Effects of exercise on the cardiovascular function of rats in a sulfur dioxide polluted environment

YANRU HU, TINGTING WU, XIAOLI LIU & DECAI QIAO

Abstract: The purpose of the study is to further explore the combined effects of exercise and sulfur dioxide (SO₂) exposure on the cardiovascular function as well as the underlying mechanisms. Rats were randomly divided into 4 groups: rest group (RG), exercise group (EG), SO₂ pollution group (SG) and SO₂ pollution + exercise group (SEG). Changes of aortic pressure and left ventricular pressure, Ang II concentration, ACE concentration and ACE activity in rats’ myocardial tissue were observed. Compared with RG, the systolic blood pressure, pulse pressure, LVSP, +dp/dtmax and -dp/dtmax of EG increased significantly, diastolic blood pressure, resting heart rate and ACE activity decreased significantly; For rats of SG, 4 weeks SO₂ exposure increased LVEDP, Ang II concentration, ACE concentration and ACE activity, decreased the +dp/dtmax and -dp/dtmax; For rats of SEG, the systolic blood pressure, pulse pressure, LVSP, +dp/dtmax and -dp/dtmax decreased significantly, HR, LVEDP, Ang II concentration, ACE concentration and ACE activity increased significantly. Results indicate that, the combination of aerobic exercise and SO₂ exposure can aggravate the negative effects of SO₂ inhalation on cardiovascular function. Renin-angiotensin system plays an important role in mediating the negative effect of SO₂ inhalation.

Key words: sulfur dioxide, exercise, haemodynamics, Ang II, ACE.

INTRODUCTION

Sulfur dioxide (SO₂) is a major component of air pollution that is artificially produced by fossil fuel combustion, although the annual levels of SO₂ has fallen in developed countries in past decades, it remains to be a severe air pollution problem in many developing countries (Wang et al. 2018, Guttikunda et al. 2003). It has been demonstrated that, inhaled SO₂ can easily be hydrated in the respiratory tract, producing sulfurous acid, then it can subsequently be decomposed into two derivatives: bisulfite and sulfite (Shapiro 1977). The derivatives can be absorbed into blood or other body fluids, as systemic toxins, they may cause various kinds of biological and toxicological impacts on cardiovascular, respiratory and other systems, the resulting symptoms may include coughing, throat irritation, breathing difficulties, cardiac arrhythmia (Dales et al. 2020), ischemic heart diseases (Torén et al. 1996), pulmonary cardiac diseases and hypertension (Yao et al. 2016, Yan et al. 2020). Studies have shown that sustained aerobic exercise can improve cardiovascular function, increase heart rate reserve (Thompson 2004), and affect anti-platelet aggregation and vascular spasm, reduce the risks of atherosclerosis (Sanchez-Muniz et al. 2003), coronary heart disease (Arita et al. 2001), hypertension (Arita et al. 2001, Waller et al. 2020) and other diseases. Because of the beneficial effects of aerobic exercise, it has been speculated whether the harmful effects of SO₂ can be offset if aerobic exercise is carried out in environments...
with SO2 pollution. However, previous studies have shown that, not only can it not be offset, but aerobic exercise under the condition of SO2 exposure will aggravate the negative effects of SO2 (Chen et al. 2018, Kleinman 1984). This may associate with the increased ventilation rates and greater doses of SO2 inhalation (Chen et al. 2018). Our previous study found that (Kleinman 1984), increased angiotensin II type 1 receptor (AT1R) and connective tissue growth factor (CTGF) expression in myocardium may contribute to the myocardial fibrosis and the decreased cardiac function for rats exercising in environments with SO2 pollution. The combined effects of exercise and SO2 exposure on the cardiovascular function, and the related mechanisms still remain to be fully understood.

Renin-angiotensin system (RAS) is an independent system in the heart, which participates in the regulation of local cellular endocrine, autocrine and paracrine, and is based on renin, angiotensinogen, angiotensin II (Ang II), angiotensin converting enzyme (ACE) and its receptors. The RAS play important role in maintaining blood pressure, water and electrolyte balance, and cardiovascular homeostasis (Cosarderelioglu et al. 2020). While, the combined effects of exercise and SO2 exposure on RAS have not been explored so far.

