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Oltipraz attenuates the progression of heart failure in rats through inhibiting oxidative stress and inflammatory response

Y. TANG, M. GUO, X.-Y. MA, W.-P. SUN, M.-H. HAO, H.-Y. ZHU

Department of Cardiology, Beijing Luhe Hospital, Capital Medical University, Beijing, China

Abstract. – OBJECTIVE: To evaluate the effect of oltipraz (OPZ) on isoproterenol-induced heart failure (HF) and heart function. We also explore the underlying molecular mechanism of OPZ.

MATERIALS AND METHODS: The rats were randomly divided into four groups, including normal control group, isoproterenol (ISO) group, ISO +100 mg/kg OPZ group, and OPZ group. Hemodynamic parameters, such as left-ventricular systolic pressure, were statistically analyzed. Besides, plasma levels of brain natriuretic peptide (BNP), pro-inflammatory cytokines and antioxidant markers were assessed by using enzyme-linked immunosorbent assay (ELISA). Moreover, histopathological examination was applied to assess the degree of cardiac interstitial fibrosis.

RESULTS: OPZ could statistically improve the hemodynamic parameters of the heart function, and could also obviously attenuate cardiac interstitial fibrosis in ISO-induced HF rats when compared with the ISO group. Besides, plasma level of BNP in ISO +100 mg/kg OPZ group dramatically decreased in comparison with that of ISO group. Moreover, compared with ISO group, OPZ treatment significantly reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). Moreover, OPZ treatment remarkably increased the levels of antioxidant markers such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in ISO-induced HF rats.

CONCLUSIONS: OPZ administration may provide experimental evidence for the possible effect of OPZ on isoproterenol-induced heart failure in rats. Moreover, OPZ administration may have potential utility for the treatment of heart failure.

Key Words:
Heart failure, Oltipraz, Brain natriuretic peptide, Antioxidant, Inflammatory.

Introduction

Heart failure (HF) affects more than 37.7 million patients throughout the world[1]. About 17.3 million people died from heart diseases in 2013, which increased by 41% since 1990[2]. The increased burden of heart diseases is mainly due to the aging of the total population[3]. Clinically, pharmacological therapies, including angiotensin receptor blockers, beta-blockers, and mineralocorticoid receptor antagonist[4], are commonly used in HF patients to control symptoms. However, there are no effective treatments for HF, and the above drugs have also exhibited extensive side effects and drug resistance[5]. Due to the high morbidity and mortality of HF, there is a tremendous need for searching new and safe treatments to improve the outcomes of HF patients. The pathogenesis and progression of HF are attributed to increased reactive oxygen species[6] and lipid peroxidation markers, such as malondialdehyde (MDA). It is also attributed to decreased antioxidant defenses, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx)[7]. Moreover, increased pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are detected in HF patients[8,9]. Nuclear factor erythroid derived 2-related factor (Nrf2) is an important transcription factor that regulates intracellular antioxidant response[10]. In recent years, many researches[11-13] have shown that Nrf2 activation can protect neurons in various diseases. Oltipraz (OPZ), an agonist of Nrf2, is a drug commonly used for schistosomiasis treatment by reducing parasite glutathione. Researchers[14,15] have shown that OPZ structure is similar to that of reduced glutathione, which is an antioxidant with scavenging effects on free radicals. However, it remains unclear whether OPZ has the ability to alleviate chronic HF. In this study, we investigated the potential therapeutic effect of OPZ on HF, and detected plasma level of brain natriuretic peptide (BNP) and the degree of cardiac interstitial fibrosis after OPZ treatment in ISO-induced HF rats. Besides, the study also examined whether

Corresponding Author: Hongyan Zhu, MM; e-mail: tangyu7810@126.com

the effect of OPZ was mediated by antioxidant and anti-inflammatory activities.

Materials and Methods

Construction of the Animal Model

Adult Sprague Dawley (SD) rats weighing 250-300 g were selected in this study for the following experiments. The rats were maintained in a comfortable environmental condition in the Animal Research Center of Capital Medical University. This study was approved by the Animal Ethics Committee of Capital Medical University Animal Center. All rats were randomly divided into 4 groups, namely control group, ISO group, OPZ group and ISO+100 mg/kg OPZ group, with 12 rats in each group. Rats in control group and ISO group were orally administrated with 5 mL/kg saline for 14 days. From the 8th day, rats in control group and ISO group were subcutaneously injected with 2 mL/kg saline and 5 mg/kg isoproterenol once at an interval of 24 h for the following 7 consecutive days. Meanwhile, rats in OPZ group and ISO+ OPZ group were orally given to 100 mg/kg OPZ for 14 days. On the 8th day, rats in OPZ group were subcutaneously injected with 2 mL/kg saline once. Rats in ISO+ OPZ group were additionally injected with 5 mg/kg isoproterenol once at an interval of 24 h for the following 7 consecutive days.

