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

Messroghli/Nordmeyer et al: Diffuse Myocardial Fibrosis

*Daniel Messroghli¹, MD; *Sarah Nordmeyer¹, MD; Thore Dietrich², PhD; Olaf Dirsch³, MD; Elena Kaschina⁴, MD; Kostas Savvatis⁵, MD; Darach O h-Ici¹, MD; Christoph Klein², MD; Felix Berger¹, MD; and Titus Kuehne¹, MD

*Both authors equally contributed to this work.

¹Department of Congenital Heart Disease and Pediatric Cardiology, Deutsches Herzzentrum Berlin, Berlin, Germany

²Department of Internal Medicine - Cardiology, Deutsches Herzzentrum Berlin, Berlin

³Institute of Pathology, University Hospital Jena, Jena, Germany

⁴Institute of Pharmacology, Charité Universitätsmedizin Berlin, Berlin

⁵Department of Cardiology and Pneumology, Charité Universitätsmedizin Berlin, Berlin

Correspondence to:
Dr. Daniel Messroghli
Kardiovaskuläre Bildgebung
Klinik für angeborene Herzfehler und Kinderrradiologie
Deutsches Herzzentrum Berlin
Augustenburger Platz 1
13353 Berlin
Phone: (+49) 30 4593-2871
Fax: (+49) 30 4593-2900
Email: dmessroghli@dhzb.de

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Abstract

Background—It has been shown that the concentration of gadopentetate dimeglumine (Gd-DTPA) in myocardium and blood can be assessed from T1 measurements, and 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 (SALLI) 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 multi-slice cine imaging as well as pre- and post-contrast SALLI T1 mapping and late gadolinium enhancement (LGE) imaging. Myocardial ECV was calculated from hematocrit and T1 values of blood and myocardium. During the course of AT2 infusion, systolic blood pressure raised from 122 +/-10.9 to 152 +/-27.5 mmHg (mean +/-standard deviation, 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. Post-contrast myocardial T1 times were shortened after treatment (median [interquartile range] 712 [63] vs. 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 micro scarring on myocardial histology. 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.

Key Words: myocardium, heart failure, collagen, magnetic resonance imaging, mapping
The development of myocardial fibrosis has been identified as an important step in the progression of congestive heart failure\(^1,2\), leading to diastolic and, at later stages, to systolic ventricular dysfunction\(^3,4\). 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, arrhythmia, as it can cause significant alteration of the electrical properties of the myocardium\(^5,6\). Therefore, accurate assessment of myocardial fibrosis is of important clinical interest.

The visualization of focal myocardial fibrosis, e.g. 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\(^4,7\), 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, as 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\(^8\). Since the concentration of Gd-DTPA is directly related to the difference between pre- and post-contrast 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 a means to identify diffuse fibrosis of the myocardium\(^9,10\). While several clinical studies have been carried out suggesting the validity of this concept\(^11,12\), experimental data are so far 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/min\textsuperscript{14}. Systolic blood pressure was measured with the tail-cuff method\textsuperscript{15} serially two days before and on the last day of AT2 infusion. In-vivo MRI was performed on the day before and one day after completion of the 2-week infusion. Upon completion of the second MRI study, animals were sacrificed 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 also underwent MRI before they were sacrificed.

**MRI protocol**

All MRI studies were carried out 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 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 heart rate constant between 280 and 320 beats per minute.

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, TR 6.8 ms, TE 3.3 ms, flip angle 15°, field of view 80 x 64 mm, acquired voxel size 0.4 x 0.4 x 1.5 mm, number of signal averages (NSA) 3, slices 7, inter-slice gap adjusted to allow for coverage of the entire LV; range -1.0 to -0.8 mm). SALLI imaging\textsuperscript{13} was performed in a mid-cavity 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 x 64 mm, acquired voxel size 0.6 x 0.6 x 3.0 mm, TR 5.2 ms, TE 2.1 ms, NSA 4, total acquisition time 3 minutes 45 seconds). A tail vein was then canulated to assess hematocrit (HCT) and administer contrast agent (gadopentetate dimeglumine; Magnevist, Bayer-Schering AG, Berlin, Germany; 0.1 mmol/kg). After 20 min, SALLI imaging was repeated at the same location with the same imaging parameters.

