Temporal and spatial vascularization patterns of unions and non-unions – role of VEGF and BMP’s

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INTRODUCTION:
Fracture healing has been intensively studied in humans and a variety of animal models. Despite our increasing knowledge on the histomorphometric and molecular basis of normal fracture healing, the cause of failure of bone healing under certain circumstances remains largely unclear. Accordingly, there is a lack of effective treatment strategies, and the clinical failure rate of bone healing still amounts up to 10% (1). Multiple factors have been reported which may affect bone regeneration. Among these, vascularization has been recognized to be crucial for successful bone healing. Atrophic non-unions have been considered as a result of an avascular and biological inert environment at the fracture side (2). Of interest established atrophic non-unions have been shown to be well vascularized (3). Because established non-unions are well vascularized, we hypothesized that lack of vascular endothelial growth factor (VEGF) expression and vascularization during the early time course of fracture healing determine atrophic non-union formation. With the use of a marine femur fracture model we therefore analyzed the temporal and spatial course of VEGF expression and vascularization in unions and atrophic non-unions.

METHODS:
In 72 CD-1 mice a femoral ostotomus with a gap size of 1.80mm (non-union group) or a gap size of 0.25mm (union group) were created and stabilized by a pin-clip technique. Healing was analyzed after 3, 7, 14, 21, 28 and 70 days by micro-CT and histomorphometry (n=6 per group and time point). Vascularization was analyzed by immunohistochemical staining of PECAM-1 (CD31). For determination of blood vessel distribution all sections were evaluated under light microscopy. The number of blood vessels was analyzed in a total of 34 high power fields (HPF) in different regions of interest according to a standardized scheme: (i) endosteal callus area, (ii) gap area, (iii) central callus area and (iv) periosteal callus area. Additional animals were analyzed after 7, 14 and 21 days for Western blot analysis of PCNA, VEGF, BMP-2 and BMP-4 expression (n=4 per group and time point).

RESULTS:
Micro-CT analysis and histomorphometric analysis of fracture healing in the union-group showed bridging of the fracture gap at day 28 and bone remodeling until day 70. In contrast, the non-union group did not heal until day 70 with very sparse callus formation. At day 70, osteotomies in the non-union group presented as atrophic non-unions with lack of bone bridging, absence of callus formation and rounded bone ends. Of interest, the medullar cavity at the femoral bone ends was capped with lamellar bone.

Quantitative analysis of vessel density in all healing zones was not significantly different between unions and non-unions. Both groups showed a similar temporal pattern of vascularization. Vessel density significantly increased between day 3 and day 7. After day 14 vessel density decreased and then remained at a lower level until the end of the observation period. In the periosteal, the central and the intercortical healing zones we also found a significant increase in vessel density from day 3 to day 7. In contrast, vessel density in the endosteal healing zone showed a different temporal pattern than the other healing zones. Vessel density remained almost constant over the observation period, without an increase in vascularization between day 3 and 7. Of interest, endosteal vessel density after 14 days was significantly greater in the non-union group compared to the union group.

Western blot analysis showed an increased proliferation in the union group compared to the non-union group at day 14, as indicated by increased expression of PCNA. In the non-union group VEGF expression was slightly greater at day 7 and significantly greater at day 14 when compared to the union group. In contrast, expression of BMP-2 was significantly lower in the non-union group compared to the union group after 2 and 3 weeks. Expression of BMP-4 also was significantly lower in non-unions compared to unions after 2 weeks. Expression of BMP-2 and BMP-4 was not significantly different within the individual groups over time.

DISCUSSION:
We hypothesized that impairment of early vascularization determines atrophic non-union formation. The data of our study indicate, however, that during early fracture healing VEGF expression and vascularization do not differ between atrophic non-unions and normally healing fractures. Thus, we have to reject our hypothesis, and conclude that non-union formation is not necessarily due to failure of VEGF-mediated vascularization.

Because non-unions showed a decreased expression of BMP-2 and BMP-4 during the early time course of fracture healing, we propose that non-union formation is most probably due to a reduced expression of osteogenic growth factors or a disturbed ratio of angiogenic to osteogenic growth factors. This is supported by a study of Peng et al. that demonstrated that over-expression of the pro-angiogenic growth factor VEGF can impair bone regeneration (4).

VEGF is recognized as the major growth factor stimulating angiogenesis and is also closely involved in the process of fracture healing. Because of its known stimulatory effects on fracture healing, one would expect decreased expression of VEGF in non-unions. However, we found an increased VEGF expression in non-unions compared to normally healing fractures. In accordance with our study, Weiss et al. and Sarahrudhi et al. found increased serum levels of VEGF in patients with non-unions compared to patients with normal fracture healing (5, 6). Because hypoxia is a strong stimulus for VEGF production, an increased hypoxic environment in the larger segmental defect of the non-union group might have caused the increased expression of VEGF in the non-union group. We hypothesize that in critical size bone defects and situations with impaired vascularization and hypoxia, the principal aim of the injured tissue is to re-establish a vascular network for adequate nutrient and oxygen supply to guarantee cell survival at the site of injury. This may be achieved by an increased VEGF production under those critical conditions. In contrast growth factors for osteoblastic cell differentiation and new bone formation are downregulated, because in this setting general cell survival at the fracture site is of principal importance.

REFERENCES: