Myocardium at risk can be determined by ex vivo T2-weighted magnetic resonance imaging even in the presence of gadolinium: comparison to myocardial perfusion single photon emission computed tomography

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Aims

Determination of the myocardium at risk (MaR) and final infarct size by cardiac magnetic resonance imaging (CMR) enables calculation of salvaged myocardium in acute infarction. T2-weighted imaging is performed prior to the administration of gadolinium, since gadolinium affects T2 tissue properties. This is, however, difficult in an ex vivo model since gadolinium must be administered for determination of infarct size by CMR. We aimed to test the ability of ex vivo T2-weighted imaging to assess MaR using myocardial perfusion single photon emission computed tomography (SPECT) as reference and to investigate whether MaR could be assessed by ex vivo T2-weighted imaging after injection of gadolinium.

Materials and methods

In 18 domestic pigs, the left anterior descending artery was occluded for either 30 or 40 min, followed by 4 h of reperfusion. After explantation of the hearts, myocardial perfusion SPECT and T2-weighted imaging were performed for determination of MaR, either with or without gadolinium. Infarct size was determined by T1-weighted imaging and by triphenyl tetrazolium chloride (TTC) staining.

Results

T2-weighted imaging agreed with myocardial perfusion SPECT, both with and without gadolinium ($r^2 = 0.70$, $P < 0.01$) with a bias of $2.6 \pm 5.1\%$ ($P = 0.04$). Infarct size was $15.4 \pm 5.3$ and $22.1 \pm 5.6\%$ with TTC and T1-weighted imaging, respectively ($P = 0.008$) in nine pigs who had both infarct measures.

Conclusion

T2-weighted CMR imaging can be used to determine MaR in an ex vivo experimental model, both with and without the presence of gadolinium. Thus, CMR alone can be used to assess myocardial salvage in experimental studies.

Keywords

Acute myocardial infarction • Myocardium at risk • Area at risk • Cardioprotection • Cardiac magnetic resonance

Introduction

In the situation of acute coronary occlusion the size of the ischaemic region, referred to as the myocardium at risk (MaR), supplied by the occluded coronary artery is a major independent variable of final infarct size.1–3 Accurate assessment of MaR enables determination of a myocardial salvage index by normalizing the infarct size to MaR.4–6 The myocardial salvage index can be used to assess the effect of different cardioprotective treatment strategies, both in experimental models7 and in clinical studies.8

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MaR can be assessed by myocardial perfusion single photon emission computed tomography (SPECT) by injection of a radioactive tracer before opening of the occluded vessel. However, there is a limited clinical and experimental applicability of myocardial perfusion SPECT to assess the MaR due to logistical problems and/or the need of access to a radioactive tracer. Therefore, alternative methods to quantify MaR need to be developed.

Myocardial oedema is associated with cell injury during acute myocardial ischaemia and reperfusion. In experimental models, the presence of oedema in ischaemic myocardium has been observed in both in vivo and ex vivo studies with the use of T2-weighted imaging. Thus, T2-weighted imaging is a promising technique for quantitative assessment of the MaR. In the clinical setting, T2-weighted imaging has been shown to enable differentiation between acute and chronic myocardial infarction. Recently, T2-weighted imaging was validated in humans against myocardial perfusion SPECT as a method to accurately determine the size of the MaR and was subsequently used to determine the rate of infarct evolution in humans with a first-time occluded myocardial infarction.

For infarct visualization by cardiac magnetic resonance (CMR), the patient is injected with a gadolinium-based contrast agent. The gadolinium is distributed in the myocardium proportional to the amount of extracellular space in the tissue, referred to as the tissue distribution volume. In regions with ischaemic injury, the distribution volume is increased and the presence of gadolinium affects the T1 tissue properties in this region, which is the basis for late gadolinium-enhanced imaging. However, gadolinium also affects the T2 tissue properties of the myocardium that are normally utilized for visualization of oedema by T2-weighted imaging. T2-weighted imaging is therefore performed prior to the administration of gadolinium in a standard clinical protocol. This is, however, difficult in an ex vivo experimental model since gadolinium has to be administered before sacrificing the animal to enable assessment of myocardial infarction.

