ELSEVIER

International Journal of Pharmaceutics 170 (1998) 109–117

international journal of pharmaceutics

# In vitro characterization and in vivo release profile of a poly (D,L-lactide-co-glycolide)-based implant delivery system for the α-MSH analog, melanotan-I

R. Bhardwaj, J. Blanchard *

*Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA*

Received 25 March 1997; received in revised form 13 April 1998; accepted 14 April 1998

**Abstract**

Melanotan-I (MT-I) is a superpotent tridecapeptide capable of stimulating melanotropic activity. In order to overcome its short half-life in the systemic circulation, the biodegradable poly(D,L-lactide-co-glycolide) (PLGA) copolymer was used to prepare an implant delivery system for MT-I. The implant was prepared by the hot melt-extrusion method. The surface morphology of the PLGA implant was assessed using scanning electron microscopy. The time-dependent changes in the molecular weight distribution of the copolymer and its erosion were monitored in order to help characterize the hydrolytic degradation processes occurring in vivo. The time required to reduce the weight-average molecular weight of PLGA to 50% of its initial value, as determined by size exclusion chromatography, was about 12 days compared to 5 weeks for 50% erosion of the copolymer mass to occur. The release of lactic acid from PLGA was also quantified simultaneously in order to characterize the degradation, and the onset of increased lactic acid release was found to coincide with the onset of the tertiary phase of the MT-I release profile in vivo in guinea pigs. The MT-I released from the depot implanted subcutaneously in guinea pigs exhibited a release profile extending over one month, in agreement with data from the in vitro studies. 

*Keywords:* Melanotan-I; Controlled-release; Poly(D,L-lactide-co-glycolide); Polymer degradation; Pharmacokinetics

## 1. Introduction

Melanotan-I (MT-I) is a synthetic analog of the pigmentary hormone, α-melanocyte stimulating hormone (α-MSH). MT-I, like α-MSH, is a tridecapeptide but it has more potent and prolonged

* Corresponding author. Tel.: +1 520 6263917; fax: +1 520 6262466; e-mail: blanchard@pharmacy.arizona.edu

0378-5173/98/$19.00 
*PII* S0378-5173(98)00149-5

melanotropic activity than the native hormone. (Sawyer et al., 1980). It is currently in Phase I trials at the Arizona Cancer Center (University of Arizona, Tucson) to evaluate its potential as a chemopreventive agent for sunlight-induced skin cancers. MT-I has a very short half life in vivo (Ugwu et al., 1997) and, as a consequence, multiple subcutaneous injections are required to achieve and maintain a tan. Hence, it seemed appropriate to develop a prolonged-action formulation for the peptide. Prolonged release of MT-I from the delivery system would likely result in a prolonged pigmentation of the skin, which is beneficial in protecting the skin from the harmful UV rays of the sun.

The overall goal of our research was to develop a controlled-release formulation of MT-I using biodegradable delivery systems based upon polyesters of poly [DL-lactide-co-glycolide] (PLGA) (Lewis, 1990). PLGA has been used commercially for the formulation of the gonadotrophin-releasing hormone analogs, e.g. goserelin (Zoladex®) (Furr and Hutchinson, 1992) and leuprolide (Leupron®) (Sanders et al., 1984). It is essential to study the degradation profile of the PLGA system in order to understand the peptide release pattern from the delivery system. The degradation rate of the polymer depends on the molecular weight, the copolymer composition and the crystallinity of the polymer, all of which control the accessibility of water to the labile ester linkages (Schmitt et al., 1993; Grizzi et al., 1995).

The objective of this study was to evaluate the in vitro degradation of PLGA formulations and to correlate the in vitro data with the release profile of the peptide in vivo in guinea pigs. The depot formulations were prepared by incorporation of MT-I into 50:50 PLGA (the actual mole ratio of lactide and glycolide in the polymer used was $0.51 \pm 0.02$ and $0.49 \pm 0.02$, respectively) using a hot melt-extrusion technique. Polymer having a viscosity of 0.6 dl/g was selected for preparing an implant designed to provide a release of MT-I extending over a 1-month period. This particular PLGA polymer was selected based upon results of a previous investigation (Bhardwaj and Blanchard, 1997). The in vitro molecular weight distribution and the mass loss of PLGA from the implants during incubation was evaluated. Lactic acid, produced by the degradation of PLGA, was quantified using an enzymatic method of analysis. The in vivo release of MT-I from the PLGA implants was studied in guinea pigs for 5 weeks to determine the release profile of the peptide from the implants.

