Assessment of Diffuse Myocardial Fibrosis in Rats Using Small-Animal Look-Locker Inversion Recovery T1 Mapping

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Background—The concentration of gadopentetate dimeglumine in myocardium and blood can be assessed from T1 measurements and can be used to calculate the extracellular volume (ECV) of the myocardium. We hypothesized that diffuse myocardial fibrosis in a small-animal model could be quantitatively assessed by measuring myocardial ECV using small-animal Look-Locker inversion recovery T1 mapping.

Methods and Results—Sprague-Dawley rats (n=10) were subjected to continuous angiotensin-2 (AT2) infusion for 2 weeks via a subcutaneously implanted minipump system. Magnetic resonance imaging (MRI) was performed both before and after AT2 infusion. The MRI protocol included multislice cine imaging and before-and-after contrast small-animal Look-Locker inversion recovery T1 mapping and late gadolinium enhancement imaging. Myocardial ECV was calculated from hematocrit and T1 values of blood and myocardium. During the course of AT2 infusion, the mean±SD systolic blood pressure increased from 122±10.9 to 152±27.5 mm Hg (P=0.003). Normalized heart weight was significantly higher in AT2-treated animals than in control littermates (P=0.033). Cine MRI documented concentric left ventricular hypertrophy. Postcontrast myocardial T1 times were shortened after treatment (median [interquartile range], 712 [63] versus 820 [131] ms; P=0.002). Myocardial ECV increased from 17.2% (4.3%) before to 23.0% (6.2%) after AT2 treatment (P=0.031), which was accompanied by perivascular fibrosis and microscarring on myocardial histological analysis. There was a moderate level of correlation between ECV and collagen volume fraction, as assessed by histological analysis (r=0.69, P=0.013).

Conclusions—In a small-animal model of left ventricular hypertrophy, contrast-enhanced T1 mapping can be used to detect diffuse myocardial fibrosis by quantification of myocardial ECV. (Circ Cardiovasc Imaging. 2011;4:636-640.)

Key Words: myocardium ■ heart failure ■ collagen ■ MRI ■ mapping

The visualization of focal myocardial fibrosis (eg, replacement fibrosis [scarring]), due to myocardial infarction, can be accurately achieved in clinical patients by magnetic resonance imaging (MRI) using the late gadolinium enhancement (LGE) approach, which is based on the contrast between tissues with different washout properties. However, the identification of diffuse myocardial fibrosis poses considerable difficulties to conventional MRI techniques because there is no normal tissue from which abnormal tissue could be delineated.

Gadolinium-based MRI contrast agents, such as gadopentetate dimeglumine (Gd-DTPA), are injected intravenously and rapidly disperse into the extracellular space, where their concentration reaches a steady state with their concentration in the blood pool. T1 mapping enables quantification of MRI signal intensity on an absolute scale and can be performed with high reproducibility in clinical settings. Because the concentration of Gd-DTPA is directly related to the difference between precontrast and postcontrast reciprocal values of T1 (ΔR1), T1 mapping can be used to quantify the concentration of Gd-DTPA in myocardium and in the blood pool. This information can be used to derive the extracellular volume (ECV) of the myocardium, which is directly related to collagen content. Thus, T1 mapping has been proposed as an important step in the progression of congestive heart failure, leading to diastolic and, at later stages, systolic ventricular dysfunction. Moreover, myocardial fibrosis plays an important role in the development of the second major factor contributing to morbidity and mortality of patients with chronic cardiovascular diseases and arrhythmia because it can cause significant alteration of the electric properties of the myocardium. Therefore, accurate assessment of myocardial fibrosis is of important clinical interest.

Editorial see p 604

Clinical Perspective on p 640

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From the Departments of Congenital Heart Disease and Pediatric Cardiology (D.R.M., S.N., D.O., F.B., T.K.) and Internal Medicine—Cardiology (T.D., C.K.), Deutsches Herzzentrum Berlin, Berlin, Germany; Institute of Pathology (O.D.), University Hospital Jena, Jena, Germany; and Institute of Pharmacology (E.K.) and Department of Cardiology and Pneumology (K.S.), Charité Universitätsmedizin Berlin, Berlin.