The aim of the present study was to test the ability of ex vivo T2-weighted imaging, with and without the presence of gadolinium, to assess MaR as compared with myocardial perfusion SPECT in pigs with experimentally induced myocardial infarction.

Methods

Experimental preparation

The experimental preparation was performed according to a previously described protocol. Healthy domestic male and female juvenile pigs weighing 42–53 kg were anaesthetized with 12.5 mg/kg thiopental (Pentothal, Abbott, Stockholm, Sweden) followed by intubation with cuffed endotracheal tubes and mechanical ventilation. A slow infusion of 1 μg/mL fentanyl (Fentanyl, Pharmalink AB, Stockholm, Sweden) in buffered glucose (25 mg/mL) was started at a rate of 2 mL/min and adjusted if needed. During balanced anaesthesia thiopental was titrated towards animal requirements with small bolus doses. The pigs were continuously monitored by electrocardiography. Heparin (200 IU/kg) was given intravenously at the start of the catheterization. A 3.0–3.5 × 15 mm Maverick monorail angioplasty balloon (Boston Scientific Scimed, Maple Grove, MN, USA) was positioned in the mid-portion of the left anterior descending artery (LAD) through a surgically exposed left carotid artery.

Figure 1 Study protocol. This image shows a schematic overview of the experimental study protocol from balloon inflation to TTC-staining. Ischaemia duration was varied (30 and 40 min) to vary the amount of infarction within the myocardium at risk. (*) After the first CMR imaging, three pig hearts were stored at 4–24 h after which they underwent a second CMR imaging session and TTC-staining. CMR, cardiac magnetic resonance; SPECT, single photon emission computed tomography; TTC, triphenyl tetrazolium chloride.
perfusion SPECT and CMR imaging for determination of MaR. Following imaging, the hearts were cut in 5 mm thick short-axis slices that were immersed in a 1% triphenyl tetrazolium chloride (TTC) solution (Sigma-Aldrich, Stockholm, Sweden) in saline at 34°C for ~5–7 min. The TTC-stained short-axis slices were then photographed under blue light to verify infarction. In order to explore what happens to the explanted heart during the first 24 h, the hearts from three pigs were placed in a refrigerator at 4°C for ~24 h, followed by a second CMR imaging session, before TTC-staining was performed.

Myocardial perfusion SPECT
The excised ex vivo heart underwent myocardial perfusion SPECT imaging with a dual head camera (Philips SKYLight, Best, the Netherlands) and a vertex high resolution collimator (ADAC Vertex, Milpitas, CA, USA) at 32 projections (40 s per projection) with a 64 × 64 matrix yielding a digital resolution of 4.24 × 4.24 × 4.24 mm. Iterative reconstruction using maximum likelihood-expectation maximization (MLEM) was performed with a low-Nyquist and order 5.0. No attenuation or scatter correction was applied. Finally, a short-axis image stack was reconstructed using commercially available software (AutoSPECT Plus, Pegasys software version 5.01, Philips).

CMR imaging
CMR imaging was performed using a 1.5T Philips Achieva (Philips, Best, the Netherlands) with a quadrature head coil. Initial scout images were acquired to locate the heart. All hearts were imaged with a T2-weighted dual inversion black blood breath hold sequence (T2-weighted turbo spin echo, T2-TSE) with an asymmetric turbo spin echo. T2-TSE images were acquired in the short- and long-axis views, covering the left ventricle from base to apex with a simulated heart rate at 60 b.p.m. Imaging parameters for the T2-TSE sequence were: field-of-view, 140 mm; echo time (TE), 60 ms; repetition time (TR), 2 heart beats; number of averages, 2; black blood inversion delay, 574 ms; acquisition matrix size, 140 × 124; image resolution, 0.625 × 0.625 mm; slice thickness, 4 mm; slice gap, 0 mm; no parallel imaging. For infarct visualization, a three-dimensional acquisition of T1-weighted images (TR = 20 ms, TE = 3.2 ms, flip angle = 70° and 2 averages) yielding a stack of ~200 images with an isometric resolution of 0.5 mm covering the entire heart.

