Long-Term Effect of Spironolactone on Cardiac Structure as Assessed by Analysis of Ultrasonic Radio-Frequency Signals in Patients With Ventricular Hypertrophy

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Background  An effect of aldosterone on ventricular fibrosis has been demonstrated in animals, but remains unclear in human patients. This study aimed to investigate (1) the relationship between left ventricular (LV) fibrosis and myocardial ultrasonic texture as assessed with myocardial radio-frequency (RF) signals analyzed from the viewpoint of their waveform with chaos theory in animals and (2) serial changes in myocardial ultrasonic texture following long-term aldosterone blockade in patients with LV hypertrophy.

Methods and Results  In an animal study, Sprague-Dawley rats were divided into 2 groups with and without adriamycin administration, and the relationship between the RF signals and LV fibrosis was assessed. In a clinical study, effects of 12-month-administration of spironolactone were assessed in patients with LV hypertrophy. The animal study revealed that the correlation dimension (CD) calculated from the RF signals inversely correlated with the area of fibrosis. The clinical study demonstrated an increase in CD following 6-month administration of spironolactone. The changes in CD positively correlated with those in the serum carboxy-terminal telopeptide of collagen type I.

Conclusion  Myocardial RF signals analyzed with chaos theory reflect the severity of LV fibrosis. Aldosterone blockade may alter myocardial ultrasonic texture with regression of LV fibrosis, at least partly through enhanced collagen degradation. 

Key Words: Aldosterone; Fibrosis; Hypertrophy; Ultrasonic tissue characterization

In animal studies aldosterone promotes left ventricular (LV) structural alteration and remodeling through the progression of LV fibrosis and the development of myocyte hypertrophy and is likely to play an important role in the development of LV dysfunction and the transition to overt heart failure! An increase in plasma aldosterone level in association with poor prognosis in patients with myocardial infarction has been reported! Benefits of aldosterone blockade in patients with chronic heart failure, as reported in recent clinical trials, partly support the experimental evidence, but the mechanisms of the beneficial effects of aldosterone blockade and the serial changes in these effects remain to be clarified in a clinical study, partly because of the lack of established indices of cardiac fibrosis.

Ultrasound returned from the myocardium is considered to reflect the characteristics of myocardial tissue, particularly the extracellular matrix. Integrated ultrasonic backscatter is a simple and widely used parameter for myocardial tissue characterization and we reported a significant correlation between myocardial integrated backscatter and LV fibrosis. However, serious problems regarding standardization limit its use. Recently, we reported that analysis of ultrasonic radio-frequency (RF) signals from the viewpoint of their waveform with chaos theory in animals and (2) serial changes in myocardial ultrasonic texture following long-term aldosterone blockade in patients with LV hypertrophy.

Methods  The animal study conformed to the guiding principles of Osaka University Graduate School of Medicine with regard to animal care and to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985). The clinical study complied with the Declaration of Helsinki.
A commercially available echo machine equipped with a 7.5 MHz transducer (Aloka SSD-5500, Aloka Co Ltd, Tokyo, Japan) to measure LV cavity size and LV wall thickness as previously described. Ejection time of the mitral E wave velocity. Time-delay was determined with reference to the carrier frequency to make the time-delay as short as possible. Systolic blood pressure was measured with a tail cuff system (BP-98A, Softron, Tokyo, Japan).

Data Collection At age 19 weeks, all rats were anesthetized with ketamine HCl (50 mg/kg) and xylazine HCl (10 mg/kg), and transthoracic echocardiography was conducted with a commercially available echo machine equipped with a 7.5 MHz transducer (Aloka SSD-5500, Aloka Co Ltd, Tokyo, Japan) to measure LV cavity size and LV wall thickness as previously described. The other 4 rats served as controls.

Following the echo study and adequate anesthesia, the heart was harvested. The LV myocardium was fixed with a phosphate-buffered 10% formalin solution for 48 h. The specimens were embedded in paraffin, and 2-μm thick transverse sections were stained with Azan Mallory stain. Two independent data-blinded observers evaluated interstitial fibrosis using the percent area of fibrosis as previously described.

