A65 5-HT RELEASE FROM PLATELETS AND ACTIVATION OF 5-HT_{2B} PLAY A CRUCIAL ROLE FOR DEVELOPMENT OF FIBROSIS IN SYSTEMIC SCLEROSIS

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Purpose Systemic sclerosis (SSc) is characterised by progressive vascular disease and organ fibrosis. Tryptophanhydroxylase-1 (tph-1) is the rate-limiting enzyme for the synthesis of 5-hydroxytryptamine (5-HT). After synthesis, 5-HT is stored in large amounts within platelets and can be released upon activation. The aim of the present study was to investigate the role of 5-HT release upon platelet activation and whether 5-HT signalling is involved in the pathogenesis of SSc.

Methods Dermal fibroblasts from SSc patients and healthy individuals were stimulated with 5-HT and incubated with selective inhibitors of different 5-HT receptors. Expression of 5-hydroxytryptamine (serotonin) receptor 2B (5-HT_{2B}) in human sections was analysed by immunohistochemistry. The role of platelet-derived 5-HT and of the 5-HT_{2B} receptor was evaluated in the mouse model of bleomycin-induced dermal fibrosis and in tight-skin-1 (tsk-1) mice.

Results Stimulation of SSc and healthy dermal fibroblasts with 5-HT significantly increased dose-dependently mRNA and protein levels of collagen by up to 3.1 ± 0.14 -fold ($p < 0.05$ for each), which was significantly reduced upon incubation with the selective 5-HT_{2B} inhibitor SB 204741. The expression of 5-HT_{2B} protein was strongly increased in the skin of SSc patients compared to healthy volunteers. In the mouse models of bleomycin-induced fibrosis and tsk-1, the concentration of 5-HT in lesional skin significantly increased from 3.2 ± 0.2 to 5.8 ± 0.5 ng/mg and from 3.5 ± 0.4 to 4.8 ± 0.2 ng/mg, respectively, as analysed by ELISA. Treatment with clopidogrel significantly reduced the 5-HT content of the skin in both models. Along with reduced levels of 5-HT, treatment with clopidogrel decreased dermal thickening upon bleomycin-challenge and prevented also hypodermal thickening in tsk-1 mice. Also selective depletion of 5-HT from platelets by knockdown of tph-1 significantly ameliorated dermal fibrosis in mice lacking tph-1 with a mean reduction in dermal thickening of $61 \pm 6\%$ ($p < 0.05$). Mice lacking 5-HT_{2B} were completely

protected from bleomycin-induced skin fibrosis with normal skin thickness. Potent anti-fibrotic effects of 5-HT_{2B} inhibitors with normalisation of histological changes and prevention of myofibroblast differentiation were also detected in the *tsk-1* model.

Conclusion We demonstrate increased levels of 5-HT in different animal models of SSc and an activation of 5-HT/5-HT_{2B} signalling in SSc patients. Platelet activation in affected vessels, the subsequent release of the profibrotic mediator 5-HT and the activation of 5-HT_{2B} might provide a novel link between vascular manifestations and tissue fibrosis in SSc.