Spironolactone alleviates late cardiac remodeling after left ventricular restoration surgery

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Objective: Although left ventricular restoration is effective for treating ischemic cardiomyopathy caused by left ventricular remodeling and redilation, the initial improvement in left ventricular function is not always sustained. We have reported that the inhibition of the renin-angiotensin-aldosterone system by angiotensin-converting enzyme inhibitors and angiotensin receptor blockers is effective in preventing late remodeling after left ventricular restoration. However, the effects of spironolactone—an aldosterone blocker—after left ventricular restoration have not been elucidated.

Methods: Myocardial infarction was induced by ligating the left anterior descending artery. The rats developed left ventricular aneurysms and underwent left ventricular restoration by the plication of the left ventricular aneurysm 4 weeks after the ligation. Thereafter, the rats were randomized into a left ventricular restoration (vehicle) group and left ventricular restoration with spironolactone (100 mg/kg/d, by mouth) group.

Results: Echocardiography revealed that in the left ventricular restoration with spironolactone group, late cardiac redilation was significantly attenuated (left ventricular end-diastolic area: 0.51 ± 0.03 cm² vs 0.63 ± 0.03 cm², P < .05) and late left ventricular function was preserved (fractional area change: 48.8% ± 3.0% vs 35.8% ± 2.4%, P < .01). Hemodynamically, rats in the left ventricular restoration with spironolactone group exhibited improved systolic function (maximal end-systolic pressure-volume relationship: 0.38 ± 0.03 mm Hg/µL vs 0.11 ± 0.04 mm Hg/µL, P < .01) and diastolic function (τ: 18.5 ± 1.5 sec vs 23.1 ± 1.4 sec, P < .01) than those in the LVR group. Histologically, interstitial fibrosis in the remote area was significantly reduced (5.6% ± 1.3% vs 12% ± 1.0%, P < .01), and fibrosis around the pledgets (near area) was also attenuated in the left ventricular restoration with spironolactone group. The myocardial messenger ribonucleic acid expressions of transforming growth factor-β1 and brain natriuretic peptide measured using the real-time polymerase chain reaction were lower in the left ventricular restoration with spironolactone group (transforming growth factor-β1: 0.13 ± 0.02 vs 0.28 ± 0.02, P < .01; brain natriuretic peptide: 0.99 ± 0.14 vs 1.54 ± 0.18, P < .05). The systemic blood pressure and heart rate did not differ between the 2 groups.

Conclusion: Spironolactone reduced the gene expression of transforming growth factor-β1 and brain natriuretic peptide and alleviated not only cardiac redilation but also the deterioration of left ventricular function late after left ventricular restoration without inducing hypotension, a major side effect of angiotensin-converting enzyme inhibitors or angiotensin receptor blocker. Spironolactone is a promising therapeutic option for alleviating remodeling after left ventricular restoration.
atrial natriuretic peptide are effective in preventing LV remodeling and improving LV function after LVR in a rat MI model.9-11 However, in the clinical setting immediately after LVR, the systemic hypotension and renal damage caused by these anti-RAAS drugs are detrimental, particularly in high-risk patients with severe LV dysfunction.12

Spironolactone—a mineralocorticoid-receptor antagonist—competes with aldosterone, which plays an important role in the RAAS. Spironolactone prevents the detrimental effect of aldosterone and, at the same time, rarely influences the blood pressure.13,14 Therefore, we tested the hypothesis that spironolactone alleviates cardiac remodeling and functional deterioration late after LVR without inducing hypotension.

Materials and Methods

Animals
In this study, we used 7-week-old male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind). All procedures performed here conformed to the “Guideline for Animal Experiments” of Kyoto University. All rats were fed with standard rat chow, given water ad libitum, and housed in a single room of the animal facility with a 12-hour light/dark cycle with independent ventilation, temperature, and humidity control.

Surgical Induction of Myocardial Infarction
Murine acute MI was established as previously described.14 Briefly, under general anesthesia, anterior wall MI was induced by the ligation of the left anterior descending artery using a 6-0 polypropylene suture near the main pulmonary artery. Rats with an infarction size of less than 30% of the LV circumference as observed on 2-dimensional echocardiography were excluded from the study.

