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European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 441–446
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Biodegradable PLGA microparticles for sustained release of a new GnRH antagonist: part II. In vivo performance

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Received 17 July 2002; accepted in revised form 29 January 2004

Abstract

Poly (DL-lactide-*co*-glycolide) microparticles (MP) containing a highly potent peptidic gonadotropin releasing hormone antagonist (degarelix) of interest in the prostate cancer indication were screened for biological performance. Efficacy was tested in a castrated male rat model at 3 doses (0.4, 1.0 and 1.5 mg/kg) and assessed as inhibition of luteinizing hormone (LH) secretion. When increasing the dose, onset of inhibition was faster, inhibition was more intense, and duration of action was prolonged. The MP type was also highly influent. If spray-dried and microextrusion particles exhibited comparable potencies, double emulsion microspheres were significantly less potent, both for onset and duration of inhibition. Interestingly, for the latter type it was found that the degarelix fraction released upon reconstitution in the solution for injection was significantly lower (max 0.3%), in comparison to spray-dried MP (max 2%) or microextrusion (max 4%). With the three types of particles, increasing peptide content was detrimental for duration of action, but only little difference was noticed between particles based on different polymers. At 1.5 mg/kg, LH inhibition was achieved over 36 days with spray-dried MP based on 75/25 lactate/glycolate copolymer. This was superior by 1 week to the performance of unformulated degarelix given at the same dose.

Keywords: Peptide drug delivery; Gonadotropin releasing hormone antagonist; Degarelix; Poly (lactic acid) and copolymers; Controlled release; Microspheres; Extrusion

1. Introduction

Synthetic gonadotropin-releasing hormone (GnRH) antagonists are recognized as potent drug candidates to treat sex hormone-dependent diseases by inhibiting the production of sex hormones from the onset of treatment [1,2]. A number of these peptides are currently in advanced clinical studies for the treatment of prostate cancer, and are also marketed for infertility. Degarelix is a new decapeptide antagonist of the pituitary GnRH receptor with increased hydrophilicity and a lower propensity to self-aggregate in aqueous solutions [3]. Therefore, unlike other antagonists with limited solubility and/or tendency to aggregate in solution [1], this novel peptide appears to be an interesting candidate for clinical investigations. Incorporation of degarelix in poly (DL-lactide-*co*-glycolide) (PLGA) microparticles (MP) was of prime interest for the development of a 3-month formulation for the prostate cancer indication. If such microspheres containing GnRH agonists are already in the market (e.g. Lupron®, Decapeptyl®) [4–6], GnRH antagonist MP have not reached this stage yet. Recently, microgranules containing the GnRH antagonist teverelix were investigated in a dog model for efficacy in terms of testosterone suppression and provided an encouraging 80-day chemical castration [7]. Orntide acetate, another GnRH antagonist, was entrapped in PLGA and PLA microspheres by solvent extraction and evaporation [8]. Biological performance in a non-castrated rat model showed a 30 and 120-day testosterone suppression. However, the doses administered as 3.0 and 8.8 mg/kg were high.

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doi:10.1016/j.ejpb.2004.01.005

This work documents the biological performance of degarelix MP previously produced with variable characteristics, good entrapment efficiency and peptide purity [9]. Biological screening was performed at low doses in a castrated rat assay recognized for its high reproducibility and ability to screen many formulations at one time [3]. The aim was to find degarelix MP able to induce a quick onset of action by down regulation of luteinizing hormone (LH) secretion to subnormal levels (approx. 1 ng/ml) in a few hours, and to maintain a consistent low level for at least 1 month. End of LH inhibition was said to occur when two consecutive increases in LH levels were found after having reached the minimum.

2. Materials and methods

2.1. Microparticles

Degarelix PLGA-microspheres were produced by double-emulsion solvent evaporation, or by spray-drying as described elsewhere [9]. Degarelix PLGA-microgranules were obtained by grinding extruded rods (2 mm external diameter) produced with a Microtruder (Randcastle Extrusion systems, Inc., Cedar Grove, NJ, USA) [9].

Degarelix content was determined by HPLC (Waters LC equipment, column YMC basic 5 μm 4.6 × 250 mm at 40 °C, mobile phase acetonitrile:water 36:64, 0.1% trifluoroacetic-acid) and particle size measurements were performed by laser light scattering (Mastersizer S long bed, Malvern Instruments Ltd, Malvern, UK). MP γ-sterilization was carried out with a ^{60}Co source, at 25 kGy, under dry ice and air atmosphere at the Swiss Federal Research Station (FAW, CH-8820 Wädenswil, Switzerland).

2.2. Methods

2.2.1. Immediate release test following microparticle resuspension

A suspension medium containing 0.2% Na-carboxymethyl cellulose (ultra low viscosity, Fluka, Buchs, Switzerland) and 0.2% Tween® 80 (Fluka, Buchs, Switzerland) was prepared in deionized water. A known amount of MP (10 mg) was suspended in the suspension medium (1 ml), and mixed on a vortex for 1 min. After centrifugation, the supernatant solution was filtered (0.22 μm), and an aliquot was analyzed by high performance liquid chromatography.

2.2.2. Bioassays

Sterile MP resuspended in the suspension medium (1 ml) were injected (200 μl) s.c. in the back of male Sprague–Dawley castrated rats ($n = 4$) (Iffa Credo, L'Arbesle, France) at a dose of 0.4, 1.0 or 1.5 mg/kg. Degarelix was given as a 10 mg/ml solution of 5% mannitol with different dosing volumes. Blood samples were taken prior to the treatment, at 3 h, days 2 and 7 and thereafter on a weekly basis until complete recovery of LH pre-treatment levels. Plasma LH levels were measured by standard radioimmunoassay with reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, Bethesda, MD), and a commercially obtained secondary antiserum. NIDDK anti-rat LH S11 sera were used. Values are expressed in terms of RP-3 reference standard. Results were expressed as mean, and for clarity, ± SD was indicated on some curves only.

3. Results and discussion

The MP investigated in this work were classified into three types according to their characteristics [9]. Table 1 describes the nature of the polymeric carrier, the particle size and the degarelix content of the various batches investigated. Briefly, type-1 MP refers to double-emulsion microspheres of large surface area due to internal and surface porosity. Type-2 MP are spray-dried microspheres, much smaller in size, but with a smooth surface. Finally, type-3 MP are non-spherical, relatively large (165 μm average diameter) and compact microgranules produced by microextrusion and grinding. The 3 MP-types are expected to elicit different degarelix release profiles and potencies because of their differences in size, shape and drug dispersion characteristics. The use of PLGA from different commercial sources was also considered for its influence on degradation rate and drug release. Polymers with different DL-lactate/glycolate composition (L/G = 50/50, 65/35, 75/25), molecular weight (M_w between 8300 and 18,800 Da) and polydispersity, but also with different purities were compared. Medisorb® copolymers contain slightly more residual monomers as determined by ^{1}H NMR (3% lactide and 0.3% glycolide) than Phusiline® and Resomer® (max 2% lactide and glycolide under limit of detection) [9].

3.1. Degarelix release during microparticles resuspension

Immediate degarelix release from the MP is important for fast absorption and binding to the GnRH receptors to provide an effective onset of inhibition of sex hormone production. Therefore, it was of interest to assess the peptide fraction washed out from the MP in the suspension medium, during resuspension.

3.1.1. Type-1 microspheres

The amount of released degarelix was very low, maximum 35 μg for 100 mg MP (Table 1, MS-1 to 10), despite the porosity exhibited by this type of particle [9]. One can therefore assume that there is little free active material available at the MP/suspension medium interface. Immediate release data were apparently not dependent on degarelix core load, since an increase from 7 to 12–14% maintained the quantities released (MS-8 to 10).

