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International Journal of Pharmaceutics 134 (1996) 37–46

international journal of pharmaceutics

In vitro and in vivo evaluation of polylactide and polylactide-co-glycolide microspheres of morphine for site-specific delivery

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Received 14 July 1995; revised 27 October 1995; accepted 13 November 1995

Abstract

The aim of using opioid drugs such as morphine in chronic pain is optimal pain relief with a minimum of side effects. After the discovery that opioids could produce analgesia when administered intrathecally or extradurally, there has been particular interest in their administration by these routes and other site-specific routes. Controlled release systems of morphine are not currently available for parenteral routes. So, the design of a controlled release system for this analgesic was undertaken. To reach this goal, morphine-loaded microspheres (MLMs) made with polylactide and polylactide-co-glycolide polymers were prepared by the solvent evaporation process. MLMs were characterized by optical and scanning electron microscopy, by their drug contents and in vitro release kinetics. MLMs were then administered via subcutaneous injection in rabbits. The in vivo results, in accordance with the in vitro release studies, showed: (i) a controlled release of morphine avoiding high plasma levels; and (ii) an extended release of morphine.

Keywords: Morphine; Solubility properties; Polylactide; Polylactide-co-glycolide; Microspheres; Controlled release; Bioavailability

1. Introduction

Morphine appears to be an opioid analgesic of choice, recommended by the World Health Organization, for the management of post-operative and cancer pain (Glare and Walsh, 1991). Parenteral administration of morphine is commonly achieved either by intravenous continuous administration or by intermittent administrations via subcutaneous, intramuscular or site-specific routes (intraarticular, perimedullar etc.) (Budd, 1989; McQuay, 1989; Finley, 1990; Glare and Walsh, 1991; Stein et al., 1991). However there are some drawbacks: i.v. infusions require the permanent use of infusion devices and the implantation of a

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SSDI 0378-5173(95)04402-V

catheter (Kwan, 1990), intermittent administration requires frequent injections and leads to transient high plasma concentrations resulting in adverse effects, such as respiratory depression (McQuay, 1989; Finley, 1990; Kwan, 1990).

Controlled release systems of morphine are not currently available for parenteral routes. Thus, there was much interest in the design of a controlled release system for this analgesic. Between the different polymers and microparticulate formulations to obtain sustained release systems (Chah and Pitt, 1989; Jalil, 1990; Smith et al., 1990; Brannon-Peppas, 1995), we designed morphine-loaded microspheres made with polylactide and polylactide-co-glycolide polymers.

The first stage of this work was to prepare microspheres by the solvent evaporation process (Aftabrouchad and Doelker, 1992). Classically, an organic polymer solution containing a drug, such as a solution or a suspension, is emulsified into an external aqueous phase (O/W). In the present case, it had to be taken into account that morphine base has intermediate solubility properties and could diffuse into the external aqueous phase.

In order to address this problem, Jalil and Nixon (1990) proposed the formation of microspheres by using an W/O emulsion in which external phase was paraffine oil with suitable surfactants so as to avoid loss of hydrophilic drug in the external phase. More recently, Bodmeier et al. (1994) achieved microencapsulation of chlorpheniramine maleate, a drug with intermediate solubility properties, by a non-aqueous solvent evaporation technique. However, the problem remained to remove the oil, solvents and surfactants from the microspheres. Although these techniques are of great interest, solvent evaporation methods using an aqueous external phase are preferable. Hence, after a study of the aqueous solubility of morphine base, morphine-loaded microspheres (MLMs) were prepared using an O/W emulsion.

The following stages of this study were to evaluate MLMs in vitro and in vivo in order to investigate the controlled and extended release of morphine.

2. Materials and methods

2.1. Materials

Poly D,L-lactic acid (PLA; Resomer R104, mol. wt. 2000 and R202, mol. wt. 9000) and poly D,L-lactic/glycolic acid (PLGA, 50:50; Resomer R503, mol. wt. 9000 and R504, mol. wt. 12 000) were supplied by Boehringer Ingelheim (Saint-Germain-en-Laye, France).

Morphine hydrochloride was supplied by Coopération Pharmaceutique Francaise (Melun, France). Morphine was encapsulated as a base which was obtained by precipitation in an alkaline medium (ammonium hydroxide) from a saturated aqueous solution of morphine hydrochloride. The purity of morphine base was assessed using HPLC by comparison with morphine hydrochloride.

The following chemicals were used as received: polyvinyl alcohol (namely Rhodoviol® 4/125) and Tween 20 (Prolabo, Paris, France); dichloromethane RPE-ACS, methanol RPE and hydrochloric acid 23% RPE (Carlo Erba, Milan, Italy); sodium carboxymethylcellulose (Cooper, Melun, France); Tris buffer and ammonium hydroxide 32% (Merck, Darmstadt, Germany). Polyamide membrane filters of 1- and 50-μm pore size were used (ZBF, Rüschliken, Switzerland).

2.2. Solubility studies of morphine base

2.2.1. Solubility in organic solvents

The solubility of morphine base was determined in dichloromethane and methanol at room temperature and was indicated by clear solutions.

2.2.2. Solubility in water

The pH solubility profile of morphine base in water was determined in order to establish an optimal pH value for the external aqueous phase, according to the phase solubility technique described by Dittert et al. (1964).

A saturated solution of morphine base was prepared with an excess of drug in distilled water and agitated for three hours at room temperature. The pH of the suspension was noted and then was lowered stepwise by drops of 1 N HCl with stirring. At each step, an aliquot of the suspension

was removed and filtered. The experiment was carried out until all of the solid phase in the system dissolved. After suitable dilution in 0.1 N HCl, the filtrates were analyzed at 213 nm by a Milton Roy (Rochester, NY) Model 1201 Spectronic spectrophotometer.

2.2.3. Intrinsic dissolution of morphine base

The intrinsic dissolution rate (IDR) of morphine base was determined according to Wood's method (Wood et al., 1965) adapted by Shah and Maniar (1993).

Morphine base powder was compressed using a Frogerais® Model AM 800 tablet press fitted with 9-mm and 12-mm flat punches and compression was executed by manual rotation of the fly wheel. Each disk was coated with wax and maintained on the extremity of a glass stick allowing a constant surface to be exposed to the dissolution medium, 0.636 and 1.131 cm^2 respectively. The system was poured into a USP XXI rotating paddle apparatus. The dissolution media (1000 ml at 37°C) were either a 0.2% NaCl aqueous solution adjusted to pH 2.0 with 25 M HCl or a pH 7.4 phosphate buffer solution. As the UV spectrum of morphine displayed absorbance maxima at 213 and 285 nm, analyses were conducted at the more sensitive wavelength i.e. 213 nm when low concentrations of morphine were expected (e.g. < 10 mg/l). Otherwise 285 nm was selected. In this experiment, the dissolution medium was analyzed continuously at 285 nm by spectrophotometry and morphine concentration recorded every 2 min. The experiments were conducted under sink conditions, in duplicate for each disk surface at each pH value, and the relative difference between individual IDR was lower than 12%.

2.3. Preparation of microspheres

2.3.1. Organic phase composition

Dichloromethane (CH_2Cl_2) and methanol (CH_3OH) were used at different CH_2Cl_2/CH_3OH volume ratios varying from 10:0 to 4:6 under a total volume of 5 ml. Morphine base was in solution or in suspension according to the CH_2Cl_2/CH_3OH ratios.

Several batches of microspheres were prepared with a similar amount (500 mg) of PLA or PLGA polymers and with different CH_2Cl_2/CH_3OH ratios (thus different amounts of morphine base). MLMs prepared with PLA mol. wt. 2000 (PLA1) and an initial morphine base amount of 200 mg dissolved in the organic phase (CH_2Cl_2/CH_3OH, ratio of 5:5) were used for the in vivo administration.

