Serial Magnetic Resonance Imaging of Microvascular Remodeling in the Infarcted Rat Heart

Christiane Waller, MD; Karl-Heinz Hiller, PhD; Elke Kahler, PhD; Kai Hu, MD; Matthias Nahrendorf, MD; Sabine Voll, MA; Axel Haase, PhD; Georg Ertl, MD; Wolfgang Rudolf Bauer, MD

Background—Alterations in the coronary circulation are important determinants of myocardial function. Few data are available, however, about microvascular changes in reactive hypertrophy. With MRI, serial determination of myocardial microcirculation after myocardial infarction (MI) is feasible.

Methods and Results—We quantitatively determined myocardial perfusion and relative intracapillary blood volume using an MRI technique. Infarct size, myocardial mass, and left ventricular volumes were determined with cine MRI. Rats were investigated at 8, 12, and 16 weeks after MI (mean MI size 24.1±2.0%) or sham operation. Vasodilation was induced by adenosine. In the infarcted group, maximum perfusion decreased significantly from 8 to 16 weeks (5.6±0.3 versus 3.5±0.2 mL·g⁻¹·min⁻¹, P<0.01) compared with sham animals (5.5±0.3 versus 5.0±0.2 mL·g⁻¹·min⁻¹, P=0.17). Myocardial mass increased significantly (559.1±20.8 mg at 8 weeks versus 690.9±42.7 mg at 16 weeks, P<0.05) compared with sham-operated animals (516.3±41.7 versus 549.2±32.3 mg). Basal relative intracapillary blood volume increased significantly to 15.7±0.5 vol% at 8 weeks after MI and remained elevated (16.8±0.6 vol%) at 16 weeks compared with 12.1±0.3 vol% (P<0.01) in sham-operated rats.

Conclusions—Our results indicate that significant microvascular changes occur during cardiac remodeling. Hypoperfusion in the hypertrophied myocardium is related to an increase in vascular capacity, suggesting a compensatory vasodilatory response at the capillary level. These microvascular changes may therefore contribute to the development of heart failure. (Circulation. 2001;103:1564-1569.)

Key Words: remodeling | microcirculation | magnetic resonance imaging

Transmural myocardial infarction (MI) induces hypertrophy and remodeling of the noninfarcted myocardium.¹-³ Alterations in the myocardial microcirculation are involved in post-MI reactive hypertrophy.⁴,⁵ Anatomic studies⁶,⁷ found a reduction in capillary surface with myocyte hypertrophy ⁴ and remodeling of the noninfarcted myocardium.¹⁰ Serial determination of these parameters is feasible. The aim of the present study was to quantify perfusion and RBV during LV remodeling from 8 to 16 weeks after MI. The parameters were measured in the rat heart at rest and during administration of intravenous adenosine.¹¹

Methods

Infarct Model and Experimental Preparation
Experiments were performed in adult female Wistar rats (Charles River, Sulzfeld, FRG) weighing 250 to 290 g. MIs were produced by left coronary artery ligation by a method described previously.² Briefly, left thoracotomy was performed after intubation under ether anesthesia. After exposure of the heart, the anterior descending branch of the left coronary artery was ligated. The thorax was instantly closed after the heart was replaced. The mortality of this procedure was 38% within 24 hours after surgical intervention. Sham-operated rats underwent the same surgical procedure but...
without coronary ligation. For MR measurements, the animals were anesthetized with sodium pentobarbital (Narcoren, Rhone Merieux GmbH; 40 mg/kg IP) and were orally intubated with a home-built tracheal cannula. Artificial ventilation was established with a small-animal respirator (BAS-7025, FMI). The respirator was controlled by the pulse program of the MR spectrometer. Respiration of the animal was automatically stopped during image acquisition to avoid respiratory motion artifacts. The ECG trigger signal was received via foreleg electrodes connected to a home-built ECG unit. Gd-DTPA–albumin was used as intravascular contrast agent and was produced according to a method described by Ogan et al. Anesthesia (sodium pentobarbital, 10 to 30 mg/kg IV) and contrast agent were administered via a tail vein. For RBV measurement, each animal received a single bolus injection of Gd-DTPA–albumin in a dose of 0.75 μmol/kg (∼0.3 mL). Animals awoke after MR measurement and were remeasured at 8, 12, and 16 weeks after operation. All experimental procedures with animals were in accordance with the European regulation on care and use of laboratory animals.