## 2. Materials and methods

### 2.1. Materials

Purified MT-I ( $\geq 99\%$ pure) was obtained from Bachem (Torrance, CA). HPLC-grade acetonitrile was from Burdick and Jackson (Muskegon, MI), oxalic acid from Aldrich (Milwaukee, WI) and tetrahydrofuran (THF) was from Fisher Scientific (Fair Lawn, NJ). Potassium phosphate (monobasic and dibasic) and potassium chloride were from J.T. Baker (Phillipsburg, NJ). The water used in the experiments was deionized and distilled using a Millipore filter system (Nylon-66 filters, 0.45 $\mu$m) (Millipore, Bedford, MA). The PLGA samples were obtained from Birmingham Polymers (Birmingham, AL). The $\alpha$-MSH kit for the radioimmunoassays was provided by INCstar (Stillwater, MN).

### 2.2. Preparation of depot formulations

Implants with or without added Melanotan-I (2.5% w/w) were prepared using a hot melt-extrusion technique (Sanders et al., 1986). Various PLGA formulations were prepared utilizing lactide:glycolide polymer with a mol ratio of 50:50 and intrinsic viscosity of 0.6 dl/g. A coarse mixture of each peptide with fine granular PLGA was prepared manually in a mortar and fed into a melt extruder. The operating temperature was maintained at 45–70°C. The extrudate produced was cylindrical and about 2 mm in diameter and 8–10 mm in length.

### 2.3. In vitro release studies

The in vitro release experiments were performed by incubating the implants containing

about 1 mg of peptide in 1 ml of ethanolic (25% v/v) aqueous isotonic phosphate buffer medium (Sanders et al., 1986) maintained at pH 7.4 and 37°C. The glass vials containing the implants were agitated at 40 oscillations/min. All release studies were performed using glass vials previously coated with Sigmacote® (Sigma, St. Louis, MO) to minimize binding of MT-I. This isotonic in vitro medium was replaced at appropriate time intervals and the aliquot removed assayed for peptide content by HPLC (Surendran et al., 1995). All experiments were performed in triplicate.

### 2.4. HPLC analysis of MT-I

The HPLC system consisted of a Thermo Separation Products (San Jose, CA) Isochrom pump, a Rheodyne (Cotati, CA) Model 9125 injector valve with 40 $\mu$l loop and a Thermo Separation Products Model 100 variable-wavelength UV detector set at 214 nm. The analytical column was a Vydac (Hesperia, CA) $C_8$ (5 $\mu$m) microbore cartridge ($150 \times 2.1$ mm I.D.) fitted with a Whatman (Clifton, NJ) $C_{18}$ (10 $\mu$m) guard column ($10 \times 4.6$ mm I.D.). The guard column was changed routinely after about 100 injections as a precautionary measure to avoid pressure build-up in the HPLC system. Peak recordings and integrations were made with a Thermo Separation Products Model 4290 integrator. All injections were made with a Hamilton (Reno, NV) Model 725-SNR syringe previously coated with Sigmacote® (Sigma, St. Louis, MO) to minimize binding of MT-I to syringe components. The mobile phase consisted of 0.1 M $K_2HPO_4$/acetonitrile (80:20% v/v) with 18 $\mu$l/l of 99% (v/v) triethylamine at pH 2.50 at a flow rate of 1 ml/min. Fresh stock solutions of MT-I (1 mg/ml) in water were prepared weekly and stored at $-20$°C until use.

### 2.5. Scanning electron microscopy (SEM)

The implants were freeze dried, cross-sectioned and then placed on steel stubs which were coated with an adhesive. The cross-sections of the implants were coated with gold under vacuum and examined under the microscope to visualize the surface characteristics of the implants.

