Effects and Mechanism of SO$_2$ Inhalation on Rat Myocardial Collagen Fibers

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDE Ping Chen
F Decai Qiao
AG Xiaoli Liu

College of Physical Education and Sports, Beijing Normal University, Beijing, P.R. China

Background: This study investigates the effects and mechanism of sulfur dioxide (SO$_2$) inhalation and exercise on rat myocardial collagen fiber.

Material/Methods: The rats were randomly divided into 4 groups: a control group (RG), an exercise group (EG), an SO$_2$ pollution group (SRG), and an SO$_2$ pollution and exercise group (SEG). Body weight, cardiac index, and left ventricular index in each group were compared. The myocardial hydroxyproline (Hyp) concentration was determined by pepsin acid hydrolysis. The interstitial myocardial collagen expression was measured by Sirius Red F3B in saturated carbazotic acid. The local myocardial angiotensin II type 1 receptor (AT1R) and connective tissue growth factor (CTGF) expression was tested by immunohistochemistry SABC method.

Results: Compared with RG, the weight growth rate of EG, SRG, and SEG decreased significantly (P<0.01). Compared with EG, the body weight growth rate of SEG significantly decreased (P<0.01) and cardiac index and left ventricular index decreased but without a significant difference. Compared with EG, myocardial Hyp and collagen concentration, myocardial collagen volume fraction (CVF), perivascular collagen area (PVCA), and the expression of AT1R and CTGF in myocardium of SEG increased significantly (P<0.01).

Conclusions: SO$_2$ inhalation and exercise will not only offset beneficial health effects of movement on the cardiovascular system, but also produce more unfavorable influences. People should pay attention to their environment when exercising, and try to avoid exercising in environments with SO$_2$ pollution.

MeSH Keywords: Collagen • Exercise • Myocardium • Sulfur Dioxide

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/904796

📄 2468   🗂 5   📊 4   📖 21

Background

It is well documented that appropriate aerobic exercise can enhance cardiac function and vascular function, and plays an important role in the prevention and treatment of cardiovascular diseases [1]. However, many people who exercise only consider the beneficial health effects of sports fitness, but overlook the harmful effects of environmental pollutants on the human body. It has been shown that the emergency hospitalization rate caused by upper-respiratory tract infections is significantly increased in people exposed to sulfur dioxide (SO_2) pollution. The incidence of cardiovascular system diseases, especially ischemic heart disease, and the risk of death due to cardiovascular and respiratory diseases, are closely related to SO_2 pollution [2]. Animal experiment results show that SO_2 is a genotoxic factor and a chromosome fragmentation agent, as it is involved in the pathophysiological process of cardiovascular disease [3]. However, the effects and mechanism of SO_2 pollution on the cardiovascular system are still unclear and relevant experiments are warranted.

Previous studies have shown that the double stimulation of SO_2 and exercise not only counteracts the effects of reducing stress after exercise and improved cardiac function, but also increases blood pressure and decreases systolic function of the heart, which have adverse effects on cardiac function. Cardiac function depends on the contraction of the heart itself and is closely related to collagen fibers. Myocardial collagen fiber expression abnormalities, and myocardial fibrosis and other clinical manifestations, are relative to the cardiac function decrease. Therefore, it is hypothesized that the changes of myocardial systolic function induced by SO_2 pollution are related to changes in myocardial morphology and myocardial collagen content. In the present study, changes in myocardial collagen fiber content, angiotensin II type 1 receptor (AT1R) content, and the expression of connective tissue growth factor (CTGF) were studied in the stimulation of SO_2 inhalation and exercise, to investigate the effects and mechanism of SO_2 inhalation and appropriate exercise on myocardial collagen fibers.

Material and Methods

Experimental animals and grouping

Twenty-four healthy male rats, age 7–8 weeks and weight 180–220 g, were provided by the Laboratory Animal Science Center, Peking University Health Science Center. The rats were randomly divided into 4 groups: a control group (RG), a simple exercise group (EG), an SO_2 pollution alone group (SRG), and an SO_2 pollution and exercise group (SEG). The RG and EG groups were placed in an environment with pollution-free fresh air, and the SRG and SEG groups were placed in an environment with artificial simulation of SO_2 pollution (concentrations of 10 mg/m³). All rats had free access to food and water and were housed at a temperature of 17–23°C and humidity of 40–60% [4]. All animal experiments were conducted according to the ethics guidelines of Beijing Normal University.

