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From Cell Biology to Tissue Engineering

Lisinopril has a cardio-protective effect on experimental acute autoimmune myocarditis in rats

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Summary. The present study investigated the effect of lisinopril on experimental autoimmune myocarditis (EAM) in rats, a histologically similar model to human acute myocarditis.

Animals and methods. Twenty four, six week-old male Wistar rats were randomly allocated into 4 groups of 6 rats each. Group I received no treatment. Group II received lisinopril at a dose of 15 mg/kg/day suspended in 1 ml of 2% gum acacia daily, from day 1 to day 21. To induce myocarditis, animals of groups III and IV were injected by 1 mg of porcine cardiac myosin on days 1 and 8. In addition, animals of group IV received lisinopril in gum acacia daily, from day 1 to day 21. All rats were sacrificed on day 21. Serum levels of creatine phosphokinase, troponin-T, tumor necrosis factor-α and interleukin-6 were estimated. Hearts were processed for histopathological, as well as immunohistochemical study for thioredoxin (TRX) immunoreactivity.

Results. The wall of hearts from rats of myocarditis-lisinopril group showed mild focal myocarditis and a significant decrease of the mean percentage of pyknotic nuclei in cardiomyocytes, coincident with a significant decrease in serum biomarkers levels and TRX immunoreactivity, compared to myocarditis group.

Conclusion. The present study suggested a cardio-protective effect of lisinopril on acute EAM in rats, probably through a mechanism related to its suppressive effect on angiotensin II formation.

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Introduction

Myocarditis is a major cause of sudden unexpected death in patients less than 40 years of age. In 30% of cases, myocarditis can give rise to dilated cardiomyopathy (DCM) with progression to heart failure (Liu and Mason, 2001). Myocarditis is defined as myocardial inflammation associated with edema, cellular infiltration, apoptosis and necrosis of cardiomyocytes (Rosenstein et al., 2000). It is likely a complex disease and its etiology has been associated with various infections, systemic diseases, drugs, and toxins. There are two types of myocarditis; lymphocytic and giant cell myocarditis (Feldman and McNamara, 2000). In acute myocarditis, an imbalance of the reactive oxygen species (ROS) and/or an inadequate cellular antioxidant defense mechanism may play a key role in myocardial injury. The increase in ROS is able to induce severe cardiovascular dysfunction by their direct attack on intercellular biomolecules such as contractile molecules or ion channels. Also, the imbalance of intracellular oxido-reductive state (redox) may lead to the activation of stress-sensitive signaling pathways, increasing apoptosis and potentially contributing to the development of heart failure (Yuan et al., 2003).

Experimental autoimmune myocarditis (EAM) induced in rats is an animal model of human myocarditis and post-myocarditis DCM. In autoimmune situations, major histocompatibility complex (MHC) class II molecules present autoantigens to T-helper lymphocytes

and direct the subsequent cardiac injury (Li et al., 2004). The cytokines interlukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are classified as pro-inflammatory cytokines and are expressed in cardiac tissue during the acute phase of EAM (Mann, 2002). Serum levels of troponin-T and Serum MB isoenzyme of creatinine kinase (CPK-mb), which are considered as strong markers for myocardial cell injury, are also increased (Bleuel et al., 1995).

Cells have developed elaborate defense systems against oxidative stresses. Among those, a pivotal role of the thiol-mediated redox systems has been recognized. Thioredoxin (TRX) is a redox regulatory protein that protects cells from various stresses by reducing activity of oxidized thiol groups on proteins. It was reported that TRX is up-regulated by acute inflammatory stimuli and may play an important protective role in the pathogenesis and development of myocarditis (Li et al., 2004).

There is some evidence for the adverse cardiac effects triggered by redox cycling of reactive oxygen species (ROS), generated in part by an NADPH oxidase dependent pathway. Reports also add the role of angiotensin II (Ang II) in triggering the oxidative stress which causes an increase in the levels of NADPH oxidase subunits in rat EAM (Seko, 2006). Angiotensin converting enzyme (ACE) inhibitors are drugs that block the formation of angiotensin II from angiotensin I, and cause the breakdown of bradykinin by binding to ACE through its peptide-binding pocket. It was found that ACE inhibitors reduce myocardial inflammation and necrosis in murine viral myocarditis (Reyes et al., 1998). They also reduce left ventricular mass when left ventricular hypertrophy is present in spontaneous hypertension (Fernandez et al., 1988). A large number of ACE inhibitors are commonly prescribed today, including captopril, enalapril and lisinopril (Daniels et al., 2007). Lisinopril is the lysine analogue of enalaprilat, the active ACE inhibitor metabolite of enalapril. In contrast to other ACE inhibitors, lisinopril requires no metabolic transformation to become active. After oral administration, about 30% of the substance is absorbed, and peak serum concentrations are reached within 6-8 hours (Fernandez et al., 1988).

In the present study, we investigated the effect of lisinopril on the acute stage of EAM in rats by testing the possible changes in cardiac injury markers and pro-inflammatory cytokines serum levels, as well as in TRX immunoreactivity, following lisinopril treatment.

Material and Methods

Experimental Animals

Twenty four, six week-old male Wistar rats, weighing about 200-250 g, were housed under standard environmental conditions with free access to standard pelleted rat chow and tap water. The study followed the International Guidelines for the Care and Use of Laboratory Animals for experimental procedures.

