Noninvasive Measurements of Infarct Size After Thrombolysis With a Necrosis-Avid MRI Contrast Agent

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Background—Gadophrin-2 is a new MRI contrast agent with high affinity for necrotic myocardium. The aim of the study was to evaluate whether noninvasive measurements of infarct size after thrombolysis are possible with gadophrin-2–enhanced MRI.

Methods and Results—Coronary artery thrombosis was induced in 3 groups of dogs by the copper-coil technique. Thrombolytic therapy together with aspirin and heparin was initiated after 90 minutes of occlusion. One day (group A), 2 days (group B), or 6 days (group C) after infarction, gadophrin-2 was injected intravenously (50 μmol · kg⁻¹). In vivo T1-weighted segmented turbo-FLASH, in vivo T2-weighted segmented half-Fourier turbo spin echo (HASTE), and T1- and T2-weighted spin-echo MRI of the excised heart were performed 24 hours after gadophrin-2 injection. Regions of strong enhancement were observed on T1-weighted images. Planimetry of short-axis MR images and of corresponding triphenyltetrazolium chloride (TTC)-stained left ventricular (LV) slices showed a close correlation between the enhanced areas and TTC-negative areas for both in vivo ($r^2 = 0.98$, $P < 0.0001$; mean difference, $0.9 \pm 2.0\%$ [SD] of the LV volume [LVV]) and postmortem ($r^2 = 0.99$, $P < 0.0001$; mean difference, $0.9 \pm 1.4\%$ of LVV) measurements. T2-weighted images overestimated the infarct size by $8.1 \pm 5.4\%$ of LVV. The mean infarct size was $10.8 \pm 11.6\%$ of LVV (group A), $22.4 \pm 11.7\%$ (group B), and $5.1 \pm 9.3\%$ (group C).

Conclusions—In this animal model, in vivo gadophrin-2–enhanced MRI could precisely determine infarct size after thrombolytic therapy. This technique may be very useful for the noninvasive evaluation of infarct size after reperfusion for AMI. (Circulation. 1999;99:690-696.)

Key Words: magnetic resonance imaging • myocardial infarction • thrombolysis • diagnosis

Noninvasive measurements of infarct size provide valuable information in clinical practice. Several methods have been used: ECG scores, cardiac enzyme measurements, and echocardiographic and scintigraphic imaging techniques.1 Although good correlations with the histological infarct size were reported, these methods have several limitations, including inability to detect small infarcts, inability to distinguish between infarcted and ischemic myocardium, and relatively poor spatial resolution.1

Cardiac MRI emerged as an alternative method for the evaluation of myocardial infarction (MI).2,3 Although T2-weighted (T2w) spin-echo sequences are able to detect the presence of necrosis, the areas of increased signal intensity (SI) are probably more related to the presence of myocardial edema, systematically overestimating infarct size.4,5 Therefore, contrast enhancement is needed for optimal contrast between viable and necrotic tissue.6,7 Gd-DTPA has been used for infarct-size quantification,2,8,9 but the enhancement with this contrast agent is nonspecific, and therefore, infarct-size measurements may be less accurate.

Gadophrin-2 (previously referred as bis-gadolinium-mesoporphyrin) belongs to a type of MRI contrast media originally developed as “tumor-seeking” agents. It was recently shown that gadophrin-2 has affinity only for necrotic tissues.10–12 The usefulness of gadophrin-2–enhanced MRI for the identification and quantification of acute MI was recently demonstrated.13–16 The aim of the present study was to evaluate whether noninvasive measurements of infarct size after thrombolysis are possible with gadophrin-2–enhanced MRI.

Methods

Experimental Model

Dogs weighing 18 to 30 kg were sedated with xylazine (Rompun, Bayer AG), anesthetized with sodium pentobarbital (Nembutal, Schering AG). The Institut für Diagnostikforschung GmbH is a subsidiary of Schering AG. Drs Marchal, Ni, and Semmler have a patent pending for gadophrin-2 usage.

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Sanoﬁ), intubated, and artiﬁcially ventilated. Occlusive thrombosis of the left anterior descending coronary artery (LAD) was induced with a copper-coil model.17 After 90 minutes of occlusion, thrombolysis was initiated with alteplase (0.1 mg · kg−1 bolus and 0.01 mg · kg−1 · min−1 infusion for 30 minutes; Actilyse, Boehringer Ingelheim GmbH), aspirin (5 mg · kg−1 bolus; Aspegic, Synthelabo), and heparin (100 IU · kg−1 bolus and 25 IU · kg−1 · h−1 infusion for 2 hours; Heparine Rorer, Rhône-Poulenc Rorer). LAD ﬂow was monitored with an electromagnetic ﬂowmeter throughout the occlusion and treatment periods. After 2 hours of heparin infusion, the copper coil was withdrawn to prevent reocclusion; the LAD patency was assessed with the methodology of Bland and Altman.19 From this, we were able to calculate the mean SI for the whole enhanced area (EA). Contrast ratio (CR) was calculated by dividing the mean SI of the EA by the mean SI in a normal region of the LV.