Image analysis
All CMR image analysis was performed using the software Segment (version 1.8, Medviso AB, Lund, Sweden, http://segment.heiberg.se). The left ventricle volume was assessed by manual tracing of the endocardial and epicardial borders of the left ventricle on the CMR images, including the papillary muscles as a part of the left ventricular wall.

To ensure co-registration of endocardial and epicardial borders of the myocardium, an image fusion module was implemented into the software Segment for registration of the endocardial and epicardial borders defined in the CMR images to be transferred to the myocardial perfusion SPECT images. The fusion module allows for co-registration by rigid body motion (translation and rotation). Fusion of the myocardial perfusion SPECT and CMR images was then performed by overlaying the myocardial perfusion SPECT images, with variable translucency, on top of the delineated CMR images. The combined fusion image was used to visually ensure optimal co-registration in all three orthogonal imaging planes. If necessary, manual adjustments were made without deviating from the LV volume as assessed by CMR imaging.

The perfusion defect was determined by an automated algorithm that considers myocardium with <55% of normal counts as being ischaemic. In some pigs minor adjustments were made in the basal short-axis slice containing a perfusion defect within the delineated left ventricle not depicted by the computer algorithm.

The MaR by CMR imaging was identified in all short-axis slices as hyperintense regions and was delineated manually after changing the window settings to maximizing the visual contrast to remote myocardium. The signal intensities between 0 (black) and 1 (white) for the resulting image on which the delineation was performed were then analysed for the different parts of the myocardium. The analysis was performed by two observers blinded to all other data according to a protocol previously validated with independent myocardial perfusion SPECT imaging. If present, an area of hypointense signal within the area of increased signal intensity, either due to no reflow/haemorrhage or the presence of gadolinium, was included in the MaR. The MaR was expressed as a percentage of the left ventricular myocardium.

The infarct size was determined on the high-resolution T1-weighted images as previously described by automatic detection of myocardium with signal intensity above the infarction threshold defined as >8 SD above the mean intensity of non-affected remote myocardium. Microvascular obstruction was defined as hypointense regions in the core of the infarction which had signal intensity less than the threshold for infarction. These regions were manually included in the infarct volume. The infarct size was expressed as a percentage of the left ventricular myocardium.

Contrast-to-noise ratio (CNR) was determined for both the hyper-enhanced part and the hypoenhanced core (for the pigs with gadolinium injected) compared to the remote myocardium. The CNR was calculated as the SNR in the affected region (hyper- or hypoenhanced regions of the MaR)—SNR within the remote myocardium.

After the MR examination the heart was sliced in 5 mm thick short-axis slices using a commercially available meat slicer. The slices were weighed and embedded in 1% TTC solution for ~5 min at 37°C. The TTC-stained short-axis slices were then photographed and the images were used to assess infarct size. The TTC-images were analysed using the software Image J (version 1.45s, National Institute of Health, Bethesda, USA, http://imagej.nih.gov/ij). The epicardium and endocardium were traced followed by the infarct size. The final infarct size was then calculated as a percentage of the left ventricular myocardium.

