Importance of single or blended polymer types for controlled *in vitro* release and plasma levels of a somatostatin analogue entrapped in PLA/PLGA microspheres

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RUNNING TITLE: VAPREOTIDE-LOADED PLGA MICROSPHERES

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Abstract

The aim of the work was to develop biodegradable microspheres for controlled delivery of the somatostatin analogue vapreotide and maintenance of sustained plasma levels over 2 - 4 weeks after a single injection in rats. Vapreotide was microencapsulated into end-group capped and uncapped low molecular weight PLA and PLGA by spray-drying and coacervation. Microspheres were prepared from single and blended (1:1) polymer types. The microparticles were characterized for peptide loading, *in vitro* release and pharmocokinetics in rats. Spray-drying and coacervation produced microspheres in the size range of 1 - 15 μm and 10 - 70 μm, respectively, and with encapsulation efficiencies varying between 46 and 87%. *In vitro* release of vapreotide followed a regular pattern and lasted more than 4 weeks, time at which 40 – 80% of the total dose were released. Microspheres made of 14 kDa end-group uncapped PLGA50:50 or 1:1 blends of this polymer with 35 kDa end-group uncapped PLGA50:50 gave the best release profiles and yielded the most sustained plasma levels above a pre-defined 1 ng/ml over approx. 14 days. *In vitro/in vivo* correlation analyses showed for several microsphere formulations a linear correlation between the mean residence time *in vivo* and the mean dissolution time (r=0.958) and also between the amount released between 6 h and 14 days and the $AUC_{6h\text{-}14d}$ (r = 0.932). For several other parameters or time periods, no *in vitro/in vivo* correlation was found. This study demonstrates that controlled release of the vapreotide is possible *in vivo* for a duration of a least two weeks when administered i.m. to rats. These results constitute a step forward towards a twice-a-month or once-a-month microsphere-formulation for the treatment of acromegaly and neuroendocrine tumors.

Keywords: Vapreotide; PLGA microspheres; Polymer blend; Controlled release; IVIV-correlation

INTRODUCTION

Over eighty peptide and protein pharmaceuticals have been approved so far [1]. An unknown and probably much higher number of therapeutically promising peptide and protein drugs have, however, failed to reach advanced clinical testing or the market, partly because of the lack of appropriate delivery systems warranting prolonged release and sustained biological effects. To achieve optimal therapeutic effects with biopharmaceuticals, drug bioavailability at the site of action must be optimized and controlled. Most protein and peptide drugs will require prolonged bioavailability to fulfill the intended effect [2]. The biodegradable poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA) have for some time proven their suitability for controlled delivery applications [3-5]. Their adjustable physico-chemical properties such as swelling and biodegradation kinetics [6,7], or molecular interaction potential with embedded drugs [8], offer numerous possibilities in the design of controlled release systems. These properties are strongly defined by structural features such as co-polymer composition, molecular weight and nature of the chain end-groups. In PLA and PLGA, the latter are hydroxyl and carboxyl moieties (so-called uncapped polymer types) or the carboxyl may be esterified (capped polymer types). Non-esterified carboxyl end-groups cause increased hydrophilicity, faster and higher polymer swelling, faster biodegradation in aqueous environments [6] and faster and sometimes more regular, i.e., less pulsatile, release of entrapped peptides or proteins. On the same line, block-*co*-polymers of PLA or PLGA and poly(ethylene oxide) [9] or branched polyesters [10] have been developed. Alternatively, physical means to control drug release from biodegradable microspheres is through *co*-entrapment of release modifying agents [11,12], or through blending different polymer types for microencapsulation [13] or blending different microsphere types [14].

For an optimization of biodegradable microspheres towards prolonged peptide and protein delivery, the knowledge of the dose to be administered and the desired time course of concentration in serum or at the site of action is essential. Various studies attempted to establish relationships between *in vitro* release kinetics and serum drug concentration after i.m. or s.c. administration of microencapsulated drugs [3, 5, 11, 15-18]. *In vivo* release kinetics were estimated either from residual drug contents in microspheres retrieved from the excised tissue at the injection site [3, 5, 11, 15-18] or from drug input rates calculated from plasma concentrations and clearance [3, 5, 11, 15-18]. Good in vitro-in vivo (IVIV)-correlation of peptide release was generally shown. However, with the exception of the study of Ogawa *et al*. [15], none of the others demonstrated such a correlation for more than one microsphere preparation, although distinct *in vitro* release profiles from several formulations were sometimes available [11,17].

In this work, PLA and PLGA microspheres containing a somatostatin octapeptide analogue, vapreotide, (DFCYDWKVCW-NH_2) were prepared. The characteristics and activity of this drug have been described previously [19, 20]. Main formulation parameters which were tested included the polymer type (end-group capped and uncapped PLA and PLGA types), polymer blends, and the size of the microspheres. The purpose of varying these parameters was to achieve a peptide entrapment efficiency of at least 70%, to control the *in vitro* burst release and prolong the sustained release over 4 weeks, and to extend plasma levels above pre-defined 1 ng/ml in rats to 2-4 weeks after a single injection of microencapsulated peptide.

MATERIALS AND METHODS

Materials

End-group capped and uncapped poly(d,l-lactide) (PLA) and various poly(D,L-lactide-co-glycolide) (PLGA) of variable molecular weight and composition were purchased from Boehringer-Ingelheim (Ingelheim, Germany). They included end-group capped 14 kDa PLGA 50:50 (Resomer® RG502), end-group uncapped 14 and 35 kDa PLGA 50:50 (Resomer® RG502H and RG503H, respectively), end-group uncapped 17 kDa PLGA 75:25 (Resomer® RG752H), and end-group uncapped 14 kDa PLA (Resomer® 202H). The somatostatin analogue vapreotide acetate, $_D$FCY$_D$WKVCW-NH_2, was synthesized by Novabiochem, Läufelingen, Switzerland. Acetic acid was from Merck (Dietikon, Switzerland), and fetal bovine serum from Gibco BRL (Basle, Switzerland).