Left Ventricular Restoration Surgery
Four weeks after the ligation of the left anterior descending artery, LVR surgery was performed in the rats as previously described.14 Briefly, rats with a large MI area underwent rethoracotomy. The border between the aneurysm and the intact myocardium was identified, and the aneurysm was plicated with 2 pledges. Polypropylene sutures (5-0 Prolene) were passed through the pledget, through the aneurysm at its border, through the opposite pledget in a horizontal mattress fashion, and then tied. An additional continuous over-and-under suture was added to plicate the LV aneurysm completely.

Study Group Profiles
After LVR surgery, the rats were randomly treated with the oral administration of spironolactone (100 mg/kg/d, dissolved in 1 mL of 2% ethanol: LVR-Sp group, n = 14) or vehicle (2% ethanol 1 mL only: LVR group, n = 14) with a metallic gastric tube. The rationale of the drug dose depended on previous studies concerning spironolactone.15,16 Concomitantly, we confirmed that 1 mL of 2% ethanol had no hemodynamic or morphologic influence on the rats. To provide appropriate frames of reference, echocardiographic data of normal rats (n = 12) and rats with MI (n = 12) were also evaluated.

Systolic Blood Pressure and Heart Rate
Systolic blood pressure and heart rate were measured with a noninvasive computerized Softron tail-cuff system (Softron, Kanagawa, Japan) without anesthesia, as previously described.7

Echocardiography
Echocardiography was performed before LVR, 1 day after LVR, and 4 weeks after LVR. Before all echocardiographic procedures, the rats were lightly anesthetized with ether and placed in a supine position. Transthoracic echocardiography was performed with a 12-MHz transducer and 2-dimensional echo Doppler system (Sonos 4500; Hewlett Packard, Andover, Mass). M-mode and 2-dimensional measurements were performed as previously described.10

Hemodynamic Evaluation
All of the rats underwent cardiac catheterization for the measurement of functional parameters 4 weeks after LVR surgery, as previously described.10 Briefly, general anesthesia was administered to the rats, and they were ventilated. A micromanometer-tipped catheter (Millar Instruments, Houston, Tex) was inserted into the right carotid artery. The catheter was advanced into the aorta and then into the left ventricle. A 3-French Fogarty balloon catheter (Edwards Life Science Corp, Irvine, Calif) was inserted via the right femoral vein into the inferior vena cava for caval occlusion. The maximal rate of LV pressure development (+LV dP/dt), maximal rate of LV pressure relaxation (−LV dP/dt), LV end-diastolic pressure, maximal end-systolic pressure-volume relationship (Emax), and time constant of isovolumic LV relaxation (tau, τ) were calculated as previously described.17,18

Histopathology
The rats were sacrificed 1 day after cardiac catheterization. Hearts and lungs were excised and weighed. LVs were sliced into 2-mm cross sections, perpendicular to the ventricular long axis at the base of the papillary muscles, and were fixed in 10% buffered formalin, embedded in paraffin, mounted on glass slides, and stained using Picrosirius red. Except for the area that was plicated using

Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACEI</td>
<td>Angiotensin-converting enzyme inhibitor</td>
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<td>ARB</td>
<td>Angiotensin receptor blocker</td>
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<td>BNP</td>
<td>Brain natriuretic peptide</td>
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<td>BW</td>
<td>Body weight</td>
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<td>Emax</td>
<td>Maximal end-systolic pressure-volume relationship</td>
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<td>FAC</td>
<td>Fractional area change</td>
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<tr>
<td>GADPH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
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<td>LV</td>
<td>Left ventricular</td>
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<tr>
<td>+LV dP/dt</td>
<td>Maximal rate of LV pressure development</td>
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<tr>
<td>−LV dP/dt</td>
<td>Maximal rate of LV pressure relaxation</td>
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<td>LVEDA</td>
<td>Left ventricular end-diastolic area</td>
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<td>LVESA</td>
<td>Left ventricular end-systolic area</td>
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<td>LVR</td>
<td>Left ventricular restoration</td>
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<td>LVR-Sp</td>
<td>Left ventricular restoration with spironolactone</td>
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<td>LVW</td>
<td>Left ventricular weight</td>
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<td>MI</td>
<td>Myocardial infarction</td>
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<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<td>RAAS</td>
<td>Renin-angiotensin-aldosterone system</td>
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<td>TGF</td>
<td>Transforming growth factor</td>
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TGF = transforming growth factor

