Activated factor X induces oxidative stress in human vascular smooth muscle cells

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Background and purpose: NADPH oxidase-derived reactive oxygen species (ROS) and inflammatory cytokines contribute to the vascular response to injury. Activated coagulation factor X (FXa) can induce vascular smooth muscle cells (SMC) proliferation and migration via protease-activated receptors PAR-1 and/or PAR-2. We investigated if FXa influences the generation of ROS and the inflammatory cytokine interleukin-6 (IL-6), which are both strongly associated with SMC proliferation and migration.

Experimental approach: Human saphenous SMC were serum-deprived prior to stimulation with FXa and/or study drugs. Intracellular ROS were detected by dihydroethidium and dichlorofluorescein fluorescence. mRNA, total protein and cell-surface expression levels were determined by realtime PCR, Western blot or immunofluorescence, and flow cytometry.

Key results: FXa significantly stimulated IL-6 expression (by 2.4±0.3 fold at 3h) and intracellular ROS generation (to maximally 196±24% at 3h) in human vascular SMC. The oxidative response to FXa was prevented by NADPH oxidase inhibition with diphenyliodinium plus apocynin and was associated with increased mRNA (to 4.1±1.0 fold at 1h) and protein expression (to maximally 3.0±0.5 fold at 24h) of the NADPH oxidase subunit NOX-1. Other NADPH oxidase subunits (NOX-4, p47-phox) were unaffected. FXa also significantly upregulated PAR-2 mRNA (1.6±0.2 fold), total protein (3.0±0.7 fold), and cell-surface expression (2.1±0.2 fold) at 24h, while PAR-1 expression remained constitutive. Accordingly, pretreatment of SMC with FXa enhanced intracellular ROS generation in response to PAR-2 activating peptide.

Conclusions: FXa induces expression of IL-6 and NOX-1 in human vascular SMC. This is accompanied by increased intracellular oxidant stress and upregulation of the FXa receptor PAR-2. Such actions are likely to support proliferative, migratory and inflammatory processes after vascular injury and contribute to vascular remodelling in vivo.