**Image Acquisition**

All images were acquired on a 7.05-T Biospec 70/21 spectrometer (Bruker). A specially adapted double probe head for rat heart measurements was used, including a whole-body coil for transmission and a circular polarized surface coil as receiver.

**Quantitative T1 Imaging**

T1 images were obtained with an inversion recovery snapshot fast, low-angle shot (FLASH) sequence. Twenty-four ECG-triggered snapshot FLASH images were recorded after global or slice-selective spin inversion. Each snapshot FLASH image (TR=2.25 ms, TE=1 ms, flip angle =3°, slice thickness 3 mm, field of view 50×50 mm) was acquired within a heart cycle (180 to 200 ms), with a resulting spatial resolution in plane of 390×780 μm². A special technique was used to improve the time resolution of the T1 measurement and to measure T1 values smaller than a heart rate: Two successive ECG-triggered T1 experiments with different delays (varying in steps of 50 to 100 ms) between the inversion pulse and the first FLASH image were recorded. For one T1 experiment, 24 snapshot FLASH images were recorded, so that the total acquisition time for one T1 image was in the range of 2×24=48 FLASH images (1 to 2 minutes).

Images were obtained in a short-axis slice perpendicular to the long axis of the heart, which was identified after axial and long-axis scout views. Infarcted myocardium of the anterior wall was regularly visible in the imaging slice. Quantification of perfusion was performed with a slice-selective and a global T1 experiment according to Equation 1:

\[
P = \frac{T1_{glob}}{T1_{blood}} \times \left( \frac{1}{(1/T1_{sel}) - (1/T1_{glob})} \right)
\]

The intracapillary blood volume may be determined from slice-selective T1 experiments by measurement of 1/T1 sel before and after application of an intravascular contrast agent (ca) according to Equation 2:

\[
RBV = \frac{1 - \frac{1}{1 - \frac{1}{T1_{sel}} \times \frac{1}{T1_{blood}}}}{\lambda} \times \frac{1}{\lambda}
\]

\[
\lambda = \frac{1}{(1 - Hct_{venous}) \times (1 - Hct_{capillaries})}
\]

\[
RBV = RBV \times \frac{1 - Hct_{capillaries}}{1 - Hct_{venous}} = 0.7 \times RBV.
\]

The theoretical model is described in detail by Bauer et al.

**MR Cine Imaging**

Imaging was performed with an ECG-triggered fast gradient echo (FLASH) cine sequence with the following imaging parameters: flip angle 40°, TE 1.2 ms, TR 4.3 ms, field of view 30 to 35 mm; slice thickness 1 mm. The acquisition matrix was 128×128, resulting in a spatial resolution in plane of 270×310 μm². Measurements were performed in the short-axis plane. Acquisition time for 1 cine image was in the range of 40 to 50 seconds, depending on heart rate. To increase the signal-to-noise ratio, the images were averaged 4 times. MR data acquisition was performed in multiple short-axis images from the apex to the base of the left ventricle with no interslice gap. Typically, 16 cine images were performed to cover the whole left ventricle, resulting in a total acquisition time of ∼15 to 20 minutes.

**Image Analysis**

**Perfusion and RBV**

Spatially resolved T1 maps were calculated from the 48 FLASH images of 2 successive inversion recovery snapshot FLASH experiments. The procedure of this calculation is described in detail by Deichmann et al.

In sham-operated animals, a midmyocardial region of interest (ROI) covering the whole left ventricle was manually delineated (170 to 200 pixels). In infarcted animals, the ROI covered the posterior, lateral, and septal hypertrophied LV regions, excluding the transient zone adjacent to the scar (140 to 180 pixels). Mean values for perfusion and RBV were obtained by averaging the pixel data in the ROI. The right ventricular wall was eliminated for further evaluation because of the thin myocardial wall and consecutive partial-volume effects.

**Infarct Size, Myocardial Function, and Myocardial Mass**

Quantitative analysis of MR infarct size and myocardial mass and function was performed by a method described by Zierahn et al. Briefly, infarct size was expressed as the ratio of scar length to total circumference determined by manual delineation of epicardial and endocardial borders of scar length and total circumference of each cine image. Histological studies have reported that the summation of these length measurements approximates the surface of the infarcted area. Scar was easily identified in the MR images by changes in myocardial wall thickness from scar to hypertrophied tissue. For LV mass measurement, epicardial and endocardial borders of all slices were delineated, and the mass was defined as the volume within the borders multiplied by a factor of 1.05, which represents the myocardial specific density (g/mL). The papillary muscles were included in the traced area. Absolute cavity volumes were calculated in end diastole (EDV) and end systole ( ESV) as the sum of all blood pool areas. Stroke volume (SV) was calculated from SV= EDV– ESV.