Table 1
Immediate release of degarelix after MP resuspension

Microparticles	PLGA (L/G) and commercial name	Degarelix content[a] (%)	Diameter D^b [3,4] (μm)	Degarelix released (μg/100 mg MP)	Fraction released (%)
Double emulsion solvent evaporation					
MS-1	$50/50_{resomer}$	5.5	108	N/D	N/D
MS-3	$50/50_{medisorb}$	6.9	195	19	0.3
MS-4	$65/35_{medisorb}$	7.3	84	N/D	N/D
MS-6	$75/25_{medisorb}$	6.9	135	18	0.3
MS-7	$75/25_{phusiline}$	6.8	84	N/D	N/D
MS-8	$50/50_{resomer}$	12.2	215	12	0.1
MS-9	$65/35_{medisorb}$	13.0	69	35	0.3
MS-10	$75/25_{phusiline}$	13.7	123	7	0.05
Spray-drying					
MS-11	$50/50_{resomer}$	3.6	26	21	0.6
MS-12	$50/50_{medisorb}$	3.8	63	20	0.5
MS-13	$65/35_{medisorb}$	3.5	74	43	1.3
MS-14	$75/25_{medisorb}$	3.7	–	27	0.7
MS-15	$75/25_{phusiline}$	4.0	29	12	0.3
MS-16	$50/50_{medisorb}$	7.3	151	66	0.9
MS-17	$65/35_{medisorb}$	7.8	70	160	2.0
MS-18	$75/25_{medisorb}$	7.2	24	88	1.2
MS-19	$75/25_{phusiline}$	7.4	83	130	1.8
Microextrusion					
MG-4	$50/50_{resomer\ (end\ capped)}$	2.8	182	90	3.6
MG-6	$50/50_{medisorb}$	2.5	162	99	4.0
MG-7	$65/35_{medisorb}$	3.6	152	123	3.4
MG-8	$75/25_{medisorb}$	2.8	159	82	2.9
MG-9	$75/25_{phusiline}$	3.4	176	113	3.3

[a] Peptide content (PC) from HPLC [9].
[b] Determined by laser light scattering [9].

Consequently, the fraction released was even lower with the higher loaded MP (max 0.3%).

3.1.2. Type-2 microspheres

Comparable amounts of active (i.e. 12–43 μg/100 mg MP) were washed out with MP bearing only half the peptide content in comparison to type-1 (Table 1, MS-11 to 15). Therefore, the fraction released was noticeably higher (max 1.3%). The atomization process applied to an emulsion may leave some peptide material on the MP surface [10]. When the MP-degarelix content was higher (~8%, MS-16 to 19), a significant increase in the amount of degarelix released was found (66–160 μg/100 mg MP). The fraction released then was between 1 and 2%.

3.1.3. Type-3 microparticles

A low degarelix content (max 3.6%) was able to induce a relatively intense immediate release comparable to type-2 particles of medium loading (Table 1, MG-4 to 9). The fraction released was in the range of 3–4% (i.e. ~100 μg/100 mg MP). In this case, one can assume that the lower surface area (large particles, no porosity) is compensated by the gross dispersion of the peptide since the active was incorporated as a physical mix [9]. Grinding the material may therefore, leave degarelix microdomains directly onto the surface of the microgranules, a situation which favors dissolution of the hydrophilic GnRH antagonist during MP resuspension.

From these results it is clear that significant amounts of free degarelix are injected following MP resuspension, particularly with MP obtained by spray-drying (type-2) and microextrusion (type-3). A short lag time in the release was shown to be detrimental to the performance of MP containing a GnRH superagonist [5]. In our case, the presence of free degarelix in the suspension for injection should trigger a quick onset of hormone down regulation. From this perspective, types 2 and 3 MP look particularly promising.

3.2. Pharmacodynamics of degarelix microparticles in castrated rats

3.2.1. Administration at a dose of 0.4 mg/kg

Double emulsion (type-1) and spray-dried (type-2) MP based on slow-degrading Phusiline® PLA37.5GA25 (MS-7, 10 and 15) and fast-degrading Resomer® RG502H (MS-1, 8 and 11) copolymers were first compared for efficacy (Fig. 1). Potency was formulation-technology-driven rather than dependent on PLGA type or degarelix core loading. This was already clear for the onset of action. Whereas a significant LH down regulation was observed within 3 h with type-2 MP ($C_pLH < 1.0$ ng/ml), LH-inhibition was

