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Myocardial Protective Effects of Nicorandil on Rats with Type 2 Diabetic Cardiomyopathy

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Study Design A
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Statistical Analysis C
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Background: Diabetic cardiomyopathy (DCM) is a common but underestimated cause of heart failure in patients with diabetes. This study investigated the myocardial-protective effects of nicorandil (Nic) on rats with DCM.

Material/Methods: A total of forty-seven 180–220 g male Wistar rats were randomly divided into 4 groups: a control group (control, n=8), a DCM group (DCM, n=13), a nicorandil-pretreated DCM group (Nic_1, n=13), and a nicorandil-treated DCM group (Nic_2, n=13). A rat model of type 2 diabetes was induced by high-fat and high-sugar diet and intraperitoneal injection of streptozotocin (STZ). Nicorandil (3 mg/kg/d) was orally administrated to rats in the Nic_1 group starting at week 4. Nicorandil (3 mg/kg/d) was orally administrated only after the induction of diabetes in the Nic_2 group. The serum lipoids, plasma glucose, insulin levels, heart weight index, serum creatine kinase (CK), lactate dehydrogenase (LDH) levels, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) were analyzed in all groups.

Results: The DCM group showed increased heart weight index, serum LDH, CK, and MDA content and decreased serum SOD activity, as compared with the control group ($P<0.05$). The DCM-induced increases in heart weight index, serum LDH, CK, and MDA content and decrease in serum SOD activity were attenuated in both Nic_1 and Nic_2 groups ($P<0.05$). However, there was no significant difference between Nic_1 and Nic_2 groups ($P>0.05$).

Conclusions: Nicorandil has protective effects on cardiac hypertrophy in DCM rats through increased SOD activity and decreased MDA content. Therefore, nicorandil may be a therapeutic method for diabetic patients with DCM.

MeSH Keywords: Diabetes Mellitus, Type 2 • Diabetic Cardiomyopathies • Nicorandil

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Background

The prevalence of diabetes mellitus is increasing rapidly in recent decades, which affects an estimated 6.4% of adults worldwide, but this is likely to increase to 7.7% by 2030. This disease is becoming an independent major risk factor for the development of cardiovascular diseases [1,2]. Diabetes mellitus and its complications have contributed tremendously to the burden of mortality and health cost worldwide. Among the various complications of diabetes, diabetes-related heart diseases are the major causes of disability and death among diabetic patients [3]. The prevalence of heart failure is high in diabetic patients and diabetic cardiomyopathy (DCM) is a common but underestimated cause of heart failure in patients with diabetes [4]. Research on the pathogenesis of DCM has focused on myocardial metabolism disorders, calcium overload, abnormal free radical production, and insulin resistance [5]. Current therapeutic strategies for DCM in diabetic patients consist of cardiac glycoside, Ca^{2+} antagonist, β-adrenergic blocking agents, angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, and diuretics [6,7]. Despite significant progress, the incidence and mortality rates of DCM still remain high. It is imperative to develop novel and effective therapeutic strategies for DCM.

Nicorandil is an ATP-sensitive potassium channel (K^+ATP) opener containing a nitric acid group. Nicorandil with vasodilatory effects can directly activate cGMP, increase the K^+ATP opening time in cell and mitochondrial membranes, increase coronary blood flow, decrease vascular spasm, and improve microcirculation. Furthermore, Nicorandil does not cause reflex tachycardia or excess oxygen consumption, and it suppresses myocardial apoptosis and enhances ischemic preconditioning [8–10]. Nicorandil has received much research attention. However, research on the effects of nicorandil on DCM development and progression is incomplete. In the present study, we investigated the myocardial-protective effect of nicorandil on DCM in streptozotocin (STZ)-induced diabetic rats by calculating heart weight index. The possible mechanism underlying the protective effect of nicorandil was also investigated in relation to serum SOD and MDA levels in rats.