2.3.2. Procedure

Microspheres were prepared according to a solvent evaporation method described by Le Corre et al. (1994). Briefly, the organic phase composed of morphine base, polymer and solvents was poured slowly into the external aqueous phase and emulsified for 5 min. To eliminate the organic solvents, the emulsion was diluted progressively (1 min) with distilled water and stirred for 60 min. During the final 55 min, the stirring was performed under vacuum. The microspheres were collected by filtration, rinced twice with distilled water, then lyophilized into a powder and stored under vacuum at 4°C.

2.4. Microsphere characterisation

2.4.1. Size and shape

Microspheres were observed under an optical microscope with transmitted light at a magnification of $\times 680$. Size distribution was assessed for all the batches at the micron level following an observation of 100 particules. Particule morphology and surface characteristics were analyzed by scanning electron microscopy (SEM). The dried microspheres were sputter-coated with a thin layer of Au/Pd using a Model JFC 1100 ion sputter (Jeol Co. Ltd, Tokyo, Japan). Observation was carried out with a Model JSM 6400 electron microscope equipped with a camera (7 kV, $\times$ 2000).

2.4.2. Drug content

Weighed amounts of MLMs (about 20 mg) were dissolved in dichloromethane (1 ml). Morphine was then extracted with 0.1N H_2SO_4 (5 ml). After shaking and centrifugation, 50 μl of the aqueous phase was diluted in 2 ml of the mobile

phase. An aliquot of 20 μl was injected into the chromatograph. Analyses were performed by using a 125 × 4 Merck Lichrocart® RP-Select B (5 μm) Lichrosorb® column and a mobile phase composed of 0.01 M KH_2PO_4, 0.1% H_3PO_4/acetonitrile (95:5, v/v) at a 1 ml/min flow rate. The HPLC system consisted in a Waters (Milford, MA) Model 6000 pump equipped with a Waters Model Wisp 710 B automatic injector, an LDC Milton Roy (Riviera Beach, FL) Model 3100 Spectromonitor detector (wavelength at 213 nm) and a Delsi (Suresnes, France) Model Enica 21 integrator.

2.5. Morphine-loaded microsphere release studies

Release studies were carried out with the USP XXI rotating paddle apparatus at 100 rpm and 37°C under sink conditions in 1000 ml of a 0.2% NaCl aqueous solution adjusted to pH 2.0 with HCl. A weighted amount of MLMs containing 10 mg of morphine base was suspended in 1 ml of an aqueous solution containing mannitol (2.5%), sodium carboxymethylcellulose (0.75%) and Tween 20 (0.05%). The suspension was poured into the release medium. Morphine concentration was measured continuously at 213 nm and recorded every 5 min during a 24-h period by using the Milton Roy Model 1201 Spectronic spectrophotometer. Each batch was analysed in duplicate and the relative difference observed between the percent drug released was lower than 8%.

2.6. In vivo biopharmaceutical study

2.6.1. Study design

In vivo biopharmaceutical study was performed on six New Zealand male rabbits (2.7–3.2 kg). Each rabbit received a single subcutaneous injection of 2 mg/kg of morphine as morphine hydrochloride solution, plain morphine suspension and MLM suspension under the same volume (1 ml) with a 1-week wash-out period between each administration. Plain morphine and MLMs were suspended in the aqueous solution containing mannitol (2.5%), sodium carboxymethylcellulose (0.75%) and Tween 20 (0.05%).

One-ml blood samples were drawn from the marginal vein of the ear immediatly before administration and at 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 h after. After centrifugation at 3000 × *g* for 5 min, plasma samples were frozen at − 18°C until analysis.

2.6.2. Morphine plasma determination

Plasma sample (0.25 ml) was loaded with nalorphine as internal standard and was alkalinized with sodium tetraborate (100 μl, 0.05 M). Then, 2 ml of ethyl acetate were added, the mixture was shaken for 2 min and centrifuged for 5 min (3000 × *g*). Fifty μl of 0.01 N sulphuric acid solution were added to the recovered organic phase. After shaking for 1 min, the tube was centrifugated at 3000 × *g* for 5 min and the organic phase was discarded. Fifty μl of the acid aqueous phase were buffered with 50 μl of a Tris, acetonitrile and distilled water solution (135 mg, 44 ml and 56 ml respectively). A 20-μl sample was injected into the chromatograph system.