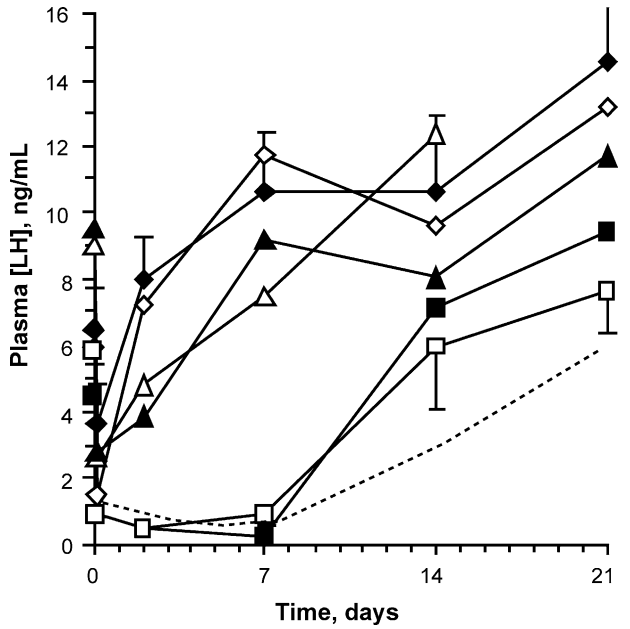

Fig. 1. Inhibition of LH after microparticles administration at 0.4 mg/kg in castrated rats. Double emulsion: MS-1 (△); MS-8 (▲); MS-7 (◇); MS-10 (◆). Spray-dried: MS-15 (□); MS-11 (■). Degarelix 10 mg/ml in 5% mannitol (- - -).

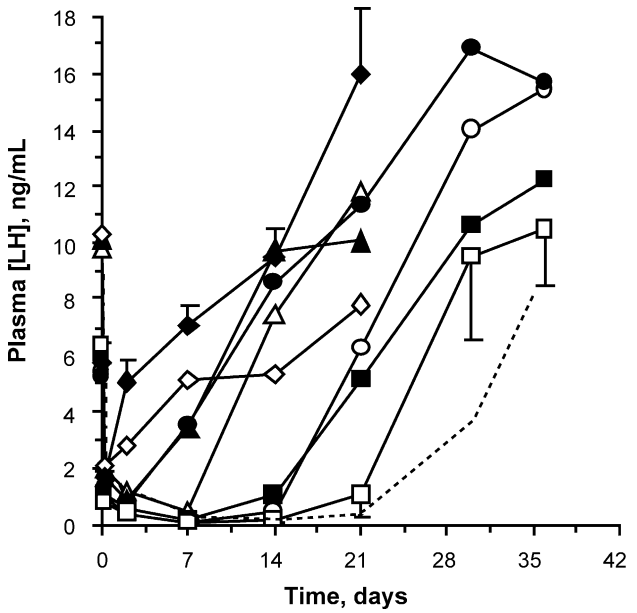

Fig. 2. Inhibition of LH after microparticles administration at 1.0 mg/kg in castrated rats. Double emulsion: MS-1 (△); MS-8 (▲); MS-4 (○); MS-9 (●); MS-7 (◇); MS-10 (◆). Spray-dried: MS-15 (□); MS-11 (■). Degarelix 10 mg/ml in 5% mannitol (- - -).

less effective with all type-1 particles. Interestingly, inhibition of hormone production was better for 5.5–6.9% loaded type-1 MP (C_pLH = 1.5–2.6 ng/ml) as compared to 12.2–13.7% loading (C_pLH = 2.7–3.7 ng/ml). Differences were also seen in duration of action. LH-inhibition was not maintained for type-1 microspheres for more than 2 days in contrast with type-2 MP for which the inhibitory effect was observed over 7 days (MS-11 and 15). This correlated with the higher degarelix fraction immediately released and the microparticle characteristics (Table 1). L/G 50/50 MP were generally more potent than L/G 75/25 MP, whatever the MP type. None of the formulation was shown to be superior in duration of action to the unformulated degarelix that was able to induce a 7-day LH down regulation comparable to MS-11 and 15 (Fig. 1).