After 1 week, the rats of EG and SEG groups performed exercise on a custom-designed, adjustable speed, electric running wheel to establish a rat animal model. The program was that for 4 weeks the rats exercised every day for 1 h every afternoon, during which they had no access to food or water, after which they were returned to their respective environments.

Collection of specimen and determination of indicators

Rats were weighed twice a week to compare body weight of the 4 groups. After the last session, the rats were fasted for 24 h and weighed, then anesthetized by 2% pentobarbital sodium (3 mL/kg). After that, the rats were laparotomized, the chest was opened, and the hearts were quickly removed. Once the great vessels were cut off, the heart was rinsed with ice-cold salt water, dried by blotting with filter paper, and weight was recorded. Then, we immediately removed the atria and large blood vessels along the junction of the atria and ventricles in ice-cold physiological saline solution, removed the right ventricular free wall, and calculated the heart weight index (HBI) and left ventricular mass index (LVMI) according to the following formula:

$$HBI = \text{heart weight (g)/body weight (g)} \times 1000,$$
$$LVMI = LVM/BW.$$

Determination of myocardial Hyp concentration

The myocardial Hyp concentration was determined by pepsin acid hydrolysis, and then the concentration of myocardial collagen (mg/g dry wt) was calculated by Hyp concentration multiplied by a factor of 7.46 [5].

Detection of myocardial collagen volume fraction

Six pieces of myocardial tissue in each group were fixed in 10% formaldehyde, embedded by paraffin, sliced continuously (thickness of about 3 µm), and finally stained by Sirius Red F3B in saturated carbazotic acid [6]. The myocardial collagen was observed and photographed using a normal microscope, and image analysis was performed using the Olympus multimedia image analysis system. Five views of each slice were randomly selected to analyze myocardial collagen volume fraction (CVF,%). Each specimen was measured with 5 arterioles in the transverse wall of the facet to analyze myocardial perivascular collagen area (PVCA,%). CVF and PVCA were calculated as follows:

Table 1. The change of rat weight in each group (mean ± standard deviation) (g).

Group	0th week	1st week	2nd week	3rd week	4th week	Growth rate (%)
RG	220.50±12.80	240.50±12.85	279.40±12.06	310.86±18.25	354.0±18.50	61%
EG	212.83±9.04	238.83±9.04	263.91±20.80	286.25±12.45	312.50±15.66**	47%
SRG	210.79±4.91	235.10±10.21	268.75±28.78	289.44±22.42	307.00±13.03**	46%
SEG	207.50±5.24	229.23±12.51	251.21±22.25	273.75±15.26	289.00±19.88**##	39%

RG – control group; EG – exercise group; SRG – SO_2 pollution group; SEG – SO_2 pollution and exercise group. Compared with RG, ** $P<0.01$; compared with EG, ## $P<0.01$.

Table 2. The heart weight, left ventricular mass, cardiac index and left ventricular index in each group (mean ± standard deviation).

Group	Heart weight (g)	Left ventricular mass (g)	Cardiac index (mg/g)	Left ventricular index (mg/g)
RG	1.16±0.09	0.72±0.15	0.327±0.018	0.204±0.04
EG	1.41±0.10**&&	0.83±0.07**&&	0.445±0.024*&&	0.267±0.02**&&
SRG	1.01±0.19##	0.66±0.04**##	0.329±0.006##	0.213±0.03##
SEG	1.20±0.22**#&	0.73±0.03**##&&	0.417±0.079*&&	0.253±0.02*&&

RG – control group; EG – exercise group; SRG – SO_2 pollution group; SEG – SO_2 pollution and exercise group. Compared with RG: * $P<0.05$, ** $P<0.01$; compared with EG: # $P<0.05$, ## $P<0.01$; compared with SRG: & $P<0.05$, && $P<0.01$.

$$CVF=collagen\ area/view\ area×100\%,$$

PVCA=the collagen area around small arterial lumen in the myocardium/Arterial lumen area×100%,

where: collagen area does not include PVCA.