The plasma concentrations of morphine were assessed by HPLC and electrochemical detection. The previously described HPLC system was used coupled with an electrochemical detector (Waters Model M460, Ework = + 0.6 V).

The mobile phase was composed of Tris (944 mg), 1 M EDTA solution (0.39 ml), acetonitrile (220 ml), fresh distilled water (780 ml) and 5 N sulphuric acid to reach a pH value of 7.45. The limit of quantification was 1 ng/ml. The between-day reproducibility determined at a morphine base concentration of 25 ng/ml was 4.8% (n = 10). The linearity was assessed in the concentration range 1–500 ng/ml (r^2 = 0.999).

3. Results and discussion

3.1. Choice of experimental conditions

Drug solubility in the external aqueous phase has to be minimized to entrap the drug within the microspheres prepared by using an O/W emulsion (Bodmeier and McGinity, 1988).

Fig. 1 displayed a proportional relationship between morphine base solubility and pH: the

solubility decreased when the pH increased. Moreover, morphine base has pK_a values of 8.21 and 9.85 (Kaufmann and Semo, 1975). Thus, the pH of the external aqueous phase was adjusted to 9.0 that allowed a low solubility of morphine base (< 0.16 g/l) and could minimize the drug partitioning from the organic phase into the external aqueous phase. Furthermore, as morphine was poorly soluble in dichloromethane (2 g/l < solubility < 4 g/l), due to its intermediate solubility properties, and more soluble in methanol (1 g dissolved in 10 ml boiling methanol), methanol was used as an hydrophilic cosolvent. The more methanol was added, the more morphine dissolved in the organic phase. The addition of water-miscible organic solvents to the organic phase was shown to enhance the drug content in the microspheres as a result of a faster precipitation of the polymer at the droplet interface (Bodmeier and McGinity, 1988). However, in our experiment, polymers could not be dissolved when the percentage of CH_3OH exceeded 60% of the total mixture.

3.2. In vitro evaluation

3.2.1. Intrinsic dissolution of morphine base

The mean (n = 2) dissolution profiles of morphine base at pH 2.0 and 7.4 are shown in Fig. 2. A non-linearity of the curves was observed at times above 60 min and could be partly explained by the modification of the disk surface at the end of the experiment. Thus, only the initial linear portion of the curves was taken into account in the evaluation

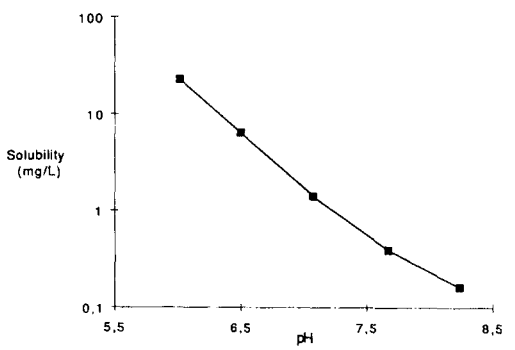

Fig. 1. The pH solubility profile of morphine base (single experiment).

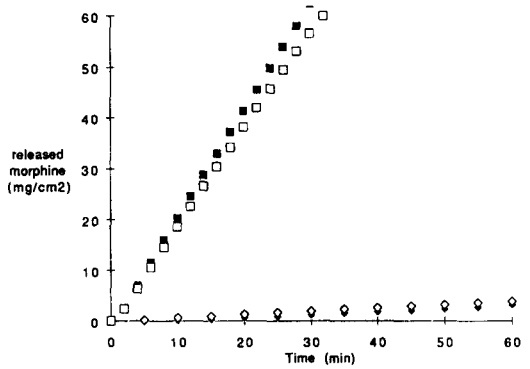

Fig. 2. Intrinsic dissolution profiles of morphine base at pH 2.0 and pH 7.4 with two different disk surface areas. pH 2.0: 0.636 cm^2 (■) and 1.131 cm^2 (□); pH 7.4: 0.636 cm^2 (◆) and 1.131 cm^2 (◇).

of the intrinsic dissolution rate (r^2 > 0.999 in all the cases).

