SPANDIDOS PUBLICATIONS

Effects of propofol on myocardial ischemia-reperfusion injury in rats with type-2 diabetes mellitus

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Abstract. The current study aimed to examine the effects of propofol on myocardial ischemia-reperfusion injury (MIRI) in rats with type-2 diabetes mellitus (T2DM) and to assess the role of inflammatory mediators. Fifty healthy male adult Sprague-Dawley rats were randomly divided into the sham, ischemia-reperfusion (IR), IR plus low, middle and high-dose (6, 12 and 24 mg/kg/h, intravenous) propofol groups. The rats of all the groups were fed a high-sugar and high-fat diet for 8 weeks and streptozotocin (30 mg/kg, intraperitoneally) was used to establish the T2DM model. Apart from the sham group rats, MIRI was induced by ligating the left anterior descending coronary artery for 30 min, followed by reperfusion for 2 h. Heart rate (HR), left ventricular systolic pressure (LVSP), and the rate of left ventricular pressure increase in early systole ($\pm$ dp/dt_{max}) were recorded. Levels of cardiac troponin T (cTnT), nitric oxide (NO), endothelin-1 (ET-1), interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were also measured. Myocardial lesions were observed under light microscopy and scanning electron microscopy. Compared with levels prior to arterial occlusion, HR, LVSP, and $\pm$ dp/dt_{max} were significantly reduced ($P<0.05$) following occlusion for 30 min and reperfusion for 2 h. The administration of propofol ameliorated the cardiac function of rats as reflected by the increase in HR, LVSP and $\pm$ dp/dt_{max}. In addition, the administration of propofol increased the serum NO concentration, and reduced ET-1 and cTnT levels, as well as levels of inflammatory mediators including IL-1β, IL-6 and TNF-α. Thus, propofol exerts protective effects against MIRI in T2DM rats by increasing NO and reducing ET-1 and the inflammatory mediators.

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Introduction

The incidence of type-2 diabetes mellitus (T2DM) is on the increase worldwide (1). The number of people succumbing to coronary heart disease is greatest in Europe, America, and urban areas of China. The number of T2DM patients succumbing to coronary heart disease is 2- to 4-fold that of non-T2DM patients (2). Previous findings have shown that the release of inflammatory mediators is closely associated with T2DM and myocardial ischemia-reperfusion injury (MIRI) (3-5). Inflammatory mediators may increase MIRI in patients with type-2 diabetes (6).

As a widely used intravenous anesthetic, propofol to some extent (7) protects the heart against IRI and reduces the expression of inflammatory mediators (8-10). Nevertheless, the protective effects of propofol against MIRI in T2DM rats and the effect of inflammatory mediators have not been investigated. Therefore, the aim of the study was to evaluate the effects of propofol on MIRI in T2DM rats and to determine the role of inflammatory mediators.

Materials and methods

A total of 50 healthy male Sprague-Dawley rats (6-8 weeks, 200-220 g) were provided by the Experimental Animal Center of Hebei Province, Shijiazhuang, China [certificate no. SCXK (Ji) 2013-1-1003]. The experiment was approved and carried out in accordance with the guidelines of Hebei University. The experimental protocols were performed in adherence with the Institutional Animal Care and Use Committee of Hebei University (Baoding, China).

All the rats were fed a high-sugar and high-fat diet at 22˚C and humidity of 50%. The feeding was provided by the Experimental Animal Center of Hebei Province (Shijiazhuang, China). After a period of eight weeks, streptozotocin 30 mg/kg body weight (Solarbio, Beijing, China) was injected into the abdomen to establish the T2DM model. It has been detected that fasting glucose is ≥14 mol/l for the preparation of a successful model.

The rats were randomly divided into five groups (n=10/group): i) Sham-operated group (sham), ii) ischemia-reperfusion (IR) and iii-v) IR plus low, middle and high-dose propofol (IR+L, M, H Pro). MIRI was induced

Table I. Hemodynamic indices of myocardial ischemia-reperfusion injury in rats with type-2 diabetes mellitus (n=10, means ± SD).