Image analysis

Using a customized image reconstruction tool written in Matlab v7.13 (Matworks, Natick MA, USA), end-diastolic and end-systolic T1 maps as well as LGE images at multiple inversion times were generated from the SALLI data sets and stored together with the multi-slice cine data in DICOM format. After completion of the study, images were transferred to a cardiac MRI analysis software package (CMR42 v3.3.1 deviation, Circle Cardiovascular Imaging Inc., Calgary, 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, end-diastolic mass. Pre- and post-contrast T1 of LV myocardium
and blood pool were recorded from regions-of-interests drawn to the end-systolic and end-diastolic SALLI T1 maps, respectively, and corrected for limited relaxation duration as previously described.  

As mentioned above, the concentration of Gd-DTPA is directly related to the difference between pre- and post-contrast reciprocal values of T1 ($\Delta R1$). Assuming steady state between myocardium and blood, extracellular volume (ECV) and T1 differences are related by 

$$ [1] \quad \frac{ECV_{myocardium}}{\Delta R1_{myocardium}} = \frac{ECV_{blood}}{\Delta R1_{blood}}. $$  

As the extracellular volume of blood can be quantified by 

$$ [2] \quad ECV_{blood} = 100 - \text{hematocrit} $$  

de the extracellular volume of myocardium can be derived from 

$$ [3] \quad ECV_{myocardium} = (100 - \text{hematocrit}) \times \frac{\Delta R1_{myocardium}}{\Delta R1_{blood}}. $$  

Hence, myocardial ECV was calculated as follows: 

$$ [4] \quad ECV_{myocardium} = (100 - \text{HCT}) \times \frac{1}{T1_{myocardium \text{ post-gd}}} - \frac{1}{T1_{myocardium \text{ pre-gd}}} \bigg/ \frac{1}{T1_{blood \text{ post-gd}}} - \frac{1}{T1_{blood \text{ pre-gd}}}. $$  

where HCT and ECV are given as percentages (%).

LGE images were assessed visually by two independent observers (D.M. and S.N.) to identify focal bright areas of the myocardium (hyper-enhancement) 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 mid-cavity sections encompassing the full width of the LV wall using ImageJ 1.42 (National
Institue of Health, http://rsbweb.nih.gov/ij). 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 (Analyse-it 2.1, Analyse-it Software Ltd., Leeds, UK). For comparison within the individual animals, paired Student’s t-test was used in the presence of normal distribution as indicated by both Shapiro-Wilk Test and visual assessment of frequency histograms, and Wilcoxon signed-rank test was used in its absence. For comparison of normalized heart weight and CVF between treatment group and controls, Mann-Whitney U test was used. Differences in the prevalence of myocardial scarring were tested using Fisher’s exact test. Pearson’s coefficient was used to assess correlation between ECV on MRI and CVF on histological examination. P-values <0.05 were regarded as significant. Results are expressed as mean±standard deviation for parametric tests, or median [interquartile range] for non-parametric tests.

**Results**

**Physiology**

During the 2-week infusion with AT2, systolic blood pressure increased from 122+/-10.9 to 152 +/-27.5 mmHg (p=0.003). No statistically significant differences were found for heart rate (288+/-12.1 vs. 287+/-8.2 bpm, p=0.859) or hematocrit (47+/-2.8 vs. 45+/-5.0%, p=0.179) during MRI before vs. after AT2. Normalized heart weight of the animals treated with AT2 was significantly higher than that of controls (3.1+/-0.28 vs. 2.7+/-0.31 mg/g, p=0.033).
**Histology**

While none of the control animals showed perivascular fibrosis on visual histological examination, 6 out of 10 AT2-treated animals did (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, CVF of AT2-treated animals was higher than that of controls (9.9+/−2.5 vs. 3.5+/−0.8%, p=0.0003; Figure 2a).

**MRI**

After the 2-week infusion of AT2, LV EDV and ejection fraction did not change significantly, but LV mass increased and ESV decreased significantly. The Table lists the results of volumetric measurements, T1 measurements and the resulting ECV_{myocardium}. T1 mapping showed a decrease in post-gadolinium myocardial T1 (712 [63] vs. 820 [131] ms, p=0.002) and an increase in ECV_{myocardium} (23.0 [6.2] vs. 17.2 [4.3]%, p=0.031; Figure 2b) after AT2 infusion. On visual assessment, there were no areas of hyper-enhancement 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 histology and MRI**

While both quantitative histology and MRI could differentiate between AT2-treated and normal states, histology yielded better separation of the two situations (Figures 2a and 2b). There was a moderate level of correlation (r=0.69, p=0.013, Figure 4) between fibrotic load as assessed by histology and MRI.
Discussion

Our study demonstrates that myocardial ECV can be used as an in-vivo marker for diffuse myocardial fibrosis.

