Urocortin attenuates myocardial fibrosis in diabetic rats via the Akt/GSK-3β signaling pathway

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Urocortin attenuates myocardial fibrosis in diabetic rats via the Akt/GSK-3β signaling pathway

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Abstract

Objective: Urocortin, a novel identified corticotropin-releasing factor-related endocrin peptide, has been shown to play an essential role in cardioprotection. Until recently, whether urocortin can protect the heart against diabetic cardiomyopathy (DCM) remained unclear. Herein, we evaluated the cardioprotective effect of urocortin on cardiac dysfunction, inflammation, and fibrosis and demonstrated the potential mechanism in a diabetic rat model.

Methods: Diabetic rats were randomly divided into 4 groups: diabetic control group, urocortin, urocortin + astressin (a selective CRF receptor 2 antagonist) and urocortin + triciribine (an Akt pathway blocker). Cardiac catheterization was performed to evaluate cardiac function. The levels of creatine phosphokinase isoenzyme (CK-MB), plasma brain natriuretic peptide (BNP), myocardial collagen volume fraction (CVF) and left ventricular mass index (LVMI) were measured. Inflammatory factors (transforming growth factor beta 1, TGF-β1; connective tissue growth factor, CTGF) and activation of signaling proteins (Akt, GSK-3β) were also detected using western blot.

Results: DCM was successfully induced by the injection of streptozotocin (STZ) as evidenced by abnormal heart mass and cardiac function as well as the imbalance of extracellular matrix homeostasis. Rats in the DCM group showed increased mRNA and protein levels of LVWI, BNP, CK-MB, CVF, TGF-β1 and CTGF compared to the control group, which were accompanied with diminished phosphorylation of Akt and GSK-3β. Interestingly, myocardial dysfunction, cardiac fibrosis, and inflammation were suppressed by urocortin in the heart of diabetic rats. Moreover, inhibition of phosphorylation of Akt and GSK-3β was also reversed by urocortin. These effects of urocortin were suppressed by astressin. In addition, triciribine partially reduced the effects of urocortin on myocardial dysfunction, inflammation, and cardiac fibrosis.

Conclusions: These results suggest that urocortin exhibits a therapeutic benefit in the treatment of DCM by attenuating fibrosis and inflammation. Furthermore, inhibition of the Akt/GSK-3β signaling pathway may be partially responsible for these effects.

Introduction

Diabetes mellitus (DM) is one of the major health problems worldwide. It is estimated that the number of diabetic patients will increase from 135 million in 1995 to 300 million by the year 2025. Diabetic cardiomyopathy (DCM), a severely disabling complication of DM, is the leading cause of mortality among adults worldwide (1). However, the cause of DCM is poorly understood. The pathophysiology of DCM has been proposed to be multifactorial (2). Current hypotheses propose that in DM, myocardial tissue structural changes and dysfunction induced by factors other than coronary artery disease and cardiac neuropathy are defined as DCM (3).

The first clinical manifestation of DCM is diastolic dysfunction, which may be followed by systolic dysfunction. Myocardial cell degeneration and necrosis are the major factors causing pathophysiological changes in DCM (4). Transforming growth factor beta 1 (TGF-β1) and connective tissue growth factor (CTGF) are highly expressed in the heart in experimental diabetes in association with cell proliferation, recognition, apoptosis, specific differentiation and extracellular matrix (ECM) accumulation. Currently, a large number of studies have shown that TGF-β1 and CTGF are increased in myocardial tissues of diabetic patients and have been proposed as new targets for the treatment of DCM (5).

