of microsphere. As a consequence of the spray-drying process, a higher amount of PEG is needed to get enough PEG loops at the surface compared to liposome or emulsion technologies. The tendency of distearate ends is to stay beneath

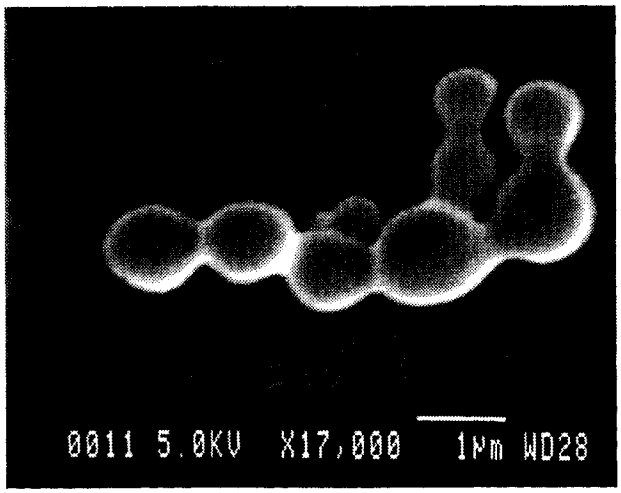

Fig. 2. SEM microphotograph of PLA micropheres after 18 hours of release.

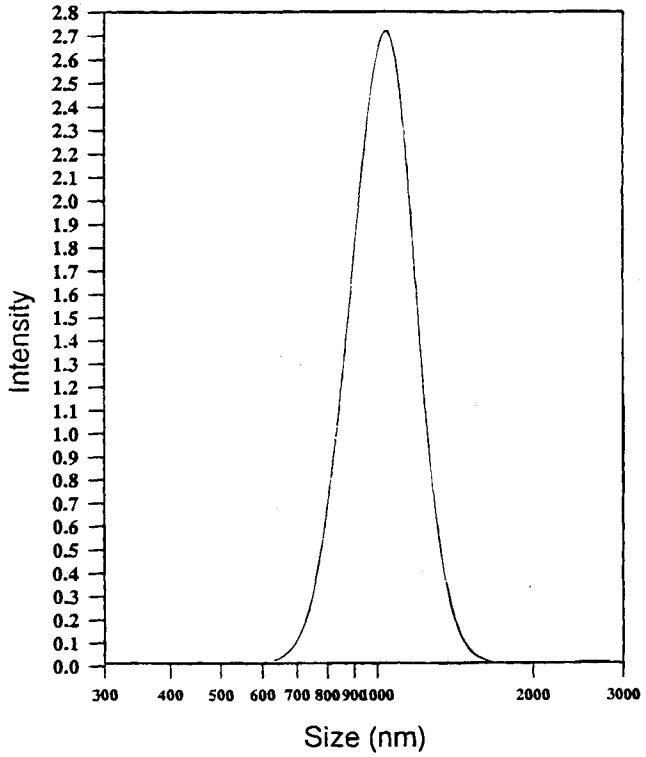

Fig. 3. Typical size distribution of PLA microspheres obtained by spray drying.

the polymer surface and the hydrophilic head to stick out of the polymer. During the spray-drying process PEG 400 distearate molecules are homogeneously dispersed in the bulk and for this reason its concentration must be high (10%) in order to insure sufficient hydrophilic heads at the surface of the microspheres, Figure 5c.

Release of L-158,809

The *in vitro* release of L-158,809 from the spray-dried microspheres are shown in Figure 6. The release is very slow and it increases with increasing percentage of low molecular weight PLA indicating that drug release from the microspheres is strongly dependent on the level of low molecular weight PLA. The sample containing 100% of PLA 82,000 shows a burst of about 15% followed by a very slow rate of release. These microspheres were still releasing at the end of the study (four months) with 60 % of the initial amount released. It is important to note that for samples with a PLA ratio (82,000/10,000) of 100/0, 90/10 and 80/20, the initial burst is followed by a near constant rate of release. As the percentage of PLA 10,000 increases, the rate of drug release increases. Although there are more than one hypothesis to explain the release kinetics from these microspheres, a multi-parameter controlled system appears to best explain the release kinetics. That is, the burst effect could be explained by the fast release of drug from surface pores which are rapidly filled with disolution medium. As these pores are filled and drug dissolves, the slow erosion of the polymer creates mores pores within the matrix and with time creating an infinite percolation network from which drug diffuses. Moreover, the overall release kinetics of a sample of a batch of microspheres is clearly the sum of many different

