Molecular MRI of myocardial angiogenesis after acute myocardial infarction

M. Oostendorp[1], K. Douma[1], A. Wagenaar[1], J. M. Slenter[1], T. M. Hackeng[1], M. A. van Zandvoort[1], M. J. Post[1], and W. H. Backes[1]

[1]Maastricht University Medical Centre (MUMC+), Maastricht, Netherlands

INTRODUCTION

Angiogenesis is a natural mechanism to restore perfusion to the ischemic myocardium after acute myocardial infarction (MI). Presently, therapeutic angiogenesis is being explored as a novel treatment option for MI patients. However, sensitive, non-invasive *in vivo* measures of therapeutic efficacy are currently lacking and need to be developed. Here, a molecular MRI method is presented to non-invasively image angiogenic activity *in vivo* in a murine model of MI using cyclic Asn-Gly-Arg (cNGR)-labeled paramagnetic quantum dots (pQDs). The tripeptide cNGR homes specifically to CD13, an aminopeptidase that is strongly upregulated during myocardial angiogenesis [1].

METHODS

Animal model. Acute MI was induced in male Swiss mice via permanent ligation of the left anterior descending coronary artery. Molecular MRI was performed 7 days after surgery, since angiogenic activity is maximal at this time point [1]. Three experimental groups were studied: MI-mice injected with cNGR-pQDs (n = 6), MI-mice injected with unlabeled, control pQDs (n = 4), and sham-operated mice injected with cNGR-pQDs (n = 5).

Contrast agent. Streptavidin coated cadmium/selenium quantum dots (585 nm emission) were purchased from Invitrogen. cNGR-pQDs were prepared by mixing QDs, biotin-cNGR ligand and biotin-Gd-DTPA-wedge (containing 8 Gd-DTPA moieties per molecule) in a molar ratio of 1:6:24, as described previously [2]. Unlabeled (no ligand) particles were prepared similarly. The contrast agent's ionic T_1 and T_2 relaxivities were 7.1 and 49 $(\text{mM Gd})^{-1}\text{s}^{-1}$ at 7 T, respectively.

Molecular MRI. Experiments were performed on a 7 T Bruker Biospec 70/30 USR. Horizontal long axis and short axis cine images were recorded using the retrospectively self-gated IntraGate protocol (Bruker). The short axis image passed through the infarction and was used for all subsequent images. Next, ECG-triggered, respiratory gated, end-diastolic bright blood gradient echo images were recorded as follows: TR 15 ms, TE 6.0 ms, flip angle 50°, 1 slice, 1 mm thickness, 4 signal averages, 4×4 cm^2 field-of-view, 256×256 matrix, in-plane resolution 0.16×0.16 mm^2. Image acquisition was started 30 minutes after contrast agent administration and was repeated every 10 minutes up to 2 hours post contrast. After MRI, mice were sacrificed by cervical dislocation and hearts were excised for validation by two-photon laser scanning microscopy (TPLSM).

Analysis. Global cardiac function was assessed using the ejection fraction, which was determined from the horizontal long and short axis cine images using the biplane ellipsoid model [3]. Local cardiac function was estimated by dividing the left ventricle (LV) myocardium into 8 radial segments [4]. In each segment, the contractile function was assessed via the endocardial radial shortening (ERS, [5]). To this extent, endocardial contours were drawn on end-systolic (ES) and end-diastolic (ED) short axis frames and the radius r was defined as the distance between the endocardial border and the LV center. The ERS was then calculated as $(r_{ED} - r_{ES}) / r_{ED} \cdot 100\%$ and plotted as function of segment number (not shown). Segments with reduced ERS were categorized as infarct/border zone by two readers in consensus. The remaining segments were considered remote myocardium. As a negative contrast was observed, the size of the hypointense area was calculated per segment by counting the number of voxels with signal intensity below a threshold value and multiplying this by the voxel size. Thresholds were defined for each image individually as the mean signal intensity in a reference region minus two times the standard deviation in this region. Reference regions were drawn manually outside the heart in (non-angiogenic) skeletal muscle tissue. Statistical analysis was performed using non-parametric tests in SPSS. $P \leq 0.05$ was considered significant.

RESULTS

The ejection fraction of MI-mice injected with cNGR-pQDs and MI-mice injected with unlabeled pQDs was 38 ± 3% and 42 ± 5%, respectively, which was significantly lower compared with sham-operated mice (58 ± 4%, $P < 0.05$). Injection of cNGR-pQDs resulted in a strong negative contrast that was mainly located in the infarct/border zone (identified based on the segmental analysis). This negative contrast was significantly less in MI-mice injected with unlabeled pQDs, and in sham-operated mice injected with cNGR-pQDs (Figs 1, 2). Validation with *ex vivo* TPLSM revealed a strong colocalization of cNGR-pQDs with vascular endothelial cells in the infarct/border zone, whereas unlabeled pQDs were mostly extravasated (Fig 3). Additionally, TPLSM demonstrated significant microvascular remodeling in the infarct/border zones compared with remote myocardium (Fig 3).

CONCLUSIONS

cNGR-labeled pQDs allowed specific detection of post-MI myocardial angiogenesis, as shown by the strong contrast observed in the infarcted mouse heart on molecular MR images, and by the colocalization of cNGR-pQDs with vascular endothelial cells as detected by TPLSM. TPLSM provided unique, detailed information on microvascular structure and remodeling in different regions of the heart. Molecular MRI with cNGR-labeled contrast agents may be applicable for the early, *in vivo* evaluation of the response to angiogenic treatments in both preclinical and clinical studies.

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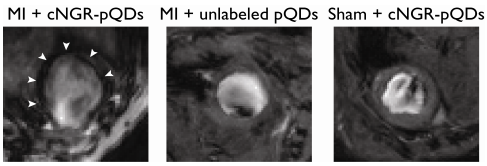

Figure 1 Short axis gradient echo images at 1 hour post contrast injection. Arrowheads: negative contrast.

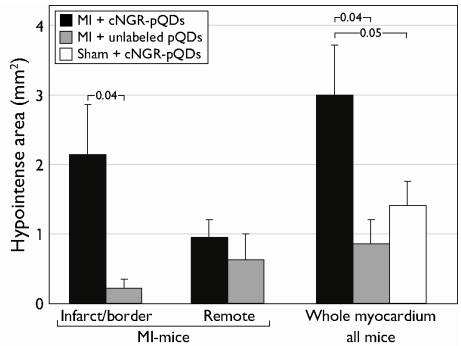

Figure 2 Size of the hypointense area for the three experimental groups at 1 hour post contrast injection. Significant P-values are also shown.

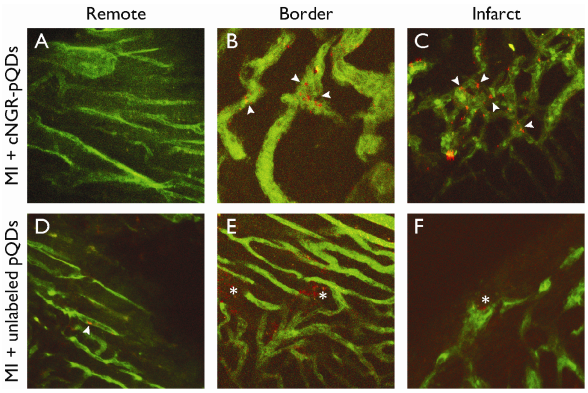

Figure 3 TPLSM results. Green: vessels stained with αCD31-FITC. Red: pQDs. Arrowheads: colocalization. Asterisks: extravasation