

The presoaking grafts in vancomycin does not exacerbate graft-bone healing in the ACLR model using rats.

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INTRODUCTION: Postoperative graft bone healing is important in anterior cruciate ligament reconstruction (ACLR) [1]. The vancomycin pre-soaking technique, in which grafts are treated with a vancomycin solution, was first reported in 2012 [2] and has been shown to reduce the rate of infection after ACLR [3][4]. However, the effect of this method on graft-bone healing has not been fully elucidated. In this study, we investigated the effect of vancomycin pre-soaking on graft-bone healing in a rat ACLR model.

MATERIALS and METHODS: ACLR was performed on the right knees of 54 male Wistar rats (9 weeks old) using the long flexor digitorum longus tendon (operated side). Rats were randomly divided into the normal saline- (NS) and vancomycin solution-treated (VS) groups. The grafts were immersed in sterile saline for 30 min in the NS group and in a 5 mg/ml vancomycin solution in the VS group. Biomechanical testing, micro-CT, and histological evaluations were performed at 4 and 12 weeks postoperatively. For biomechanical testing, all soft tissue structures other than the graft were dissected. The surgical clip and soft wire used for the initial graft fixation were removed. The samples were fixed to a biomechanical testing machine using bone cement and were subjected to tensile testing. The tibia was imaged using micro-CT, and the bone tunnel area was measured in cross sections vertical to the bone tunnel. For histological evaluation, the 5µm sagittal sections of the tibia were stained with hematoxylin and eosin. The degree of bone-tendon healing was assessed using the tendon-bone tunnel healing (TBTH) score [5] by three individuals, and the average and intraclass correlation coefficients (ICC) were calculated. Statistical analysis was performed using Graph pad prism version 9 using the Mann-Whitney U test, with $P < 0.05$ considered a significant difference. Data are shown as mean \pm standard deviation.

RESULTS: Biomechanical testing: The mean maximum failure load (N) was 13.7 ± 8.2 in the NS group and 11.6 ± 4.8 in the VS group at 4 weeks ($p = 0.95$), and 23.2 ± 13.2 in the NS group and 30.4 ± 18.0 in the VS group at 12 weeks ($p = 0.35$). Mean stiffness (N/mm) was 5.4 ± 2.1 in the NS group and 5.5 ± 3.1 in the VS group at 4 weeks ($p = 0.86$), and 7.0 ± 3.3 in the NS group and 10.5 ± 6.0 in the VS group at 12 weeks ($p = 0.19$). **Micro CT:** The mean bone tunnel area (mm^2) was 1.82 ± 0.46 in the NS group and 2.15 ± 0.58 in the VS group at 4 weeks ($p = 0.21$) and 1.69 ± 0.38 in the NS group and 1.84 ± 0.36 in the VS group at 12 weeks ($p = 0.36$). **Histological evaluation:** The mean score was 8.0 ± 1.7 in the NS group and 7.8 ± 0.8 in the VS group at 4 weeks ($p = 0.49$), and 9.5 ± 1.1 in the NS group and 10.5 ± 0.5 in the VS group at 12 weeks ($p = 0.40$). ICC (3,1) was 0.83 (> 0.7).

DISCUSSION: Previous *in vitro* experiments have shown cytotoxicity in osteoblasts and the inhibition of bone regeneration at vancomycin concentrations of 0.125 mg/mL [6]. However, our study showed that graft strength and mechanical properties are not adversely affected immediately after pre-soaking. Our *in vivo* study also showed no adverse effects on graft-bone healing. These results could support the findings of clinical studies showing that the re-tear rate decreases or does not change [7][8].

SIGNIFICANCE/CLINICAL RELEVANCE: Pre-soaking of grafts in vancomycin had no apparent adverse effects on graft-bone healing in an ACLR rat model.

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