International Journal of Pharmaceutics, 99 (1993) 263–273

IJP 03255

Development of biodegradable microspheres and nanospheres for the controlled release of cyclosporin A

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(Received 1 December 1992)
(Modified version received 26 February 1993)
(Accepted 12 March 1993)

Key words: Poly(DL-lactide-co-glycolide); Microsphere; Nanosphere; Controlled release; Peptide delivery system; Cyclosporin A; Immunosuppressive agent

Summary

With the aim to develop a new controlled release dosage form of administration for cyclosporin A (CyA), several formulations of CyA-loaded microspheres and nanospheres based on poly(DL-lactide-co-glycolide) (PLGA) were designed. Microspheres and nanospheres, prepared by the solvent evaporation process, were characterized with respect to morphology, size distribution, drug content, internal structure and in vitro drug release. The micro- and nanospheres designed displayed various sizes (from 0.2 to 30 μm), spherical shape and smooth surface. The loading capacity was high for all formulations although it was influenced by the microsphere size. Results obtained by reversed-phase high-performance liquid chromatography, gel-permeation chromatography, differential scanning calorimetry, X-ray powder diffraction and Fourier transform infrared spectroscopy revealed that the peptide forms a molecular dispersion in the co-polymer matrix and no chemical interaction between co-polymer and drug occurs. Release profiles of CyA from the microspheres developed displayed a biphasic shape. The duration and intensity of each phase were affected by the microsphere size and the molecular weight of PLGA. Consequently, using PLGA it is possible to design micro- and nanospheres which allow the controlled release of CyA over a prolonged period of time. This may represent an interesting approach to provide therapeutic levels of the drug for extended periods of time.

Introduction

Cyclosporin A (CyA), a highly lipophilic cyclic peptide, is the immunosuppressant of choice for the prevention of allograft rejection after transplantation of bone marrow, kidney, liver, heart, lung, pancreas and skin (Matzke and Luke, 1988). In addition, clinical investigations suggest that CyA is efficacious in the treatment of autoimmune diseases such as insulin-dependent diabetes mellitus and rheumatoid arthritis (Mauric, 1982; Borel and Gunn, 1985). However, in spite of the great therapeutic interest of this drug, several drawbacks have been reported for the currently available dosage forms of CyA (Sandimmun® intravenous and oral solution). At present, these forms are administered orally and intravenously, athough none are considered satisfactory. CyA absorption following oral administration is slow

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and highly variable (Kahan et al., 1983). This is a very important limitation since clinical experience has shown that the therapeutic index for this drug is low (Wood and Lemaire, 1985). On the other hand, to provide a loading dose before organ transplantation and during the first week of therapy, an oleous solution of this drug is administered daily, as a slow intravenous infusion (over approx. 2–6 h). This intravenous formulation contains polyoxyethylated castor oil (Cremophor EL®), which has been reported to be nephrotoxic (Luke et al., 1987) and to cause anaphilactoid reactions (Cavanak and Sucker, 1986). However, due to the difficulty of dissolving CyA in an aqueous medium no commercial alternatives to Cremophor EL® have been found.

To overcome these problems and, in an attempt to increase the therapeutic efficacy of CyA, alternative dosage forms have been suggested, including liposomes for intravenous administration (Venkataram et al., 1990) and lipophilic carriers for oral and intravenous administration (Yanagawa et al., 1989; Venkataram et al., 1990). Nevertheless, these dosage forms have not yet been widely applied for clinical use as they deliver CyA for short periods of time and have a limited in vitro and in vivo stability (Yanagawa et al., 1989). On the other hand, ethylene-vinyl acetate copolymer microspheres were also described for the controlled release of CyA (D'Souza, 1988). However, the main disadvantage of this system is related to the non-biodegradability of the polymer and, consequently, the necessity to remove it once the release process has concluded (about 1 week). In addition, these beads, due to their large size (up to 780 μm), cannot readily be administered by injection (Tice et al., 1989) and require a surgical implantation. Consequently, the development of a long-term controlled release parenteral system, which delivers CyA for an extended period following a single administration, is of considerable therapeutic and commercial interest.

Among injectable systems designed to provide controlled drug release, biodegradable microspheres based on polymers of lactic and glycolic acids and its co-polymers (PLGA) are the most acceptable. Long experience with these polymers has shown that these materials are inert and biocompatible in the physiological environment and degrade to toxicologically acceptable products (Wise et al., 1979; Visscher et al., 1987). Furthermore, at present, several formulations of injectable microspheres based on these polymers are being investigated in clinical trials and some of them are already on the market (Tice et al., 1989).

Even though a great deal of attention has recently been focused on microsphere preparation for peptide delivery, very little work concerning the physico-chemical characterization of the formulations designed has been carried out. In fact, generally the stability of the encapsulated macromolecule and its physical state in the polymeric matrix have scarcely been investigated.