Variable	Group	Before ligation	After 30 min of ischemia	After 2 h of reperfusion
Heart rate	Sham	424±15	433±27	421±8
	IR	423±19	384±18[a,d]	217±28[a,d]
	IR+low-dose propofol	418±19	401±38[a,b,d]	242±37[a,b,d]
	IR+middle-dose propofol	420±18	411±10[a,c,d]	262±39[a,c,d]
	IR+high-dose propofol	419±16	398±45[a,b,d]	245±26[a,d,b]
LVSP (mmHg)	Sham	124±12	120±9	116±9
	IR	123±9	98±6[a,d]	68±7[a,d]
	IR+low-dose propofol	122±11	106±4[a,b,d]	78±5[a,b,d]
	IR+middle-dose propofol	123±10	112±10[a,c,d]	80±8[a,c,d]
	IR+high-dose propofol	121±15	110±5[a,b,d]	75±10[a,b,d]
+ dp/dt_{max}	Sham	3409±177	3384±141	3453±180
	IR	3348±167	2583±315[a,d]	2243±359[a,d]
	IR+low-dose propofol	3385±246	2735±436[a,b,d]	2696±217[a,b,d]
	IR+middle-dose propofol	3392±321	2881±356[a,c,d]	2755±312[a,c,d]
	IR+high-dose propofol	3378±259	2801±345[a,b,d]	2522±215[a,b,d]
- dp/dt_{max}	Sham	3819±144	3582±268	3548±396
	IR	3883±93	2620±186[a,d]	2232±427[a,d]
	IR+low-dose propofol	3887±174	2830±484[a,b,d]	2781±430[a,b,d]
	IR+midle-dose propofol	3822±124	2845±320[a,c,d]	2788±562[a,c,d]
	IR+high-dose propofol	3857±152	2785±182[a,b,d]	2635±352[a,b,d]

Compared with sham group, [a]P<0.05; compared with IR group, [b]P<0.05, [c]P<0.01; compared with before,[d]P<0.05. IR, ischemia-reperfusion; SD, standard deviation.

by ligating the left anterior descending coronary artery for 30 min, followed by reperfusion for 2 h. The rats in the sham group received an intravenous infusion of physiologic (0.9%) saline (3 mg/kg/h) for 10 min without ligation. In the IR group, the rats received an intravenous infusion of physiologic saline (3 mg/kg/h) for 10 min before IR. In the IR+L, M, H Pro group rats, Pro (6, 12 and 24 mg/kg/h, intravenous) was respectively administered for 10 min before IR. The rats were sacrificed after IR in the treatment groups.

The rats were anesthetized, and a polyethylene Millar catheter was inserted into the right common carotid artery and then further advanced into the left ventricular chamber, after which the cannula was connected to a pressure transducer. The heart rate (HR), left ventricular systolic pressure (LVSP), and the rate of left ventricular pressure increase in early systole (± dp/dt_{max}) were recorded by an 8-channel polygraph system (Powerlab 8s; ADInstruments, Castle Hill, New South Wales, Australia). The levels of cardiac troponin T (cTnT), nitric oxide (NO), endothelin-1 (ET-1), interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α were respectively measured using an enzyme-linked immunosorbent assay. Myocardial lesions were observed under light microscopy and scanning electron microscopy.

Statistical analysis. SPSS v16.0 (IBM, Armonk, NY, USA) was used for all the analyses. Hemodynamics were compared and analyzed within groups and between groups by multivariate analysis of variance. cTnT, NO, ET-1, IL-1β, IL-6, and TNF-α levels were compared using one-way ANOVA. The 95% confidence interval was used for significance.

Results

Changes in cardiac function. The HR, LVSP and ± dp/dt_{max} were significantly decreased after IR compared with those before ligation (P<0.05). No significant difference was observed in HR, LVSP and ± dp/dt_{max} before ligation. Compared with that of the sham group, HR, LVSP and ± dp/dt_{max} were significantly decreased in the IR group rats (P<0.05). Compared with those of the IR group, HR, LVSP and ± dp/dt_{max} were significantly increased in the IR+L, M, H Pro group rats (P<0.05; Table I).

Changes in NO, ET-1 and cTnT in the serum. Compared with those of the sham group, NO was reduced, and ET-1 and cTnT were significantly increased in the IR group rats (P<0.05). Compared with those of the IR group, NO was increased, and ET-1 and cTnT were significantly reduced in the IR+L, M, H Pro group rats (P<0.05; Table II).

Changes in IL-1β, IL-6 and TNF-α in the serum. Compared with those of the sham group, IL-1β, IL-6 and TNF-α were significantly increased in the IR group rats (P<0.05). Compared with those of the IR group, IL-1β, IL-6, TNF-α

Table II. Serum concentrations of NO, ET-1and cTnT (μmol/l) in the different groups (n=10, means ± SD).