Microencapsulation of vapreotide

Vapreotide acetate was microencapsulated into single polymer types or into 1:1 (w/w) mixtures of two polymer types. For this, both the peptide (0.5%, w/w) and polymer (5%, w/w) were co-dissolved in 40 ml acetic acid, and this solution was spray-dried through a 0.7 mm nozzle installed in a Mini Spray-Dryer 190 (Büchi, Flawil, Switzerland) [21]. The product feed was 3 ml/min, inlet and outlet temperatures were at 50 and 40 °C, respectively, aspirator setting at 40 m^3/h, and spray-flow at 450 Nl/h.

Vapreotide acetate was also microencapsulated by coacervation [22]. Briefly, 2 ml of aqueous peptide solution (10%, w/w) was dispersed by ultrasonication in 38 ml of 5% (w/w) polymer solution in ethyl acetate. The mixture was cooled to 10 °C and kept under vigorous stirring by an impeller stirrer in a closed reactor. Upon

addition of approx. 40 ml of silicone oil (1070 mPas; Fluka, CH-Buchs) , polymer phase separation was induced, and the coacervate droplets engulfing the peptide were finally hardened in 400 ml of octamethylcyclotetrasiloxane (Abil K-4; Goldschmidt, D-Essen).

In both procedures, the microspheres were washed first with 0.1% (w/w) poloxamer 188 solution and, subsequently, with distilled water, and collected on a 0.2 μm cellulose acetate filter. After drying under reduced pressure (approx. 10 mbar) at room temperature for 24 hours, the particles were re-dispersed in hexane to break up any aggregated particles, and dried again under vacuum (10 mbar) for 12 hours.

Peptide assay

The intact peptide was analyzed by HPLC (Column Licrospher® RP-18, 4 x 250 mm, Merck, Darmstadt, Germany). The elution phase consisted of a gradient of solvent A (triethylammonium phosphate buffer of pH 2.3; TEAP) and solvent B (acetonitrile/TEAP pH 2.3, 60/40), with B increasing from 30 to 80% (v/v) within 25 min. Detection was at 215 nm.

Size distribution of microspheres

Size and size distribution of the microspheres were analyzed by light microscopy (Wild, Heerbrugg, Switzerland) and laser light diffraction (Mastersizer®, Malvern Instruments, Malvern, UK) [22, 23].

Peptide encapsulation efficiency

Vapreotide content in the microspheres was determined by HPLC [23]. The microspheres (approx. 20 mg, accurately weighed) were dissolved in 3 ml of acetonitrile, to which 2 ml of chloroform were added. The mixture was stirred

vigorously, and the peptide extracted three times with 2 ml of triethylamino phosphate buffer of pH 2.3. The loading efficiency of vapreotide was determined by the ratio of the actual amount of encapsulated peptide over the amount of peptide used to prepare the microspheres (actual loading/nominal loading).

Residual solvent in the microspheres

100 mg of dried microspheres were weighed into 5 ml screw cap vials and dissolved in 0.5 ml of 1,4-dioxane (Merck). The polymer was precipitated by adding 1.0 ml isooctane (Scharlau, EGT, Chemie, Tägerig, Switzerland), containing 2-butoxy-ethanol (Fluka) as internal standard. The coacervate dispersion was centrifuged at 3000 rpm for 5 min, and the samples cooled to –10º C for 15 min. The clear supernatant was assayed in duplicate by gas chromatography (Varian 3600 CX, Walnut Creek, CA, USA), as specified previously [24].

***In vitro* release**

Microspheres (approx. 10 mg, accurately weighed, n=3) were incubated in 4.0 ml of fetal bovine serum preserved with 0.02% (w/w) of thiomersal. Incubation took place in rotating vials at 37 °C. Due to the instability of the peptide in the release medium [21], the amount of drug released was determined indirectly by measuring the amount of drug remaining in the microspheres. At regular intervals, three vials for each microsphere formulation were withdrawn, and the polymer particles separated by centrifugation. After removal of the supernatant, the polymer mass was dried overnight at 10 mbar and room temperature. The amount of remnant vapreotide in the microspheres was determined by HPLC [23] following the aforementioned extraction procedure.

***In vivo* evaluation**

A pharmacokinetic study was performed in male Sprague-Dawley rats weighing 380-400 g (C.E.R.J., Les Genest St. Isle, France). The animals were taken care of in accordance with the UFR des Sciences Pharmaceutiques et Biologiques Local Ethical Committee and the NIH Guidelines for the Care and Use of Laboratory Animals (1985). Selected microsphere formulations were suspended in 0.5 ml of an aqueous injection vehicle (the dose was 1.5 mg of peptide per animal, calculated as vapreotide base) and injected intramuscularly in six animals per group. The animals were maintained under constant environmental conditions (22 ± 1°C; 50 ± 5% relative humidity). Food and water were available *ad libitum*. Plasma levels of vapreotide (base) were determined by double-radioimmuno assay [25] with a sensitivity of 50 pg/ml. The pharmacokinetic parameters were calculated by non-compartimental methods using the pharmacokinetic software WinNonlin 1.5 (Pharsight Corporation, Mountain View, USA). The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule from time zero to the last observed concentration and was extrapolated after the last point. Maximum plasma concentration (C_{max}) and time to peak concentration (T_{max}) were registered from the observed plasma concentration-time data. Mean residence time (MRT) was computed by moment analysis. A statistical analysis was performed in order to evaluate the influence of chemical characteristics of PLA, the microencapsulation procedure and the formulation composition (single or polymer blends) on the *in vivo* profile of vapreotide. The differences between the pharmacokinetic parameters were evaluated by a paired t-test (two-tailed) or a Wilcoxon signal-rank test. A p-value below 0.05 was considered to be statistically significant.