On the other hand, in spite of the high potential of nanospheres, very little work has been published dealing with the preparation of PLGA nanospheres (Julienne et al., 1992). These colloidal carriers may represent a promising form for both oral and parenteral administration of CyA. In fact, the modification of the tissue distribution and pharmacokinetic parameters of a drug following intravenous injection of drug-loaded nanospheres can be exploited in terms of better drug specificity and, consequently, a reduction in the side-effects and toxicity of the drug. In addition, PLGA microspheres smaller than 5 μm have been shown to be a viable system for the targeted delivery of vaccines to the immune system when orally administered (Eldridge et al., 1990). The latter authors suggested that these microspheres were taken up by the Peyer's patches of the gut-associated lymphoid tissue and transported to the mesenteric lymph nodes. In this way, Jani et al. (1989, 1990) have shown that microspheres larger than 1 μm are taken up by the Peyer's patches less efficiently than smaller particles. Thus, according to the previously reported results, PLGA nanospheres may represent a new interesting oral dosage form for the targeted delivery of CyA.

In this study, in an attempt to develop a controlled-delivery system for CyA, microspheres and nanospheres of PLGA containing CyA were prepared by a solvent evaporation method. The in-

fluence of the polymer type and processing conditions on the physical and physico-chemical properties of these systems was carefully examined. Finally, the relationship between these characteristics and the drug release profiles was also investigated.

Materials and Methods

Materials

PLGA with a DL-lactide : glycolide molar ratio of 1 : 1 was supplied by Boehringer Ingelheim (Germany). Two samples designated as Resomer® RG503 and RG506 were chosen. Inherent viscosities in chloroform were, respectively, 0.44 and 0.80 dl/g. Polyvinyl alcohol (PVA) of average molecular weight 10 000–30 000 was purchased from Sigma (France). CyA was obtained from Sandoz (Switzerland). Other materials were reagent grade.

Preparation of PLGA microspheres and nanospheres

Empty and CyA-loaded PLGA microspheres and nanospheres were prepared by a solvent evaporation method previously described (Julienne et al., 1992). Briefly, 1.080 g of PLGA and 0.108 g of CyA were dissolved in 15 ml of methylene chloride. This solution (organic phase) was then emulsified in 135 ml of an aqueous PVA solution (0.5%) using a stirring motor with a stainless-steel propeller (Ika RW20 DZM, Janke & Kunke, Ika-werk, Germany) for 30 min or a high speed homogenizer (Polytron, Kinematica GmbH, Switzerland) for 1 min, as indicated in Table 1. Finally, the solvent was evaporated at 20°C under reduced pressure. The resulting suspensions were washed with distilled water using a filtration system (microspheres) or a tangential ultrafiltration system (Minitan®, Millipore, U.S.A.) (nanospheres) and freeze-dried.

Determination of surface morphology and microsphere size

The shape and surface characteristics of the formulations were examined by scanning electron microscopy (SEM) (Cambridge Instruments, 250 MK, AMR1000A, U.S.A.) at 20 kV. Samples were coated with gold to a thickness of 200–500 Å prior to SEM examination.

The mean diameter and the particle size distribution of the microspheres and nanospheres were measured by phase-contrast light microscopy (Olimpus BHS, Japan), according to a reference scale, and by photon correlation spectroscopy (PCS) (Zetasizer III, Malvern Instruments, U.K.), respectively.

TABLE 1

Encapsulation efficiency and mean size of CyA-loaded microspheres prepared according to the processing conditions

Formulation	PLGA i.v. [a] (dl/g)	Emulsification speed (rpm)	E.E. [d] (%)	Mean size (μm)
1	0.44	1,000 [b]	79.0 ± 0.2 [c]	28.5 ± 17.2 [2]
2	0.80	1,000 [b]	79.1 ± 0.3	30.3 ± 18.1
3	0.44	3,000 [c]	74.0 ± 0.4	0.960 ± 0.164
4	0.80	3,000 [c]	73.9 ± 0.3	1.009 ± 0.172
5	0.44	10,000 [c]	59.6 ± 0.2	0.285 ± 0.052
6	0.80	10,000 [c]	59.8 ± 0.2	0.298 ± 0.071

[a] Co-polymer inherent viscosity in chloroform.
[b] Stirring for 30 min.
[c] Homogenization for 1 min.
[d] Encapsulation efficiency.
[e] Standard deviation (6 determinations).

Evaluation of the encapsulation efficiency

The amount of CyA encapsulated was determined by reversed-phase high-performance liquid chromatography (RP-HPLC), using a procedure previously reported (Sawchuk and Cartier, 1981) and conveniently modified. A specific amount of CyA-loaded microspheres was dissolved in acetonitrile and diluted adequately prior to injection in the HPLC system (Spectra-Physics, U.S.A.). The HPLC system consisted of a P1500 pump, and a UV-1000 ultraviolet detector. Separation was achieved by using a reversed-phase column (Spherisorb ODS-2, Tracer, Teknokroma, Spain) thermostated at 75°C and a flow rate of the mobile phase of 1.0 ml/min. The mobile phase consisted of acetonitrile/water (65 : 35).

Physico-chemical characterization of CyA-loaded micro- and nanospheres

Differential scanning calorimetry (DSC)

Thermograms were obtained in the temperature range from 35 to 200°C using a Perkin-Elmer DSC-4 (Perkin-Elmer, U.S.A.). Samples in sealed aluminium pans were heated at 10°C/min in a nitrogen atmosphere.