The intrinsic dissolution rates, i.e. the slope of the linear curves, were dependent on the pH value of the dissolution medium but independent of the surface disk area exposed to the medium. At pH 2.0, intrinsic dissolution rates for 0.636 and 1.131 cm^2 disk areas were very close 2.089 and 1.933 mg/min/cm^2, respectively. At pH 7.4, intrinsic dissolution rates were found to be of 0.060 and 0.063 mg/min/cm^2, respectively, showing a great decrease in the intrinsic dissolution rate with increasing pH. Such a magnitude of fall in dissolution may affect the release characteristics of morphine base from a drug delivery system. Indeed, intrinsic dissolution rate less than 0.1 mg/min/cm^2 could be a limiting factor of drug absorption rate, especially for oral administrations (Kaplan, 1972).

3.2.2. Morphine-loaded microspheres

3.2.2.1. Shape and size. Size ranged from 1 μm to 30 μm and was compatible with a further parenteral non-intravascular administration. SEM showed spherical-shaped particules with a smooth surface coating a porous matrix for microspheres prepared with morphine as a solution (MSOLM) (Fig. 3). Microspheres prepared with morphine as a suspension (MSULM) had an particular aspect: they were less regular and morphine crystals were embedded in the polymer (Fig. 4).

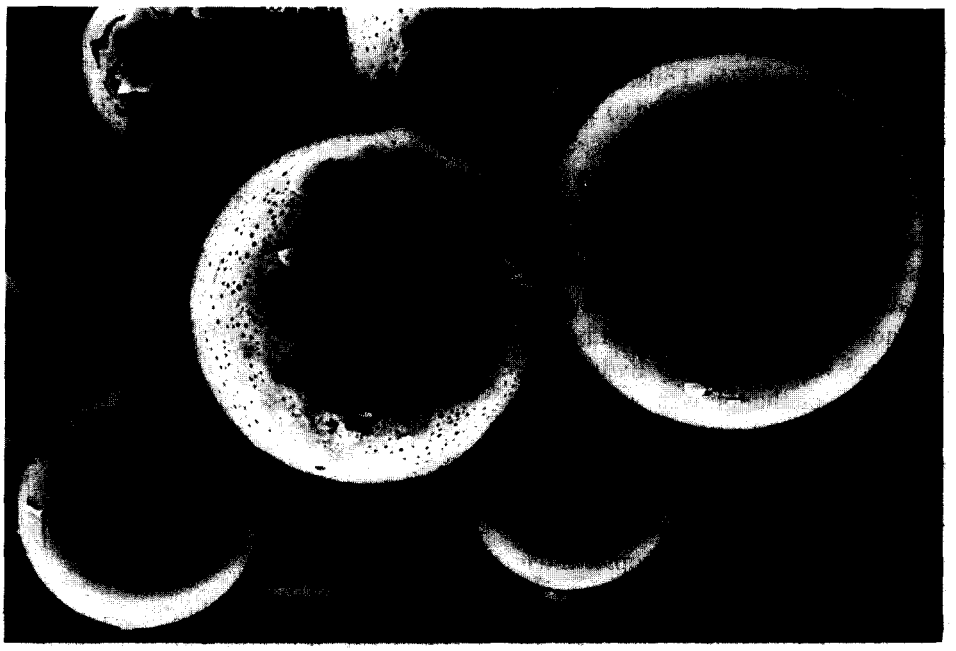

Fig. 3. Scanning electron micrograph of morphine-loaded microspheres prepared with morphine as a solution (MSOLM) in the organic phase (bar = 10μm).