Analysis of *in vivo* / *in vitro* data correlation (IVIVC)

The data generated in the *in vitro* release studies and *in vivo* evaluation of microsphere formulations were used to develop the IVIV-correlation (IVIVC). Two levels of correlation, B and C, were studied according to the FDA regulations [26]. Level B IVIVC was based on statistical moment analysis. The mean *in vitro* dissolution time (MDT), an model-independent in vitro parameter that shows the mean time for vapreotide to release from the microspheres under in vitro release conditions, was compared to the mean residence time *in vivo* (MRT). The MDT was calculated according to the equation:

$$MDT = \frac{ABC_{in\ vitro}}{M_{\infty}}$$

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

Level C IVIVC represents single-point correlation between one dissolution time point and one pharmacokinetic parameter. In this work the parameters considered at this IVIVC level were: (i) amount of peptide released *in vitro* within the initial 6 hours (so-called burst release); (ii) amount released *in vitro* between time zero and day 14; (iii) amount released *in vitro* between 6 hours (after burst) and day 14; (iv) *in vivo* C_{max}; (v) area under the plasma level curves between time zero and day 14 ($AUC_{0\text{-}14d}$); (vi) AUC between 6 hours and day 14 ($AUC_{6h\text{-}14d}$). Under each IVIVC level, a linear regression analysis using an ordinary least squares method was applied to estimate the regression parameters. Correlation coefficient (r) was evaluated and the F-statistic was estimated if the slope was significantly different from zero ($P<0.05$).

RESULTS AND DISCUSSION

Microsphere size, encapsulation efficiency, residual solvent

Both microencapsulation methods of spray-drying and coacervation produced microspheres with particle diameters in the range of 1-15 μm (with the majority of particles below 5 μm) and 10-70 μm, respectively. The various polymer types affected only negligibly the particle size.

The encapsulation efficiency for the different preparations varied between 46 and 87% (Table 1). Generally, the encapsulation was higher in the microspheres made with the end-group uncapped polymer types, in agreement with data on microencapsulated capsaicin [27, 27a].

Microspheres were also prepared from blends of end-group uncapped polymers consisting of equal amounts of 14 kDa PLGA50:50 (RG502H) and either 35 kDa PLGA50:50 (RG503H), or 17 kDa PLGA75:25 (RG752H), or 14 kDa PLA (R202H). These formulations were prepared because we expected them to release the peptide in a regular pattern over a period of 3-4 weeks. With the blended polymer types, the highest encapsulation efficiency of 87% was attained with the most hydrophilic mixture of RG502H/RG503H, whereas the less hydrophilic RG502H/ RG752H or RG502H/ R202H yielded more modest entrapment (Table 1). These differences may be best explained by the differing polarity of the polymers, which is higher for uncapped than for capped PLGA; the increased polarity affords stronger peptide-polymer interactions, thereby improving vapreotide acetate encapsulation (27a).

Solvent residues in microspheres for parenteral administration may be of safety concern in therapeutic use, depending on the duration, frequency and amount of particles to be administered (Ph.Eur. III). In this work, acetic acid, a safety class-3

solvent (Ph.Eur. III) was used as common solvent for both the peptide and the polymers. Acetic acid residues in all microsphere formulations lay in the very low ppm-range, i.e., from 10 to 35 ppm. This low amount of residual acetic acid and the toxicologically uncritical nature of this solvent should obviate any safety concern.

In vitro release kinetics

Serum was used as *in vitro* release medium, because previous release experiments in different media revealed that serum mirrored best the pharmacokinetics in rats [21]. The vapreotide release profile from the spray-dried uncapped PLGA50:50 (RG502H) microspheres (size: 1-15 μm) was composed of a burst release during the first 6 hours of approx. 42% of the total dose, followed by a continuous release of additional 40% of the dose until day 28 when the experiment was terminated (Table 1; Fig. 1a). The uncapped PLGA50:50 microspheres prepared by coacervation (size: 10-70 μm) exhibited a lower burst release (14% of the total dose), while the remaining release kinetics until day 28 followed the same pattern as the spray-dried particles. The different burst release of the two differently sized microspheres (Table 1) may have been caused by the different particle surface areas [28, 29], or by a different drug distribution inside the microspheres arising from the two processes of particle formation. In agreement with previous data [30], the kinetics after the burst was not affected by the particle size, suggesting that peptide-polymer interactions and related restricted peptide diffusion mainly controlled peptide release.

