Life Sciences 92 (2013) 719–726

Contents lists available at SciVerse ScienceDirect

Life Sciences

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

Cystamine ameliorates ventricular hypertrophy associated with modulation of IL-6-mediated signaling in lupus-prone mice

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ARTICLE INFO

Article history:
Received 15 October 2012
Accepted 25 January 2013

Keywords:
Cystamine
Lupus-prone mice
Left ventricular hypertrophy
IL-6
MEK5

ABSTRACT

Aims: The aim of this study is to investigate the protective effects of cystamine on lupus-associated cardiac hypertrophy.
Main methods: Balb/c and lupus-prone NZB/W-F1 mice were individually randomized into sham group (saline, n = 16) and cystamine group (n = 16). Mice received saline or cystamine (100 mmol in 100 μL saline) by daily intraperitoneal injection for 2 consecutive weeks. Morphological, histological, and biochemical alterations were investigated.
Key findings: Cystamine decreased both left ventricular (LV) mass and LV mass/tissue-to-blood ratio (TBR) in NZB/W-F1 mice ($p<0.05$), whereas slight effects were observed in Balb/c mice. Moreover, cystamine reduced levels of atrial natriuretic peptide (ANP), C-reactive protein (CRP), heart type-fatty acid binding protein (h-FABP), creatine kinase-MB (CK-MB) and IL-6 in LV tissues of NZB/W-F1 mice ($p<0.05$). Additionally, in LV tissues of NZB/W-F1 mice, suppression of hypertrophic signaling mediated by IL-6 in response to administration of cystamine was revealed, including phosphorylation of MEK5, ERK5, c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38) ($p<0.05$).
Significance: Cystamine alleviated LV hypertrophy in NZB/W-F1 mice as a result of decrease in hypertrophic mediators and suppression of IL-6 mediated hypertrophic signaling.

Introduction

Prevalence of left ventricular (LV) hypertrophy and dysfunction is significant in patients with systemic lupus erythematosus (SLE) (Cervera et al., 1992; Crozier et al., 1990; Fujimoto et al., 1994; Sturfelt et al., 1992). Various etiological mechanisms underlying cardiac abnormalities associated with SLE have been postulated and investigated. Previous studies have reported that cardiac tissue injury caused by autoantibodies, immune complexes, complement activation and inflammation-related arterial stiffening is attributable to high cardiac morbidity and mortality in SLE patients (Kyttaris et al., 2005; Leung et al., 1990; Pieretti et al., 2007).

Elevated levels of antibodies against anionic phospholipids such as cardiolipin and lupus anticoagulants have been reported for their association with fetal loss, autoimmune thrombocytopenia and thrombosis (Wilson et al., 1999). Prevalence of arterial stiffness in SLE patients is correlated with chronic inflammation, suggesting that ventricular remodeling and subsequent hypertrophy is attributable to increased inflammation-mediated vascular stiffness (Roman et al., 2003). Moreover, preclinical coronary artery disease is considered as a contributing factor to LV structural changes, given the premature development of atherosclerosis in SLE patients (Manzi et al., 1997; Roman et al., 2003).

Cystamine is known as an inhibitor of transglutaminase 2 (TG2) and tissue transglutaminase (tTG) through formation of mixed disulfide (Jeitner et al., 2005; Lorand and Conrad, 1984). Recent studies have reported that cystamine exerts beneficial effects on various diseases via suppressing production of inflammatory mediators including tumor necrosis factor-alpha (TNF-α) and IL-6 (Elli et al., 2011;

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0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2013.01.027

Hsu et al., 2008a). Some animal studies also demonstrate that cystamine improves neuroprotection, prolongs cell survival and reduces abnormal cell movements in the transgenic model of Huntington disease (Karpuj et al., 2002). Our previous studies have also shown that cystamine treatment alleviated liver, brain and cardiac tissue injuries via inhibition of apoptosis in lupus-prone mice (Hsu et al., 2008a, 2008b; Tzang et al., 2012). Furthermore, cystamine has been demonstrated to have therapeutic potential for neuropsychiatric SLE through attenuation of inflammatory responses (Wang et al., 2009). However, whether cystamine treatment has beneficial effects on lupus-associated cardiac hypertrophy remains sketchy.

In the present study, we aimed to investigate the effects of cystamine on lupus-associated left ventricular (LV) hypertrophy by using a lupus-prone NZB/W-F1 animal model. In parallel, the effects of cystamine on LV tissues of Balb/c mice were also determined and referred as sham control. Hypertrophy was assessed by mass change. Tissue morphology was examined by histochemical staining and Masson's trichrome staining. We examined the factors associated with hypertrophy, cardiomyopathy and cardiovascular diseases, including atrial natriuretic peptide (ANP) (Yasuda et al., 1993), creatine kinase-MB (CK-MB) (Lygate et al., 2007), C-reactive protein (CRP) (Iwashima et al., 2007) and heart type-fatty acid binding protein (h-FABP) (Naraoka et al., 2005), as well as expression and phosphorylation of protein by immunoblotting incorporating specific antibodies and densitometric analysis.

Materials and methods

Lupus-prone mouse models and cystamine treatment

Control female mice Balb/c and lupus-prone mice NZB/W-F1 were purchased from the Animal Center, National Taiwan University, Taiwan and were maintained under supervision of the Institutional Animal Care and Use Committee at Chung Shan Medical University. Lupus index proteinuria was monitored biweekly using Albustix test strips (Bayer Diagnostics Ltd, Hong Kong, China) as described previously (Hsu et al., 2008a, 2008b). Balb/c and NZB/W-F1 mice aged 26 weeks were randomly divided into two groups (eight mice for each group). Cystamine administration was performed by daily intraperitoneal injection (100 μL of 10 mM cystamine) (Sigma, Saint Louis, MO, USA) in normal saline or 100 μL of normal saline for 14 days as described previously (Karpuj et al., 2002). After the 14-day administration, the treated mice were sacrificed by CO_2 asphyxiation and the LV cardiac tissues were dissected and stored at −80 °C until their use.

Characteristics of hearts

All mice were weighed by digital weight scales and decapitated. The hearts were surgically excised and rinsed with distilled water. The left ventricle was isolated and weighed. To normalize the whole heart weight, the right tibias were also separated and tibia length (TBL) was measured by the electronic digital vernier caliper as previously described (Lee et al., 2007). The ratios of the whole heart mass (WHM) to TBL, the LV mass (LVM) to the TBL, and the LVM to WHM were calculated.

Histochemical staining and Masson trichrome staining

Tissues were fixed with 10% formol saline. Each slice was divided into eight contiguous LV tissue blocks and prepared for routine processing to paraffin wax. Two consecutive tissue sections (5 μm) were performed for each individual block. Two slides were respectively stained with hematoxylin–eosin and Masson's trichrome stain, which stained collagen blue and myocytes red. Images were obtained using Carl Zeiss Axiophot microscopes (Carl Zeiss AG, Oberkochen, Germany).

Crude protein extraction

LV tissues were excised and washed with ice-cold PBS followed by homogenization into ice-cold lysis buffer A [PBS, pH 7.2; containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/mL aprotinin, 1 mg/mL leupeptin, 1 mM NaF and 1 mM $Na_4P_2O_7$] at a ratio of 200 mg tissue to 1 mL lysis buffer A. After 15 min incubation with gentle agitation, the extracts were centrifuged at 12,000 *g* for 30 min to remove the pellets, and the supernatant was collected and stored at −80 °C for further analyses.

For nuclear protein extraction, PBS-washed LV tissues were homogenized into lysis buffer B (10 mM HEPES, pH 7.6; containing 0.05%v/v Igepal CA-630, 15 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM PMSF, 1 mM Na_3VO_4, 50 mM NaF, 10 μg/mL leupeptin, and 10 μg/mL aprotinin) for 10 min. The homogenates were collected by a centrifugation at 2500 *g* for 10 min at 4 °C. The pellets containing nuclei were washed with PBS, resuspended in nuclear extraction buffer (25 mM HEPES, pH 7.6; containing 0.1%v/v Igepal CA-630, 1 M KCl, 0.1 mM EDTA, 1 mM PMSF, 1 mM Na_3VO_4, 2 mM NaF, 10 μg/mL leupeptin, and 10 μg/mL aprotinin), and centrifuged at 10,000 *g* for 15 min at 4 °C. The resulting supernatants were collected, namely nuclear fraction. Concentration of crude protein was determined by using the BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA).

Gel electrophoresis and immunoblot

Twenty micrograms of crude protein was subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel and electrophoresed at 140 V for 1.5 h. After electrophoresis, the proteins were transferred onto a nitrocellulose membrane (Amersham Hybond-C Extra Supported, 0.45 μm; GE-Health, USA) by Bio-Rad scientific instruments transphor unit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The blots were blocked with 2%w/v bovine serum albumin in PBS followed by 1 h incubation with 1000-fold diluted primary antibodies [ANP, CK-MB, CRP, h-FABP, IL-6, phosphorylated-extracellular signal-regulated kinase 5 (p-ERK5), phosphorylated-mitogen-activated protein kinase kinase 5 (MEK5), phosphorylated-p38 mitogen-activated protein kinase (p-p38 MAPK), phosphorylated-c-Jun N-terminal kinase (p-JNK) or c-Jun (Santa Cruz Biotechnology, Inc., Santa Cruz, CA)]. After their being washed with PBS, the reacted blots were incubated with 2000-fold diluted peroxidase-conjugated secondary antibodies (BioSource International Inc., Camarillo, CA, USA). Antigen–antibody complexes were revealed using ECL chemiluminescence (Supersignal West Dura HRP Detection Kit, Pierce Biotechnology, Inc., Rockford, IL, USA). The photographic density was quantitated by an image analysis system (Fujifilm, Tokyo, Japan) and the density of α-tubulin (α-TN) was used as internal control.

Statistical analysis

Data were presented as means ± SD of three independent experiments. Statistical significance analysis was determined by using one-way ANOVA followed by Dunnett for multiple comparisons with the control. Differences were considered significant for $p<0.05$.

Results

Effect of cystamine treatment on ventricular architecture changes

To examine whether cystamine ameliorates LV hypertrophy in NZB/W-F1 mice, physical parameters including body weight (BW, g), whole heart mass (WHM, g), LV mass (LVM, g) and tibia length (TBL, mm) were measured. Our results revealed that cystamine treatment significantly decreased LVM and LVM/TBL ($p<0.05$) but it did not affect BW, WHM, WHM/TBL and LVM/WHM (Table 1).