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Biomaterials 22 (2001) 563–570

Biomaterials

In vivo–in vitro study of biodegradable methadone delivery systems

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Received 30 November 1999; accepted 19 June 2000

Abstract

Three one-week controlled-release methadone formulations: polylactic acid microspheres (F-PLA) and poly(lactide-*co*-glycolide) microspheres (F-PLGA) with 24 and 30% methadone content, respectively, and an implant of 50:50 poly(lactide-*co*-glycolide): methadone, were evaluated in vitro and in vivo. The implant released the total amount of methadone in vitro while microsphere formulations released the methadone incompletely, 63% from F-PLA and 85% from F-PLGA in a week. Methadone release in vivo was estimated by deconvolution, F-PLGA giving a bioavailability >99% (methadone was totally released in 48 h), while the estimated bioavailability of F-PLA was lower than expected. The bioavailability of the implant by deconvolution was around 60%, but absence of methadone in the implant indicated its complete release. These differences are due to an increase in methadone clearance after 72 h of the in vivo experimental period had passed, disturbing a good in vivo–in vitro correlation. A linear correlation between in vitro methadone release and in vivo release calculated from the amount of drug remaining within the implant, was found until the drug was completely released.

Keywords: In vivo–in vitro correlation; Microspheres; Implants; Controlled release; Polylactic acid; Poly(lactide-*co*-glycolide)

1. Introduction

The use of methadone in maintenance programs for opiate-addiction began in 1965 when Dole and Nyswander [1] described the blocking effect of methadone on both the euphoria effects elicited by heroin administration and its withdrawal syndrome. These methadone programs required regular visits to treatment centers, which in most cases ended in a lack of patient compliance.

In order to lessen the frustrating effect of these continuous visits, Choulis et al. [2,3] introduced longer-acting methadone tablets made of different proportions of carbomer and cellulose acetate phthalate to prolong their effect. However, they could achieve sustained methadone levels for only short periods of time. Therefore, to avoid these problematic daily visits, patients would be provided with a large stock of tablets that could unfortunately be misused extracting the methadone and injecting it intravenously, to reach higher methadone levels and subsequently achieve a heroin-like state of euphoria. To prevent or at least diminish this methadone abuse, another oral dosage form, methadone-resin-salt (sulphuric acid resin and methadone HCl) was proposed by De Leede [4]. This new dosage form could not easily be extracted and at the same time permitted a sustained methadone release, depending on the cation concentration in the gastrointestinal tract.

Moreover, the use of a controlled release dosage form for long periods of time avoids daily administration, and is therefore the best way to improve the efficacy of the treatment and patient compliance. Thus, Cha and Pitt [5] developed biodegradable L-methadone microspheres using three different polymers. A mixture of these microspheres (50–500 μm in size) results in a system able to release about 80% of the drug during a 7-day period in vitro, following a near zero-order release kinetics. However, this system was never tested in vivo.

In previous work in our laboratory [6] we studied the design and optimization of DL-polylactic acid (DL-PLA) microspheres containing DL-methadone base to be delivered over a week. We used one polymer (DL-PLA) instead of three, to simplify the manufacture process: the polymer fraction was limited to a maximum of 85% in order to keep the microsphere dose within reasonable

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0142-9612/01/$ - see front matter
PII: S0142-9612(00)00214-3

limits, and microsphere size was reduced to facilitate parenteral administration and polymer degradation after drug release. According to these results, the optimal formulation should be obtained using high Mw DL-PLA and low methadone load.

In the present work, three different one-week controlled methadone release formulations were tested in vivo: the optimal microsphere formulation for controlled methadone delivery prepared according to Delgado et al. [6], a PLGA microsphere formulation and a PLGA implant. For this purpose, we have calculated the in vivo methadone dosage delivered from each system to obtain an in vitro–in vivo correlation. This is of aid in modifying these formulations in order to achieve the serum methadone levels required to avoid the withdrawal syndrome.

