Systemic Inflammation Alters the Cellular Composition of the Early Fracture Callus and Impairs Bone Healing

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ABSTRACT INTRODUCTION:
There is clinical evidence, that fracture healing is impaired in polytraumatic patients. One reason might be the complex posttraumatic systemic inflammatory response, being characterized by the rapid release of proinflammatory cytokines, a massive activation of the complement system and the resulting overactivation of inflammatory cells, together frequently leading to secondary tissue and organ damage after severe trauma. Therefore, the question arises, whether the rapid and transient systemic inflammation after a severe trauma could also influence the recruitment and the function of immune cells locally at the fracture site, thus disturbing the inflammatory balance during the early healing stage and consequently resulting in impaired fracture healing.

METHODS:
58 male Wistar rats received a femur osteotomy, which was stabilized with an external fixator. Half of the animals underwent an additional blunt chest trauma (TXT) by the application of a standardized blast wave centered on the middle of the thorax. Blood was taken from the animals in order to evaluate the early inflammatory response as measured by the interleukin-6 (IL-6) serum levels. To investigate the influence of the systemic inflammation on the cellular composition of the early fracture callus, animals were harvested after 3 and 7 days, and slices of the fracture callus were stained and analyzed histologically for polymorphonuclear neutrophils (PMN, N-ASD-C staining), macrophages (CD68), osteoclasts (TRAP) and IL-6. For evaluating the fracture healing outcome the animals were harvested after 35 days and the fracture callus was tested mechanically by a three point bending test and structurally by µCT measurements as well as by histology and histomorphometry. The animal experiment was performed according to international regulations for the care and use of laboratory animals, and approved by the local ethical committee (Regierungspräsidium Tübingen, Germany). Statistics: Student’s t-test. Level of significance: p<0.05.

RESULTS SECTION:
The blunt chest trauma led to an approximate 3-fold increase of the IL-6 serum levels after 6h (p=0.02) and 24h (p=0.03), and to an approximate 2-fold increase after 72h (p=0.04) compared to preoperative values. In contrast, rats with an isolated fracture did not show a significant difference in IL-6 serum values at any time point compared to preoperative values (Fig. 1). During the early inflammatory phase of fracture healing, the blunt chest trauma led to an approximate 4-fold immigration of PMN into the fracture callus at day 3 (p=0.02) compared to animals with isolated fractures (Fig. 2). In contrast, macrophages were significantly less present in the fracture callus after the blunt chest trauma at 3 (p=0.03) as well as at 7 (p=0.01) days postoperation (Fig. 3). After 3 days, the blunt chest trauma led to a significantly stronger expression of IL-6 (p=0.01) within the periosteal callus in zones of intramembranous ossification (Fig. 4). The number of osteoclasts considerably increased during healing, with the amount not influenced by the blunt chest trauma (results not shown). During the time of cortical bridging after a healing period of 35 days, the blunt chest trauma led to an impaired fracture healing, which was shown by a significantly inferior bending stiffness (-63%; p=0.03; Fig.5) and maximum moment of inertia (-50%; p=0.04) associated with a significantly decreased callus volume (p=0.03) and a diminished relative bone surface (p<0.01). Furthermore, the blunt chest trauma reduced the area of new bone (-29%; p=0.02; Fig. 6) in the fracture callus as evaluated by histomorphometry.

DISCUSSION:
We were able to show, that the blunt chest trauma and the resulting systemic inflammation, as determined by increased IL-6 serum levels, strongly disturbed the finely tuned inflammatory phase of the early fracture callus, being characterized by a stronger recruitment of PMN, an enhanced interleukin-6 expression within areas of newly formed bone and a diminished number of macrophages locally at the fracture site. In confirming the clinical evidence, this study shows for the first time, that the impairment of the initial inflammatory phase resulted in delayed fracture healing at a later stage, as shown by a significantly reduced flexural rigidity (three-point-bending) in combination with a significantly diminished callus volume, moment of inertia and relative bone surface (µCT analysis) as well as decreased amounts of newly formed bone (histomorphometry) in fracture callus of animals subjected to a thoracic trauma.

Fig. 1: Serum IL-6 concentrations of rats subjected either to a blunt chest trauma in combination with a femur fracture or an isolated femur fracture at 0 (pre-operative value), 6, 24 and 72 h after surgery. * = p<0.05 compared to pre-operative values; $ = p < 0.05 compared to animals with isolated fractures.

Fig. 2: Polymorphonuclear neutrophils (PMN) within the periosteal callus of rats with (grey columns) or without (white columns) blunt chest trauma after 3 and 7 days. n=6-10, *p=0.05

Fig. 3: Macrophages within the periosteal callus of rats with (grey columns) or without (white columns) blunt chest trauma after 3 and 7 days. n=5-7, *p=0.05

Fig. 4: IL-6 staining within the newly formed bone, cartilage or soft tissue after a healing period of 3 days of rats with (grey columns) or without (white columns) blunt chest trauma. n=6-8; *p=0.05

Fig. 5: Flexural rigidity (FR) of the fracture callus of rats with or without blunt chest trauma in comparison to the contra-lateral intact bone. n=7-8, *p=0.05

Fig. 6: Absolute values of the callus area (CA), bone (TOT), cartilage (Cg) or fibrous tissue (FT) within the total callus after a healing period of 35 days of rats with (grey columns) or without (white columns) blunt chest trauma. n=7-8, *p=0.05

SIGNIFICANCE:
This study provides new insights into the pathophysiology and potential therapy approaches of compromised bone healing in polytraumatic patients.

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REFERENCES: