

# SDF-1 and its receptor in the ventricles of rat with monocrotaline-induced pulmonary hypertension

Original research article/Review

Veteskova J.✉, Obsivan M., Kmecova Z., Radik M., Srankova J., Malikova E., Klimas J., Krenek P.

Comenius University in Bratislava,  
Faculty of Pharmacy, Department  
of Pharmacology and Toxicology,  
Bratislava, Slovak Republic

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**Abstract** Aim: Chemokine stromal cell derived factor-1 (SDF-1) plays an important role in many processes such as apoptosis, proliferation, migration and angiogenesis, and these effects are mediated mostly by the receptor CXCR4. The aim of this study was to determine the expression of SDF-1 and CXCR4 in the ventricles of rats with monocrotaline-induced pulmonary hypertension. Methods: 10–12 weeks old male Wistar rats were injected with monocrotaline (s. c., 60mg/kg; MON) or vehicle (CON). Rats were sacrificed 1 week (1W-MON, 1W-CON), 2 weeks (2W-MON, 2W-CON) and 4 weeks after monocrotaline administration (4W-MON, 4W-CON). Gene expression of SDF-1 and CXCR4 was determined by qRT-PCR. Results: We observed a decrease in the SDF-1 expression on mRNA level in the right ventricle in 2W-MON and 4W-MON rats without any changes in the left ventricles and a decrease in CXCR4 expression in 1W-MON in both ventricles with an increase of CXCR4 expression in 4W-MON in the left ventricle (\*P < 0.05). Conclusion: SDF-1/CXCR4 axis is affected in both ventricles of rats with monocrotaline model of pulmonary hypertension. Keywords: Pulmonary hypertension, monocrotaline, stromal cell-derived factor 1, ventricles

**Keywords** Pulmonary hypertension – monocrotaline – stromal cell derived factor-1 – ventricles

## INTRODUCTION

Pulmonary hypertension (PH) is defined as an increase in the mean pulmonary arterial pressure over 25 mmHg at rest measured by the right heart catheterization (Hoepfer et al., 2013). PH is an orphan disease (European PH prevalence is 15–60 subjects per million population) (Galiè et al., 2015); however, its development is quite progressive and fatal (almost 50% mortality rate in 3 years without treatment) (Lai et al., 2014). Elevation of pulmonary arterial pressure along with remodelling of the pulmonary arterial vessels increase the right ventricular (RV) afterload, thus contributing to the development of RV dysfunction and failure (Bogaard et al., 2009). Current therapeutic approach for PH has evolved with increase in complexity, which arises from the complex molecular mechanism of PH development (Galie et al., 2015). Recently, it has been noticed that stromal cell derived factor-1 (SDF-1) concentration were elevated in the plasma of patients with pulmonary arterial hypertension compared to healthy controls. Furthermore, the elevated circulating SDF-1 seems to be an independent risk factor for reduced survival in these patients (McCullagh et al., 2015). SDF-1 was originally

identified as a molecule secreted in the bone marrow stromal cell lines attracting and stimulating the growth of B-cells (Bleul et al., 1996). As a ligand for chemokine (C – X – C motif) receptor 4 (CXCR4), SDF-1 plays a role in the recruitment of stem cells to areas of tissue injury in multiple organ system. CXCR4 receptor has been described as an essential chemokine receptor for development, haematopoiesis, organogenesis and vascularization (Wang et al., 2014). Increase in expression of CXCR4 and SDF-1 in lungs of hypoxia-induced PH mice (Gambaryan et al., 2011) or pulmonary arterial pressure reduction, attenuation of right ventricle hypertrophy and wall thickness of pulmonary arteries with CXCR4 inhibition in hypoxic-induced PH rats (Yu et al., 2011) indicate that of SDF-1/CXCR4 axis also takes part in hypoxic model of PH. Hypoxia stimulates the expression of CXCR4 in endothelial cells and also potentiates migratory response of endothelial cells to exogenous SDF-1 (Schioppa et al., 2003). Increased SDF-1 levels were also demonstrated in the decompensated right ventricle of monocrotaline-induced PH in rats (Sutendra et al., 2013).