3.2.2. Administration at a dose of 1.0 mg/kg

The inhibitory effect of degarelix on LH production was dose dependent as illustrated when the dose was increased to 1.0 mg/kg with the same formulations (Fig. 2). Two Medisorb® L/G 65/35-based microspheres of type-1 were investigated as well (MS-4 and 9). Increasing the dose resulted in an immediate onset of LH down regulation in all cases (C_pLH = 0.8–2.1 ng/ml after 3 h), and the inhibitory effect was generally maintained over time, still with the exception of type-1 particles based on L/G 75/25 copolymer (MS-7 and 10) that escaped very rapidly. Maintenance of LH inhibition was seen over variable time periods, ranging from 2 to 14 days (MS-4 and 15). There was, first, an influence of the formulation technology, since as for the 0.4 mg/kg dose, the rule was that type-1 MP were generally less potent than type-2, with the exception of MS-4. At this dose, again a medium loading was more efficient than a higher loading (type-1 MP). The comparison with the unformulated degarelix (21-day LH inhibition) showed again no superiority of the PLGA MP.

3.2.3. Administration at a dose of 1.5 mg/kg

Dose dependence and MP were further investigated at 1.5 mg/kg with particles belonging to the three types, i.e. types 1-2 (Fig. 3A) and 3 (Fig. 3B). As for the 1.0 mg/kg dose, LH secretion was effectively down regulated at 3 h (C_pLH = 0.8–2.5 ng/ml) in all cases. Type-1 MP was able to maintain LH inhibition over a 2-, 7- or 14-day period depending on the batch. The best performance was exhibited by the L/G = 65/35 batch (MS-4). With type-2 MP, LH-inhibition was maintained over a longer time period, with a maximum of 36 days (MS-14). This was superior to the unformulated degarelix (~28-day LH inhibition). In this case, potency followed copolymer composition since duration of LH inhibition for 50/50, 65/35 and 75/25 Medisorb® PLGA-based microspheres were of 14, 21 and 36 days, respectively. The effect of higher degarelix content was generally found again to be negative on LH inhibition. Therefore, it was assumed that concentration-dependent physico-chemical properties of the drug were potentially involved (e.g. self-aggregation, interaction with polymeric carrier). Such side reactions have been described for several other GnRH analogues when encapsulated in PLGA carriers or after reconstitution in aqueous solution [4,11,12]. However, additional investigations are needed to clarify this point with degarelix.