Urocortin, a peptide that belongs to the corticotrophin-releasing hormone (CRH) family, represents a novel inotropic agent that has a multifaceted effect on the body with significant effects on the cardiovascular, hemodynamic, neurohormonal, and renal systems (6). Urocortin was first described by Vaughan as a 40-amino-acid peptide related to the corticotrophin-releasing factor (CRF) family and can bind to and activate both type 1 and type 2 CRF receptors (7). Urocortin is distributed in both the central nervous system and periphery tissue, in sites such as the Edinger–Westphal nucleus, adipose tissue, heart, kidney and immunological tissue (8). It is a cardioactive peptide exhibiting beneficial effects in normal and various cardiovascular diseases. Endothelial urocortin suppressed the generation of angiotensin II-induced reactive
oxygen species (ROS) production in endothelial cells (9). Urocortin-induced endothelium-dependent relaxation of the rat artery has been reported (10). This peptide was found in the heart and produced a marked vasodilatation of the aorta (11). Four-day administration of urocortin had sustained beneficial hemodynamics, hormonal, and renal effects in experimental heart failure (12). It was previously demonstrated that urocortin might play a protective role in ischemia/reperfusion injury in rat hearts against oxidative stress by inhibiting the activities of free radicals (13). Urocortin was also found to have an inhibitory effect on the activity of serum angiotensin converting enzyme (14). Given these capabilities, urocortin is increasingly recognized as an endogenously produced peptide hormone with a promising future as a potential drug for the treatment of heart disease. Members of the urocortin family are known to act as potent regulators of cardiac and vascular functions via the activation of CRF receptors, which are highly expressed in the heart and peripheral tissues. Taken together, these reports strongly suggested that urocortin might have a beneficial effect on DCM.

Thus, in this study, we examined the role of urocortin in the progression of DCM and the relevant mechanisms involving the Akt/GSK-3β (glycogen synthase kinase-3β) signaling pathway. Cardiac function evaluation and the levels of creatine phosphokinase isoenzyme (CK-MB), plasma brain natriuretic peptide (BNP), myocardial collagen volume fraction (CVF) and left ventricular mass index (LVWI) were also measured. Inflammatory factors, including transforming growth factor beta 1 (TGF-β1) and connective tissue growth factor (CTGF) were selected as candidate effectors to estimate the effect of urocortin on DCM and the underlying molecular mechanisms.

Materials and methods

Animals and treatments

Animal care and experimental protocols were performed according to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and approved by the ethics committee of Liaoning Medical University. Fifty male Wistar rats were used (weight, 250–300 g; age, 18–20 weeks, purchased from Experimental Animal Centre of Shanghai Animal Institute, Shanghai, China).

Diabetes was induced in 40 rats by intraperitoneal injection of streptozotocin (STZ, Sigma Chemicals, MO) at the dose of 55 mg·kg−1 dissolved in 0.1 M citrate buffer, pH 4.5. The remaining 10 animals were treated with vehicle and were used the control group. After 3 days of STZ injection, the rats’ blood glucose levels were measured using a glucometer (AccuCheck; Roche, Germany). Rats with blood sugar values >200 mg·dl−1 were used for this study. The diabetic rats were randomly divided into 4 groups (10 rats per group) as follows: diabetic group (DM), urocortin - treated group (UCN), urocortin + astressin group (UCN + AST) and urocortinin + triciribine group (UCN + TRI). Astrinssin, a CRF receptor 2 antagonist, and triciribine, an inhibitor of Akt, were used. There was no significant difference in the non-fasting blood glucose levels among these four groups. Urocortin, astressin and triciribine in these regimens were all administered daily i.p. at dosages of 7, 35, and 0.5 mg·kg−1, respectively, for a period of 16 weeks. The rats in the DM group received the same volume of normal saline. The normal control and diabetic rats were housed in a room with a 12 h artificial light cycle and had free access to a high-fat diet (18% fat; the standard rat diet has 8% fat) and water. Body weight and non-fasting blood glucose were measured weekly. After 16 weeks of treatments, the animals were weighed, examined for heart function and sacrificed. Then, the serum and plasma were separated and the tissues were harvested and processed for biochemical measurements.

Measurements of BW, LVW and LVWI

After the rats were weighed and anesthetized with intraperitoneal urethane (1 g·kg−1), the thorax was rapidly opened and the heart was excised and washed in 0.01 mol·L−1 PBS. The LVW was detected and LVWI was defined as LVW/BW.

Measurements of CK-MB and BNP

The CK-MB and BNP levels in the rats were measured using a RA-50 semi-auto analyzer.

Cardiac function evaluation

Cardiac function was determined using invasive hemodynamic evaluation methods. A pressure-tip catheter was positioned into the left ventricle (LV) to record LV pressure changes representative of cardiac systolic and diastolic performance. LV diastolic function was quantified by the left ventricular end-diastolic pressure (LVEDP) and the maximum rate of fall of left ventricle pressure (−dP/dt max). LV systolic function was quantified by the left ventricular end-systolic pressure (LVSP) and maximum rate of the rise of left ventricle pressure (+dP/dt max).