X-ray powder diffraction

X-ray powder diffractograms were recorded using a Philips PW1710 system (Philips, U.S.A.) with Cu-$K_{2\alpha}$ radiation (40 kV, 30 mA). The scan speed was 1°/min.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of the formulations were obtained with a Cygnus 100 spectrometer (Mattson, U.S.A.). Potassium bromide pellets containing the samples were prepared prior to FT-IR analysis.

In vitro CyA release studies

A variable amount of micro- and nanospheres containing 0.48 mg of CyA were suspended in 400 ml of phosphate buffer pH 7.4 at 37°C, in order to keep the system under sink conditions during the release studies. The system was maintained under magnetic stirring and, at successive time intervals, aliquots (5 ml) of micro- and nanosphere suspensions were collected and centrifuged (Centrikon, Kontron, U.S.A.) for 1 h at 150 000 × *g*. CyA released was measured by the analytical method described above (RP-HPLC). Simultaneously, the morphology of microspheres and nanospheres collected at different times during the release study was determined by phase-contrast light microscopy and PCS, as mentioned above, and polymer molecular weight was determined by gel-permeation chromatography (GPC).

In vitro polymer degradation

The molecular weight of CyA and polymers, before and after microsphere preparation and also during in vitro release studies, was determined by GPC, using polystyrene standards (Supelco, U.S.A.) as reference samples. A GPC system (Spectra-Physics, U.S.A.) was used with a refractive index detector. The samples were freeze-dried, redissolved in chloroform, filtered and eluted with chloroform through a GPC colum (Phenogel linear, Phenomenex, U.S.A.) thermostated at 35°C and a flow rate of 1.0 ml/min.

Results and Discussion

In the present work, in order to overcome several problems associated with the use of the currently available dosage forms of CyA, we chose to explore the possibility of developing biodegradable microspheres and nanospheres.

PLGA microspheres and nanospheres containing CyA were prepared by a solvent evaporation method using different formulation conditions (as specified in Table 1). As shown in Fig. 1, microspheres were spherical in shape and had smooth surfaces. No drug crystals were observed on the surface of any of the formulations developed. Photographs also illustrated the influence of the emulsification speed on the microsphere size. As indicated in Table 1, when the emulsification speed was increased, the microspheres became smaller and the size distribution narrower. Thus, microspheres of controlled size (from 0.2 up to 30 μm) may be prepared by modifying the emulsification procedure and the agitation speed. For high emulsification rates, energetic conditions are appropriate for the maximum separation of the organic phase, producing very small droplets and, thus, allowing the formation of nanospheres (0.2 μm). On the other hand, the co-polymer molecular weight, which is directly related to the viscosity of the inner phase, was found to affect microsphere size. Consequently, high co-polymer molecular weight led to a greater particle size, due to larger drops formed during emulsification. This fact could be explained by the higher energy level that is necessary to disperse high viscosity solutions.

Table 1 also shows that, for all the formulations developed, the encapsulation efficiency (defined as the percentage of CyA encapsulated in respect to the total amount of CyA used to prepare the microspheres) was very high and was independent of the polymer molecular weight. Since CyA is a very poorly water soluble drug, it was preferentially partitioned in the organic phase of the emulsion and, consequently, very small