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pledgets, the LV wall was divided into 4 areas. The 2 areas adjacent to the pledgets were called the “near areas,” and the other 2 areas were called the “remote areas.”

The 2-dimensional echocardiography data before LVR and 1 day after LVR were statistically significant. However, this difference was not observed between the 2 groups (from 0.92 ± 0.02 cm² to 0.32 ± 0.04 cm² in the LVR-Sp group [P < .05] and from 0.91 ± 0.03 cm² to 0.34 ± 0.03 cm² in the LVR group [P < .05]). The left ventricular end-systolic area (LVESA) also decreased in both groups (from 0.70 ± 0.02 cm² to 0.11 ± 0.04 cm² in the LVR-Sp group [P < .05] and from 0.66 ± 0.03 cm² to 0.12 ± 0.05 cm² in the LVR group [P < .05]).

Four weeks after LVR, the LVEDA and LVESA values demonstrated an increase in comparison with the values recorded immediately after surgery in the LVR group (LVEDA: from 0.34 ± 0.03 to 0.63 ± 0.03 cm² [P < .05] and LVESA: from 0.12 ± 0.05 to 0.40 ± 0.08 cm² [P < .05]). Although the LVEDA and LVESA values also increased in the LVR-Sp group, theLVESA value was lower in the LVR-Sp group than in the LVR group at 4 weeks after LVR (0.51 ± 0.03 cm² vs 0.63 ± 0.03 cm², P < .05).

Immediately after LVR, the fractional area change (FAC) demonstrated an increase (from 24.2% ± 1.0% to 65.7% ± 3.1% in the LVR-Sp group [P < .05] and from 26.6% ± 1.6% to 66.5% ± 2.6% in the LVR group [P < .05]). The time course of the FAC is shown in Figure 1. The FAC decreased gradually after LVR in both groups. However, the FAC at 4 weeks after LVR was significantly higher in the LVR-Sp group than in the LVR group (48.8% ± 3.0% vs 35.8% ± 2.4%, respectively; P < .01).

Statistical Analysis
All data are presented as means ± standard deviation. To analyze echocardiographic data, multiple group comparisons were performed using 1-way analysis of variance. In multiple comparisons among independent groups in which analysis of variance indicated significant differences, the statistical value was determined according to the Bonferroni/Dunn post hoc test. All data comparing LVR and LVR-Sp groups were analyzed with the Wilcoxon rank-sum test. Statistical analyses were performed with StatView for Windows (version 5.0; SAS Institute Inc, Cary, NC).

Results
All of the rats survived LVR surgery. There was no difference in systolic blood pressure levels between the 2 groups at 4 weeks after LVR (109.5 ± 5.8 mm Hg vs 111.3 ± 8.6 mm Hg). The heart rate was higher in the LVR-Sp group than in the LVR group (367 ± 39 beats/min vs 400 ± 38 beats/min, respectively). However, this difference was not statistically significant.

Echocardiography
The 2-dimensional echocardiography data before LVR and 1 day and 4 weeks after LVR are summarized in Table 1. Before LVR and 1 day after LVR, no differences were observed between the echocardiographic data of the 2 groups. The akinetic area was 35% ± 6% in the LVR group and 33% ± 4% in the LVR-Sp group. One day after LVR, the left ventricular end-diastolic area (LVEDA) decreased compared with the preoperative values in both groups (from 0.92 ± 0.02 cm² to 0.32 ± 0.04 cm² in the LVR-Sp group [P < .05] and from 0.91 ± 0.03 cm² to 0.34 ± 0.03 cm² in the LVR group [P < .05]).

