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The effect of traumatic brain injury on bone healing: an experimental study in a novel *in vivo* animal model

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ABSTRACT

Introduction: Among many factors determining the outcome of complex fractures in polytrauma patients, the role of traumatic brain injury (TBI) remains only partly understood. The aim of the present study was to examine the effect of traumatic brain injury on bone healing through the establishment of a novel standardised animal model that sequentially combines traumatic brain injury (TBI) with a long bone injury.

Materials and methods: Thirty-six female twelve-week old C57/BL6 mice were randomised in two groups (fracture (Fx)-group and combined-trauma (Fx/TBI) group). The methods of the Control Cortical Impact Injury for induction of TBI and of the femoral osteotomy, fixed with an external fixator for the simulation of the long bone fracture, were combined. No TBI was induced in the Fx-group. Bone healing was examined using *in vivo* micro-CT measurements over a period of three weeks.

Results: The severity of the TBI was sufficient to stimulate a significantly increased callus formation in the Fx/TBI-group with an acceptable mortality rate. The micro-CT analysis of fracture healing displayed a significantly increased callus volume in the Fx/TBI-group already from the second postoperative week. This difference remained significant throughout the entire study period.

Discussion: The successful and standardised combination of TBI and fracture in a mouse model allows systematic and quantitative *in vivo* analysis of underlying pathways that trigger the mutual interaction between musculoskeletal trauma and brain injury, as well as, corresponding differences in fracture healing using micro-CT methods.

Conclusion: The present study offers three new aspects: a standardised model for combined injury of TBI and femoral osteotomy; direct and serial *in vivo* imaging and quantification of fracture healing response using micro-CT; testing of potentially beneficial therapeutic regimens for fracture treatment in presence of TBI. Thus this model provides a valuable basic approach for the study of the amplifying effect of TBI on callus formation seen in patients with craniocerebral injury and concomitant skeletal trauma.

Introduction

Among many factors determining the outcome of complex fractures in polytrauma patients, the role of traumatic brain injury (TBI) remains only partly understood. The underlying pathophysiological mechanisms of exacerbated callus formation in TBI patients are now just being comprehended [1–3]. Factors limiting our understanding of the effect of TBI on bone metabolism include: (i) the heterogeneous injury pattern and variable severity in polytrauma patients, (ii) the limited number and possibility of clinical trials, (iii) the absence of appropriate animal models of clinical relevance, and (iv) the yet unidentified biochemical, endocrine and neuronal pathways triggering bone healing and progressive callus formation in presence of TBI as noted in clinical practice. The management of severe TBI and complex extremity fractures is an exceptional clinical challenge [4–8]. There is now clear evidence that specific and efficient therapeutic interventions

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must be directed to early stages of this interaction because the end stage, which is complicated by extensive callus formation, skeletal muscle involvement, heterotopic periarticular ossifications with articular ancylosis being the maximum deleterious consequence, is minimally responsive to treatment [5,8,9]. To the opposite, understanding why callus formation is accelerated in TBI patients may assist in promoting therapeutic regimens aimed at improvement of impaired bone healing response, *i.e.* following severe accompanying soft tissue damage, delayed- and non-unions. To date, a valid and clinical relevant model of standardised TBI and long bone extremity fracture, as typically seen in high-energy accident victims (polytrauma), remains to be established.

The aim of the present study was to sequentially combine both injuries, *i.e.* the Controlled Cortical Impact Injury (CCI) for induction of TBI with a femur osteotomy, in a standardised mouse model. The objective was to establish a polytrauma-model in mice that allows testing the hypothesis that progressively accelerated callus formation is secondary to TBI. A successful and standardised combination of those two injuries in a mouse model would serve as an experimental proof of principle and allow both, systematic *in vivo* analysis of underlying pathways that trigger the mutual interaction and dependency between musculoskeletal trauma and brain injury, as well as, the quantitative assessment of corresponding differences in fracture healing using micro-CT methods. Successful use of that model could therefore have potential therapeutic value, as it would provide an essential precondition to perform studies aimed to effectively prevent escalation in exacerbated/non-directional callus formation, as well as, utilisation of the tremendous TBI-triggered callus formation for conditions associated with impaired fracture healing, *i.e.* delayed-/non-unions, pathological fractures. All experiments were carried out with ethical permission according to the policies and principles established by the Animal Welfare Act, the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the National Animal Welfare Guidelines and were approved by the local legal representative animal rights protection authorities (Landesamt für Gesundheit und Soziales Berlin: G 0009/12; March 2012).

Materials and methods

The animals were housed under conditions of controlled temperature (20 ± 2 °C) with a 12 h light/dark circle and food and water *ad libitum*. Prior to study inclusion, all animals were kept for one week in the laboratory premises in order to allow acclimatisation.

