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Diethylcarbamazine: A potential treatment drug for pulmonary hypertension?

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ABSTRACT

The present study demonstrated the potential effects of diethylcarbamazine (DEC) on monocrotaline (MCT)-induced pulmonary hypertension. MCT solution (600 mg/kg) was administered once per week, and 50 mg/kg body weight of DEC for 28 days. Three C57Bl/6 male mice groups (n = 10) were studied: Control; MCT_{28}, and MCT_{28}/DEC. Echocardiography analysis was performed and lung tissues were collected for light microscopy (hematoxylin-eosin and Masson's trichrome staining), immunohistochemistry (αSMA, FADD, caspase 8, caspase 3, BAX, BCL2, cytochrome C and caspase 9) western blot (FADD, caspase 8, caspase 3, BAX, BCL2, cytochrome C and caspase 9) and qRt-PCR (COL-1α and αSMA). Echocardiography analysis demonstrated an increase in the pulmonary arterial blood flow gradient and velocity in the systole and RV area in the MCT28 group, while treatment with DEC resulted in a significant reduction in these parameters. Deposition of collagen fibers and αSMA staining around the pulmonary arteries was evident in the MCT28 group, while treatment with DEC reduced both. Western blot analysis revealed a decrease in BMPR2 in the MCT28 group, in contrast DEC treatment resulted in a significant increase in the level of BMPR2. DEC also significantly reduced the level of VEGF compared to the MCT28 group. Apoptosis extrinsic and intrinsic pathway markers were reduced in the MCT28 group. After treatment with DEC these levels returned to baseline. The results of this study indicate that DEC attenuates PH in an experimental monocrotaline-induced model by inhibiting a series of markers involved in cell proliferation/death.

1. Introduction

Pulmonary hypertension (PH) is a life-threatening progressive disorder associated with abnormally elevated pulmonary pressure and right heart failure. It is a disease of a complex etiology and pathobiology that results from interactions between the genetic make-up of an individual and the surrounding environment (VV et al., 2009; Rosenkranz, 2015).

The initial pathological events of the pulmonary artery dysregulation involve the proliferation of the smooth muscle cells. Several lines of evidence suggested that increased proliferation and decreased apoptosis of the pulmonary arterial smooth muscle cells can mediate thickening of the pulmonary vasculature, which would subsequently lead to a reduced inner diameter and increased pulmonary vascular resistance (Maron and Loscalzo, 2013).

Endothelial cell (EC) apoptosis and apoptosis resistance seems to play crucial roles in the development of plexiform lesions that feature in the pathogenesis of PH. Subsequently, EC injury associated with smooth muscle cell (SMC) proliferation facilitates vascular remodeling and eventually leads to narrowed vascular lumen, increased pulmonary vascular resistance, increased pulmonary arterial pressure, and right heart failure (Jin and Choi, 2012). The imbalance between cell death and proliferation occurs in every stage of pulmonary vascular remodeling and the pathogenesis of PH, and involves every cell type in the vasculature including, but not limited to ECs, SMCs, and fibroblasts (Guignabert and Dorfmuller, 2013).

Intriguingly, PH pathogenesis involves both inappropriate apoptosis and over-proliferation. Apoptosis in ECs, after initial environmental insults, has been recognized as one of the crucial events that trigger

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Table 1
Pulmonary artery blood velocity after DEC treatment: Velocity-time integral (VTI, cm) mean and peak gradient (mm Hg) and mean and peak velocity (mm/s) of blood flow in the pulmonary artery and area of the right ventricle (mm^2) were measured from Doppler waveforms acquired by ultrasound imaging. N = 5. Mean ± SD. $p < 0.05$ vs SHAM.

	VTI, cm	Mean gradient, mm Hg	Peak gradient, mm Hg	Mean velocity, mm/s	Peak velocity, mm/s	Area RV (mm^2)
SHAM	2.27 ± 0.4	0.29 ± 0.1	0.84 ± 0.22	268.0 ± 49.49	473.4 ± 86.49	11.40 ± 0.52
MCT_{28}	4.28 ± 0.35*	1.19 ± 0.22*	3.40 ± 0.69*	565.5 ± 42.39*	959.2 ± 67.87*	17.57 ± 0.97*
$MCT_{28/DEC}$	3.15 ± 0.24#	0.84 ± 0.15#	2.42 ± 0.45#	461.8 ± 40.48#	775.1 ± 75.80#	12.78 ± 1.63#

* $p < 0.05$ vs SHAM.
\# $p < 0.05$ vs MCT28.