Clinical Study

Study Subjects Patients were eligible for enrollment if they had LV hypertrophy that was defined by echocardiography as described below and were clinically stable for at least 3 months. Patients were excluded from the study if they had congenital heart disease, valvular heart disease, a history of coronary artery disease, renal failure (serum creatinine concentration >2.0 mg/dl) or hyperkalemia (serum potassium >5.5 mmol/L).

The study population comprised 15 patients who met the criteria (Table 1). Medications were unchanged during the study, and spironolactone was added at 25 mg once daily. Data were collected before and at 6 and 12 months after the administration of spironolactone.

Data Collection Two-dimensional (D) M-mode echo and Doppler ultrasound recordings were obtained for each patient as previously described. A commercially available echo machine equipped with a 3 MHz transducer (Aloka SSD-5500) was used to measure LV cavity size and LV wall thickness as previously described. Ejection fraction was calculated by a modification of the method of Quinones et al and LV mass was calculated following the formula derived from the data of the American Society of Echocardiography as previously described. LV hypertrophy was diagnosed if the ratio of LV mass to body surface area (LV mass index) was >130 g/m² in men and >110 g/m² in women. In patients with sinus rhythm, the pulsed Doppler transmitral flow velocity curve was measured to estimate a ratio of peak mitral E wave velocity to peak mitral A wave velocity (E/A ratio) and deceleration time of the mitral E wave velocity.

Following conventional echocardiographic data collection, ultrasonic RF signals were obtained by a parasternal approach at a pulse-repetition frequency of 1 kHz along an M-mode beam intersecting the ventricular septum at end-diastole as previously described. After the echocardiographic study, phlebotomy was performed. The serum concentration of the carboxy terminal telopeptide of collagen type I (CITP) was measured using radioimmunoassay with a commercially available kit (Orion Diagnostica, Espoo, Finland), and plasma concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured using immunoradiometric assay with commercially available kits (Shionogi Co Ltd, Osaka, Japan).

Analysis of RF Signals

Deterministic behavior means that the present state of a signal is determined by its previous states. To test such a relationship, each successive value of a signal variable is plotted against its previous value, which is known as a time-delay embedding technique. Random or noisy behavior constructs formless structure, and a signal with nonrandom elements forms a distinct structure. If the signal shows a sine curve, its continuous 3-D trajectories represent a circle. A typical quasiperiodic signal that contains 2 oscillatory motions with different frequencies forms torus. Addition of a third oscillatory motion leads to chaos. The combination of many oscillatory motions causes noisy and random behavior.

In the data analysis, 50 sample points from the region-of-interest per ultrasound pulse were stacked 10 times serially for 10 ms, yielding 500 points, and these were provided for the analysis. Digitized RF signals returned from the ventricular septum were analyzed by the time-delay embedding technique to construct an end-diastolic 3-D trajectory. Time-delay was determined with reference to the carrier frequency to make the time-delay as short as possible (7.5 MHz in the animal study and 3 MHz in the clinical study). Poincaré sections of the trajectories were obtained by sectioning the torus with planes transverse from the center of the torus at the given degree (theta). To mathematically estimate a fractal dimension of the trajectories, its correlation dimension (CD) was calculated as previously described. CD is defined as $C(r) = r^{CD}$, where $C(r)$ is the cumulative number of all rank-ordered vector differences within a range, r, that begins with the smallest and ends with the largest vector difference. An individual vector is made by taking time steps beginning at a reference point and using each value encountered as 1 coordinate of the multidimensional vector. The next vector is made by moving the beginning reference point to a new location in the time...
series and then using the same number of time steps. Vectors of varying time steps, that is, embedding dimensions, are made when increasing the embedding dimensions no longer increases the CD. The mean values of interobserver and intraobserver variability of the measurement of CD values were examined in 5 patients and were 0.05±0.19 and 0.30±0.31, respectively.

Statistical Analysis
Results are expressed as mean±SEM. Comparisons between the 2 rat groups were assessed using unpaired t-test. The serial data of the clinical study were assessed with analysis of variance for repeated measures followed by Fisher's test. Linear regression analysis was used to assess a correlation between 2 indices. Discrete variables were summarized by frequency percents and were analyzed with χ² test. All statistical analyses were performed using commercially available statistical software (STATVIEW version 5, SAS Institute Inc, Cary, NC, USA). A p-value <0.05 was considered statistically significant.