Group	NO	ET-1	cTnT
Sham	83±3.2	3.90±0.25	14.60±1.0
IR	55±2.5[a]	8.45±0.32[a]	25.56±1.3[a]
IR+low-dose propofol	72±3.8[a,b]	5.52±0.31[a,b]	18.89±2.1[a,b]
IR+middle dose propofol	81±3.2[a,c]	4.33±0.42[a,c]	17.12±0.9[a,c]
IR+high dose propofol	75±3.5[a,b]	5.32±0.38[a,b]	19.75±1.2[a,b]

Compared with sham group, [a]P<0.05; compared with IR group, [b]P<0.05, [c]P<0.01. NO, nitric oxide; ET-1, endothelin-1; cTnT, cardiac troponin T; SD, standard deviation.

Table III. Serum concentrations of IL-1β, IL-6, and TNF-α (μmol/l) in the different group (n=10, means ± SD).

Group	IL-1β	IL-6	TNF-α
sham	173.19±23	177.38±15	198.21±15
IR	259.86±18[a]	253.48±16[a]	562.58±19[a]
IR+low-dose propofol	238.68±18[a,b]	216.36±19[a,b]	385.15±20[a,b]
IR+middle dose propofol	194.20±20[a,c]	191.13±18[a,c]	334.59±21[a,c]
IR+high dose propofol	217.58±21[a,b]	215.23±18[a,b]	362.31±25[a,b]

Compared with sham group, [a]P<0.05; compared with IR group, [b]P<0.05, [c]P<0.01; IR, ischemia-reperfusion; TNF-α, tumor necrosis factor-α; IL, interleukin; SD, standard deviation.

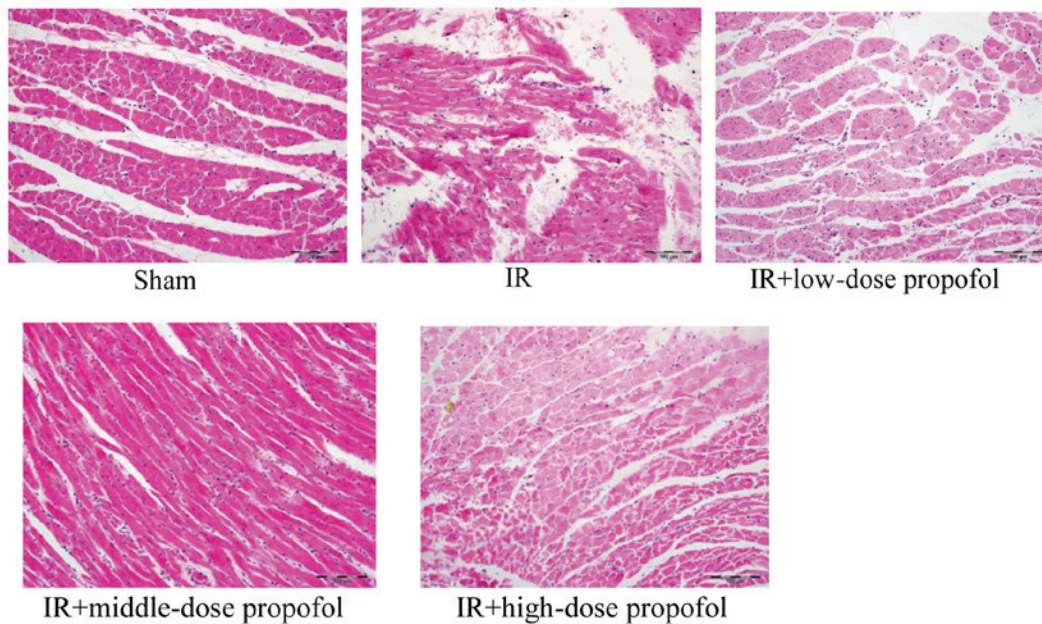

Figure 1. Pathologic alterations in heart of rats with hematoxycylin and eosin staining (magnification, x20; scale bar, 100 μm). IR, ischemia-reperfusion.

were significantly reduced in the IR+L, M, H Pro group rats (P<0.05; Table III).

Morphologic changes under light microscopy. No myocardial fibrosis and normal nuclear morphology was uniformed in the sham group. Myocardial fibers and stromal necrosis, eosinophil-enhanced muscle cells, elongated wavy/fragmented myocardial fibrosis, with most nuclei showing fragmentation and degeneration were observed in the IR group. Eosinophil-enhanced myocardial fibrosis was evident, along with elongated wavy/fragmented cardiac muscle fibres arranged in an orderly manner, with most nuclei showing pyknosis and fragmentation in the IR+low-dose propofol and IR+high-dose propofol groups. Eosinophil-enhanced myocardial fibrosis, elongation of myocardial fibers and hyperchromatic nuclei were observed in the IR+middle-dose propofol group (Fig. 1).