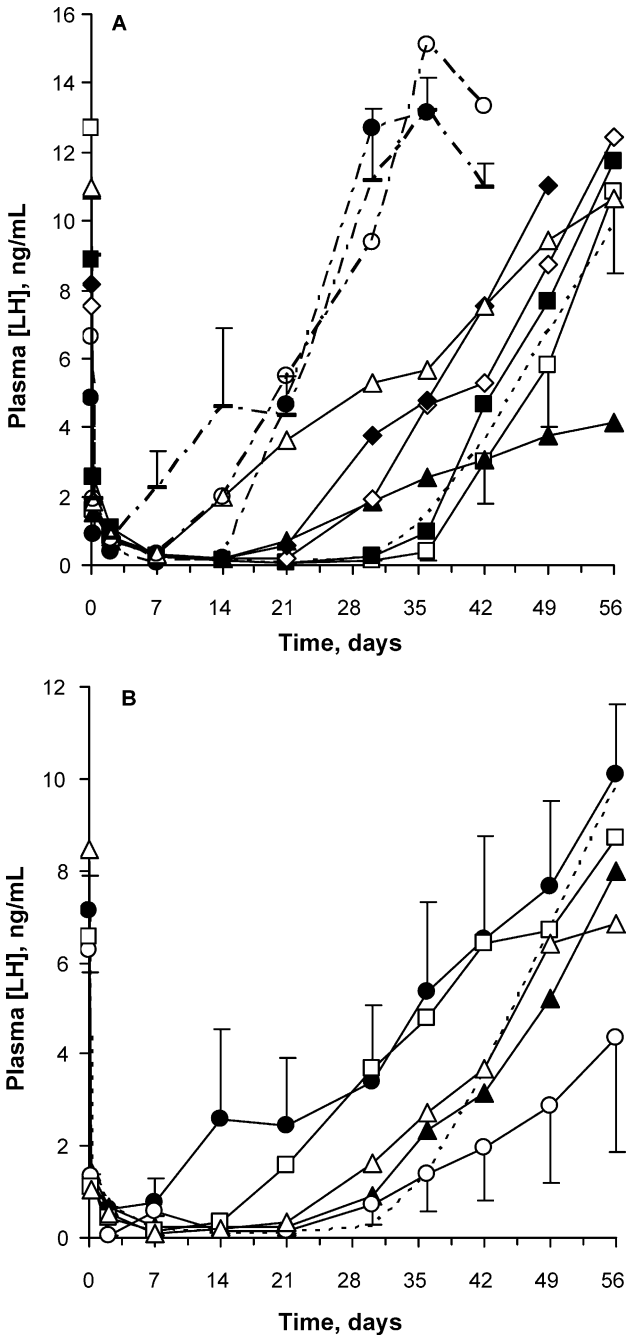

Fig. 3. Inhibition of LH after microparticles administration at 1.5 mg/kg in castrated rats. A. Double emulsion. MS-3 (–); MS-4 (○); MS-6 (●). Spray-dried: MS-12 (△); MS-16 (▲); MS-13 (◇); MS-17 (◆); MS-14 (□); MS-18 (■). B. Microextrusion: MG-6 (○); MG-7 (□); MG-8 (△); MG-4 (●); MG-9 (▲). Degarelix 10 mg/ml in 5% mannitol (- - -).

In comparison to the other MP-types, type-3 exhibited a 21-day LH inhibition for three batches, namely MG-6, MG-9 and MG-8 (Fig. 3B). It should be noted that MG-4 based on the 'hydrophobic' L/G 50/50 copolymer (end-capped) induced effective LH down regulation over 7 days only in comparison to the 'hydrophilic' copolymer of the same composition and overall characteristics which induced a 21-day LH inhibition (MG-6). This was in agreement with the better efficacy of hydrophilic copolymers described for testosterone suppression in rats with orntide GnRH antagonist microspheres based on free and end-capped PLGA 50/50 (Resomer® RG503 vs. RG503H) [8]. End-capped copolymers are expected to reduce polymer/peptide interactions if they take place at the COOH end of PLGA as suggested in the literature [4]. Our results may therefore indicate that such an interaction does not apply to degarelix.

3.2.4. Formulation technology vs. polymeric carrier composition

LH inhibition results collected after injection at 1.5 mg/kg of degarelix-MP based on PLGA taken from the same manufacturer were averaged (Fig. 4). It was obvious that type-1 MP were less effective on average than type-2 and 3 MP, thus following the immediate release test data, at least in part, since type-3 MP, although releasing the double amount of peptide, were not significantly more potent than type-2 MP. Surprisingly, potency modulation by polymeric carrier composition was not very influencing in our case (Fig. 5). This was in disagreement with other data that have shown the importance of a proper PLA/PLGA selection to achieve a prolonged hormone down regulation with GnRH agonists or antagonists [4,8]. On average, the trend was to observe a less effective onset of LH down regulation and a shorter duration of action with L/G 50/50 when compared to L/G 65/35 or 75/25, which were shown to be equivalent. One explanation for this poor incidence could be that the polymer selection was not broad enough to trigger marked differences. One other explanation may be that the intrinsic microparticle characteristics, such as peptide dispersion or local peptide

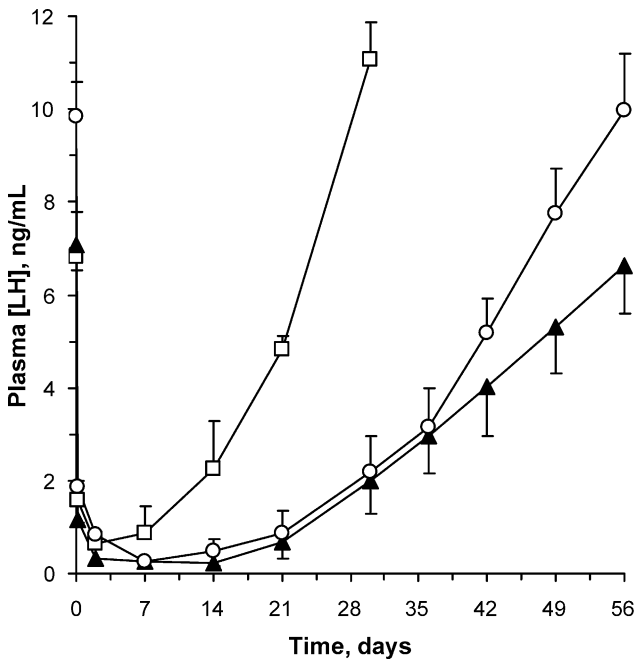

Fig. 4. Dependence on formulation technology at 1.5 mg/kg, mean LH plasma levels (all Medisorb® copolymers). Average double emulsion (□): MS-3, MS-4 and MS-6. Average spray-dried (○): MS-12, MS-13, MS-14, MS-16, MS-17 and MS18. Average microextrusion (▲): MG-6, MG-7, MG-8.