**Experimental Protocol**

MR perfusion, RBV, and cine imaging were performed on 12 infarcted animals (group 1). Ten sham-operated animals served as controls (group 2). Animals were measured 3 times at 8, 12, and 16 weeks after operation. At each time point, a measurement protocol was observed, as follows: (1) cine imaging, (2) perfusion at rest, (3) adenosine, (4) perfusion under adenosine, (5) RBV under adenosine, (6) withdrawal of adenosine, and (7) RBV at rest. Animals received adenosine in a continuous dose of 2.5 to 3 mg·kg⁻¹·min⁻¹. It was recently shown that this dose leads to a maximum vasodilatory response without critical bradycardia. At 16 weeks, a final hemodynamic measurement of groups 1 and 2 was performed after MR measurement. Because of the serial character of the study, 2 additional groups (groups 3a and 3b) were needed for hemodynamic studies at 8 and 12 weeks.
Hemodynamic Studies
A terminal hemodynamic study was performed 16 weeks after sham operation or coronary artery ligation. In addition, hemodynamic studies were performed in a group of 16 animals with 8-week-old MI (n=8, group 3a) and 12-week-old MI (n=8, group 3b). Rats were prepared as described above. A polyethylene catheter (Portex) connected to a Millar micromanometer (Millar Instruments) was used to measure mean aortic pressure and heart rate. Hemodynamic data were recorded at rest and during infusion of adenosine as in the experimental setup of groups 1 and 2. Coronary resistance (CR) was determined from the mean aortic pressure of group 3 and perfusion data of group 1 according to CR=mean aortic pressure/perfusion.

Infarct size determination of the additional group was determined histologically according to the method described by Pfeffer et al.²

Statistical Analysis
The data are expressed as mean±SEM. Statistical tests were evaluated by ANOVA (InStat, GraphPad). Significant difference was determined by the Bonferroni test. The probability level of statistical significance was P<0.05. Correlation coefficients were computed by a least-squares linear regression analysis.

Results
Perfusion and RBV
Perfusion and RBV maps of a representative animal of group 1 (MI 22.4%) at rest and during administration of 3 mg · kg⁻¹ · min⁻¹ of adenosine are shown in Figure 1, a through d. Corresponding maps of perfusion and RBV of an animal of group 2 at rest are illustrated in Figure 1, e and f. Images were generated 8 weeks after induction of MI. The scar tissue, LV dilation, and hypertrophy of the noninfarcted myocardium are visible. All animals developed MI in the size range of 10% to 35% (mean 24.1±2.0%) of the LV area. Data of myocardial perfusion and RBV of the infarcted (group 1) and the sham-operated (group 2) animals during this time are demonstrated in Figures 2 and 3. Perfusion in surviving myocardium decreased significantly (P<0.01) from 8 to 16 weeks compared with the sham-operated animals. Coronary reserve was reduced significantly at 16 weeks after MI (P<0.01) compared with 8 weeks after MI (Figure 2). Basal RBV in surviving myocardium was increased significantly at 8 weeks after MI (P<0.01) and remained elevated during the observation time, whereas maximum RBV during adenosine was the same in the MI and the sham-operated groups (Figure 3).

MR Myocardial Mass and Functional Parameters
Data for LV mass and volumes of the infarcted group and the sham-operated animals are summarized in Table 1. There was
no significant change in body weight with time or with respect to the sham-operated animals at each time point. LV mass of the infarcted animals of group 1 increased significantly from 8 to 16 weeks after MI ($P < 0.05$). LV mass of the sham-operated animals at 16 weeks was significantly lower ($P < 0.01$) than the values of the infarcted animals. LV volumes remained elevated compared with their respective noninfarcted controls ($P < 0.001$) and did not increase further with time from 8 to 16 weeks. LV weight–to–body weight ratio (mg/g) relative to changes in RBV and perfusion of group 1 are illustrated in Figure 4. Perfusion at rest and during adenosine decreased significantly ($P < 0.01$) with increasing relative LV heart weight, whereas RBV at rest and during adenosine showed no significant change. LV weight–to–body weight ratio of the sham-operated group was $1.66 \pm 0.09$, $1.64 \pm 0.08$, and $1.74 \pm 0.09$ mg/g at 8, 12, and 16 weeks, respectively. Linear regression analyses of relative heart weight and CR at rest and during adenosine are shown in Figure 5. A significant correlation ($P < 0.001$) was found between the 2 parameters.