All surgical procedures were performed on a heating pad (37 °C, feedback controlled by rectal temperature) in anesthetised and spontaneously breathing (isoflurane 1.5 vol.%, N_2O 0.5 L/min and O_2 0.3 L/min) 36 female 12-week old C57/BL6 mice (Charles Rivers, Sulzfeld, Germany, body weight: 20–22 g) [10]. The animals were randomised in two groups: fracture (Fx)-group ($n = 19$) and combined-trauma (Fx/TBI) group ($n = 17$). The eyes of the animals were covered with moistening ointment (Bepanthen Eye Ointment, Bayer Vital GmbH, Germany). Preoperative analgesia with a single subcutaneous shot of buprenorphine (TEMGESIC®, RB Pharmaceuticals Limited, Germany) was applied (0.1 mg/kg body weight) prior to surgery. The animals were antibiotically covered with a perioperative single-shot of clindamycin (0.02 ml s.c.).

First, in the Fx/TBI-group TBI was induced using the CCI-method [11,12]. After prone positioning of the mouse and fixation of the skull in a stereotactic device (Stoelting, Wood Dale, IL) (Fig. 1A), the operation field was shaved and disinfected. Bupivacain 1% (1 ml/kg body weight) was injected subcutaneously for local anaesthesia before sagittal incision of the scalp.

All operative steps were microsurgically performed under microscopic control. Following skin mobilisation and preparation of Musc. temporalis, a left craniotomy of the parietotemporal region was performed using a micro drill (Fig. 1A) within the anatomic barriers outlined by the sagittal, lambdoid and coronal sutures, as well as, the zygomatic arc (Fig. 1B). After completion of the craniotomy a 7×7 mm bone window was gently lifted with a custom-made blunt orthogonal instrument (Fig. 1C). Craniotomy was meticulously performed and special care has been taken in order to avoid any injury of the dura mater (Fig. 1D). The animals were moved to the impact device and the skull was again stereotactically fixed under continuous anaesthesia. The animal position on the impactor was manually adjusted, so that the impaction took place in the centre of the bone window. TBI was performed with the high-pressure computer-assisted CCI-technique by accelerating a pneumatically driven 3 mm bolt with a flat tip to an impact velocity of 3.5 m/s (13 km/h) in 45-degree angle, inducing a penetration depth of 0.25 mm perpendicular to the surface of the cerebral convexity at a contact time of 0.15 s. (Fig. 2) These settings resulted in a reproducible non-lethal focal injury (blunt trauma) to the parietal mouse dura mater and cerebral cortex. Finally, the preserved piece of cranial bone was repositioned and fixed with dental cement (Hoffmann Dental Manufaktur, Berlin, Germany). Thereafter, the skin was closed by 6.0 running suture (Ethilon 6.0; Ethicon®, Norderstedt, Germany)

Directly after completion of CCI, the animals were removed from the stereotactic fixator, turned back to the heating pad and underwent the procedure of open femoral osteotomy with external fixation for the simulation of long bone fracture. In the same prone position, the operation area was shaved and disinfected. For the mid-diaphyseal approach to the femur, a lateral longitudinal incision of the skin (2 cm length) along an imaginary line, from knee to hip joint, was performed. The femoral bone was exposed by dissection of fascia lata and by blunt preparation of Musc. vastus lateralis and Musc. biceps femoris, carefully sparing the sciatic

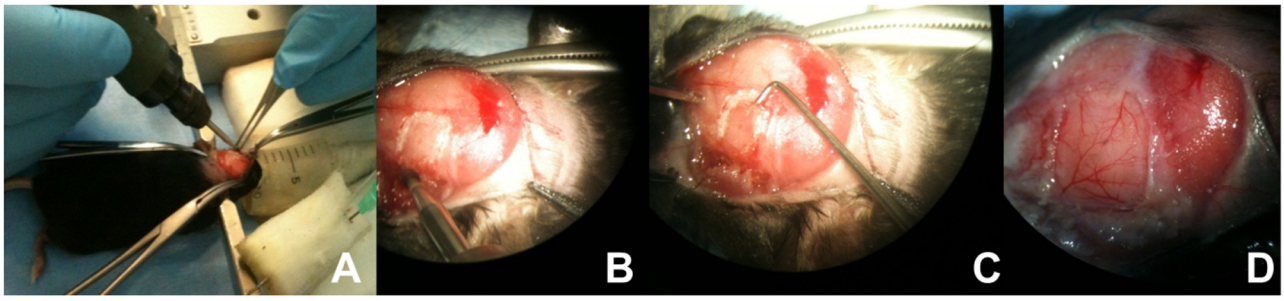

Fig. 1. (A) Prone positioning on the stereotactic fixation device. Anaesthesia is performed through a custom-made inhalation mask. Craniotomy with hand-drill at the left parietotemporal region. (B) The craniotomy is located within the anatomical barriers outlined by the sagittal, lambdoid and coronal sutures, and the zygomatic arc. (C) Surgical preparation of a 7×7 mm bone window. Meticulous removal of the bone piece with a custom-made blunt orthogonal instrument. (D) The dura mater remains intact after the removal of the bone window.