Release mechanisms of tacrolimus-loaded PLGA and PLA microspheres and immunosuppressive effects of the microspheres in a rat heart transplantation model

Ryo Kojima a,b,*, Takatsune Yoshida a, Hiroaki Tasaki a, Hiroyuki Umejima a, Masashi Maeda c, Yasuyuki Higashi c, Shunsuke Watanabe a, Naoto Oku b

a Pharmaceutical Research & Development Labs., Astellas Pharma Inc., 180, Ozumi, Yaizu-shi, Shizuoka 425-0072, Japan
b Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
c Research Portfolio & Science, Astellas Pharma Inc., 21, Miyakiguchi, Tsukuba-shi, Ibaraki 305-8585, Japan

**A R T I C L E   I N F O**

Article history:
Received 24 March 2015
Received in revised form 9 June 2015
Accepted 2 July 2015
Available online 6 July 2015

Keywords:
Tacrolimus
PLGA
PLA
Microsphere
Controlled release
Heart transplantation

**A B S T R A C T**

The objective of this study was to elucidate the release and absorption mechanisms of tacrolimus loaded into microspheres composed of poly(lactic-co-glycolic acid) (PLGA) and/or polyactic acid (PLA). Tacrolimus-loaded microspheres were prepared by the o/w emulsion solvent evaporation method. The entrapment efficiency correlated with the molecular weight of PLGA, and the glass transition temperature of PLGA microspheres was not decreased by the addition of tacrolimus. These results indicate that intermolecular interaction between tacrolimus and the polymer would affect the entrapment of tacrolimus in the microspheres. Tacrolimus was released with weight loss of the microspheres, and the dominant release mechanism of tacrolimus was considered to be erosion of the polymer rather than diffusion of the drug. The whole-blood concentration of tacrolimus in rats was maintained for at least 2 weeks after a single subcutaneous administration of the microspheres. The pharmacokinetic profile of tacrolimus following subcutaneous administration was similar to that following intramuscular administration, suggesting that the release and dissolution of tacrolimus, rather than the absorption of the dissolved tacrolimus, were rate-limiting steps. Craft-survival time in a heart transplantation rat model was prolonged by the administration of tacrolimus-loaded microspheres. The microsphere formulation of tacrolimus would be expected to precisely control the blood concentration while maintaining the immunosuppressive effect of the drug.

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1. Introduction

Tacrolimus (FK506) is an immunosuppressive drug that inhibits calcineurin through complex formation with the FK506-binding protein (Schreiber and Crabtree, 1992). Tacrolimus has been used for prophylaxis of organ rejection and many other immunological disorders (Braun and Behrend, 2006; Simpson and Noble, 2005). The blood trough concentration of tacrolimus correlates with not only the immunosuppressive effect but also the toxicity (Kershner and Fitzsimmons, 1996). The blood concentration of the drug is both intra- and inter-individually variable, and therapeutic drug monitoring is recommended for an immunosuppressive therapy with tacrolimus (Scott et al., 2003). In addition, glucose intolerance, one of the adverse effects of tacrolimus, depends on the maximum blood concentration of the drug (Ishibashi et al., 2001; Mecule et al., 2010). Therefore, a reduction in the maximum blood concentration would be preferable. It is also anticipated that advanced tacrolimus formulations could improve medication adherence. About one-fifth of all transplant patients do not take their medications correctly (Hofmann and Bunzel, 2000). For example, the 6-month persistence rate for renal transplant patients is 71.9% for a tacrolimus twice-daily formulation and 81.5% for a once-daily one (Kuypers et al., 2013).

