Drug-Releasing Behavior and Immunosuppressive Effects of Tacrolimus-Loaded PLGA and PLA Microspheres

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Purpose
The objective of this study was to elucidate the release and absorption mechanisms of tacrolimus from poly(lactic-co-glycolic acid) (PLGA) and/or polylactic acid (PLA) microspheres. Immunosuppressive effects of the microspheres were also evaluated to investigate whether blood concentration could be precisely controlled while maintaining the pharmacological effects of tacrolimus.

Methods
Tacrolimus-loaded PLGA and PLA microspheres were prepared by the o/w emulsion solvent evaporation method. The weight ratio of PLGA/PLA was set as 100/0, 75/25, 50/50, 25/75, or 0/100. Physicochemical properties of the microsphere formulation such as entrapment efficiency, particle diameter, and morphology were evaluated. Release profiles of tacrolimus from PLGA and PLA microspheres were determined by in vitro release test. Weight change of the microspheres was also measured after the release test. Pharmacokinetic profile of the microsphere formulation was compared to that of tacrolimus solution in rats. Dispersion of tacrolimus-loaded microspheres was administered subcutaneously or intramuscularly to the rats. Immunosuppressive effects of the microspheres were investigated using rat heart transplantation models. Heart transplantation was performed by using the heterotopic heart transplantation technique. The dispersion of tacrolimus-loaded microspheres was postoperatively administered in a single dose into the subcutaneous tissue of the recipient. Graft survival was monitored by daily palpation, and graft rejection was defined as cessation of palpable cardiac graft beats.

Results
By the o/w emulsion solvent method, 12-19 μm sized microspheres were obtained. The entrapment efficiency of tacrolimus was in the range of 81-103%. Tacrolimus was released with weight loss of the microspheres, suggesting that the dominant release mechanism of tacrolimus from the microsphere formulation was considered to be erosion of the polymer rather than diffusion of the drug. The in vitro release rate of tacrolimus from PLGA/PLA microspheres was increased with an increase of the PLGA ratio. This result corresponded to the degradation rate of PLGA and PLA polymer itself. Therefore, the in vitro release profile could be controlled by changing the PLGA/PLA ratio. Tacrolimus in blood was rapidly eliminated when tacrolimus solution was administered to the rat. On the other hand, flat pharmacokinetic profile was sustained for at least 14 days after a single subcutaneous administration of the microspheres. The pharmacokinetic profile of tacrolimus following subcutaneous administration was similar to that following intramuscular administration, and the release and dissolution of tacrolimus, rather than the absorption of the dissolved tacrolimus, were considered to be rate-limiting steps. Graft-survival time in a heart transplantation rat model was improved by the administration of tacrolimus-loaded microspheres. The graft-survival time was prolonged as the trough concentration of tacrolimus increased, and the survival time reached 14 days after the transplantation at more than approximately 2 ng/mL.

Conclusion
The microsphere formulation of tacrolimus should contribute to further pharmacokinetic optimization of tacrolimus and improvement of medication adherence.