Testing of myocardial AT1R and CTGF expression

Paraffin sections were embedded again after the immunohistochemical process. The 2nd, 5th, 8th, 11th, 14th, and 16th slices of each the specimens were selected from the consecutive sections for AT1R immunohistochemical staining, and the 3rd, 6th, 9th, 12th, 15th, and 18th slices were selected for CTGF immunohistochemical staining [7]. Ten high-power fields (×200) of each slice were randomly selected to analyze brown-yellow area by use of a computer image analysis system. The positive area, which is the area ratio of the brown-yellow area to the field of view, was considered as the AT1R or CTGF concentration of myocardial tissue slice. Finally, the average concentration of AT1R or CTGF in 6 slices represented the expression of AT1R protein or CTGF protein in the myocardium tissue.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 13.0 (Chicago, IL, USA). Quantitative data are expressed as mean ± standard deviation. One-way ANOVA was used to compare the differences. $P<0.05$ was considered a statistically significant difference.

Results

The change of body weight

To observe changes in body weight in each group after SO_2 inhalation and exercise, the rats were weighed twice a week, and the growth rate was calculated. As shown in Table 1, the rats in each group had a general increase in body weight after the experimental period, which was due to natural growth. However, compared with RG, the rat body weight growth rate of EG, SRG, and SEG were significantly decreased ($P<0.01$), and SEG decreased most significantly. Compared with EG, the rat body weight growth rate of SEG was significantly decreased ($P<0.01$).

The change of cardiac index and left ventricular index

To assess the effects of SO_2 inhalation and exercise on rat cardiac index and left ventricular index, we assessed heart weight, left ventricular mass, cardiac index, and left ventricular index in each group, as listed in Table 2. Compared with the RG, heart weight, left ventricular mass, cardiac index, and left
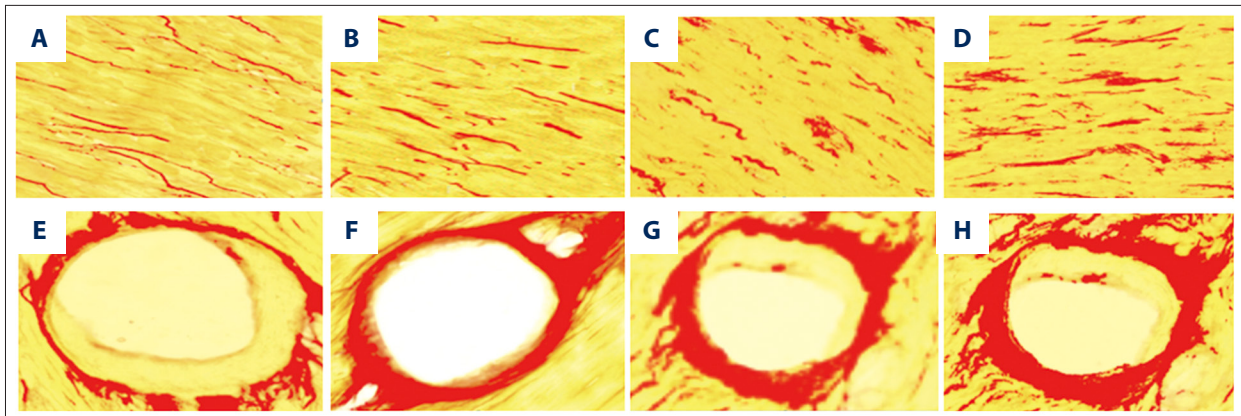
Figure 1. Morphological changes in collagen fibers (**A–D**) and changes in collagen fibers (**E–H**) in peripheral arteries. **A, E**: RG, **B, F**: EG, **C, G**: SRG, **D, H**: SEG. The myocardial tissue was yellow and collagen was red (picrosirius red stain, ×200). RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group.

Table 3. Changes of hydroxyproline content and collagen concentration in myocardium of rats in each group (mean ± standard deviation) (ug/mg).

Group	Hydroxyproline content	Collagen concentration
RG	0.359±0.008	2675.7±58.7
EG	0.394±0.013**	2944.2±84.2**
SRG	0.386±0.031*	2911.9±127.0**
SEG	0.430±0.028**##&&	3207.8±216.0**##&&

RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group. Compared with RG: * P<0.05, ** P<0.01; compared with EG: # P<0.05, ## P<0.01; compared with SRG: & P<0.05, && P<0.01.

ventricular index of EG and SEG were significantly increased (P <0.05), but left ventricular mass of SRG was decreased significantly (P <0.01), and heart weight, cardiac index, and left ventricular index were not significantly different. Compared with EG, heart weight and left ventricular mass in SEG were significantly decreased (P<0.01), while cardiac index and left ventricular index were not significantly different.