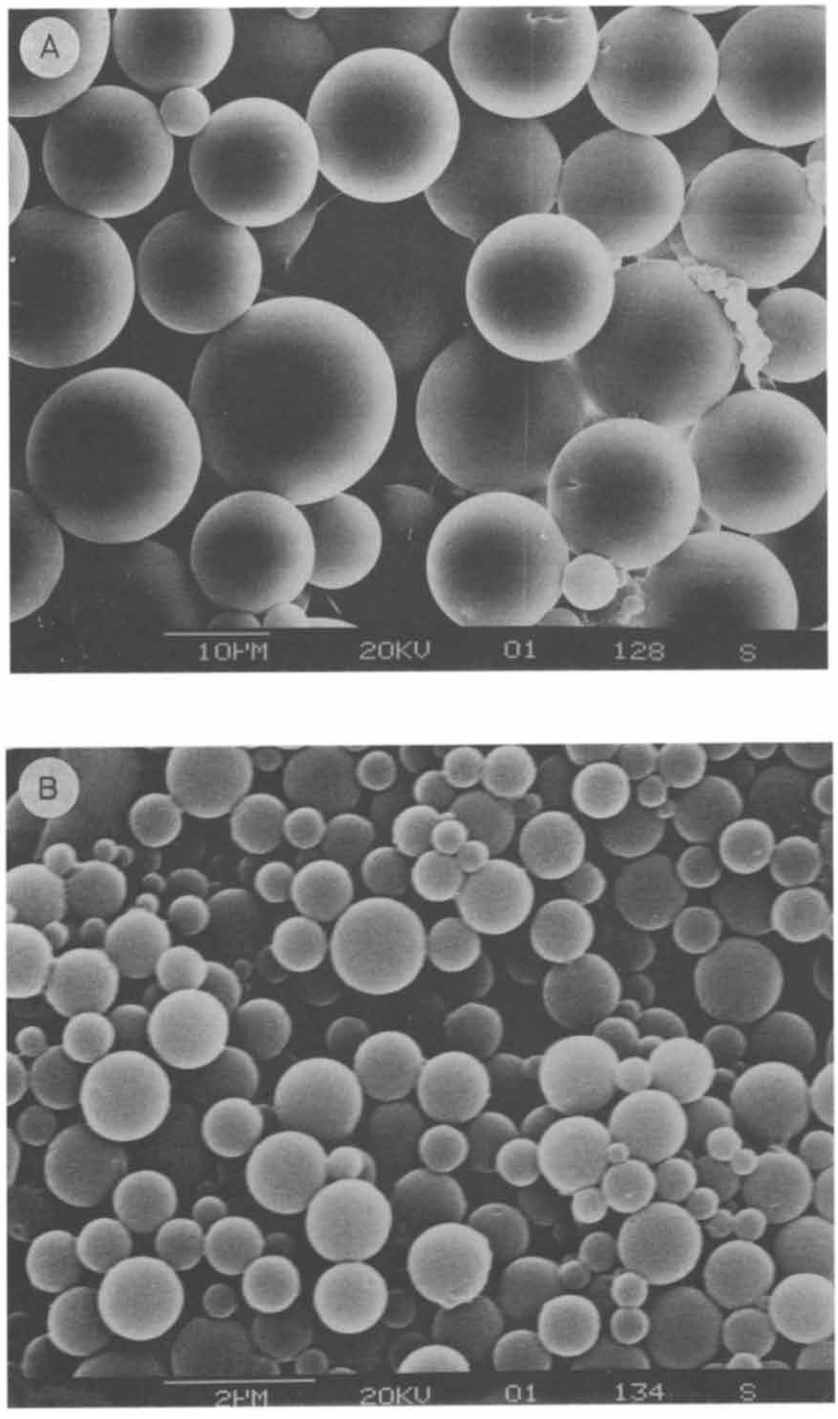

Fig. 1. Scanning electron micrographs of PLGA microspheres and nanospheres containing CyA: (A) formulation 1; (B) formulation 3.

amounts of drug are lost in the aqueous phase. In addition, the data reported in Table 1 show that the amount of CyA encapsulated increased simultaneously with the increase in microsphere size. In fact, higher loading (80%) was achieved for microspheres of larger size. This suggests that the larger surface area of the nanospheres results in greater drug loss during the microencapsulation process and washing steps. On the other hand, the yield of the process (defined as the ratio of the weight of microspheres to the initial weight of polymer and drug employed) was, in all cases, higher than 96% and was independent of the polymer molecular weight.

A second step in the development of these new CyA formulations was the characterization of the solid dispersion state of the peptide into the polymer matrix. Since CyA is completely soluble in the PLGA solution (organic phase) and slightly soluble in the aqueous phase, it could precipitate within the microspheres during polymer precipitation in either a crystalline or amorphous form, or it could be present in a molecularly dispersed state within the PLGA matrix. DSC and X-ray analysis were performed in order to characterize the physical state of the polymer and drug in the microspheres. The X-ray powder diffraction scans of the formulations based on PLGA RG503 are shown in Fig. 3. The X-ray diffraction peaks characteristic of CyA were only detected in the pattern obtained for the physical blend, whereas they were not visible in the diffraction scans corresponding to CyA-loaded microspheres and nanospheres. These results indicate that the drug is not present in its crystalline state in the formulations developed, suggesting that CyA is dispersed either molecularly or in an amorphous form in the polymer network. The same results (not shown) were obtained with PLGA RG506. This situation was previously observed for another hydrophobic drug, such as hydrocortisone (Benoît et al., 1986; Cavalier et al., 1986). On the other hand, Fig. 2 displays DSC thermograms obtained for PLGA RG503, CyA-PLGA physical mixture, and blank and loaded microspheres prepared according to the conditions specified in Table 1. PLGA showed an endotherm at 48°C corresponding to its glass

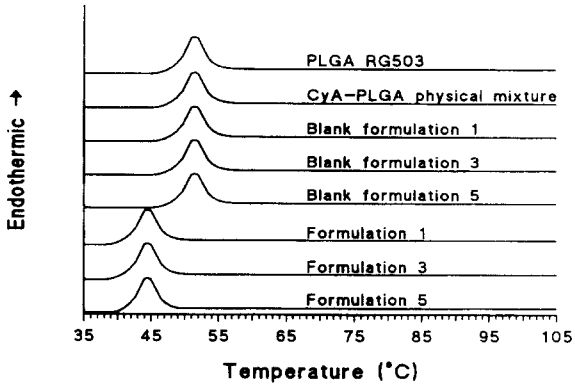

Fig. 2. DSC thermograms of PLGA, CyA-PLGA physical mixture, blank micro- and nanospheres and CyA-loaded micro- and nanospheres (formulations 1, 3 and 5).

transition temperature (T_g). No modification of the T_g of PLGA was observed for blank (non-loaded) microspheres, however, a significant decrease in the PLGA's T_g was observed for CsA-loaded microspheres and nanospheres. The same results (not shown) were obtained with the co-polymer with a higher inherent viscosity (PLGA RG506). Consequently, these data suggest that the peptide forms a molecular dispersion in the co-polymer matrix.