### 2.6. Molecular weight analysis

Average molecular weights of the PLGA were determined by size-exclusion chromatography (SEC). The chromatography system consisted of a Waters 590 pump (Waters, Milford, MA), Waters WISP 710 Injector (autosampler), a Waters differential refractometry detector, a PL gel mix column (5 $\mu$m, 30 cm). The mobile phase consisted of THF and oxalic acid (99.5:0.5) at a flow rate of 0.5 ml/min. The injection volume was 50 $\mu$l and detection was based on differential refractometry. Molecular weights of the samples were determined relative to polystyrene standards having molecular weights ranging from 500 to 1290000 Da. The PLGA samples were prepared in HPLC grade THF at a concentration of 15 mg/ml and the run time was 35 min. Peak slicing (Polymer Laboratory Scientific Software, Amherst, MA) was used to calculate the weight average molecular weights ($M_w$).

### 2.7. Weight loss analysis

The weight loss was determined gravimetrically (Hausberger and DeLuca, 1995). Individual implants were weighed initially and after degradation in vitro. The initial and the final weights were used to calculate the percentage of mass remaining after the in vitro degradation, according to the following equation:

$$\% \text{ Mass Remaining} = \frac{m_t}{m_i} * 100$$

where $m_i$ and $m_t$ are the implant weights determined initially and after degradation for time $t$, respectively.

### 2.8. Lactic acid determination

The lactic acid concentration in the release medium was measured using a Paramax lactate reagent assay kit (Dade Diagnostics of Puerto Rico, Aguada, PR). The assay was based on an enzymatic reaction involving NADH oxidation by lactate dehydrogenase (LDH).

### 2.9. In vivo studies

The rate of release pattern of the peptide from the PLGA formulations was evaluated in vivo using a guinea pig model. Six male Hartley guinea pigs (SASCO, Wilmington, MA) weighing about 800 g each were used. The guinea pigs were fasted for 24 h prior to the experiment and water was allowed ad libitum. Each animal was anesthetized using an intramuscular injection of a mixture containing acepromazine 20 mg, ketamine 500 mg and xylazine 160 mg. Catheters (made of PE-50 polypropylene tubing) were inserted into the carotid artery of each animal for periodic blood sampling. An injection port was inserted at one end of the cannula and a 1-ml polypropylene syringe with a 23-gauge needle was used for blood collection. The implants containing 10% w/w peptide were injected subcutaneously in the abdominal area. Blood was collected on a daily basis for up to 5 weeks and analyzed for MT-I content by radioimmunoassay (Kreutzfeld and Bagnara, 1989). Blood samples (approximately 0.4 ml) were collected into chilled polypropylene tubes containing 10 $\mu$l of EDTA (0.7 mg $K_3$EDTA in 10 $\mu$l saline) and 10 $\mu$l aprotinin (18000 Kallikrein inhibitory units/ml saline). Following centrifugation at 4°C (10 min, $2000 \times g$), the plasma samples collected were stored at $-20$°C until the day of analysis.

## 3. Results and discussion

The goal of our study was to develop a 1-month depot for MT-I using PLGA polymer. The PLGA polymers meet the requirements needed for a matrix delivery system, including mechanical properties, biodegradability, tissue compatibility and ease of processing. The need for a continuous tanning effect for the prevention of skin cancer by MT-I makes the use of a prolonged-release delivery system appropriate for this peptide.

The PLGA implants were transparent initially and later became translucent and white in color. The SEM examination of the cross-section of the PLGA implant at zero time showed a compact and smooth structure with pores which would be filled with water upon incubation (Fig. 1). The degradation of the surface of the implants was observed after 1 week of incubation in the release medium. After 2 weeks of incubation, the implants still maintained their shape and integrity, however after the longer period of incubation (i.e. 4 weeks) the implants became very porous and lost their shape due to degradation of PLGA and the decrease in the PLGA molecular weight to 5% of its initial value.

The release of MT-I from the PLGA polymer by partition-dependent diffusion is expected to be minimal as most peptides and proteins are insoluble in these polymers. Consequently, the degradation of PLGA is a critical factor in determining the release of the peptide from these matrices. The degradation of PLGA was quantified by measuring the reduction in the molecular weight of the PLGA, the decrease in mass of the implants, and the release of lactic acid. The size-exclusion chromatographic analysis showed that the weight-average molecular weight decreased with increasing in vitro incubation times. Fig. 2 shows representative $M_w$ data for the PLGA depot. The hot melt-extrusion of the polymer did not affect the molecular weight of PLGA as shown by the initial MW of the polymer. In addition, the implant fabrication procedure did not affect the stability of MT-I as evidenced by the fact that the MT-I released after 24 h from implants injected subcutaneously into guinea pigs exhibited 100% of its melanotropic activity compared with a freshly prepared MT-I standard solution using a frog-skin bioassay (Castrucci et al., 1984). The molecular weight profile of PLGA showed an initial rapid decrease, which slowed at longer incubation times (Helder et al., 1990). The break in the rate of molecular weight reduction occurs at approximately the time of onset of the tertiary phase of MT-I release, i.e. about 3 weeks. This is followed by the degradation of PLGA into low molecular weight oligomers which become more soluble and diffuse into the release medium (Park, 1995). The molecular weight decreased to about 1000 at the end of a one month incubation period.

Fig. 3 shows the in vitro mass degradation profile of PLGA implants. A lag phase characterized by a constant mass was observed until about

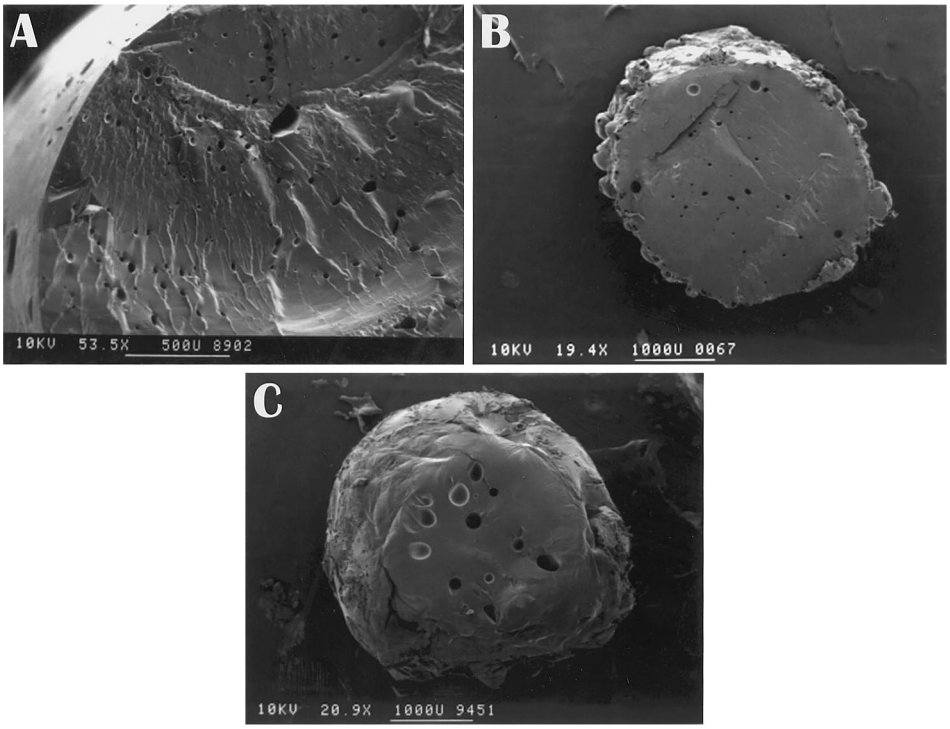


Fig. 1. SEM scan of PLGA implants at time 0 (A), 1 week (B), and 2 weeks (C).