Hemodynamic Measurements

At the end of the experiment, all rats were anesthetized with urethane (1.2 g/kg, i.p). To evaluate the function of left ventricle, polyethylene catheters (PE50) filled with heparin saline (500 U/mL) were inserted into the right carotid artery to flow into the left ventricle. Meanwhile, PE50 filled with heparin saline were linked to BL-420E+ Biological Data Acquisition & Analysis Class (Chengdu TME Technology Co, Ltd. Sichuan, China). The maximum rate of the left ventricular pressure (LV dP/dt_{max}), the minimum rate of the left ventricular pressure (LV dP/dt_{min}) and the left-ventricular systolic pressure (LVSP) were continually recorded for 10 min. Subsequently, the catheters were pushed into the carotid artery to monitor systemic arterial pressure (SAP) and mean arterial pressure (MAP).

Reagents and Drugs

Dimethyl sulfoxide (DMSO), potassium phosphates, propidium iodide, Tris-HCl and Triton X-100 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Oltipraz (purity > 99.9%) and isoproterenol hydrochloride were purchased from Shanghai Pharmaceutical Co. (Shanghai, China).

Histopathological Examination

Cardiac apex of the rats were harvested, washed with normal saline immediately and then fixed in neutral formalin. Subsequently, tissues were embedded in paraffin wax, cut into 5 mm sections and finally stained with hematoxylin and eosin (HE) (R&D Systems, Minneapolis, MN, USA). The ventricular cross sections were randomly divided into four fields, and one of the four quadrants was selected and counted. The counting protocol was proceeded to compare the difference of fibrosis among different groups[16].

Measurement of Plasma Biomarkers

Plasma levels of BNP, MDA (Bioassay Technology Laboratory, Shanghai, China), TNF-α (Glory Science, Del Rio, TX, USA), and IL-1β (Boster Biological Technology, Wuhan, China) were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions. Meanwhile, plasma levels of SOD and GRx were measured by using the relative chemical kit (Biorexfars, Shiraz, Iran).

Statistical Analysis

Statistical analysis was performed by using the Statistical Product and Service Solutions (SPSS) 22.0 Software (IBM, Armonk, NY, USA). All data were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance was used to compare the difference among groups, followed by Bonferroni-Dunn correction. p values < 0.05 were considered statistically significant.

Results

ISO Induced Heart Dysfunction in Rats

After subcutaneous injection of 5 mg/kg ISO in rats, the minimal decrease rate of the left ventricular pressure (-LVd P/dt_{min}) increased, while heart rate, SAP, MAP, LVSP and the maximal increase rate of the left ventricular pressure (+$LVdp/dt_{max}$) significantly decreased (Table I). Besides, plasma level of BNP in ISO-treated rats dramatically increased than that of the control rats (Table I). The-

Table I. Haemodynamic parameters and BNP between the ISO group and the control group.

Group	Heart rate	SAP (mmHg)	MAP (mmHg)	LVSP (mmHg)	+LVdp/dt$_{max}$ (mmHg/s)	LVdp/dt$_{min}$ - (mmHg/s)	BNP (ng/L)
Control	409±21	130.80±5.45	94.45±2.45	142.35±8.23	6635.45±460.13	-4620.34±785.66	120.75±5.76
ISO	312±18*	114.82±6.85*	74.32±3.12*	125.32±6.93*	5637.75±433.45*	-3422.21±543.65*	154.34±7.65*

*$p<0.05$, compared with the control group.

se results indicated the successful construction of ISO-induced HF model in rats, manifesting as systolic and diastolic dysfunction in rats.