Once we concluded that a drug-polymer molecular dispersion existed, it was important to determine whether or not this physical dispersion can promote a chemical interaction between drug and polymer. To investigate this possibility, microsphere formulations developed were analyzed

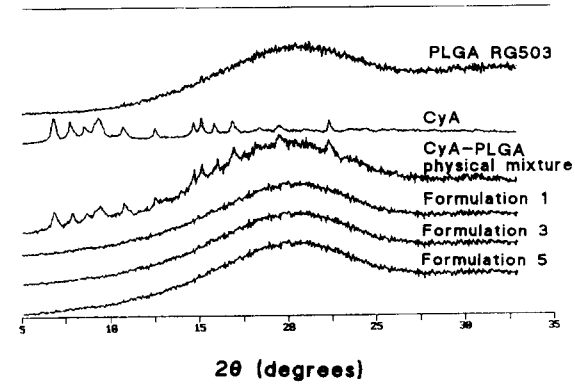

Fig. 3. X-ray powder diffractograms of PLGA, CyA, CyA-PLGA physical mixture and CyA-loaded micro- and nanospheres (formulations 1, 3 and 5).

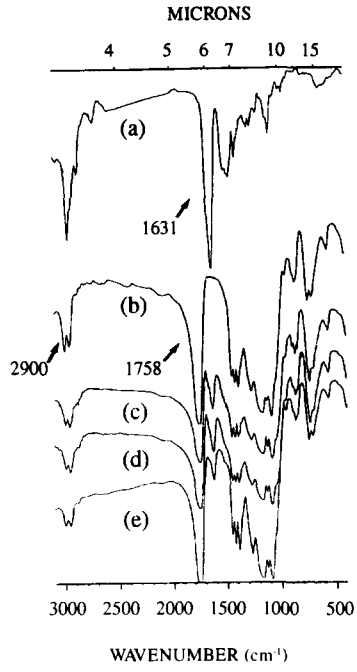

Fig. 4. Infrared spectra of CyA (a), PLGA (b) and CyA-loaded micro- and nanospheres (formulations 1 (c), 3 (d) and 5 (e)).

by FT-IR. Fig. 4 displays the infrared spectra over the range 400–3100 cm^{-1} for CyA, PLGA RG503 and the formulations based on this copolymer (formulations 1, 3 and 5). The CyA IR spectrum shows an intense amide carbonyl band at 1631 cm^{-1} (a). On the other hand, the polymer FT-IR spectrum shows the carbonyl band and the alkyl group band at 1758 and ~2900 cm^{-1}, respectively (b). FT-IR spectra corresponding to CyA-loaded micro- and nanospheres displayed the typical bands of both ingredients (drug and polymer) without any spectral shifts in either band. The same results (not shown) were obtained with PLGA RG506. This indicates the absence of chemical interaction between the components of the microspheres.

The importance of the above information is revealed in the fact that the internal structure of these systems has a significant influence on the release patterns of the drug (Benoît et al., 1986; Maulding, 1987; Izumikawa et al., 1991). In fact, when the drug is dispersed in the polymer matrix at a molecular level, the release rate should be strictly controlled by the erosion rate of the matrix. In addition, since the usefulness of FT-IR spectroscopy in the detection of conformational changes in peptides has been demonstrated (Chen, 1992), the results obtained using this technique also suggest that the physico-chemical integrity of CyA is mantained in the encapsulated form. To confirm these results, the formulations developed were also analyzed by RP-HPLC and size exclusion chromatography, two analytical methods which are often used to monitor peptide

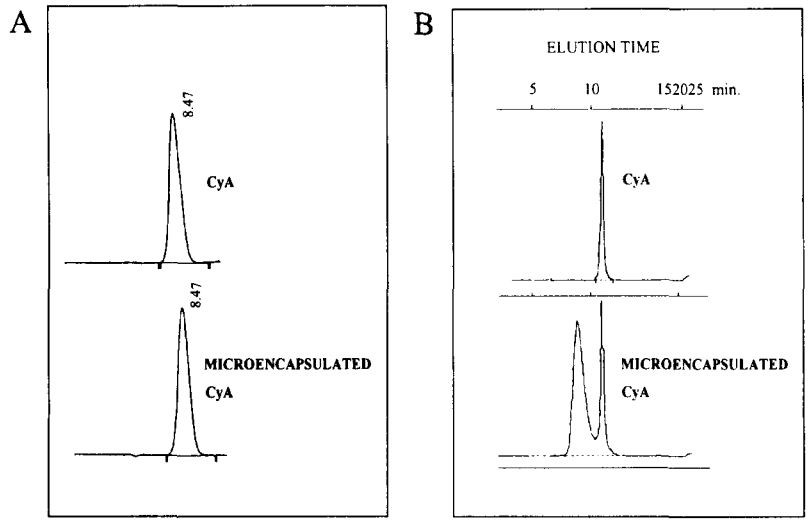

Fig. 5. Chromatograms of CyA and microencapsulated CyA (formulation 1) obtained by RP-HPLC (A) and GPC (B).