The spray-dried end-group capped PLGA50:50 (RG502) microspheres released more slowly the entrapped peptide (Table 1; Fig. 1a), with a burst release of approx. 27% of the total dose (42% for uncapped PLGA50:50 microspheres). By contrast, the coacervated end-group capped PLGA50:50 microspheres produced a

higher initial burst (35%) than the spray-dried end-group capped PLGA50:50 (27%) or the coacervated end-group uncapped PLGA50:50 microspheres (14%). This does not support a relationship between microsphere size and burst release, as suggested above, but emphasizes the likely importance of peptide distribution inside the particles. For illustration, the peptide will be more evenly distributed throughout the matrix if the peptide interacts more strongly with the polymer than with the disappearing solvent. Conversely, the peptide will locate preferentially at the particles' periphery, if the affinity for the solvent (or solvent mixture in coaceravtion) is high. Release kinetics between 6 hours and day 7 for was similar for the uncapped and capped PLGA50:50 type microspheres, whereas between days 7 and 30, the latter released only 10% of peptide at an almost constant rate. Obviously, the less hydrophilic nature of the end-group capped PLGA type restricted the initial hydration of the polymeric matrix, resulting in a lower burst and a reduced polymer degradation rate, causing a slower release particularly during the time when polymer degradation rate accelerated, i.e., between days 7 and 30 [30]. This view is supported by previous data showing that the initial water uptake (up to day 14) was much greater and the initial degradation rate 6.5 times higher for the end-group uncapped PLGA50:50 than for the end-group capped PLGA50:50 type [6].

Vapreotide release profiles from the spray-dried uncapped polymer blend microspheres (Fig. 1b) resembled the profiles obtained with the single uncapped PLGA50:50 microspheres (Fig. 1a). The major differences between the two groups of formulations resided in the initial burst release, which decreased (from 42% for the RG502H) with the admixture of the higher molecular weight PLGA50:50 (RG503H) (31% burst), or the more hydrophobic PLGA 75:25 (RG752H) or PLA (R202H) (both approx. 5% burst). Interestingly, there was not significant difference in the release

rates up to day 14 between the RG502H/RG752H and the RG502H/R202H microspheres.

The selection of appropriate polymer types and encapsulation parameters (solvent, method) allowed us to meet largely the initially set criteria regarding encapsulation efficiency and *in vitro* release over one month. In particular, the microspheres prepared from RG502H alone or from a 1:1 mixture of RG502H/RG503H satisfied fully our set targets.

Pharmacokinetic study in rats

For pharmacokinetic testing, the microsphere formulations were injected intramuscularly into rats. Vapreotide serum concentrations were monitored for 28 days. The common feature of most plasma level profiles was an initial increase within 1 to 6 hours, an irregular phase between 6 hours and 7 days, and a final drop to the lower limit set at 1 ng/ml between days 7 and 14 (Fig. 2). The mean vapreotide pharmacokinetic parameters of microsphere formulations are summarized in Table 2. The obtained results showed that there are discernible differences between the microsphere formulations. Typically, spray-dried and coacervated uncapped PLGA50:50 (RG502H) microspheres produced pharmacologically relevant drug serum levels for slightly less than 14 days, whereas spray-dried capped PLGA50:50 (RG502) particles produced such levels for only two days (Fig. 2a). MRT was significantly shorter ($p=0.01$) with vapreotide formulated in microspheres using end-group capped polymers (Table 2). Further particle preparations made from other types of end-group capped PLA and PLGA gave similarly unsatisfactory plasma profiles (data not shown). In the case of coacervated RG502H, the above mentioned irregular profile phase between 1 h and 7 days consisted in a C_{max} of 16.4 ng/ml at t_{max} of 1 h, a sharp drop to 5 ng/ml between 1 and 6 h, and a slow re-increase to 12

ng/ml between days 2 and 7. For the spray-dried RG502H particles, the initial increase leveled off at 9.2 ng/ml after 6 h and remained almost constant for up to day 7. Nevertheless, there was not a significant difference in the MRT and AUC values from spray-dried and coacervated microspheres using the same polymer (p=0.1) (Table 2), suggesting that the rate and extent of vapreotide release was comparable *in vivo*, despite the differences in the plasmatic concentration-time profiles of vapreotide between the two formulations.

As promising *in vivo* results were obtained only with uncapped polymer type microspheres, we tested the feasibility of extending vapreotide plasma levels by using blends of the uncapped 14 kDa PLGA50:50 (RG502H) with more slowly degradable uncapped PLGA and PLA types. With the 50% admixture of the 35kDa uncapped PLGA50:50 (RG503H), the 17 kDa PLGA75:25 (RG752H) or the 14 kDa PLA (R202H) the MRT of vapreotide was much higher than that observed with the single polymer type microspheres (p=0.001), suggesting a longer prolonged action when vapreotide is formulated with these polymer blends. Indeed, pharmacologically relevant plasma levels were detected over an entire fortnight after drug administration (Fig. 2b). The RG502H/RG503H and RG502H/RG752H particles produced a C_{max} of 4-6 ng/ml after t_{max} of 6 hours, and the levels were kept above the desired 1 ng/ml over 14 days. The RG502H/RG503H microspheres produced a slight re-increase in plasma level between days 2 and 4, while those made of RG502H/RG752H microspheres showed this re-increase between days 4 and 7. The particles made from the polymer blend RG502H/R202H gave a slightly different profile with a C_{max} of 6 ng/ml after a t_{max} of 2 days. No re-increase in plasma levels was observed at later time points, and the levels decreased to 0.2 ng/ml within 15 days.

Analysis of *in vivo* / *in vitro* data correlation

The few reports in the literature that attempted to demonstrate a relationship between *in vitro* and *in vivo* release restricted the analysis to a single microsphere formulation exhibiting a specific *in vitro* release under adjusted release conditions [3-4, 11, 16-18], with one exception [15]. Although an IVIVC can be defined with different batches of the same formulation, two or more preparations with different release rates and consequently different absorption profiles of the drug, are recommended to obtain a more consistent IVIVC [26]. Many more studies have certainly been undertaken to test IVIVC for microencapsulated peptide drugs, but were not published, at least in some cases because of lack of correlation. The reasons for the lack of correlation may be manifold. For example, the fate of the released peptide *in vivo* affects greatly plasma levels of intact and free peptide. The kinetics and pathways of degradation, protein binding and other clearance processes, e.g. lymphatic, re-adsorption to the polymer, may differ between peptide released from microspheres injected intramuscularly and peptide injected in solution. Particularly lymphatic clearance (particles can be readily phagocytosed) and peptide re-adsorption onto the remnant polymer may alter long-term bioavailability.