**Systemic Hemodynamics**

Hemodynamic data of group 1 (16 weeks after MI), group 2 (16 weeks after sham operation), group 3a (8 weeks after MI), and group 3b (12 weeks after MI) at rest and during adenosine are shown in Table 2. In all groups, mean aortic pressure and heart rate decreased significantly ($P < 0.001$) during adenosine showed no significant change. LV weight–to–body weight ratio of the sham-operated group was $1.66 \pm 0.09$, $1.64 \pm 0.08$, and $1.74 \pm 0.09$ mg/g at 8, 12, and 16 weeks, respectively. Linear regression analyses of relative heart weight and CR at rest and during adenosine are shown in Figure 5. A significant correlation ($P < 0.001$) was found between the 2 parameters.

### Table 1. MR Cine Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Week</th>
<th>MI, %</th>
<th>BW, g</th>
<th>LV mass, mg</th>
<th>LV EDV, µL</th>
<th>SV, µL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>$24.1 \pm 3.0$</td>
<td>$297 \pm 14$</td>
<td>$559.1 \pm 20.8$</td>
<td>$528.2 \pm 78.5$</td>
<td>$131.4 \pm 16.3$</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>$23.6 \pm 3.4$</td>
<td>$313 \pm 10$</td>
<td>$566.0 \pm 35.0$</td>
<td>$469.9 \pm 60.4$</td>
<td>$112.2 \pm 12.1$</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>$24.4 \pm 2.9$</td>
<td>$319 \pm 12$</td>
<td>$690.9 \pm 42.7^*$</td>
<td>$473.4 \pm 57.6$</td>
<td>$121.9 \pm 12.4$</td>
</tr>
<tr>
<td>Group 2</td>
<td>12</td>
<td>$294 \pm 16$</td>
<td>$300 \pm 12$</td>
<td>$516.3 \pm 41.7$</td>
<td>$158.9 \pm 10.0^†$</td>
<td>$95.6 \pm 9.7$</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>$309 \pm 18$</td>
<td>$309 \pm 18$</td>
<td>$478.3 \pm 48.3$</td>
<td>$185.4 \pm 14.6^†$</td>
<td>$121.5 \pm 19.1$</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>$549.2 \pm 32.3^†$</td>
<td>$549.2 \pm 32.3^†$</td>
<td>$549.2 \pm 32.3^†$</td>
<td>$221.6 \pm 12.6^†$</td>
<td>$114.6 \pm 12.0$</td>
</tr>
</tbody>
</table>

BW indicates body weight; EDV, end-diastolic volume; and SV, stroke volume.

* $P < 0.05$ vs 8 weeks.
† $P < 0.05$ vs group 1.
is based on the application of microspheres. This method is invasive, however, and serial measurements during cardiac remodeling are not feasible. Myocardial blood volume may be determined by morphometric techniques or from radioactive labeling of red blood cells. Unfortunately, the 2 methods cannot be used for longitudinal studies in the same animal because of ex vivo data evaluation. By echocardiography or CT imaging, myocardial blood volume can be determined after bolus injection of a contrast agent. Because of the underlying first-pass imaging technique, however, repeated measurements under steady-state conditions are not possible.

We recently developed a non–contrast agent–based MR technique to measure myocardial perfusion. This method has been validated by the microsphere technique for the isolated rat heart and for in vivo studies. In addition, we evaluated MRI of the RBV with an intravascular contrast agent. These data showed a favorable correlation with data for RBV obtained by the MR first-pass technique.

Because of the serial character of this study, simultaneous invasive determination of blood pressure is not possible at each time point. Therefore, we introduced reference groups with comparable infarct sizes for the measurement of blood pressure at baseline and after vasodilation at 8 and 12 weeks after the induction of MI. This allowed calculation of derived parameters as CR.

