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Inhibitory Effects of Methylsulfonylmethane on Ventricular Hypertrophy Related Gene Expression

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Abstract: Methylsulfonylmethane (MSM) is naturally accruing organic sulphur that is known as a potent anti-inflammatory compound. The aim of this study was to investigate the effect of MSM on mRNA expressions of angiotensinogen, endothelin-1 (ET-1) and Transforming Growth Factor (TGF)-β1 in rats with monocrotaline (MCT)-induced pulmonary arterial hypertension. Wistar rats were randomly assigned to 38-days pretreatment or 28-days treatment. MSM was administered to either 10 days before or 14 days after a single dose of MCT. Right Ventricle (RV) tissue samples were obtained to evaluate changes in the inflammatory genes expression using RT-PCR assay. The expression levels of angiotensinogen, ET-1 and TGF-β1 significantly were reduced ($p<0.01$) at efficient dose of MSM in MCT-induced pulmonary arterial hypertensive rats. Results suggest that harmful effects of MCT induced PAH on the RV function could be attenuated by anti-inflammatory actions through the suppression of local RAAS along with associated growth-promoting factors TGF-β1 and ET-1.

Key words: Inflammation, right ventricle, monocrotaline, endothelin-1, TGF-β1, angiotensinogen

INTRODUCTION

Pulmonary Arterial Hypertension (PAH) is a pathophysiological state characterized by a progressive increase in pulmonary vascular resistance. In addition to inducing myocardial hypertrophy, it also induces marked interstitial fibrosis to compensate for the increased ventricular workload. These adaptive changes often clinically lead to heart failure and sudden cardiac death (Simonneau *et al*., 2009). Despite all, current progress in the diagnosis and therapeutics, PAH continues to be a devastating disease with a high morbidity and mortality (Benza *et al*., 2010). Angiotensin II has been recognized as an important contributor to cardiac hypertrophy and inflammation in PAH (Huggins *et al*., 2003). Angiotensin II increases endothelin 1 (ET-1) expression which stimulates the conversion of angiotensinogen to angiotensin II and promotes myocardial inflammation by recruiting leukocytes and releasing of fibrogenic cytokines, like Transforming Growth Factor (TGF)-β (Gray *et al*., 1998; Jun *et al*., 2007). Angiotensin II also up-regulates TGF-β synthesis by cardiac fibroblasts occurring due to systemic hypertension (Huntgeburth *et al*., 2011).

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Methylsulfonylmethane (MSM) is naturally occurring organic sulphur that is known as a potent anti-oxidant/anti-inflammatory compound (Beilke *et al*., 1987; Ebisuzaki, 2003). MSM is widely used as an arthritis remedy with inhibitory activities on proinflammatory cytokines (Alam and Layman, 1983; Debbi *et al*., 2011). Despite, an increasing clinical use, the mechanisms by which MSM exert their effects remain largely unknown.

Recent findings have linked anti-inflammatory effects of MSM to modifications in proinflammatory gene expression. Kim *et al*. (2009) have recently shown that MSM inhibits LPS-induced release of pro-inflammatory mediators in macrophages through downregulation of NF-κB signaling. MSM may also be beneficial in PAH due to its anti-inflammatory and anti-proliferative effects (Lim *et al*., 2012). It has been shown that sulfur dioxide (SO_2) and its derivatives play a protective role in PAH (Jin *et al*., 2008). Thus, it is possible that MSM exerts its effect on PAH by interfering with inflammatory processes that may be associated with the heart failure. Monocrotaline (MCT) is a toxic pyrrolizidine alkaloid and has a selective toxic effect on pulmonary vessels without an effect on systemic vessels. To investigate whether MSM may provide therapeutic or preventive effects on PAH, an experimental study was conducted examining the gene expression of the angiotensinogen, ET-1 and TGF-β1 in the Right Ventricles (RV) from rats with MCT-induced PAH subjected to pretreatment or post treatment.

