Effects of Qili Qiangxin capsule on renal aquaporin-2 expression in rats with chronic heart failure

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Qili Qiangxin is a traditional Chinese medical formulation, which can improve cardiac function, urine volume, and subjective symptoms in patients with chronic heart failure (CHF). We sought to investigate whether Qili Qiangxin could suppress renal aquaporin-2 (AQP2) expression in a rat model of CHF. High, medium, and low dosages of Qili Qiangxin (1.0, 0.5, and 0.25 g.kg⁻¹.d⁻¹, respectively) were administered intragastrically to rats with CHF. The continuous administration of Qili Qiangxin for 1 week increased urine volume while the indexes of cardiac function were unchanged after treatment for 1 week. Interestingly, after treatment with Qili Qiangxin for 4 weeks, daily urine output, ejection fraction, and fractional shortening values were significantly increased in rats with CHF, with more significant changes in the high dosage group. Levels of plasma B-type natriuretic peptide and renal V2R mRNA, AQP2 mRNA, and protein expression were significantly decreased. Treatment with Qili Qiangxin for 4 weeks can attenuate AQP2 expression in the kidneys and improve the cardiac function in rats with CHF.

KEYWORDS
Chronic heart failure; Qili Qiangxin; Aquaporin-2; Vasopressin type 2 receptor

Introduction

Chronic heart failure (CHF) is a major public health burden worldwide and associated with high morbidity, mortality, and cost. Epidemiological evidence indicated that the incidence of CHF among Chinese adults is 0.9%. Drugs, including diuretics, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, beta-blockers, aldosterone receptor antagonists, and digitalis, should be used as standard treatments for CHF. Although the drug treatment strategies for CHF were improved, the mortality of CHF remains high.

Qili Qiangxin capsule is a traditional Chinese medical formulation, which consists of 11 types of herbs, including Radix Astragali, Radix et Rhizoma Ginseng, Radix Preparata Aconiti Lateralis, Radix et Rhizoma Salvia Miltiorrhiza, Semen Lepidii Semen Descurainiae, Rhizoma Alismatis, Rhizoma Polygonati Odorati, Ramulus Cinnamomi, Flos Carthami, Cortex Periplocae, and Pericarpium Citri Reticulatae. The formula of Qili Qiangxin can improve cardiac function, urine volume, and subjective symptoms in CHF patients with good efficacy and safety. However, the underlying mechanisms have not been well identified. Activation of several neurohormonal compensatory systems, including the sympathetic nervous system, the renin-
angiotensin–aldosterone system (RAAS) and arginine vasopressin (AVP) is the important feature of CHF. Several studies had demonstrated that the levels of serum AVP and renal aquaporin-2 (AQP2) expression are increased in CHF, indicating that these biomarkers may associated with the underlying mechanisms of CHF.6–8 The AVP is involved in free water reabsorption (vasopressin type 2 receptor, V2 receptor) and arteriolar vasoconstriction (vasopressin V1a receptor) in CHF patients.9,10 AVP exerts these effects primarily through the regulation of renal AQP2 expression and redistribution, which plays a critical role in the homeostasis of water in the body.11–13 We reported that elevated renal AQP2 expression plays an important role in CHF in rats,14 and the urinary concentration of the AQP2 water channel protein increased significantly in rats with CHF.15 Qili Qiangxin has been reported to improve heart function by inhibition of RAAS and suppression of pro-inflammatory cytokine release.15,16 However, whether the improvement of heart function by Qili Qiangxin is associated with regulation of the AVP signalling pathway and AQP2 expression remains unknown.

In this study, we used a post-myocardial infarction heart failure model of rats to determine whether Qili Qiangxin capsules could improve cardiac function via regulating the expression of renal AQP2.

Methods

Animals, chemicals, and reagents

Male Sprague–Dawley (SD) rats (age ranged from 12 to 14 weeks, mean body weight 235 g) were purchased from Nanfang Hospital Laboratory, Southern Medical University (Guangzhou, China). Rats were maintained on a standard pellet diet and acclimated in a quarantine room for 2 weeks before any experiments. All experiments were approved by the Animal Care and Research Committee of Southern Medical University. Qili Qiangxin powder was kindly provided by Yiling Pharmaceutical Co. Ltd (Shijiazhuang, Hebei, China). Protein assay reagents and electrochemiluminescence (ECL) immunoblotting substrate were purchased from Pierce (Rockford, IL, USA, Catalog number 32106). The rabbit anti-rat AQP2 polyclonal antibody was prepared by using the method as described previously.8 Briefly, the rabbit polyclonal antibody against AQP2 was prepared by immunizing rabbit with a synthetic peptide (CELSHPQSLPRGSKA), which was conjugated to keyhole limpet haemocyanin (KHL), from the C-terminus of AQP2. The final titres were reported to be >1:100,000 as measured by ELISA (Genemed Biotechnologies Inc., San Francisco, CA, USA).