Long-acting injection (LAI) such as depot, implant or injectable gel sustains the efficacy for weeks or months by a single administration. Among various kinds of LAI, biodegradable microspheres have certain advantages: encapsulation of a variety of drugs, biocompatibility, high bioavailability, less-invasive administration, and controllable long-term release (Varde and
Poly(lactic-co-glycolic acid) (PLGA) and polyactic acid (PLA) are well-known biodegradable materials, and the release rate of an entrapped drug can be controlled by changing the polymer composition (Miller et al., 1977). There are 2 anticipated effects of an application of PLGA and PLA microsphere technology to tacrolimus. One is a reduction in adverse effects associated with a change of pharmacokinetic profile. A flat pharmacokinetic profile obtained by the controlled release of tacrolimus does not necessarily improve the safety but may optimize the immunosuppressive therapy with tacrolimus (Ishibashi et al., 2001; Meucle et al., 2010). The other is improved medication adherence. It is reported that the adherence of LAL is better than that of oral formulations (Olivares et al., 2009). A PLGA and PLA microsphere formulation of tacrolimus would reduce the administration frequency and contribute to improvement of the medication adherence.

There are some reports about PLGA microsphere or nanosphere formulations of tacrolimus. For example, it was reported that tacrolimus-loaded PLGA microspheres prolong the survival time in a liver transplantation rat model (Miyamoto et al., 2004) or graft-survival time in an islet transplantation mouse model (Wang et al., 2004). Shin et al. (2010) reported that the lympatic targeting efficiency of tacrolimus is improved by its encapsulation in PLGA or PEG–PLA nanospheres. Affifi et al. (2011) also reported an increase in the liver distribution of tacrolimus by the entrapment of it in PLA nanospheres. In spite of the above-mentioned examples, there are few reports about controlling the release rate of tacrolimus from PLGA microspheres or nanospheres in the long term. Especially, the release and absorption mechanisms of tacrolimus loaded into PLGA microspheres have not been revealed to the best of our knowledge.

In this study, the objective was thus to elucidate the release and absorption profiles of tacrolimus formulated in PLGA and PLA microspheres. We characterized the physicochemical properties of the microspheres and investigated the in vitro and in vivo release patterns of the drug. The effect of the microsphere formulation on graft survival after transplantation was also studied by using a rat heterotopic heart transplantation model to ensure that the immunosuppressive property of tacrolimus from this formulation was maintained.

2. Materials and methods

2.1. Materials and animals

Tacrolimus and Prograf® injection (tacrolimus formulated for injection, 5 mg/mL) were manufactured by Astellas Pharma Inc. (Tokyo, Japan). PLGA and PLA (RG502H, RG503H, RG504H, RG752H, and R202H) were purchased from Boehringer Ingelheim Pharma GmbH & Co., KG (Ingelheim, Germany). Polyvinyl alcohol (GOHSEONOL™ EG-05) was obtained from The Nippon Synthetic Chemical Industry Co., Ltd. (Osaka, Japan). Aminoalkyl methacrylate copolymer E (Eudragit® E) was kindly provided by Evonik Japan Co., Ltd. (Tokyo, Japan). All materials were of analytic reagent grade and used as received.

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. The Tsukuba Research Center and Yaizu Pharmaceutical Research Center of Astellas Pharma Inc. are accredited by AAALAC International.

2.2. Preparation of tacrolimus-loaded microspheres

PLGA and PLA microspheres were prepared by the o/w emulsion solvent evaporation method (Elkharrar et al., 2006; Sansdrap and Moes, 1993). Specifically, 215 mg of tacrolimus and a 500-mg mixture of PLGA and PLA were dissolved in 20 mL of dichloromethane. This solution was slowly added to 300 mL of 0.5 w/v% polyvinyl alcohol aqueous solution with 1000-rpm agitation by a propeller mixer (MAZELA Z-2100, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). After overnight agitation, the solution was centrifuged (1870 × g, 10 min); and the precipitate was then dispersed in distilled water. After the aqueous dispersion had been filtered, the residue was freeze-dried. The placebo PLGA and PLA microspheres were prepared by the same procedure without tacrolimus loading.