Morphological changes in collagen fibers in myocardium and arterioles

To explore morphological changes in collagen fibers in myocardium and arterioles, myocardial collagen was observed and photographed by normal microscopy. As illustrated in Figure 1, the collagen of RG rats had uniform distribution and the arteriolar wall was smooth, with no increase in collagen fibers. Compared with RG, myocardial collagen fiber of rat in EG was thickened and neatly arranged, and the arteriole collagen fibers increased. The collagen of rats in SRG was greatly increased

and arranged in bundles. The collagen fibers around the small arteries significantly increased, and the surrounding muscles were deep and had burrs formed. The sarcoplasmic reticulum fibers of SEG rats were clustered and disordered. The collagen fibers around the arteries were deeply invaded by myocardial parenchyma and formed large collagenous deposition areas with collagen fibers as the center.

Changes in myocardial Hyp and collagen concentration

To assess the effects of SO$_2$ inhalation and exercise on rat collagen concentration, the myocardial Hyp concentration of each group was tested (Table 3). As shown in Figure 2, compared with RG, rat myocardial Hyp and collagen concentration of EG, SRG, and SEG were increased significantly (P<0.01; P<0.05). Compared with EG, rat myocardial Hyp and collagen concentration in SEG were increased significantly (P<0.01).

Changes in CVF and PVCA

The myocardial CVF and the PVCA in each group are shown in Table 4. Compared with RG, the rat myocardial CVF and the PVCA of SRG and SEG were increased significantly (P<0.01; P<0.05), but not significantly increased in EG (Figure 3). Compared with EG, the rat myocardial CVF and the PVCA in SEG were increased significantly (P<0.01).

Effects of SO$_2$ inhalation on expression of AT1R and CTGF

To identify the effects of SO$_2$ inhalation on expression of AT1R and CTGF, we compared RG, SG, SRG, and SEG, as shown in Table 5. Figure 4 shows anti-AT1R immunohistochemical staining and anti-CTGF immunohistochemical staining of each group at 10 high-power fields (×200). Compared with RG, the expression of AT1R and CTGF in myocardium in SRG and SEG were increased significantly (P<0.05), but in EG they were decreased
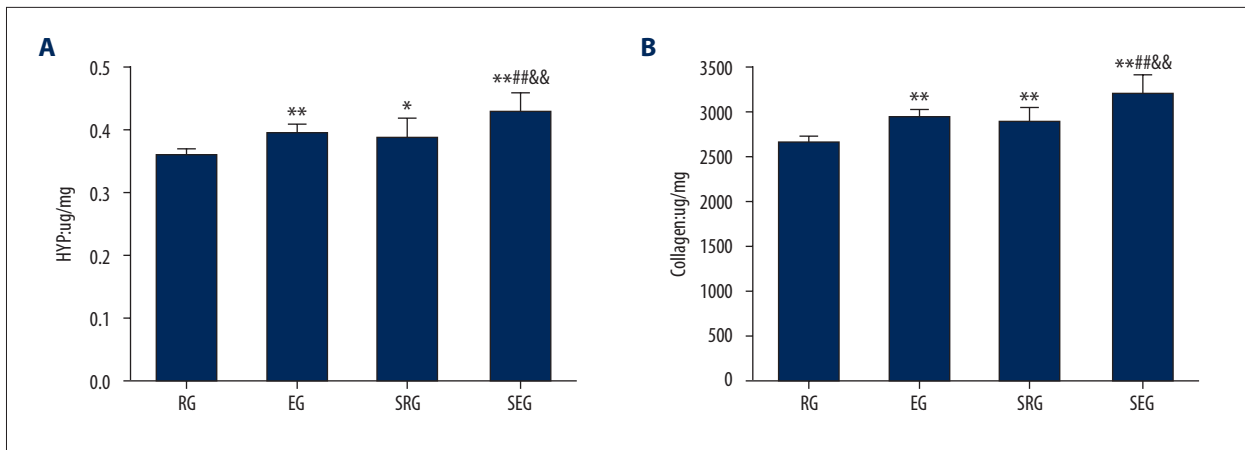
Figure 2. Changes in myocardial Hyp content (**A**) and collagen content (**B**) in rats of each group. RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group.