Drug absorption from a particulate delivery system after intramuscular administration depends on the release of the drug from the formulation, the permeability across the tissue barriers, and the dissolution of the drug under physiological conditions. In the case of vapreotide-loaded microparticles, drug dissolution may be the rate-limiting step for the *in vivo* ADME and a linear relationship between *in vivo* disposition and the *in vitro* release from microspheres can be expected. Two levels of IVIVC (B and C) were investigated in this work, using parameters obtained from all the developed formulations. Linear correlation plots for mean *in vitro* dissolution time and *in vivo* mean residence time are shown in Fig. 3.

An acceptable correlation (r= 0.958; filled triangles in Fig. 3) was obtained with spray-dried uncapped PLGA50:50 (RG502H), coacervated uncapped PLGA50:50 (RG502H), spray-dried microspheres made from uncapped 14 + 17 kDa PLGA50:50 (RG502H/RG503H), and 14 kDa PLGA50:50 + 17 kDa PLGA75:25 (RG502H/RG752H) microspheres. However, a poor correlation coefficient was obtained when spray-dried capped PLGA50:50 (RG502) and 14 kDa PLGA50:50 + 14 kDa PLA (RG502H/R202H) formulations were subjected to IVIVC analysis (r =0.098; unfilled triangles in Fig. 3). For these latter two formulations, the MRT lagged substantially behind MDT-values, and AUC-values were approximately two-fold lower than the other microparticle formulations tested (Table 2). Thus, as MRT and AUC are pharmacokinetic parameters reflecting the residence time and amount of free vapreotide, respectively in systemic circulation, the *in vivo* vapreotide release must have been significantly delayed compared with the *in vitro* release from spray-dried capped PLGA50:50 (RG502) and 14 kDa PLGA50:50 + 14 kDa PLA (RG502H/R202H) microspheres.

On the other hand, the correlation between *in vitro* burst and either C_{max}, $AUC_{0\text{-}6h}$ or $AUC_{0\text{-}14d}$ was very poor, i.e. with r-values < 0.2. Similarly, the total amount released *in vitro* between time 0 and day 14 correlated poorly (r = 0.467) with the $AUC_{0\text{-}14d}$. The best correlation was obtained when the amount released *in vitro* between 6 h (after burst) and 14 days was compared to the $AUC_{6h\text{-}14d}$ (r = 0.932) (Fig. 4). The results of this study highlight the influence of burst effect on the development of an IVIV study for microencapsulated peptide drugs. Generally, the phase of rapid release of microencapsulated peptide drugs may be related to the onset of bulk erosion of the polymer, providing additional pores for diffusion of the entrapped peptide [5]. In this process drug release is not a rate-limiting step of *in vivo* disposition of the peptide, and a fast distribution or degradation of the drug at this

early stage can be expected. Usually, the IVIVC models failed to accurately predict the *in vivo* drug performance under burst release conditions, where the rate-limiting step for drug availability is the drug permeability across the tissue barriers, a non-linear kinetic process. Nonetheless, the development of an IVIVC model with the exclusion of the burst release data allows the accurate determination of the *in vivo* peptide release after intramuscular administration of microencapsulated peptide drugs. It is interesting to note that the second release pulse, as observed between 2 and 7 days with some formulations, does not appear to hamper the IVIVC. This may be due to the rather low release rate of the peptide during this second release pulse.

The lack of IVIVC for longer time periods (14-28 days) may be related to the different ways the peptide was detected in the *in vitro* and *in vivo* studies. Indeed, *in vitro*, the peptide that remained in the microspheres was quantified, whereas in vivo, the peptide released and detectable in serum was determined. Since vapreotide degrades relatively fast in biological fluids, it appears that the small amounts released at later time points *in vivo* were degraded before the peptide reached the central compartment.

CONCLUSION

The objective of this study was to develop vapreotide-loaded microspheres that would deliver biologically active vapreotide over a period of 2 to 4 weeks. The parameter C_{max} should be kept below approx. 20 ng/ml, and the minimum serum drug concentration to be maintained over 2 - 4 weeks should be above 1 ng/ml. The main strategies employed to control the *in vitro* release kinetics of vapreotide from the microspheres were encompassed the utilization of different types of PLA and PLGA, blending of the most promising polymer types, and variation of the microspheres size as done by using either spray-drying and coacervation methods for peptide

encapsulation. The IVIVC reported here was excellent for predicting vapreotide AUC between 6 hours and 14 days for all PLGA microspheres formulations tested. For the formulations with low burst, this should allow to predict *in vivo* results from *in vitro* observations, permitting a more rapid and more efficient screening of different preparations. With the best formulation, twice-a-month injectable delivery system for vapreotide was finally achieved.

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Figure legends

Figure 1. *In vitro* release profiles of vapreotide acetate from microspheres made from single polymer types (A) and polymer blends (B). A: Spray-dried uncapped PLGA50:50 (RG502H) microspheres (q); Coacervated uncapped PLGA50:50 (RG502H) microspheres (m); Spray-dried capped PLGA50:50 (RG502) microspheres (**+**). B: Spray-dried microspheres made from uncapped 14 + 17 kDa PLGA50:50 (RG502H/RG503H) (u); 14 kDa PLGA50:50 + 17 kDa PLGA75:25 (RG502H/RG752H) (t);14 kDa PLGA50:50 + 14 kDa PLA (RG502H/R202H) (s).