2. Materials and methods

2.1. Polymers

Poly(DL-lactic acid) of weight average molecular weight (Mw) 220 kD and polydispersivity (pd) 1.63, (PLA-220) was synthesized using the ring opening method [7] with 0.1% tetraphenyl tin (Merck) as catalyst at 160°C for 4.25 h, according to previous data [8].

Poly(DL-lactic-*co*-glycolic acid) (Resomer® RG 506), Mw 100 kD and pd 1.56 (PLGA-100), and poly(DL-lactic-*co*-glycolic acid) (Resomer® RG502), Mw 14 kD and pd 1.52 (PLGA-14), both with a molar ratio of 50 : 50 were purchased from Boehringer Ingelheim, KG, Germany.

Polymer molecular weights were determined by gel permeation chromatography (GPC) as described previously [6]. Briefly, the conditions of GPC analysis were: three columns (Ultrastyragel) of 1000, 10 000, and 100 000 Å pore size, an oven temperature of 31°C, and a tetrahydrofuran (Panreac) flow rate of 0.9 ml/min. The system was calibrated using monodisperse polystyrene (Tokyo Soda Ltd.).

2.2. Formulation preparation

Microspheres were prepared using the solvent evaporation technique as previously described [6]. Briefly, polymer (2.38 g of PLA-220 and 2.03 g of PLGA-100) and DL-methadone base (0.771 and 0.900 g, respectively) were dissolved in 20 ml of dichloromethane (Merck) and poured into 800 ml of a 0.1% aqueous polyvinyl alcohol (Mw 30 000–70 000, Sigma) solution. Emulsification by turbine homogenizer (IKA mod. Ultra-Turrax T-25, head type KR) at 8000 rpm for 2 min. Solvent evaporation was completed after 4 h, then the microspheres were collected by centrifugation and freeze-dried.

Tablets (3×12 mm) were prepared by compressing 500 mg of a 50 : 50 mixture of PLGA-14 and methadone base at a force of 5 tons using a Carver hydraulic press at room temperature, for 5 min. These tablets were cut to obtain implants ($3 \times 2 \times 1$ mm) of 10 ± 0.1 mg.

2.3. Characterization of formulations

Accurately weighed implant or freeze-dried microspheres were dissolved in CH_2Cl_2, and the methadone content determined by spectrophotometry at 290 nm. Each sample was assayed in triplicate.

The non-dissolved methadone content in the microsphere samples was determined by DSC analyses (Mettler Toledo DSC821°), using a methadone standard for quantification. Samples in sealed aluminum pans were heated at 5°C/min in a nitrogen atmosphere.

Microspheres suspended in aqueous 0.1% PVA solution were sized by laser diffractometry using a Coulter® LS100, after bath sonication for 30 s to deaggregate.

The microsphere morphology was examined by scanning electron microscopy (JEOL JSM-6300, Tokyo).

2.4. In vitro release assay

Microspheres (20 mg) or an implant (10 mg) were suspended in 100 ml in 0.066 M phosphate buffer pH 7.4 ($\mu = 0.264$) containing 0.001% Tween 80, at 37°C with magnetic stirring (50 rpm). Each assay was replicated three times. Methadone release was assayed by spectrophotometry ($\lambda = 207$ nm) in acid medium.

2.5. In vivo release assay

The experiments were carried out in male Swiss mice (28–32 g) purchased from the Central University Animal House. The animals had free access to food and water before and during the experimental period.

To determine the pharmacokinetics of methadone in mice, a solution of methadone chloride, corresponding to 12 mg/kg of methadone base, in 50 µl of saline solution was injected subcutaneously between their shoulder-blades. The appropriate microsphere dose was administered in 50 µl of aqueous 1% Pluronic® F-68 solution, by the same route or the intraperitoneal route using a 20-gauge needle. The implants were surgically placed in the back of the mice, previously anesthetized with ether.

For each time point sampled, a group of four mice were anesthetized with ether and blood samples obtained by cardiac puncture. The serum was separated by centrifugation and stored at −80°C for TDX analysis. The specific modification [9] was used, to adapt the commercially available TDX kit (ABBOT) for urinary methadone assay to the serum determinations.