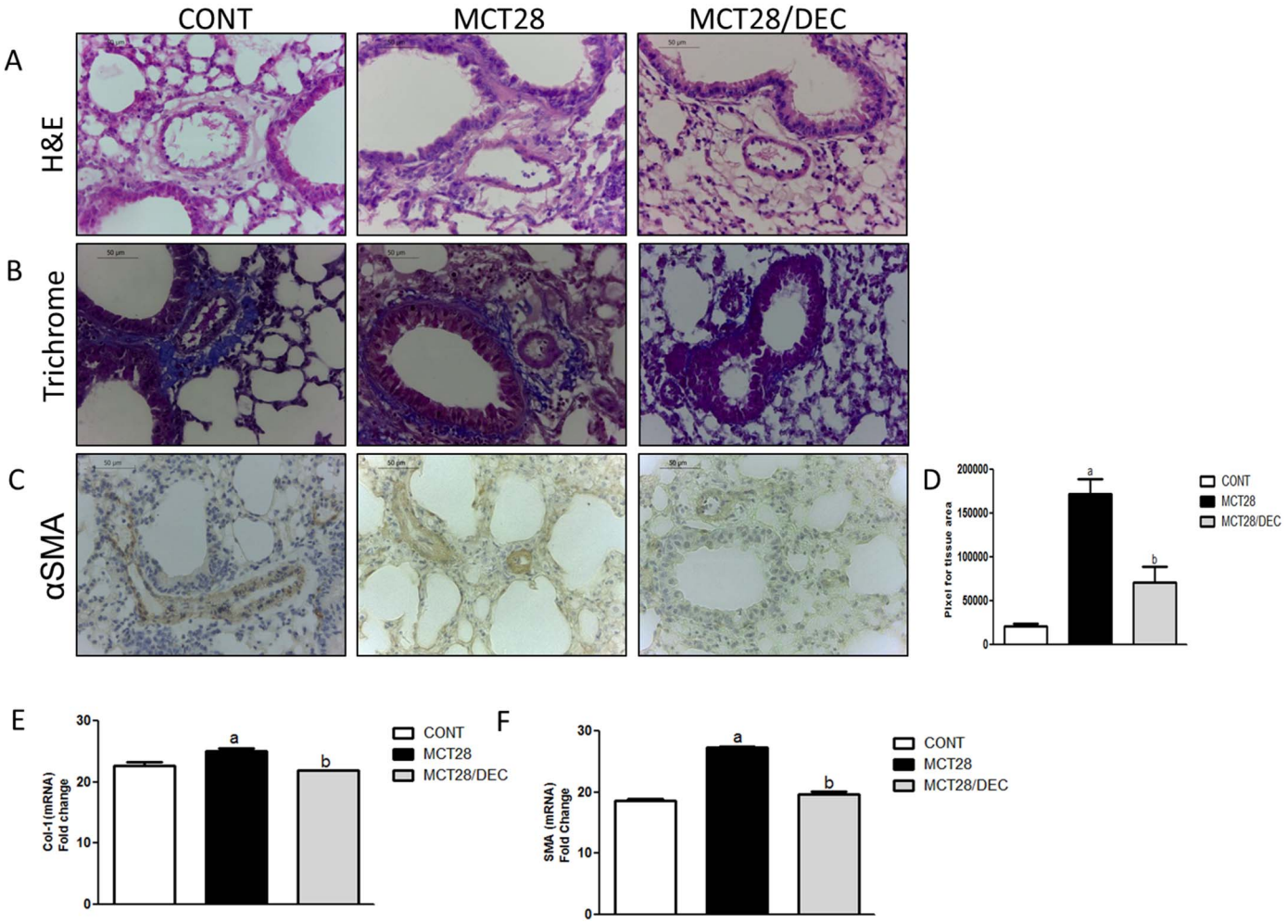

Fig. 1. Effect of DEC treatment on histological alterations in lung after MCT-induced pulmonary hypertension. A: Representative images of H & E staining demonstrating, interstitial edema with thickening of the septum alveolar, infiltrates of inflammatory cells with the presence of activated macrophages and plexiform lesions of pulmonary arteries and emphysema in MCT28 groups. Administration of DEC significantly attenuated the lung damage. Histological analysis of the control group did not reveal any morphological changes. B: Representative images of Masson's trichrome staining demonstrating significantly increased collagen deposition and MCT28 group. Administration of DEC significantly reduced collagen. C: Immunohistochemical localization of αSMA labeling around the arteries, vessels and bronchioles and MCT28 group. After treatment with DEC there was a reduction of immunostaining for α-SMA. D: Quantitative densitometry analysis (GIMP2 analyzed) α-SMA. Data is expressed as mean ± S.D. from n = 5 mice for each group. E e F: Relative expression of mRNA COL-1α and αSMA showing a significant increase in the MCT28 group. After treatment with DEC there was a reduction of expression COL-1α and αSMA respectively. Data is expressed as mean ± S.D. from n = 5 mice for each group. The letter at the top of the columns represents the groups where there was a significant difference with the cited group: a - CONT; b – MCT28; c- MCT28/DEC ($p < 0.05$).

pulmonary vascular remodeling in PH (Rajagopalan et al., 2009). Despite extensive studies, a detailed understanding of the cellular and molecular mechanisms involved in the transition from initial apoptosis to apoptosis resistant proliferation of ECs and SMCs has yet to be established (Jin and Choi, 2012).

The MCT model is considered by some to be a toxic model, as it is suggested that MCT rats die from hepatic veno-occlusive disease with liver failure, instead of right ventricle failure (Ruiter et al., 2013). MCT is known to cause pulmonary endothelial injury and pulmonary hypertension in humans and rats (Price et al., 2012; Schultze and Roth, 1998), but has little effect on mice (Molteni et al., 1989).

The drug diethylcarbamazine (DEC) is used throughout the world against lymphatic filariasis. However, in recent years many studies have described other pharmacological activities of DEC. It has been established that DEC interferes with the cyclooxygenase and lipoxygenase pathways, reducing eicosanoid production and acting as an anti-inflammatory drug (Peixoto and Silva, 2014). Furthermore, DEC inhibits the activation of NF-kB, suppressing target genes involved in pulmonary inflammatory