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a means to identify diffuse fibrosis of the myocardium.\textsuperscript{9,10} Although several clinical studies have been performed suggesting the validity of this concept,\textsuperscript{11,12} experimental data are lacking. Small-animal Look-Locker inversion recovery (SALLI) has been proposed as a tool to generate cardiac T1 maps from small animals at high heart rates.\textsuperscript{13} The aim of our study was to investigate the ability of cardiac T1 mapping using SALLI to detect diffuse myocardial fibrosis in a small-animal model of diffuse myocardial fibrosis.

**Methods**

**Study Protocol**
All animal studies were approved by the local animal care authorities. In male Sprague-Dawley rats (n = 10; age, 16 weeks; weight, 485 ± 21 g), miniosmotic pumps (Alzet 2ML2; Charles River, Sulzfeld, Germany) filled with angiotensin-2 (AT2; Sigma Aldrich, Taufkirchen, Germany) were subcutaneously implanted to enable continuous AT2 infusion for 2 weeks at a rate of 500 ng/kg per minute.\textsuperscript{14} Systolic blood pressure was measured with the tail-cuff method\textsuperscript{15} serially 2 days before and on the last day of AT2 infusion. In vivo MRI was performed on the day before and 1 day after completion of the 2-week infusion. On completion of the second MRI study, animals were euthanized and their hearts were excised and weighed. After fixation with buffered formaldehyde (4%), hearts were sectioned and stained using Van Gieson stain. Seven additional littermates served as controls to assess normal heart weight and normal histological prevalence of myocardial fibrosis; 4 of these littermates also underwent MRI before they were euthanized.

**MRI Protocol**
All MRI studies were performed on a 3-T clinical MRI system (Achieva; Philips Healthcare, Best, The Netherlands) equipped with a dedicated solenoid coil for rat hearts. After induction of inhalative anesthesia (isoflurane-oxygen, 2.5%) and weighing, animals were shaved on the chest and abdomen to attach MRI-compatible electrocardiogram (ECG) electrodes. The animals were then placed on and fixed to a dedicated animal bed, and the bed was positioned within the coil. Throughout the examination, anesthesia was maintained via inhalation of isoflurane-oxygen (0.8–1.5%). Care was taken to keep the heart rate constant between 280 and 320 beats/min.

After generation of survey images and of a long-axis set of cine images, a stack of left ventricular (LV) short-axis cine images was acquired (phases, 30; repetition time (TR), 6.8 ms; echo time (TE), 3.3 ms; flip angle, 15°; field of view, 80 × 64 mm; acquired voxel size, 0.4 ÷ 0.4 × 1.5 mm; number of signal averages, 3; slices, 7; interslice gap adjusted to allow for coverage of the entire LV range, −1.0 to −0.8 mm). The SALLI imaging\textsuperscript{13} was performed in a midcavity short-axis view of the left ventricle using a radial acquisition scheme (acquisition duration, 5000 ms; relaxation duration, 500 ms; phases, 4; flip angle, 10°; field of view, 64 × 64 mm; acquired voxel size, 0.6 ÷ 0.6 × 3.0 mm; TR, 5.2 ms; TE, 2.1 ms; number of signal averages, 4; total acquisition time, 3 minutes 45 seconds). A tail vein was then cannulated to assess hematocrit (HCT) and administer contrast agent (gadopentetate dimeglumine [Magnevist]; Bayer-Schering AG, Berlin, Germany), 0.1 mmol/kg. After 20 minutes, SALLI imaging was repeated at the same location with the same imaging parameters.