Results
Animal Study
Characteristics of the Adriamycin and Control Rats There was no significant difference in systolic blood pressure (136±2 vs 129±3 mmHg), interventricular septal thickness (1.2±0.1 vs 1.2±0.1 mm), LV end-diastolic dimension (8.1±0.1 vs 8.6±0.5 mm) or fractional shortening (31±3 vs 32±1%) between the adriamycin-treated rats and the control rats. The area of fibrosis was significantly higher (4.5±0.6 vs 1.6±0.1%, p<0.05) in the rats subjected to adriamycin than in the control rats. The absence of a difference in lung weight (1.42±0.06 vs 1.57±0.08 g) between the 2 groups indicates that pulmonary congestion did not occur in the rats treated with adriamycin.

Correlation Between the RF Signals and LV Structural and Functional Indices Attractors constructed by the time-delay embedding techniques formed a thin ring-like structure consisting of a distinct empty circular core region, indicating a quasi-periodic behavior in the rats subjected to adriamycin (Fig 1A). In the control rats, they had a distinctive pattern: a thick ring-like structure consisting of a relatively empty, roughly circular core region, indicating a chaotic pattern in a rat not receiving adriamycin. Mean value of CD of this rat was 3.39. The value of CD was significantly lower in the rats receiving adriamycin than in the control rats (2.55±0.13 vs 3.09±0.15, p<0.05).

Taking the data of the 8 rats together, CD inversely correlated with the area of fibrosis (r=0.729, p<0.05, Fig 1C), but did not correlate with systolic blood pressure, interventricular septal thickness, LV end-diastolic dimension or fractional shortening.

Clinical Study
Effects of Aldosterone Blockade on Hemodynamic, Conventional Echocardiographic and Serum Parameters All
hemodynamic and echocardiographic parameters are summarized in Table 2. Blood pressure, heart rate, LV end-diastolic dimension, LV wall thickness, ejection fraction, LV mass index, E/A ratio and deceleration time of the mitral E wave velocity did not change after spironolactone administration. There were no significant changes in CITP, ANP and BNP following spironolactone administration.

**Effects on RF Signals** Attractors changed from quasi-periodic behavior to a chaotic pattern following the administration of spironolactone (Fig 2). Our previous study showed that the value of CD was significantly higher in healthy normal subjects than in patients with dilated cardiomyopathy (mean value: 3.4 vs 2.6)12 In this study, CD significantly increased in the patients with LV hypertrophy following the administration of spironolactone for 6 months (Fig 3). There was no further increase in association with the drug for an additional 6 months.

![Fig2](image.png)

**Fig2.** Attractors of myocardial radio-frequency (RF) signals before (A) and at 6 months (B) and 12 months (C) after spironolactone administration. Attractors changed from a quasi-periodic behavior to a chaotic pattern following the administration of spironolactone. X(t) indicates the amplitude of the RF signals at the time, t. ∆t in A–C=583 ns.

![Fig3](image.png)

**Fig3.** Serial changes in the correlation dimension (CD) in all patients following spironolactone administration.

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**Table 2 Hemodynamic and Neurohumoral Factors Before and at 6 and 12 Months After Spironolactone Administration**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Before</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>135±7</td>
<td>127±6</td>
<td>130±6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78±3</td>
<td>74±3</td>
<td>77±3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>65±3</td>
<td>67±4</td>
<td>67±4</td>
</tr>
<tr>
<td>LV end-diastolic dimension (mm)</td>
<td>5±1</td>
<td>49±2</td>
<td>49±2</td>
</tr>
<tr>
<td>Interventricular septum thickness at end-diastole (mm)</td>
<td>14±1</td>
<td>14±1</td>
<td>14±1</td>
</tr>
<tr>
<td>LV posterior wall thickness at end-diastole (mm)</td>
<td>11±1</td>
<td>11±1</td>
<td>12±1</td>
</tr>
<tr>
<td>LV mass index (g/m2)</td>
<td>196±15</td>
<td>175±12</td>
<td>195±15</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>63±5</td>
<td>64±5</td>
<td>68±5</td>
</tr>
<tr>
<td>E/A</td>
<td>1.0±0.1</td>
<td>1.1±0.2</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Deceleration time of the mitral E wave velocity (ms)</td>
<td>191±8</td>
<td>185±9</td>
<td>206±22</td>
</tr>
<tr>
<td>CITP (ng/ml)</td>
<td>3.7±0.2</td>
<td>3.8±0.4</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td>ANP (ng/ml)</td>
<td>52.5±17.0</td>
<td>36.2±16.2</td>
<td>55.1±33.1</td>
</tr>
<tr>
<td>BNP (ng/ml)</td>
<td>147.6±82.3</td>
<td>108.0±58.1</td>
<td>109.2±52.6</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
LV, left ventricular; E/A, ratio of peak mitral E wave velocity to peak mitral A wave velocity; CITP, carboxy terminal telopeptide of collagen type I; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.
CD did not increase at 6 months after the administration in all of the study subjects. It increased in 9 patients but not in the other 6 patients. Angiotensin-converting enzyme inhibitor was administered to 6 of the 9 patients with increased CD and in 3 of the 6 patients without the increase. Angiotensin II type 1 receptor blocker was administered to 2 patients in each group. There was no significant difference in the rate of administration of these medicines, which are known to affect LV fibrosis, between the 2 groups.