Therefore, in this study, by using on-line hemodynamic parameters monitoring method, we observed the changes of aortic pressure and left ventricular pressure for rats exercising in environments with SO2 pollution, the concentration of Ang II in myocardial tissue were measured by using immunoradiometric assay method, the concentration and activity of ACE were detected with western blotting method and hippuroyl-glycyl-glycocoll (HGG) decomposition method respectively, the purpose of the study is to further explore the combined effects of exercise and SO2 exposure on the cardiovascular function as well as the underlying mechanisms.

MATERIALS AND METHODS
Experimental animals and grouping
Eight-week-old male Sprague-Dawley rats (180-200g) used for this study were purchased from Experimental Animal Department of Peking University Health Science Center [License No.: SCXK (Jing) 2006-0008]. All experiments were conducted following the “Guide for the Care and Use of Laboratory Animals” and approved by the Ethics Committee of Beijing Normal University (LUNLI20130901). Rats were maintained on a 12 h-light /12 h-dark schedule with food and water ad libitum. 24 rats were divided into 4 groups randomly: rest group (RG), exercise group (EG), SO2 pollution group (SG) and SO2 pollution + exercise group (SEG) (n=6 in each group). The sample size used in this study is calculated by the “resource equation” method (Charan & Kantharia 2013). According to this method, a value “E” is measured by following formula: E = Total number of animals-Total number of groups, the value of E should lie between 10 and 20. In this study E will be E= (6×4)-4, which is the acceptable limit and hence can be considered as adequate sample size. Rats of RG and EG were placed in an environment with pollution-free fresh air, while rats of SG and SEG were placed in an environment with artificial simulation of SO2 pollution (concentration=10 mg/m3, 1 h / day×7 days/week×4 weeks) (Chen et al. 2018), the concentration of SO2 in the simulation chamber was controlled by adjusting the ratio of pure SO2 (purchased from Beijing Hua-Yuan Industrial Gases Co., Ltd.) to air, and tested with a portable SO2 detector.
Exercise protocol
Rats of EG and SEG were required to conduct exercise on a custom-designed electric running wheel under their respective environments, running speed was 8m/min, exercising for 1 h/day, 7 days a week for 4 weeks (Liu et al. 2009).

Monitoring of cardiac hemodynamic parameters
All rats undertook measurement of cardiac hemodynamic parameters 48 h after 4 weeks’ treatment. Rats were anesthetized with 2% sodium pentobarbital (3ml/kg), and placed supine on an operating board, the neck skin was incised, blunt separation of subcutaneous tissue and muscle was conducted. After isolating the right common carotid artery, we ligated the artery near the distal end and clamped the proximal end, a polyethylene tube filled with sodium citrate (3.8g/mL) was guided into the aorta through an incision, the other side of the polyethylene tube was connected to the multi-channel physiological signal acquisition and processing system (RM6240B, Chengdu Instrument Factory, China), the clamp was removed, 1 min after stabilization, the systolic blood pressure, diastolic blood pressure, pulse pressure and heart rate (HR) were recorded for 2 min with a sampling frequency of 500 Hz. Then the polyethylene tube was inserted into the left ventricle, the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum rising rate of left intraventricular pressure (+dp/dtmax), and maximum falling rate of left intraventricular pressure (-dp/dtmax) were recorded with the same recording parameters. After measuring, the hearts of rats were collected and stored at -20 °C until use.

Determination of Ang II concentration in myocardial tissue
One hundred mg left ventricular tissue was homogenized in ice-cold 0.9% saline solution, put the homogenate into a precooled test tube containing 3.0 mmol/L disodium EDTA, 3.4 mmol/L 8-hydroxyquinoline and 3.2 mmol/L dimercapropanol, the mixture was boiled for 10 min, then it was cleared by centrifugation at 3000rpm for 10 min; the supernatant was collected and stored at -20 °C. The concentration of Ang II in the supernatant was tested by immunoradiometric assay method, following the protocols provided by the kits manufacturer (Beijing North Institute of Biological Technology, Beijing, China).