MATERIALS AND METHODS

Animals: Two-month-old male Wistar rats (200±20 g) were, fed with standard laboratory chow *ad libitum*, used in the experiment, as previously described by (Garjani *et al*., 2009). Animals obtained from the Pasteur Institute of Iran (Tehran, Iran). Tabriz University of Medical Sciences animal ethics committee approved the study protocol. Injections were all administered intraperitoneally (i.p.) to rats. Invasive experimental procedures were carried out on pentobarbital anaesthetized rats (60 mg kg^{-1} b.wt. i.p.). RV tissue samples were harvested and snap frozen in liquid nitrogen for further RT-PCR analysis of angiotensinogen, ET-1 and TGF-β1 genes.

Experimental protocols: MCT (Sigma, MO, USA) was dissolved in 1 M HCl, diluted with physiologic saline and neutralized to pH 7.4 with 1 M NaOH. MSM (Sigma, MO, USA) used for systemic administration were dissolved in physiological saline. PAH was induced by means of a single dose of MCT (60 mg kg^{-1}). The effective dose of MSM was determined according to a significant improvement in hemodynamic status of MCT-induced PAH rats. In a separate series of experiments, rats were subjected to post-treatment with MSM (400 mg kg^{-1} day^{-1}) from the third week after injection of MCT (MSM-post treated group, n = 22) or pre-treatment with MSM (400 mg kg^{-1} day^{-1}) from the 10th day prior to injection of MCT and for 4 weeks after the MCT (MSM-pretreated group, n = 22). Saline was used as vehicle in control experiments. MSM was well tolerated by the rats and no abnormal behavior was observed.

RT-PCR analysis: Total RNAs were purified with trizol reagent (Invitrogen, Carlsbad, CA). One microgram of RNA was reverse-transcribed using the First Strand (Promega, Madison, WI) as per the manufacturer's instructions. Semiquantitation of the cDNA was performed by RT-PCR using specific primers (Table 1) according to a previous report (Park *et al*., 2001). The reverse transcripts of candidate genes were amplified with GAPDH as an internal control, using standard conditions of PCR.

Quantitative analysis: For comparing the amount of PCR product between samples, a gel digitizing software, Uviphotomw (UVItec, Cambridge, UK; version 11.01), was used for estimating the intensity of each band on the gel. Each experiment was repeated four times. The Coefficients of Variation (CV) were about 5-8%.

Statistical analysis: Data presented are the Mean±SD of four separate experiments. Calculation of significance between groups was done according to Student's t-test and a p-value <0.05 was considered statistically significant.

RESULTS

To explore possible mechanisms by which development of PAH is inhibited by MSM treatment, at a dose effective in significantly improving hemodynamic (data not shown); the effects of MSM pretreatment and posttreatment on mRNA expressions of the angiotensinogen, ET-1 and TGF-β1 were examined in MCT-induced pulmonary hypertensive rats. Levels of angiotensinogen, ET-1 and TGF-β1 mRNAs in the hypertrophied RV were significantly increased at week 4 of MCT treatment. The angiotensinogen, ET-1 and TGF-β1 mRNAs levels were significantly decreased in both MSM-pretreated and -post treated rats when compared with those in the saline-treated pulmonary hypertensive rats ($p<0.05$; Fig. 1). Levels of ET-1 mRNA transcript in MSM-treated pulmonary hypertensive rats decreased, even as compared to the values corresponding to normotensive control rats ($p<0.01$; Fig. 1).