Observation of myocardial pathology and myocardial CVF

Heart tissues were paraffin-embedded, cut into sections and stained with hematoxylin. In addition, collagen in the heart was specifically stained using ponceau red, and the CVF was determined. Briefly, sections were obtained and analyzed using the Image-Pro plus 6.0 image analysis system. Five fields were randomly selected and the CVF was calculated as the collagen area/total area followed by averaging. The area of collagen surrounding the vessels was not included in the CVF. Four arterioles in the ventricular wall were selected and the cross-section area was measured.

Measurement of TGF-β1 and CTGF in plasma

To investigate the protective mechanism of urocortin on myocardial fibrosis in diabetic rats, we focused on TGF-β1 and CTGF because they have been shown to be involved in myocardial fibrosis. TGF-β1 and CTGF levels in the plasma were determined by the ELISA method using a commercially available kit BD Opt-EIA ELISA Set (BD Biosciences, U.S.). All appropriate controls and standards as specified by the manufacturer’s kit were used, and the data were expressed as picogram per milliliter plasma.
Table 1. Effect of urocortin on LVWI, CK-MB, BNP and CVF in the diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LVWI (mg/g)</th>
<th>CK-MB (μg/l)</th>
<th>BNP (ng/l)</th>
<th>CVF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.9 ± 0.2</td>
<td>12.1 ± 2.4</td>
<td>30.2 ± 10.6</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>DM</td>
<td>2.8 ± 0.2**</td>
<td>68.1 ± 9.8**</td>
<td>310.1 ± 91.4**</td>
<td>11.2 ± 1.8**</td>
</tr>
<tr>
<td>UCN</td>
<td>2.1 ± 0.2**</td>
<td>30.1 ± 6.9**</td>
<td>152.9 ± 76.3**</td>
<td>6.5 ± 1.5**</td>
</tr>
<tr>
<td>UCN+AST</td>
<td>2.7 ± 0.3**</td>
<td>61.5 ± 8.7**</td>
<td>301.1 ± 72.6**</td>
<td>10.9 ± 1.6**</td>
</tr>
<tr>
<td>UCN+TRI</td>
<td>2.6 ± 0.3**</td>
<td>51.4 ± 4.7**</td>
<td>236.6 ± 49.2**</td>
<td>9.0 ± 1.6**</td>
</tr>
</tbody>
</table>

LVWI, left ventricular mass index; CK-MB, creatine kinase isoenzyme; BNP, brain natriuretic peptide; CVF, collagen fraction volume; DM, diabetes mellitus; UCN, urocortin; AST, astressin.

**p < 0.01 in UCN + TRI versus UCN.**

RT-PCR analysis of TGF-β1 and CTGF mRNA expression

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) and purified with QIA-GEN RNeasy (Qiagen, Valencia, CA) according to the manufacturer’s instructions. Reverse transcription of 500 ng total RNA was performed in a total volume of 20 μl using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). One microliter of cDNA was amplified by PCR in 20 μl reactions containing specific primers and iQ SYBR Green Supermix (Bio-Rad). PCR was performed for 40 cycles consisting of 95 °C for 15 s, 94 °C for 5 s, 58 °C for 15 s and 72 °C for 15 s using iCycler iQ Real Time Detection System (Bio-Rad). Data were expressed as the percentage of control, which was set to 100%.

Western blotting analysis

Proteins were extracted from heart homogenates, and aliquots (50 μg) were subjected to SDS-PAGE (7.5% gel) and transferred onto nitrocellulose membranes. Equal protein was used for western blotting analysis using the following antibodies: TGF-β1, CTGF, phospho- Akt (Ser473), Akt, phospho-GSK-3β (Ser9), GSK-3β, and β-actin. The immunoreactive bands were detected using an ECL kit.

Statistical analysis

Statistical analysis was performed with SPSS version 14.0 statistics software package and values were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using Dunnett’s t-test or one-way ANOVA analysis of variance followed by Bonferroni’s post-hoc test. Differences with a value of p < 0.05 were considered statistically significant.

Results

Urocortin attenuated diabetes-induced changes in LVWI, CK-MB, BNP and CVF

Compared to the control group, the levels of LVWI, CK-MB, and BNP were all increased in DM, UCN, UCN + AST and UCN + TRI groups (p < 0.05). Urocortin significantly decreased the levels of LVWI, CK-MB, and BNP in the diabetic rats. The effects of urocortin on these parameters were partially attenuated in the presence of triciribine (p < 0.01 in UCN + TRI versus UCN). In addition, the presence of the CRF-2R inhibitor (astressin) also reduced the beneficial effect of urocortin on LVWI, CK-MB, and BNP in DCM rats (p < 0.05) (Table 1).