Planimetric Measurements

Planimetry was performed with the Adobe Photoshop 4.0 software. LV area (LVA) (on MR images and TTC-stained slices), infarct area (IA) (on TTC-stained slices), and in vivo and postmortem EA (on MR images) were measured, and the results were converted from pixels squared to millimeters squared. To evaluate infarct volume (IV), enhanced volume (EV), and left ventricular volume (LVV), we ﬁrst calculated the slice averages (arithmetic mean of IA, EA, and LVA on the 2 sides of the TTC-stained slices and corresponding MR images). The volumes in 1 slice were obtained by multiplying the corresponding average by the slice thickness. The total volumes (IV, EV, and LVV) were calculated by summation of all slice volumes of 1 heart. The delineation of the LV cavity was not possible on in vivo T1w images; therefore, LVA was calculated only on T2w images for in vivo MRI. The enhanced regions on MRI were compared with the TTC-labeled infarct regions in 2 ways: slice-by-slice (IA and EA were compared; 1 slice gives 1 data point) and globally (IV and EV were compared; 1 heart gives 1 data point).

Statistical Analysis

The agreement between MRI and TTC measurements of infarct size was assessed with the methodology of Bland and Altman.19 The SI of EAs and of nonenhanced regions was compared by paired t tests; the CRs in the 3 groups of dogs were compared by unpaired t tests. A value of P≤0.05 was considered signiﬁcant. The normal distribution was tested with the Shapiro-Wilk statistic,20 and logarithmic transformations were used when appropriate. All calculations were performed with the SAS procedures GLM, UNIVARIATE, and TTEST.21 Data are presented as mean±SD.

Results

A total of 32 experiments were performed. Twelve dogs died before MRI (during thrombolysis, 6; not recovered from anesthesia, 2; during the postinfarction period, 4) and were excluded. Initially, 18 dogs were divided into 3 groups of 6. The group allocation was based on the availability of the catheterization and MRI laboratories. This nonrandomized design resulted in a selection bias (see below); therefore, 2 more dogs were added to group C. Thrombolysis induced reperfusion in 17 dogs; reocclusion occurred in 6 dogs during the initial 2 hours of therapy. All dogs showed patent LADs 1 hour after removal of the copper coil. T1w and T2w MR images of 189 in vivo slices, of 800 postmortem slices, and
189 pictures of the TTC-stained LV slices were obtained. In 7 dogs, the first apical slice on in vivo MRI was excluded from the analysis because of artifacts (partial volume effects with fatty tissue, poor delineation of the ventricle). The remaining 182 slices were used for the comparison of in vivo MRI and TTC staining. Of the 800 postmortem slices, only the 182 images corresponding to the TTC-stained slices were selected for comparisons (Figure 2).

Anatomic Infarct Size
Planimetry of the TTC-stained slices showed a broad range for infarct size (0% to 98% of LVA, 0% to 36% of LVV). Eight dogs had infarctions of <5% of LVV. Infarcted zones were present on 134 of 182 slices. The infarcts were significantly smaller in group C than in group B (Table 1).

MRI Evaluation of Infarct Size
The results of MRI evaluation of infarct size are summarized in Tables 1 and 2. T1w EAs were observed on 118 slices at in vivo MRI and on 147 slices at postmortem MRI. Both slice-by-slice and global analyses showed an excellent correspondence between T1w images and TTC staining with regard to the quantification of infarct size (correlation coefficients were $r^2=0.98$ between in vivo MRI and TTC and $r^2=0.99$ between postmortem MRI and TTC; $P<0.0001$ for both). The limits of agreement calculated with the methodology of Bland and Altman were narrow; the mean difference between TTC and MRI measurements of infarct size was almost zero (Table 2, Figure 3). No significant differences between groups were observed for the TTC-MRI agreement.