Study design

Animals were randomly allocated into 4 groups of 6 rats each:

1. Group I (untreated control group): rats received neither lisinopril nor myosin.
2. Group II (control-lisinopril group): rats received lisinopril suspended in 1 ml of 2% gum acacia, orally, at the dose of 15 mg/kg/day (Dubey et al., 2003) from day 1 to day 21.
3. Group III (myocarditis group): rats were immunized with porcine cardiac myosin (Shioji et al., 2000; Futamatsu et al., 2003; Matsui et al., 2004; Otsuka et al., 2009).
4. Group IV (myocarditis-lisinopril group): rats were immunized with porcine cardiac myosin and received lisinopril suspended in 1 ml of 2% gum acacia, orally, at the dose of 15 g/kg/day from day 1 to day 21.

All animals were euthanized on day 22. Truncal blood was collected and serum was separated from each animal for biochemical assay. The hearts were excised and processed for histological and immunohistochemical studies.

Induction of EAM by Immunization

The rats were injected subcutaneously in the back on days 1 and 8 with 1 mg (0.1 ml) purified porcine cardiac myosin (10 mg/ml) (M 0531-10MG, Myosin, calcium activated, from porcine heart. Buffered aqueous glycerol solution 1.05 ml; 9.5 mg protein/ml, Sigma-Aldrich Chemical Company, St. Louis, MO, USA) emulsified in an equal volume of complete Freund's adjuvant supplemented with mycobacterium tuberculosis H37Ra (Difco lab., Detroit, MI, USA). Control rats were injected with complete Freund's adjuvant alone.

Histology

The excised hearts were fixed in 10% buffered formalin at 4°C for 24 hours and processed to prepare transverse mid-ventricular 5-μm-thick paraffin sections. These sections were stained with hematoxylin and eosin (H&E). Myocarditis was determined by identifying both mononuclear cellular infiltration and cardiomyocyte necrosis (Liu et al., 2004). The percentage of myocarditis was assessed semiquantitatively (Liu et al., 2004; Li et al., 2005) according to the scale: 0, normal; 1, mild (0 to 5% of heart cross-section involved); 2, moderate (5 to 10% of cross-section involved); 3, marked (10 to 25% of cross-section involved); and 4, severe (more than 25% of cross-section involved).

Immunohistochemistry

Immunostaining of mid-ventricular paraffin sections of the heart for detection of thioredoxin (Shioji et al., 2000) was performed using streptavidin-biotinylated horseradish peroxidase (S-ABC) method (Novalink Max

Polymer detection system, Novocastra). The procedure involved the following steps: endogenous peroxidase activity was inhibited by 3% H_2O_2 in distilled water for 5 minutes, and then the sections were washed in Tris buffered saline (Sigma, T 5030-100 TAB, pH 7.6) for 10 minutes. Non-specific binding of antibodies was blocked by incubation with protein block (Novocastra) for 5 minutes. Sections were incubated with rabbit polyclonal anti-rat thioredoxin (TRX) (Abcam, 100 μg, 0.4 mg/ml) diluted 1:200 for 1 hour at room temperature. Sections were washed in Tris buffer for 3 times each for 3 minutes then incubated with biotinylated antirabbit IgG (Novocastra) for 30 minutes. This was followed by washing in Tris buffer 3 times, each for 3 minutes, and then incubated with Novolink polymer (Novocastra) for 30 minutes. Then, sections were washed in Tris buffer 3 times, each for 3 minutes. Peroxidase was detected with a working solution of Diaminobenzedine (DAB) substrate (Novocastra) for 10 minutes. Finally, sections were washed in distilled water for 10 minutes, nuclei were stained with Mayer's hematoxylin, and sections were mounted in DPX. For negative control sections, the same procedure was followed with omission of incubation in rabbit polyclonal anti-rat TRX. Thioredoxin immunoreactivity was assessed semi-quantitatively according to the scale: +, weak; ++, medium; and +++, strong.

Serum biochemical analysis

Determination of Cardiac Injury Marker levels: Serum troponin-T concentration was determined using a Siemens Dimension Xpand® Plus instrument (IL, USA). Serum CPK-mb level was measured with an auto-