Compared with the control group, the CVF levels in the DM, UCN, UCN+AST and UCN+TRI groups were significantly increased (p < 0.05). However, this effect was partially inhibited in the presence of triciribine (p < 0.01 compared with urocortin alone). In addition, the effect of urocortin could be reversed by astressin (Table 1).

Urocortin improved hemodynamic function in DCM

LVSP and +dp/dt max were used as the main indices of reflex of the left ventricular systolic function. The diabetic group exhibited markedly impaired systolic function as shown by a decrease in LVSP and +dp/dt max. Compared with the DM group, LVSP was decreased and +dp/dt max was significantly increased in the UCN group.

Similarly, LVEDP and −dp/dt max were the main indices of reflex of the diastolic function of the left ventricle. LVEDP was increased and −dp/dt max decreased in the DM group (p < 0.01). Compared with the DM animals, LVEDP was decreased and −dp/dt max was increased significantly in rats in the UCN group (p < 0.01).

These effects of urocortin were reduced by astressin and partially reversed by triciribine (p < 0.01) (Table 2).

Urocortin alleviated aberrant myocardial tissue architecture of DCM

The cardiomyocytes in the normal rats presented a regular arrangement, clear stripes without myofilament fracture, and uniform intercellular space (Figure 1A). In contrast, the cardiomyocytes in the DM group showed myofibrillar disarray and myocardial fibrosis, as evidenced by scattered muscle fiber degeneration, coarse granules in the cytoplasm, nucleus swelling and deformation, loss of cardiac muscle fiber stripes, interstitial edema and hyperplasia, and lymphocytic infiltration (Figure 1B). The myocardial cell arrangement in the diabetic rats treated with urocortin was more regular than that of the diabetic group (Figure 1C, D, E).

Urocortin decreased the levels of TGF-β1 and CTGF in the plasma of diabetic rats

Hyperglycemia is known to activate several cytokines by oxidative stress, which might contribute to the development of DCM. Compared with the control group, TGF-β1 and CTGF
levels were significantly increased in the plasma of the diabetic rats \( (p < 0.01) \). The elevation of these two cytokines was distinctly reduced by urocortin. The presence of triciribine partially attenuated the inhibition of urocortin on these cytokines \( (p < 0.01 \text{ compared with urocortin alone}) \). In addition, astressin treatment also reversed the inhibitory effect of urocortin on cytokine production in the diabetic rats \( (p < 0.01) \) (Figure 2).

### Table 2. Diabetes–induced left ventricular dysfunction were improved by urocortin treatment.

<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>Control</td>
<td>120.6 ± 9.7</td>
<td>6.1 ± 2.1</td>
<td>1908 ± 254</td>
<td>1452 ± 214</td>
</tr>
<tr>
<td>DM</td>
<td>72.4 ± 8.6**</td>
<td>18.6 ± 4.9**</td>
<td>1326 ± 175**</td>
<td>794 ± 138**</td>
</tr>
<tr>
<td>UCN</td>
<td>102.1 ± 9.4*</td>
<td>7.2 ± 3.0*</td>
<td>1795 ± 209*</td>
<td>1274 ± 202*</td>
</tr>
<tr>
<td>UCN+AST</td>
<td>80.2 ± 8.0**</td>
<td>16.4 ± 3.9**</td>
<td>1393 ± 278**</td>
<td>801 ± 125**</td>
</tr>
<tr>
<td>UCN+TRI</td>
<td>91.4 ± 8.2**</td>
<td>13.5 ± 3.6**</td>
<td>1601 ± 310**</td>
<td>989 ± 169**</td>
</tr>
</tbody>
</table>

LVSP, left ventricular end – systolic pressure; −dp/dtmax, maximum rate of fall of left ventricle pressure; +dp/dtmax, maximum rate of rise of left ventricle pressure; LVEDP, left ventricular end – diastolic pressure.

Sig: LVSP, +dp/dtmax and LVEDP were improved by urocortin and triciribine treatment, while astressin reversed the effect of urocortin. \( *p < 0.05 \) and \( **p < 0.01 \), vs. control; \( ^{\Delta}p < 0.05 \) and \( ^{\Delta\Delta}p < 0.05 \), vs. DM group and UCN + AST; \( ^{\Delta}p > 0.05 \), vs. DM group; \( ^{\Delta\Delta}p < 0.05 \), vs. UCN group.