Animals treatment and sample collection

The rats were randomized into the following five groups: control, high-dose Qili Qiangxin (QH, 1.0 g/kg/d), medium-dose Qili Qiangxin (QM, 0.5 g/kg/d), low-dose Qili Qiangxin (QL, 0.25 g/kg/d), and sham group. Myocardial infarction was induced by coronary ligation. Animals were anaesthetized by intraperitoneal injection of pentobarbital (40 mg/kg). When the rats became unconscious, they were placed on a heating pad to maintain their body temperature at 37°C. Endotracheal intubation was performed with a 22 or 20 gauge cannula according to the body weight of the animal. Animals were ventilated with a rodent ventilator at a rate of 60 breaths/min and a tidal volume of 2–3 mL, using 100% oxygen.

A left thoracotomy was performed to expose the heart. Myocardial infarction model was induced by ligation of left coronary. The major anterior descending branch of the left coronary artery was ligated with a 6-0 silk suture at a position ~2.0 mm distal to the lower edge of the left atrium. For the rats assigned to the sham group, the same operation was performed without ligation of the left coronary artery.

All animals received treatment for 1 or 4 weeks via intragastric administration starting on the day of coronary ligation or sham operation. The rats in control and sham groups received equal volume of normal saline via intragastric administration. All rats were given free access to tap water and food, and their urine was collected for 24 h. The urine volume and urinary AQP2 were determined daily. At the end of 1 or 4 weeks, all groups of rats were anaesthetized with isoflurane to evaluate the cardiac function with echocardiography. After echocardiography, the rats were euthanized by exposure to CO2. Blood sample from the aorta was centrifuged and plasma was collected for measurements of sodium (Na+), potassium (K+), and creatinine with a Beckman AU680 biochemical analyser. Kidneys were dissected on ice for isolation of the inner medullary regions. One of the two inner medullary regions was used to detect AQP2 mRNA and V2R mRNA by real-time reverse transcriptase–polymerase chain reaction (RT–PCR), and the other was used to measure AQP2 protein by immunoblotting.17

Immunoblotting

Immunoblotting was applied to detect AQP2 protein expression. The protein samples were separated on 4 and 12% SDS gels and transferred onto nitrocellulose membranes. Membranes were blocked overnight at 4°C with 5% dry milk in TBPS (PBS containing 0.1% Tween 20), and then incubated with the appropriate primary antibody (AQP2 antibody was diluted 1:500) for 2 h at room temperature. After washing with TBPS, membranes were incubated with horseradish peroxidase (HRP)-linked secondary antibodies (1:5000 dilution with TBPS containing 5% dry milk, Catalog number BA1055) at room temperature for 1–2 h. Bands were developed using ECL and exposed on X-ray films. Band density was analysed using Image pro plus software.

ELISA

Enzyme-linked immunosorbent assay (ELISA) kit (Catalog number 42020) was used to detect AQP2 level in urine. Rat urine was centrifuged at 3000 rpm for 10 min at 4°C in an Eppendorf centrifuge. Supernatant was collected, and recombinant rat AQP2 was used to construct standard curves. Absorbance of standards and samples was determined spectrophotometrically at 450 nm using a microplate reader. Results were plotted against the linear portion of a standard curve.