Previous experimental studies focusing on focal fibrosis induced by myocardial infarction have shown that myocardial ECV accurately reflects the extracellular volume of myocardial tissue\textsuperscript{17-19}, allowing ECV to be used as a marker for the extent of interstitial space and thus collagen content. AT2 infusion is an established model for the generation of myocardial hypertrophy and fibrosis. 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 histology. While there was no hyper-enhancement 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. The increase in ECV on MRI correlated with an increase of CVF as assessed by histology. The inability of LGE to detect myocardial fibrosis in our model could be expected due to the lack of an internal “reference standard” (non-affected myocardium), which is a prerequisite for the hyper-enhancement phenomenon in LGE. Another reason might be the relatively mild changes of ECV in our study. While previous rat studies\textsuperscript{18,19} on myocardial infarction found ECV values in remote areas (18\%) that were in very 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 hyper-enhancement. 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\textsuperscript{11}.
The assessment of myocardial ECV is based on the measurement of gadolinium concentration in the myocardium at steady state between myocardium and blood, normalized to gadolinium concentration within the blood pool. Since hematocrit and thus gadolinium 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, as 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 multi-slice cine measurements were used for this study as multi-slice T1 mapping was not necessary and would have required longer scan times.

All T1 maps were reconstructed in end-systole in order 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. Of those, the inner layer was carefully avoided during analysis. While 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 should be small and be present in all animals, and therefore be 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, e.g. using AT2 receptor antagonists\textsuperscript{20}.

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. MRI quantification of myocardial ECV might be used for in-vivo monitoring of experimental myocardial fibrosis. Demonstrating the validity of this concept in an experimental setting, our study also supports the use of myocardial ECV assessment in clinical applications.
Acknowledgments

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Disclosures

None.
References


Table.

Results of LV volumetric measurements, T1 measurements, and the resulting FDV as assessed by MRI. Results are given as mean+/−standard deviation for data with normal distribution, or median [interquartile range] for data with non-normal distribution. EDV = end-diastolic volume, ESV = end-systolic volume, EF = ejection fraction, ECV = extracellular volume, ns = non-significant.

<table>
<thead>
<tr>
<th></th>
<th>pre AT2</th>
<th>post AT2</th>
<th>difference (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDV (ml)</td>
<td>609+/−73</td>
<td>560+/−90</td>
<td>0.075 (ns)</td>
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<tr>
<td>ESV (ml)</td>
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<td>157+/−30</td>
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<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>pre-gd myocardial T1 (ms)</td>
<td>917 [139]</td>
<td>877 [40]</td>
<td>0.084 (ns)</td>
</tr>
<tr>
<td>post-gd myocardial T1 (ms)</td>
<td>820 [131]</td>
<td>712 [63]</td>
<td>0.002</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>
Figure Legends

Figure 1. Representative histological images (Van Gieson stain). While the control heart on the left panel (magnification x200) is composed of regularly arranged and homogenous myocardium, the AT2-treated heart on the middle/ right panels (magnification x100/ x400) is pierced by streaky scarring and perivascular fibrosis.

Figures 2a and 2b. Skeletal boxplots with median marked in the center and whiskers extending to minimum and maximum observations, illustrating fibrotic load as assessed by collagen volume fraction (CVF) on histology (Figure 2a) and by extracellular volume (ECV) on MRI (Figure 2b). Both techniques enable differentiating AT2-treated (AT2) from normal states (controls for histology, pre-treatment for MRI). CVF on histology better separates AT2-treated animals from controls than ECV on MRI separates post- from pre-treatment states.

Figure 3. Representative mid-cavity short-axis T1 maps (before and after application of contrast agent), cine images (diastolic and systolic), and LGE images from a single animal before (upper row) and after (lower row) treatment with AT2. T1 relaxation times are shortened by the contrast agent, resulting in lower signal intensities on the post-gd T1 maps. Cine images show concentric LV hypertrophy. There is no hyper-enhancement on LGE images. Gd = gadopentetate dimeglumine.

Figure 4. Scatter plot illustrating the relationship of ECV on MRI and CVF on quantitative histology. There is moderate correlation between the two techniques (Pearson correlation coefficient r=0.69, p=0.013). Circles = AT2-treated animals, triangles = controls, dashed line = 95% prediction interval.
native T1 map  post-gd T1 map  diastolic cine  systolic cine  LGE

pre AT2

post AT2
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