Figure 1. (A) Effect of urocortin on myocardial fibrosis in DCM (HE stain. ×400). Animal groups, (B–E); control rats without diabetes, (B) diabetic rats treated with vehicle, (C) urocortin, (D) urocortin + astressin, and (E) urocortin + triciribine.

**Urocortin reduced TGF-β1 and CTGF expression in diabetic rat hearts**

As shown in Figure 3, the mRNA levels of TGF-β1 and CTGF in the DM, UCN, UCN + AST and UCN + TRI groups were higher than those of the control group \( (p < 0.05) \). We found that urocortin decreased both mRNA levels in the diabetic rats and that this effect could be partially attenuated by the
administration of triciribine \((p < 0.01\) compared with urocortin alone). Furthermore, a similar pattern of changes in TGF-\(\beta\) and CTGF protein levels was observed with urocortin treatment in the presence and absence of triciribine in the diabetic rats. Importantly, the inhibitory effect of urocortin on TGF-\(\beta\) and CTGF overexpression was completely abolished using astressin (Figure 3).

**Urocortin activates the Akt/GSK-3\(\beta\) signaling pathway**

Our results showed that activation of Akt was significantly suppressed in the diabetic hearts according to western blotting analysis and was accompanied by a marked reduction in activated GSK-3\(\beta\). In contrast, urocortin treatment significantly increased the phosphorylation of Akt and GSK-3\(\beta\) in diabetic myocardium, and this effect was inhibited in the presence of astressin \((p < 0.01\) compared with urocortin alone) (Figure 4).

**Discussion**

DCM is one of the most severe cardiovascular complications and can cause cardiac dysfunction in diabetic patients. The main pathological changes include focal myocardial cell hypertrophy, degeneration, necrosis, apoptosis, and myocardial remodeling. A series of pathophysiological changes caused by myocardial interstitial remodeling play an important role in the pathogenesis of DCM (15).

Our major finding was that urocortin could be beneficial in reversing the effects of DCM. Consistent with previous reports, we found that untreated diabetic rats were characterized by excess collagen accumulation and cardiac hypertrophy. The untreated diabetic hearts demonstrated increased BNP and CK-MB accumulation, elevated LVWI and CVF, and enhanced myocardial expression of TGF-\(\beta\) and CTGF coupled with inactivation of the Akt/GSK-3\(\beta\) signaling
pathway, subsequently resulting in cardiac fibrosis. In contrast, urocortin prevented the development of such characteristic alterations of DCM, and these beneficial effects involved the CRF receptor 2. The subsequent downstream mechanisms also involved inhibition of the expression and secretion of TGF-β1 and CTGF via activation of the Akt/GSK-3β signaling pathway in diabetic hearts.

This study focused on examining the effects of urocortin on myocardial remodeling during DCM. First, we found that 16 weeks of intervention with urocortin significantly reduced the LVWI, BNP, CK-MB, myocardial CVF, and TGF-β1 and CTGF mRNA and protein levels in diabetic rats. Second, urocortin significantly promoted phosphorylation of Akt and GSK-3β in diabetic myocardium. However, in the presence of astressin, the effects of urocortin were inhibited, suggesting that the effects were closely related to CRF receptors. Third, triciribine partially abolished the effects of urocortin on DCM, which suggested that the Akt/GSK-β pathway was partially involved in mediating the effects of urocortin.

In response to high levels of glucose, the output of the potent profibrotic factor, TGF-β1, was significantly increased, which resulted in fibrotic consequences. TGF-β1 is an important fibrogenic factor that promotes the synthesis and secretion of collagen I and III by myocardial fibroblasts and induces myocardial fibrosis (16). In the development of ECM accumulation, CTGF may act as a downstream mediator of TGF-β1 (17). In this study, the LVWI, BNP, CK-MB, CVF, TGF-β1 and CTGF mRNA and protein levels in the diabetic group were all significantly higher than those in the control group, indicating that the increase in TGF-β1 and CTGF in the DM rats was closely associated with myocardial remodeling.

