

Summary

Pulmonary arterial hypertension (PAH) is a severe, progressive disease, which harbors a high mortality. Underlying pathomechanisms are multifactorial and to date only partly understood. An imbalance of vasoconstrictive and vasodilative agents, as well as elevated levels of growth factors, pro-coagulative and inflammatory mediators lead to progression and worsening of this fatal disease, contributing to the establishment of a permanently increased pulmonary arterial pressure and the development of right heart hypertrophy. Vascular remodeling of small pulmonary arteries caused by aberrant proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs) play a substantial role in the pathogenesis of PAH.

Recent studies indicate that, proliferation and migration of SMCs can be mediated by the activation of protease-activated receptors (PARs) via cleavage by serine proteases. Particularly, the isoforms PAR-1 and PAR-2 have been found to be involved in pathologic vascular remodeling processes. In the context of PAH, PAR-1 and PAR-2 are up-regulated in the lungs of PAH patients. Current data implicate that the key coagulation proteases FXa and thrombin exert direct, coagulation-independent effects on cells via proteolytical processing of PAR-1 and PAR-2. Therefore, we tested the hypothesis that FXa and thrombin contribute to the

pathogenesis of PH via activation of PAR-1 and PAR-2 promoting aberrant proliferation and migration of PSMCs. Specifically, the impact of PAR-1 and PAR-2 and the involvement of their potential activators FXa and thrombin on proliferation and migration of PSMCs *in vitro* and experimental PH *in vivo* was systematically investigated.

First, FXa- and thrombin-induced cellular effects and downstream signaling were characterized in human and murine PSMCs. Here, FXa and thrombin were identified as potent mitogens for PSMCs. Particularly, FXa induced proliferation via activation of PAR-2, whereas thrombin mediated cellular mitogenesis via PAR-1. Chemotactic effects of both coagulation proteases were observed, but were less pronounced. Consistently, analysis of downstream signaling events revealed that FXa mediated activation of ERK1/2 and AKT via PAR-2 in murine PSMCs. However, in human PSMCs only MEK activation was involved in FXa-induced proliferation. In contrast, thrombin mediated ERK1/2 and AKT activation via PAR-1 in murine PSMCs. Accordingly, both MEK and PI3K emerged as important signaling

molecules for thrombin-mediated mitogenic effects in human PSMCs. Moreover, SPHK-1 was identified as a common mediator of FXa- and thrombin-induced proliferation in PSMCs. Based on the above *in vitro* findings, the relevance of PAR-1 and PAR-2, and

the role of FXa and thrombin for pulmonary vascular remodeling and the progression of PH were investigated *in vivo*. To this end, FXa inhibition using rivaroxaban and thrombin inhibition by dabigatran etexilate were analyzed in WT mice in the model of hypoxia- induced PH. Finally, the impact of PAR-1 and PAR-2 on the progression of experimental PH was investigated in the same animal model by utilizing mice harboring targeted gene deletion of either receptor.

Unexpectedly, FXa inhibition had no beneficial effects on the progression of hypoxia- induced PH. Likewise, thrombin inhibition with dabigatran etexilate had no significant impact on right ventricular systolic pressure and vascular remodeling when compared to the control group.

Analysis of PAR-1 and PAR-2 deficient mice subjected to chronic hypoxia revealed that both receptors are relevant for disease progression *in vivo*. PAR-1 deficient mice exhibited significantly decreased PH and diminished muscularization of the small pulmonary arteries in comparison to hypoxic WT controls. The same was true for PAR-2 deficient mice, where a protective effect against the establishment of PH and vascular remodeling was observed.

Taken together, these results indicate that FXa and thrombin are activators of PAR-2 and PAR-1, respectively, initiating PASM

proliferation and migration. However, with respect to the *in vivo* situation, the inhibition of either FXa or thrombin was not sufficient to reduce the progression of PH, at least in the applied model of hypoxia-induced PH in mice. This might be due to additional serine proteases, which are also capable of activating PAR-1 and PAR-2 *in vivo*. Hence, inhibition at the receptor level emerges as a promising approach to prevent the progression of PH.

Conclusively, this study indicates a pathogenic potential of the coagulation proteases FXa and thrombin, and proposes a crucial role for PAR-1 and PAR-2 for the progression of pulmonary vascular remodeling and PH, offering a novel therapeutic target for the treatment of PH.