Table 4. The myocardial collagen volume fraction and the collagen volume fraction around small arteries in each group (mean ± standard deviation).

Group	Myocardial collagen volume fraction (%)	Collagen volume fraction around small arteries (%)
RG	0.037±0.006	0.507±0.060
EG	0.045±0.005	0.596±0.045&
SRG	0.049±0.013**	0.673±0.217**#
SEG	0.067±0.016**##&&	0.946±0.023**##&

RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group. Compared with RG: * P<0.05,** P<0.01; compared with EG: # P<0.05, ## P<0.01; compared with SEG: & P<0.05, && P<0.01.

Table 5. The expression of AT1R and CTGF in each group (mean ± standard deviation).

Group	Expression of AT1R	Expression of CTGF
RG	0.101±0.024	0.112±0.035
EG	0.062±0.019**&&	0.076±0.022**&&
SRG	0.113±0.013##	0.132±0.036##
SEG	0.130±0.014**##&	0.151±0.043**##

RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group. Compared with RG: * P<0.05, ** P<0.01; compared with EG: # P<0.05, ## P<0.01; compared with SEG: & P<0.05, && P<0.01.
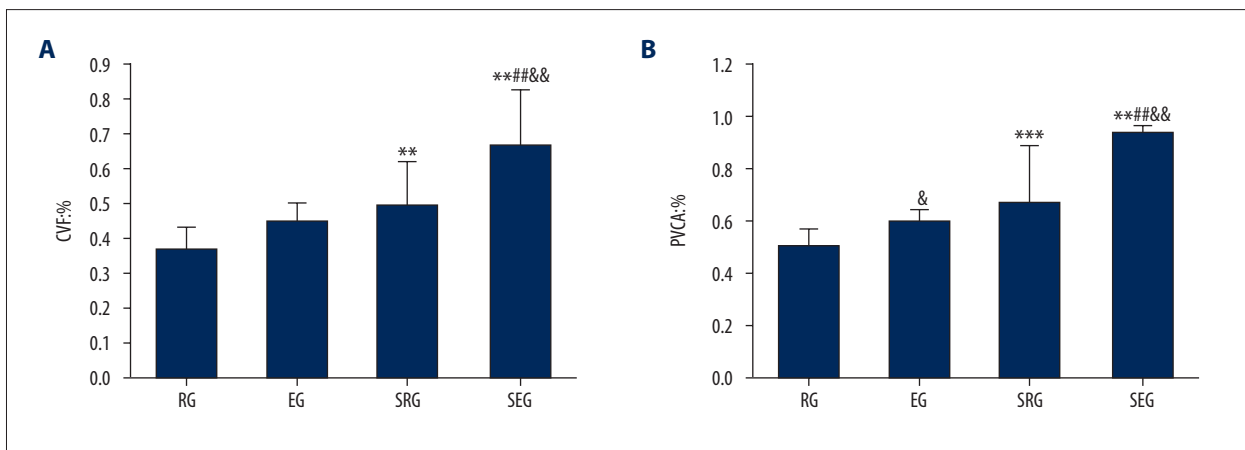