**Image Analysis**
By using a customized image reconstruction tool written in Matlab version 7.13 (Matworks, Natick, MA), end-diastolic and end-systolic T1 maps and LGE images at multiple inversion times were generated from the SALLI data sets and stored together with the multislice cine data in DICOM format. After completion of the study, images were transferred to a cardiac MRI analysis software package (CMR42 version 3.3.1 deviation; Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada) and analyzed in a blinded fashion to assess the following LV parameters from the stack of short-axis cine slices: end-diastolic volume, end-systolic volume, ejection fraction, and end-diastolic mass. Precontrast and postcontrast T1 of LV myocardium and blood pool were recorded from regions of interest drawn to the end-systolic and end-diastolic SALLI T1 maps, respectively, and corrected for limited relaxation duration, as previously described.\textsuperscript{13} As previously mentioned, the concentration of Gd-DTPA is directly related to the ΔR1. Assuming steady state between myocardium and blood, ECV and T1 differences are related as follows\textsuperscript{16}:

\[
(1) \quad \frac{ECV_{myocardium}}{ECV_{blood}} = \frac{\Delta R1_{myocardium}}{\Delta R1_{blood}}
\]

The ECV of blood can be quantified as follows:

\[
(2) \quad ECV_{blood} = 100 - HCT
\]

The ECV of myocardium can be derived as follows:

\[
(3) \quad \frac{ECV_{myocardium}}{ECV_{blood}} = \frac{(100 - HCT) \times \Delta R1_{myocardium}}{\Delta R1_{blood}}
\]

Hence, myocardial ECV was calculated as follows:

\[
(4) \quad ECV_{myocardium} = \left( \frac{100 - HCT}{\Delta R1_{myocardium}} \right) \times \left( \frac{1}{\Delta R1_{blood}} \right)
\]

where HCT and ECV are given as percentages.

The LGE images were assessed visually by 2 independent observers (D.M. and S.N.) to identify focal bright areas of the myocardium (hyperenhancement) on those images that provided best nulling of the myocardium.

**Histological Analysis**
Qualitative analysis of histological samples treated with Van Gieson stain was performed by a blinded pathologist (O.D.), who assessed the presence of perivascular and interstitial fibrosis. Quantitative histological analysis was performed on digitalized images of the same samples by automated color deconvolution of representative myocardial segments from midcavity sections encompassing the full width of the LV wall using ImageJ 1.42 (National Institutes of Health; http://rsbweb.nih.gov/ij). The collagen volume fraction (CVF) was calculated as the percental fraction of the pink-colored collagen from the total area.

**Statistical Analysis**
All statistical analysis was performed using a statistics software package (Analyze-it 2.1; Analyze-it Software Ltd, Leeds, UK). For comparison within the individual animals, a paired Student t test was used in the presence of normal distribution, as indicated by both the Shapiro-Wilk test and visual assessment of frequency histograms; a Wilcoxon signed-rank test was used in its absence. For comparison of normalized heart weight and CVF between treatment and control groups, a Mann-Whitney U test was used. Differences in the prevalence of myocardial scarring were tested using the Fisher exact test. The Pearson coefficient was used to assess correlation between ECV on MRI and CVF on histological examination. P < 0.05 was regarded as significant. Results are expressed as mean ± SD for parametric tests or median (interquartile range) for nonparametric tests.

**Results**

**Physiological Data**
During the 2-week infusion with AT2, the systolic blood pressure increased from 122 ± 10.9 to 152 ± 27.5 mm Hg (P = 0.003). No statistically significant differences were found for heart rate (288 ± 12.1 versus 287 ± 8.2 beats/min; P = 0.859) or HCT (47 ± 2.8% versus 45 ± 5.0%; P = 0.179) during MRI before versus after AT2. The normalized heart weight of the animals treated with AT2 was significantly higher than that of controls (3.1 ± 0.28 versus 2.7 ± 0.31 mg/g; P = 0.033).

**Histological Data**
Although none of the control animals showed perivascular fibrosis on visual histological examination, 6 of 10 AT2-treated animals did show fibrosis (P = 0.033); 5 of these 6
animals (but none of the controls) also exhibited interstitial fibrosis, presenting as streaky scarring. Figure 1 shows representative images of animals with and without fibrosis. On quantitative analysis, the CVF of AT2-treated animals was higher than that of controls (9.9 ± 2.5% versus 3.5 ± 0.8%; \( P < 0.0003 \); Figure 2A).