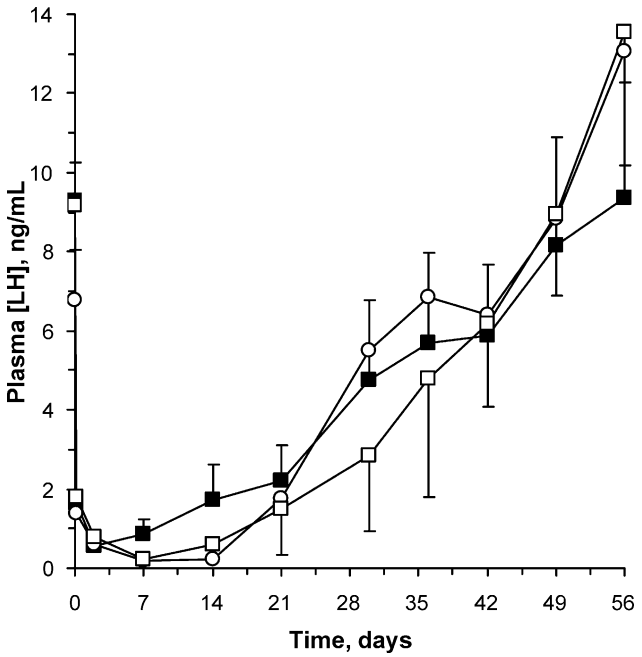

Fig. 5. Dependence on polymeric carrier composition at 1.5 mg/kg, mean LH plasma levels (all Medisorb® copolymers). Average L/G 50/50 (■): MS-3, MS-12, MS-16 and MG-6. Average L/G 65/35 (○): MS-4, MS-13, MS-17 and MG-7. Average L/G 75/25 (□): MS-6, MS-14, MS-18 and MG-8.

concentration effects played a key-role in the case of degarelix.

4. Conclusions

PLGA-MP containing degarelix were evaluated for immediate release following resuspension in the suspension vehicle, and for biological performance in castrated rats. Microparticle characteristics induced by selected formulation technologies, namely double emulsion solvent evaporation, spray-drying and microextrusion, were shown to be very important for immediate release characteristics more than drug loading or PLGA characteristics. The largest fraction released was found for microextrusion MP (4%), followed by spray-dried MP (max 2%, depending on core loading) and finally by double-emulsion (max 0.3%). Differences were explained in terms of size/shape and degarelix dispersion/accessibility in the different MP. The fraction washed out during resuspension was important for the onset and level of hormone down regulation. At the lower dose (0.4 mg/kg) double-emulsion MP were unable to completely down regulate LH. In comparison, spray-dried MP was shown to be more effective, and maintained LH inhibition over 7 days. Performance of degarelix-MP was dose dependent and improved efficacy both in onset and duration of action was evident at 1.0 and 1.5 mg/kg for all particles. At the higher dose, distinction between the different types of particles was confirmed, in the order spray-dried (36-day LH inhibition) > microextrusion (21-day LH inhibition) > double emulsion (14-day LH inhibition). A formulation showing a 1-week longer duration of action than the unformulated degarelix was found. Increasing the MP peptide content decreased potency in general, suggesting a relationship with the physico-chemical properties of the antagonist in terms of self-aggregation and/or interactions with the polymeric carrier. However, further investigations are required to clarify this point. A weak modulation of the biological performance was induced by the polymeric carrier. For instance, only small differences in potencies were exhibited by polymers differing in lactate/glycolate composition. However, end-capped copolymer was shown to be less potent than similar PLGA bearing free chain ends. Optimization of the type-2 (spray-dried) and type-3 (microextrusion) formulations for longer duration of action for the prostate cancer treatment will constitute the next important challenge, paying special attention on the physico-chemistry of degarelix in terms of self-aggregation and/or interaction with the polymeric carrier.

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