ENHANCEMENT AND ACCELERATION OF FRACTURE HEALING BY SEX STEROIDS

Haberland, M; Tibba, J; Gesicki, M; Nguyen, CK; Beil, FT; Rueger, JM; Amling, M
Dept. of Trauma Surgery, Hamburg University School of Medicine, Germany

Introduction: Fractures of the skeleton belong to the most common medical problems. Over 6 million fractures occur in the United States each year, and it is estimated that 10 % of these are complicated by disrupted patterns of bone healing. Although most fractures heal appropriately, they still lead to pain, disability or confinement of patients, which in turn leads to a tremendous loss of productivity and income. Recent years have shown rapid progress in the molecular understanding of fracture healing, leading to advanced experimental therapies including the application of bone morphogenetic proteins and growth factors. However, till today, no therapy exists that could routinely be given to patients suffering from fractures in order to enhance or accelerate fracture healing.
Here we report the beneficial influence of sex steroids (estrogen and testosterone) on fracture healing. As these drugs have been safely administered to patients in the past, e.g. in hormone replacement therapy, they may be a therapeutical option for enhancing and accelerating fracture healing for a broad group of patients, thereby reducing the amount of patients days lost due to incapacity; and may be used for the prevention and therapy of disturbed fracture healing seen in delayed and non-union.

Methods: Fracture healing was studied in 150 female and male C57/Bl6 mice. All mice were eleven weeks old when sacrificed for analysis. An Einhorn fracture device with slight modifications was used to induce closed femoral fractures. The fractures were stabilized by intramedullary nailing. At different points of time biomechanical testing was performed by using a three point bending test. Additional morphological analysis included x-ray evaluation, histology, histomorphometry and microCT analysis. Drugs were administered by subcutaneously implanted slow release pellets in a dosage equivalent to human hormone replacement therapy. Two groups of mice were ovariectomized (ovx) to induce acute and chronic estrogen deficiency. Statistical differences were detected by using Student`s *t* test. Data are expressed as means, standard deviation (SD) is shown in brackets. All animal procedures were approved and conducted in accordance with the Hamburg University Institutional Animal Care and Use Committee.

Results: In an initial experiment the development of fracture stability was determined in untreated mice. Additionally the biomechanical stability of the contralateral femur was determined for each mouse (n=15 for each point of time). At day 20 the breaking strength of the callus was equal to that of the contralateral femur. This point of time was therefore defined as biomechanically completed fracture healing (Fig.1).

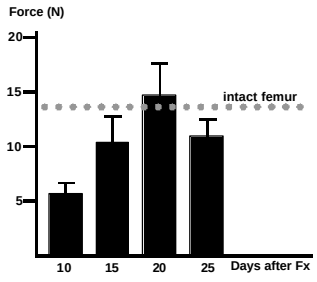

Fig.1: Timecourse of bio-mechanical competence of fractured femur in controls (Force to failure, female)

To determine the influence of sexsteroids, female mice were treated either with estrogen or testosterone and after 20 days compared to control and ovariectomized mice. As can be seen in Fig. 2, estrogen is essential for fracture healing in female mice, as ovariectomized mice have a significantly decreased biomechanical stability. But, more importantly, it can be seen that treating eugonadal female mice with either estrogen or testosterone leads to a significant increase in biomechanical stability (Fig. 2). To determine if this enhancement of fracture healing is sex-specific, male mice were treated with testosterone and fracture stability was determined at day 20. As in female mice, testosterone significantly enhances the stability of the fractured femur (Force to failure: Control 13,2 N vs. 25,6 N testosterone treated, $p<0,01$, n=10 each). To determine if the application of estrogen can overcome the deleterious effects of chronic estrogen deficiency mice were ovariectomized at 4 weeks of age and after another 4 weeks the femur was fractured. These mice showed a severe defect in fracture healing (Force to failure: 4,2 N). If these mice received estrogen at the time of fracture a clear rescue of the impaired fracture healing could be demonstrated (22,8 N, $p<0,05$, n=10 each).

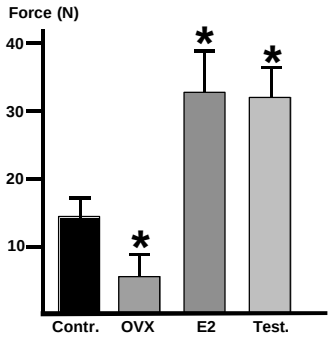

Fig.2: Influence of ovx, estrogen (E2) and testosterone (Test.) on biomechanical stability (Force to failure, female mice, day 20, n=10 for each group, *$p<0,01$ vs. control).

Next we determined if sex steroids, in addition to enhance fracture healing, can also accelerate fracture healing. Female mice were treated with estrogen, and callus stability was compared to controls at 10, 15 and 20 days (n=10 for each group).

Table 1: Acceleration of fracture healing by estrogen, force to failure.

days	10	15	20
control	6,0 (1,7)	10,5 (2,4)	**14,1** (3,4)
estrogen	6,4 (2,7)	**15,6** (3,9)	33,9 (4,2)

The data in table 1 demonstrates that estrogen treatment causes a significant acceleration of fracture healing. While an untreated fracture needs 20 days to reach a stability that equals a normal femur, an estrogen treated fracture obtains this stability 5 days earlier. To assess the morphological correlate to this improved stability, callus of ovx, control and estrogen treated female mice was analyzed by histomorphometric analyis. As seen in table 2, estrogen had a significant influence on cortical width, cortical mineralization, and amount of intramedullary callus. These changes could also be visualized by microCT analysis.

Table 2: Influence of estrogen on histomorphometric properties of fracture callus. Cortical width (Ct.Wi., in mm), mineralized area/cortical bone area (Md.Ar./Ct.B.Ar., in %), mineralized area/intramedullary callus area (Md.Ar./Im.Cl.Ar, in %).

	Ct.Wi.	Md.Ar./Ct.B.Ar.	Md.Ar./Im.Cl.Ar.
control	0,11 (0,03)	48,9 (6,1)	17,5 (4,5)
ovx	0,08 (0,01)	49,8 (2,4)	10,7 (2,0)
estrogen	0,20 (0,06)	72,4 (16,2)	48,8 (4,6)

Discussion: The results of this study show, that 1) estrogen is essential for fracture healing in female mice, 2) estrogen and testosterone can enhance the biomechanical stability of fractures in eugonadal female mice, 3) testosterone enhances fracture healing in eugonadal male mice, 4) short term estrogen application can overcome the deleterious effects of chronic estrogen deficiency on fracture healing and 5) that estrogen accelerates fracture healing in eugonadal female mice by 25 %.
Taken together these data reflect the profound impact of sex steroids on fracture healing. Inasmuch as fracture healing reenacts many of the morphogenetic events in bone development and remodeling, the influence of these hormones is not altogether surprising, given the well established role of sex steroids in skeletal homeostasis. However, although many advanced and sophisticated fracture modulating studies have been performed, the influence of sex steroids on fracture healing has, to our knowledge, never been fully evaluated, neither in an animal model, nor in humans. Given the proven safety of (short term) application of sex steroids in humans, it is conceivable that these drugs might be useful for treating patients at risk for delayed fracture healing (such as osteoporotic patients) or even to be generally used in order to reduce the patients days lost due to incapacity.

49th Annual Meeting of the Orthopaedic Research Society
Poster #0517