3 weeks. This was followed by the pseudo-zero order decrease in polymer mass for another 2 weeks. The time required to reach 50% of the initial mass was about 5 weeks. The onset of the polymer erosion coincides with the tertiary phase of MT-I release from the implants. During the degradation of PLGA, an almost immediate decrease in molecular weight occurs as the labile polymer chains are cleaved. This decrease in molecular weight shown in Fig. 2, did not reflect any change in the overall mass of the implant, as

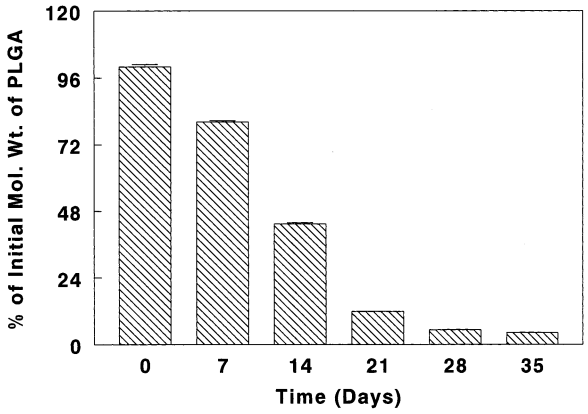


Fig. 2. In vitro molecular weight breakdown of PLGA implants. The error bars represent S.E.M. ($n = 3$).

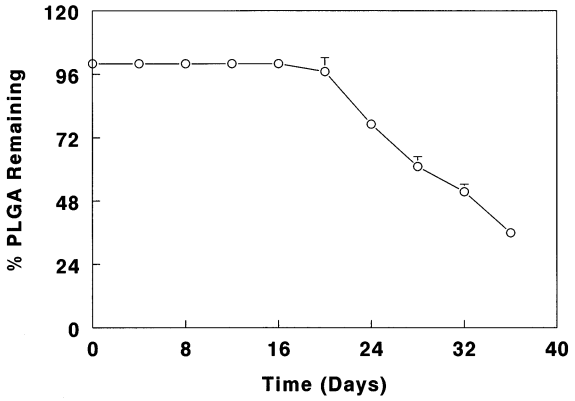


Fig. 3. In vitro mass loss profile of PLGA implants. The error bars represent S.E.M. ($n = 3$).

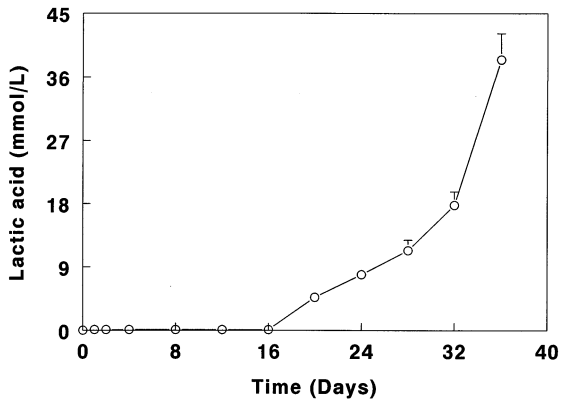


Fig. 4. Lactic acid released in vitro from the PLGA implants. The error bars represent S.E.M. ($n = 3$).

observed in Fig. 3. This phenomenon occurs because there is a critical MW of polymer (i.e. about 4000 Daltons in this study) above which no dissolution of oligomers occurs into the medium and consequently no mass loss is observed. A similar critical MW for PLGA polymer dissolution has been reported elsewhere (Park, 1995). After 3 weeks of incubation, the onset of degradation of PLGA becomes obvious as an 88% decrease in the initial molecular weight is observed, as shown in Fig. 2 (Kenley et al., 1987).

The degradation of the implant upon aging was also monitored for 5 weeks using lactic acid measurements. Lactic acid and glycolic acid are the two major degradation products of PLGA hydrolysis resulting from the breakdown of the lactide–glycolide bond (Park, 1995). Hence, the measurement of either of these two compounds should accurately reflect the degradation of PLGA. Fig. 4 shows the lactic acid concentration in the release medium as analyzed by the LDH-based enzymatic assay. Lactic acid concentrations were less than 0.05 mmol/l for about 16 days, indicative of a negligible amount of polymer mass loss. The sharp increase in lactic acid release thereafter indicates the rapid erosion of the polymer (Vert and Garreau, 1995). The lactic acid release pattern was in good overall agreement with the polymer mass loss profile (Fig. 3).