Material and Methods

Drugs

Nicorandil was supplied by Chugai Pharmaceutical Company (Japan). STZ was supplied by Sigma-Aldrich, Inc., St. Louis, MO, USA.

Induction of diabetes

Forty-seven male Wistar rats, 180–220 g, were purchased from the Animal Center of Shandong University (Jinan, China) and allowed 2 weeks for environmental and trainer-handling acclimation. The animals were housed under controlled temperature (26–28°C) and a 12 h/12 h light/dark cycle with food and water available ad libitum. All procedures complied with the protocols approved by the Institutional Animal Care Committee of Shandong University. All rats were randomly divided into 4 groups: the control group (control, n=8), the type 2 diabetic cardiomyopathy group (DCM, n=13), the nicorandil-pretreated type 2 diabetic cardiomyopathy group (Nic_1, n=13), and the nicorandil-treated type 2 diabetic cardiomyopathy group (Nic_2, n=13). Control rats were fed standard rat chow. The other 3 diabetic group rats were fed high-fat chow (ingredients including 10% refined lard, 20% sucrose, 2% cholesterol, 1% sodium cholate, and 67% standard rat chow). At 6 weeks, rats in the DCM, Nic_1, and Nic_2 groups were given a peritoneal injection of a low dose of streptozotocin (STZ, 30 mg/kg body weight; Sigma, St. Louis, MO, USA. 1% STZ diluted by 0.1 mM pH 4.2 sodium citrate buffer solution). Control group rats were injected with citrate buffer alone to serve as a normal control group. Fasting plasma glucose (FPG) was tested at 3 and 5 days after the streptozotocin injections and the rats with FPG ≥16.7 mM were considered to be diabetic. Thirty-seven rats with diabetes were established successfully (2 failed). Nicorandil 3 mg/kg/d was administered from 2 weeks before STZ injection to the end of week 16 in the Nic_1 group. Nicorandil 3 mg/kg/d was administered from the DCM model establishment to the end of week 16 in the Nic_2 group. Control and DCM group rats were given a peritoneal injection of 1 ml/d normal saline to the end of week 16.

Observation of general behavior

The general behavior of rats was observed during the experiment. The mental state, activity, coat luster, food intake, water intake, urine output, mortality, and body weight changes were recorded.

Measurement of biochemical parameters

At the end of week 4, rats were fasted for 8–12 h. Blood samples (0.5–1.0 ml) were collected from the angular vein. Glucose was measured using a Johnson Surestep glucometer. Insulin levels were detected by enzyme-linked immunosorbent assay (ELISA) according to the kit instructions. The total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined by use of an automatic biochemical analyzer.

Measurement of heart weight index

At the end of week 16, rats were fasted for 12 h and body weight (BW) was measured. Following anesthesia of rats with an intraperitoneal injection of 30 mg/kg pentobarbital sodium,

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Table 1. Comparison of serum TC, TG, and LDL-C levels and FINS and IRI between diabetic rats and control rats at the end of week 4 (means ±SD).

Groups	N	FPG (mmol/L)	FINS (ng/mL)	IRI	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Diabetic	39	5.29±0.93	1.91±0.54*	2.25±0.46*	5.89±1.88*	2.50±1.00*	1.34±0.39*	1.11±0.37
Control	8	5.02±0.72	1.04±0.28	1.59±0.26	1.84±0.48	0.81±0.33	0.31±0.09	1.09±0.19

* P<0.05 compared with control.

2 ml of blood was collected from the femoral vein and centrifuged for 15 min at 3000 rpm. The supernatant was stored at 80°C in a freezer. Rats were quickly killed with 10% potassium chloride injection and hearts were removed. Through the heart's pumping function, residual blood was pumped out into heparin normal saline at 37°C. The vessels and atrial, epicardial adipose, and fibrous tissue were cut and washed in cold saline. Along the septum, the right ventricular free wall was cut and the left ventricle (the interventricular septum was reserved) was separated. The left ventricular mass (LVW) was weighed. The heart weight index (LVW/BW) was calculated.