2.6. Degradation assay

In vitro, the microspheres were filtered and dried in vacuum dessicator conditions for 24 h and for the in vivo

assay, microsphere samples were recovered from the subcutaneous tissue and freeze-dried before analysis. Dried samples were dissolved in THF and analyzed by GPC.

2.7. Pharmacokinetic analysis

The pharmacokinetic model used consists of two compartments as shown in Scheme 1.

The input function for the sustained release systems include two processes: the release kinetics, $I(t)$, and a first-order absorption. The model for methadone solution administered subcutaneously is given by Eq. (1).

$$C_p(t) = \frac{FX_0k_a}{V_1}\left(\frac{k_{22}-k_a}{(k_a-\alpha)(k_a-\beta)}e^{-k_at} + \frac{\alpha-k_{22}}{(k_a-\alpha)(\alpha-\beta)}e^{-\alpha t} + \frac{k_{22}-\beta}{(k_a-\beta)(\alpha-\beta)}e^{-\beta t}\right). \quad (1)$$

As information from IV administration is not available, the exponential terms k_a, α and β are not distinguishable, it was expressed by Eq. (2).

$$c_p = \sum_{i=1}^{3} A_i e^{-\lambda_i t}. \quad (2)$$

Since $A_1 + A_2 + A_3 = 0$, and

$$\lambda_1 + \lambda_2 + \lambda_3 = k_{11} + k_{22} + k_a,$$
$$\lambda_1\lambda_2 + \lambda_1\lambda_3 + \lambda_2\lambda_3 = k_a(k_{11} + k_{22}) + k_{11}k_{22} - \gamma_2$$
$$\lambda_1\lambda_2\lambda_3 = k_a(k_{11}k_{22} - \gamma_2), \quad (3)$$

the identifiable parameter combinations for the two compartment model with IV bolus administration [10] are

$$k_{11} = k_{10} + k_{12},$$
$$k_{22} = k_{20} + k_{21},$$
$$\gamma_2 = k_{12}k_{21}.$$

However, after extravasal administration, only k_{22} is identifiable and can be estimated by Eq. (6).

To develop the equation corresponding to $I(t)$ we have applied the method described by Hwang et al. [11]. Let $G(t)$ be the weighting function obtained by dividing coefficient A_i (2) by the dose

$$G(t) = \sum_{i=1}^{3} a_i e^{-\lambda_i t}. \quad (4)$$

Since

$$G'(0) = -\sum_{i=1}^{3} a_i\lambda_i \quad (5)$$

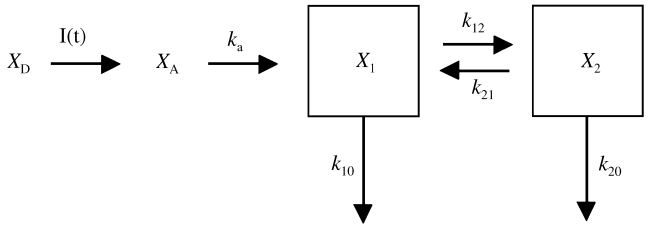
and

$$k_{22} = \frac{1}{G'(0)}\sum_{\substack{i=1\\ i\neq j\neq k}}^{3} a_i\lambda_j\lambda_k. \quad (6)$$

The solution for $I(t)$ is given by Eq. (7).

$$\int_0^t I(\tau)\,d\tau = \frac{1}{A}\left[\frac{dc_p}{dt} + (\lambda_1+\lambda_2+\lambda_3-k_{22})c_p + \frac{\lambda_1\lambda_2\lambda_3}{k_{22}}\int_0^t c_p\,d\tau + (\lambda_1\lambda_2 + \lambda_1\lambda_3 + \lambda_2\lambda_3 - k_{22}(\lambda_1+\lambda_2+\lambda_3-k_{22}) - \frac{\lambda_1\lambda_2\lambda_3}{k_{22}}e^{-k_{22}t}\int_0^t c_p e^{k_{22}\tau}\,d\tau\right]. \quad (7)$$

Eq. (7) has some advantages over previously published expressions. There is no assumption made about which λ_i is the absorption rate, nor about the elimination compartment. Moreover, it is easy to implement the calculations in a spreadsheet.