OPZ Improved the Heart Function of ISO-Induced HF Rats

Heart rate, SAP, MAP, LVSP, -LVd P/dt$_{min}$ and +LVdp/dt$_{max}$ of OPZ group (100 mg/kg) were not significantly different from those of /normal control group (Table II). Meanwhile, rat heart failure was interfered by ISO treatment. After OPZ intervention, -LVdp/dt$_{min}$ significantly decreased, whereas heart rate, SAP, MAP, LVSP and +LVdp/dt$_{max}$ dramatically increased when compared with those of ISO group (Table II). Moreover, plasma level of BNP in ISO+OPZ 100 mg/kg group significantly decreased in comparison with that of ISO group. However, no significant differences were found in the plasma level of BNP between control group and OPZ group (100 mg/kg) (Table II).

OPZ Attenuated Cardiac Interstitial Fibrosis in ISO-Induced HF Rats

Isoproterenol can result in significant proliferation of myocardial collagen fibers. As shown in our study, ISO treatment could significantly aggravate the degree of cardiac interstitial fibrosis in rats when compared with that of control group. Besides, histopathological examination also demonstrated that OPZ could attenuate cardiac interstitial fibrosis in ISO-induced HF rats in comparison with ISO group. However, no significant difference in cardiac interstitial fibrosis was found between control group and OPZ group (100 mg/kg) (Figure 1).

OPZ Altered the Plasma Level of IL-1β and TNF-α in Rats

As shown in Figure 2, plasma levels of IL-1β and TNF-α in ISO group were significantly higher than those of normal control group. Meanwhile, ELISA results also demonstrated that OPZ could reduce the levels of IL-1β and TNF-α in ISO-induced HF rats when compared with those of ISO group. Moreover, no differences in plasma levels of IL-1β and TNF-α were found between control group and OPZ group (100 mg/kg).

OPZ Changed the Plasma Levels of SOD, GRx and MDA in Rats

As shown in Figure 3, plasma levels of SOD and GRx significantly decreased in ISO group than those of control group, whereas MDA was significantly elevated in ISO group. Besides, ELISA also demonstrated that OPZ could increase the levels of SOD and GRx, and reduce MDA level in ISO-induced HF rats when compared with those of ISO group. However, there was no significant differences in plasma levels of SOD, GRx and MDA between the control group and the OPZ group (100 mg/kg).

Table II. Haemodynamic parameters and serum BNP among the four groups.

Group	Heart rate	SAP (mmHg)	MAP (mmHg)	LVSP (mmHg)	+LVdp/dt$_{max}$ (mmHg/s)	LVdp/dt$_{min}$ - (mmHg/s)	BNP (ng/L)
Control	409±21	130.80±5.45	94.45±2.45	142.35±8.23	6635.45±460.13	-4620.34±785.66	120.75±5.76
OPZ	413±34	125.85±5.18	104.99±4.43	138.35±9.83	6487.45±560.83	-4721.45±685.45	114.57±6.86
ISO	312±18*	114.82±6.85*	74.32±3.12*	125.32±6.93*	5637.75±433.45*	-3422.21±543.65*	154.34±7.65*
ISO+OPZ	388±23#	131.33±5.98#	96.44±5.82#	145.25±7.94#	6617.45±513.34#	-4426.65±743.56#	116.38±5.95#

*$p<0.05$, compared with the control group; #$p<0.05$, compared with the ISO group.

