Effect and mechanism of Qishen Yiqi Pills on adriamycin-induced cardiomyopathy in mice

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[ABSTRACT] AIM: To study the effect and probable mechanism of Qishen Yiqi Pills on adriamycin (ADR)-induced cardiomyopathy in mice. METHODS: Sixty-four mice were randomly divided into (1) the ADR group: saline (1 mL/100 g) administered every day by intragavage, ADR (4 mg·kg⁻¹) administered to each mouse by intraperitoneal injection twice a week for four weeks; (2) the ADR + Qishen Yiqi Pills I group: ADR (4 mg·kg⁻¹) administered to each mouse by intraperitoneal injection twice a week for four weeks, and at the beginning of the third week Qishen Yiqi Pills (3.5 mg/100 g) administered by intragavage every day for four weeks; (3) the ADR + Qishen Yiqi Pills II group: ADR (4 mg·kg⁻¹) administered to each mouse by intraperitoneal injection twice a week for four weeks, and at the same time Qishen Yiqi Pills (3.5 mg/100 g) administered by intragavage every day for four weeks; (4) the control group: saline (1 mL/100 g) administered every day by intragavage. RESULTS: 1. The left ventricular diastolic diameter and the left ventricular systolic diameter were significantly increased (P < 0.05) and the left ventricular ejection fraction was significantly decreased (P < 0.05) in the ADR group, and the cardiac function of both the ADR + Qishen Yiqi Pills I group and the ADR + Qishen Yiqi Pills II group improved. 2. Myocardial morphologic observation showed that the myocardial fibers were disordered, there was cell edema, and gap widening in the ADR group. The degree of myocardial cell injury was reduced in the ADR + Qishen Yiqi Pills I group and ADR + Qishen Yiqi Pills II group compared with the ADR group. 3. The expression of Bax in the ADR group was significantly up-regulated, and the expression of Bcl-2 was significantly down-regulated compared with the ADR + Qishen Yiqi Pills I group, the ADR + Qishen Yiqi Pills II group, and the control group (P < 0.05). CONCLUSIONS: Qishen Yiqi Pills can effectively improve the cardiac function of ADR-induced cardiomyopathy, and the earlier it is used is better. The probable mechanism of action may be the inhibition of the apoptosis of myocardial cells.

[KEY WORDS] Adriamycin-induced cardiomyopathy; Qishen Yiqi Pills; Apoptosis; Bcl-2; Bax


1 Introduction

Cardiomyopathies are diseases based on myocardial injury and necrosis, and present with arrhythmia and cardiac dysfunction. There is no effective therapy of cardiomyopathies as yet, except for symptomatic treatment which is unable to reverse the progress. In addition, the clinical prognosis of cardiomyopathy is very poor. Drug-induced myocardial damage is an important risk factor for cardiomyopathy, and adriamycin (ADR) is one of the most common chemotherapy drugs. Therefore, it is important to explore the effective prevention and treatment for the myocardial damage of ADR. Qishen Yiqi Pills are now widely used in coronary heart disease, but lack of basic research and clinical application on cardiomyopathy. This experimental study is aimed at investigating the efficacy and mechanisms of Qishen Yiqi Pills in the treatment of ADR-induced cardiomyopathy in mice, which may provide a new way for the treatment of cardiomyopathies, espe-
cally for the drug-induced myocardial injury.

2 Materials and Methods

2.1 Animal model establishment and grouping
Sixty-four mice [male, (30 ± 5) g, 8 weeks old, provided by the Shanghai Animal Experiments Center, qualified number (scxk2009-0001)] were randomly divided into the ADR group (n = 16), ADR + Qishen Yiqi Pills I group (n = 16) and ADR + Qishen Yiqi Pills II group (n = 16). In the ADR group, saline (1 mL/100 g) was administered every day by intraga- vage, and ADR (Hydrochloric doxorubicin star injection) (4 mg·kg⁻¹) was administered to each mouse by intraperitoneal injection twice a week for four weeks. In the ADR + Qishen Yiqi Pills I group, ADR was administered at 4 mg·kg⁻¹ to each mouse by intraperitoneal injection twice a week for four weeks, and at the beginning of the third week Qishen Yiqi Pills (Tasly Pharmaceutical Co., Z20030139) at 3.5 mg/100 g was administered by intragavage every day for four weeks. In the ADR + Qishen Yiqi Pills II group, ADR at 4 mg·kg⁻¹ was administered to each mouse by intraperitoneal injection twice a week for four weeks, and at the same time Qishen Yiqi Pills at 3.5 mg/100 g were administered by intragavage every day for four weeks. In the control group, saline (1 mL/100 g) was administered every day by intragavage, and saline (1 mL·kg⁻¹) was also administered to each mouse by intraperitoneal injection twice a week for four weeks. The general status of the mice was observed daily, and changes in weight and mortality were observed weekly.