Scheme 1. Pharmacokinetic model. X_D and X_A are the amounts of drug in the delivery system and in the absorption site, respectively.

3. Results and discussion

Following our previous in vitro optimization results, a similar formulation to that previously described [6] was prepared using high Mw PLA-220 with low methadone load (24%). The mean diameter of these microspheres was 23 μm.

To check the extent to which the new microspheres were similar we carried out an in vitro methadone release assay. The cumulative release profile is shown in Fig. 1, about 14% is released in 24 h and 63% in 1 week. Afterwards, the release rate dropped dramatically reaching only 73% in 2 weeks. Thus, our results indicated that these new microspheres were similar enough to those previously prepared to assess them in vivo.

To calculate the appropriate dose of microspheres needed to achieve therapeutic methadone levels in serum, we determined the pharmacokinetics of methadone in mice. Serum methadone concentrations after subcutaneous administration of methadone hydrochloride solution, equivalent to 12 mg/kg of methadone base, were fitted to a bicompartmental model (Eq. (1)) ($C_{serum} = 4018.2e^{-4.138t} + 920.0e^{-0.535t} - 4938.2e^{-8.689t}$) using a non-linear regression program (Fig. 2),

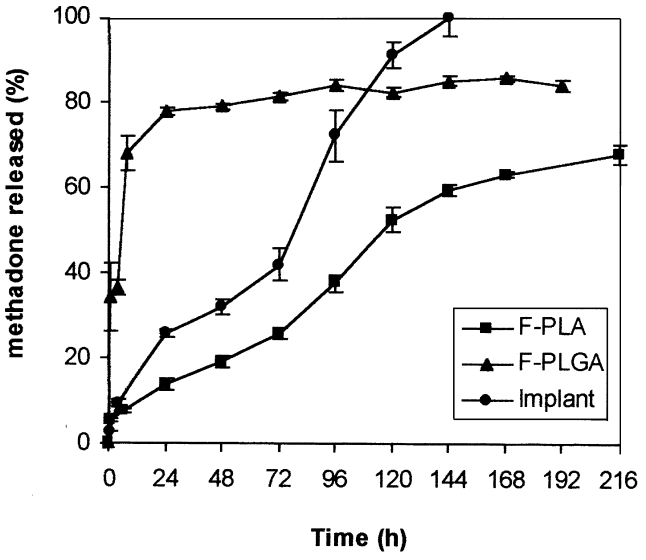


Fig. 1. Cumulative in vitro release profiles of the PLA, PLGA microspheres and the implant.

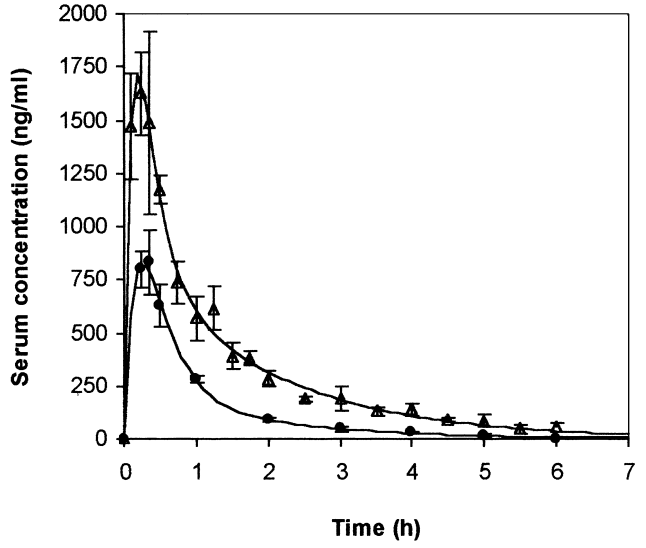


Fig. 2. Serum level curves of methadone obtained after a single subcutaneous administration of 12 mg/kg to non-metabolic induced mice (▲) and to previous metabolic induced mice (12 mg/kg each 12 h during four days) (●).