Ultrastrucral changes under electron microscopy. The integrity of the membrane and cristae of mitochondria and myocardial fibrosis were clearly visible, and the integrity of inner and outer nuclear membranes of nuclei was retained in the sham group rats. In the IR group, swelling of mitochondria, rupture and disappearance of mitochondrial membranes, dissolution of edema between mitochondria, absence of chromatin, disappearance of the outer nuclear envelope, disappearance of some inner and outer nuclear envelopes, and perinuclear edemawere observed. Mitochondrial degeneration and necrosis of myocardial fibers, indistinct sarcomeres, and an absence of muscle-fiber structure were revealed. In the IR+low-dose propofol and IR+high-dose propofol groups, mitochondrial edema, mitochondrial films, and the partial disappearance of mitochondria were observed. Additionally, high-density chromatin, partial disappearance of the outer layer of nuclear films (but not of nuclear film

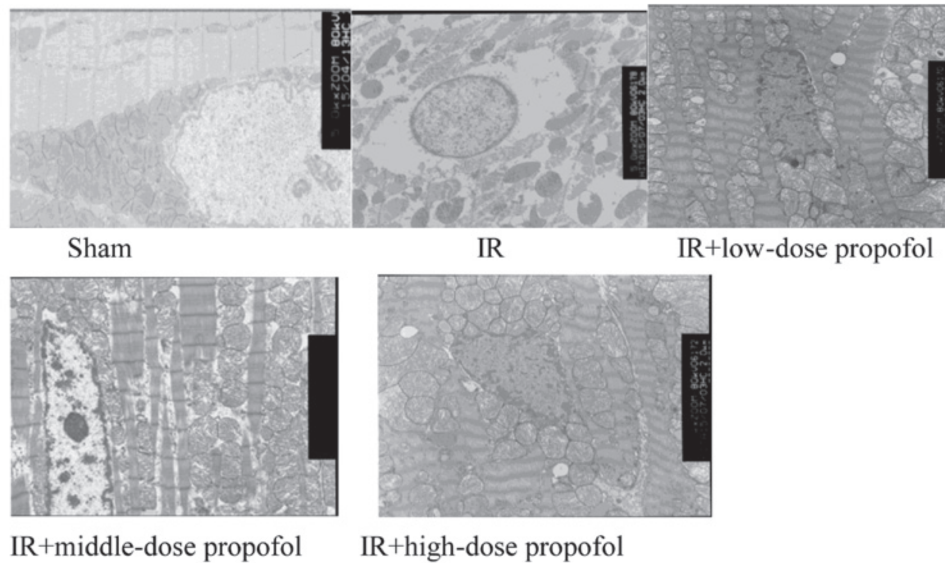

Figure 2. Ultrastructural changes in the heart of rats with electron microscopy (magnification, x3,500). IR, ischemia-reperfusion.

within layers), mild edema between muscle fibers, mild damage to myocardial fibers, and comparatively neat muscle arrangement, were observed. In the IR+middle-dose propofol group, some mitochondrial edema was present, along with chromatin-dense masses but no obvious perinuclear edema or lighter myocardial fibrosis was observed, and sarcomeres were aligned (Fig. 2).

Discussion

In the present study, our results demonstrated that propofol ameliorated cardiac function, increased serum NO and decreased ET-1 and inflammatory mediators against MIRI in T2DM rats.

In 1966, Jennings *et al* first suggested the concept of IR injury which involves destruction of the tissue structure and metabolic disorders (11). For instance, in clinical heart surgery, infarction after coronary artery ligation is observed after MIRI. Furthermore, type-2 diabetes is one of the most common endocrine metabolic diseases, and myocardial injury was increased in type-2 diabetes resulting in diabetic cardiovascular disease having the highest morbidity and mortality (12,13). In the present experiment, we used the traditional preparation methods of a type-2 diabetes model. Fasting glucose ≥14 mol/l was considered as the successful model. HR, LVSP, and ± dp/dt_{max} were significantly reduced after IR was compared with those before ligation. These results indicate that the MIRI model was successfully established.