2.2 Cardiac function measurement
The left ventricular systolic function of each group was assessed and compared in the sixth week using small animal ultrasound echocardiography (Vevo 770TM, VisualSonics, Toronto, Canada) by measuring the left ventricular diastolic diameter (LVEDD), the left ventricular systolic diameter (LVESD), and the left ventricular ejection fraction (LVEF).

2.3 Myocardial tissue pathological examination
Left ventricular myocardial tissue was collected from mice in each group in the sixth week. The tissue was fixed with 4% paraformaldehyde solution, embedded in paraffin, sliced, and HE stained. The myocardial cell morphology was observed and compared.

2.4 Bcl-2 and Bax protein detection
Total protein was prepared from the infarcted myocardial tissues. Two hundred micrograms of protein was electrophoresed on a 12% SDS polyacrylamide gel and transferred to a PVDF membrane.

Diluted first antibody was added and incubated on the shaker at 4 °C overnight, and then the membrane was washed three times, horseradish peroxidase-labeled goat anti-mouse IgG (H + L) was added and incubated for 1 h. The film was washed and fixed for darkroom light-emitting developing. Bandscan 4.3 software was used for grayscale analysis.

2.5 Statistical analyses
All results are expressed as mean ± SD. Results were compared by means of one-way Anova and Student Newman Keuls test. Statistical analysis was performed using SPSS 17.0 software. A value of P < 0.05 was considered to be statistically significant.

3 Results

3.1 Observation of survival and general status of the mice
Mice administered ADR showed hair loss, activity and appetite reduction, and weight loss. There ten of the mice died in the six week period. Four mice died in the ADR group, four mice died in the ADR + Qishen Yiqi Pills I group, and two mice died in the ADR + Qishen Yiqi Pills II group.

3.2 Observation of cardiac function
As shown in Table 1 and Fig. 1, LVEDD and LVESD were significantly increased (P < 0.05), and LVEF was significantly decreased (P < 0.05) in the ADR group compared with the ADR + Qishen Yiqi Pills I group, the ADR + Qishen Yiqi Pills II group, and the control group, respectively. LVEDD and LVESD were significantly increased (P < 0.05) and LVEF was significantly decreased (P < 0.05) in the ADR + Qishen Yiqi Pills I group compared with the ADR + Qishen Yiqi Pills II group. These results indicate that the establishment of the cardiomyopathy model by the administration of ADR is successful, and that the cardiac function of ADR + Qishen Yiqi Pills I group improves more than the ADR + Qishen Yiqi Pills II group.

3.3 Observation of myocardial morphology
As shown in Fig. 2, HE staining showed that in the control group the myocardial fibers were arranged neatly, with no swelling or degeneration, uniform cytoplasm, and normal cell gap. However, in the ADR group the myocardial fibers were disordered, there was broken cell edema, the cell gap was widened, and there was partial vacuolar degeneration. The degree of myocardial cell injury is reduced in the ADR + Qishen Yiqi Pills I group and ADR + Qishen Yiqi Pills II group than ADR group.

