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5-HT2 and 5-HT2B Receptor Antagonism Abrogates Fibrotic Potential of Human Renal Allograft Fibroblasts by Targeting STAT3 Pathway

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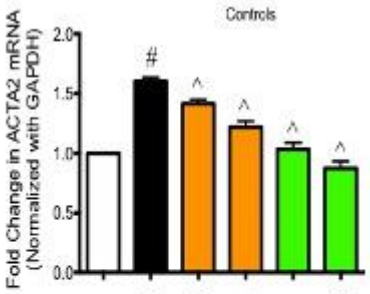
Objectives: Despite improvements in immunosuppressive therapy, long-term allograft survival after kidney transplantation remains as low as 50%. The primary cause of chronic allograft nephropathy is "interstitial fibrosis and tubular atrophy" (IF/TA). Serotonin (5-HT; 5-Hydroxytryptamine) produces extracellular matrix proteins in presence of TGF-β1 dependent manner. TGF-β1 activates resident fibroblasts, and trans-differentiate into myo-fibroblasts, which is the hallmark of the pathogenesis of fibrosis. We aimed to evaluate the anti-fibrotic role of inhibitors of 5-HT and 5-HT (Terguride and SB204741), respectively in human renal fibroblasts (HRFB) isolated from renal allograft rejection patients.

Methods: Renal fibroblasts isolated from renal allograft rejection patients (n=6) and controls (n=3), were incubated with 5-HT (1μM)/TGF-β1 (10ng/ml) for 1 hour and later with 5-HT (1μM)/TGF-β1 (10ng/ml) and terguride or SB204741 (1μM, each) for 24 hours (Post-treatment strategy). In the pre-treatment strategy, cells were pre-treated with terguride or SB204741 (1μM, each) for 1 hour and later with only 5-HT (1μM)/TGF-β1 (10ng/ml) for 24 hours. Real time PCR for pro-fibrotic (TGFB1, COL1A1, COL1A2, ACTA2, CTGF and FN1) and anti-fibrotic genes (MMP2/TIMP1) expression was performed. Type I collagen and α-SMA, the phosphorylation status of Smad-3, ERK1/2, Src, and STAT-3 was examined by western blotting.

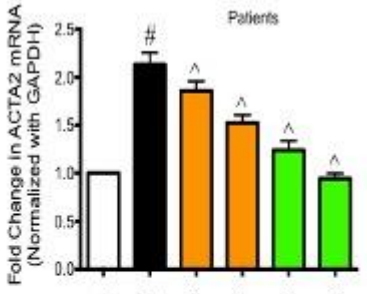
Results: In 5-HT/TGF-β1 stimulated HRFB, upregulated pro-fibrotic gene expression was observed, which significantly reduced co-culture with 5-HT /5-HT inhibitors, with no effect on anti-fibrotic genes mRNA expression (Figure 1). In 5-HT stimulated HRFB, treatment with both 5-HT inhibitors decreased type 1 collagen and α-SMA with reduced ERK1/2 phosphorylation, however, Smad-3 phosphorylation remains unaltered. In 5-HT/TGF-β1 simulated HRFB, 5-HT inhibitors decreased STAT3 phosphorylation, without affecting Src phosphorylation.

Conclusions: TGF-β1 mediated non-canonical pathways, ERK1/2 and STAT3 have been implicated in the development of fibrosis. 5-HT receptor antagonists might reduce the fibrotic potential of HRFB via suppression of TGF-β1 mediated non-canonical pathways.

Figure 1



	1	2	3	4	5	6
Pretreatment	0	0	5-HT	5-HT	Ter	SB
5-HT	-	+	+	+	+	+
Terguride	-	-	+	-	-	-
SB204741	-	-	-	+	-	-
p value		**	**	**	**	**



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Pretreatment	0	0	5-HT	5-HT	Ter	SB
5-HT	-	+	+	+	+	+
Terguride	-	-	+	-	-	-
SB204741	-	-	-	+	-	-
p value		*	*	*	-	-