The implant prepared with PLGA having an intrinsic viscosity of 0.6 dl/g showed an initial release of 3% of the loaded peptide (Fig. 5). The small initial 'burst' of MT-I indicates that there is very little surface-located peptide and that the majority of the peptide is present within the interior of the implant (Bhardwaj and Blanchard, 1997). This was followed by a lag period during which little or no peptide was released. During this 'secondary' phase the hydrated polymer is undergoing hydrolysis, but the molecular weight has not been lowered to the point at which the degradation products are sufficiently soluble to dissolve. Once the polymer molecular weight reaches a critical point, it becomes water soluble and the third phase begins at about three weeks after incubation of the implant. This phase is due to hydrolysis of the polymer leading to the disintegration of the matrix (Bodmer et al., 1992). Thus, the release of MT-I from the implant delivery system appeared to be triphasic and lasted for more than a month, as previously observed with similar polymeric implant systems (Sanders et al., 1986; Furr and Hutchinson, 1992).

The plasma concentrations of MT-I after subcutaneous administration of implants containing 10% w/w MT-I to guinea pigs are shown in Fig. 6. The release profile showed an initial peak at day 2 as water diffused into the matrix and released MT-I from the surface regions of the device. The initial level of peptide release is controlled by factors such as peptide/polymer ratio, particle size

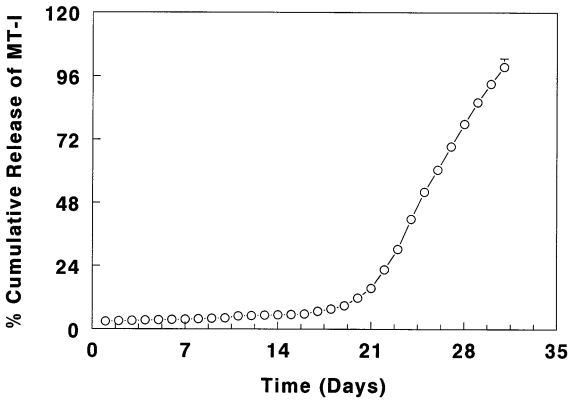


Fig. 5. In vitro release profile of MT-I from PLGA implants containing 2.5% w/w peptide. The error bars represent S.E.M. ($n = 3$).

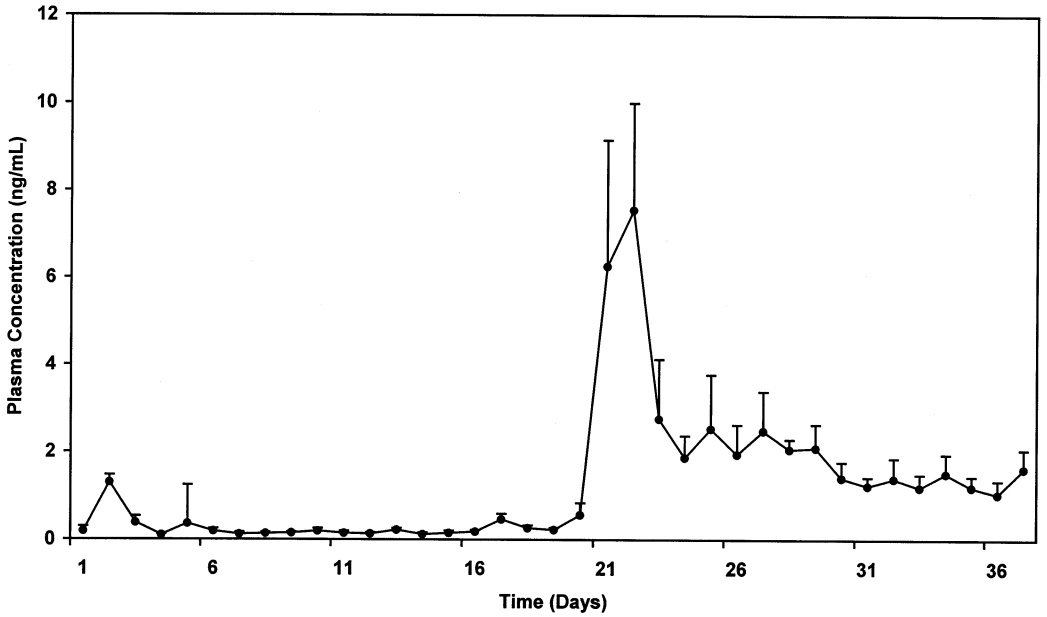


Fig. 6. In vivo profile of MT-I released from PLGA implants containing 10% w/w peptide in guinea pigs. Each point represents the mean ± S.E.M. ($n = 6$).

