Granulocyte stimulating factor (G-CSF) Contributes to Medial Collateral Ligament Healing

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INTRODUCTION:
It has been reported that the mechanical properties of the healing ligament do not return to normal (1). Revascularization plays an essential role in the initial phase of ligament healing (2). Granulocyte colony-stimulating factor (G-CSF) is recently proved to contribute to angiogenesis under appropriate environment (3). It has been previously reported that G-CSF mobilized CD34 cells promotes fracture healing via vasculogenesis and osteogenesis (4), and also reported enhancement of tendon-bone osteointegration of anterior cruciate ligament graft using G-CSF (6). Therefore, we performed a series of experiments to prove a hypothesis that ligament healing may be supported by G-CSF via angiogenesis.

MATERIALS and METHODS:
Animal Model
36 female athymic Sprague-Dawley (SD) rats (24W) were used in this study. The institutional animal care and use committees of Kobe University approved all animal procedures including human cell transplantation. The surgical procedure has been previously reported (6). After creating medial collateral ligament injury (MCL), animals were divided into two groups of 18 animals each. In group A, only biodegradable gelatin hydrogel was applied in the injury site of MCL. In group B, 0.05ug of G-CSF was applied in the injury site with biodegradable gelatin hydrogel was applied in the injury site of MCL. In future studies, we should investigate the mechanism of G-CSF to recruitment of stem cells in the peri-injury site of ligament.

Assessment of MCL healing
To evaluate MCL healing, in 6 knees from each group euthanized at 2 and 4 weeks after surgery, the cut portions of MCLs were inspected macroscopically. Real time RT-PCR analysis was performed with rat VEGF at week 1 in each group. And molecular biologically, real time RT-PCR analysis was performed with rat VEGF at week 1 in each group. All soft tissue spanning the knee, except for the MCL, was sharply transected. The femur-MCL-tibia complex was mounted in a specially designed device using acrylic resin, and fixed in a mechanical testing machine (Autograph AGS-5KN; Shimadzu Corp., Kyoto, Japan). The femur and tibia were oriented at angles of 60° and 0° from the loading axis, respectively, so that the load was directed along the longitudinal axis of the MCL. A tensile load was then applied at a rate of 0.25 mm/second until gross failure of the MCL occurred. In addition to the tensile tests on the healing ligaments, mechanical tests were also performed on a sham-exposed MCL to obtain normal control values. The four normal, uninjured ligaments were prepared, tested, and measured in the same way as the experimental specimens.

RESULTS:
Evaluation of neovascularization
Neovascularization assessed by capillary density was significantly enhanced in group B compared with group A (Fig.1A,B). Furthermore, Gene expression of rVEGF was significantly increasing in group B compared with group A (Fig.1C).

Assessment of MCL healing
Assessment of ligament healing by macroscopic inspection demonstrated that the ligament was significantly healed in group B compared with group A (Fig.2). Gene expressions of both rTeM and Col1A2 were significantly higher in group B compared with group A.

DISCUSSION:
This study demonstrated that a local application of G-CSF significantly accelerates ligament healing. This indicate that G-CSF contribute to angiogenesis at least in part.
In conclusion, our findings suggest therapeutic potential of G-CSF in promoting an environment conducive to angiogenesis in the peri-injury site of MCL. In future studies, we should investigate the mechanism of G-CSF to recruitment of stem cells in the peri-injury site of ligament.

REFERENCES:
5. Sasaki K et al. AJSM 2008.