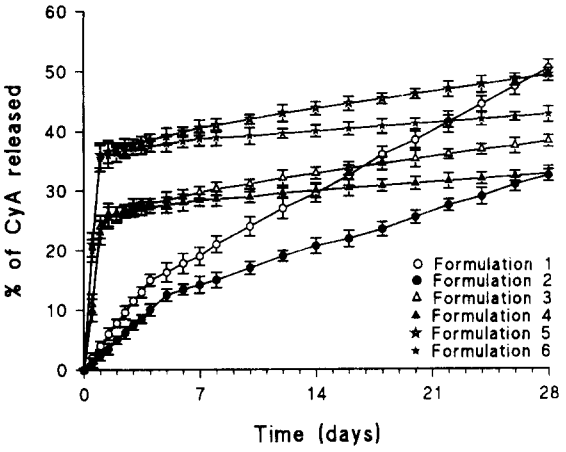

Fig. 6. Cumulative percent of CyA released from PLGA micro- and nanospheres as a function of time.

stability (Benedek and Swadesh, 1991; Randall et al., 1991; Chen, 1992). The chromatograms obtained via both techniques (Fig. 5A and B) also support the integrity of CyA in the microspheres. The GPC chromatograms presented in Fig. 5B also show the peak corresponding to the polymer, the principle of this technique being based on a size-exclusion process. In both series of chromatograms it can be seen that the peak corresponding to CyA was unchanged after the microencapsulation process, suggesting the absence of physical or chemical modification in the molecule of CyA. The same results (not shown) were obtained for formulations 2–6. This is of great importance, since the frequent conformational fragility of peptides and proteins renders them susceptible to denaturation during the encapsulation process (Sanders, 1991). However, due to the multiplicity of protein degradation pathways, no single test method can be guaranteed to be stability indicating (Chen, 1992). Only the combined information from various methods can provide confirmation that the physico-chemical integrity of a peptide is retained after the microencapsulation process.

When formulations were tested for release in vitro, it was found that CyA was released in a biphasic manner, characterized by an initial and variable rapid release period followed by a continuous and slower release thereafter (Fig. 6). The initial release, usually referred to as an initial burst, was found to be greatly affected by the microsphere size. Thus, a modest initial burst (between 10 and 15%) in the first 5 days followed by a slower release was observed from 30 μm microspheres (formulations 1 and 2). In contrast, a considerable release of drug of 25–35% within the first day followed by an extremely slow release was observed from all nanosphere formulations (0.2–1 μm). The initial burst was greater for nanospheres of smaller size. This first phase corresponds to the release of the peptide located on or near the surface of the delivery system and therefore available for immediate release. Thus, as expected, small microspheres displayed more rapid initial release of drug than larger ones due to their greater surface area per unit mass and, consequently, the number of CyA molecules close to the surface being higher.

To investigate the mechanism of peptide release after the initial burst, we determined the in vitro degradation profiles of the polymers forming the microspheres by GPC. The chromatograms obtained (Fig. 7) showed a progressive near-linear decrease of the number-average polymer molecular weight over an experimental duration of 1 month. This near-linear decrease and the almost constant release of the drug from all the formulations suggested that drug release in the second phase occurs by homogeneous

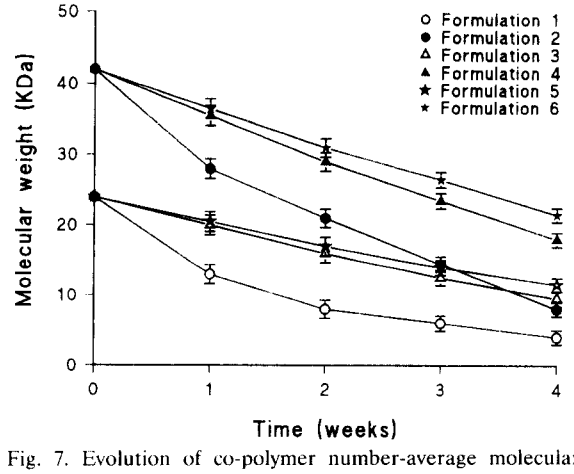

Fig. 7. Evolution of co-polymer number-average molecular weight of CyA-loaded PLGA micro- and nanospheres during in vitro release studies.

