**Normobaric Oxygen Treatment Improves Fracture Healing After A Blunt Chest Trauma In Mice**

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**Introduction:** A frequent injury in polytrauma patients is a blunt chest trauma (TXT), frequently accompanied by long bone fractures. Fracture healing in these patients is considerably delayed and the risk for non-unions is increased (1). In previous studies from our group has confirmed the clinical reports demonstrating impaired fracture healing after TXT in rats, due to a complex interaction between the posttraumatic systemic inflammation and the tightly regulated healing process at the fracture site (2, 3). TXT leads to acute lung injury, alveolar hypoxia and hypoxemia, which trigger and modulate local and systemic posttraumatic inflammatory reactions (4, 5). The present study investigates the effects of a normobaric, intermittent 100% O2-therapy on the pathophysiology of fracture healing after TXT in a mouse model in order to assess if impaired bone healing after TXT is caused by systemic inflammation, and if 100% O2 ameliorates the inflammatory response and improves fracture healing in this poly-trauma model.

**Methods:** 12-week old, male C57Bl/6 mice received a standardized osteotomy of the femur (Fx), stabilized by an external fixator. Directly after surgery, one group was subjected to a TXT using a blast wave generator. Half of the TXT-group immediately underwent normobaric, intermittend O2-therapy (2x 3h 100% O2 with 1x 3h 21% air in between), while the others did not receive a special treatment after TXT induction. Mice were sacrificed after 3, 9, and 24h, and after 3, 14, and 21d. Arterial blood gas analysis was performed, and serum and lung homogenates were analyzed for pro- and anti-inflammatory cytokines such as interleukin (IL)-6, and IL-10 via Multiplex ELISA. Femurs and lungs were subjected to immunohistochemistry (IHC) and adequate sections were stained for neutrophils using a specific Ly-6G-antibody. Fracture calli were stained with Safranin-O to evaluate histomorphological changes 14 and 21d post-surgery. After a healing period of 21d, femurs underwent biomechanical testing (three-point-bending) and micro-computed tomography (µCT). Data were analyzed using either Kruskall-Wallis, or by one-way ANOVA and Fishers LSD post-hoc test. The level of significance was set at \( p<0.05 \).

**Results:** Blood gas analysis of arterial blood revealed a lower pO2 in the TXT-group (-27%) in comparison to mice with isolated fracture 3 h post-surgery. O2-therapy provoked a significant pO2 increase compared to TXT-mice without O2 application (+74%, \( p=0.001 \)) and mice without TXT (+48%; \( p=0.014 \)). pCO2 after TXT was increased by trend (+38%) but did not reach significance compared to the other groups.

Serum IL-6 and IL-10 levels were significantly elevated in the TXT-group, compared to mice with isolated fracture (IL-6: +196%, \( p=0.000 \); IL-10: + 78%, \( p=0.017 \)) and O2-treated TXT-mice (IL-6: +151%, \( p=0.002 \); IL-10: +96%, \( p=0.004 \)) 3h after trauma. Cytokine measurements of lung homogenates also revealed
significant differences of the TXT-group in comparison to mice with isolated fracture (IL-6: +52%, p=0.008; IL-10: +45%, p=0.017) and to the O2-group (IL-6: +42%, p=0.031; IL-10: +38%, p=0.037). Neutrophil staining of lung tissue 3 h post-surgery revealed a higher percentage of neutrophils/mm² in mice of the TXT-group compared to mice without TXT (+300%, p=0.022). In bones, neutrophil counts were also significantly higher in animals with TXT but without O2-treatment in comparison to mice with isolated fracture (+59%, p=0.037) 3 d after injury. Additional O2-therapy after TXT led to a reduction in neutrophil counts in lung (-248%, p=0.12) and bone tissue (-46%) compared to the TXT-group after 3 h or 3 d respectively. Histomorphological analysis 14 d after surgery revealed significantly more cartilage in the TXT-group than in O2-treated animals (+71%, p=0.009), while comparisons after 21 d did not reveal any differences. Mice with TXT showed a significantly reduced bending stiffness (-46%, p=0.001), moment of inertia (-33%, p=0.032) and apparent Young’s modulus (-43%, p=0.021) compared to mice without TXT (Fig. 1 A-C). The O2-treatment after TXT led to a significantly higher bending stiffness (+36%, p=0.023) and apparent Young’s modulus (+46%, p=0.023) in the O2-group compared to the TXT-group without O2 treatment (Fig. 1 A-C).

Discussion: This study confirmed earlier results performed in rats, which demonstrated impaired fracture healing after TXT (3). Here, we observed no significant changes in blood analysis after TXT for pCO2 an pO2, but elevated levels of pro- and anti-inflammatory mediators in serum and lung homogenate samples indicating a strong activation of systemic inflammatory reactions. The increase of IL-6 after TXT is in accordance to clinical studies, which identified a positive correlation between elevated IL-6 concentrations after major TXT and mortality in polytrauma patients (6). Increased concentrations of IL-10 indicate a simultaneous activation of anti-inflammatory mechanisms at the same time, as was observed in other studies as well (7). Neutrophils belong to the first cells that invade the injury site and therefore, we expected a significant increase in the neutrophil population in the lungs shortly after the TXT, which perfectly corresponds to the observed increase 3 h after surgery. Regarding the fractured femurs, we found higher neutrophil counts in the TXT-group only after 3 d. After 21 d, bending stiffness, moment of inertia and apparent Young’s modulus of fractured bones were decreased, suggesting a reduced callus quality in mice after TXT. The short intermitted O2-therapy positively influenced bone healing. Other studies already reported beneficial properties of a short O2-treatment after shock, such as anti-inflammatory and anti-apoptotic effects (8). In our current study, O2-therpy increased arterial pO2 levels shortly after TXT, and the systemic inflammatory response could be attenuated, demonstrated by reduced cytokine concentrations and neutrophil counts locally in lung-, and systemically in serum samples. The O2-
therapy improved fracture healing after TXT, confirmed by a significantly increased bending stiffness and apparent Young’s modulus compared to TXT-animals without O2-therapy.

In conclusion, we showed that fracture healing was impaired when compounded by TXT, due to the post-traumatic systemic inflammatory response. Systemic inflammation and its deleterious effect on bone healing could be attenuated by a treatment with O2 in the early posttraumatic phase.

Significance: The results of this study delineate new aspects of the compromised pathophysiology of fracture healing in multiple injury and suggests a new therapy of improved fracture healing in polytrauma patients.

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