European Journal of Pharmacology 713 (2013) 89–93

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

Diminazene aceturate improves autonomic modulation in pulmonary hypertension

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ARTICLE INFO

Article history:
Received 22 August 2012
Received in revised form
12 April 2013
Accepted 18 April 2013
Available online 9 May 2013

Keywords:
ACE2
Diminazene
Pulmonary hypertension
Autonomic nervous system

ABSTRACT

We have previously demonstrated that diminazene aceturate (DIZE), a putative angiotensin 1–7 converting enzyme activator, protects rats from monocrotaline (MCT)-induced pulmonary hypertension (PH). The present study was conducted to determine if the beneficial effects of DIZE are associated with improvements in autonomic nervous system (ANS) modulation. PH was induced in male rats by a single subcutaneous injection of MCT (50 mg/kg). A subset of MCT rats were treated with DIZE (15 mg/kg/day) for a period of 21 days, after which the ANS modulation was evaluated by spectral and symbolic analysis of heart rate variability (HRV). MCT administration resulted in a significant ($P<0.001$) increase in the right ventricular systolic pressure (62 ± 14 mmHg) when compared with other experimental groups (Control: 26 ± 6; MCT+DIZE: 31 ± 7 mmHg), while DIZE treatment was able to decrease this pressure. Furthermore MCT-treated rats had significantly reduced total power of HRV than the controls. On the other hand, although not significant, a trend towards increased HRV was observed in the MCT+DIZE group (Control: 108 ± 47; MCT: 12 ± 8.86 and MCT+DIZE: 40 ± 14), suggesting an improvement of the cardiac autonomic modulation. This observation was further confirmed by the low-frequency/high-frequency index of spectral analysis (Control: 0.74 ± 0.62; MCT: 1.45 ± 0.78 and MCT+DIZE: 0.34 ± 0.49) which showed that DIZE treatment was able to recover the ANS imbalance observed in the MCT-induced pulmonary hypertensive rats. Collectively, our results demonstrate that MCT-induced PH is associated with a significant increase in sympathetic modulation and a decrease in HRV, which are markedly improved by DIZE treatment.

1. Introduction

Pulmonary hypertension (PH) is a fatal lung disease that negatively affects the patient's quality of life. Endothelial dysfunction, vasodilator/vasoconstrictor imbalance (Bradford et al., 2010) and disturbances in the autonomic nervous system (ANS) (Dimopoulos et al., 2009; Wensel et al., 2009) are some of the known factors that have been linked to the pathogenesis of PH. Although considerable efforts have been directed towards understanding the physiopathology of PH, management and cure of this disease still remains elusive.

Recent evidences suggest that PH may be associated with changes in heart rate (HR) dynamics. Studies involving pulmonary hypertensive patients have shown either an increase (Sanyal and Ono, 2002) or decrease (Hessel et al., 2006) in the HR mean value. On the other hand, it is widely recognized that PH is associated with increased sympathetic tone (Ciarka et al., 2007; Velez-Roa et al., 2004). Also, numerous studies have revealed a reduced spectral power of HR variability (HRV) (Sanyal and Ono, 2002) and increased low-frequency (LF) versus high-frequency (HF) spectral power ratio (LF/HF ratio) in pulmonary hypertensive subjects (Rosas-Peralta et al., 2006). These findings suggest that the cardiac ANS modulation is altered in PH, resulting in an increased sympathetic and decreased parasympathetic modulation. All of these changes can have profound effects on many organ systems leading to disease severity (Gillman et al., 1993).

Clinically, ANS function can be easily evaluated by performing HR measurements in the supine position or during postural changes (Petrofsky et al., 2009; Robinson et al., 1966). The ECG is a low cost diagnostic tool that provides essential information about Q–T series (Molnar et al., 1997). Moreover, the spectral and

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http://dx.doi.org/10.1016/j.ejphar.2013.04.017

symbolic analyses are simple methods that would allow the physicians and researchers to collect valuable information about ANS modulation. Measurement of the ANS function in PH patients may be clinically relevant, since an improvement in the ANS modulation could potentially lower the risk factors for adverse cardiovascular events (Gillman et al., 1993).

Experimental studies from our group have demonstrated that diminazene aceturate (DIZE) treatment significantly prevented the development of PH probably due to an increase in the vasoprotective axis of the lung renin-angiotensin system, decreased inflammatory cytokines, improved pulmonary vasoreactivity and enhanced cardiac functions. These beneficial effects were abolished by C-16, an ACE2 inhibitor. Furthermore, initiation of DIZE treatment after the induction of PH arrested disease progression (Shenoy et al., 2013). DIZE is structurally similar to the previously reported angiotensin-(1–7) converting enzyme (ACE2) activator compound XNT, (1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one), but has better physicochemical characteristics. Therefore, we hypothesized is that DIZE activates ACE2 to directly or indirectly modulate the cardiac ANS to produce its beneficial effects.