Figure 3. Changes in myocardial CVF (**A**) and myocardial CVF (**B**) in rats of each group. RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group.
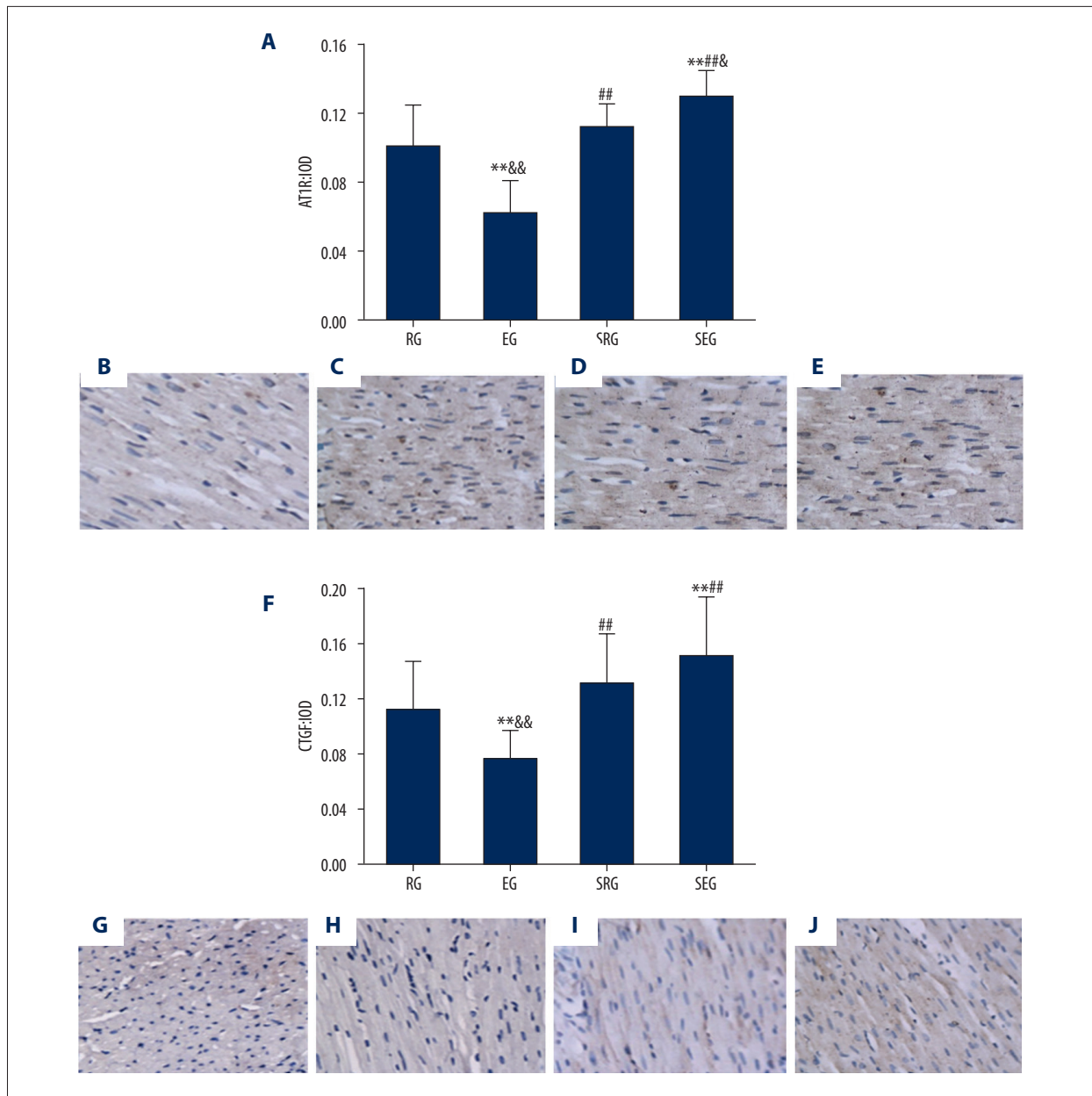
Figure 4. AT1R and CTGF immunohistochemical staining of rat myocardium (× 200). Changes in AT1R expression of myocardium of rats in each group (**A**). AT1R immunohistochemical staining of RG (**B**), EG (**C**), SRG (**D**), and SEG (**E**). Changes in myocardial CTGF expression in rats of each group (**F**). CTGF immunohistochemical staining of RG (**G**), EG (**H**), SRG (**I**), and SEG (**J**). RG – control group; EG – exercise group; SRG – SO$_2$ pollution group; SEG – SO$_2$ pollution and exercise group.

significantly (P<0.05). Compared with EG, the expression of AT1R and CTGF in myocardium in SEG was increased significantly (P<0.01).

Discussion

This study showed that the rats in each group had natural growth. However, the body weight of rats exercising in the

SO$_2$ pollution environment was significantly lower than in the other groups, which might be because that the rats developed limb weakness, lack of energy, and decreased appetite after the stimulation of exercise and SO$_2$ pollution. Rats were exposed to a 10 mg/m^3 concentration of SO$_2$, which was higher than the SO$_2$ concentration we measured in the air environment. The concentration of SO$_2$ used in the experiment was 100 times that of the annual average of level 3 standard of China's air quality standards, which is 0.1 mg/m^3. The reason

of we chose the concentration of 10 mg/m^3 is that the respiratory system of rodents, including rats, was 100 times more sensitive than in humans.