of the dispersed peptide and the size of the delivery device. To compare the concentration of MT-I at the onset of polymer degradation and the $C_{max}$ achieved by the administration of an MT-I solution, a pilot study was performed in which one-half and one-tenth of the implant dose of MT-I (i.e. 4 mg) were injected subcutaneously, into two guinea pigs. The $C_{max}$ values observed at 60 min were about 90 and 20 ng/ml, respectively. These results indicate that the $C_{max}$ observed in the pharmacokinetic profile of the implant represents a controlled-release of MT-I by the PLGA polymer as opposed to an 'uncontrolled' burst release.

The initial release was followed by a phase of very low peptide release, the duration and amount of which is dependent on the physicochemical properties of the peptide and polymer as well as the peptide loading. At about 3 weeks, an increase in the level of MT-I due to erosion of the polymer matrix was observed which continued for another 2 weeks (Okada et al., 1991; Dutta et al., 1993). Since very low concentrations of α-MSH, ranging from 24–72 pg/ml, have been detected in human cerebrospinal fluid (Krieger et al., 1980) and because MT-I is highly potent (i.e. 'superpotent') and has a prolonged biological activity despite its short plasma half life, the low peptide release (below the 39 pg/ml detection limit of the RIA assay) during the slow release (secondary) phase was adequate to provide a continuous pharmacological effect as observed in recently completed studies with pigmented guinea pigs (Bhardwaj et al., unpublished results). In these studies it was observed that the melanogenic effects of the MT-I implants persisted for about three months. This conclusion was based on the fact that the skin pigmentation, as measured using a reflectometer (Chroma Meter), showed evidence of skin darkening in hairless guinea pigs for up to 3 months. In addition, measurement of eumelanin, the black/brown melanin pigment, in guinea pig skin biopsies, showed a 2.5-fold increase after 1 month, and elevated levels for up to 3 months. The initial release of MT-I during the first week probably acted like a loading dose. A good correlation was observed between the in vitro and in vivo release profiles as the duration of peptide release was greater than 1 month and the tertiary phase in both cases occurred at about 3 weeks.

## 4. Conclusion

The thermoplastic copolymers of poly(lactic acid) and poly(glycolic acid) have many advantages, including biocompatibility and lack of toxicity. The formulation chosen was designed to release the peptide for one month. The implant offers the potential for high drug loading and a good probability of achieving a controlled release. Synchronization of pore-diffusion and the polymer erosion process will produce a constant release of MT-I from the PLGA implants. The in vivo studies with guinea pigs demonstrated a prolonged peptide release into the systemic circulation for at least 36 days, which illustrated the potential of the implanted delivery system to increase the therapeutic efficacy of Melanotan-I. This, in turn, provides a prolonged pharmacological activity of MT-I (Bhardwaj et al., unpublished results) and should overcome the need for daily administration of MT-I. PLGA implants are a promising delivery device for providing a prolonged and controlled release of MT-I over a 1-month period.

## Acknowledgements

We are thankful to David Bentley for his help with SEM and Stephanie Cameron for her help with the animal surgery. Support from a National Cancer Institute Grant # 2 P01 CA27502 is gratefully acknowledged.

## References

Bhardwaj, R., Blanchard, J., 1997. In vitro evaluation of poly(DL-lactide-co-glycolide) polymer-based implants containing the α-melanocyte stimulating hormone analog, Melanotan-I. J. Control. Release 45, 49–55.

Bodmer, D., Kissel, T., Traechslin, E., 1992. Factors influencing the release of peptides and proteins from biodegradable parenteral depot systems. J. Control. Release 21, 129–137.

Castrucci, A.M., Hadley, M.E., Hruby, V.J., 1984. Melanotropin bioassays: in vitro and in vivo comparisons. Gen. Comp. Endocrinol. 55, 104–111.