2.3. Characterization of tacrolimus-loaded microspheres

2.3.1. Drug content

Ten milligrams of tacrolimus-loaded microspheres was dissolved in 25 mL of acetonitrile. Subsequently, 5 mL of the solution was diluted with 15 mL of 20% acetonitrile. This solution (100 µL) was then injected into a HPLC (2695 Separation module, Waters Co., MA, USA) with a C8 column (4.6 mm × 150 mm, 5 µm) at 50°C. The mobile phase was a mixture of acetonitrile/water/methanol/ 6% phosphoric acid = 460/360/180/1 (volume ratio), and the flow rate was constant at 0.45 mL/min. Tacrolimus was detected at a wavelength of 210 nm.

The drug-loading ratio and entrapment efficiency were determined as follows: drug-loading ratio (%) = (weight of tacrolimus in microspheres/weight of microspheres) × 100 and entrapment efficiency (%) = (weight of tacrolimus in microspheres/weight of tacrolimus used for microsphere preparation) × 100.

2.3.2. Particle size

The particle diameter of tacrolimus-loaded microspheres was measured by use of a particle size distribution analyzer (LA-920, Horiba, Ltd., Kyoto, Japan). The sample was dispersed in distilled water and sonicated before the measurement.

2.3.3. Thermal analysis

The glass transition temperature (Tg) of the microspheres was determined by using differential scanning calorimetry (DSC8230 S-1199A, Rigaku Co., Tokyo, Japan). Three milligrams of PLGA microspheres was scanned from room temperature to 220°C at the rate of 10°C/min.

2.4. In vitro release studies

The microspheres (10 mg as whole microspheres) and phosphate-buffered saline, pH 7.4, containing 1 w/v% freeze-dried Eudragit® E (20 mL) were added to a 50-mL tube. In order to make a sink condition, freeze-dried Eudragit® E (freeze dry of 0.8 w/v% hydrochloric acid containing 11.0 w/v% of Eudragit® E) was used (Yoshida et al., 2012). The dispersion was sonicated for a few seconds before the release studies. The tubes were placed in a shaker bath with the water temperature maintained at 37°C and were shaken horizontally at a rate of 120 strokes per minutes (Kamijo et al., 1996). At predetermined intervals, the dispersion was centrifuged (1870 × g, 10 min) and the supernatant was discarded. After freeze-drying, the residue was weighed to determine the amount eroded. The amount of released tacrolimus was calculated from that of tacrolimus remaining in the microspheres. Morphology of the microspheres at each time point was also observed by use of a scanning electron microscope (S-800 U-0095A, Hitachi, Ltd., Tokyo, Japan).

2.5. In vivo pharmacokinetic studies

Six-week-old Lewis rats were obtained from Japan SLC, Inc. (Shizuoka, Japan). Tacrolimus-loaded microspheres were dispersed in an aqueous solution consisting of 0.5 w/v% of...
carboxymethyl cellulose sodium and 10 w/v% of sucrose. Solid contents in the dispersion of Form. 1–1 and Form. 1–3 were 1.5 and 3 w/v%, respectively. The dispersion was sonicated for a few seconds before administration. Tacrolimus solution (Prograf® injection diluted with saline to 0.3 mg/mL) was used as control. The samples were subcutaneously or intramuscularly administered to the rats (body weight: 200 ± 30 g) at 7 weeks of age. At specific time points, whole-blood samples were collected from the heart by using heparinized syringes during anesthesia effected by the inhalation of isoflurane. The rats were sacrificed by cervical dislocation after the blood sampling.