Table 1: Primer sequences used for reverse transcription-polymerase chain reaction (RT-PCR)

Gene	Gene symbol	GenBank accession	Forward primer sequence	Reverse primer sequence	Amplicon size (bp)
Angiotensinogen	AGT	NC_000001.10	TTCAGGCCAAGACCTCCC	CCAGCCGGGAGGTGCAGT	308
Endothelin-1	EDN1	NC_000006.11	ATGGATTATTTTCCCGTGA	GGGAGTGTTGACCCAGATGA	231
Transforming growth factor-β1	Tgfb1	NC_000073.6	AATACGTCAGACATTCGGGAAGCA	GTCAATGTACAGCTGCCGTACACA	498
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	NC_000012.11	ATCAAATGGGGTGATGCTGGTGCTG	CAGGTTTCTCCAGGCGGCATGTCAG	505

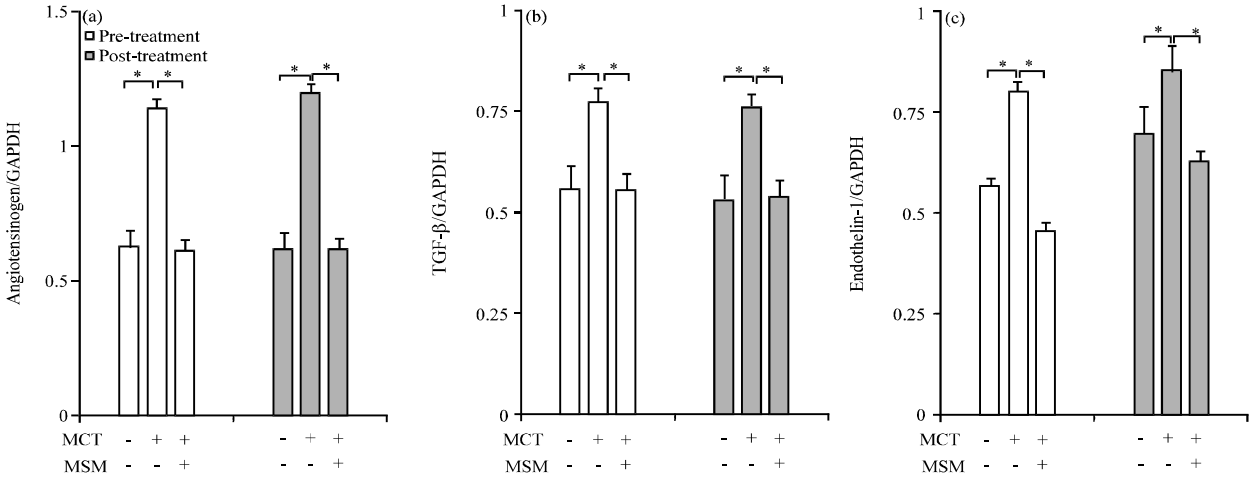

Fig. 1(a-c): Effects of methylsulfonylmethane on right ventricle (RV) hypertrophy related gene expression of (a) Angiotensinogen, (b) Transforming growth factor (TGF)-β1, (c) Endothelin-1 (ET-1), RV tissue samples were harvested 28 days after MCT administration and processed for RT-PCR analysis. The mean values±SD of four independent experiments are given. *$p<0.01$ via student's t-test, MCT: Monocrotaline, MSM: Methylsulfonylmethane

DISCUSSION

In this study, significant changes in angiotensinogen, ET-1 and TGF-β1 gene expression were observed in MCT-induced pulmonary hypertensive rats and these changes were reduced with MSM in two distinct experimental treatments for the first time. Consistent with these findings, several studies have reported that cardiovascular system of MCT-induced pulmonary hypertensive rats showed inflammatory alterations similar to those observed in human PAH (Akhavein *et al*., 2007). The effects of PAH on myocardium include hypertrophy and interstitial inflammatory cell infiltration. The RV tissue in the rat model of PAH was generally characterized by inflammatory reaction and showed alterations in contractile and regulatory protein expression (Bogaard *et al*., 2009). The expression changes observed in patients with chronic heart failure included markedly increased serum levels and myocyte expression of the pro-inflammatory cytokines Tumor Necrosis Factor (TNF)-α, interleukin (IL)-1 and IL-6 (Bogaard *et al*., 2009).

