Long-term and zero-order release of basic fibroblast growth factorfrom heparin-conjugated poly(L-lactide-co-glycolide) nanospheres and fibrin gel

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Statement of Purpose: The administration of basic fibroblast growth factor (bFGF) has shown therapeutic potential for angiogenesis and tissue regeneration[1]. However, bFGF undergoes rapid degradation when injected into the body in soluble form, because of its short half-life *in vivo*. Delivery systems that release bFGF over a long period in a controlled manner may increase the efficacy of bFGF for angiogenesis and tissue regeneration. Methods to sustain the release of bFGF have been reported previously. These methods include the impregnation of bFGF in alginate beads[2] and gelatin hydrogel[3]. However, bFGF release from these delivery systems was completed within only a few days. In this study, we investigated whether bFGF release from fibrin gel can be prolonged by heparin-conjugated PLGA nanospheres (HCPNs) suspended in fibrin gel, and whether the angiogenic efficacy of sustained bFGF delivery is further enhanced compared to bFGF delivery using a fibrin gel. The rate and duration of bFGF release from the delivery system were controlled with the concentration of fibrinogen. The bioactivity of bFGF released from the delivery system was examined by measuring HUVEC growth *in vitro* in a medium containing the bFGF delivery system. The therapeutic potential of the bFGF delivery system was evaluated using a mouse ischemia model.

Methods: PLGA copolymer was prepared by ring-opening polymerization of L-lactide and glycolide. Novel HCPNs were prepared by using a coupling reaction between amino-terminated PLGA nanospheres and heparin in the presence of 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide. The amount of heparin conjugated to the nanosphere surface was determined with the toluidine blue method. The efficiency of bFGF complexation to HCPNs and bFGF release profile were determined with enzyme-linked immunosorption assay (ELISA). The bioactivity of bFGF released from HCPNs was assessed *in vitro* by determining its ability to stimulate the proliferation of human umbilical vein endothelial cells (HUVECs) cultured in endothelial cell basal medium-2 without fetal bovine serum. The angiogenic efficacy of bFGF released from HCPNs was evaluated using a mouse (C57BL/6) limb ischemic model. Angiogenic efficacy of each therapy was evaluated by immunohistological examinations and microvessel density determination in the ischemic areas. All quantitative data were expressed as the mean ± standard deviation. Statistical analysis was performed by unpaired Student's *t* test. A value of $p<0.05$ was considered to be statistically significant.

Results / Discussion: The amount of heparin conjugated to the PLGA nanospheres was increased up to 29-fold by using nanospheres made from lower molecular weight PLGA, or star-shaped PLGA, as compared to nanospheres made from higher molecular weight PLGA, or linear PLGA. The release of bFGF from HCPNs was sustained for three weeks with no initial burst release. The bFGF release period was increased to more than four weeks using a delivery system of HCPNs suspended in fibrin gel. The release was nearly zero order. The rate of bFGF release from HCPNs in fibrin gel was controlled by the fibrinogen concentration in the fibrin gel. As the fibrinogen concentration increased, the bFGF release rate decreased. The bioactivity of bFGF released from HCPNs in fibrin gel was assessed using HUVEC culture. Basic FGF released from HCPNs in fibrin gel exhibited HUVEC growth for 15 days, similar to that of cultures to which bFGF in free form was added daily, suggesting that the delivery system of HCPNs in fibrin gel can release bFGF in a bioactive form for a long period. Immunohistological analysis of mouse ischemic limbs indicated that the microvessel density was much higher in the ischemic limbs treated with bFGF delivery using HCPNs in fibrin gel than in the ischemic limbs treated with daily injections of bFGF or with bFGF delivery using fibrin gel.

Conclusions: This study demonstrates that a delivery system of HCPNs in fibrin gel can sustain bFGF release for more than one month at nearly zero-order, and that the release rate can be controlled with the fibrinogen concentration. The bFGF delivery system showed therapeutic potential for angiogenesis in a mouse ischemic limb model. The local, sustained, and controllable delivery system for bFGF developed in this study may provide a powerful modality for a variety of therapeutic interventions, such as cardiac ischemia, limb ischemia, wound healing, and bone regeneration.

References:

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