In order to determine the tacrolimus concentration in whole blood, we mixed 0.5 mL of whole-blood sample, 0.1 mL of distilled water, 0.1 mL of internal standard (50 ng/mL of ascomycin, Wako Pure Chemical Industries, Ltd., Osaka, Japan) in methanol solution, and 3 mL of deproteinization solution (2.8 w/v% zinc sulfate/methanol/acetoneitrile = 20/12/8) and then centrifuged the mixture (1870 × g, 15 min). The supernatant was applied to a C18 column (Bond Elut C18, 100 mg/1 mL, Agilent Technologies, Inc., CA, USA). After having been washed with 1 mL of 40% methanol and 1 mL of hexane, the column was eluted with 2 mL of methanol. The eluent was evaporated and dissolved in 0.4 mL of 50% acetoneitrile. Finally, the amount of tacrolimus in 20 μL of the solution was measured by use of an HPLC-MS (CBM-20A, LC-20A, DGU-20A5, SCL-20A, CTO-20AC, Shimadzu Co., Kyoto, Japan and TSQ Quantum Access Max, Thermo Fisher Scientific Inc., MA, USA) equipped with a C18 column (2.1 mm × 150 mm, 5 μm) at 55 °C. A gradient flow of ammonium acetate aqueous solution (2 mM) and ammonium acetate methanol solution (1 mM) was used as the mobile phase.

The absorption rate of tacrolimus from the tacrolimus-loaded microspheres in rats was estimated by using the WinNonlin program for a two-compartment model. The pharmacokinetic parameters of the two-compartment model were calculated by reference to pharmacokinetic data for a rat intravenously administered a tacrolimus solution at a dose of 0.1 mg/kg (n = 3).

### 2.6. ACI-to-Lewis rat heterotopic cardiac transplantation

Seven-week-old Lewis (RT1) and ACI (RT1 av1) rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and Japan SLC, Inc. (Shizuoka, Japan), respectively. ACI and Lewis rats at 8 weeks of age were used as cardiac donors and recipients, respectively. Heart transplantation was performed by using the heterotopic heart transplantation technique previously described (Ono and Lindsey, 1969). Briefly, hearts were transplanted from male ACI donors into male Lewis recipients. The donor aorta and pulmonary artery were anastomosed, end to side, to the recipient’s abdominal aorta and inferior vena cava,

respectively. The day of transplantation was designated as day 0. The dispersion of tacrolimus-loaded microspheres was postoperatively administered in a single dose into the subcutaneous tissue of the recipient. The dispersion medium was the same as described earlier, and the solid content of the microspheres was 1 w/v%. Graft survival was monitored by daily palpation until day 14, and graft rejection was defined as cessation of palpable cardiac graft beats. Rats that had lost palpable contraction of the graft within 3 days or had died postoperatively were excluded from the analysis. The graft-survival rate at 14 days after the heart transplantation was calculated.

Blood samples were collected from the abdominal aorta by using a heparinized syringe at day 14, and then the model rats were sacrificed. The blood concentration of tacrolimus was measured by the above-mentioned procedure.

### 3. Results and discussion

#### 3.1. Characterization of tacrolimus-loaded microspheres

Table 1 presents the molecular weight and lactic acid/glycolic acid (L/G) ratio of PLGA and PLA used in this work. In order to evaluate the effect of the molecular weight and L/G ratio on entrapment and release of tacrolimus, we investigated various polymer species and the blend of 2 polymers. Physicochemical properties of PLGA and PLA microspheres are summarized in Table 2. The particle diameters of PLGA microspheres were comparable over a range of 12–19 μm.

The entrapment efficiency was plotted against the sum of the numbers of PLGA and PLA molecules (Fig. 1). The X-axis shows sum of the molecular numbers calculated: weight of PLGA or PLA used for microsphere preparation divided by the molecular weight of the polymer. The entrapment efficiencies of all formulations were more than 80%. These high efficiencies are probably due to the hydrophobicity of tacrolimus: hydrophobic drugs hardly diffuse from organic phase to continuous phase in o/w emulsion, and also may interact with hydrophobic part of PLGA and PLA (Ishihara and Mizushima, 2010; Li et al., 2008). In addition, the entrapment efficiency increased with an increase in the number of the polymer molecules. All PLGA and PLA employed here were uncapped, and