$Cl_{(serum)} = 5.66\,l/h\,kg$ and $t_{1/2} = 1.29\,h$. The absorption–disposition function obtained should allow us to calculate the amount of methadone delivered in vivo from the controlled release formulation using the deconvolution procedure proposed by Hwang et al. [11], according to the pharmacokinetic model described in Material and Methods.

To maintain the recommended 200 ng/ml of methadone in serum [12] for 7 days in mice of 30 g body weight, the microsphere dose of F-PLA to be administered was 20 mg. Methadone serum levels obtained after the subcutaneous administration of this dose were much lower than those expected from the in vitro results. Thus, the serum methadone concentrations after the first few hours dropped to values 4 times lower than those needed for inhibition of the withdrawal syndrome. The bioavailability of this formulation (31.3%) was calculated from the quotient of the cumulative amount of methadone released from the microspheres, estimated by deconvolution (Eq. (7)), and the methadone dose administered via the microspheres.

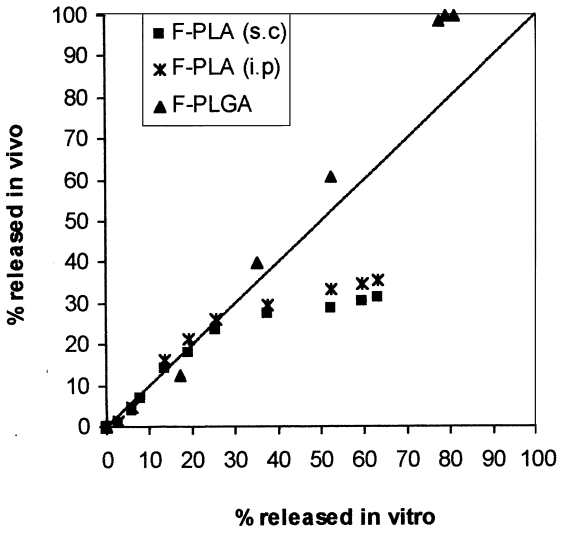


Fig. 3. In vivo–in vitro correlation obtained with the microsphere formulations.

To improve the bioavailability of methadone microspheres, a new polymer, PLGA, was used to prepare another formulation (F-PLGA). We chose PLGA because it is more hydrophilic than PLA and shows a faster hydrolytic degradation in aqueous media that might be further increased by the catalytic effect of a basic drug like methadone [13,14]. The degradation of PLGA polymer results in a loss of mass, giving a more porous microsphere structure and consequently an enhanced methadone release.

Formulation F-PLGA, prepared with PLGA (Mw 100 000) and 30% of methadone load (mean diameter 15 μm), showed an in vitro methadone release of 81% in 3 days and a burst effect of 34% (Fig. 1). The bioavailability of this formulation was 99.7%, but methadone levels were only detected during the first 48 h, making this formulation unsuitable for sustained drug release. However, the in vivo–in vitro correlation [15] for the percentage released from this formulation seemed close to the ideal (Fig. 3), while that for F-PLA showed a linear relationship up to 72 h, after which a high deviation was observed (Fig. 3).

To better understand the in vitro and in vivo methadone release results from the microspheres, more