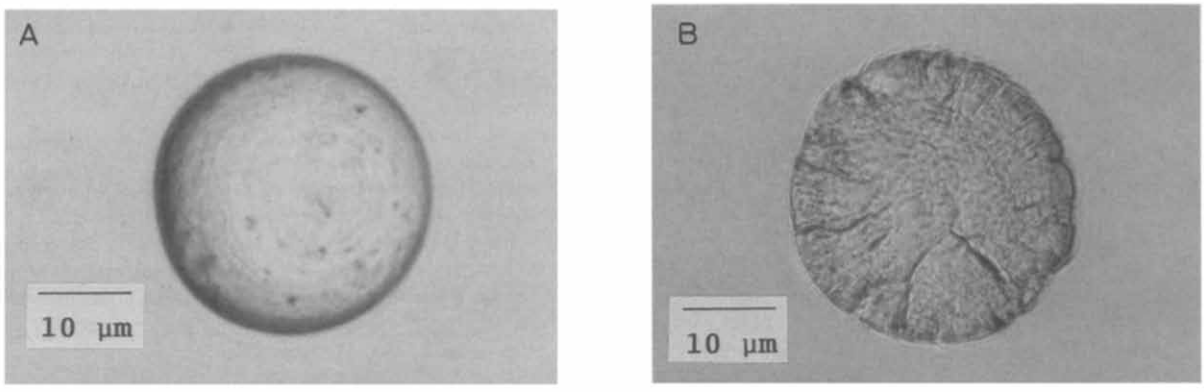

Fig. 8. Photomicrographs of PLGA microspheres containing CyA as observed by phase-contrast light microscopy: (A) before in vitro degradation and (B) after 14 days in vitro degradation (formulation 1).

degradation of the PLGA matrix. Thus, bulk hydrolysis of the polyester leads to erosion of the co-polymer matrix, followed by release of the molecularly dispersed peptide. As the process of erosion is time dependent (as observed during GPC studies), a gradual but continuous release occurs which is governed by various factors. These factors include co-polymer molecular weight and particle size.

The release rate from these systems is dependent on the co-polymer molecular weight, the release rate being faster from microspheres prepared with a lower co-polymer molecular weight (RG503), as noted from the slopes of the dissolution plots for the second phase. Similar results have been obtained for hydrophilic peptides included in PLGA microspheres (Sanders et al., 1984).

Significant differences between the microspheres and nanospheres can also be seen in this phase of the release profiles. The extremely slow release from 1 μm microspheres and nanospheres may be due to their dense matrix structure. Indeed, the slower degradation rate of the nanospheres with respect to microspheres (as demonstrated in Fig. 7) may be explained by a more dense matrix, resulting in very little release of CyA.

Microspheres prepared with PLGA RG503 immediately after preparation and after a 14 day incubation period are shown in Fig. 8. Sustained erosion of the microspheres, that may be related to the polymer degradation and subsequent peptide release, was observed. Similar results were obtained with PLGA RG506.

The results presented here are very encouraging, since they show the possibility of designing CyA-loaded microspheres with pre-programmed durations of action, by selecting the desired polymer properties and formulation parameters. Consequently, optimal long-term controlled-release systems for CyA delivery should be possible to achieve. On the other hand, CyA-loaded PLGA nanospheres offer several other potential uses. First, these systems may be administered intravenously, thereby allowing one to modulate the distribution pattern of CyA and, probably, to reduce the toxicity of this peptide. Second, after oral administration these systems provide a means of targeted delivery of CyA to the immune system. Consequently, this new approach of using biodegradable micro- and nanospheres as CyA carriers is a promising step toward the development of a clinically useful new dosage form of this drug.

Conclusions

This work has demonstrated the feasibility of efficiently encapsulating CyA into PLGA micro- and nanospheres of controlled size by using a solvent evaporation method. The drug carrier consists of a biodegradable, biocompatible co-

polymer in which the drug is dispersed at a molecular level and no drug interaction with the polymer exists. The characteristic shape of the drug release profiles and degradation studies by GPC suggest that drug release from these systems is controlled, essentially, by matrix erosion, following an initial diffusive release from the surface. Thus, different release profiles (i.e., near-constant or biphasic) and release rates can be achieved by modifying the particle size and by selecting the appropriate co-polymer molecular weight. Consequently, using PLGA it is possible to design a biodegradable controlled-release system which, after a single dose, can maintain the drug at the desired concentrations, achieving the ideal mode of CyA delivery: an initial burst ('induction therapy' phase), followed by a prolonged continuous release, to attain a 'maintenance therapy' phase.

Current in vivo experiments should allow us to determine which specific release profile can optimize the immunosuppression, in terms of duration and intensity, in order to provide adequate long-term immunosuppressive therapy while avoiding potential toxicity.

Acknowledgments

This work was supported by a grant from the C.I.C.Y.T. (FAR91-0664). We are grateful to Sandoz Inc., Basel, Switzerland, for the gift of CyA.

References

Benedek, K. and Swadesh, J.K., HPLC of proteins and peptides in the pharmaceutical industry. In Fong, G.W. and Lam, S.K. (Eds), *HPLC in the Pharmaceutical Industry*, Dekker, New York, 1991, pp. 241–303.

Benoît, J.P., Madoule, P., Trampont, P., Benita, S., Puisieux, F. and Thies, C., Les technologies d'embolisation selective, l'utilisation des microspheres. *Innov. Tech. Biol. Med.*, 7 (1986) 137–151.

Borel, J.F. and Gunn, H.C., Cyclosporine as a new approach to therapy of autoimmune diseases. *Ann. N.Y. Acad. Sci.*, 475 (1985) 307–319.

Cavalier, M., Benoît, J.P. and Thies, C., The formation and characterization of hydrocortisone-loaded poly(DL-lactide) microspheres. *J. Pharm. Pharmacol.*, 38 (1986) 249–253.

