Therapeutic Effect of Local Administration of Low Dose Simvastatin-conjugated Gelatin Hydrogel for Fracture Healing

1 Tomoaki Fukui; 1,2 Masaaki Ik; 1,2 Yutaka Mifune; 1,2 Taro Shoji; 1,2 Tomoyuki Matsumoto; 1,2 Yohei Kawakami; 1,2 Tomoya Kuroda;
1 Hiroshi Akimaru; 1 Atsuhiko Kawamoto; 1 Junichiro Jo; 1 Yasuhiro Tabata; 1 Masahiro Kurosaka; 1 Ryoosuke Kuroda; 1 Takayuki Asahara

1, Department of Vascular Regeneration Research, Institute of Biomedical Research and Innovation, Kobe, Japan. 2 Department of Orthopaedics, Kobe University Graduate School of Medicine, Kobe, Japan. 3 Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan. 4 Department of Pharmacology, Osaka Medical College, Osaka, Japan. tomoakifukui@yahoo.co.jp

INTRODUCTION

Defective bone formation in fracture sites is mainly caused by impairment of neovascularization resulting in delayed union or persistent nonunion. Statins have been reported to demonstrate a pleiotropic effect as well as a cholesterol lowering effect, specifically, including effect for bone formation or favorable effects on ischemic tissue with enhanced neovascularization via an activation of the endothelial progenitor cells (EPCs) (1-6). We recently reported the therapeutic potential of circulating EPC administration for fracture healing via angiogenesis and osteogenesis (7-9). These evidences led us to hypothesize the therapeutic potential of Statins for fracture healing, which also requires sufficient blood perfusion. Although several reports have shown a certain therapeutic effect of Simvastatin on bone formation with neovascularization (1,2,4-6), systemic administration of Simvastatin made the effect minimum because of the clearance in liver, and high dose administration therefore may cause systemic adverse side effects (10). In order to overcome the problem of low efficacy/frequent side effects by high dose of Statin treatment, we utilized biodegradable gelatin hydrogels as a tool for drug delivery system (11) for fracture healing. In the present study, we evaluated the therapeutic efficacy of local Simvastatin-conjugated gelatin hydrogel treatment in a rat unhealing fracture model.

MATERIALS AND METHODS

Animal model:
A reproducible model of femoral fracture was created in Sprague-Dawley rats with periosteum cauterization, which leads to nonunion at 8 weeks post-fracture (12).

Drug delivery system:
Rats received local administration of following materials after fracture creation;
(a) 250 µg Simvastatin conjugated with gelatin hydrogel (ST-Gel)
(b) Gelatin hydrogel (Gel) alone.

All experimental procedures were conducted in accordance with the Japanese Physiological Society Guidelines for the care and Use of Laboratory Animals and the study protocol was approved by the Ethics Committee in RIKEN Center for Developmental Biology.

RESULTS

Radiographical and histological findings in fracture healing
In about 90 % of animals receiving ST-Gel, fracture healed with bridging callus formation radiographically, while no animals receiving Gel showed bridging callus formation (Table 1). In histological evaluation, the degrees of fracture healing assessed by Allen’s classification (13) were significantly greater in the ST-Gel group than the Gel group at week 8.

<table>
<thead>
<tr>
<th>Period after fracture</th>
<th>2 wks</th>
<th>4 wks</th>
<th>6 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-Gel</td>
<td>0/5</td>
<td>1/8</td>
<td>7/8</td>
</tr>
<tr>
<td>Gel</td>
<td>0/5</td>
<td>0/8</td>
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Functional bone healing assessed by three point bending test
To confirm the functional recovery of fractured bone, biomechanical evaluation by a three-point bending test was performed at week 8 in both groups. The percent ratios of all parameters in fractured femur to contralateral intact femur were significantly greater in the ST-Gel group than those in the Gel group.

Enhancement of angiogenesis and osteogenesis in sites of fracture:
Enhanced angiogenesis and osteogenesis by paracrine effect of the transplanted materials on recipients’ cells were examined by immunostaining with rat-specific markers. Capillary density and osteoblast density were significantly greater in the ST-Gel group compared with the Gel group, suggesting the enhancement of intrinsic angiogenesis and osteogenesis following Simvastatin transplantation.

To further explore molecular mechanism for the enhanced angiogenesis and osteogenesis by Statin treatment, we have performed real time RT-PCR to quantify the expressions of angiogenesis- and osteogenesis-related cytokines, such as vascular endothelial growth factor (VEGF) and bone morphogenic protein 2 (BMP-2) in peri-fracture sites 2 weeks after surgery. The mRNA expressions of VEGF and BMP-2 were significantly greater in the ST-Gel group compared with the Gel group.

Serial improvement of blood perfusion in sites of fracture:
Local blood perfusion was evaluated by Laser Doppler Imaging (LDI) system one hour, 1, 2 and 3 weeks after surgery. There were no significant differences in the blood perfusion ratio (fractured site/contralateral site) one hour after fracture creation between 2 groups, while the ratio was significantly higher in the ST-Gel group compared with the Gel group at 1 week and 2 weeks after surgery. Finally, the ratio reached to similar level in both groups 3 weeks after surgery.

DISCUSSION

A number of previous animal studies investigated and showed the therapeutic effect of Statins on bone formation with enhanced angiogenesis. However, few clinical studies have demonstrated sufficient therapeutic effect of Statin for bone formation. This discrepancy might be explained by a big difference of systemically administered dose of Statins to animals versus human ranging from 5-70 mg/kg versus 80-400 µg/kg, respectively. In the current study, we locally applied Simvastatin with gelatin hydrogel to the fracture site at a dose of 750 µg/kg, which is close to the permissible amount in clinical use, and successfully induced complete fracture union in a rat delayed union bone fracture model.

Further investigations will be performed to assess the effect of Simvastatin on EPC kinetics as another mechanism for fracture healing.

CONCLUSION

Local administration of low dose Simvastatin-conjugated gelatin hydrogel accelerated a delayed fracture healing via the effect on both angiogenesis and osteogenesis. These findings indicated the possibility of local Statin therapy for fracture healing avoiding major side effect in clinical settings.

REFERENCES