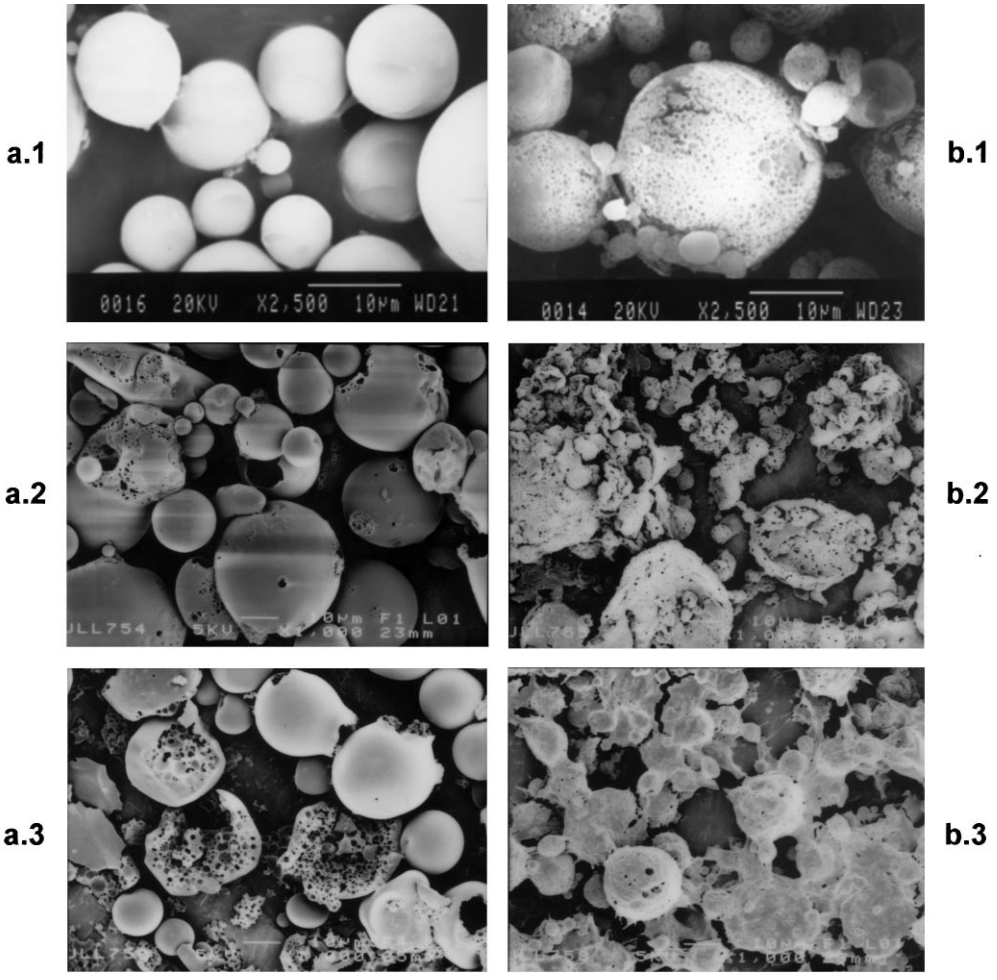


Fig. 4. Microphotograph of microspheres: freshly prepared for: (a1) F-PLA; (b1) F-PLGA. After 1 week in vitro incubation (a2) F-PLA; (b2) F-PLGA. After 1 week s.c. administration (a3) F-PLA; (b3) F-PLGA.

detailed characterization studies were performed. These studies included: polymer degradation during the release assays, morphological monitoring by SEM, and DSC analysis to measure the drug distribution in the formulations.

When microphotographs of each formulation were obtained by SEM (Fig. 4), F-PLA microsphere surfaces were apparently smooth and non-porous whereas F-PLGA microspheres were porous. No methadone crystals were adsorbed or included on the microsphere surfaces.

To quantify the amount of non-dissolved methadone in the polymer, DSC measurements were carried out. The results are shown in Fig. 5 and Table 1. Methadone base melting point (T_m) and PLGA vitreous transition temperature (T_g) are clearly identifiable, whereas PLA T_g was not detected. The non-dissolved methadone was calculated by the peak area ratio at its melting point. Results indicated that 10 and 48 % of methadone was not dissolved in the polymer for F-PLA and F-PLGA, respectively. Methadone released in the first few hours was probably dependent on non-dissolved methadone in the polymer.

In vivo and in vitro microsphere degradation studies were carried out to elucidate if any difference in the degradation process could be the cause of the in vivo–in vitro release rate difference observed in F-PLA. In both F-PLA and F-PLGA the in vivo and in vitro degradation profiles were similar (Fig. 6). SEM of microspheres after in vitro and in vivo release (Fig. 4) are similar and they lose their shape in both cases, so the degradation process could not be the cause of the observed differences.

To determine the possible effect of administration route on methadone absorption, intraperitoneal (i.p.) injection was chosen as an alternative parenteral route for microsphere administration. When F-PLA was administered by the i.p. route following the same schedule as the s.c. administration, both serum curves were similar. The bioavailability after i.p. administration estimated by deconvolution (35.7%) was similar to that by s.c. injection (31.3%).