In this study, gene expression investigations showed elevated angiotensinogen, ET-1 and TGF-β1 in RV myocardium of PAH rats compared with control. A similar observation was previously made by Park *et al*. (2001) in another study of gene expression patterns in rats with RV hypertrophy. The Renin-angiotensin-aldosterone System (RAAS) is deregulated in PAH and suggested to potentially contribute to the pathogenesis of PAH. Both ET-1 and TGF-β1 are autocrine/paracrine growth factors and mediate ventricular dysfunction associated with elevated RAAS activity (Rosenkranz, 2004).

The mechanism for toxic effects of MCT on the lung has not yet been fully explained. MCT itself can affect adversely metabolic activity *in vitro* (Fu *et al*., 2004). However, as the animal model of PAH is induced using a single-dose over several weeks it seems unlikely that MCT contributes to such an effect *in vivo*. In animals exposed to agents that induce inflammation, MSM provides substantial protection against tissue destruction and chronic inflammation (Amirshahrokhi *et al*., 2011). In addition, MSM has been shown to protect against undesirable inflammatory state induced by exposure to PS, potent allergens and certain parasitic infections (Barrager *et al*., 2002; Kim *et al*., 2009). Comparable results were observed in the present study as angiotensinogen, ET-1 and TGF-β1 gene expression were increased in

MCT-induced pulmonary hypertensive rats and these alterations decreased following treatment with MSM.

In this study, ET-1 expression level was lower in the RV tissue homogenates of MSM-treated pulmonary hypertensive rats than in either the normotensive controls or the MCT-induced pulmonary hypertensive rats. Similarly, An elevated level of ET-1 in plasma and lung tissues of pulmonary arterial hypertension patients has been detected (Giaid *et al*., 1993) and the endothelin receptor antagonists have been suggested as effective treatments for this condition (Channick *et al*., 2001; Garjani *et al*., 1995). According to study results, MSM was found to prevent and reverse effectively ET-1 response in MCT-induced PAH. These results are consistent with previous reports showing antiinflammatory effects of MSM through inhibition of proinflammatory cytokine production (Kim *et al*., 2009).

Previous clinical and experimental reports have implicated increased oxidative stress as a mediator in the pathogenesis and the development of PAH (Farahmand *et al*., 2004; Li *et al*., 2002). Accordingly, antioxidant therapy has been effective in the treatment of RV dysfunction in PAH (Redout *et al*., 2010). RAAS and ET-1 are known as prooxidant agents that induce Reactive Oxygen Species (ROS) production through the activation of ROS-generating enzymes (Cheng *et al*., 2003; Touyz *et al*., 2004). The finding of increased RAAS and ET-1 in rats with MCT-induced pulmonary hypertension supports the presence of enhanced oxidative stress in the RV of PAH rats. The PAH-induced increase in RAAS and ET-1 were reverted by pre- and post-treatment of MSM, indicating an improvement in the oxidative status of the cells. Moreover, MSM has been shown to act directly as free radical scavenger which would further add to the efficiency of MSM as an antioxidant (Parcell, 2002).

In both pre- and post-treatment assessments, a decrease in transcriptional elevation of proinflammatory agents with MSM treatment was evident. No documented evidence is available regarding the effect of MSM on inflammation-related parameters in myocardial tissue of pulmonary hypertensive rats. Although, no mechanistic interpretation can be made at this point, the results obtained in the present study provide evidence for the first time that the MSM could limit inflammatory response in the RV following to pulmonary hypertension. However, the experimental model used in this study may not be analogous to the clinical situation and could not be used in a clinical setting. Future studies to clarify how MSM affects RV function will help in designing MSM therapy for the treatment of PAH.

CONCLUSION

In conclusion, these results suggest that MCT-induced PAH could induce harmful effects on the RV function, probably due to an increase in proinflammatory molecules and oxidative damage. In addition, it was demonstrated that MSM could exert protective antiinflammatory effects through the suppression of local RAAS along with associated growth-promoting factors, such as TGF-β1 and ET-1.

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