Figure 2. Plasma levels of vapreotide acetate after intramuscular injection (1.5 mg of peptide, base equivalent) of microspheres made from single polymer types (A) and polymer blends (B). A: Spray-dried uncapped PLGA50:50 (RG502H) microspheres (q); Coacervated uncapped PLGA50:50 (RG502H) microspheres (m); Spray-dried capped PLGA50:50 (RG502) microspheres (**+**). B: Spray-dried microspheres made from uncapped 14 + 17 kDa PLGA50:50 (RG502H/RG503H) (u); 14 kDa PLGA50:50 + 17 kDa PLGA75:25 (RG502H/RG752H) (t);14 kDa PLGA50:50 + 14 kDa PLA (RG502H/R202H) (s).

Figure 3. Relationship between the mean dissolution time of peptide (MDT) and the mean residence time of plasma levels (MRT) calculated for the same period of time. Analyzed data were from microsphere formulations made of spray-dried uncapped PLGA50:50 (RG502H), uncapped 14 + 17 kDa PLGA50:50 (RG502H/RG503H), 14 kDa PLGA50:50 + 17 kDa PLGA75:25 (RG502H/RG752H) and coacervated uncapped PLGA50:50 (RG502H) (r=0.958) (all s), and from spray-dried capped

PLGA50:50 (RG502), and 14 kDa PLGA50:50 + 14 kDa PLA (RG502H/R202H) (r=0.098) (8).

Figure 4. Relationship between the amount of peptide released after the initial burst until day 14 and the AUC of plasma calculated for the same period of time (r=0.932).

Table 1: Formulation parameters of various microsphere (MS) types loaded with vapreotide. The nominal drug loading was 10%, relative to the microsphere mass.

Polymer type (Resomer®)[1]	Preparation Method [2]	Encapsulation efficiency (%)	Burst release within initial 6 h (ng/mg MS)	Amount released from 6h to 14 d (ng/mg MS)
RG502H	SD	77 ± 6	42.5 ± 4.5	30.5± 5.2
RG502H	CO	75 ± 5	14.2 ± 1.7	28.5 ± 1.5
RG502	SD	46 ± 2	27.0 ± 2.6	14.0 ± 2.7
RG502	CO	66 ± 2	35.5 ± 2.3	0.5 ± 1.6
RG502H + RG503H	SD	87 ± 2	31.6 ± 2.4	23.4 ± 2.5
RG502H + RG752H	SD	63 ± 3	7.0 ± 2.6	23.0 ± 4.3
RG502H + R202H	SD	65 ± 2	8.0 ± 4.1	20.0 ± 3.8

[1] RG502H: 14 kDa end-group uncapped PLGA50:50; RG502: 14 kDa end-group capped PLGA50:50; 503H: 35 kDa end-group uncapped PLGA50:50; RG752H: 17 kDa end-group uncapped PLGA75:25; 14 kDa end-group uncapped PLA.

[2] SD: Spray-drying; CO: Coacervation

Table 2: Vapreotide pharmacokinetic parameters after intramuscular administration of different microsphere formulation types loaded with vapreotide. The dose was 1.5 mg of peptide per animal, calculated as vapreotide base.

Polymer type (Resomer®)[1]	Preparation Method [2]	C_{MAX} (ng/ml)	t_{MAX} (h)	AUC (ng*day/ml)	MRT (h)
RG502H	SD	9.2 ± 2.8	3.0 ± 2.6	77.6 ± 24.5	4.8 ± 0.6
RG502H	CO	16.4 ± 2.5	0.042 ± 0	68.7 ± 34.2	5.0 ± 0.7
RG502	SD	28.3 ± 4.5	0.042 ± 0	20.3 ± 2.9	2.2 ± 0.7
RG502H + RG503H	SD	5.2 ± 0.5	0.042 ± 0	43.7 ± 6.4	6.5 ± 0.3
RG502H + RG752H	SD	5.1 ± 3.1	2.9 ± 3.5	40.2 ± 27.2	6.5 ± 2.2
RG502H + R202H	SD	7.5 ± 4.3	0.87 ± 0.9	28.4 ± 17.6	7.5 ± 1.8

[1] RG502H: 14 kDa end-group uncapped PLGA50:50; RG502: 14 kDa end-group capped PLGA50:50; 503H: 35 kDa end-group uncapped PLGA50:50; RG752H: 17 kDa end-group uncapped PLGA75:25; 14 kDa end-group uncapped PLA.