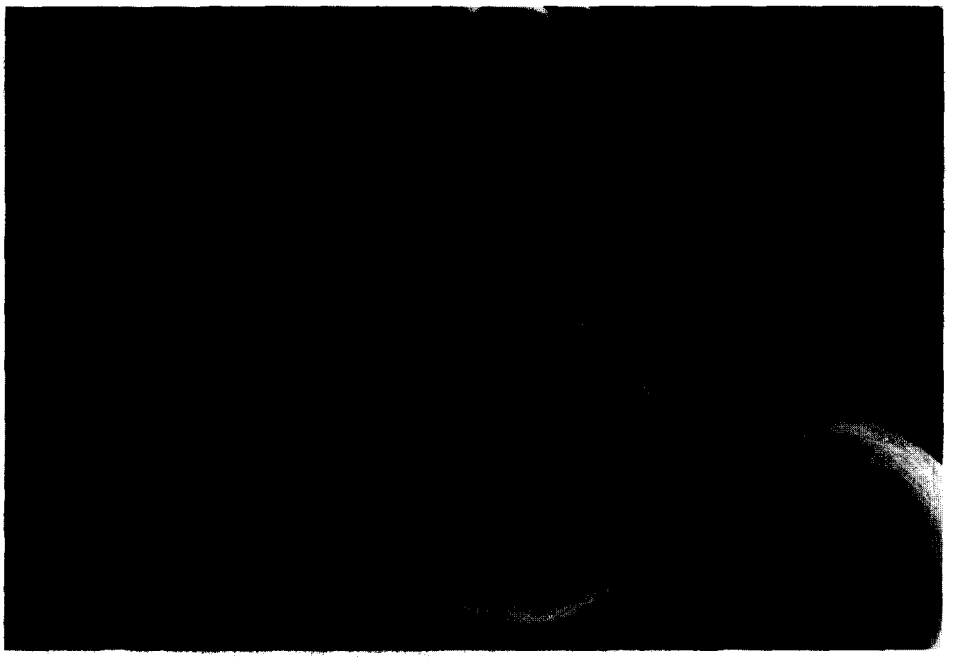

Fig. 4. Scanning electron micrograph of morphine-loaded microspheres prepared with morphine as a suspension (MSULM) in the organic phase (bar = 10μm).

3.2.2.2. Drug content. The drug content data showed that morphine was differently entrapped within polylactide and polylactide-co-glycolide microspheres according to the polymer molecular weight and according to the physical state of morphine in the organic phase (Table 1). The highest drug loading was obtained for MSULM (11,1%). For the batches prepared with a CH_2Cl_2/CH_3OH organic mixture (MSOLM), the most successful encapsulation of morphine was obtained with PLA MW 2000 (PLA1) and varied according to the initial amount of morphine (6.9–9.5%). However, morphine was not entrapped within microspheres made with PLA 9000 and drug content values of PLGA microspheres were lower than those of PLA1 (1.2–1.7% for PLGA 9000 and 1.2–1.4% for PLGA 12000). Rapid polymer precipitation at the droplet surface was of primary importance for the successful encapsulation of drug (Bodmeier and McGinity, 1988). The low drug contents observed with PLGA and PLA 9000 could be attributed to a low rate of precipitation: morphine base could diffuse across the non-precipitated droplet surfaces into the external aqueous phase. Thus, these polymers appeared to be unsuitable for the design of morphine-loaded microspheres. Hence, experiments were performed only with PLA1: the encapsulation efficiency within PLA1 microspheres decreased when the initial amount of morphine increased (50.2–29.4%), indicating that the drug content reached a maximum (around 10%). Furthermore, the microsphere recovery yield decreased (65.9–37.8%) when drug content increased (6.9–9.5%).

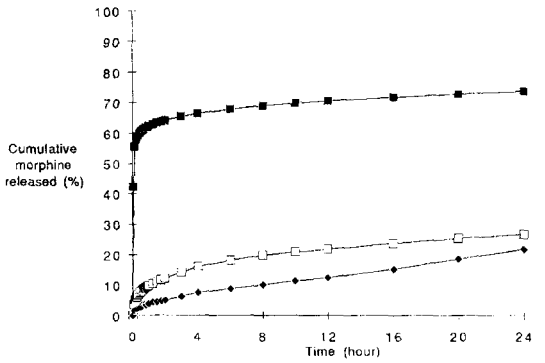

Fig. 5. Morphine release profiles from PLA1 microspheres prepared with 120 mg of morphine as a solution (MSOLM, □) or as a suspension (MSULM, ■) and from PLGA microspheres prepared with 120 mg of morphine as a solution (MSOLM, ◆) (n = 2)

3.2.2.3. Morphine-loaded microsphere release studies. In vitro release kinetics of PLA and PLGA microspheres displayed significant differences as a function of the type of polymers and of the state of morphine base in the microspheres (Fig. 5).