Measurement of serum CK, LDH, SOD activity, and MDA content

At the end of the experiment, CK and LDH enzyme activity was measured using a colorimetric assay. SOD enzyme activity was measured using the xanthine oxidase assay and thiobarbituric acid (TBA) colorimetric assay for the determination of MDA content. The operation was performed in accordance with the kit's instructions.

Statistical analysis

All analyses were performed using SPSS v11.0 (SPSS, Inc., Chicago, IL, USA). All data are represented as means ±SD. The data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant for values of P<0.05.

Results

The effects of nicorandil on the general conditions of DCM rats

The eating, drinking, normal growth, activity, responsiveness, and coat luster was observed in control rats. Initially, body mass of diabetic rats was significantly increased. After intraperitoneal STZ injection, the listlessness, dry and dull fur, and polydipsia, polyphagia, polyuria, significant weight loss, lethargy, and decreased immunity were observed. The litter needed to be changed 1 to 2 times every day. In the Nic1 and Nic2 groups, the diabetes-related syndromes were reduced compared with the DCM group. The rats in the Nic_1 and Nic_2 groups moved naturally with improved mental status.

Changes in biochemical parameters in DCM rats

There was no statistically significant difference in FPG and HDL-C levels between control and diabetic groups at the end of week 4 (P>0.05). However, the serum TC, TG, and LDL-C levels and FINS and IRI in diabetic rats were higher compared with the control rats (P<0.05, Table 1).

Effects of nicorandil on FPG and heart weight index in DCM rats

The FPG levels at the end of week 16 in DCM, Nic_1, and Nic_2 rats were remarkably increased compared with control rats (P<0.05). The heart weight index in DCM, Nic_1, and Nic_2 rats was higher than in control rats. Furthermore, the heart weight index in both Nic_1 and Nic_2 rats was lower than in DCM rats (P<0.05, Table 2).

Effects of nicorandil on serum CK, LDH, and SOD enzyme activity and MDA content in DCM rats

Compared with the control rats, the serum CK, LDH enzyme activity, and MDA content in DCM rats were enhanced at the end of week 16 (P<0.05). Compared with the DCM rats, the serum CK and LDH enzyme activity, and MDA content was reduced in Nic_1 and Nic_2 rats. The MDA content was obviously reduced in Nic_1 and Nic_2 rats (P<0.05). The SOD enzyme activity was reduced in DCM rats compared with the control rats (P<0.05). The SOD enzyme activity was enhanced in Nic_1 and Nic_2 rats compared with the DCM rats (P<0.05, Table 3).

Discussion

Diabetes is currently a serious global issue. Although it cannot be explained by hypertension, coronary disease, valvular heart disease, or other heart diseases, in diabetic patients, DCM is considered to be the primary cause of mortality among all the complications of diabetes [8]. Apart from diabetic symptoms,

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Table 2. Comparison of FPG levels and heart weight index among DCM, Nic_1, and Nic_2 rats and control rats at the end of week 16 (means ±SD).

Groups	n	FPG (mmol/L)	Heart weight index (mg/g)
Nic_1	12	22.71±3.22*	2.60±0.16*#
Nic_2	12	21.76±2.85*	2.64±0.15*#
DCM	13	23.69±3.41*	3.02±0.13*
Control	8	5.15±0.63	2.17±0.14

* P<0.05 compared with control; # P<0.05 compared with DCM.

Table 3. Comparison of serum CK, LDH, and SOD enzyme activity and MDA content among DCM, Nic_1, and Nic_2 rats and control rats at the end of week 16 (means ±SD).

Groups	n	LDH (U/L)	CK (U/L)	SOD (U/mL)	MDA (nmol/mL)
Nic_1	12	1077.25±46.89	1383.84±86.27	113.36±14.35#	7.89±0.62#
Nic_2	12	1187.43±34.99	1457.70±83.05	109.82±20.07#	8.11±1.39#
DCM	13	1612.27±52.33*	1985.07±62.91*	74.97±17.12*	10.52±1.22*
Control	8	850.37±33.40	1007.90±59.42	139.41±11.20	5.98±1.41

*P<0.05 compared with control; # P<0.05 compared with DCM.

