Effects of Ligustuzaine on Myocardial Fibrosis in Rats with Pressure Overload

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ABSTRACT
Objective: To investigate the effects of ligustuzaine (Li) on myocardial fibrosis in rats with pressure overload. Methods: Pressure overload rat models were established by constricting the abdominal aorta. Sixty-three SD rats were divided into 3 groups: Sham operated group (SOG, n = 21), operated group (OG, n = 21) and operated combined with ligustuzaine group (OG+Li, n = 21). Each group was randomly assigned to seven time points: The 1st, 2nd, 4th, 7th, 14th, 21st and 30th day after operation. Three rats were included in each time point. Serial sections of cardiac tissues were examined and the morphological or morphometric analysis of the SOG, OG and OG+Li done by histopathological and computer image analyzer technique. Results: (1) It showed that there were reactive fibrosis (from the 4th day on after operation) and reparative fibrosis (from the 21st day on after operation) in the OG, while myocardial fibrosis in the OG+Li was alleviated. Though reactive fibrosis (from the 21st day on after operation) was shown, reparative fibrosis wasn't seen. (2) Perivascular collagen area (PVCA) in the OG (2.09 ± 0.45) was significantly higher than SOG (0.83 ± 0.06) from the 1st day on after operation and then steadily increased, while in the OG+Li (1.16 ± 0.06), it was significantly lower than OG at the same time; collagen volume fraction (CVF) in the OG (3.08 ± 0.56) significantly increased compared with the SOG (2.78 ± 0.64) from the 2nd day on after operation and showed a trend of rapid ascending from the 21st day on after operation; and in the OG+Li (4.69 ± 0.85), it was significantly decreased compared with the OG (7.56 ± 0.88) from the 21st day on after operation, with all P < 0.05. Conclusion: Ligustuzaine could alleviate and postpone the accumulation of myocardial collagen and has protective effects on the heart.

KEY WORDS ligustuzaine, pressure overload, myocardial fibrosis

It has been recognized that myocardial remodeling is the essential mechanism of heart failure, and the remodeling of myocardial collagen is one among these. The key to treat heart failure at the present time is to inhibit myocardial remodeling. It was reported that ligustuzaine (Li) had therapeutic effect on pathological changes of fibrosis, such as atherosclerosis, pulmonary fibrosis and hyperplasia scar (1, 2). As for its protective effect on myocardial fibrosis with myocardium hypertrophy and cardiac failure, there has been so far few reports about researches on it. The present study tries to observe the effect of Li on the dynamic changes of myocardial fibrosis in rats with pressure overload, and to discuss its possible mechanism in expectation of providing a foundation in treating myocardial fibrosis.

METHODS

Animal Models and Grouping
Sixty-three Sprague-Dawley rats, (provided by Experimental Animal Center of Nanjing Jinling Hospital), chosen regardless of their sex and weight 200 ± 20 g, were randomly divided into 3 groups; (1) Sham operated group (SOG, n = 21); except without abdominal aorta constriction, the rats were treated in the same way as those in the operated group; (2) Operated group (OG, n = 21); the rats were made into models with pressure overload produced by abdominal aorta constriction. The rats were anesthetized by intraperitoneal injecting soluble pentobarbitone 40 mg/kg. After laparotomy, the abdominal aorta was separated and a silk thread was inserted between left and right renal artery. A short needle with an external diameter of 0.45 mm was placed along aortic long axis and the abdominal aorta, which was ligated together with the needle, and finally the needle was drawn out and abdominal aortic constriction was finished; (3) Operated combined with ligustuzaine group (OG+Li, n = 21); the rats were intraperitoneally given ligustuzaine injection (Wuxi 7th Pharmaceutical Co., Ltd., batch number: 010518) 40 mg/kg each day from the day before operation on until they were sacrificed, with other treatments the same as those in the OG. Each group was randomly assigned to seven time points: The 1st, 2nd, 4th, 7th, 14th, 21st and 30th day after operation. Three rats were included in each time point.

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Physiological Studies

After the rats were anesthetized by intraperitoneal soluble pentobarbitone 35 mg/kg, its abdominal aorta was cannulated beyond the ligating spot. Abdominal aortic pressure was directly recorded by SIRECUST 960 physiological recorder (produced by Germany SIEMENS Co., Ltd.) with the rats in the slightly anesthetized state. Main abdominal aortic pressure (MAP) was equal to diastolic aortic pressure (DAP) + 1/3[systolic aortic pressure (SAP) + DAP]. The chest was opened by median sternotomy, and the heart was removed and weighed. Cardiac weight index was equal to cardiac wet weight/body weight (CW/BW, mg/g).

Morphology and Morphometry

Cross sections were obtained from its middle segments, which were prepared for light microscopy as routine, the collagen-specific sirius red stain was used on 4 μm thick, paraffin-embedded sections. One entire cross-section of the myocardium was used for the observation with light microscopy and morphometric analysis with HPIAS-1000 highly defining power and color pathological image analyzer (Qianping Image Engineering Co. produced, version 7.0).

The left ventricular (including interventricular septum) collagen volume fraction (CVF) and perivascular collagen area (PVCA) were determined on the sirius red stained section (10×) with the image analyzer described by Brilla, et al[10]. Stained by sirius red, myocardial cells showed yellow and the collagen presented scarlet. CVF was equal to the sum of all collagen areas of the field/all areas in the field, while perivascular collagen areas are excluded from this measurement. Four fields were randomly selected from left ventricular epicardium, endocardium and interventricular septum respectively, of course with the blood vessels avoided. There were twelve visual fields for each specimen, and one mean value for CVF was calculated for each specimen. The ratio of arteriole perivascular collagen area to arterial luminal area was also determined. For each left ventricle, an average of 5 cross sections of intramural coronary arteries was found to be appropriate for morphometric analysis. One average value for PVCA was calculated for each specimen.

Statistical Analysis

The data were analyzed with SPSS 11.0 for Windows. One-way ANOVA was used in comparison of means. The differences between each two groups were evaluated using multiple comparisons of LSD or Dunnett’s T3 post hoc test, with comparison of pairwise variances.

RESULTS

Determination of MAP and Cardiac Weight Index

The rat models with pressure overload were produced successfully in this study. MAP in the OG significantly increased compared with the SOG on the 1st day after operation, and CW/BW also began to increase markedly on the 7th day after operation compared with SOG. Moreover, these indexes were increased persistently henceforth (Fig. 1, 2).

Fig 1. Results of MAP of Each Group (x±s)
Note: *P<0.05, vs SOG at the same time; ▲P<0.05, vs OG at the same time

Fig 2. Results of CW/BW of Each Group (x±s)
Note: *P<0.05, vs SOG at the same time; ▲P<0.05, vs OG at the same time

Effect of Morphologic Analysis of Myocardial Collagen

On the sirius red stained section of the SOG, it was seen that a little collagen accumulated inside or around the intramural coronary arterial adventi-