<table>
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<tr>
<th>Form.</th>
<th>Tacrolimus (mg)</th>
<th>RG502H (mg)</th>
<th>RG503H (mg)</th>
<th>RG504H (mg)</th>
<th>RG752H (mg)</th>
<th>R202H (mg)</th>
<th>Drug-loading ratio (%)</th>
<th>Entrapment efficiency (%)</th>
<th>Median particle diameter (μm)</th>
</tr>
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<tbody>
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<td>0</td>
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<td>0</td>
<td>29.3 ± 0.0</td>
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<td>16.3</td>
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<td>500</td>
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<td>0</td>
<td>29.5 ± 0.3</td>
<td>98.3</td>
<td>16.1</td>
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<tr>
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<td>500</td>
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<td>125</td>
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<td>0</td>
<td>26.4 ± 0.4</td>
<td>88.0</td>
<td>17.0</td>
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the polymers ended with a carboxyl group. This result suggests that terminal carboxyl group of the polymer enhanced the encapsulation of tacrolimus. It was earlier reported that capping the carboxyl group of PLGA diminishes the loading efficiency of active compounds, especially basic drugs, in PLGA microspheres. On the other hand, the effects of the molecular weight of a polymer on entrapment efficiency are dependent on the characteristics of drugs; because a decrease in the molecular weight means not only an increase in the number of terminal groups of the polymer per unit weight but also a decrease in its inherent viscosity (Gao et al., 2006; Makino et al., 2004; Su et al., 2009). Regarding tacrolimus-loaded microspheres prepared in this study, the carboxyl group of the polymer would be more important than the viscosity for entrapment of tacrolimus in microspheres. Two factors of carboxyl group are considered to increase the entrapment efficiency. One is rigidity of PLGA and PLA. The uncapped terminal groups would make the polymer rigid and easily interact with tacrolimus (Samadi et al., 2013). The other is hydrogen bonding between tacrolimus and the polymer. Considering the chemical structure of tacrolimus, carboxyl groups of PLGA and PLA may interacts with tacrolimus through hydrogen bonding rather than ionic interaction.

In order to properly investigate the interaction between tacrolimus and PLGA, the Tg of microspheres was measured by DSC. Tg of Form. 6 (PLGA, L/G = 50/50), Form. 1–1 (PLGA, L/G = 75/25), Form. 5 (PLA), placebo of Form. 6, placebo of Form. 1–1, and placebo of Form. 5 were 49.0 °C, 52.1 °C, 57.1 °C, 47.9 °C, 50.9 °C, and 55.2 °C, respectively. Almost same values of Tg were also confirmed by differential thermal analysis (data not shown). The Tg was raised by an increase in the L/G ratio of PLGA, and this result was consistent with the Tg of the polymer itself (Okada et al., 1994). In addition, the inclusion of tacrolimus in all the microsphere formulations did not decrease the Tg from that of the placebo microspheres. A small molecule added to a polymer matrix usually acts as a plasticizer that diminishes intermolecular interaction of the polymer and depresses Tg of the mixture from Tg of the pure polymer (Freed, 2011). Therefore, we considered that the DSC results in this study indicated an interaction between tacrolimus and PLGA (Achim et al., 2008; Presmanes et al., 2011; Sanchez et al., 1993). Based on the results for the entrapment efficiency and DSC, hydrophobic interaction and hydrogen bonding would be important for the entrapment of tacrolimus in microspheres.

3.2. In vitro release studies

In vitro release profiles of tacrolimus loaded into microspheres are shown in Fig. 2. The amount of tacrolimus released was measured at 1, 3, 7, 14, 21, and 28 days. The microspheres were prepared with blend of PLGA (L/G molar ratio was 75/25) and PLA. PLGA/PLA weight ratios of Form. 1, 2, 3, 4, and 5 were 100/0, 75/25, 50/50, 25/75, and 0/100, respectively. The release rate of tacrolimus from the microspheres decreased with an increase in the PLA content, and this result corresponded to the degradation rates of PLGA and PLA. The release periods of tacrolimus from the microspheres could be controlled from 2 weeks to more than 1 month by changing the PLGA/PLA weight ratios. Furthermore, the initial bursts of tacrolimus release from all formulations in 24 h were less than 10%. Regarding the microsphere formulation, dose dumping of tacrolimus seemed to hardly occur.