This study also found that heart weight, left ventricular mass, left ventricular index, and cardiac weight index of rats who exercised on the running wheel were significantly higher than the rats who did not exercise, which is consistent with previous studies.

Heart weight index is the ratio of heart weight to body weight, which is an important indicator of cardiac hypertrophy [8]. The results of this study showed that the heart weight and heart weight index of rats were increased after long-term aerobic exercise, indicating that long-term appropriate exercise induced formation of hypertrophy. The cardiac weight index of SRG was significantly higher than in RG, which was caused by the decrease of body weight after rat exposure to the SO$_2$ pollution environment. In addition, myocardial AngII can promote the release of catecholamines of the sympathetic nerve endings, promote the synthesis of mitochondrial and expression of proto-oncogene in cardiomyocytes through the receptors on the nucleus, and stimulate the growth and proliferation of cardiomyocytes, leading to cardiac hypertrophy [9]. The significantly increased heart weight index of SRG and SEG rats may be related to the increase in AngII.

Previous studies have shown that in the rat model of renal hypertension, the total CVF and collagen content in the myocardium was increased [10]. This study showed that Hyp concentration of EG had an increasing trend compared with RG rats, and was significantly increased in SRG and SEG, indicating that exercise in the SO$_2$ pollution environment could cause rat myocardial interstitial collagen accumulation. Studies have shown that cardiac function is not only closely related with the systolic and diastolic function of cardiomyocyte, but also with the myocardial interstitial collagen fibers.

In this study, the myocardial CVF and the CVF around small arteries of EG rats were increased compared with RG rats, and the groups of SRG and SEG were increased significantly. Therefore, the negative reaction of the rat cardiac function in the SO$_2$ pollution environment may be caused by excessive accumulation of collagen in the rat myocardia and around small arteries.

In the physiological state, the synthesis and degradation of extracellular matrix (ECM) is in a dynamic balance, which involves a series of regulatory factors [11]. The renin-angiotensin system (RAS) is an independent system in the heart, which is involved in the regulation of the local cell endocrine, autocrine,

and paracrine, and is based on the renin, angiotensinogen, vascular conversion enzyme, and angiotensin II (Ang II) and its receptors. At present, AT1R, AT2R are the most studied receptors of Ang II. The present study found that AngII can significantly stimulate the expression of primary response gene and embryonic gene of cardiomyocytes, thus promoting protein synthesis and DNA amplification of myocardial cells [12]. Ang II also participates in the hypertrophy of motor cardiomyocytes through activating MAPK (mitogen-activated protein kinase) [13]. Ang II can promote cardiomyocyte hypertrophy and growth and proliferation of fibroblasts, and also promotes the synthesis and accumulation of collagen, but studies show these biological activity functions of Ang II are primarily mediated by its receptor, AT1R [14–16]. Polypeptide protein CTGF is an important mediator in formation of fibrosis in various tissues and organs. It has a strong regulatory effect on the differentiation and proliferation of fibroblasts and kidney cells, as well as the synthesis and degradation of ECM [17,18]. A study found that the expression of CTGF mRNA in the left ventricle was significantly increased in acute myocardial infarction rats [19], which was caused by AT1R [20]. Once AT1R antagonist acted on AT1R, myocardial fibrosis was reduced and the expression of CTGF was decreased, suggested that the reduced myocardial fibrosis was related to decreased expression of CTGF in the myocardium [21].

Conclusions

This study found that the expression of AT1R and CTGF in the SO$_2$ pollution environment was higher than in the control group, suggesting that the high expression of AT1R and CTGF in myocardial tissue may be one of the causes of excessive deposition of collagen in the SO$_2$ pollution environment. It was also found that the expression of CTGF changed with the expression of AT1R, indicating that CTGF may be a biological downstream effect factor of AngII for cardiac fibroblasts. Ang II promotes changes in myocardial interstitial collagen fibers, possibly in part by the CTGF pathway. Ang II regulates the formation of CTGF in cardiac fibroblasts through the AT1R-mediated mechanism, further affecting the occurrence of fibrosis of myocardial interstitial collagen. SO$_2$ inhalation and exercise will not only offset beneficial health effects of exercise on the cardiovascular system, but also produce more unfavorable influences.

