The angiogenesis effect of a novel long-acting prostacyclin agonist loaded-PLGA microspheres prepared using different molecular weight in murine sponge model

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ABSTRACT SUMMARY

The purpose of this study was to evaluate effect of angiogenesis after topical application of three types of ONO-1301-loaded poly(lactide-co-glycolide) microspheres (PLGA MS) prepared using difference of molecular weight of PLGA.

In drug release test in vitro and ONO-1301 plasma level in rats after administration of ONO-1301 PLGA MS prepared using three types PLGA for 28 days in vivo, the sustained release of ONO-1301 PLGA MS and its dependency to molecular weight of the polymer was confirmed.

In murine sponge model study, three types of PLGA MS were singly administered into sponge. Hemoglobin and HGF levels in sponge removed from the back of mice for 28 days were measured. The obtained hemoglobin and HGF levels after single administration of PLGA MS in sponge were significantly higher than those after daily administration of ONO-1301 powder.

EXPERIMENTAL METHODS

A sustained-release formulation of ONO-1301 was prepared by loading ONO-1301 into PLGA MS using the oil-in-water emulsion / solvent evaporation method described in a previous report. Three types of PLGA (PLGA 5010, wt average molecular weight 10 000, co-polymer ratio of DL-lactide to glycolide 50/50; 5020, wt average molecular weight 20 000, co-polymer ratio of DL-lactide to glycolide 50/50; PLGA 5050, wt average molecular weight 50 000, co-polymer ratio of DL-lactide to glycolide 50/50) were used as the substrate of the MS.

In drug release test, ONO-1301 PLGA MS were suspended in phosphate-buffered saline (pH 6.8) containing 0.2% Tween-80 to adjust the concentration of ONO-1301 to 100 µg/mL. Aliquots of this solution (1 mL) were incubated at 37°C. At various time intervals, one of the aliquots was centrifuged at 12,000 rpm for 5 min, and the supernatant was discarded. The pellet was dissolved in DMSO, and the amount of ONO-1301 remaining was analyzed by HPLC.

In assay of ONO-1301 plasma level in rats, ONO-1301 were measured 1, 6 h, and 1, 3, 7, 10, 14, 18, 21, 24, and 28 days after a single subcutaneous (s.c.) administration of 200, 400, 800 µg/mL ONO-1301 PLGA5010, 5020, 5050 MS, respectively. Plasma ONO-1301 levels were measured by LC with tandem MS assay.

Male ddY mice were used in sponge model. Polyurethane sponge discs, 5 mm thick and 13 mm diameter, were used as matrices in monitoring for angiogenesis. The sponge discs were implanted aseptically into s.c. pouch through a 1-cm long dorsal mid-line incision with curved artery forceps. The incision wound after sponge implantation was secured by a 5-0 silk suture, followed by glue.

INTRODUCTION

ONO-1301 was developed as a novel long-acting prostacyclin agonist with thromboxane synthase inhibitory activity. Recently, previous study was reported that ONO-1301 enhances HGF expression and augments angiogenesis. However, there are still no reports about angiogenesis of ONO-1301 PLGA MS in long term experimental period in murine sponge model.

In this study, three types of ONO-1301 PLGA MS were prepared using PLGA varied in the molecular weight. ONO-1301 PLGA MS were evaluated its effect of angiogenesis in topical during sustained drug release period to show the possibility to apply as angiogenesis formulation.
On the 7, 14, 28th day post-implantation, the sponge disc was carefully excised, and weighed. Each implant was homogenized in distilled water for sponge weight and centrifuged at 12,000 g for 30 min. The supernatants were used for the assessment of hemoglobin and HGF content. The hemoglobin was quantified colorimetrically at 540 nm in a microplate reader. HGF concentrations in sponge were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the protocol supplied by manufacturer.

RESULTS AND DISCUSSION

The drug release rate of ONO-1301 PLGA MS was changed in a molecular weight-dependent manner of PLGA. From these results, ONO-1301 PLGA MS using PLGA 5010, 5020 and 5050 was seemed to be effective as slow release drug for 7, 14 and 28th day, respectively.

The plasma levels of ONO-1301 in rats after a single s.c. administration of three types of ONO-1301 PLGA MS remained in the range 1–10 ng/mL for 28 days after application. These results suggested that three types of ONO-1301 PLGA MS using PLGA 5010, 5020 and 5050 are useful as the 7, 14 and 28 day-sustained release injectable formulation, respectively.

Effective concentration of ONO-1301 for angiogenesis could be maintained by single administration of ONO-1301 PLGA MS and daily administration of ONO-1301. Figure 1 shows results of hemoglobin concentrations of three types of ONO-1301 PLGA MS administration groups. The hemoglobin concentrations of three types of ONO-1301 PLGA MS were significantly increased compared with vehicle for experimental period (p<0.01, n=3-5).

HGF concentrations of all ONO-1301 PLGA MS were significantly increased compared with vehicle for experimental period (p<0.01, n=3-5). These data supported that HGF enhanced angiogenesis in murine sponge model. However, the hemoglobin concentration of PLGA 5010 MS group at 28th day was lower than other group (PLGA 5020 MS or PLGA 5050 MS). These results suggested that ONO-1301 PLGA MS as sustained release formulation was useful to obtain highly efficient angiogenesis.

CONCLUSION

In conclusion, three types of ONO-1301 PLGA MS stimulate angiogenesis by topical application and seem useful to reduce clinical symptoms conditions of vascular diseases.

REFERENCES