DCM patients also have symptoms of restrictive cardiomyopathy, while the coronary arteriography results are negative [11]. Abnormal glucolipid metabolism and many other factors are involved in the morbidity of DCM [12]. In the present study, we established abnormal glucolipid metabolism model in rats by high-fat and high-carbohydrate diet and a small dose of STZ by intraperitoneal injection. We found that the levels of TINS, IRI, serum TC, TG, and LDL-C in diabetic rats were higher than those in control rats, which indicated that we successfully created insulin resistance and glucose and lipid metabolism disorders in the rat model, and it was consistent with the disease course of type 2 diabetes. At the end of the experiment, we found that the heart weight indexes of Nic_1, Nic_2, and DCM rats were increased compared to that of control group rats, which suggested that we successfully established DCM model with cardiac hypertrophy reflecting damage of diabetes to myocardium.

Nicorandil is one of K^+ATP channel openers developed in recent years with nitric acid ester function. Numerous protective effects of nicorandil on the cardiovascular system have been reported, including reduction in preload and afterload, improvement of myocardial perfusion, prevention of Ca^{2+} overload by opening K^+ATP channels, anti-inflammatory and anti-apoptosis effects, protection of mitochondria and energy-modulating function, and preservation of kidney function [13–15]. However, research on the effect of nicorandil on DCM development and progression has not been investigated in detail. The results of this study showed that the general behavior of rats in the Nic_1 and Nic_2 groups were improved by nicorandil treatment compared with that in the DCM group. Meanwhile, the heart weight index in Nic_1 and Nic_2 rats was significantly decreased, while serum LDH and CK were slightly decreased compared with that in DCM rats, which suggests that nicorandil can prevent cardiac muscle hypertrophy in rats with DCM.

Hyperglycemia can cause oxidative stress and oxygen free radical generation. The interaction between insulin resistance and oxidative stress induces the development of diabetes in a vicious cycle. The rise of redox intermediates of reactive oxygen species (ROS) levels can cause severe heart dysfunction. The increase of heart weight index in DCM rats indicated that heart cardiac hypertrophy had occurred. In the present study the redox indicators (serum SOD and MDA levels) in DCM rats showed obvious abnormalities. After application of nicorandil (Nic_1 group, Nic_2 group), the serum SOD activity was increased and the MDA content was decreased, suggesting that nicorandil protects cardiac muscle in rats with DCM by inhibition of oxidative stress. A study by Serizawa et al. showed that NADPH oxidase and nitric oxide synthase activity in STZ-induced diabetic rats can be improved by nicorandil treatment [16].

Elevated blood glucose levels caused an increase in ATP, leading to K^+ATP channel closure, resulting in cell depolarization and Ca^{2+} influx and insulin release. However, in the present study, after application of nicorandil (Nic_1 group, Nic_2 group), there was no significant deterioration in fasting blood glucose compared with the DCM group. Studies showed that potassium ion

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channels consist of different subunits and receptors [17]. The potassium channels can be regulated according to the different sensitivity in various tissues. Since the receptors of nicorandil are different from those of the pancreas potassium channel, it can be inferred that nicorandil has little effect on islet β cell K^+ATP channel and insulin secretion. Therefore, nicorandil has good tolerability in diabetic patients. Kasono et al. found that in STZ-induced diabetic rats after nicorandil intervention, there was a slight decrease in ROS *in vivo*, and islet β cells were susceptible to apoptosis due to oxidative stress and its products [18].

Conclusions

In summary, our data show that nicorandil prevents cardiac hypertrophy resulting from DCM induced by diabetes and that its protective effects are mediated by inhibition of oxidative stress. Given that it has good tolerability, nicorandil may be a therapeutic method for patients with DCM induced by diabetes.

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