Perfusion During LV Remodeling

At 8 weeks after MI, baseline perfusion was slightly elevated, whereas the maximum perfusion was comparable to control animals. In the following period (12 and 16 weeks) of remodeling, baseline and maximum perfusion declined significantly (Figure 2). Because blood pressure was not significantly different between the sham-operated and the infarcted animals of group 1 (Table 2), this decrease is due to an increase in CR. In contrast to our findings, Karam et al observed an increase of myocardial baseline perfusion, mainly due to a reduction of CR, whereas maximum perfusion was lower and minimum CR higher than in control animals. Those authors, however, performed measurements 4 weeks after infarction, whereas the decrease of baseline perfusion in our study was observed from 12 weeks on after MI. CR is affected by vascular and extravascular factors. Karam et al found a significant increase in minimum CR, which was related to reactive LV hypertrophy. The positive correlation between CR and relative heart weight (Figure 5) and the time course of both parameters in our study suggest that parameters that are related to myocardial hypertrophy may explain the elevation of baseline and minimum CR. Our values of an increase in LV diameter and relative heart weight are in good accordance with data of Pfeffer et al for animals with small MI size. Structural alterations of the resistance vessels, eg, increased total arteriolar length or arteriolar wall thickening, may be responsible for this increase in CR.

RBV During LV Remodeling

RBV at baseline is elevated in residual myocardium of post-MI hearts, whereas the RBV after vasodilation does not differ from that of control hearts. In contrast to myocardial perfusion, baseline and maximum regional blood volume

**TABLE 2. Hemodynamic Data**

<table>
<thead>
<tr>
<th>Week</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
<th>BW, g</th>
<th>MI size, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>At rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (Group 3a)</td>
<td>107.3±3.7</td>
<td>355±16</td>
<td>302±9</td>
<td>23.9±2.0</td>
</tr>
<tr>
<td>12 (Group 3b)</td>
<td>117.9±5.1</td>
<td>348±20</td>
<td>314±4</td>
<td>24.2±1.9</td>
</tr>
<tr>
<td>16 (Group 1)</td>
<td>109.9±3.3</td>
<td>320±21</td>
<td>318±8</td>
<td>24.0±2.0</td>
</tr>
<tr>
<td>16 (Group 2)</td>
<td>118.8±2.8</td>
<td>378±14</td>
<td>314±7</td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>41.0±2.5*</td>
<td>223±23*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At rest</td>
<td>42.6±3.6*</td>
<td>178±20*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>44.0±1.7*</td>
<td>225±21*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MAP indicates mean aortic pressure; HR, heart rate; and BW, body weight.

*P<0.001 at rest vs adenosine.
(RBV) remain almost constant during the observation time. This implies that the increase in baseline RBV must have occurred earlier than 8 weeks after MI. This elevation of RBV may be considered as a compensatory mechanism at the capillary level to an increased workload of the residual myocardium due to LV dilation. Olivetti et al. found a preservation of the volume percent of capillaries within the surviving myocardium as a result of an increase in capillary diameter that compensated for the reduction of capillary density. This was found also for hearts with small MI size and is congruent with our observation when we relate the morphometric RBV to our maximum RBV data. Other groups have described that insufficient vascular growth relative to myocyte hypertrophy results in a vasodilatory response of microvessels to maintain tissue oxygen needs due to hypertrophy. Our results indicate that the RBV is independent of changes in CR during cardiac remodeling, because RBV at rest and during hyperemia remains constant over time, whereas CR increases.

Our study has shown that significant adaptation of the myocardial microcirculation in hearts with small MI size occurs during LV remodeling. We found significant hyperfusion from 8 to 16 weeks after MI with increasing CR and an increase in vascular capacity, suggesting a compensatory vasodilation at the capillary level. Our results suggest that microvascular remodeling due to myocyte hypertrophy may play an important role in the development of heart failure also in patients with chronic MI.

Acknowledgments

This work was supported by grant SFB 355 and Graduiertenkolleg 178 NMR 178 HA 1232/8-1 of the Deutsche Forschungsgemeinschaft and of the Forschungsfonds der Universitätsklinik Mannheim/Heidelberg (Projekt 42). The authors gratefully acknowledge the dedicated work of T. Lanz, A. Weisser, and E. Rommel. The authors also thank F. Wiesmann for help with the experimental preparation.

References

Serial Magnetic Resonance Imaging of Microvascular Remodeling in the Infarcted Rat Heart

Circulation. 2001;103:1564-1569
doi: 10.1161/01.CIR.103.11.1564
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/103/11/1564

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/