The contrast between infarcted and normal myocardium in black-blood images was poor; these images were used only

<table>
<thead>
<tr>
<th>Group</th>
<th>TTC</th>
<th>In Vivo T1w</th>
<th>Postmortem T1w</th>
<th>Postmortem T2w</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=6)</td>
<td>10.8±11.6</td>
<td>12.3±11.5</td>
<td>11.4±11.7</td>
<td>18.7±16.7*</td>
</tr>
<tr>
<td>B (n=6)</td>
<td>22.4±11.7</td>
<td>24.3±12.6</td>
<td>24.9±12.9</td>
<td>33.6±16.4*</td>
</tr>
<tr>
<td>C (n=8)</td>
<td>5.1±9.3†</td>
<td>5.7±9.3</td>
<td>5.0±8.2</td>
<td>10.9±10.8*</td>
</tr>
<tr>
<td>Total (n=20)</td>
<td>12.0±12.3</td>
<td>12.9±13.0</td>
<td>12.9±13.3</td>
<td>20.0±16.8*</td>
</tr>
<tr>
<td>CR, %</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>A (n=6)</td>
<td>...</td>
<td>168.8±29.1</td>
<td>139.2±11.3</td>
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</tr>
<tr>
<td>B (n=6)</td>
<td>...</td>
<td>176.7±35.2</td>
<td>137.6±13.2</td>
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<tr>
<td>C (n=8)</td>
<td>...</td>
<td>149.8±20.6‡</td>
<td>123.2±12.9</td>
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<tr>
<td>Total (n=20)</td>
<td>...</td>
<td>166.9±31.9</td>
<td>133.3±14.2</td>
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</tr>
</tbody>
</table>

Data are presented as mean±SD.
*P<0.05 vs TTC measurements; †P<0.05 vs group B; ‡P<0.05 vs groups A and B.
TABLE 2. Agreement Between MRI- and TTC-based Measurements of Infarct Size

<table>
<thead>
<tr>
<th>Analysis and Group</th>
<th>In Vivo T1w</th>
<th>Postmortem T1w</th>
<th>Postmortem T2w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slice-by-slice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference MRI-TTC, % of LVA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n=55)</td>
<td>−0.9±4.5</td>
<td>0.2±2.3</td>
<td>8.7±10.8</td>
</tr>
<tr>
<td>B (n=54)</td>
<td>−0.9±2.9</td>
<td>0.3±2.3</td>
<td>7.0±6.5</td>
</tr>
<tr>
<td>C (n=73)</td>
<td>0.2±2.1</td>
<td>−0.2±1.0</td>
<td>6.1±7.0</td>
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<tr>
<td>Total (n=182)</td>
<td>−0.1±2.4</td>
<td>0.0±2.0</td>
<td>7.3±8.1</td>
</tr>
<tr>
<td>Limits of agreement MRI-TTC, % of LVA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A (n=55)</td>
<td>(−9.7, +8.0)</td>
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<td>(−12.9, +30.3)</td>
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<td>(−2.3, +1.9)</td>
<td>(−7.9, +20.2)</td>
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<tr>
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<td>(−3.9, +4.0)</td>
<td>(−8.9, +23.5)</td>
</tr>
<tr>
<td>Global</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean difference MRI-TTC, % of LVV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n=6)</td>
<td>−0.5±1.2</td>
<td>0.7±0.9</td>
<td>8.0±6.5</td>
</tr>
<tr>
<td>B (n=6)</td>
<td>0.9±3.0</td>
<td>1.6±1.4</td>
<td>8.6±4.8</td>
</tr>
<tr>
<td>C (n=8)</td>
<td>0.6±1.1</td>
<td>−0.1±0.5</td>
<td>5.9±4.4</td>
</tr>
<tr>
<td>Total (n=20)</td>
<td>0.9±2.0</td>
<td>0.9±1.4</td>
<td>8.1±5.4</td>
</tr>
<tr>
<td>Limits of agreement MRI-TTC, % of LVV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (n=6)</td>
<td>(−1.9, +2.9)</td>
<td>(−1.1, +2.5)</td>
<td>(−5.0, +21.0)</td>
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<td>(−5.1, +6.9)</td>
<td>(−1.2, +4.4)</td>
<td>(−1.0, +18.2)</td>
</tr>
<tr>
<td>C (n=8)</td>
<td>(−1.6, +2.8)</td>
<td>(−1.1, +1.0)</td>
<td>(−2.9, +14.6)</td>
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<tr>
<td>Total (n=20)</td>
<td>(−3.0, +4.9)</td>
<td>(−1.9, +3.8)</td>
<td>(−2.8, +18.9)</td>
</tr>
</tbody>
</table>

n indicates number of slices or hearts. Data are presented as mean±SD and as intervals. The limits of agreement are given within parentheses.

