The revascularization and bone healing following fracture with severe soft tissue injury: A new closed fracture model in the rat

Shohei Iwabu, Peter Augat, Lutz Claes
Institute of Orthopedic Research and Biomechanics, University of Ulm, Germany

Introduction: The purpose of this study was to elucidate the vascular response following fracture with severe soft tissue injury. For this study we established a new closed tibia fracture model with severe soft tissue injury in the rat.

Material & Methods: We modified the standard closed fracture model in the rat's tibia made by a guillotine device in the following subjects: 1) an addition of a soft tissue crush before fracture, 2) intramedullary nailing "after" fracture, 3) an additional pin to increase rotational stability. Male Wistar rats (350 to 450g) were divided into two groups; group A: only fracture, group B: soft tissue injury and fracture. Ten animals each were sacrificed 1, 2, 5, 10, and 28 days postoperatively. The legs of rats killed between day 1 and 10 were used for the histological analysis and the edema analysis. The tibias of rats killed at day 28 were used for mechanical testing with three-point bending. The analysis of angiogenesis was performed by counting microvessels on the histological sections assisted by immuno-staining for factor VIII. The local blood flow was measured with laser Doppler flowmetry. For the edema analysis, water content of the leg was determined by gravimetry after dehydration and the edema index was calculated by the ratio of water content weight to dry weight in the experimental compared with contralateral leg.

Results: In group A, the blood flow of the tibia severely decreased just after the operation, but small changes occurred in the muscle (n=10, bone: 59% of contralateral leg, p=0.044, muscle: 87%, p=0.11). In group B, the blood flow of both the tibia and the crushed muscle distinctly decreased after the operation (n=10, bone: 55%, p=0.001, muscle: 76%, p=0.047). After 5 days blood flow recovered to a higher level than that before operation (Fig.). The histological analysis showed active new microvessel formation around the callus at 5 and 10 days. As compared with group A, in group B was less callus formation and a smaller number of microvessels under the crushed muscle. There was little difference in the maximum strength of the fractured tibia after 28 days between group A (46.9±15.1N) and B (45.7±12.0N). The edema index was higher in group B than group A at day 1 (1.26±0.15, 1.18±0.26, P=0.006) and day 2 (1.23±0.32, 1.18±0.40, p=0.13)(n=3).

Discussion: The histological findings, the edema index, and the blood flow showed a more pronounced severer soft tissue damage in group B, in the very early phase of healing. In the later phase, in spite of this damage, active angiogenesis occurred and the early recovery of blood flow was observed. We could see an apparently disturbed healing response only in the area underneath the crushed muscle. The vigorous vascular response restored the healing process of the severely damaged fracture to the level of the less damaged fracture. Hence, little differences were found in the mechanical properties of the healed tibias at day 28.