Regulation of SDF-1 and CXCR-4 in fracture healing

Jan-Tobias Weitkamp¹, Peter Behrendt¹, Sabine Fuchs², Andreas Seekamp¹, Sebastian Lippross¹, Tim Klütér¹
¹Experimental Trauma Surgery, University Medical Center Schleswig-Holstein, Kiel, Germany.

Disclosures: Jan-Tobias Weitkamp (N), Sabine Fuchs (N), Andreas Seekamp (N), Sebastian Lippross (N), Peter Behrendt (N), Tim Klütér (N)

INTRODUCTION: Critical fracture healing is a serious problem in multiple injured patients and in the elderly population (Cole, Dennison, & Cooper, 2008). To avoid the increasing costs of long-term treatment and the risk of hospital-acquired infections, it is of general interest to develop new strategies to accelerate bone healing. More recently, focus has been placed on the regulatory processes that provide stem cell homing to the fracture site. In particular, the stromal derived factor-1 (SDF-1) – CXCR-4 chemokine signal axis is currently being investigated for cell homing in connection with a wide range of tissue healing processes including fracture healing (Yellowley, 2013). Although it is known that the signaling axis attracts hematopoetic (HSCs) and mesenchymal stem cells (MSCs) to the fracture site, the homing mechanism in detail remains unclear. The aim of this study was to investigate the regulation of SDF-1 in serums of multiple injured patients. In a second experimental approach we tried to verify the biological effect of SDF-1 and CXCR-4-7 induction in fractured compared to healthy vertebral bodies.

METHODS: We analyzed serum samples from 5 donors over a period of 9 days after multiple trauma (ISS>16) and compared them to a control group with 3 healthy donors. Quantification of SDF-1 serum levels was performed with ELISA. In an independent experiment bone marrow aspirates (BMA) were collected from 18 donors including samples from fractured (9) versus healthy vertebral bodies (9). SDF-1 was measured in the BMA serum of 12 donors using an ELISA. Bone marrow mononuclear cells (MNCs) were isolated with density centrifugation and scanned for CXCR-4, -7 and SDF-1 using real-time PCR.

RESULTS: Multiple trauma: From day 2-4 after multiple trauma SDF-1 serum levels were upregulated significantly (P < 0.05; day 2: 1130pg/ml ± 512pg/ml; day 3: 1212pg/ml ± 409.2pg/ml; day 4: 1399pg/ml ± 334.2pg/ml) compared to the control group (146.6pg/ml ± 168.9pg/ml; figure 1).

Vertebral bodies fracture: The mRNA expression of the chemokine receptor CXCR-4 and CXCR-7 was induced in MNCs of fractured vertebral bodies (CXCR-4: control 1.07 ± 0.43 vs. fracture 1.125 ± 0.43; P = 0.803; CXCR-7: control 1.03 ± 0.25 vs. fracture 1.135 ± 0.66; P = 0.715). BMA serum of fractured vertebral bodies contained higher amounts of SDF-1 (control: 94.04 ± 28.39pg/ml vs. fracture: 123.96 ± 54.39pg/ml; P = 0.24), but there was no detectable mRNA expression of SDF-1 in MNCs (figure 2).

DISCUSSION: Chemokine signaling is a key event in tissue regeneration. In this study we showed SDF-1 induction in serums of multiple inured patients for the first time. This patient group often suffers from multiple fractures. The source of SDF-1 in patient’s serums remains unclear. In a second experiment we investigated the biological effect of the systemic SDF-1 induction, seen in experiment one, localized around fractures of vertebral bodies. The serum samples of the bone marrow aspirates indicated rising chemokine levels in the surrounding of fractures. CXCR-4 and -7 showed a trend for higher expression at the fracture site. We therefore hypothesize that SDF-1 and CXCR-4-7 are secreted after trauma to promote tissue regeneration. Patients underwent surgery within two weeks after fracture and sample collection could only be performed during surgery. The multiple injured patients showed significant higher SDF-1 levels from day 2 to 4. Hence, it can be hypothesized that the chemokine signaling is mostly important during the first few days after trauma. In further investigations we will analyze the BMA at different time point and search for further sources of SDF-1 in multiple injured patients.

SIGNIFICANCE: Calculation and statistical analysis was performed using ANOVA (GraphPad PRISM Software). Quantitative data are presented as mean and SD. Differences were considered significant at P < 0.05.


1.

Fig 1: ELISA of SDF-1 serum levels in multiple trauma patients over 9 days (n= 5). Day 2-4 showed a significant increase compared to the control group (n=3), * = p< 0.05

Fig 2: ELISA of SDF-1 bone marrow serum levels. There is a tendency of higher SDF-1 levels in fractured vertebral bodies (n=12), * = p< 0.05

ORS 2017 Annual Meeting Poster No.1234