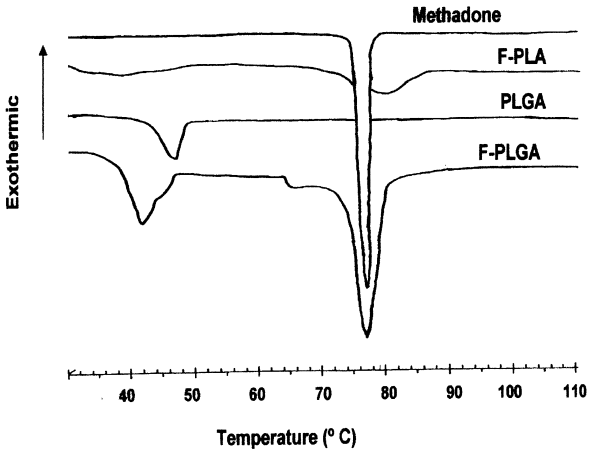


Fig. 5. The DSC thermograms of methadone base, the PLGA polymer and the F-PLA and F-PLGA microsphere formulations.

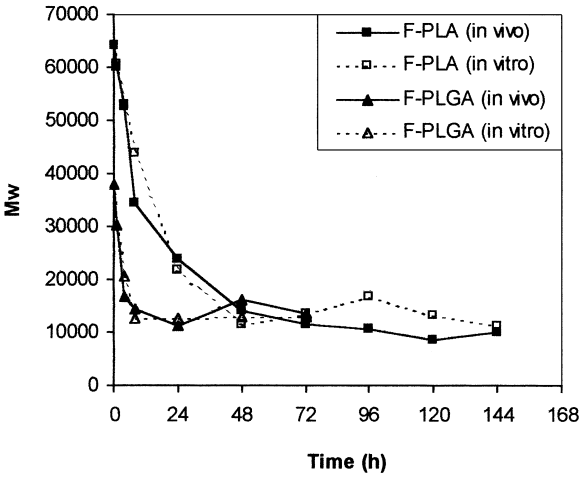


Fig. 6. In vivo and in vitro degradation profiles of microsphere formulations during the release assays.

Table 1
Results of DSC analyses

	Peak (°C)	Area (J/g)	Peak (°C)	Area (J/g)	No dissolved methadone (%)
Methadone			78.7	87.3	
PLA + methadone			77.4	85.8	98.2
F-PLA			84.0	9.1	10.4
PLGA polymer	47.4	4.8			
F-PLGA	42.1	6.7	77.0	42.1	48.2

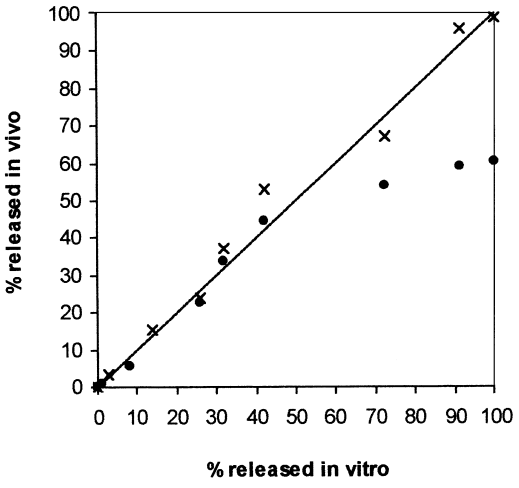


Fig. 7. In vivo–in vitro correlation obtained for the implant from the percentage of in vivo.

A histological analysis of subcutaneous tissue was performed. A non-specific mild inflammatory process during the initial hours was observed. Ten hours after administration an abscess was formed, and from the second day on a delimitation of the area was detected, becoming a cyst on the sixth day. However, these tissue reactions did not seem strong enough to prevent methadone absorption from the microspheres since a similar process was observed with F-PLGA. Furthermore, it has already been reported to occur with this kind of biodegradable polymer formulations, and drug release still occurred [16].

