Life Sciences 88 (2011) 493–503

Contents lists available at ScienceDirect

Life Sciences

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

Functional and molecular effects of imidazoline receptor activation in heart failure

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ARTICLE INFO

Article history:
Received 3 August 2010
Accepted 27 December 2010

Keywords:
Moxonidine
Hamster
Cardiomyopathy
Apoptosis
Fibrosis
Echocardiography

ABSTRACT

Aims: Heart failure is a progressive deterioration in heart function associated with overactivity of the sympathetic nervous system. The benefit of inhibition of sympathetic activity by moxonidine, a centrally acting imidazoline receptor agonist, was questioned based on the outcome of a failing clinical trial. The following studies measured cardiac structure and hemodynamics and mechanisms underlying moxonidine-induced changes, in cardiomyopathic hamsters, where the stage of the disease, dose, and compliance were controlled.

Main methods: Male BIO 14.6 hamsters (6 and 10 months old, with moderate and advanced heart failure, respectively) received moxonidine at 2 concentrations: low (2.4 mg/kg/day) and high (9.6 mg/kg/day), or vehicle, subcutaneously, for 1 month. Cardiac function was measured by echocardiography, plasma and hearts were collected for histological determination of fibrosis and apoptosis, as well as for measurement cytokines by Elisa and cardiac proteins by Western blotting.

Key findings: Compared to age-matched vehicle-treated BIO 14.6, moxonidine did not reduce blood pressure but significantly reduced heart rate and improved cardiac performance. Moxonidine exerted anti-apoptotic effect with differential inflammatory/anti-inflammatory responses that culminate in attenuated cardiac apoptosis and fibrosis and altered protein expression of collagen types. Some effects were observed regardless of treatment onset, although the changes were more significant in the younger group. Interestingly, moxonidine resulted in upregulation of cardiac imidazoline receptors.

Significance: These studies imply that in addition to centrally mediated sympathetic inhibition, the effects of moxonidine may, at least in part, be mediated by direct actions on the heart. Further investigation of imidazolines/imidazoline receptors in cardiovascular diseases is warranted.

Introduction

Chronic heart failure, a progressive deterioration in heart function, is characterized by an enhanced sympathetic tone, with increased circulating concentrations of catecholamines (Kaye et al. 1995) that initially tend to improve cardiac output and maintain tissue perfusion. However, long-term exposure to norepinephrine causes cardiomyocyte apoptosis, fibroblast proliferation, and cardiac fibrosis (Colucci et al. 2000). Ultimately, progressive cardiac myocyte loss, via apoptotic cell death, and the development of interstitial fibrosis contribute to progressive cardiac remodeling, arrhythmias, impaired cardiac function and development of heart failure (Foo et al. 2005; Kaye et al. 1995). Genetically engineered mice that are unable to synthesize norepinephrine exhibit less cardiac hypertrophy and preserved ventricular function after aortic banding, demonstrating the deleterious effects of sustained catecholamine excess (Esposito et al. 2002). The remodeling process is also associated with stimulated inflammatory cytokines (Fedak et al. 2005). Plasma levels of IL-1β, IL-6, and TNF-α are elevated in heart failure patients and have been linked to disease severity and poor prognosis (Aukrust et al. 1999; Rauchhaus et al. 2000). These cytokines may stimulate both anti- and pro-apoptotic pathways in the myocardium and exert negative inotropic effects, thus aggravating already impaired left ventricular function (Kubota et al. 2001; Yokoyama et al. 1993). Antagonism of excess exposure to norepinephrine within the failing heart by beta blockers (β-blocker) reduces elevated serum cytokine levels and improves cardiac function in patients with dilated cardiomyopathy (Kurum et al. 2007; Mayer et al. 2005). These

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doi:10.1016/j.lfs.2011.01.008

Table 1
Hemodynamic and physical parameters in normal (F1B) hamsters and in 6-month-old cardiomyopathic (BIO 14.6) hamsters after 4 weeks of treatment with vehicle or moxonidine at low (2.4 mg/kg/day) or high (9.6 mg/kg/day) concentrations.

Hemodynamic and physical parameters	F1B n = 10	BIO 14.6		
		Vehicle n = 12	Moxonidine low n = 12	Moxonidine high n = 8
BW, g	127 ± 3	113 ± 3*	113 ± 4*	109 ± 3*
MAP, mm Hg	108 ± 3	78 ± 4†	75 ± 4†	68 ± 4*
Lung/BW, mg/g	0.45 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.41 ± 0.01
RA/BW, mg/g	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.00
LA/BW, mg/g	0.17 ± 0.00	0.12 ± 0.02	0.09 ± 0.01	0.10 ± 0.01
RV/BW, mg/g	0.65 ± 0.03	0.75 ± 0.03†	0.73 ± 0.03†	0.83 ± 0.06†
LVM, mg	300 ± 22	359 ± 8*	355 ± 12*	369 ± 16*
LVM/tibia length, mg/mm	8.9 ± 0.7	10.7 ± 0.3	10.7 ± 0.3	11.2 ± 0.5
LVM/BW, mg/g	2.4 ± 0.1	3.1 ± 0.1*	3.1 ± 0.1*	3.1 ± 0.2*
LV-CSA, μm^2	450 ± 17	662 ± 28*	557 ± 45†‡	517 ± 12‡

BW, body weight; MAP, mean arterial pressure; RA, right atria; LA, left atria; LVM, left ventricular mass; *P<0.01; †P<0.05 vs. normal F1B; ‡P<0.05 vs. vehicle-treated hamsters.

findings form the cornerstone of the rationale for β-adrenoceptor antagonist (β-blocker) therapy of heart failure. However, treatment with a centrally acting sympatholytic imidazoline compound, moxonidine, did not show favorable results in a clinical trial on patients with advanced heart failure (Cohn et al. 2003; Swedberg et al. 2002), despite its established benefits in hypertensive patients (Ollivier and Christen 1994) and experimental animals (Amann et al. 1992; Paquette et al. 2008). This reflects incomplete understanding of the mechanisms of action of these drugs. Better understanding of the cellular and molecular mechanisms underpinning imidazoline compounds would improve treatment of heart failure. Therefore, the aim of these studies was to evaluate cardiac structure and performance in relation to altered molecular processes induced by moxonidine, with a focus on cardiac imidazoline receptors (El-Ayoubi et al. 2002) as well as cytokines and downstream mechanisms involved in myocardial remodeling. Because treatment benefit/adverse effects can be influenced by disease etiology, stage of heart failure, treatment dose, and/or compliance, moxonidine was given at 2 concentrations to cardiomyopathic BIO 14.6 hamsters, at 2 stages of heart failure: the hypertrophic (6 months old) and the overt heart failure (10 months old) stages.

Materials and methods

Experimental animals and drug treatment

Male BIO 14.6 hamsters were purchased from Bio Breeders (Fitchburg, MA, USA) at 6 and 10 months of age (n = 32, each). Age-matched F1B hamsters (n = 10, each) served as normal controls. Hamsters were housed in a pathogen free environment in a temperature and light controlled room, with food and water ad libitum and were allowed 1 week to acclimatize before experimentation. Procedures were performed following the approval of the Institutional Bioethics Committee, according to the Canadian Guidelines and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Cardiac structure and function were analyzed by transthoracic echocardiography (Reffelmann and Kloner 2003). Then, cardiomyopathic hamsters were randomly assigned to treatment with moxonidine at 2 concentrations: low (2.4 mg/kg/day) and high (9.6 mg/kg/day), or normal saline vehicle, for 4 weeks, via Alzet osmotic minipumps (model 2ML4, Alzet Corporation), implanted subcutaneously under isoflurane anesthesia, as previously described (Paquette et al. 2008; Mukaddam-Daher et al. 2009). After 4 weeks of treatment, echo-Doppler measurements were repeated; then, intracarotid blood pressure and heart rate were recorded. The hamsters were euthanized under isoflurane anesthesia. Blood was collected; hearts and lungs were excised, blotted dry, and weighed. To avoid circadian variability, all hemodynamic measurements and animal sacrifice were performed in the morning, between 8:00 and 12:00 a.m. Also, hamsters representing each group were investigated simultaneously.

The low and high concentrations of moxonidine (sub-hypotensive and antihypertensive, respectively) were chosen from previous studies showing regression of left ventricular hypertrophy in hypertensive rats (Paquette et al. 2008; Mukaddam-Daher et al. 2009).

Cardiac function

Echocardiography was performed under 2% isofluorane anesthesia, as previously described (Reffelmann and Kloner 2003; Mukaddam-Daher et al. 2009), using a Sonos 5500 (Philips, Andover,

Table 2
Hemodynamic and physical parameters in normal (F1B) hamsters and in 10-month-old cardiomyopathic (BIO 14.6) hamsters after 4 weeks of treatment with vehicle or moxonidine at low (2.4 mg/kg/day) or high (9.6 mg/kg/day) concentrations.

Hemodynamic and physical parameters	F1B n = 6	BIO 14.6		
		Vehicle n = 12	Moxonidine low n = 12	Moxonidine high n = 6
BW, g	140 ± 2	110 ± 3*	115 ± 3*	117 ± 3*
MAP, mm Hg	97 ± 4	57 ± 4*	56 ± 4*	51 ± 4*
Lung/BW, mg/g	0.48 ± 0.02	0.49 ± 0.03	0.48 ± 0.02	0.44 ± 0.02
RA/BW, mg/g	0.06 ± 0.00	0.21 ± 0.05	0.21 ± 0.03	0.16 ± 0.02
LA/BW, mg/g	0.07 ± 0.00	0.17 ± 0.03†	0.2 ± 0.03†	0.14 ± 0.03
RV/BW, mg/g	0.67 ± 0.04	1.02 ± 0.05*	0.89 ± 0.06*	1.06 ± 0.04*
LVM, mg	358 ± 1	390 ± 20	391 ± 20	410 ± 10
LVM/tibia length, mg/mm	10.4 ± 0.1	11.8 ± 0.4*	11.7 ± 0.4*	12.4 ± 0.3*
LVM/BW, mg/g	2.6 ± 0.1	3.6 ± 0.2*	3.4 ± 0.1*	3.5 ± 0.1*
LV-CSA, μm^2	463 ± 3	733 ± 56*	588 ± 22‡	587 ± 21‡

BW, body weight; MAP, mean arterial pressure; RA, right atria; LA, left atria; LVM, left ventricular mass; *P<0.01, †P<0.05 vs. normal F1B; ‡P<0.05 vs. vehicle-treated hamsters.