Evidence indicates that the intravenous anesthesic propofol may inhibit lipid peroxidation, improve mitochondrial function (14), protect the myocardium and reduce MIRI in rats (15). Furthermore, it improves the function of vascular endothelial cells and promotes the expression of anti-apoptotic proteins, thus reducing MIRI in T2DM. Several clinical and biochemical indices may be used for the diagnosis of myocardial injury. cTnT is considered to be the 'gold standard' for the diagnosis of myocardial injury. In the present study, propofol decreased the cTnT concentration in serum and ameliorated cardiac function, as reflected by an increase in HR, LVSP and ± dp/dt_{max}. In addition, the myocardial damage degree was significantly decreased after the administration of propofol. Therefore, propofol has myocardial protection for type-2 diabetes rat myocardial IR.

Since 1980, Furchgott and Zawadzki identified the endothelial diastolic factor (EDRF) (16). In 1988, Yanagisiawa *et al* (17) first extracted ET from swine aortic endothelial cultures and found that vascular endothelial cells between blood circulation and vascular smooth muscle cells play an important role in regulating cardiovascular activity. EDRF is NO and it has a strong function of diastolic blood vessels and inhibits vascular smooth muscle cell proliferation and thrombosis (18). At the time of myocardial ischemia, the change of NO level was controversial. Previous findings showed that NO release was increased in coronary artery myocardial ischemia (19-21). By contrast, other authors found that NO release was decreased (22,23). The function of endothelium plays an important role in maintaining stability and normal blood flow dynamics. The key factor of its function is the NO. Vascular endothelial often constantly release NO into the vascular smooth muscle cells so as to maintain vascular tension in a moderate degree of relaxation state (24). NO exerts anti-inflammatory effects by inhibiting the neutrophil adhesion to endothelial cells and decreasing the release of inflammatory factors. An appropriate amount of NO could protect cardiomyocytes, reduce damage, inhibit intimal hyperplasia and ameliorate the heart function following IR injury. As an important regulator of cardiovascular function, ET plays a significant role in maintaining vascular tension and cardiovascular system steady state (25). Endothelial cells stimulate the synthesis and release of ET-1. ET-1 is responsible for endothelial dysfunction and inflammation and contributes to atherosclerotic plaque formation (26). ET-1 induces the left ventricular afterload increase and participates in the fibrotic process of the myocardium (27,28). Adrenaline,

thromboxane, angiotensin, insulin, inflammatory factors and hypoxia stimulated the synthesis of ET-1, and the inhibitors of ET-1 synthesis included NO, PGI2, atrial natriuretic peptide and heparin. This experiment showed that propofol could increase the NO level and decrease the serum ET-1 concentration, thus exerting the cardioprotective effects on MIRI in T2DM rats.

Studies have shown that T2DM and MIRI are associated with inflammatory mediators (6-8). The inflammatory factors potentially cause the myocardial damage in T2DM patients. Thus, inhibition of the release of inflammatory cytokines is an important strategy to protect heart against myocardial damage in T2DM patients. As an important inflammatory cytokine, TNF-α is produced mainly by the activation of monocytes/macrophages, and participates in certain autoimmune diseases (29-33). Thus, it could stimulate the NO synthase (i-NOS) to synthesize and release a large number of NO. NO and the ultra oxygen anion reaction occurs rapidly, and subsequently the oxidation ability stronger light free radicals was generated. Which could make the cell membrane lipid peroxide and the tissue damage was aggravating (34). IL-1β is produced mainly by macrophages and it was found that systemic reactions resulted from injection and secretion of large amounts of IL-1 (35). IL-6 is mainly generated by macrophages, T-cells, B-cells and other cell types. IL-6 contributed to the cachexia induced by TNF-α and IL-1, and promotes glucocorticoid synthesis. IL-6 has an important role against infection as reflected by increasing the effects of other cytokines and regulating the immune response, acute-phase response and hematopoiesis. Inflammation factor occupies an important position on MIRI. Inflammation caused by myocardial ischemia and hypoxia promotes the release of large quantities of ILs from monocytes and macrophages. As neutrophil chemotactic factors with high specificity, the ILs cause adhesion and gathering of numerous white blood cells (the obstacles against mini-circulation), an increase of active oxygen and damage myocardial cells (36-38). Inflammatory factors resulted in neutrophils releasing cytotoxicity, aggravating the inflammatory reaction, blocking blood capillaries, vascular active substances, thereby leading to acute tissue damage (38). The results of the present study have shown that propofol decreased the expression of inflammatory cytokines, such as IL-1β, IL-6, TNF-α in serum.

In conclusion, our data provide strong supportive evidence that propofol has protective effects on MIRI in T2DM rats as reflected by ameliorating cardiac function, the increasing serum NO and decreasing serum ET-1, IL-1β, IL-6 and TNF-α.

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