Dutta, A.S., Furr, B.J.A., Hutchinson, F.G., 1993. Discovery and development of Goserelin (Zoladex®). Pharm. Med. 7, 9–28.

Furr, B.J.A., Hutchinson, F.G., 1992. A biodegradable delivery system for peptides: preclinical experience with the gonadotrophin-releasing hormone agonist Zoladex®. J. Control. Release 21, 117–127.

Grizzi, I., Garreau, H., Li, S., Vert, M., 1995. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 16, 305–311.

Helder, J., Dijkstra, P.J., Feijen, J., 1990. In vitro degradation of glycine/DL-lactic acid copolymers. J. Biomed. Mater. Res. 24, 1005–1020.

Hausberger, A.G., DeLuca, P.P., 1995. Characterization of biodegradable poly(DL-lactide-co- glycolide) polymers and microspheres. J. Pharm. Biomed. Anal. 13, 747–760.

Kenley, A.A., Lee, M.O., Mahoney, T.R., Sanders, L.M., 1987. Poly(lactide-co-glycolide) decomposition kinetics in vivo and in vitro. Macromolecules 20, 2398–2403.

Kreutzfeld, K.L., Bagnara, J.T., 1989. Application of an alpha-melanocyte stimulating hormone (alpha MSH) radioimmunoassay to the detection of the superpotent analog [Nle$^4$-DPhe$^7$]α-MSH. Pigment Cell Res. 2, 65–69.

Krieger, D.T., Liotta, A.S., Brownstein, M.J., Zimmerman, E.A., 1980. ACTH, β-lipoprotein, and related peptides in brain, pituitary, and blood. Rec. Prog. Horm. Res. 36, 277–344.

Lewis D.H., 1990. Controlled release of bioactive agents from lactide/glycolide polymers. In: Chasin, M., Langer, R. (Eds), Biodegradable Polymers as Drug Delivery Systems. Marcel Dekker, New York, pp. 1–14.

Okada, H., Inoue, Y., Heya, T., Ueno, H., Ogawa, Y., Toguchi, H., 1991. Pharmacokinetics of once-a-month injectable microspheres of leuprolide acetate. Pharm. Res. 8, 787–791.

Park, T.G., 1995. Degradation of poly(lactic-co-glycolic acid) microspheres:effect of copolymer composition. Biomaterials 16, 1123–1130.

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

Sanders, L.M., Kell, B.A., McRae, G.I., Whitehead, G.W., 1986. Prolonged controlled-release of nafarelin, a luteinizing hormone-releasing hormone analogue, from biodegradable polymeric implants: influence of composition and molecular weight of polymer. J. Pharm. Sci. 75, 356–360.

Sawyer, T.K., Sanfilippo, P.J., Hruby, V.J., Engel, E.H., Heward, C.B., Burnett, B., Hadley, M.E., 1980. [Nle$^4$-DPhe$^7$]α-melanocyte stimulating hormone: a highly potent α-melanotropin with ultralong biological activity. Proc. Natl. Acad. Sci. USA 77, 5754–5758.

Schmitt, E.A., Flanagan, D.R., Linhardt, R.J., 1993. Degradation and release properties of pellets fabricated from three commercial poly(DL-lactide-co-glycolide) biodegradable polymers. J. Pharm. Sci. 82, 326–329.

Surendran, N., Ugwu, S.O., Sterling, E.J., Blanchard, J., 1995. High-performance liquid chromatographic assay for Melanotan-I ([$Nle^{4}$-$DPhe^{7}$]$\alpha$-melanocyte-stimulating hormone) in biological matrices. J. Chromatogr. B 670, 235–242.

Ugwu, S.O., Blanchard, J., Dorr, R.T., Levine, N., Brooks, C., Hadley, M.E., Aickin, M., Hruby, V.J., 1997. Skin pigmentation and pharmacokinetics of melanotan-I in humans. Biopharm. Drug Dispos. 18, 259–269.

Vert, M., Garreau, H., 1995. Recent advances in the field of lactic acid/glycolic acid polymer-based therapeutic systems. Macromol. Symp. 98, 633–642.