Fig 4. Correlation between the changes in the correlation dimension (\(\Delta CD\)) and those in the serum concentration of carboxy-terminal telopeptide of collagen type I (\(\Delta CITP\)), ratio of peak mitral E wave velocity to peak mitral A wave velocity (E/A) (\(\Delta E/A\)) and deceleration time (\(\Delta DT\)). The changes in these parameters were calculated as the value at 6 months after spironolactone administration minus that before the administration.

Discussion

The current animal study revealed that the RF signals of the myocardium showed quasi-periodic behavior in adriamycin-treated rats with progressive LV fibrosis and chaotic behavior in control rats, and that the value of average CD decreased with the progression of LV fibrosis. There was no significant difference in blood pressure, LV end-diastolic diameter, interventricular septal thickness, fractional shortening or lung weight between the 2 groups. In the clinical study, RF signals changed from quasi-periodic to chaotic behavior after 6-month administration of spironolactone without changes in the hemodynamic or conventional echocardiographic parameters in patients with LV hypertrophy. The mean value of CD of the study subjects increased after drug administration for 6 months, and the spironolactone-induced changes in CD positively correlated with those in CITP, which is a serum marker of collagen degradation. The changes in CD did not correlate with those in any of the echocardiographic parameters. Thus, analysis of myocardial RF signals from the viewpoint of the waveform with chaos theory may provide an index of the severity of LV fibrosis, which is partly compatible with the results of a previous in vitro study. We previously reported that the waveform of RF signals differed between normal subjects and patients with dilated cardiomyopathy, and that CD was significantly lower in the patients with dilated cardiomyopathy. The current findings may at least partly explain those results.

The spironolactone-induced increase in CD and the positive correlation between CD and CITP in the current clinical study also suggest that aldosterone blockade alters the myocardial ultrasonic texture with regression of LV fibrosis, at least partly through enhanced collagen degradation. Matsumoto et al demonstrated that aldosterone blockade inhibited the gene expression of collagens in rats with myocardial infarction. A substudy of the Randomized Aldosterone Evaluation Study (RALES) attributed the benefits of aldosterone blockade to suppression of collagen synthesis by demonstrating that spironolactone decreased the serum levels of procollagen type I carboxy-terminal peptide, procollagen type I amino-terminal peptide and procollagen type III amino-terminal peptide in patients with heart failure. Thus, aldosterone blockade is likely to attenuate LV fibrosis through promotion of collagen degradation and suppression of collagen synthesis.