\* E-mail: veteskova3@uniba.sk

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In our study, we aim to determine the involvement of SDF-1 and CXCR4 receptor in the ventricles of monocrotaline-induced PH rats in development of the disease.

## METHODS

### Experimental Design

10–12 weeks old male Wistar rats were administered a single dose of monocrotaline (Sigma Aldrich, USA) injection (s. c., 60mg/kg, MON) or vehicle (CON). Animals were handled under standard conditions with free access to food and drinking water. All experimental procedures involving experimental animals were approved by a local Ethical Committee and the State Veterinary and Food Administration of the Slovak Republic. Rats were sacrificed 1, 2 or 4 weeks after monocrotaline administration (1W-CON, 1W-MON; 2W-CON, 2W-MON; 4W-CON, 4W-MON) and tissue samples from right and left ventricle were isolated.

### Gene Expression

Total RNA was isolated from the samples by phenol/chloroform extraction using Tri-Reagent (Sigma Aldrich, USA). Quality of isolated RNA was verified by agarose gel electrophoresis and spectrophotometric analysis (NanoDropND-1000, Thermo Scientific, USA). Total RNA were reverse-transcribed to cDNA (High capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA) and real-time PCR (StepOne Plus System, Applied Biosystem, USA) was performed using SYBR green PCR Master Mix kit (Applied Biosystems, USA). Expression of SDF-1 and CXCR4 were determined using gene specific primers (Srankova et al., 2016). All primers were verified to yield a single PCR product with the correct molecular weight. Beta-2-microglobulin was used as endogenous reference gene and results were calibrated to the control groups.

### Statistical Analysis

Results are expressed as average  $\pm$  standard error of the mean. Mean PCR efficiency estimates per amplicon and quantification cycle (Cq) values per sample were determined with LinRegPCR software (version 2015.0) and relative expression were calculated (Doka et al., 2017). Statistical significance was determined by a non-parametric Mann-Whitney test or parametric t-test after Shapiro-Wilk's test of normality. Differences were considered significant at  $P < 0.05$ . Results were analysed by GraphPad Prism 4.0 (GraphPad Software, California).

## RESULTS

We observed a decrease in the relative expression of SDF-1 on mRNA level in the right ventricle in 2W-MON and 4W-MON rats, however, without any changes in the left ventricle in all

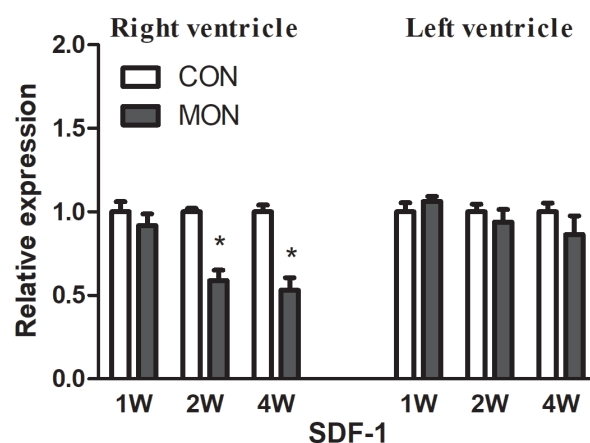


Figure 1. Relative expression of SDF-1 determined by qRT-PCR in the right and the left ventricle in 1, 2 and 4 weeks after monocrotaline administration. Results are expressed as average  $\pm$  SEM. \* $P < 0.05$  vs. CON.

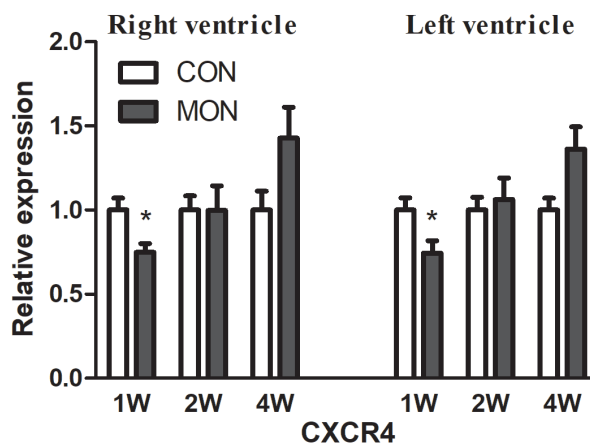


Figure 2. Relative expression of CXCR4 receptor determined by qRT-PCR in the right and the left ventricle in 1, 2 and 4 weeks after monocrotaline administration. Results are expressed as average  $\pm$  SEM. \* $P < 0.05$  vs. CON.

observed weeks (Figure 1). A small, but significant decrease of expression of receptor CXCR4 were determined in 1W-MON rats in both ventricles. Elevated expression of CXCR4 was observed in 4W-MON animals in the left ventricle (Figure 2).