Despite the fact that autonomic nervous system imbalance is a common finding of many diseases, its treatment is still unmentioned. Moreover, once the right ventricular is the major determinants of the functional capacity and prognosis of the PH, the relevance of evaluating the ANS function in PH and the evidence that angiotensin converting enzyme 2 plays a key role in the physiopathology of this disease (Ferreira et al., 2009), our goal was to investigate whether DIZE, an ACE2 activator (Gjymishka et al., 2010), improves the autonomic modulation in the MCT-model of PH using the spectral and symbolic analysis methods. MCT-induced PH has been extensively used as a hemodynamically relevant animal model to study this disease. This model mimics several key aspects of the human disorder (Werchan et al., 1989).

We propose that DIZE treatment of the pulmonary hypertensive rats will not only decrease the sympathetic modulation and the sympathetic/parasympathetic ratio, but also reduce the ANS dysfunction and pulmonary pressure.

2. Materials and methods

2.1. Animals

Seven-week old male Sprague Dawley rats were housed in a temperature-controlled room (25 ± 1 °C) and were maintained on a 12:12-h light/dark cycle with free access to water and food. All procedures involving experimental animals were approved by the Institutional Animal Care and Use Committee at the University of Florida and complied with National Institutes of Health guidelines.

2.2. MCT-induced pulmonary hypertension and DIZE or saline treatment

PH was induced in two groups of rats by a single subcutaneous injection of MCT (50 mg/kg). One group was co-treated with DIZE subcutaneously (15 mg/kg/day) for 21 days. The other experimental group was age matched control rats that were injected daily with saline (500 μl, subcutaneously).

2.3. Cardiovascular evaluation

All the procedures and measurements were carried out on anesthetized rats. The right ventricular systolic pressure was measured using a silastic catheter inserted into the right descending jugular vein and advanced to the right ventricle, under the influence of a rodent anesthetic cocktail comprising of ketamine (70 mg/kg), xylazine (8 mg/kg), and acepromazine (1.5 mg/kg).

The data were recorded (4000 Hz/sample rate) after stabilization of the tracing using a liquid pressure transducer, which was interfaced to a PowerLab (AD Instruments, Colorado Springs, CO) signal transduction unit. The appearance of a waveform was used to confirm the positioning of the catheter in the right ventricle. Data were analyzed by using the Chart program supplied along with the PowerLab system.

2.4. Autonomic evaluation

After detecting the pulse intervals, the heart period was automatically calculated on a beat-to-beat basis as the time interval between two consecutive systolic peaks or pulse interval (PI). All detections were carefully checked to avoid erroneous or missed beats. Sequences of 200–250 beats were randomly chosen (Dabire et al., 1998; Dias da Silva et al., 2006) and if they presented non-stationary episodes, they were discarded and a new random selection was performed. Stationarity of the series was tested as previously reported (Porta et al., 2004). Frequency domain analysis of HRV was performed with an autoregressive algorithm (Porta et al., 2004) on the PI interval sequences (tachogram). The power spectral density was calculated for each time series.

In this study, two spectral components were considered: low frequency (LF), from 0.25 to 0.75 Hz; and high frequency (HF), from 0.75 to 3.00 Hz (Dabire et al., 1998; Dias da Silva et al., 2006; Murasato et al., 1998; Soares et al., 2004; Waki et al., 2006). The spectral components (ms^2) were expressed in absolute (a) and normalized (nu) units. Normalization consisted of dividing the power of a given spectral component by the total power, then multiplying the ratio by 100. In a reduced variability condition, linear methodologies have poor applicability (Montano et al., 1994). Thus, the non-linear approach provides a new perspective in the investigation of neural control of the cardiovascular system (Casali et al., 2008; Porta et al., 2008). Symbolic analysis is a powerful tool already validated to detect changes in autonomic modulation of cardiovascular variability (Guzzetti et al., 2005; Porta et al., 2007) that transforms a time series into short, three beat long patterns. The sequences are spread on six levels and all possible patterns are divided into four groups, consisting of patterns with: (i) no variations (0V, three symbols equal); (ii) one variation (1V, two symbols equal and one different); (iii) two like variations (2LV); and (iv) two unlike variations (2UV) (Guzzetti et al., 2005).

2.5. Statistical analysis

The method used for statistical analysis was the non-parametric variance test of Kruskal-Wallis, complemented by Dunn test. Data are presented as the mean $\pm$ S.D. and $P < 0.05$ was considered statistically significant.

3. Results

Animals treated with MCT exhibited a significant increase in right ventricle systolic pressure when compared to control rats (Table 1), while rats treated with DIZE showed a decrease in this pressure. Heart rate was not different among the experimental groups.

MCT treatment significantly decreased the total power of HRV, HFa and LFa versus control rats. Although not significant, there was a trend towards an increase in HFa, the parasympathetic component, when comparing MCT+DIZE with MCT group. However, DIZE treatment of MCT-treated rats resulted in a significant