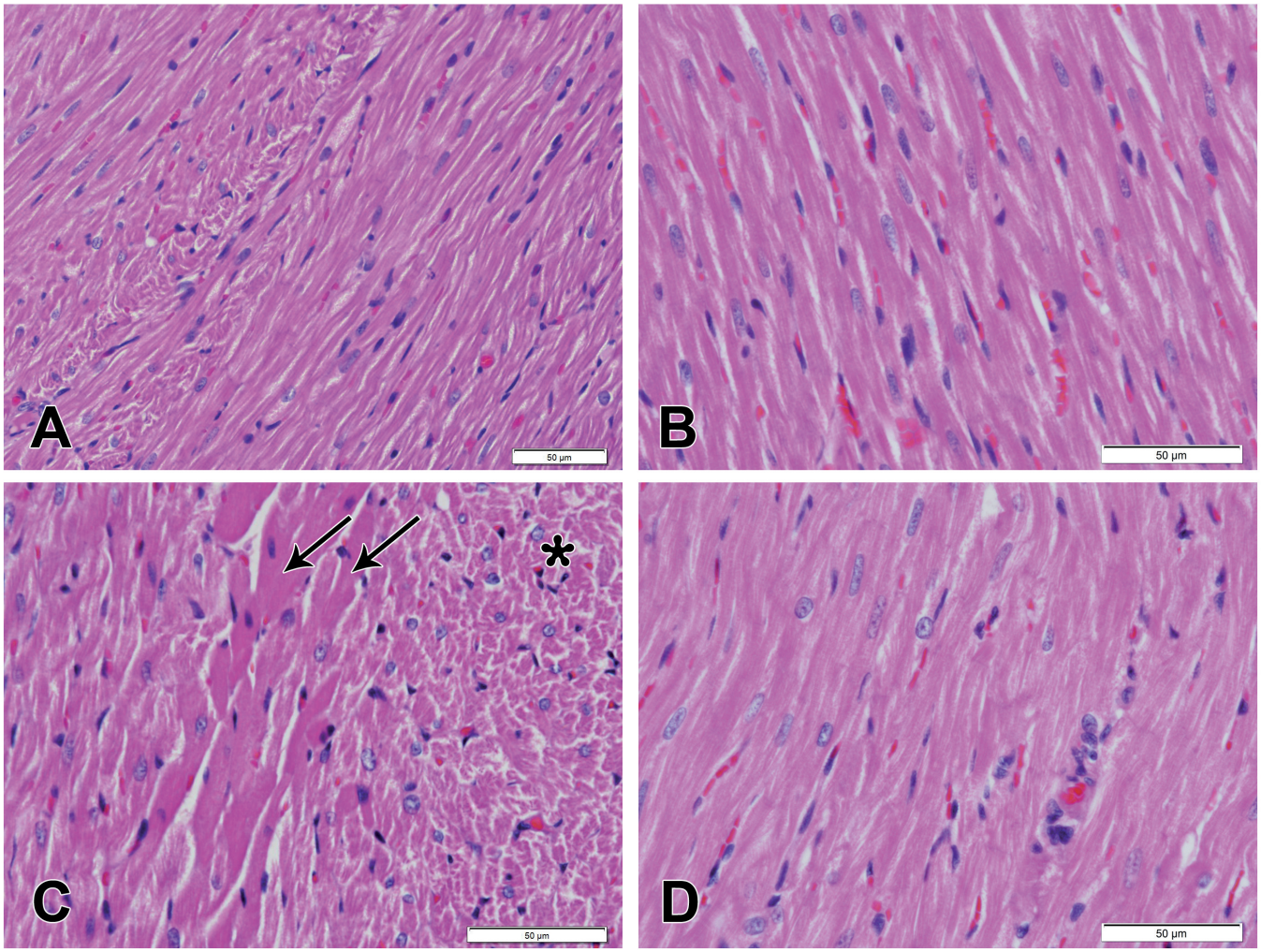

Fig. 1. Photomicrographs of heart sections stained with H&E. Heart of a rat from untreated control group **(A)** and heart of a rat from control-lisinopril group **(B)**, showing normal architecture of cardiomyocytes and interstitium. **C.** Heart of a rat from myocarditis group showing swollen cardiomyocytes with pyknotic nuclei and deeply acidophilic sarcoplasm (arrows). The interstitium is infiltrated with mononuclear inflammatory cells (asterisk) with vascular congestion. **D.** Heart of a rat from myocarditis-lisinopril group showing apparently normal cardiomyocytes and interstitium. Scale bars: 50 μm.

analyzer (ILab-300 bioMérieux Diagnostics, Milan, Italy).

Determination of Pro-inflammatory Cytokine levels: The concentration of TNF-α in serum was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits following the instructions supplied by the manufacturer (DuoSet kits, R&D Systems; Minneapolis, MN, USA). The results were shown as pg of cytokine per ml. IL-6 levels were measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) with an analytical CV of 6.3% and a detection level of 0.04 pg/ml (Blauwet and Cooper, 2010).

Statistical analysis

Data collected were subjected to statistical analysis using IBM SPSS Statistics version 22 software. The homogeneity of the obtained numerical data was first checked with Levene test and the homogeneity of variance assumption was met. Analysis of variance (ANOVA) was used for an overall comparison between the study groups followed by Bonferroni as a post-hoc test for pairwise comparisons. Differences were considered significant when p was equal to or less than 0.05.

Results

Animals of all groups survived all through the experiment till the time of sacrifice. The body weight of animals of myocarditis group decreased, while the body weight of the animals of the other groups markedly increased. The daily food and water consumption was reduced in animals of myocarditis group compared to the animals of the other groups. There was a marked decrease in the movements and activities of animals of myocarditis group compared to the animals of the other groups.

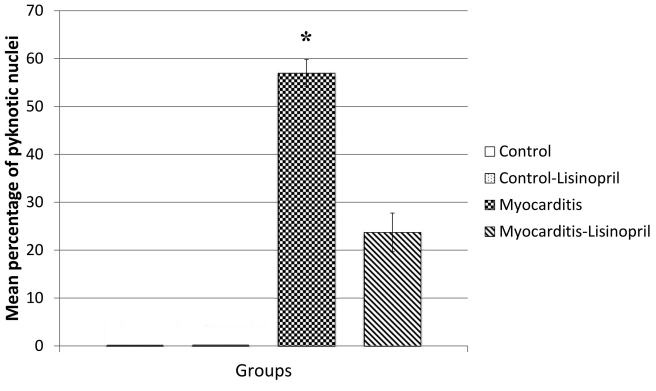

Fig. 2. Percentage of pyknotic nuclei in cardiomyocytes. *significant difference (p≤0.05) compared to any of the other groups.