## DISCUSSION

We aimed to determine the expression of SDF-1 during the development of monocrotaline-induced PH. In this model, the severe PH stage with elevated RV pressure, RV hypertrophy and clinical signs of PH (dyspnoea, cachexia, loss of social interactions) develops approximately 4 weeks after monocrotaline administration. In the early stages of the model (1 or 2 weeks after monocrotaline administration), none of these features occur (data not shown), despite the supposed endothelial cell injury (Gomez-Arroyo et al., 2012).

We found that SDF-1 expression on mRNA level was decreased 2 and 4 weeks after monocrotaline administration. Sutendra et al. linked SDF-1 expression in the developed stages of monocrotaline PH to RV function as they recognised the elevation of SDF-1 expression in the compensated RV failure and SDF-1 decrease in decompensated right ventricular failure with high mortality rate and severe PH symptoms (Sutendra et al., 2013).

Influence of SDF-1 on heart tissue has been seen in the models of systemic hypertension or cell culture systems. Increase of SDF-1 was described in spontaneously hypertensive rats, which further increased with aging (Shao et al., 2015). SDF-1 on cultures of myofibroblast increased the migration of myofibroblasts and wound healing (Shao et al., 2015), treatment with exogenous SDF-1 has concentration-dependent effects on proliferation, hypertrophy and collagen production in activated cardiac fibroblasts from normotensive and hypertensive rats (Jackson et al., 2017). All these effects were almost completely blocked with antagonist of CXCR4 receptor AMD3100 (Shao et al., 2015; Jackson et al., 2017). Whether SDF-1 is associated with improvement of cardiac function or further drives pathological changes in the hearts in PH models remains to be elucidated.

Data from the models of PH are almost completely from work on hypoxic models and effects of SDF-1 on lung vasculature. Not only SDF-1, but also CXCR4 was elevated in lungs of hypoxic-induced PH mice and antagonism with AMD3100 partially improved right ventricular pressure (Gambaryan et al., 2011). In hypoxic rat model of PAH, an increase in SDF-1 preceded the development of complex inflammatory microenvironment in lungs and pulmonary artery and an increase in the expression of SDF-1 was parallel to an increase in the CXCR4 expression (Burke et al., 2009). In our study, CXCR4 expression seems to be independent on the SDF-1 mRNA level as we have seen a decrease in the CXCR4

expression in 1W-MON animals in both ventricles, where SDF-1 was not changed. Treatment with CXCR4 inhibitor in hypoxia-induced PH rats improved RV pressure, RV hypertrophy and prevented increased wall thickness of pulmonary arteries. Also, electroporation of bone marrow cells with the CXCR4 shRNA inhibited the development of hypoxia-induced PH and lung vasculature remodelling (Yu et al., 2011).

SDF-1 can act beside CXCR4 receptor also on CXCR7 receptor, but data about CXCR7 in PH models are to this date limited to one study from Gambaryan et al., where they noticed the elevation of CXCR7 in lungs of hypoxic-induced PH mice and antagonism of this receptor only partially improved RV pressure. However, in combination with CXCR4 antagonism significant pressure and RV hypertrophy reduction were determined (Gambaryan et al., 2011).

## CONCLUSION

In conclusion, we determined a decrease in SDF-1 in right ventricle in monocrotaline model of PH and also changes in CXCR4 expression. Involvement of SDF-1/CXCR4 axis in the development of PH is undeniable as we can see not only from our study, but also from elevation of SDF-1 in plasma of patients with pulmonary arterial hypertension. Since SDF-1/CXCR4 axis is pharmacologically modifiable, it remains to be further studied in PH.

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