Discussion

Evaluation of Infarct Size on Gadophrin-2–Enhanced Images

This report represents the first study of infarct-size measurements with gadophrin-2–enhanced MRI after thrombolytic therapy. The experimental design mimicked the clinical setting of an acute MI treated with thrombolysis. Furthermore, MRI was performed at different intervals, corresponding to the time points at which patients are generally stabilized and can be discharged from the coronary care unit (2 and 3 days after infarction) or from the hospital (7 days after infarction).

The results of this study support our previous findings that gadophrin-2–enhanced MRI is a reliable, noninvasive method for infarct-size evaluation. We were able to locate and quantify infarcts as small as 1% or as large as 36% of LVV with similar accuracy. The methodology used allows systematic in vivo quantification of the infarct size: the LV was completely imaged by 9 to 11 parallel slices, 96% of the images being included in the analysis. The mean difference between TTC-labeled infarct size and gadophrin-2 enhanced areas in T1w images was almost zero, with limits of agreement of less than ±5% of the LV for both slice-by-slice and global comparison, indicating a high accuracy of the MRI measurements.

The gadophrin-2–induced enhancement of necrosis was good, with an in vivo CR averaging 166.9%. The CR was similar in dogs monitored 2 or 3 days after acute MI but significantly lower in dogs monitored after 7 days. The lower uptake of gadophrin-2 may be related to the process of healing, during which denatured tissue components responsible for contrast “trapping”10–13 are progressively replaced by scar. This hypothesis is supported by the findings of a lack of enhancement with gadophrin-2 in the presence of strong enhancement with Gd-DTPA 1 month after infarction in pigs,22 indicating that gadophrin-2 is a sensitive marker of necrosis in the early but not in the late stages of MI.

MRI was performed 24 hours after gadophrin-2 administration for a number of reasons. First, early imaging after gadophrin-2 was already reported.15 Second, a protocol with for LVA measurements. Postmortem T2w images showed the presence of myocardial injury. Both hyperintense and hypointense areas were present in most of these images; when these areas were summed, the results systematically overestimated the TTC-measured infarct size by 8.1±5.4% of LVV (range, 1.2% to 18.3%; P<0.0001). The interval of agreement with the TTC technique was large for T2w imaging.
both early and delayed imaging would not allow a period of recovery for the animals, especially in group A. Finally, and most importantly, considering that the highest mortality rates after acute MI are observed during the first 24 hours and that the present technology does not allow monitoring of the patient in the MRI laboratory at standards comparable to those in a coronary care unit, a pragmatic solution is to evaluate infarct size with MRI outside this critical period. Because of its persistence in the necrotic tissue, gadophrin-2 may offer the unique possibility to obtain a “fingerprint” of the infarct size as it was at the time of administration (ie, early after thrombolysis or direct angioplasty) with an MRI study performed as soon as the patient is stabilized. Furthermore, although an enhancement of the infarcts can be observed as early as 1 to 2 hours after injection, the best images are obtained after the agent is cleared from the blood.

The mechanisms responsible for contrast trapping in the areas of necrosis remain to be elucidated. The observation that gadophrin-2 enhancement of MI persists for a long period despite complete clearance from the blood (in an unpublished experiment, the enhancement was observed during the first 2 weeks but was lost at 4 weeks) suggests a binding or precipitation of gadophrin-2 in the necrotic areas. We have previously shown that the gadophrin-2 content is 10 times higher in infarcted than in normal myocardium. As for safety, no alterations were observed in the ECG, LV or aortic pressure, or dP/dt during intracoronary administration of gadophrin-2 in a previous experiment (unpublished data). Other studies with metalloporphyrins also suggest that these agents are stable compounds.

Figure 3. Agreement between MRI and TTC. Top, Plots of enhanced volumes (as percentage of LV) on MRI versus TTC-labeled infarct volumes (as percentage of LV). Line of identity is presented. Bottom, Plots of differences between methods against their mean; horizontal lines represent limits of agreement between TTC and MRI evaluations of infarct size.