Histological Study

H&E stained sections of the wall of the heart at the mid-ventricular level, from all rats of both untreated control and control-lisinopril groups, showed normal architecture of cardiomyocytes and interstitium (Fig. 1A,B). There were no pyknotic nuclei in the cardiomyocytes of either groups (Fig. 2). H&E-stained sections of the wall of the heart from myocarditis group showed evidence of severe myocarditis: swollen cardiomyocytes with pyknotic nuclei and deeply acidophilic sarcoplasm. The interstitium showed edema, vascular congestion, and mononuclear cellular infiltration. No fibrosis was detected (Fig. 1C). The mean percentage of pyknotic nuclei was 56.992±2.816 of the total number of cardiomyocytes (Fig 2). H&E-stained sections from myocarditis-lisinopril group showed normal architecture in almost all cardiomyocytes and interstitium (Fig. 1D). Mild focal myocarditis (less than 5% of the total areas of the examined sections) was evident in the sections of this group. The mean percentage of pyknotic nuclei of myocardiocytes showed a significant decrease to 23.703±4.049 compared to myocarditis group (Fig. 2).

Immunohistochemical study

Immunohistochemistry was performed to determine the histological localization of TRX in the heart. Sections of the heart from untreated control and control-lisinopril groups showed medium (++) immunoreactivity for TRX in the sarcoplasm of cardiomyocytes, while their nuclei were not immunostained (Fig. 3A,B). Sections of the heart from myocarditis group showed strong (+++) immunoreactivity for TRX in the sarcoplasm and almost all nuclei of cardiomyocytes (Fig. 3C). Sections of the heart from myocarditis-lisinopril group showed medium (++) immunoreactivity for TRX similar to that of untreated control and control-lisinopril groups (Fig. 3D).

Biochemical study

Serum cardiac injury markers, namely troponin-T and CK-MB groups were significantly increased in myocarditis group compared to both untreated control and control-lisinopril groups. The intake of lisinopril in myocarditis-lisinopril group significantly down-modulated the deviation of these markers compared to myocarditis group. However, the levels were still significantly higher than those of untreated control and control-lisinopril groups. No significant differences were observed between untreated control and control-lisinopril groups (Fig. 4).

Serum pro-inflammatory biomarker levels (TNF-α and IL-6) were elevated significantly in sera of myocarditis group as compared to untreated control and control-lisinopril groups. The administration of lisinopril to rats with myocarditis significantly reduced the pro-

inflammatory cytokine levels compared to myocarditis group. However, their levels were still significantly higher than those of untreated control and control-lisinopril groups. No significant differences were observed between the untreated control and control-lisinopril groups (Fig. 5).

Discussion

In the present study, we investigated the effect of lisinopril on acute EAM in rats. To the best of our knowledge, no study has systematically examined such effect. Our findings suggest that lisinopril administration ameliorates acute EAM in rats.

EAM in rats is characterized by severe myocardial damage and the appearance of multinucleated giant cells. It is used as an animal model of human giant-cell myocarditis (Kodama and Izumi, 1991). Previous studies reported that EAM was induced by T-cell activation and consisted of two stages. The first stage was characterized by a focal inflammatory process with macrophage infiltration and lasted up to day 14. The second stage was characterized by a more diffuse inflammatory process consisting of both macrophages and CD4-positive T-cell infiltration and lasted up to day 21 (Kodama et al., 1992).

Angiotensin II, the principal effector peptide of the renin–angiotensin system (RAS), has been reported to induce both immune and inflammatory responses in various cardiac disease conditions, including

