Role of Platelet-released growth factors in detoxification of reactive oxygen species in osteoblasts

Mersedeh Tohidnezhad¹, Christoph-Jan Wruck¹, Alexander Slowik¹,², Nisreen Kweider¹, Rainer Beckmann¹, Andreas Bayer³, Holger Jahr⁴, Daike Varoga⁵, Sebastian Lippross³, Thomas Pufe¹

¹Department of Anatomy and Cellbiology, RWTH Aachen University, Aachen, Germany, ²Institute of Neuroanatomy, RWTH Aachen University, Aachen, Germany, ³Department of Trauma Surgery, University Hospital of Schleswig Holstein, Campus Kiel, Kiel, Germany, ⁴Department of orthopaedic surgery, RWTH Aachen University, Aachen, Germany

Introduction: Oxidative stress can impair fracture healing. To protect against oxidative damage, a system of detoxifying and antioxidative enzymes works to reduce the cellular stress. The transcription of these enzymes is regulated by antioxidant response element (ARE). The nuclear factor (erythroid-derived 2)-like2 (Nrf2) plays a major role in transcriptional activation of ARE-driven genes [1]. Recently it has been shown that vascular endothelial growth factor (VEGF) prevents oxidative damage via activation of the Nrf2 pathway in vitro [2]. Platelet-released growth factor (PRGF) is a mixture of autologous proteins and growth factors prepared from platelet-rich plasma (PRP), which has already been frequently used to enhance fracture healing in vivo.

Aim: The aim of the present study was to elucidate if platelets can up-regulate VEGF expression and if platelets can regulate the activity of the Nrf2-ARE system in primary human osteoblasts (hOB).

Material and methods: Platelets and PRGF were obtained from healthy human donors. A dual luciferase assay was used to demonstrate ARE-activation at different concentrations of PRGF. We used real-time RT-PCR to analyse gene expression of VEGF and two established Nrf2 target genes, carrying AREs in their promoter regions: heme Oxygenase-1 (HO-1) and NAD(P)H quinine oxidoreductase-1 (NQO1).

Results: PRGF concentrations of 2.5% and 5% led to the largest relative ARE activation in hOB cells (1.53±0.38 and 1.28±0.27 respectively, n=6, p<0.05; Fig. 1A). Both concentrations also resulted in the most prominent VEGF induction (5.93±3.66, n=12 and 8.14±6.87, n=10, respectively, p<0.05; Fig.1B). Furthermore, relative expression of HO-1 and NQO1 genes increased upon addition of 5% and 10% PRGF (HO-1: 2.27±1.94 and 2.84±1.73 n≥7, respectively; Fig.1C; NQO1: 1.85±0.85 and 1.39±0.31, n≥9, respectively; p<0.05; Fig 1D)

Conclusions: These results provide a new insight into PRGF’s mode of action in osteoblasts. PRGF increased endogenous VEGF synthesis, which is a crucial factor to stimulate angiogenesis in vivo. PRGF may further be involved in preventing oxidative cell damage through regulating Nrf2-ARE signaling. Nrf2 activation via PRGF may therefore have great potential as an effective therapeutic drug target to stimulate fracture healing.

References:

**Keywords:** Nrf2; PRP; VEGF; Fracture healing