Immunofluorescent staining

Immunofluorescent staining was performed to examine AQP2 in renal medulla. Renal sections were fixed in 0.01 mol/L PBS (PH7.4), incubated with a rabbit anti-rat AQP2 antibody (diluted 1:200) at 4°C for 12 h, and then incubated with FITC-tagged secondary goat anti-rabbit IgG or goat anti-rabbit IgG (imaged in the green channel, Catalog number sc-2012). Photography was performed with a fluorescent digital microscope (OLYMPUS BX 51, Japan). Under ×400 magnifications, AQP2 fluorescence was determined from five randomly selected areas.
Results

To determine whether Qili Qiangxin can improve cardiac function in CHF rats, echocardiograms were performed after treatment with Qili Qiangxin for 1 or 4 weeks. The results showed that the left-ventricular end-diastolic dimensions (LVEDd) and the left-ventricular end-systolic dimensions (LVESd) were increased, while the fractional shortening (FS) and ejection fraction (EF) were decreased in rats with CHF after coronary artery ligation for 1 or 4 weeks. Interestingly, the FS and EF were markedly improved in a dose-dependent manner after treatment with Qili Qiangxin for 4 weeks but was not significant in the group with Qili Qiangxin treatment for 1 week (Figure 1C). These results indicate that Qili Qiangxin can improve cardiac function in CHF rats, indicating by increasing fractional shortening (FS) and ejection fraction (EF) were decreased in rats with CHF after coronary artery ligation for both 1 and 4 weeks in rats, the plasma levels of BNP were significantly increased (Figure 1D). The left-ventricular end-diastolic dimensions (LVEDd) and the left-ventricular end-systolic dimensions (LVESd) were increased, while the fractional shortening (FS) and ejection fraction (EF) were decreased in rats with CHF after coronary artery ligation for both 1 and 4 weeks. After coronary artery ligation for 1 or 4 weeks, the plasma levels of BNP were significantly increased (Figure 1D). Interestingly, the FS and EF were greatly improved in a dose-dependent fashion after treatment with Qili Qiangxin for 4 weeks but was not significant in the group with Qili Qiangxin treatment for 1 week (Figure 1C). These results indicate that Qili Qiangxin can improve cardiac function in CHF rats, indicating by increasing fractional shortening (FS) and ejection fraction (EF) were decreased in rats with CHF after coronary artery ligation for both 1 and 4 weeks. After coronary artery ligation for 1 or 4 weeks, the plasma levels of BNP were significantly increased (Figure 1D). Interestingly, the FS and EF were greatly improved in a dose-dependent fashion after treatment with Qili Qiangxin for 4 weeks but was not significant in the group with Qili Qiangxin treatment for 1 week (Figure 1C). These results indicate that Qili Qiangxin can improve cardiac function in CHF rats, indicating by increasing fractional shortening (FS) and ejection fraction (EF) were decreased in rats with CHF after coronary artery ligation for both 1 and 4 weeks. After coronary artery ligation for 1 or 4 weeks, the plasma levels of BNP were significantly increased (Figure 1D).

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Table 1 Qili qiangxin improved cardiac function in rats with chronic heart failure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham (n = 10)</th>
<th>Control (n = 9)</th>
<th>QH (n = 8)</th>
<th>QM (n = 8)</th>
<th>QL (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment with Qili Qiangxin for 1 week</td>
<td>HR (beats/min)</td>
<td>377.2 ± 36.8</td>
<td>349.2 ± 35.9</td>
<td>362.8 ± 50.5</td>
<td>371.3 ± 61.7</td>
</tr>
<tr>
<td></td>
<td>LVESd (mm)</td>
<td>8.1 ± 1.5</td>
<td>12.4 ± 2.8A</td>
<td>12.1 ± 2.3A</td>
<td>12.6 ± 2.6A</td>
</tr>
<tr>
<td></td>
<td>LVEDs (mm)</td>
<td>4.7 ± 0.8</td>
<td>9.8 ± 2.2A</td>
<td>9.9 ± 1.7A</td>
<td>9.7 ± 1.5A</td>
</tr>
<tr>
<td></td>
<td>FS (%)</td>
<td>56.4 ± 5.9</td>
<td>16.2 ± 2.5A</td>
<td>14.1 ± 3.3A</td>
<td>15.3 ± 3.8A</td>
</tr>
<tr>
<td>Treatment with Qili Qiangxin for 4 weeks</td>
<td>HR (beats/min)</td>
<td>376.1 ± 39.0</td>
<td>348.9 ± 36.2</td>
<td>356.9 ± 33.8</td>
<td>349.5 ± 31.6</td>
</tr>
<tr>
<td></td>
<td>LVESd (mm)</td>
<td>8.1 ± 1.4</td>
<td>12.3 ± 2.3A</td>
<td>11.7 ± 1.4A</td>
<td>12.0 ± 1.6A</td>
</tr>
<tr>
<td></td>
<td>LVEDs (mm)</td>
<td>4.7 ± 0.7</td>
<td>9.8 ± 1.5A</td>
<td>9.7 ± 1.4A</td>
<td>9.9 ± 1.1A</td>
</tr>
<tr>
<td></td>
<td>FS (%)</td>
<td>56.6 ± 5.1</td>
<td>15.0 ± 2.0A</td>
<td>22.9 ± 3.7**</td>
<td>20.1 ± 3.1**</td>
</tr>
</tbody>
</table>