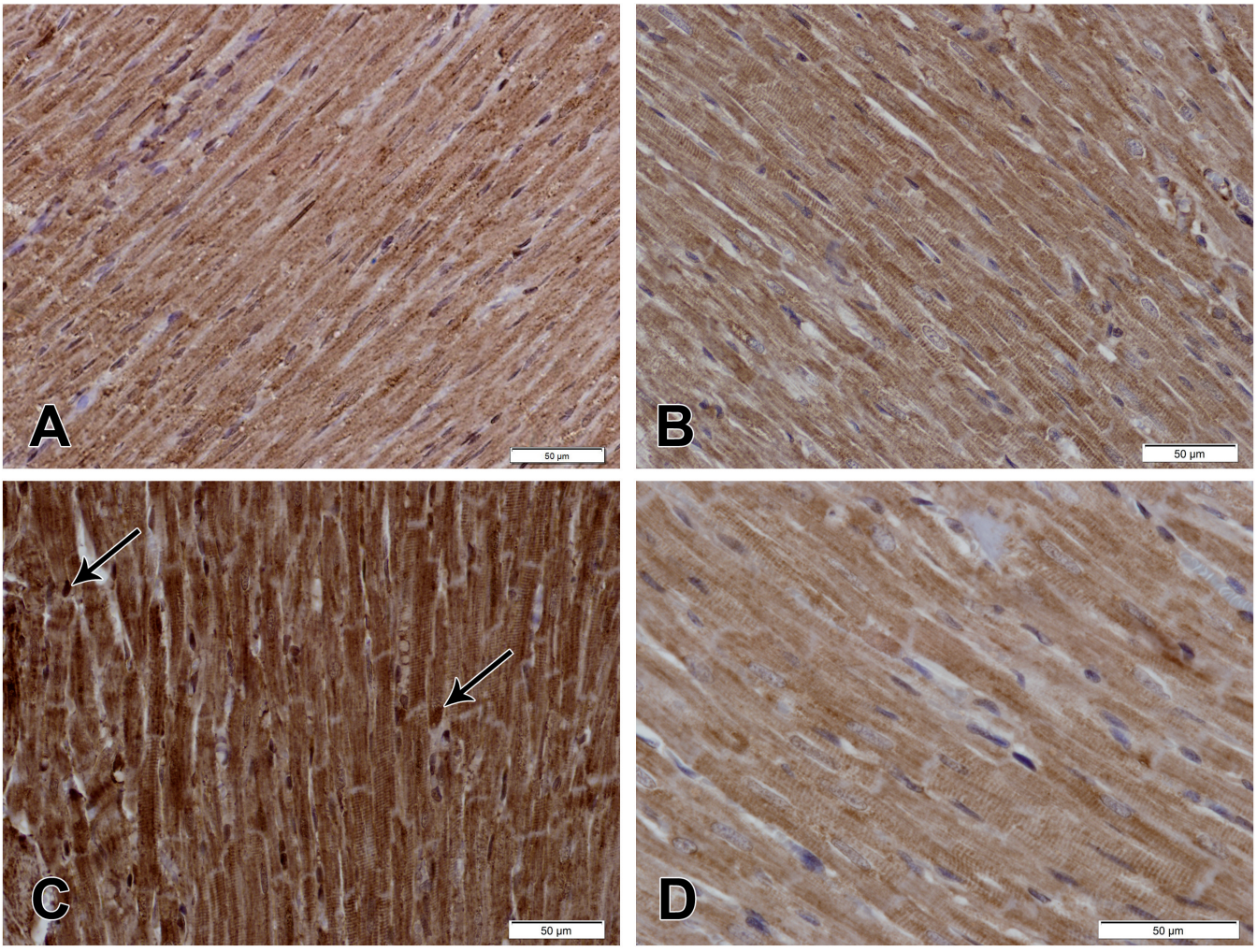

Fig. 3. Photomicrographs of heart sections immunostained with anti-thioredoxin antibody. Heart of a rat from untreated control group **(A)** and heart of a rat from control-lisinopril group **(B)**, showing moderate immunoreactivity of sarcoplasm of all cardiomyocytes. All the nuclei are negatively immunostained. **C.** Heart of a rat from myocarditis group showing intense immunoreactivity in the sarcoplasm of cardiomyocytes and almost all nuclei (arrows). **D.** Heart of a rat from myocarditis-lisinopril group showing moderate immunoreactivity of the sarcoplasm of all cardiomyocytes. Scale bars: 50 *µ*m.

atherosclerosis, hypertension, left ventricular hypertrophy, myocardial infarction, heart failure and myocarditis. The cellular effects of Ang II appeared to be mediated by ROS generated by NADPH oxidase (Koumallos et al., 2011). In the present study, the increase in serum levels of TNF-α and IL-6 observed in myocarditis group could be explained by T-cells production of Ang II, which then exerts an autocrine action to stimulate production of superoxide. Superoxide, in turn, promotes T-cell production of both

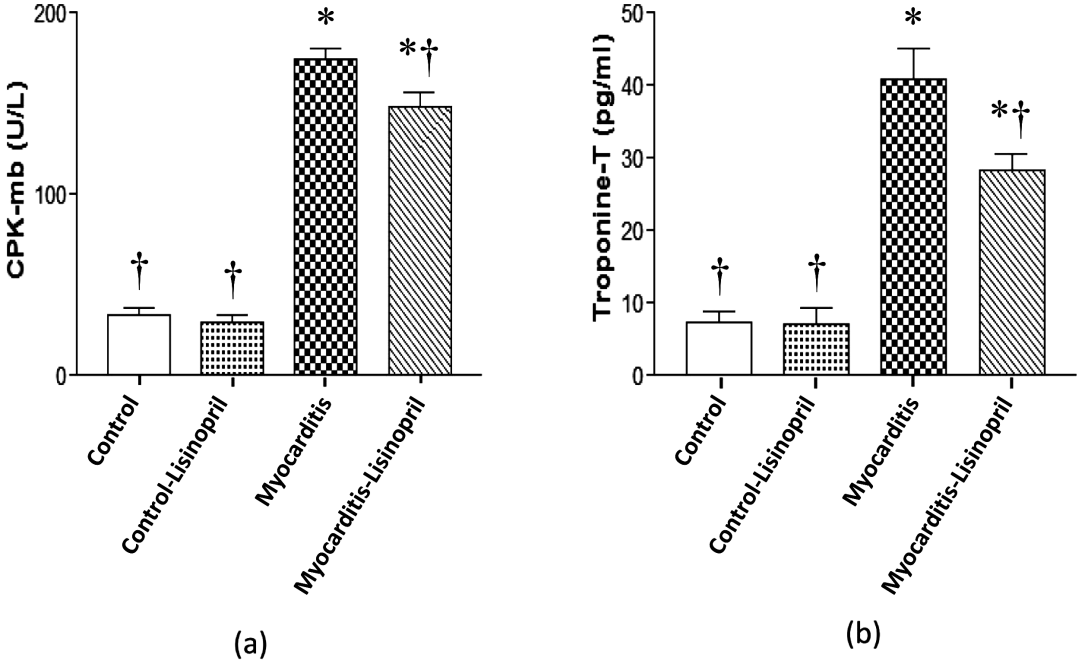

Fig. 4. Effect of lisinopril on serum level of creatine phosphokinase-mb (CPK-mb) **(a)** and troponin-T **(b)** in control rats and rats with myocarditis. Data are presented as mean ± S.D. *: Significant versus untreated control group ($p<0.05$). †: Significant versus myocarditis group ($p<0.05$).

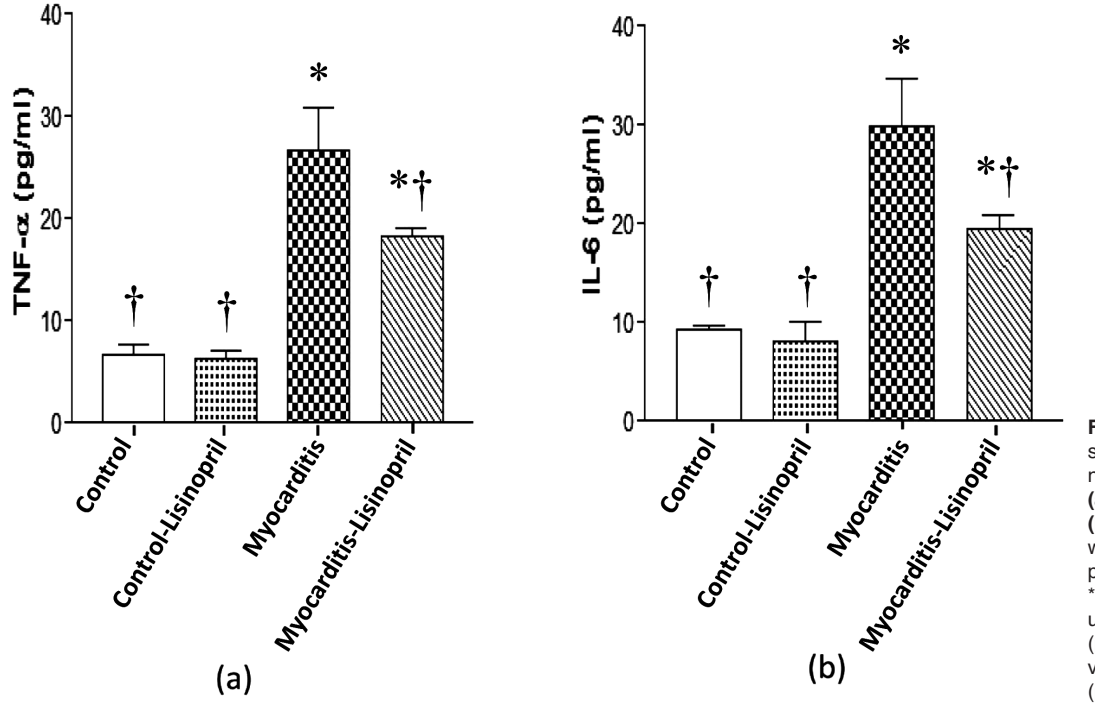

Fig. 5. Effect of lisinopril on serum level of tumor necrosis factor-α (TNF-α) **(a)** and interleukin-6 (IL-6) **(b)** in control rats and rats with myocarditis. Data are presented as mean ± S.D. *: Significant versus untreated control group ($p<0.05$). †: Significant versus myocarditis group ($p<0.05$).

cytokines (Hoch et al., 2009). TNF-α and IL-6 are capable of modulating cardiac and peripheral vascular functions by a variety of mechanisms, including abnormal regulation of nitric oxide synthase (NOS) expression, overproduction of oxygen free radicals and induction of cardiac myocyte and endothelial cell apoptosis (Ferrari et al., 1998; Meldrum, 1998) or by triggering apoptosis in myocardial and endothelial cells through oxidative stress (Keith et al., 1998). Previous studies reported the involvement of TNF-α and IL-6 in the impairment of cardiac contractility; IL6 may induce hypertrophy of myocytes whereas TNF-α may play a role in the development of myocardial fibrosis (Meléndez et al., 2010). In the present study, the walls of hearts from rats of myocarditis-lisinopril group showed mild focal myocarditis and a significant decrease of the mean percentage of pyknotic nuclei in cardiomyocytes, coincident with a significant decrease in the serum levels of TNF-α and IL-6, compared to myocarditis group. Such findings might indicate that the mechanism of action of lisinopril on acute EAM was through its suppressive effect on Ang II formation.

In the present study, rats of myocarditis group showed an increase in the serum levels of troponin-T and CPK-mb, which are considered as strong markers for myocardial cell injury (Bleuel et al., 1995). Such an increase was due to the loss of integrity of myocardial-cell membranes with the resulting release, into the circulation, of proteins of the cardiac contractile apparatus, such as the regulatory contractile protein troponin-T. Katus and Kubler (1990) and Bachmaier et al. (1995) demonstrated that the determination of serum troponin-T and CPK-mb potentially provides valuable diagnostic information in inflammatory heart disease. The significant decrease in serum levels of both markers, observed in rats of myocarditis-lisinopril group, confirmed a protective effect of lisinopril on acute EAM.

In the present study, immunohistochemistry for TRX was performed to examine the possible involvement of a redox regulating mechanism in the pathogenesis of immune-mediated myocarditis. Sections of the heart from myocarditis group showed a significant increase in the mean area percent of strong positivity for TRX compared to untreated control and control-lisinopril groups. Enhanced TRX expression might be induced by ROS produced by infiltrating inflammatory cells in acute immune-mediated myocarditis which, in turn, induces translocation of TRX from the cytoplasm into the nucleus (Shioji et al., 2000). Accordingly, TRX might have a protective role against the progressive myocardial damage in acute immune-mediated myocarditis through its ability to scavenge ROS that would oxidize proteins, lipids and DNA (Arner and Holmgren, 2000). Sections of the heart from myocarditis-lisinopril group showed that the mean area of TRX expression was significantly decreased compared to myocarditis group, while no significant difference was shown between myocarditis-lisinopril and both control groups. The present results suggest a strong anti-ROS effect of lisinopril that might prevent cellular protein oxidation. The reduced TRX expression observed in myocarditis-lisinopril group might be due to a form of competitive inhibition between lisinopril and TRX, both of them compete for degradation of ROS. The results of the present study contradict those of Yuan et al. (2003), who reported that temocapril, one of the ACE inhibitors, reduced the severity of myocardial inflammation and necrosis in rat EAM due to its ability to enhance redox regulatory protein TRX expression. In accordance with the present results, Bahk et al. (2008) reported that administration of the ACE inhibitor-captopril significantly reduced inflammation, necrosis and fibrosis in myosin-immunized mice, and concluded that captopril reduced myosin-specific delayed-type hypersensitivity and myosin-specific autoantibody production. Because lisinopril has a faster action on the heart compared to other ACE inhibitors (Fernandez et al., 1988), we recommend that it should be the drug of choice in acute EAM cases.

Regarding the limitations of the present study, measurement of the serum levels of TRX would be more accurate than TRX expression in the wall of the heart performed in the present study. Also, the present study did not measure oxidative stress parameters. Future studies will be focused to test the cardioprotective effect of lisinopril by measuring cardiac functional parameters using echocardiography and hemodynamic experiments.

In conclusion, the present study suggested a cardio-protective effect of lisinopril on acute EAM in rats. Such an effect may be attributed to the significant suppression of pro-inflammatory cytokines IL-6 and TNF-α, apart from a wide range of biological activities (angiotensin inactivation, anti-proliferative, antioxidant and immunomodulatory). The lisinopril effect was evidenced by the significant decrease of serum levels of troponin-T and CPK-mb, as well as the significant decrease of cardiac TRX expression. The present study would recommend the use of lisinopril as a prophylactic and therapeutic agent for acute autoimmune myocarditis in human. Furthermore, the present study encourages the estimation of serum level of TRX as a relevant indicator for the evaluation of the progress of acute myocarditis cases.

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Disclosure. The authors report no conflicts of interest in this work.

References

Arner E. and Holmgren A. (2000). Physiological functions of thioredoxin and thioredoxin reductase. Eur. J. Biochem. 267, 6102-6109.

Bachmaier K., Mair J., Offner F., Pummerer C. and Neu N. (1995). Serum cardiac troponin T and creatine kinase-MB elevations in murine autoimmune myocarditis. Circulation 92, 1927-1932.

Bahk T.J., Daniels M.D., Leon J.S., Wang K. and Engman D.M. (2008). Comparison of angiotensin converting enzyme inhibition and angiotensin II receptor blockade for the prevention of experimental autoimmune myocarditis. Int. J. Cardiol. 125, 85-93.

Blauwet L. and Cooper L. (2010). Myocarditis. Prog. Cardiovasc. Dis. 52, 274-288.

Bleuel H., Deschl U., Bertsch T., Bölz G. and Rebel W. (1995). Diagnostic efficiency of troponin T measurements in rats with experimental myocardial cell damage. Exp. Toxicol. Pathol. 47, 121-127.

Daniels M.D., Hyland K.V. and Engman D.M. (2007). Treatment of experimental myocarditis via modulation of the renin-angiotensin system. Curr. Pharm. Des. 13, 1299-1305.

Dubey K., Balani D.K. and Pillai K.K. (2003). Potential adverse interaction between aspirin and Lisinopril in hypertensive rats. Hum. Exp. Toxicol. 22, 243-247.

Feldman A.M. and McNamara D. (2000). Myocarditis. N. Engl. J. M. 343, 1388-1398.

Fernandez D., Bolli P., Snedden W., Vasdev S. and Fernandez P.G. (1988). Modulation of left ventricular hypertrophy by dietary salt and inhibition of angiotensin converting enzyme. J Hypert. Suppl. 6, S145-147.