MRI Data
After the 2-week infusion of AT2, LV end-diastolic volume and ejection fraction did not change significantly, but LV mass increased and end-systolic volume decreased significantly. The Table lists the results of volumetric measurements, T1 measurements, and the resulting ECV\textsubscript{myocardium}. T1 mapping showed a decrease in post-Gd myocardial T1 (712 [63] versus 820 [131] ms; \( P = 0.002 \)) and an increase in ECV\textsubscript{myocardium} (23.0 [6.2%] versus 17.2 [4.3%]; \( P = 0.031 \); Figure 2B) after AT2 infusion. On visual assessment, there were no areas of hyperenhancement on LGE images at baseline or after treatment with AT2. Figure 3 shows representative cine MR images and T1 maps from a single animal before and after treatment with AT2.

Comparison Between Histological and MRI Data
Although both quantitative histological and MRI data could differentiate between AT2-treated and normal states, histological data yielded better separation of the 2 situations (Figure 2A and 2B). There was a moderate level of correlation (\( r = 0.69, P = 0.013 \), Figure 4) between fibrotic load, as assessed by histological and MRI data.

Discussion
Our study demonstrates that myocardial ECV can be used as an in vivo marker for diffuse myocardial fibrosis. Previous experimental studies\textsuperscript{17–19} focusing on focal fibrosis induced by myocardial infarction have shown that myocardial ECV accurately reflects the ECV of myocardial tissue, allowing ECV to be used as a marker for the extent of interstitial space and, thus, collagen content. The AT2 infusion is an established model for the generation of myocardial hypertrophy and fibrosis. After choosing a moderate infusion rate in combination with a relatively short infusion time of 14 days in our study, a moderate increase in myocardial mass was achieved, as documented by both in vivo MRI and ex vivo weighting of the hearts. This concentric hypertrophy was accompanied by a mild degree of perivascular fibrosis and streaky scarring on histological analysis. Although there was no hyperenhancement detectable on LGE MRI, myocardial ECV, as derived from T1 mapping MRI, significantly increased from 17.5% to 22.8%, demonstrating the superiority of ECV assessment over LGE imaging for the assessment of diffuse myocardial fibrosis.

Table. Results of LV Volumetric Measurements, T1 Measurements, and the Resulting EDV, as Assessed by MRI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-AT2</th>
<th>Post-AT2</th>
<th>Difference, P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDV, µl</td>
<td>609±73</td>
<td>560±90</td>
<td>0.075 (NS)</td>
</tr>
<tr>
<td>ESV, µl</td>
<td>182±36</td>
<td>157±30</td>
<td>0.023</td>
</tr>
<tr>
<td>EF, %</td>
<td>70 (5.4)</td>
<td>73 (2.2)</td>
<td>0.322 (NS)</td>
</tr>
<tr>
<td>Mass, mg</td>
<td>706±63</td>
<td>815±84</td>
<td>0.004</td>
</tr>
<tr>
<td>Gd myocardial T1, ms</td>
<td>Before 917 (139)</td>
<td>877 (40)</td>
<td>0.084 (NS)</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>820 (131)</td>
<td>712 (63)</td>
</tr>
<tr>
<td>Myocardial ECV, %</td>
<td>17.2 (4.3)</td>
<td>23.0 (6.2)</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Results are given as mean±SD for data with a normal distribution or median (interquartile range) for data with a nonnormal distribution. EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; NS, nonsignificant.
dial fibrosis. The increase in ECV on MRI correlated with an increase of CVF, as assessed by histological analysis. The inability of LGE to detect myocardial fibrosis in our model could be expected because of the lack of an internal “reference standard” (nonaffected myocardium), which is a prerequisite for the hyperenhancement phenomenon in LGE. Another reason might be the relatively mild changes of ECV in our study. Although previous rat studies on myocardial infarction found ECV values in remote areas (18%) that were in good agreement with baseline ECV in our study (17.5%), ECV in infarcted areas reached up to 88%, allowing LGE to translate these differences into the typical black/white contrast of hyperenhancement. Accordingly, Flett et al could show that ECV calculations, based on T1 mapping, have the potential to detect similar diffuse processes in clinical patients with aortic stenosis and hypertrophic cardiomyopathy.