Echocardiographic data demonstrate that treatment with Qili Qiangxin for 1 week had no effect on HR, LVEDd, LVEDs, and FS in rats with CHF, whereas treatment with Qili Qiangxin for 4 weeks markedly increased FS in rats with CHF but had no effect on HR, LVEDd, and LVEDs in rats with CHF.

*P < 0.05 vs. sham.

*p < 0.05 vs. Control.
Qili Qiangxin increased urinary output and reduced urinary AQP2 excretion

To determine whether Qili Qiangxin has a diuretic effect, urinary volume in rats was measured before and after treatment with Qili Qiangxin for 1 or 4 weeks. As shown in Figure 2A and B, urinary volume was markedly increased in a dose-dependent manner after treatment with Qili Qiangxin, beginning on Day 1. Although treatment with Qili Qiangxin for 1 week had no effect (Figure 3A) on the levels of urinary AQP2 excretion, treatment with Qili Qiangxin for 4 weeks significantly reduced urinary AQP2 excretion in rats with CHF (Figure 3B).

Qili Qiangxin suppressed renal AQP2 mRNA and protein expression

To elucidate the mechanisms of diuretic effect by Qili Qiangxin, we examined whether Qili Qiangxin plays a role in the expression of AQP. To determine whether Qili Qiangxin suppressed the expression of renal AQP2, we examined AQP2 protein expression using immunoblotting and immunofluorescence staining and AQP2 mRNA expression using RT–PCR in the medulla of rats at 1 and 4 weeks after coronary ligation. The real-time RT–PCR data and the representative immunoblots demonstrated that the
results suggest that CHF induced by coronary artery ligation for 4 weeks attenuated urinary AQP2 excretion in rats with CHF (Figure 3A and B). In addition, the reduction of AQP2 expression was greater in CHF rats treated with high dose than in those treated with lower dose of Qili Qiangxin. To confirm the results obtained via immunoblotting and RT–PCR, we examined renal AQP2 in all groups of rats using immunofluorescence staining. The representative immunofluorescence images showed that AQP2 expression was markedly attenuated in CHF rats treated with Qili Qiangxin for 4 weeks (Figure 4C). This finding was consistent with the immunoblotting results. Therefore, these results suggest prolonged administration of oral Qili Qiangxin to rats with CHF markedly reduced the levels of renal AQP2 expression.

Qili Qiangxin reduced renal V2R mRNA expression

To determine whether Qili Qiangxin could alter the expression of renal V2R, we examined the V2R mRNA expression in the medulla 4 weeks after coronary artery ligation. We found that rats with CHF expressed higher levels of renal V2R mRNA than sham rats after 4 weeks. After treatment with Qili Qiangxin for 4 weeks, the levels of V2R mRNA were markedly reduced. The levels of V2R mRNA were much lower in rats with CHF that were treated with a high dose of Qili Qiangxin than in those that were treated with lower dosage of Qili Qiangxin (Figure 5). Thus, these results suggest that CHF induced by coronary artery ligation up-regulates renal V2R mRNA expression, and renal AQP2 mRNA and protein expression. Prolonged administration of Qili Qiangxin to rats with CHF can markedly attenuate the elevated renal V2R mRNA expression, AQP2 mRNA and protein expression.

Discussion

In the present study, we found that prolonged treatment with Qili Qiangxin can markedly improve cardiac function in rats with CHF. Furthermore, Qili Qiangxin increased urine volume via the down-regulation of renal AQP expression and V2R expression. These results provide new insights into the underlying mechanisms for improvement of cardiac function by Qili Qiangxin and highlight the important role of the down-regulation of AQP2 in this process.