Four weeks after LVR, the body weight (BW) of the rats in the LVR-Sp group (407.2 ± 52.8 g) was similar to that of the rats in the LVR group (390.3 ± 44.3 g). The left ventricular weight (LVW) and lung weight normalized to BW were lower in the LVR-Sp group compared with the LVR group (LVW/BW: 2.14 ± 0.1 g/kg vs 2.96 ± 1.2 g/kg, P < .05; lung weight/BW: 8.6 ± 3.1 g/kg vs 10.9 ± 1.7 g/kg, respectively, P < .05) (Figure 2). Right ventricular weight/BW did not differ between the 2 groups (1.1 ± 0.3 for LVR-Sp vs 1.3 ± 0.3 for LVR).

Histopathology
Representative photographs of histopathologic findings are shown in Figures 3 and 4. The interstitial fibrosis in the remote
area was attenuated in the LVR-Sp group (Figure 3). A lower percentage of myocardial fibrosis in the remote area was observed in the LVR-Sp group compared with the LVR group (5.6% ± 1.3% vs 12% ± 1.0%, respectively, P < .05). In the LVR group, interstitial fibrosis was thick and partially composed of a reticular structure surrounding the myocardial fibers. On the other hand, the fibrosis was thin and scattered among the myocardial fibers in the LVR-Sp group. Severe fibrosis developed around the pledgets (near area) in the LVR group. However, the fibrosis around the pledgets was attenuated in the LVR-Sp group.

Reverse Transcriptase Polymerase Chain Reaction

The results of reverse transcriptase polymerase chain reaction are shown in Figure 5. Spironolactone inhibited the expressions of TGF-β1 and BNP mRNA (TGF-β1/GAPDH: 0.13 ± 0.02 vs 0.28 ± 0.02, P < .005; BNP/GAPDH: 0.99 ± 0.14 vs 1.54 ± 0.18, P < .05; LVR-Sp vs LVR, respectively).

Discussion

Major Findings

By using hemodynamic, histologic, and molecular methods, we showed that spironolactone attenuated cardiac remodeling and preserved systolic and diastolic function late after LVR. LVEDD and LVEDA values were lower; FAC, Emax, and +LV dP/dt values were higher; and the τ value was lower in the LVR-Sp group than in the LVR group. Lower values of LVW/BW and lung weight/BW in the LVR-Sp group indicate that spironolactone attenuated LV hypertrophy and lung congestion induced by LV dysfunction. Histopathologically, spironolactone prevented the accumulation of connective tissue in both the near and remote areas. The expressions of TGF-β1 mRNA, which is related to adverse remodeling, and BNP mRNA, which indicates LV wall stress, were inhibited. One of the most impressive results of this study was that spironolactone did not induce severe hypotension in the rats exposed to such a critical condition after LVR surgery.

Renin-Angiotensin-Aldosterone System and Cardiac Remodeling After Left Ventricular Restoration

LVR is an effective treatment for LV aneurysm and ischemic cardiomyopathy after MI. However, several clinical and experimental studies have reported cardiac redilation and functional deterioration late after LVR. Although the precise mechanism of LV redilation after LVR has not been fully elucidated, we and others have demonstrated that progressive fibrosis in areas both near and remote from the

![Figure 1. The time course of FAC calculated from the echocardiographic data obtained from LVR (closed circles) and LVR-Sp (closed squares) groups. *P < .05 versus LVR. Values are represented as mean ± standard deviation. LVR, Left ventricular restoration; LVR-Sp, left ventricular restoration with spironolactone.](Image)
plicated region is one of the key factors responsible and that
the RAAS plays an important role in an autocrine/paracrine
manner. Therefore, the inhibition of the RAAS may be
important in preventing persistent remodeling after the ex-
clusion of the aneurysm by LVR and in reducing postopera-
tive morbidity and mortality.