Some authors [17–20] have suggested that methadone could produce tolerance, due probably to an induction of its own metabolism, while others reported that this tolerance is not clear [21,22]. To check if this phenomenon was the cause of the lack of in vivo–in vitro correlation of formulation F-PLA, an implant was inserted, as above. The advantage of the implant is that it can be removed from the back of the mice and the amount of methadone remaining within it after different periods be determined at the same time as the serum methadone levels. The percentages released in vivo and in vitro were 98.7 and 100% respectively, whereas the estimated percentage released in vivo by deconvolution was only 60.1%. Fig. 7 shows a good correlation between the in vitro and in vivo percentages released, calculated from the methadone remaining in the implant. The correlation between in vitro and in vivo release estimated by deconvolution failed after approximately 72 h, due probably to the metabolic induction process.

To apply deconvolution methods, the drug absorption–disposition function must be linear and time invariant. To test this metabolic induction, another experiment was designed. To 3 groups of 9 mice, 12 mg/kg of methadone was injected subcutaneously at 12-h intervals over four days. After a 15-h wash period ($10t_{1/2}$) a new injection of 12 mg/kg was administered and blood samples were taken over the next 6 h. The results and the fitted curve ($C_{\text{serum}} = 7876.8e^{-3.685t} + 282.9e^{-0.590t} - 8159.7e^{-4.642t}$) are shown in Fig. 2, together with the

Table 2
Results of the parameters, correlation concordance coefficient (ρ_c), lower limit of the $1-\alpha$ ($\alpha=0.05$) confidence interval for ρ_c, Pearson's correlation coefficient (r) and similarity factor f_2, to evaluate the in vivo–in vitro correlation for the first 72 h, except for implant which was for 144 h

Formulation	ρ_c	Lower limit	r	f_2
F-PLA (s.c)	0.985	0.961	0.993	87.603
F-PLA (i.p)	0.985	0.947	0.991	85.658
F-PLGA (s.c)	0.927	0.904	0.997	43.156
Implant (deconvolution)	0.992	0.970	0.995	82.053
Implant (release)	0.991	0.970	0.992	65.379

serum level curve obtained with a single dose. The metabolic induction was evident, serum clearance increased from 5.66 to 14.22 l/h kg.

Due to the time-dependent process involved in methadone elimination, as has been shown, methadone clearance increases with time (Fig. 2). The deconvolution method gives us an underestimation of the real amount of methadone released and consequently an unreliable bioavailability.

To evaluate how far the linear in vivo–in vitro relationship differs from the optimum: slope = 1 and intercept = 0, we have used two parameters to compare in vitro release profiles. The similarity factor (f_2), proposed by Moore and Flanner [23] and the correlation concordance coefficient (ρ_c), proposed by Llabrés [24] to assess the equivalence of in vitro dissolution profiles fixing the lower limit of the $1-\alpha$ ($\alpha=0.05$) confidence interval for $\rho_c > 0.944$. This means that 10% is the maximum average difference accepted for each experimental point, with a prediction capability $\geqslant 95\%$. Table 2 shows the results obtained applying these procedures to the in vivo–in vitro relationship for up to 72 h for all the formulations; except for the in vivo–in vitro correlation obtained from the percentage of drug remaining within the implant, until the drug was completely released (144 h). The values for the $1-\alpha$ confidence interval for ρ_c are > 0.944 and $f_2 > 50$, indicating a good correlation, except for formulation F-PLGA, which did not achieve the minimum required values.

4. Conclusions

We have shown that the deconvolution method can predict the amount of drug released from such delivery systems and that it does not work when a time-dependent process is involved. High values of the concordance coefficient indicated an in vivo–in vitro correlation close to the optimum. The total dose of methadone, in vivo and in vitro, is released from the implants, whereas despite the induction phenomenon the results seem to indicate an incomplete release of methadone from F-PLA microspheres. Both kinds of formulations could be useful delivery systems for methadone maintenance treatment to achieve constant levels in tolerant mice or humans.

Acknowledgements

This work was supported by the Consejería de Educación Cultura y Deportes de la Comunidad Autónoma de Canarias (PI 1998/004) Spain.

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