Statistical methods
Continuous variables are presented as mean ± SD. The Spearman’s rank correlation was used to determine the relationship between MaR assessed by T2-weighted imaging and myocardial perfusion SPECT and myocardial infarct size by TTC-staining. The interobserver variability was expressed as mean difference ± SD. The agreement between T2-weighted imaging and myocardial perfusion SPECT and myocardial infarct size by TTC-staining was expressed as mean difference ± SD, and the limits of agreement were expressed as mean ± 2 SD. A Wilcoxon Signed Rank test was performed to determine bias between the two different methods for assessment of MaR. SPSS version 18.0 software package (Chicago, IL, USA) was used for all statistical analyses. A P-value < 0.05 was considered statistically significant.
Results

All pigs

All 18 pigs, both with and without the presence of gadolinium, had a hyperintense signal present on the T2-weighted images. The mean MaR by myocardial perfusion SPECT was $33 \pm 7\%$ of the LV. The mean MaR assessed by T2-weighted imaging was $35 \pm 8\%$ of the LV. The MaR assessed with T2-weighted imaging correlated significantly with the MaR measured with myocardial perfusion SPECT ($r^2 = 0.70, P < 0.01$) with a bias of $2.6 \pm 5.1\%$ ($P = 0.04$; Figure 2).

Without gadolinium

For the seven pigs without gadolinium, the mean MaR by myocardial perfusion SPECT was $30 \pm 7\%$ of the LV. The mean MaR assessed by T2-weighted imaging was $34 \pm 6\%$ of the LV, with an interobserver variability of $1.6 \pm 4.0\%$. The MaR assessed with T2-weighted imaging correlated significantly with the MaR measured with myocardial perfusion SPECT ($r^2 = 0.78, P < 0.01$) with a bias of $4.3 \pm 2.9\%$ ($P = 0.02$). Figure 3 shows an example of a single pig where the MaR is visualized on short-axis images by T2-weighted imaging and myocardial perfusion SPECT. In this particular pig, the MaR was 33 and 29% of the LV, respectively. In three of seven pigs there were hypointense regions within the MaR. The CNR between the hyperenhanced MaR and the remote myocardium was $7.2 \pm 1.8$ (Table 1). The signal intensity for the MaR was $0.65 \pm 0.21$ and for the remote myocardium $0.01 \pm 0.10$ on the images at which the delineation was performed after adjusting the brightness and contrast ($0 = $ black and $1 = $ white, Table 1).

With gadolinium

For the 11 pigs with gadolinium, the mean MaR by myocardial perfusion SPECT was $35 \pm 6\%$ of the LV. The mean MaR-assessed T2-weighted imaging after injection of gadolinium was $36 \pm 10\%$ of the LV, with an interobserver variability of $2.2 \pm 5.2\%$. There was a significant correlation between MaR measured by T2-weighted imaging and myocardial perfusion SPECT ($r^2 = 0.64, P < 0.01$), and no significant bias between the two methods was observed ($1.5 \pm 6.0\%, P = 0.63$).

For demonstration of the effect of gadolinium on T2-weighted imaging, Figure 4 shows an example of short-axis slices of T2-TSE in one pig without injection of gadolinium and in one pig with injection of gadolinium. On the T2-TSE image without the presence of gadolinium, a hyperintense region could be seen that corresponded well with the MaR by myocardial perfusion SPECT. On the T2-TSE image with the presence of gadolinium, hyperintense as well as hypointense regions were seen within the MaR. The hypointense regions within the MaR closely related to the infarcted regions seen on the TTC-stained and T1-weighted short-axis slices, whereas the hyperintense regions within the MaR by T2-TSE showed no sign of infarction on the TTC-staining and T1-weighted short-axis slices (Figure 5). In 3 of the 11 pigs with gadolinium injected, hypointense regions were seen within the infarcted myocardium on the T1-weighted images. The CNR for the hyperenhanced periphery and hypointense core of the MaR compared to remote myocardium was $8.1 \pm 2.4$ and $-2.8 \pm 2.4$, respectively (Table 1). The signal intensity for the hyperenhanced periphery of the MaR, the hypointense core of the MaR and the remote myocardium was $0.43 \pm 0.12$, $-0.14 \pm 0.08$, and $0.02 \pm 0.05$, respectively, on the images at which the delineation was performed.
performed after adjusting the brightness and contrast (0 = black and 1 = white, Table 1).