Measurement of ACE concentration
The concentration of ACE in myocardial tissue was determined by western blotting. 200 mg tissue samples were homogenized in lysis buffer (2ml/100mg), then it was centrifuged at 4°C, 12000rpm for 10 min. We collected the supernatant, protein quantitation was determined by using bicinchoninic acid method (BCA) according to the procedure described by the kits manufacturer (Wuhan Boster Biological Technology Co., Ltd.), 20 μg of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred overnight to nitrocellulose membranes. Nonspecific binding of antibodies was blocked with 5% non-fat dry milk. Membranes were then probed with goat anti-rat ACE polyclonal antibody (1:500, Santa Cruz Biotechnology, USA). After three washes for 10 minutes each in PBS-TW20, horseradish peroxidase (HRP) labeled rabbit anti-goat secondary antibody (1:1000, Beijing Zhong Shan Golden Bridge Biotech Co., Ltd, China) was applied for 1 h. The section was stained with diaminobenzidine tetrahydrochloride (DAB),
and analyzed with Quantity One software (Bio-Rad Laboratories, USA).

**Measurement of ACE activity**
One hundred mg left ventricular tissue was homogenized in ice-cold PBS, then it was centrifuged at 4°C, 4000rpm for 10 min. The activity of ACE was measured by hippuroylglycyl-glycocoll (HGG) decomposition method following the procedure provided by kits manufacturer (Navy General Hospital, China).

**Statistical analysis**
All the data were presented as mean ± SD, statistical analysis was performed using SPSS Statistics 13.0, the normality of the data distribution was assessed by the Shapiro-Wilk’s test, the data showing normal distribution was assessed with one-way analysis of variance and post hoc Tukey’s test. For variables with non-normal distribution, Kruskal-Wallis test and post hoc Dunn’s test were used. *P*<0.05 was considered to be statistically significant.

**RESULTS**

**The effects of exercise on aortic pressure, left ventricular pressure, Ang II concentration, ACE concentration and ACE activity**
As shown in Table I, compared with RG, the aortic systolic blood pressure and pulse pressure of EG increased significantly (*P*<0.01), while diastolic blood pressure and HR decreased significantly (*P*<0.01, *P*<0.05). In Table II, the LVSP, +dp/dtmax, -dp/dtmax of EG are significantly higher than that of RG (*P*<0.01). In Figure 3, the ACE activity of EG is significantly lower than that of RG (*P*<0.05).

**The effects of SO2 exposure on aortic pressure, left ventricular pressure, Ang II concentration, ACE concentration and ACE activity**
As seen in Table II, Figure 1, Figure 2 and Figure 3, compared with RG, the LVEDP of SG increased significantly (*P* <0.05), the +dp/dtmax, -dp/dtmax of SG are significantly lower than that of RG (*P*<0.01), the Ang II concentration, ACE concentration and ACE activity of SG all increased significantly compared with that of RG (*P*<0.01). Compared with EG, the systolic blood pressure and pulse pressure of SG decreased significantly (*P*<0.01), the diastolic blood pressure and HR of SG increased significantly (*P*<0.01, *P*<0.05), the LVEDP of SG increased significantly (*P*<0.01). The LVSP, +dp/dtmax, -dp/dtmax of SG are significantly lower than that of EG (*P*<0.01). Compared with EG, the Ang II concentration, ACE concentration and ACE activity of SG all increased significantly (*P*<0.01).