Table 1  Assessment of left ventricular systolic function of each group (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LVEDD/mm</th>
<th>LVESD/mm</th>
<th>LVEF/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADR group</td>
<td>12</td>
<td>3.826 ± 0.04</td>
<td>2.418 ± 0.06</td>
<td>39.98 ± 1.32</td>
</tr>
<tr>
<td>ADR + Qishen Yiqi Pills I group</td>
<td>12</td>
<td>3.568 ± 0.03*</td>
<td>2.345 ± 0.05*</td>
<td>60.41 ± 0.59*</td>
</tr>
<tr>
<td>ADR + Qishen Yiqi Pills II group</td>
<td>14</td>
<td>3.472 ± 0.10*</td>
<td>2.224 ± 0.10*</td>
<td>63.77 ± 0.68*</td>
</tr>
<tr>
<td>Control group</td>
<td>16</td>
<td>2.008 ± 0.07</td>
<td>1.607 ± 0.05</td>
<td>74.15 ± 2.41</td>
</tr>
</tbody>
</table>

*P < 0.05 vs ADR group
Fig. 1 Assessment of left ventricular systolic function of each group in the sixth week with small animal ultrasound echocardiography. Cardiograms A-D, respectively represent the ADR group, the ADR + Qishen Yiqi Pills I group, the ADR + Qishen Yiqi Pills II group, and the control group

![Cardiograms](image1)

Fig. 2 Myocardial morphology by HE staining of each group. Stained samples A-D, respectively represent the ADR group, the ADR + Qishen Yiqi Pills I group, the ADR + Qishen Yiqi Pills II group, and the control group

![Stained samples](image2)

3.4 Expression of Bcl-2, Bax

As shown in Table 2 and Fig. 3, the expression of Bcl-2 in the ADR group was significantly lower than in the ADR + Qishen Yiqi Pills I group, the ADR + Qishen Yiqi Pills II group and the control group ($P < 0.05$). The expression of Bcl-2 in the ADR + Qishen Yiqi Pills I group is lower than the ADR + Qishen Yiqi Pills II group ($P < 0.05$). The expression of Bax in the ADR group was significantly higher than in the ADR + Qishen Yiqi Pills I group, the ADR + Qishen Yiqi Pills II group, and the control group ($P < 0.05$). The expression of Bax in the ADR + Qishen Yiqi Pills I group is higher than in the ADR + Qishen Yiqi Pills II group ($P < 0.05$).

Table 2 Expression of Bcl-2 and Bax in each group (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Bcl-2</th>
<th>Bax</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADR</td>
<td>12</td>
<td>0.108 ± 0.016</td>
<td>0.796 ± 0.023</td>
</tr>
<tr>
<td>ADR + Qishen Yiqi Pills I</td>
<td>12</td>
<td>0.217 ± 0.018*</td>
<td>0.505 ± 0.029*</td>
</tr>
<tr>
<td>ADR + Qishen Yiqi Pills II</td>
<td>14</td>
<td>0.489 ± 0.018*</td>
<td>0.391 ± 0.020*</td>
</tr>
<tr>
<td>Control group</td>
<td>16</td>
<td>0.593 ± 0.022</td>
<td>0.201 ± 0.021</td>
</tr>
</tbody>
</table>

* $P < 0.05$ vs ADR group
Drug-induced myocardial injury is a particular type of cardiomyopathy, among which myocardial damage caused by chemotherapy drugs is very common. The anthracycline anticancer chemotherapy drugs possess significant cardiotoxicity, and doxorubicin produces the most significant myocardial damage \cite{1-2}. Coenzyme Q10, vitamin E \cite{3}, vitamin C, ATP, acetyl cysteine, and verapamil can protect the myocardium, and prevent and reduce cardiac toxicity. Whether a traditional Chinese medicine can be applied to overcome myocardial damage caused by chemotherapy, and a possible mechanism of action is not reported yet.