Ferrari R., Agnoletti L., Comini L., Gaia G., Bachetti T., Cargnoni A., Ceconi C., Curello S. and Visioli O. (1998). Oxidative stress during myocardial ischemia and heart failure. Eur. Heart J. 19 (Suppl. B), B2-11.

Futamatsu H., Suzuki J., Kosuge H., Yokoseki O., Kamada M., Ito H., Inobe M., Isobe M. and Uede T. (2003). Attenuation of experimental autoimmune myocarditis by bocking activated T cells through inducible co-stimulatory molecule pathway. Cardiovas. Res. 59, 95-104.

Hoch N.E., Guzik T.J., Chen W., Deans T., Maalouf S.A., Gratze P., Weyand C. and Harrison D.G. (2009). Regulation of T-cell function by endogenously produced angiotensin II. Am. J. Physiol. Regul. Integr. Comp. Physiol. 296, R208-R216.

Katus H.A. and Kubler M.A. (1990). Detection of myocardial cell damage in pateints with unstable angina by serodiagnostic tools. In: Unstable angina. Bleifeld W. Hamm C.W. and Braunwald E. (eds). Springer-Verlag. Berlin, Germany. pp 92-100.

Keith M., Geranmayegan A. and Sole M.J. (1998). Increased oxidative stress in patients with congestive heart failure. J. Am. Coll. Cardiol. 31, 1352-1356.

Kodama M. and Izumi T. (1991). Experimental autoimmune myocarditis. Acta Med. Biol. 39, 1-10.

Kodama M., Matsumoto Y. and Fujiwara M. (1992). In vivo lymphocyte-mediated myocardial injuries demonstrated by adoptive transfer of experimental autoimmune myocarditis. Circulation 85, 1918-1926.

Koumallos N., Nteliopoulos G., Paschalis A., Dimarakis I. and Yonan N. (2011). Therapeutic interventions to renin-angiotensin-aldosterone system, and vascular redox state. Recent Pat. Cardiovasc. Drug. Discov. 6, 115-122.

Li Y., Heuser J.S., Kosanke S.D., Hemric M. and Cunningham M.W. (2004). Cryptic epitope identified in rat and human cardiac myosin S2 region induces myocarditis in the Lewis rat. J. Immunol. 172, 3225-3234.

Li Y., Heuser J.S., Kosanke S.D., Hemric M. and Cunningham M.W. (2005). Protection against experimental autoimmune myocarditis is mediated by interleukin-10-producing T cells that are controlled by dendritic cells. Am. J. Pathol. 167, 5-15.

Liu P.P. and Mason J.W. (2001). Advances in the understanding of myocarditis. Circulation 104, 1076-1082.

Liu W., Nakamura H., Shioji K., Tanito M., Oka S., Ahsan M.K., Son A., Ishii Y., Kishimoto C. and Yodoi J. (2004). Thioredoxin-1 ameliorates myosin-induced autoimmune myocarditis by suppressing chemokine expressions and leucocyte chemotaxis in mice. Circulation 110, 1276-1283.

Mann D.L. (2002). Inflammatory mediators and the failing heart: past, present and the foreseeable future. Circ. Res. 91, 988-989.

Matsui Y., Okamoto H., Jia N., Akino M., Uede T., Kitabatake A. and Nishihira J. (2004). Blockade of macrophage migration inhibitory factor ameliorates experimental autoimmune myocarditis. J. Mol. Cell. Cardiol. 37, 557-566.

Meldrum D.R. (1998). Tumor necrosis factor in the heart. Am. J. Physiol. 274, R577-595.

Meléndez G.C., McLarty J.L., Levick S.P., Du Y., Janicki J.S. and Brower G.L. (2010). Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. Hypertension 56, 225-231.

Otsuka K., Terasaki F., Ikemoto M., Fujita S., Tsukada B., Katashima T., Kanzaki Y., Sohmiya K., Kono T., Toko H., Fujita M. and Kitaura Y. (2009). Suppression of inflammation in rat autoimmune myocarditis by S100A8/A9 through modulation of the pro-inflammatory cytokine network. Eur. J. Heart Failure 11, 229-237.

Reyes M.P., Khatib R., Khatib G., Ho K.L., Smith F. and Kloner R.A. (1998). Prolonged captopril therapy in murine viral myocarditis. J. Cardiovascul. Pharmacol. Ther. 3, 43-50.

Rosenstein E., Zucker M. and Kramer N. (2000). Giant cell myocarditis: most fatal of autoimmune disease. Semin. Arthritis Rheum. 30, 1-16.

Seko Y. (2006). Effect of the angiotensin II receptor blocker olmesartan on the development of murine acute myocarditis caused by coxsackievirus B3. Clin. Sci. (Lond). 110, 379-386.

Shioji K., Kishimoto C. and Nakamura H. (2000). Up-regulation of thioredoxin (TRX) expression in giant cell myocarditis in rats. FEBS Lett. 472, 109-113.

Yuan Z., Kishimoto C., Shioji K., Nakamura H., Yodoi J. and Sasayama S. (2003). Temocapril treatment ameliorates autoimmune myocarditis associated with enhanced cardiomyocyte thioredoxin expression. Mol. Cell. Biochem. 248, 185-192.

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