[2] SD: Spray-drying; CO: Coacervation

Fig. 1

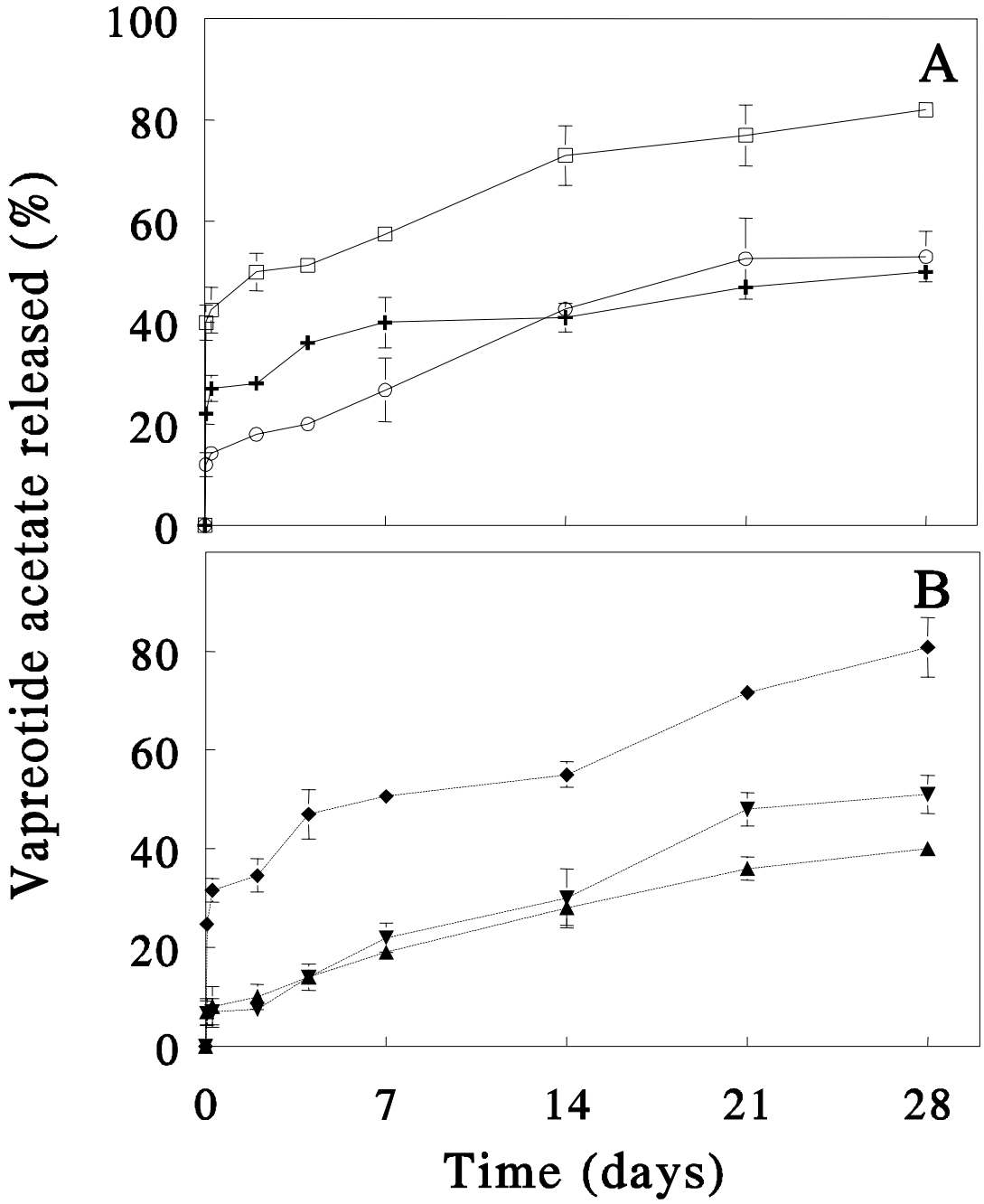

A
B
Vapreotide acetate released (%)
Time (days)
0
20
40
60
80
100
0
7
14
21
28

Fig. 2

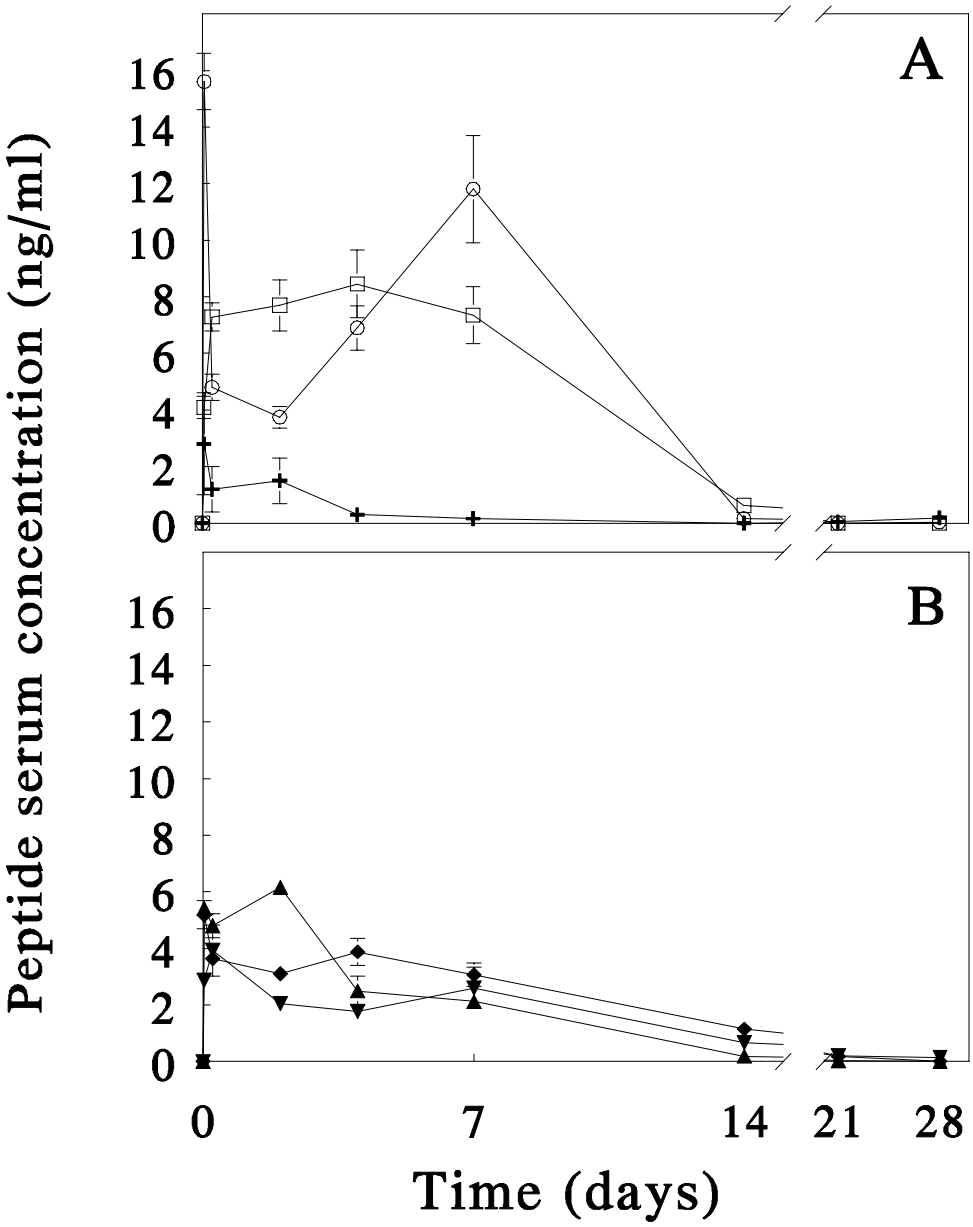

A
B
16
14
12
10
8
6
4
2
0