Conflict of interests

None.

References:

1. Liu T., Xu S., Ding H: [Effects of aerobic exercise on cardiovascular functions and blood indexes.] Journal of Clinical Rehabilitative Tissue Engineering Research, 2008; 12: 2965–68 [in Chinese]

2. Liu Y: Damage effects of SO$_2$ and its epidemiology and toxicology research. Asian Journal of Ecotoxicology, 2007; 2: 225–31

3. Meng Z, Wang S: SO$_2$-A secret biological small molecule: The biphasic roles of SO$_2$ and its derivatives on the blood vessel tension. Asian Journal of Ecotoxicology, 2007; 2: 158–63

4. Tian Z: Sports heart biology. Science Press, 2006

5. Heineke J, Molkentin JD: Regulation of cardiac hypertrophy by intracellular signaling pathways. Mechanisms of Disease, 2006; 7: 223–29

6. Lu YJ, Lu Y, Zhu YM et al: Preliminary histological and biomechanical study about the timing of surgical repair for acute rotator cuff tears in rabbits. Chin J Surg, 2012; 50: 560–65

7. Van der Loos, Chris M, Teeling P: A generally applicable sequential alkaline phosphatase immunohistochemical double staining. Journal of Histotechnology, 2008; (3): 119–27

8. Ma Y: [Histologic changes of different exercise patience on myocardial collagenous fibers in rats.] Journal of Shandong Institute of Physical Education and Sports, 2005; 21: 58–61 [in Chinese]

9. Wang Y, Xiong Y, Chen Z, Li L: Effects of simvastatin on myocardial fibrosis and connective tissue growth factor in renovascular hypertensive rats. Chin J Clin Pharm Therap, 2004; 9: 1101–4

10. Zhen L, Pan S: [Relationship between local RAS of heart and remold of heart at exercise.] Journal of Shanghai University of Sport, 2000; 24: 33–36 [in Chinese]

11. Wu F, Yang F: Connective tissue growth factor and myocardial fibrosis. Chin Heart, 2006; 18: 715–17

12. Cheng Z, Xu B, Dou L et al: [Effects and mechanisms of PNS on myocardial fibrosis in chronic viral myocarditic mice.] Journal of Zhejiang University of Traditional Chinese Medicine, 2008; 32: 23–28 [in Chinese]

13. Yamazaki T, YazakiY: Role of tissue angiotensin II in myocardial remodeling Induced by mechanical stress. Hum Hypertens, 1999; 13: S43–47

14. Sun Y, Weber KT: Cardiac remodelling by fibrous tissue: Role of local factors and circulating hormones. Ann Med, 1998; 30: S3–8

15. Huang R, Liu T, Pang Y et al: [Collagen network remodeling in rats with dilated cardiomyopathy and the changes of angiotensin II and it's type I receptor.] South China Journal of Cardiovascular Diseases, 2004; 10: 299–302 [in Chinese]

16. Kuwahara K, Saito Y, Harada M, et al. Involvement of cardiotrophin-1 in cardiac myocyte-nonmyocyte interactions during hypertrophy of rat cardiac myocytes *in vitro*.Circulation, 1999; 100: 1116–24

17. Paradis P, Dali-Youcef N, Paradis FW et al: Overexpression of angiotensin II type I receptor in cardiomyocytes induces cardiac hypertrophy and remodeling. Mol Cell Cardiol, 2000; 97: 930–36

18. Lv S, Wu M, Li M et al: Effect and ,echanism of QiShenYiQi Pill on experimental autoimmune myocarditis rat. Med Sci Monit, 2016; 22: 752–56

19. Perbal B: NOV (nephroblastoma overexpressed) and the CCN family of genes: Structural and functional issues. Mol Pathol, 2001; 54: 57–79

20. Perbal BL CCN proteins: Multifunctional signaling regulations. Lancet, 2004; 363: 62–64

21. Iwanciw D, Rehm M, Porst M, Goppelt-Struebe M: Induction of connective tissue growth factor by angiotension II: Integration of signaling pathways. Arterioscler Thromb Vasc Biol, 2003; 23: 1782–85