Cavanak, T. and Sucker, H., Formulation of dosage forms. *Prog. Allergy*, 38 (1986) 65–72.

Chen, T., Formulation concerns of protein drugs. *Drug Dev. Ind. Pharm.*, 18 (1992) 1311–1354.

D'Souza, M.J., Controlled release of cyclosporine from microspheres. *Drug Dev. Ind. Pharm.*, 14 (1988) 1351–1357.

Eldridge, J.H., Hammond, C.J., Meulbroek, J.A., Staas, J.K., Gilley, R.M. and Tice, T.R., Controlled vaccine release in the gut-associated lymphoid tissues: I. Orally administered biodegradable microspheres target the Peyer's patches. *J. Controlled Release*, 11 (1990) 205–214.

Izumikawa, S., Yoshioka, S., Aso, Y. and Takeda, Y., Preparation of poly(l-lactide) microspheres of different crystalline morphology and effect of crystalline morphology on drug release rate. *J. Controlled Release*, 15 (1991) 133–140.

Jani, P., Halbert, G.W., Langridge, J. and Florence, A.T., The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. *J. Pharm. Pharmacol.*, 41 (1989) 809–812.

Jani, P., Halbert, G.W., Langridge, J. and Florence, A.T., Nanoparticle uptake by the rat gastrointestinal mucosa: Quantitation and particle size dependency. *J. Pharm. Pharmacol.*, 41 (1990) 821–826.

Julienne, M.C., Alonso, M.J., Gómez Amoza, J.L. and Benoît, J.P., Preparation of poly(DL-lactide/glycolide) nanoparticles of controlled particle size distribution: Application of experimental designs. *Drug Dev. Ind. Pharm.*, 18 (1992) 1063–1077.

Kahan, B.D., Ried, M. and Newburger, J., Pharmacokinetics of cyclosporine in human renal transplantation. *Transplant. Proc.*, 15 (1983) 446–453.

Luke, D.R., Kasiske, B.L., Matzke, G.R., Awni, W.M. and Keane, W.F., Effects of cyclosporine on the isolated perfused rat kidney. *Transplantation*, 43 (1987) 795–799.

Matzke, G.R. and Luke, D.R., Dialysis and renal transplant therapy. In Herfindal, E.T., Gourley, D.R. and Hart, L.L. (Eds), *Clinical Pharmacy and Therapeutics*, Williams & Wilkins, Baltimore, 1988, pp. 229–242.

Maulding, H.V., Prolonged delivery of peptides by microcapsules. *J. Controlled Release*, 6 (1987) 167–176.

Mauric, C., Sandimmun(e) (cyclosporin) in autoimmune diseases. A selection of publications. *Med. Documentation Sandoz*, Basel, Switzerland, 1982.

Randall, C.S., Malefyt, T.R. and Sternson, L.A., Approaches to the analysis of peptides. In Lee, V.H.L. (Ed.), *Peptide and Protein Drug Delivery*, Dekker, New York, 1991, pp. 785–806.

Sanders, L.M., Controlled delivery systems for peptides. In Lee, V.H.L. (Ed.), *Peptide and Protein Drug Delivery*, Dekker, New York, 1991, pp. 785–806.

Sanders, L.M., Kent, J.S., McRae, G.I., Vickery, B.H., Tice, T.R. and Lewis, D.H., Controlled release of leutinizing hormone releasing hormone analogue from poly(d,l-lactide-co-glycolide) microspheres. *J. Pharm. Sci.*, 73 (1984) 1294–1297.

Sawchuk, R.J. and Cartier, L.L., Liquid-chromatographic determination of cyclosporin A in blood and plasma. *Clin. Chem.*, 27 (1981) 1368–1371.

Tice, T.R., Mason, D.W. and Gilley, R.M., Clinical use and future of parenteral microsphere delivery systems. In Prescott, L.F. and Nimmo, W.S. (Eds), *Novel Drug Delivery*, Wiley, U.K., 1989, pp. 223–235.

Venkataram, S., Awni, W.M., Jordan, K. and Rahman, Y.E., Pharmacokinetics of two alternative dosage forms for cyclosporine: liposomes and intralipid. *J. Pharm. Sci.*, 79 (1990) 216–219.

Visscher, G.E., Robison, R.L. and Argentieri, G.I., Tissue response to biodegradable injectable microcapsules. *J. Biomat. Appl.*, 2 (1987) 118–131.

Wise, D.L., Fellmann, T.D., Sanderson, J.E. and Wentworth, R.L., Lactid/glycolic acid polymers. In Gregoriadis, G. (Ed.), *Drug Carriers in Biology and Medicine*, Academic Press, London, 1979, pp. 237–270.

Wood, A.J. and Lemaire, M., Pharmacologic aspects of cyclosporine therapy: Pharmacokinetics. *Transplant. Proc.*, 17 (1985) 27–32.

Yanagawa, A., Iwayama, T., Saotome, T., Shoji, Y., Takano, K., Oka, H., Nakagawa, T. and Mizushima, Y., Selective transfer of cyclosporin to thoracic lymphatic systems by the application of lipid microspheres. *J. Microencapsulation*, 6 (1989) 161–164.