Evaluation of Infarct Size on T2w Images
Delineation of infarcts on T2w HASTE images was in practice not feasible. On postmortem images obtained with a high-resolution coil, T2w imaging did not accurately estimate infarct size. The analysis was complicated by the simultaneous presence of hyperintense and hypointense areas on the same slice, by the smaller CR, and by the difficult delineation. Typically, areas with increased SI at the borders and decreased SI in the center were observed (“hyperintense” and “hypointense” were defined in comparison to the SI of normal myocardium). The hypointensity may be related to the presence of gadophrin-2 and/or hemoglobin in the center of necrosis. Like those of previous reports, our results show that T2w imaging overestimates the infarction in the first week after acute MI.
Potential Advantages of Gadophrin-2 Over Gd-DTPA

There are several potential advantages of gadophrin-2 over Gd-DTPA–enhanced MRI in the clinical settings of acute MI. Gadophrin-2 is an agent with high affinity for necrosis and therefore should be able to distinguish between irreversibly and reversibly injured myocardium. Good correlations were also reported between infarct size and Gd-DTPA imaging, but an analysis of agreement was not performed. Therefore, it is not known how much the quantification of infarct size with TTC staining and Gd-DTPA–enhanced MRI differ. Furthermore, Gd-DTPA allows only a narrow time window for MRI after contrast delivery. It was shown that infarct size is overestimated on MR images obtained early after Gd-DTPA injection. The contrast agent is then rapidly washed out, with a speed depending on many local factors. In contrast, gadophrin-2 uptake into the necrotic area is stable, and our study shows that MRI measurements of infarct size are accurate 24 hours after injection. We have previously reported the same accuracy for early imaging after intracoronary injection of a minimal dose of gadophrin-2.

Clinical Implications

Our results show that gadophrin-2–enhanced MRI can be used as a reliable noninvasive method to distinguish necrotic from ischemic and normal myocardium. This methodology may allow a better understanding of complex processes, such as stunning, hibernation, and infarct healing. Furthermore, considering that MRI can provide anatomic and functional information within a single imaging session, it may be very useful for the evaluation of the effects of reperfusion and the need for additional revascularization. Furthermore, in vivo infarct-size measurements may be a valuable efficacy end point in clinical trials of new reperfusion strategies.

Study Limitations

Our study has several limitations. First, LVA was calculated only from T2w images for in vivo MRI. Second, the TTC staining was not confirmed by histology. Third, the infarcts were significantly smaller in group C, probably as a result of a selection bias (dogs with severe hemodynamic impairment at the end of thrombolysis were generally not assigned to group C because of lower chances of survival). However, after we added 2 dogs to this group, the infarct size range (0% to 24%) was comparable to those of groups A (0.5% to 31%) and B (0.6% to 36%). It is difficult to assess whether changes in gadophrin-2 uptake over time or changes in the intrinsic properties of the necrotic area were responsible for the lower CR values in group C. Fourth, LAD patency was not systematically evaluated at the time of MRI for practical reasons (availability of the catheterization laboratory). Nevertheless, the homogeneous enhancement pattern observed on T1w images suggests that the LAD was patent at the time of gadophrin-2 injection in all dogs. Furthermore, the LAD was patent 1 hour after removal of the thrombogenic copper coil in all animals. Finally, no dogs with persistent occlusion were studied with this protocol. Thus, no conclusion can be drawn regarding the accuracy of the method for nonreperfused infarcts.

Conclusions

In this canine model, in vivo gadophrin-2–enhanced MRI could precisely evaluate infarct size after thrombolysis. The
contrast-induced enhancement was lower after 1 week. This technique may be very useful in the evaluation of infarct size after reperfusion in patients with an acute MI.

Appendix

The black-blood HASTE technique is an ultrafast MR sequence that combines a preparation pulse to suppress possible artifacts from flow in the cavity with an ultrafast acquisition scheme. The preparation pulse consists of a nonselective 180° inversion recovery pulse followed by a slice-selective 180° pulse (Figure 4A). The succession of these 2 pulses has no effect on the myocardial tissue. The spins of the blood in the cavity are in general not completely realigned with the axis of the main magnetic field due to flow into or out of the slice. A time delay (TI) is kept between the inversion recovery pulses and the start of the acquisition process. Ideally, its timing must be adjusted to the zero crossing of the signal in the cavity. Usually, TI is set to 600 ms, and it may be further adjusted in case of an incomplete suppression of the cavity signal.

The black-blood pulse is followed by a fast spin-echo acquisition with a long echo train. In the single-shot HASTE technique, 1 echo train fills half of the k space plus 8 additional lines (Figure 4B). The segmented HASTE uses 2 preparation pulses followed by echo trains that fill the same k space. The effective echo time is 43 ms. Images have a moderate T2 weighting. A preliminary study in patients has shown that this particular acquisition is very robust.28

Acknowledgments

This study was supported in part by research grant G.0304.97N of the National Fund for Scientific Research, Belgium. We are also grateful to Bert Bippen for his help with the computers and to Françoise Iweins for her help in the statistical analysis.

References

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Circulation. 1999;99:690-696
doi: 10.1161/01.CIR.99.5.690

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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