Akt promotes cell survival by inhibiting several targets involved in apoptotic signaling cascades. GSK-3β, a major substrate of Akt, not only has central functions in glycogen metabolism and insulin action but also plays a crucial role in transmitting the apoptotic signal during diabetes-induced inflammation and fibrosis (18). Previous studies have demonstrated that activation of GSK-3β plays an important role in diabetes-induced energy metabolic derangement and consequently in pathological remodeling in the heart (19). In diabetes, Akt phosphorylation could be reduced by elevated circulation of free fatty acids and inflammatory cytokines, which results in activation of GSK-3β (20). In addition, Akt has a clearly defined role in regulating cardiovascular functions, such as cardiac growth, contractile function and coronary angiogenesis (21). Our results demonstrated that triciribine, an inhibitor of Akt, partially weakened the effects of urocortin on diabetic rat hearts, thereby indicating the involvement of Akt/GSK-β signaling in mediating the effects of urocortin on DCM.

Urocortins are endogenous vasoactive peptides known to exert powerful beneficial neurohormonal, hemodynamic, and renal actions in experimental heart failure (22). Urocortins act predominantly via 2 receptor subtypes, CRF-1 and CRF-2. Receptors exhibit 7 transmembrane domains and are G-protein coupled. CRF-2(a) receptors constitute the dominant peripheral CRF-2 receptor form, particularly in the heart and vasculature. Receptor concentrations are high in the left ventricle and intramyocardial vessels (23). However, recent studies have shown that urocortins have more potent effects on the cardiovascular system than the CRF (24). Urocortin enhances cardiac contractility, coronary blood flow, heart rates and cardiac output. Some in vitro and ex vivo investigations have shown that in case of ischemia/reperfusion, atherosclerosis and hypertension, urocortin could protect cardiac cells from severe injury (25). Urocortin exhibits potent vasodilatory effects in arteries from different species. These effects are related to its capability to regulate intracellular Ca²⁺ concentrations via different molecular mechanisms. In addition, urocortin increases heart contractility and evokes positive inotropic and lusitropic effects via mechanisms involving different kinase signaling pathways. Furthermore, heart protection by urocortin involves regulation of the transcription of specific genes, which act to minimize mitochondrial damage, cell death, and oxidative stress (26).

As a new small molecular active peptide, urocortin exerts its protective effects via urocortin 2 causes activation of cAMP-PKA, PI3K-Akt, and...
MEK1/2-ERK1/2 signaling. The former two pathways, cAMP-PKA and PI3K-Akt, converge on endothelial nitric oxide synthase (eNOS) phosphorylation and result in pronounced and sustained cellular NO production with subsequent stimulation of cGMP signaling (30). Hearts of diabetic patients have lower basal urocortin levels, which fail to increase after cardioprotective arrest. In healthy volunteers, urocortins 2 and 3 cause potent and prolonged arterial vasodilatation without tachyphylaxis. These vasomotor responses are at least partly mediated by endothelial NO and cytochrome P450 metabolites of arachidonic acid (31). It has also been shown that urocortin directly activated AMPK in ex vivo-perfused mouse hearts and diminished injury and contractile dysfunction during ischemia/reperfusion. Consistent with our findings, several studies have revealed that stimulation of CRFR2 by CRF and urocortin induced the release of BNP, which had an inotropic action on the heart (32,33).

In summary, our results provided evidence that urocortin could effectively inhibit the development of cardiac dysfunction, myocardial fibrosis and inflammation in diabetic rats. The main mechanism was that urocortin could bind to CRFR2, inhibit TGF-β1 and CTGF expression, and inactivate the Akt/GSK-β pathway. These findings provided a new therapeutic option for the clinical treatment of DCM. Intriguingly, a more direct link has been established between urocortin and diabetes, which is supported by the evidence that urocortin can modulate glucose utilization and insulin sensitivity in the skeletal muscle, suggesting its use as a molecular target for the treatment of insulin-resistant disorders, such as type 2 diabetes (34). However, it is still unknown whether these findings have bearing on the potential clinical application of urocortin and its downstream molecular effectors as targets for the treatment of human DCM. Moreover, it remains unclear whether alterations in the effectors of this pathway will lead to the development of cardiovascular diseases.

Conclusions
The present study demonstrated that urocortin could significantly improve cardiac function, and inhibit myocardial fibrosis and inflammation in diabetic rats. Further studies have shown that Akt/GSK-β signaling pathway-mediated inhibition of TGF-β1 and CTGF expression could contribute to the beneficial effects of urocortin.

Declaration of interest
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References


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