The progressive reduction of left-ventricular function is the feature of CHF that results in the activation of several neurohormonal compensatory systems, including the sympathetic nervous system, RAAS, and AVP. The prognosis of patients with CHF has been greatly improved by the use of neurohormonal antagonists, such as angiotensin-converting enzyme inhibitors, beta-blockers, and aldosterone antagonists. However, the mortality and morbidity of patient with CHF remain high. Therefore, new therapeutic approaches must be developed. Qili Qiangxin has been reported to improve cardiac function in patients with heart failure. We found that Qili Qiangxin markedly increases EF and FS in rats with CHF. Several studies had reported that the dynamic changes of BNP levels reflect the condition of CHF. Our results demonstrated that the ligation of the coronary artery induced the elevation of BNP levels in rats and that Qili Qiangxin reduced BNP levels at 4 weeks. The results of this study suggested that Qili Qiangxin can improve cardiac function in rats with CHF. However, the mechanism that underlies the improvement of cardiac function in CHF conditions must be further identified. Zou et al. found that Qili Qiangxin capsules inhibited myocardial inflammation and cardiomyocyte death and promoted cardiomyocyte proliferation in a mouse model of pressure overload. The potential mechanisms responsible for these effects may involve the inhibition of the angiotensin II type one receptor and the activation of ErbB receptors. Xiao et al. reported that Qili Qiangxin may improve the cardiac function of rats with myocardial infarction by regulating the balance between pro-inflammatory and anti-inflammatory cytokines in cardiomyocytes. A study reported by Liu et al. demonstrated that Qili Qiangxin capsules improve both systolic and diastolic cardiac function in spontaneously hypertensive rats by down-regulating the cardiac chymase signalling pathway and chymase-mediated AngII production. Wei et al. reported that Qili Qiangxin may affect L-type Ca2+ channels and block ICa-L to affect cardiac function. Qili Qiangxin also exhibits biphasic action, which means that this formulation acts as either a class IV antiarrhythmic agent or an agent that improves cardiac function. In addition, the protective effect on energy metabolism and myocardial mitochondria might be involved in the mechanisms underlying the improvement of cardiac function by Qili Qiangxin. In our previous study,
we found that the level of AQP2 was associated with CHF. The present study identified a new mechanism underlying the improvement of cardiac function by Qili Qiangxin: the down-regulation of AQP2 levels. AQP2 is the most important aquaporin and plays an important role in CHF. AQP2 has an effect on the balance of free water clearance and reabsorption through a cycle of water endocytosis and exocytosis. Fluid retention and congestion are important features of CHF, and AQP2 is involved in these features. We found that treatment with Qili Qiangxin increases urinary output and decreases urinary osmolality. The results of the present study demonstrated that prolonged treatment with Qili Qiangxin has a greater effect on the improvement of cardiac function than 1-week treatment. In addition, a high dose of Qili Qiangxin has a greater effect on the improvement of cardiac function than medium and low doses of Qili Qiangxin. Thus, Qili Qiangxin improves cardiac function in a time- and dose-dependent fashion. However, Qili Qiangxin markedly increased urinary output in rats with CHF but failed to significantly elevate the levels of AQP2 in rats with CHF after treatment for 1 week. In this regard, the increase of urinary output observed in rats with CHF that were treated with Qili Qiangxin for 1 week did not occur due to the reduction of AQP2 levels. The direct effect on the improvement of cardiac function might be the main cause of the increased urinary output observed in CHF rats, and this need to be confirmed by future studies.

In the present study, we found that treatment with Qili Qiangxin down-regulates AQP2 mRNA and protein levels in rats with CHF. Our results demonstrated that Qili Qiangxin reduces the expression of AQP2 at the gene level. Urinary AQP2 excretion positively correlated with renal expression and was increased in CHF conditions. This reduction of urinary AQP2 excretion reflected the down-regulation of renal AQP2 expression by Qili Qiangxin in rats with CHF. Further, we found that Qili Qiangxin also down-regulates mRNA levels of V2R, which is the upstream regulators of AQP2 expression. Thus, the predominant mechanism that underlies the reduction of AQP2 expression is the down-regulation of AVP signalling pathway.
Conclusions

In conclusion, the present study demonstrated that treatment with Qili Qiangxin for 4 weeks markedly improves cardiac function in a rat CHF model. This effect is associated with the attenuation of AQ2P2 expression in the kidneys.

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Conflict of interest: none declared.

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