Studies on the signalling of oxidative stress induced by metallic corrosion products in human endothelial cells *in vitro*

Kirsten Peters, Roman Tsaryk, Ronald E. Unger, Maike Fischer, C.J. Kirkpatrick.
Institute of Pathology, Langenbeckstr. 1, 55101 Mainz, Germany

Statement of Purpose: It is known that metallic implants can cause high concentrations of corrosion products in the peri-implant tissue (e.g. up to 0.9 mM Co-ions and up to 2.1 mM Cr-ions) [1]. Metallic corrosion products (particles and ions) have the potential to induce the production of reactive oxygen species (ROS) and thus to induce oxidative stress by disturbing the prooxidant/antioxidant balance of the cells. ROS (e.g. $O2^-$/superoxide radical, H_2O_2) have the potential to induce damage to all biomolecules (e.g. DNA, proteins). Thus, ROS has been implicated in several roles in the pathogenesis of chronic-degenerative conditions [2].

The complex process of wound healing following implantation of a biomaterial involves an acute inflammatory response followed by reparative processes. Vascular endothelial cells (EC), which cover the inner surface of blood vessels, are involved in both inflammation and reparation. EC release pro-inflammatory factors and express cell adhesion molecules and are furthermore the driving cell type in the formation of blood vessels, a process called angiogenesis. During angiogenesis EC migrate towards an angiogenic stimulus (e.g. soluble factors like vascular endothelial growth factor/VEGF) [3]. Thus, EC viability and function are a prerequisite for an adequate early tissue response to the implanted material and long-term implant stability, so that the impairment of cellular and EC functions by implant-derived ROS could impair implant acceptance.

This study examines the effects of different inducers of oxidative stress (Co^{2+}-ions, H_2O_2 and oxygen deficiency) in EC *in vitro* with respect to the signalling pathways of oxidative stress, DNA repair, cell cycle and cell death.

Methods: Human dermal EC (HDMEC) were isolated from juvenile foreskin and cultured in Endothelial Cell Basal Medium MV (PromoCell) supplemented with 15% FCS, basic fibroblast growth factor (bFGF), Na-heparin, 1% Pen/Strep at 37°C, 5% CO_2 and used in passage 4. Cell proliferation status was analysed by flow cytometry and immunocytochemical detection of Ki67 staining. Total RNA preparations were collected after Co^{2+}-exposure (0.1 and 0.7 mM) and oxygen deficiency (3% O_2 and anoxia) of 1 and 40 h. The PIQOR™ Skin Microarray (cDNA-array, ~1308 genes) was performed by Memorec/Miltenyi. p53- and p21-ELISA were performed according to the manufacturer's instructions (R&D-Systems).

Results / Discussion: All conditions tested (Co^{2+}-ions, H_2O_2 and oxygen deficiency) induced a decrease in proliferative activity of EC. This proliferative reduction by high Co^{2+}-concentrations and anoxia was mediated by the p53/p21 pathway (upregulation of p53 protein amount, upregulation of p21 mRNA and protein). Whereas Co^{2+}-ions induced a significant pro-inflammatory stimulation, H_2O_2-treatment led to minor changes in the pro-inflammatory response (e.g. IL-8 release, E-selectin). Oxygen deficiency induced no effect on pro-inflammatory parameters tested.

The effects on the oxidative stress response were pronounced with high Co^{2+}-concentrations (0.7 mM) in that e.g. the expression of SOD1 (Cu/Zn-superoxide dismutase), SOD2 (Mn-superoxide dismutase), glutathione peroxidase (plasma GPX3) and glutathione synthetase was significantly increased indicating a response to oxidative stress. Low Co^{2+}-concentrations (0.1 mM) and oxygen deficiency induced no significant effects on the expression of these genes.

It is known that high Co^{2+}-concentrations induce apoptosis in EC [4]. Therefore, the activation of DNA repair genes (e.g. FANCA, NBS1) by Co^{2+} might indicate the induction of apoptosis by DNA damage signalling pathways. Oxygen deficiency showed an ambiguous response regarding DNA damage in that mild hypoxia induced no DNA-repair genes, whereas anoxia led to the activation of further DNA repair genes (NBS1, Rad17, Rad30B, Rad50).

Conclusions: The tested stimuli known to be involved in the development of oxidative stress stimulated different signalling pathways in human EC *in vitro*. Since the cellular processes in wound healing are also known to develop different ROS, it is likely that a co-occurrence of these different oxidative stress-stimuli will occur. This co-occurrence can influence the *in vivo* situation in a cumulative, possibly non-predictable way. As a consequence, the release of transition metal ions from implants (e.g. Co^{2+}) could increase the oxidative stress situation in the peri-implant tissue and thus aggravate the already existing oxidative stress of wound healing leading to cellular damage, which again can impair implant acceptance.

References: [1] Blumenthal NC et al. A new technique for quantitation of metal particulates and metal reaction products in tissues near implants. J Appl Biomaterials 1994;5(3):191-193.
[2] Galli F et al. Oxidative stress and reactive oxygen species. Contrib Nephrol 2005;149:240-260.
[3] Folkman J. Fundamental concepts of the angiogenic process. Curr Mol Med 2003;3(7):643-651.
[4] Peters K et al. Induction of apoptosis in human microvascular endothelial cells by divalent cobalt ions. Evidence for integrin-mediated signalling via the cytoxkeleton. J Mater Sci Mater Med 2001;12(10-12):955-958.
[5] Peters K et al. Paradoxical effects of hypoxia-mimicking divalent cobalt ions in human endothelial cells *in vitro*. Mol Cell Biochem 2005;270(1-2):157-166.

This work was supported by the German Research Foundation (Priority Programme Biosystem 322 1100, KI 601/1-3).