The assessment of myocardial ECV is based on the measurement of Gd concentration in the myocardium at steady state between myocardium and blood, normalized to Gd concentration within the blood pool. Because HCT and, thus, Gd concentration within the blood pool was similar in our animals, differences due to myocardial fibrosis were already detectable through comparison of myocardial T1 alone. The use of ECV rather than myocardial T1 alone still seems advantageous for general application in less standardized situations, because it more robustly reflects changes due to myocardial fibrosis by correcting for potential confounding processes, such as anemia. Moreover, myocardial ECV provides an indirect measure for collagen content, which was demonstrated by a moderate level of correlation and agreement between ECV and CVF in our study. This might allow for quantitative comparison of different models of myocardial fibrosis.

In this study, conventional cine MRI was used for the in vivo assessment of LV volumes and mass. As expected in the AT2 infusion model, the decrease of LV volumes in combination with an increase of LV mass demonstrated concentric hypertrophy of the heart. In principle, the SALLI technique used for T1 mapping and LGE can also be used to generate high-resolution cine images. However, additional conventional multislice cine measurements were used for this study because multislice T1 mapping was not necessary and would have required longer scan times.

All T1 maps were reconstructed in end systole to yield maximum wall thickness and minimum contamination of myocardial T1 by signal from cavity blood. Typical end-systolic wall thickness ranged from 2.4 to 3.0 mm, corresponding to 4 to 5 pixels. The inner layer was carefully avoided during analysis. Although these measures were taken to minimize the influence of cavity blood on myocardial signal, we cannot rule out that there were residual partial volume effects in the regions of interest. However, these effects should be small and present in all animals and, therefore, negligible.

This study demonstrated the ability of T1 mapping MRI to detect diffuse myocardial fibrosis by assessment of myocardial ECV. In the future, myocardial ECV might be used as a marker for regression of myocardial fibrosis in interventional studies (eg, using AT2 receptor antagonists).

In summary, myocardial ECV calculations from T1 values generated by SALLI MRI allow for the detection of diffuse...
myocardial fibrosis in a pharmacological small-animal model of LV hypertrophy. Magnetic resonance imaging quantification of myocardial ECV might be used for in vivo monitoring of experimental myocardial fibrosis. By demonstrating the validity of this concept in an experimental setting, our study also supports the use of myocardial ECV assessment in clinical applications.

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Disclosures
None.

References

CLINICAL PERSPECTIVE
The development of diffuse myocardial fibrosis has been identified as a crucial step in the progression of myocardial disease toward heart failure. Despite this knowledge, fibrotic burden of the heart is not a routine component of clinical decision making because noninvasive quantification of diffuse myocardial fibrosis has not been routinely possible. Extracellular volume (ECV) can be determined by the use of T1 mapping magnetic resonance imaging (MRI) and has been proposed as a marker for collagen content of the myocardium. During the past few years, robust T1 mapping techniques for human applications have become available for most MRI systems. Initial clinical studies showed promising results, suggesting the clinical potential of this approach. The results of our study on ECV changes in a small-animal model of mild angiotensin-2–induced diffuse myocardial fibrosis further support the validity of this concept, demonstrating significant increases of ECV in rats subjected to a 2-week infusion of angiotensin 2. There was a moderate correlation of ECV and collagen volume fraction, as assessed by histological analysis. The MRI-derived ECV can be noninvasively estimated in both humans and rodents, supporting serial in vivo studies of fibrosis in suitable preclinical models of disease to test novel therapeutic strategies that can be translated to clinical evaluation.
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