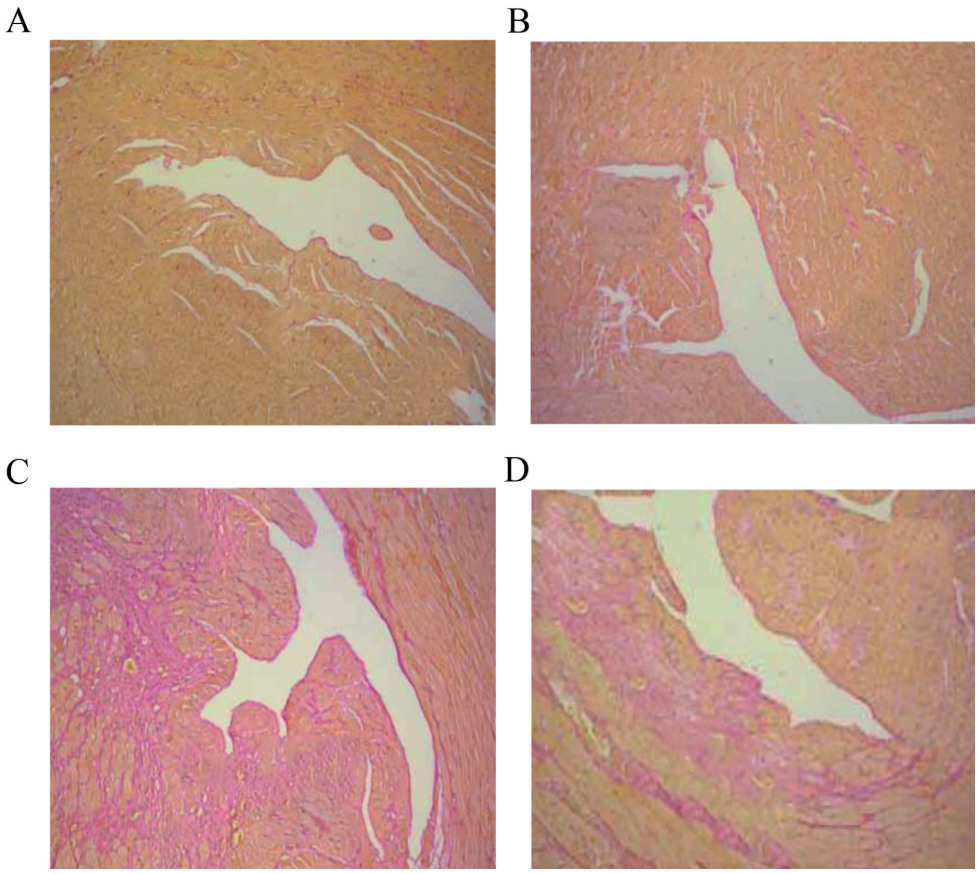

Figure 1. Effects of OPZ on cardiac interstitial fibrosis. Histopathological examination was performed to detect the effect of OPZ on attenuating cardiac interstitial fibrosis in ISO-induced HF rats in comparison with the ISO group (*x*20). ***A,*** control group. ***B,*** OPZ 100 mg/kg group. ***C,*** ISO group. (D) ISO+100 mg/kg OPZ group.

Discussion

In the present study, we evaluated the potential therapeutic effect of OPZ on HF. Our results suggested that OPZ treatment could attenuate HF. It was found that OPZ significantly reduced -LVdp/dt_{min} and plasma level of BNP, while dramatically increased heart rate, SAP, MAP, LVSP and +LVdp/dt_{max} when compared with those of ISO group. Besides, histopathological examination also demonstrated that OPZ could attenuate cardiac interstitial fibrosis in ISO-induced HF rats in comparison with ISO group. Moreover, OPZ treatment significantly reduced the levels of pro-inflammatory cytokines such as TNF-α and IL-1β, whereas remarkably increased the levels of antioxidant markers, including SOD and GRx in ISO-induced HF rats when compared with ISO group. No significant differences were found in hemodynamic parameters, plasma BNP level, cardiac interstitial fibrosis, inflammation and oxidative stress related indicators between control group and OPZ group (100 mg/kg). HF is a major clinical problem with high morbidity and mortality worldwide, which is characterized by impaired contraction and/or the relaxation of the affected ventricles[17]. Many therapeutic targets have been explored for the treatment of HF. However, current exploration of these targets remains insufficient. Isoproterenol has been used to induce heart failure in rats for many years[18]. Cardiac morphologic and pathophysiological alterations in HF rat model are comparable with human HF. Previous studies have shown that 7-day subcutaneous injection of 5 mg/kg isoproterenol can lead to cardiac dysfunction, hypertrophy and severe myofibrillar degeneration[19]. Serum concentrations of oxidative stress markers and pro-inflammatory cytokines can predict the severity of HF because they are correlated with the severity of HF[20,21]. Our findings showed that the present HF model is associated with increased oxidative stress and the release of pro-inflammatory cytokines. It is suggested

