

Abstract

The role of plasmin in the regulation of VEGF activity during wound healing: Studies in the db/db mouse model

Die Rolle von Plasmin in der Regulation der VEGF Aktivität während der Wundheilung: Untersuchungen im db/db Mausmodell

Dissertation von Detlev Roth, Universität Köln, 2004

A functional and coordinated vascular system is essential for maintaining the integrity and barrier function of the skin. In wound healing a well balanced regulation of stimulating and inhibitory factors play an important role in re-establishing an intact vascular system. A disruption in angiogenesis regulation appears to be the cause for the formation and maintenance of most wound healing disorders. So far the cellular and molecular mechanisms that lead to chronic wound healing impairment are not fully understood.

This study tries to identify some underlying defects. Vascular endothelial growth factor (VEGF) is an endothel-specific, highly potent mediator of angiogenesis and functions as a key molecule in wound healing. Previous studies of our group in the human system showed that VEGF₁₆₅ protein is degraded and its bioactivity reduced by the serine protease plasmin in chronic wound healing disorders. One goal of this study was to investigate whether topically applied VEGF₁₆₅ could rescue an impaired wound healing phenotype. The second goal was to investigate the role of plasmin in the regulation of VEGF₁₆₅ activity during wound healing disorders. The diabetic db/db mouse model was used for our studies which shows a similar wound healing impairment as found in chronic human wounds with reduced angiogenesis and VEGF availability.

Macroscopic and histologic studies of the VEGF treated mouse wounds showed that topic application of VEGF₁₆₅ protein and *in vivo* transfection with a VEGF₁₆₅ expression plasmid by particle bombardment were able to accelerate wound closure and improve angiogenesis. The acceleration of wound closure is associated with the induction of a highly vascular granulation tissue and an accelerated reepithelialization of the wound. Stabilization of VEGF₁₆₅ against proteolytic degradation leads to a prolonged angiogenic response with a delayed regression of endothelial structures in late granulation tissues as seen in wounds transfected with the plasmin-resistant VEGF₁₆₅-MutPro₁₁₁. A plausible explanation for the delayed regression might be the prolonged anti-apoptotic effect of the stabilized VEGF mutant. Investigations considering the protein stability of the VEGF₁₆₅ wildtype and the VEGF₁₆₅ mutant in wound lysates of db/db mice showed an increased stability of the mutant form thus supporting the thesis of an increased *in vivo* stability leading to a prolonged biological activity of the plasmin resistant VEGF₁₆₅.

The described studies support the findings in the human system concerning the proteolytic degradation of VEGF₁₆₅ and the subsequent loss of its bioactivity in the pathogenesis of the impaired wound healing. A stabilization of the integrity of the VEGF₁₆₅ molecule - especially in the context of a protease rich environment - can lead to an increased angiogenic activity of VEGF₁₆₅. This study shows the therapeutic potential of the plasmin-resistant VEGF₁₆₅ mutant in chronic wound healing disorders.