As shown in Fig. 3, weight changes of Form. 1 (PLGA) and Form. 5 (PLA) were also measured. The weight-loss rate of PLGA microspheres was higher than that of PLA microspheres, and the profiles were similar to the release patterns of tacrolimus. Fig. 4 presents the erosion behavior of the tacrolimus-loaded and placebo microspheres. PLGA microspheres, but not the PLA ones, showed a porous structure. This behavior was consistent with the result of weight change. Based on these results, we considered the degradation property of PLGA and PLA to contribute to the controlled release of tacrolimus from the microspheres.

PLGA microspheres generally show a triphasic release profile of the encapsulated drug (O’Donnell and McGinity, 1997; Siepmann and Gopferich, 2001). Initially, a burst of release of the drug located near the microsphere surface is observed. The second phase is slow release derived from diffusion of the drug out of the microspheres. Finally, the release rate is increased with erosion of the polymeric matrix. The initial bursts of tacrolimus loaded into microspheres were low, and tacrolimus was released in association with the weight loss of PLGA microspheres. These release profiles and
dissolution behaviors indicate that the dominant release mechanism of tacrolimus loaded into PLGA and PLA microspheres would be erosion of the polymer rather than diffusion of tacrolimus.

3.3. In vivo pharmacokinetic studies

Fig. 5 shows blood concentration profiles of tacrolimus in rats. Compared with tacrolimus solution, the microsphere formulation sustained the blood concentration. Furthermore, significantly high blood concentration derived from initial burst of tacrolimus was not observed within 24 h of administration in spite of a relatively high dose (5.0 mg/kg as tacrolimus). Blood concentration of tacrolimus was also measured for 14 days following administration of the microspheres (Fig. 6). Tacrolimus in Form. 1–3 was subcutaneously administered at a dose of 6.75, 13.5, 27.0 or 54.0 mg/kg. Blood concentrations of tacrolimus increased as the dose level was increased. Almost steady blood concentrations of tacrolimus were maintained for 2 weeks after a single subcutaneous injection of the microspheres.

The administered microspheres formed aggregates in the subcutaneous tissue. It was reported that aggregates of PLGA microspheres are degraded faster than the dispersed ones (Sansd rap and Moes, 1997). Another group also indicated that the rate of drug release from PLA implants in water-limited in vivo conditions is faster than in vivo. The administered microspheres formed aggregates in the subcutaneous tissue. It was reported that aggregates of PLGA microspheres are degraded faster than the dispersed ones (Sansd rap and Moes, 1997). Another group also indicated that the rate of drug release from PLA implants in water-limited conditions is faster than in vivo.

Fig. 4. Erosion behavior of microspheres with or without tacrolimus. After the release studies, the freeze-dried microspheres were observed with a scanning electron microscope. The magnification was 3000×.
tissue is higher than in vitro (Okabe et al., 2003). These phenomena are considered to be due to a decrease in the pH inside the matrix in conditions with fewer water channels and acidic autocatalysis of the polymer hydrolysis. However, the in vivo absorption rates of tacrolimus loaded into PLGA microspheres were not faster than the in vitro release rate and decelerated with an increase in the administered dose (Fig. 7). Tacrolimus is a drug with poor water solubility, and its dissolution rate would be lower in water-limited subcutaneous tissue in vivo than under the sink condition in vitro. The result indicates that not only the hydrolysis of PLGA but also the dissolution of tacrolimus were important factors for the release of the drug from the microspheres in vivo.

Fig. 8 shows changes in the blood concentration of tacrolimus following subcutaneous or intramuscular administration of the microspheres. These time-course changes following intramuscular administration were similar to those following subcutaneous administration. The intramuscular absorption rate of a drug is generally higher than the subcutaneous one because of less fat and greater vascularity of the dense muscles (Peters, 2012). Consequently, these results suggest that the release and dissolution of tacrolimus, rather than the absorption process, were rate-limiting for tacrolimus-loaded PLGA microspheres.

Fig. 9 shows a schematic model for the absorption mechanism of tacrolimus-loaded microspheres. The results of the in vitro release study indicated that the erosion of PLGA was more dominant than the diffusion of tacrolimus. In addition, the absorption of dissolved tacrolimus would not be rate-limiting based on the comparison between subcutaneous and intramuscular administration. Therefore, the degradation of PLGA and dissolution of tacrolimus were important processes, and regulating the erosion rate of PLGA would be one useful approach to control the absorption rate of tacrolimus from the microspheres.

Fig. 5. Blood concentration profiles of tacrolimus in normal rats after a single subcutaneous administration of the tacrolimus solution or the microsphere formulation. Each value represents the mean ± SD (n = 3–6).

Fig. 6. Blood concentration profiles of tacrolimus in normal rats after a single subcutaneous administration of the PLGA microspheres. Administered doses of tacrolimus were 6.75, 13.5, 27.0, and 54.0 mg/kg. Each value represents the mean ± SD (n = 3).

Fig. 7. Comparison between in vitro release profile and in vivo absorption profile of tacrolimus from the PLGA microspheres. The closed symbols show the release rate in vitro; and open symbols, the absorption rates in vivo.
3.4. ACI-to-Lewis rat heterotopic cardiac transplantation

Blood concentration profiles of the formulation used for pharmacological study are shown in Fig. 10. Tacrolimus in Form. 3 was subcutaneously administered to normal rats at a dose of 0.75, 3.0 or 7.5 mg/kg. This formulation also showed a dose-dependent increase in the blood tacrolimus concentration for 2 weeks.

The graft-survival time and survival rate of the rat heart transplantation model administered tacrolimus in Form. 3 are summarized in Table 3. The beat of the transplanted heart stops within 5–6 days without treatment in this model (Nakanishi et al., 2010). The graft-survival rates were 0, 57, and 100% at a dose of 0.75, 3.0, and 7.5 mg/kg, respectively. Thus, a dose-dependent increase in the graft-survival rate was observed.

Fig. 11 shows the blood concentration of tacrolimus of individual rats at day 14 and the individual plotted against their graft-survival time. The graft-survival time was prolonged as the trough concentration of tacrolimus increased up to 2 ng/mL, and the survival time reached 14 days after the transplantation at more than approximately 2 ng/mL. The area under the curve (AUC) of the blood concentration of tacrolimus correlates with rejection episodes of renal transplant patients treated with current oral tacrolimus formulations (Undre et al., 1999). Although the AUC is generally considered to be the best marker of drug exposure, it is difficult for practical reasons to measure the AUC of every patient (Wallemacq et al., 2009). Therefore, the trough concentration is monitored in clinical practice. The result of this study indicates that the trough concentration could also be used an index for treatment with tacrolimus-loaded microspheres in spite of its flat pharmacokinetic profile.
4. Conclusions

In this study, we characterized tacrolimus-loaded PLGA microspheres in vitro and in vivo. Intermolecular interaction between tacrolimus and the polymer was considered to affect the entrapment efficiency. The period of tacrolimus release from the microspheres in vitro could be controlled by changing the PLGA/PLA ratio in the long term. Pharmacokinetic profiles were maintained for at least 2 weeks by a single subcutaneous or intramuscular administration. The results of the in vitro release and in vivo absorption studies indicated that the erosion of PLGA and the dissolution of tacrolimus were dominant aspects of the release mechanism rather than the diffusion of tacrolimus from PLGA microspheres and the absorption of the dissolved tacrolimus. The microsphere formulation of tacrolimus prolonged the graft survival in the rat model of heart transplantation. This formulation should contribute to further pharmacokinetic optimization of tacrolimus and improvement of medication adherence.

References