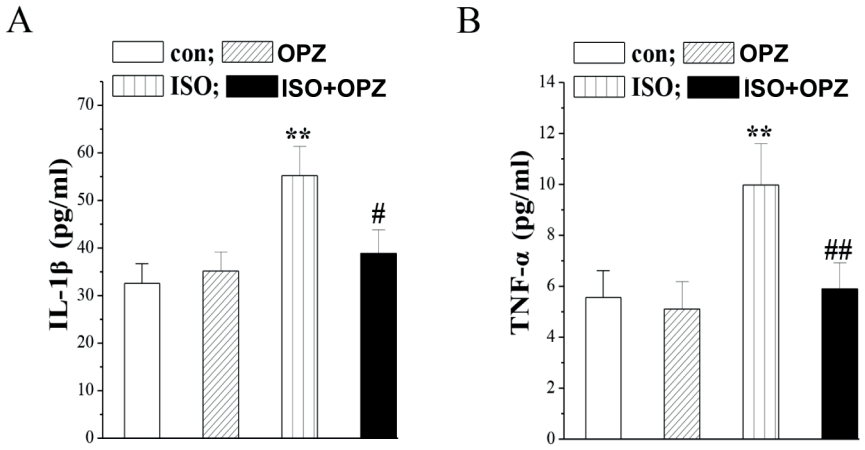

Figure 2. Effects of OPZ on plasma levels of IL-1β and TNF-α in rats. ELISA was applied to assess plasma levels of IL-1β (***A***) and TNF-α (***B***) in the four groups. Significant differences were observed when compared with the control group (**p<0.01, *p<0.05) and compared with the ISO group ($^{\#\#}p$<0.01, $^{\#}p$<0.05).

that oxidative stress contributes to the development of HF *via* numerous mechanisms, including direct cytotoxicity and negative inotropic action[22], cytokine-stimulation[23], and apoptosis[24]. Moreover, pro-inflammatory cytokines greatly influence cardiac structure and contractile function during the disease progression of HF[25,26]. Our study found for the first time that OPZ treatment in ISO-induced HF rats could attenuate heart dysfunction, such as changes of hemodynamic parameters and plasma level of BNP. Besides, OPZ treatment could alleviate cardiac interstitial fibrosis. Levels of pro-inflammatory cytokines such as TNF-α and IL-1β were significantly downregulated, whereas levels of antioxidant markers such as SOD and GRx were upregulated in ISO-induced HF rats when compared with ISO group. The close correlation between heart dysfunction and OPZ administration may provide experimental evidence for improving clinical outcomes of HF patients.

Conclusions

We observed that OPZ could ameliorate isoproterenol-induced HF in rats. However, the clear underlying mechanism of OPZ and its utility in the treatment of human HF still need to be further investigated.

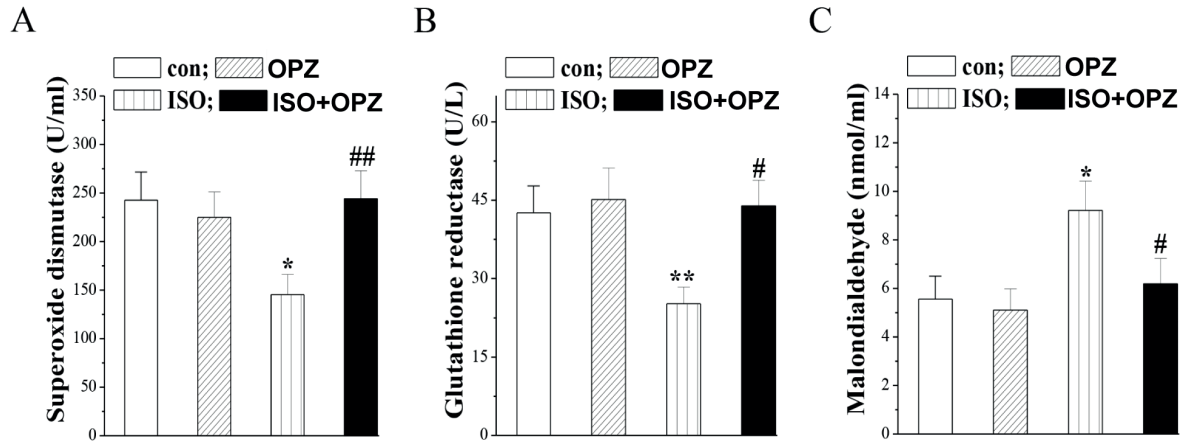

Figure 3. Effects of OPZ on plasma levels of SOD, GRx and MDA in rats. ELISA was applied to assess plasma levels of SOD (***A***), GRx (***B***) and MDA (***C***) in the four groups. Significant differences were observed when compared with the control group (**p<0.01, *p<0.05) and compared with the ISO group ($^{\#\#}p$<0.01, $^{\#}p$<0.05).

Conflict of Interest

The Authors declare that they have no conflict of interest.

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