For the three hearts that were imaged directly after excision and 24 h later after being stored at a temperature of 4°C, no difference was observed between the two assessments of MaR (−0.33 ± 2.89%).

Table 1  Contrast to noise values for the MaR compared to remote and intensities of the different parts of the myocardium after window levelling

<table>
<thead>
<tr>
<th>CNR compared to remote</th>
<th>Mean intensity after adjusting window settings (1 = white, 0 = black)</th>
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<td>MaR (hyperenhanced periphery)</td>
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With gadolinium

Case 1  10.1  −1.0  0.38  −0.09  −0.05
Case 2  7.6  −3.9  0.44  −0.17  0.04
Case 3  11.6  −2.9  0.55  −0.18  −0.03
Case 4  8.2  −2.4  0.37  −0.08  0.02
Case 5  5.2  −7.4  0.35  −0.44  0.02
Case 6  7.7  −0.6  0.28  −0.01  0.01
Case 7  9.3  −3.2  0.32  −0.10  0.01
Case 8  7.7  −1.9  0.51  −0.14  −0.01
Case 9  7.6  −2.7  0.40  −0.08  0.05
Case 10 5.4  −5.9  0.50  −0.40  0.07
Case 11 8.0  1.5  0.60  0.14  0.04
Mean ± SD  8.1 ± 2.4  −2.8 ± 2.4  0.43 ± 0.12  −0.14 ± 0.08  0.02 ± 0.05

Without gadolinium

Case 12  7.9  na  0.64  na  −0.01
Case 13  9.8  na  0.68  na  0.03
Case 14  6.6  na  0.55  na  0.07
Case 15  6.3  na  0.77  na  −0.13
Case 16  4.2  na  0.59  na  0.06
Case 17  6.5  na  0.73  na  −0.06
Case 18  8.8  na  0.58  na  0.14
Mean ± SD  7.2 ± 1.8  na  0.65 ± 0.21  na  0.01 ± 0.10

CNR, contrast-to-noise ratio; MaR, myocardium at risk; na, not applicable.

Infarct size

The mean myocardial infarct size by TTC staining was 12.4 ± 6.4% of the LV. Nine of the 11 pigs with gadolinium injected had infarct size assessed with both TTC staining and T1-weighted MRI, which correlated well ($r^2 = 0.90$). The infarct size was, however, significantly

Figure 3  MaR by T2-weighted imaging and myocardial perfusion SPECT in one of the study subjects without injection of gadolinium. Short-axis slices are shown at the same ventricular level for T2-weighted imaging and myocardial perfusion SPECT in a pig subjected to 30 min of coronary artery occlusion followed by 4 h of reperfusion. The epicardium is traced in green, the endocardium is traced in red, and the MaR is traced in white. Note the similarities of the MaR by the two methods. MaR, myocardium at risk; SPECT, single photon emission computed tomography.
smaller with TTC (15.4 ± 5.3%) compared to T1-weighted MRI (22.1 ± 5.6%) in these pigs (p = 0.008). The MaR by T2-TSE was larger than the infarct size measured with TTC staining and T1-weighted MRI for all animals. Comparison of the MaR by T2-weighted imaging with the infarct size by TTC staining in all pigs resulted in a myocardial salvage index of 66 ± 16% (range 38–100).

Discussion

This study shows that MaR can be determined by T2-weighted CMR imaging in an ex vivo experimental model both with and without the presence of gadolinium, using myocardial SPECT perfusion imaging as a reference standard.

In humans, T2-weighted imaging has previously been validated against MaR assessed by myocardial perfusion SPECT, showing a mean difference of −2.3 ± 5.7%.

