The vitamin-k-cycle-enzyme VKORC1L1, but not its isoenzyme VKORC1, reduces inflammation in coronary endothelial cells

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Introduction: Vascular inflammation is a crucial contributor to atherosclerosis, in which oxidative stress, endoplasmic reticulum (ER) stress and transition of endothelial to mesenchymal cells (EndMT) are of critical importance. Vitamin-K-antagonists (VKA) promote vascular dysfunction, while vitamin K intake was proposed to reduce incidence of coronary artery disease. Key enzyme for regeneration of vitamin K is the Vitamin K epoxide reductase complex subunit 1 (VKORC1), which also represents the pharmacological target of VKA. VKOR-like1 (VKORC1L1) is an isoenzyme of VKORC1, which resides at the ER-membrane, exerts antioxidative properties and is involved in vitamin K maintenance.

Aim of this study was to investigate the role of VKORC1 and VKORC1L1 in inflammation of human coronary artery endothelial cells (HCAEC).

Methods and Results: In silico analyses of the proteome of human coronary atherosclerosis revealed increased expression of VKORC1L1, but not of VKORC1. In vitro induction of oxidative stress (H2O2) and ER-Stress (tunicamycin) in HCAEC promoted time- and dose-dependent enhanced expression of VKORC1L1, without altering VKORC1 expression (H2O2: 2.3-fold vs. 0.8-fold after 40 minutes, p=0.04; tunicamycin: 1 μg/ml: 1.43-fold vs. 0.8-fold, p<0.01). Moreover, inducing EndMT by treatment with a differentiation medium (containing TGF-β2, IL-1β) resulted in a reduction of VKORC1L1, but not of VKORC1 (Fig.1).

To analyze the relevance of the VKOR-enzymes on endothelial cell function, VKORC1 and VKORC1L1 were downregulated by siRNA. VKORC1L1 downregulation increased formation of reactive oxygen species (ROS) (DCFDA signal: 0.98-fold vs. 1.31-fold, p = 0.03) and reduced proliferation (EdU signal: 0.56 ± 0.02 vs. control, p < 0.01), whereas viability of HCAEC remained unchanged. ELISA and qPCR experiments revealed enhanced expression of a variety of markers of vascular inflammation after VKORC1L1 downregulation (e.g., IL-6: 3.42-fold ± 0.53, NF-κB: 1.95-fold ± 0.13, VCAM-1: 1.59-fold ± 0.12, vs. control). Additionally, expression of ER-stress markers was increased (e.g., GRP78: 1.32-fold ± 0.04 vs. control, p<0.01) (Fig.1).

In contrast, VKORC1 downregulation significantly promoted proliferation (EdU signal: 3.9 ± 1.7 vs. control, p<0.01) as well as viability (alamarBlue® absorbance: 1.07 ± 0.08 vs. control, p=0.04), and decreased ROS (0.88 ± 0.17 vs. control, p=0.03). Pro-inflammatory and ER-stress proteins were either unaltered or decreased after VKORC1 knockdown (e.g., GRP78: 0.89-fold, VCAM-1: 0.68-fold) (Fig.2).

To further elucidate these findings, we will perform overexpression experiments and stable knockdown of the VKOR-enzymes.

Conclusion: VKORC1 and its isoenzyme VKORC1L1 exhibit divergent effects on endothelial cell inflammation. Further studies are warranted to shed light on the regulation of the enzymes to improve our understanding regarding vascular side effects of VKA treatment and beneficial effects of vitamin K supplementation.