Table 1
Drug content, encapsulation efficiency and recovery yield of different batches of microspheres

State of morphine in organic phase	Polymer	Morphine (mg)	CH_2Cl_2/CH_3OH ratio	Drug content (%)	Encapsulation efficiency (%)	Recovery yield (%)
Solution	PLA (mol. wt. 2000, PLA 1)	80	8:2	6.9	50.2	65.9
		120	7:3	7.8	40.3	58.9
		160	6:4	9.2	37.0	53.9
		200	5:5	8.4	29.4	50.6
		240	4:6	9.5	29.4	37.8
	PLGA (mol. wt. 9000)	80	8:2	1.2	9.0	58.1
		120	7:3	1.7	8.3	42.8
Suspension	PLA 1	120	10:0	11.1	56.2	43.9

Microspheres were prepared with PLA and PLGA polymers, different initial amounts of morphine in solution or in suspension according to the organic phase composition (n = 2 for each batch).

With MSULM, release of morphine reached about 80% of the 24-h cumulated release within a few minutes. This release likely resulted from dissolution of embedded crystalline morphine observed by scanning electron microscopy. Such a rapid release kinetics should not be suitable for a controlled delivery system. A continuous increasing release was achieved with MSOLM, but drug release profiles were different between PLA and PLGA microspheres. A drug burst around 50% of the 24-h cumulated release was observed with PLA1 microspheres whereas at the same time only 25% of the 24-h cumulated release was reached with PLGA microspheres. The release profile of PLA1 microspheres displayed two phases: a rapid release (i.e. drug burst) during the first 4 h followed by an apparent linear phase. Initial release may result from dissolution of the drug within the pores located near the surface of the microspheres. Indeed, scanning electron microscopy displayed a porous matrix for MSOLM. The second phase may be due to the polymer degradation and/or drug diffusion through the matrix.

The cumulative drug release of the different batches of PLA1 microspheres seemed to be influenced by the initial drug loading (i.e. morphine base amount in the organic mixture). Indeed, the percent release obtained with the formulation made with the highest drug loading was much higher (38.5% released at 24 h) than those measured for the three other formulations whose percent release at 24 h ranged from 23.3 to 26.1%. Based on drug content and in vitro release kinetics, morphine-loaded microspheres made of PLA1 were chosen to conduct the in vivo evaluation.

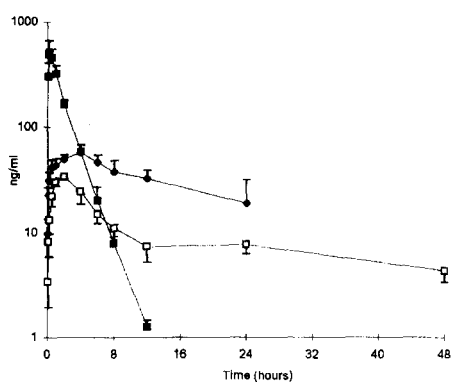

Fig. 6. Mean morphine plasma concentrations following subcutaneous administration of morphine hydrochloride solution (■), plain morphine (◆) and morphine-loaded microspheres (□) suspensions at a dose of 2 mg/kg in rabbits (n = 6).

3.3. *In vivo biopharmaceutical study*

The mean (n = 6) plasma concentrations versus time curves following subcutaneous administration of 2 mg/kg of morphine such as morphine hydrochloride solution, plain morphine suspension and MSOLM suspension are illustrated in Fig. 6. The biopharmaceutic data are summarized in Table 2.

The data obtained after administration of morphine hydrochloride solution showed high values of maximum plasma concentrations (C_{max}) (520 ± 151 ng/ml) with significant variation (C.V. of 29%). Morphine plasma concentrations at 24 h were under the limit of quantification (below 1 ng/ml). By using plain morphine and MSOLM suspensions, C_{max} were significantly lower (58 ± 10 and 34 ± 2 ng/ml respectively). However, C_{max} had a higher variability with plain morphine compared to MSOLM (C.V. of 17.5% vs. 4.4%).

Morphine plasma concentrations at 48 h after administration of plain morphine were detected only in two rabbits whereas concentration values were measurable in all the six animals after MSOLM administration, indicating that a sustained release of morphine over a 48-h period at least could be achieved with MSOLM. At physiological pH, the low morphine base intrinsic dissolution rate may be the rate limiting factor to absorption (Kaplan, 1972) and could explain the low C_{max} obtained with plain morphine and morphine-loaded microspheres. Indeed, this low intrinsic dissolution rate of morphine base could affect the drug release rate from the microspheres which may not control the overall release rate of the drug. But the in vivo results displayed a real sustained release of morphine after administration of the microspheres in contrast with plain morphine suspension, suggesting that the rate limiting

Table 2
Apparition time (T_{max}) of maximum plasma concentrations (C_{max}), area under the curve (AUC) and relative bioavailability (F) of morphine hydrochloride solution, plain morphine suspension (PM) and morphine-loaded microspheres suspension (MLM) administered at a dose of 2 mg/kg by subcutaneous injection in rabbits

Rabbit	T_{max} (h)			C_{max} (ng/ml)			C 48 h (ng/ml)			AUC (ng·h/ml) 0–24 h			F (%)		
	Solution	PM	MLM	Solution	PM	MLM	Solution	PM	MLM	Solution	PM	MLM	MLM/Solution	PM/Solution	MLM/PM
1	0.50	6	2	391.3	52.7	34.9	—	—	3.71	1052	868	318	30	82	37
2	0.25	4	1	761.2	58.3	33.7	—	—	6.05	1191	864	285	24	73	33
3	0.17	4	2	579.1	55.8	35.5	—	—	3.52	919	699	288	31	76	41
4	0.25	4	4	591.8	78.7	34.5	—	—	5.10	1029	905	367	36	88	41
5	0.25	4	2	401.8	51.8	31.2	—	1.44	3.65	847	716	255	30	85	36
6	0.50	4	2	393.7	53.3	34.5	—	4.02	4.32	906	882	294	32	97	33
Mean	0.32	4.33	2.17	519.8	58.4	34.1	—	—	4.39	990	823	301	31	84	37
S.D.	0.14	0.82	0.98	150.5	10.2	1.5	—	—	1.00	125	90	38	4	9	3
C.V. (%)	44.6	18.8	45.4	29.0	17.5	4.4	—	—	22.8	13	11	13	13	11	9

Dashes indicate not detectable (<1 ng/ml).

factor was the microspheres and not the drug dissolution rate. Indeed, the drug diffusion through the polymer matrix of the microspheres might be the rate limiting step allowing such a sustained release.

The relative area under the curve (AUC) for MSOLM, i.e. index for relative bioavailability, was lower than for plain morphine (31 ± 4 versus 84 ± 9%) over a 24-h period, but this parameter increased to reach 44 ± 5% over a 48-h period with MSOLM (data not shown). Nevertheless, the relative bioavailability of this drug delivery system should be improved.

In conclusion, these results, in accordance with the in vitro release studies, showed: (i) a controlled release of morphine avoiding high plasma levels; and (ii) an extended release of morphine. Such features obtained with the microspheres should avoid adverse effects such as respiratory depression and should allow less frequent administrations of morphine. This preliminary work highlights the interest of this controlled delivery system of morphine. Pharmacodynamic studies should be carried out in order to evaluate the reduction in drug toxicity. Moreover, this controlled delivery system should be evaluated by using site-specific routes of administration because of the peripheral antinociceptive effects of the opioid agonists, such as morphine.

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