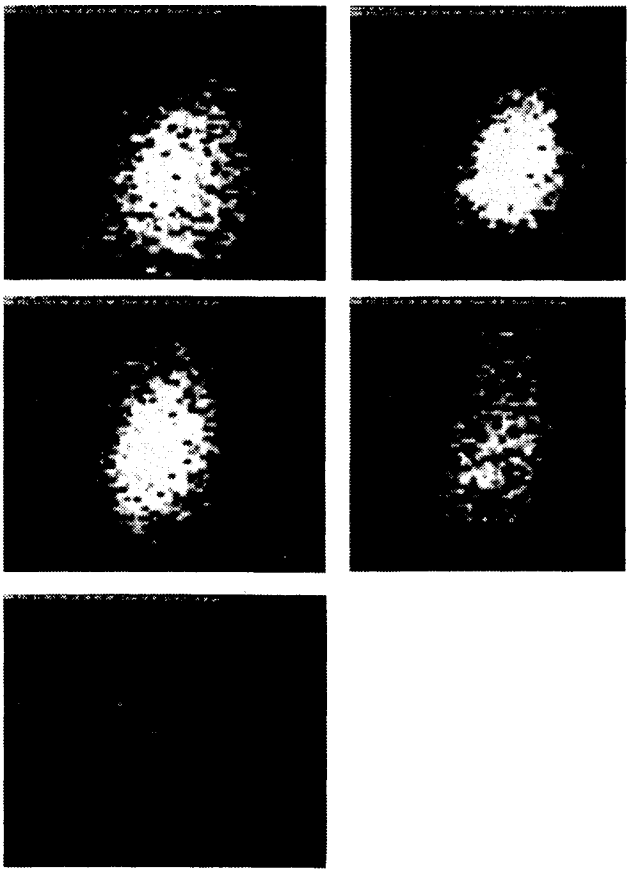

Fig. 4. Confocal microphotographs of PLA microspheres containing L-158,809. a), b), c), d) and e) are virtual slices (200 nm thickness) of one microsphere.

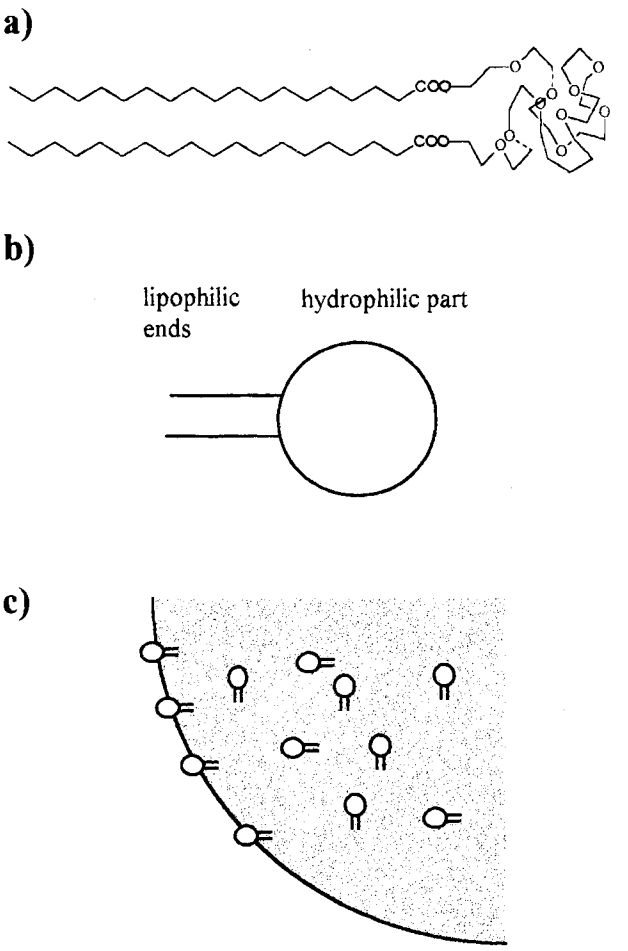

Fig. 5. Scheme of PEG 400 distearate. a) molecular representation, b) simplified representation, c) Assumed dispersion of PEG 400 distearate in microsphere.

individual release kinetics. To the knowledge of the authors, there is no model that takes this statistical fact into account. There is also an increasing permeability of the microspheres by erosion of PLA and by elution of PEG 400 distearate. It as been shown that the erosion of PLA is a bulk erosion process (3) but it has not been proven in this case that the erosion is a limiting factor although the degradation time of low molecular weight PLA is shorter than for the high molecular weight PLA. In this case, the erosion would not be the limiting factor of the release because the effective diffusion coefficient of the drug changes as the other parameters change. After an initial burst, a near constant release kinetic is observed for the 100% PLA 82,000, the 10% and 20% PLA 10,000 blends. The other samples do not show a constant release over the period of the release study suggesting Fickian's kinetics. Another hypothesis of the mechanism of constant release is that small pores are present in the matrix which are saturated by the drug but this hypothesis must be confirmed by further study of the microsphere structure and porosity.