Laviades et al showed that depressed collagen degradation plays a crucial role in progressive organ fibrosis in patients with LV hypertrophy. Brilla et al demonstrated that aldosterone did not affect collagenase activity in cultured rat cardiac fibroblasts which conflicts with our finding. Recently, Harada et al showed that aldosterone induced angiotensin-converting enzyme gene expression in cultured neonatal rat cardiomyocytes. Brilla et al also showed that angiotensin II inhibited collagenase activity. Although aldosterone blockade may not directly promote collagen degradation, it might enhance collagenase activity by inhibiting the expression of tissue angiotensin-converting enzyme and the production of tissue angiotensin II. Despite a significant increase in CD in the study subjects at 6 months after the administration of spironolactone, the changes in CITP were not significant, which may be partly explained by the fact that promotion of collagen degradation contributes to regression of LV fibrosis less than suppression of collagen synthesis, and the parameters of...
collagen synthesis were not assessed in this study. This study showed a lack of further changes in CD in any patient after 12 months’ administration of spironolactone. CTIP tended to decrease after 12 months administration as compared with 6-month administration, suggesting that collagen turnover declined. Previous studies have reported that in patients with heart failure spironolactone changed the serum parameters of collagen synthesis and neurohumoral parameters within 6 months of therapy initiation;24,28,29 however, data about subsequent changes in the parameters were absent. It is notable in the substudy of the RALES that spironolactone changed neurohormonal parameters after 3-month administration and did not show further changes at the 6-month time point.29 Our findings are partly compatible with the previous studies, and suggests that the effectiveness of spironolactone at the prescribed dose reaches a plateau, if any, within 6 months after the initiation of its administration. The current study also suggests that the analysis of the RF signals from the viewpoint of their waveform is a useful marker of the drug’s effectiveness. To confirm the clinical significance of the current findings, we need to clarify in a future study the relationship between prognosis and the changes in CD induced by 6-month administration.

Previous studies have shown that long-term aldosterone blockade decreases LV volume, LV mass index and plasma BNP level and increases ejection fraction in patients with chronic heart failure caused by systolic dysfunction but such effects were not observed in the present study. This may be partly explained by the fact that ejection fraction and LV size were normal in our subjects apart from 2 patients with dilated cardiomyopathy. Thus, the current results do not conflict with the described effects of aldosterone blockade in patients with systolic dysfunction and LV dilatation. Although the administration of spironolactone was considered to cause LV fibrosis to regress and to improve LV compliance in this study, the transmural flow velocity curve did not change, which may be because almost all of the study subjects were free of heart failure symptoms and the LV filling pressure may not have been elevated. In addition, the values of CD were not completely normalized. Thus, the spironolactone-induced changes in LV fibrosis were not enough to change the LV filling dynamics in the present subjects.

Study Limitations
First, the small number of study subjects and second, we used only 1 dosage of spironolactone, and the dose–response effects on serial changes remain to be clarified. Third, we did not increase the dose of spironolactone after 6 months and thus, it also remains to be clarified whether an increase in the dose at that time would provide additional effects on myocardial ultrasonic texture. Fourth, we could not directly assess changes in LV fibrosis by ventricular biopsy for ethical reasons, or compare the CD value with indices derived from integrated ultrasonic backscatter. Fifth, we did not measure serum markers of collagen synthesis such as procollagen type I carboxy-terminal peptide, procollagen type I amino-terminal peptide and procollagen type III amino-terminal peptide. Thus, we could not assess which markers best correlate with changes in CD. Sixth, the method of adriamycin administration induced progressive LV fibrosis but did not change LV end-diastolic dimension, LV wall thickness or LV systolic function. It still remains to be clarified whether myocardial RF signals analyzed from the viewpoint of their waveform with chaos theory are affected by factors other than LV fibrosis. For example, the mean value of CD was 2.6 in both the subjects of this study before the administration of spironolactone and in the patients with dilated cardiomyopathy in our previous study;12 however, this does not necessarily indicate that the degree of fibrosis was similar between these different study subjects. A study to investigate the effects of spironolactone in adriamycin-administered rats may be informative. However, spironolactone did not significantly change LV end-diastolic dimension, LV wall thickness or LV systolic function in the clinical study. Thus, the current conclusion on the effects of aldosterone blockade may not be affected, even if the RF signals are altered by LV structural or functional indices other than LV fibrosis. Seventh, the characteristics of the RF signals may not be consistent among ultrasound machines. If so, CD values may be affected and further studies are required to clarify this issue.

Conclusions
The present animal and clinical studies suggest that myocardial RF signals analyzed from the viewpoint of their waveform with chaos theory at least partly reflect the severity of LV fibrosis and may become a useful marker for noninvasively assessing therapeutic effects on cardiac structure. Aldosterone blockade alters the myocardial ultrasonic texture with regression of LV fibrosis at least partly through enhanced collagen degradation, but its effect on cardiac structure may reach a plateau within 6 months after initiation of drug therapy.

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