<p>| Table I. Comparison of aortic pressure between different groups (n=6). |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Pulse pressure (mmHg)</th>
<th>HR (b/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>106.19±3.49</td>
<td>85.51±4.58</td>
<td>20.69±2.29</td>
<td>424±17</td>
</tr>
<tr>
<td>EG</td>
<td>119.81±4.20**</td>
<td>64.18±3.24**</td>
<td>55.64±5.41**</td>
<td>409±12*</td>
</tr>
<tr>
<td>SG</td>
<td>107.79±3.78▲▲</td>
<td>84.16±5.20▲▲</td>
<td>23.64±2.93▲▲</td>
<td>428±14▲▲</td>
</tr>
<tr>
<td>SEG</td>
<td>97.04±2.94***▲▲</td>
<td>85.28±2.27▲▲</td>
<td>11.76±2.21***▲▲</td>
<td>466±12***▲▲</td>
</tr>
</tbody>
</table>

RG=rest group; EG=exercise group; SG=S02 pollution group; SEG=S02 pollution + exercise group; HR=heart rate. *P*<0.05 vs RG, **P*<0.01 vs RG, ***P*<0.01 vs EG, ▲▲P*<0.01 vs SG.
Table II. Comparison of left ventricular pressure between different groups (n=6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>+dp/dtmax (mmHg/s)</th>
<th>-dp/dtmax (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>114.55±2.29</td>
<td>2.69±0.70</td>
<td>3320.86±140.01</td>
<td>-3424.35±159.60</td>
</tr>
<tr>
<td>EG</td>
<td>130.19±3.09**</td>
<td>2.33±0.51</td>
<td>4717.46±242.71***</td>
<td>-4917.17±576.72**</td>
</tr>
<tr>
<td>SG</td>
<td>114.81±2.98▲▲</td>
<td>3.95±0.90*▲▲</td>
<td>2975.62±255.22***</td>
<td>-2629.47±101.71***</td>
</tr>
<tr>
<td>SEG</td>
<td>100.88±3.26**▲▲</td>
<td>4.97±1.06***</td>
<td>2473.25±200.61***</td>
<td>-2244.44±259.23***</td>
</tr>
</tbody>
</table>

RG=rest group; EG=exercise group; SG=SO2 pollution group; SEG=SO2 pollution + exercise group; LVSP=left ventricular systolic pressure; LVEDP=left ventricular end-diastolic pressure. *P<0.05 vs RG, **P<0.01 vs RG, ▲▲P<0.01 vs EG, #P<0.05 vs SG, ##P<0.01 vs SG.

Figure 1. The comparison of Ang II concentration in rats’ myocardial tissue between different groups. RG=rest group, EG=exercise group, SG=SO2 pollution group, SEG=SO2 pollution + exercise group (each group, n=6). **P<0.01 vs RG, ▲▲P<0.01 vs EG, ##P<0.01 vs SG.

Figure 2. Western blot (WB) analysis of the angiotensin converting enzyme (ACE) concentration in rats’ myocardial tissue. a) Representative gel electrophoresis images of ACE. b) Statistical comparison of ACE concentration between different groups. RG=rest group, EG=exercise group, SG=SO2 pollution group, SEG=SO2 pollution + exercise group (each group, n=6). **P<0.01 vs RG, ▲▲P<0.01 vs EG, ##P<0.01 vs SG.
The combined effects of exercise and SO$_2$ exposure on aortic pressure, left ventricular pressure, Ang II concentration, ACE concentration and ACE activity

For aortic pressure (Table I), compared with RG, EG and SG, the systolic blood pressure, pulse pressure of SEG all decreased significantly ($P<0.01$), HR increased significantly ($P<0.01$). Compared with EG, diastolic blood pressure of SEG increased significantly ($P<0.01$). For left ventricular pressure (Table II), compared with RG, EG and SG, the LVSP, +dp/dtmax and -dp/dtmax of SEG all decreased significantly ($P<0.01$), while the LVEDP increased significantly ($P<0.01$). Compared with RG, EG and SG, the Ang II concentration, ACE concentration and ACE activity of SEG all increased significantly ($P<0.01$) (Figure 1, Figure 2 and Figure 3).