In Vivo Studies

In order to compare the effect of the free drug versus the encapsulated drug considering that L-158,809 has a plasmatic half-life of 7.6 hours with a long terminal half-life in the rat (27), microspheres made respectively with 40% of PLA 10,000 and 30% of PLA 10,000 were used for the *in vivo* studies since the time for 50% drug release was demonstrated to be respectively 24 hours and 250 hours *in vitro*. Figure 6 shows the shift of angiotensin dose-response curves 17 hours and 85 hours after treatment. A significant right shift (about three log scales) appears demonstrating that L-158,809 is more effective in prolonging the activity using these microsphere formulations by comparison to the free form at the same dosage level. Previous work (28) demonstrated a controlled release of an ACE inhibitor (Delapril hydrochloride) which was encapsulated in polyglycerol esters of fatty acids. The authors demonstrated that, by oral administration, there was an inhibition of blood pressure increase to angiotensin I injection. The maximum inhibition was induced at 1 hour then decreased rapidly. L-158,809 *in vivo* studies demonstrated a controlled blood pressure inhibition after 17 hours for the 30% PLA 10,000 and 85 hours for the 40% PLA 10,000 after treatment.

In this work the authors demonstrated that spray-drying is a promising technique that can be used to produce injectable microspheres from various blends of (DL)-PLA with a mean diameter of 1 μm. As demonstrated, confocal microscopy can be a useful tool in the elucidation of microsphere structure. The study clearly demonstrates that the release kinetics of this system are strongly dependent on the molecular weight ratio

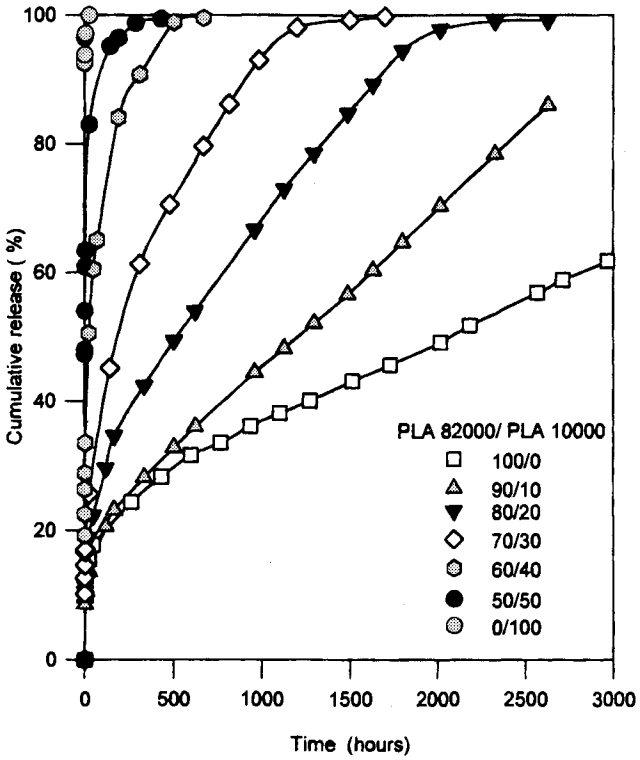

Fig. 6. Cumulative releasease of L-158,809 from PLA microspheres with different ratio of low molecular weight PLA.

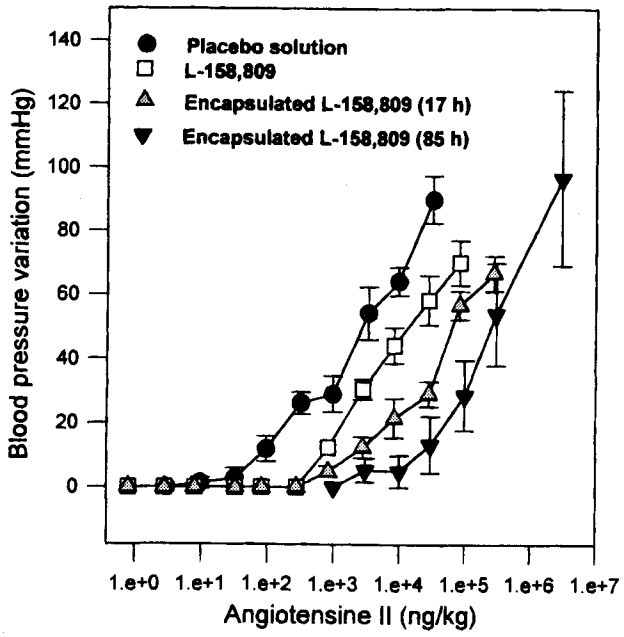

Fig. 7. Blood pressure response of Sprague-Dawley rats to angiotensin II in presence of L-158,809. Placebo solution (n = 3), Saline solution of L-158,809 (n = 3), encapsulated L-158,809 at 17h (n = 3), encapsulated L-158,809 at 85h (n = 3).

in the polymer blend. *In vivo* studies have shown that it was possible to have a prolonged release with these microspheres. This corroborates the tendency that long-acting preparations of drug for treatment of hypertension have been and have to be developed because the avoidance of high plasma concentration of the drugs decreases the risks of undesirable side-effects.

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