0
7
14
21
28
Peptide serum concentration (ng/ml)
Time (days)

Fig. 3

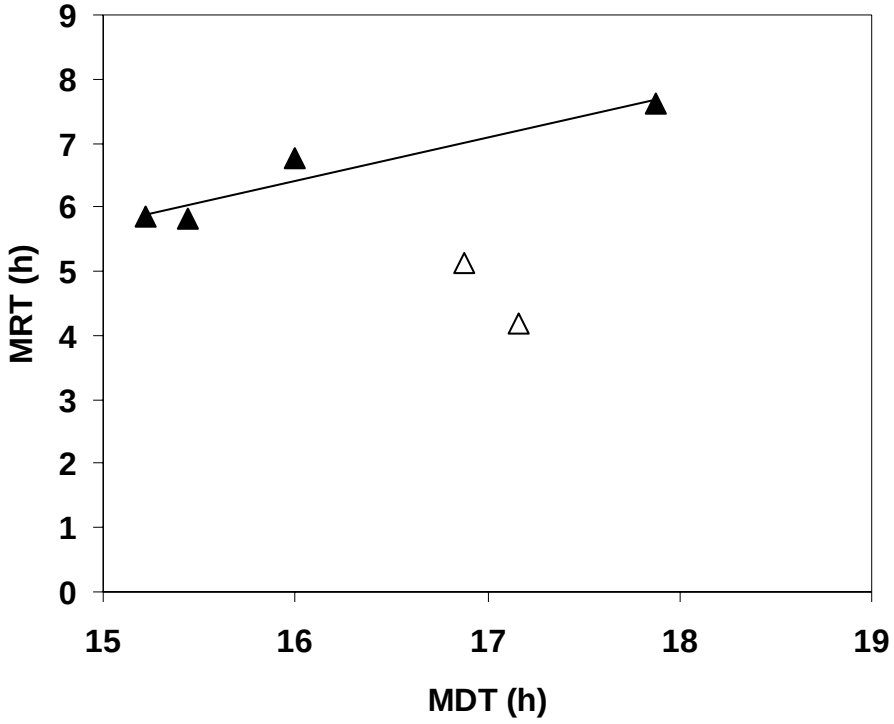
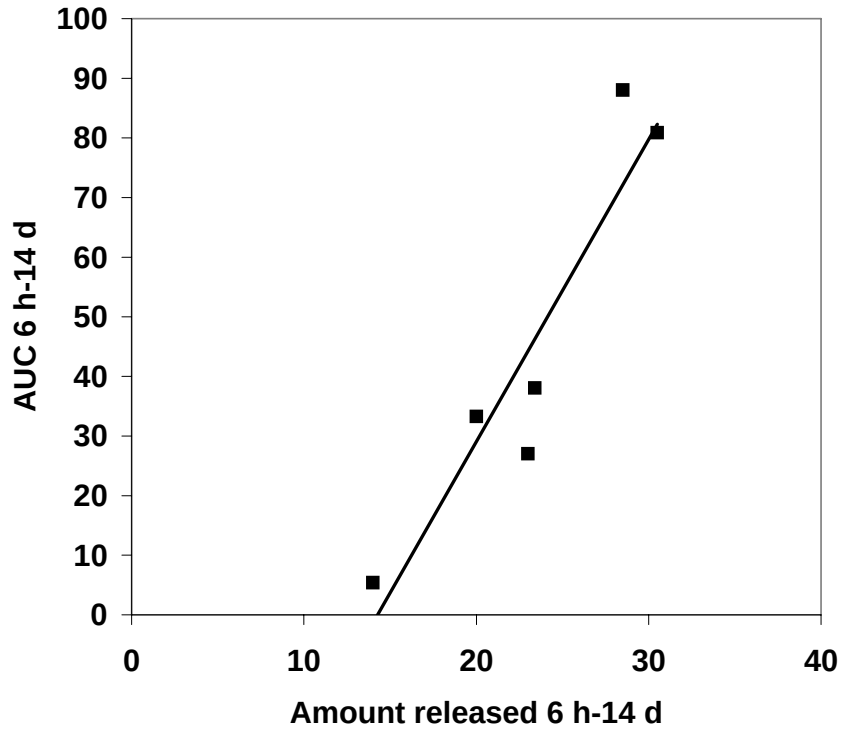


Fig. 4


100
90
80
70
60
50
40
30
20
10
0
AUC 6 h-14 d
0
10
20
30
40
Amount released 6 h-14 d