DISCUSSION

Previous studies have found that aerobic exercise can lead to adaptive changes in cardiac structure and function, manifested as cardiac hypertrophy, the decrease of resting heart rate and blood pressure, the improvement of cardiac function and the enhancement of myocardial contractility (Wang 2002). Our study found that, the systolic blood pressure, pulse pressure, LVSP, +dp/dtmax and -dp/dtmax of SG increased significantly (Table I, Table II), while the diastolic blood pressure and resting heart rate decreased significantly after 4 weeks of aerobic training, indicating that the cardiovascular function was significantly improved. These changes may be related to exercise-induced increase of myocardial glycogen reserve (Wang 2001), increase of myocardial blood flow and the formation of collateral circulation (Huang et al. 2002). Guan’s study shows that, the expression of Ang II in plasma and myocardium of rats decreased significantly after 8 weeks of swimming training, in the present study we find decreased ACE activity in rats’ myocardial tissue (Figure 3). It has been demonstrated that (Fernandes et al. 2011), blockade of RAS can promote the therapeutic benefits to patients with essential hypertension and cardiovascular disease, decreased RAS levels following exercise training may be an important factor leading to the beneficial changes of cardiac function.

Our study found that (Table II), 4 weeks SO$_2$ exposure increased LVEDP of rat, decreased the +dp/dtmax and -dp/dtmax, which implies the decline of cardiovascular function.

Figure 3. The comparison of angiotensin converting enzyme (ACE) activity in rats’ myocardial tissue between different groups. RG=rest group, EG=exercise group, SG=SO$_2$ pollution group, SEG=SO$_2$ pollution + exercise group (each group, n=6). * $P<0.05$ vs RG, ** $P<0.01$ vs RG, *** $P<0.01$ vs EG, **** $P<0.01$ vs SG.
Currently, there are no studies that have clearly demonstrated the biological pathway of SO₂ exposure leading to the decline of cardiovascular function. It has been proved that, myocardial contraction significantly depends on extracellular calcium, within a certain range, the higher calcium concentration in extracellular fluid, the more calcium flow into the cell, when intracellular calcium overload occurs, it may lead to the decline of cardiac function (Valverde et al. 2006). Nie once reported that (Nie & Meng 2007), SO₂ derivatives can increase the intracellular calcium in rat myocytes, thus cause a negative inotropic effect on cardiac function, the mechanism may be related to the inhibition of Na/Ca exchanger current and enhancement of L-type calcium channel. Some studies also found that SO₂ exposure can significantly affect the activities of antioxidative enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Yan et al. 2020), resulting in oxidation/reduction imbalance. Provocation of systemic inflammation and oxidative stress may be another potential pathway for decline of cardiovascular function affected by SO₂ exposure. In our study (Figure 1, Figure 2 and Figure 3), we found that, SO₂ exposure cause increase of the Ang II concentration, ACE concentration and ACE activity in rats' myocardial tissue, since ACE can catalyze the conversion of Ang I to Ang II, therefore, the increase of Ang II concentration in this study may be the result of the increase of ACE concentration and activity. The increase of Ang II concentration may attach to the specific receptors of smooth muscle cells and endothelial cells, stimulate endothelin to induce coronary artery contraction, reduce capillary density and increase myocardial ischemia and hypoxia injury, and eventually leads to the decline of cardiac function.

Our results show that, among the four groups, rats of SEG have the lowest values of systolic blood pressure, pulse pressure, LVSP, +dp/dtmax and -dp/dtmax, and the highest values of HR and LVEDP. Results of RAS levels in this study show that, rats of SEG have the highest values of Ang II concentration, ACE concentration and ACE activity, this may be closely related to the increased ventilation rates and greater doses of SO₂ inhalation (Chen et al. 2018). To the best of our knowledge, this is the first demonstration of the combined effects of exercise and SO₂ exposure on RAS. The results further demonstrate that, compared with single SO₂ exposure, 4 weeks of SO₂ exposure combined with exercise can aggravate the negative effects of SO₂ inhalation on cardiovascular function, changes of RAS play important role in mediating the negative effect.

CONCLUSIONS

By observing the changes of aortic pressure and left ventricular pressure in rat, the present study found that the combination of aerobic exercise and SO₂ exposure can aggravate the negative effects of SO₂ inhalation on cardiovascular function. The increased ACE concentration and activity in myocardial tissue may result in increase of Ang II concentration, which plays an important role in mediating the negative effect of SO₂ inhalation.

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