Qishen Yiqi Pills is a newly researched and developed drug, which combines the extracted active ingredients of Astragalus, Salvia, and Panax, and is prepared by modern technology. It is a combination of traditional Chinese medicine theory and modern preparation techniques. Recent studies suggest that Qishen Yiqi Pills have a good effect on the secondary prevention of coronary heart disease \cite{4}, and there are also some auxiliary effects in the treatment of heart failure and arrhythmia \cite{5}. At present, the main indication of the drug is the treatment of coronary heart disease. It was noticed that its main ingredient, Astragalus, has the effect of protecting the myocardium, and is also anti-apoptotic and can regulate the immune system, which indicates that the drug can protect the myocardium and may slow chemotherapy drug-induced myocardial injury. Therefore, the purpose of this study was to observe the effects and possible mechanism of Qishen Yiqi Pills on chemotherapy-induced myocardial injury. It is considered that there are a variety of mechanisms which participate in the process of doxorubicin cardiomyopathy, of which cardiac myocyte apoptosis is one of the most important mechanism, and Bcl-2 family play an important role \cite{6-7}. Kitta et al. \cite{8} found that ADR can cause myocardial apoptosis by down-regulating the expression of Bcl-2 and up-regulating the expression of Bax. Thus, the expression of Bcl-2 and Bax was used to investigate the degree of myocardial apoptosis within each group of mice. The results showed that the down-regulated expression of Bcl-2 and up-regulated expression of Bax is very apparent in the ADR group, and that the situation improves in the ADR + Qishen Yiqi Pills groups. It is speculated that the myocardial protection of Qishen Yiqi Pills may be related to the anti-oxygen free radicals, membrane lipid peroxidation and the inhibition of cardiomyocyte apoptosis of Astragalus. Recent studies have shown that Astragalus can increase the activity of the oxygen radical scavenger superoxide dismutase, can decrease lipid peroxidation, and reduce myocardial injury caused by free radicals, thereby protecting the myocardium \cite{9}. Danshen, Sanqi, and other ingredients of Qishen Yiqi Pills can also increase coronary blood flow, reduce blood viscosity, reduce ischemic myocardial injury, and protect the vascular endothelium, inhibiting the inflammatory response and anticoagulation and inhibition of platelet aggregation.

In summary, these experiments in mice showed that it is effective to apply the traditional Chinese medicine Qishen Yiqi Pills to treat the ADR-induced cardiomyopathy, which may relate to the reduction of myocardial cell apoptosis. Further studies are needed to determine whether Qishen Yiqi Pills can protect the myocardium from other chemotherapy drug-induced injury and non-drug-induced myocardial damage. Following studies in humans, which are now in progress, Qishen Yiqi Pills may provide a new option for the treatment of drug-induced myocardial injury.

References

应用芪参益气滴丸治疗心肌病的效应与机制研究

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【摘 要】 目的: 通过构建小鼠阿霉素心肌病模型, 观察芪参益气滴丸对小鼠心肌病的治疗作用, 并初步探讨其机制。方法: 取健康雄性昆明小鼠 64 只, 分为 1. 阿霉素组(ADR 组, 16 只): 生理盐水 1 mL/100 g/d 灌胃, ADR 4 mg·kg−1 腹腔注射, 每周两次, 连续 4 周。2. 阿霉素+芪参益气滴丸 1 组(16 只): ADR 4 mg·kg−1 腹腔注射, 每周两次, 连续 4 周。第 3 周开始予芪参益气滴丸 3.5 mg/100 g/d, 灌胃给药, 累计 4 周。3. 阿霉素+芪参益气滴丸 2 组(16 只): ADR 4 mg·kg−1 腹腔注射, 每周两次, 连续 4 周, 同时予芪参益气滴丸 3.5 mg/100 g/d, 灌胃给药。4. 对照组(16 只): 生理盐水 1 mL/100 g/d 灌胃, 同时生理盐水 1 mL·kg−1 腹腔注射, 每周两次, 累计 4 周。实验第六周末测定小鼠心功能, 制作病理 HE 切片, 用光镜行形态学观察, western 法检测小鼠 Bcl-2, Bax 蛋白的表达。结果: 1. ADR 组小鼠 LVESD、LVEDD 增大, LVEF 明显降低。芪参益气滴丸和芪参益气滴丸 2 组小鼠心功能较 ADR 组均有改善。2. 病理结果显示 ADR 组心肌细胞变性水肿、排列紊乱, 用药组及 2 组病理结果均有不同程度的改善。3. Bcl-2 蛋白表达阿霉素组、用药组及 2 组病理结果均有不同程度的改善。结论: 芪参益气滴丸可有效改善阿霉素心肌病小鼠的心功能, 纠正心脏, 减轻心肌损害作用, 且早期用药效果佳, 其机制与抑制小鼠心肌细胞凋亡有关。

【关键词】 阿霉素心肌病; 芪参益气滴丸; 效应与机制; 凋亡

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