In accordance with these results, the present experimental study found a small bias of 2.6 ± 5.1% when ex vivo T2-weighted imaging was compared to myocardial perfusion SPECT.

Cellular and extracellular osmolarity as well as plasma membrane permeability are altered as a result of a shift from aerobic metabolism to anaerobic glycolysis in ischaemic myocardium due to an acute coronary occlusion. In combination with a reduced Na/K ATPase activity, these alterations cause cell swelling and interstitial oedema. In addition, reperfusion leads to an inflammatory-like response, increasing the amount of extracellular fluid. This increased content of free water (intracellular and extracellular) in the affected myocardium is likely to explain the increased signal intensity compared to the non-affected myocardium as seen by T2-weighted imaging.

T2-weighted imaging has been used to assess the MaR in several in vivo studies and only a few studies have validated T2-weighted imaging for measuring MaR ex vivo. Recently, Carlsson et al. validated T2-weighted imaging for the measurement of MaR against myocardial perfusion SPECT in humans. In accordance with this study, only a small bias was seen between the MaR by T2-weighted imaging and myocardial perfusion SPECT in the present study. The current findings are also in accordance with previous experimental studies where the MaR by T2-weighted imaging was compared against microspheres and fluorescein, respectively. Aletras et al. found a small bias between the two methods with a slightly larger standard deviation, whereas Garcia-Dorado et al. showed only a small overestimation of the MaR by T2-weighted imaging.
Gadolinium is known to have a strong influence on the T1 relaxation times of hydrogen protons. The clinically used triple inversion recovery sequence (T2-TRIR) is highly sensitive to the presence of gadolinium, due to the introduction of inverse T1-weighting by the third inversion pulse, and it is therefore recommended that T2-weighted images should be acquired prior to the administration of a gadolinium-based contrast agent, to avoid signal loss caused by gadolinium. However, the double-inversion recovery sequence used in this study is less sensitive to the presence of gadolinium, since it does not introduce a third inversion pulse that generates inverse T1-weighting. The double-inversion recovery sequence might therefore be useful to assess MaR after injection of gadolinium.

The current study showed that T2-TSE had both hyperintense and hypointense regions within the MaR in the hearts from the pigs that had been injected with gadolinium prior to excision of the heart. The hypointense regions were shown to be closely related to the infarcted regions seen by TTC-staining and T1-weighted imaging, whereas no myocardial infarction could be seen by TTC-staining in those regions showing a hyperintense region by T2-TSE imaging. Thus, T2-TSE can therefore be used to assess myocardial salvage in an experimental ex vivo model since this method enables determination of MaR after the administration of gadolinium used to assess infarct size. Of note is that the infarct size by T1-weighted imaging was significantly larger than that assessed by TTC. This is in line with the findings of Saeed et al. showing a significant overestimation of infarct size when comparing nonspecific extracellular gadolinium-DTPA with a necrosis-specific MR contrast media (mesoporphyrin) early after reperfused infarction.

The current study showed that a 24 h storage of the heart in 4°C did not affect the measurements of MaR by T2-weighted imaging compared to T2-weighted imaging performed ~1 h after the heart was excised. This finding indicates that the CMR can be performed within the first 24 h after excision of the heart making the experimental protocol more logistically flexible.

**Study limitations**

The findings in the present study should be interpreted in the light of some limitations. No automatic method with fixed standard deviations from remote was used to determine MaR by CMR since the difference between ischaemic and normal myocardium varies between slices and between subjects. Manual delineation, however, as in this paper is the only method that has been validated against independent methodology using myocardial perfusion SPECT.

**Conclusions**

T2-weighted CMR imaging can be used to determine MaR in an ex vivo experimental model, both with and without the presence of gadolinium. Thus, CMR can be used alone to assess myocardial salvage in experimental studies without the use of histopathologic staining, microspheres or myocardial perfusion SPECT.

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**Conflict of interest:** none declared.

**References**