**Spironolactone**

A number of clinical and experimental studies have dem-
onstrated the efficacy of spironolactone in the treatment
of heart failure. Nonetheless, other RAAS inhibi-
tors, such as ACEI and/or ARB (rather than spironolac-
tone), are chosen in the clinical setting for the treatment
of patients with chronic heart failure because these drugs
are effective against hypertension, which induces cardiac
hypertrophy and plays an important role in the vicious
cycle of heart failure. However, these antihypertensive
drugs may not be as well tolerated in acute and unstable
settings after LVR surgery as they are in chronic heart
failure.

One of the rationales behind the choice of spironolactone
as a treatment option after LVR was that it does not act as
a vasodilator and rarely induces hypotension. In this study,
treatment with spironolactone for 4 weeks did not induce hy-
potension. Although the higher heart rate observed in the rats
treated with spironolactone could be caused by the differ-
ences in the LV output, these differences were not statisti-
cally significant.

Further, recent studies have indicated that the once-
inhibited production of aldosterone often increases and
that the anti-remodeling effects of ACEI and ARB therapy
decrease during their long-term administration (aldosterone
escape phenomenon). Although the precise mechanism of
this phenomenon has not been well elucidated, the induc-
tion of angiotensin-converting enzyme, the conversion of
angiotensin II from angiotensin I through enzymatic path-
ways other than angiotensin-converting enzyme (eg, chym-
ase), or both may play important roles. Therefore, some
investigators have suggested that ACEI or ARB treatment
is not adequate, and a combination of ACEI and ARB
with an agent that inhibits aldosterone could be effective
in preventing remodeling. The important point in this
study is that spironolactone inhibited the expression of the
gene that was related to adverse remodeling and the severity

**Figure 2.** LV weight (A) and lung weight (B) normalized to the BW (gray bar: LVR group, white bar: LVR-Sp group). \( P < .05 \) versus LVR. Values are represented as mean ± standard deviation. LV, left ventricular; LVR, left ventricular resto-
ration; LVR-Sp, left ventricular restora-
tion with spironolactone.

**Figure 3.** Histologic findings. High-
power photomicrographs (×200) of Pic-
rosirius red-stained LV cross-section
obtained from LVR (A) and LVR-Sp (B)
rats. In the LVR group, the interstitial
fibrosis (arrows) partially forms a retic-
ular structure surrounding the myocar-
dial fibers. In the LVR-Sp group, the
fibrosis is thin and scatters among the
myocardial fibers.
of heart failure (TGF-β1 and BNP). Moreover, spironolactone inhibited connective tissue aggregation both in the near and remote areas, and further attenuated LV redilation and preserved LV function without inducing hypotension.

Clinical Implications
As shown in this study, spironolactone may be well tolerated in critical and unstable settings immediately after surgery. In addition, spironolactone can be administered intravenously in patients who are unable to take drugs orally. We recommend the following with respect to spironolactone therapy. Spironolactone is the first choice immediately after LVR because these patients are often in low cardiac output states and hypotension. ACEIs and/or ARBs should be administered and titrated after patients recover from such severe conditions and are able to take drugs orally. The proper drug dose and timing of administration of each drug should be considered individually.

Study Limitations
There were several study limitations in the present study. First, the LVR method used in this study is similar to that of linear closure but not identical. In the clinical setting, the endoventricular circular patch plasty technique (first described by Dor and colleagues) is more widely performed than the linear closure methods. However, the aneurysm was completely excluded by plication, and we think that this method adequately simulated clinical LVR because the LV size decreased and LV function improved.

Second, we used the dose of 100 mg/kg/d according to the previous studies that used spironolactone for an animal MI model. However, in the surgical settings used in the present study, the plasma renin level might be higher than that in the MI model. Therefore, if we had used a larger amount of spironolactone, LV redilatation would have been alleviated more.
Conclusions

Spironolactone (an aldosterone blocker) attenuated the expression of mRNA responsible for cardiac fibrosis, LV remodeling, and the deterioration in LV function after LVR without hypotension. Spironolactone is a possible therapeutic option after LVR.

References


