



^{68}Ga -DOTA chelate, a novel imaging agent for assessment of myocardial perfusion and infarction detection in a rodent model

Anu Autio, PhD,^{a,b} Sauli Uotila, MD,^a Max Kiugel, PhD,^a Ville Kytö, MD, PhD,^c Heidi Liljenbäck, MSc,^{a,d} Nobuyuki Kudomi, PhD,^e Vesa Oikonen, MSc,^a Olli Metsälä, MSc,^a Semi Helin, MSc,^a Juhani Knuuti, MD, PhD,^{a,f} Antti Saraste, MD, PhD,^{a,c,f} and Anne Roivainen, PhD^{a,d,f}

^a Turku PET Centre, University of Turku, Turku, Finland

^b MediCity Research Laboratory, University of Turku, Turku, Finland

^c Heart Center, Turku University Hospital and University of Turku, Turku, Finland

^d Turku Center for Disease Modeling, University of Turku, Turku, Finland

^e Department of Medical Physics, Faculty of Medicine, Kagawa University, Kagawa, Japan

^f Turku PET Centre, Turku University Hospital, Turku, Finland

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Background. Magnetic resonance imaging (MRI) with Gadolinium 1,4,7,10-tetraazacyclododecane- N' , N'' , N''' , N'''' -tetraacetic acid (Gd-DOTA) enables assessment of myocardial perfusion during first-pass of the contrast agent, while increased retention can signify areas of myocardial infarction (MI). We studied whether Gallium-68-labeled analog, ^{68}Ga -DOTA, can be used to assess myocardial perfusion on positron emission tomography/computed tomography (PET/CT) in rats, comparing it with ^{11}C -acetate.

Methods. Rats were studied with ^{11}C -acetate and ^{68}Ga -DOTA at 24 hours after permanent ligation of the left coronary artery or sham operation. One-tissue compartmental models were used to estimate myocardial perfusion in normal and infarcted myocardium. After the PET scan, hearts were sectioned for autoradiographic detection of ^{68}Ga -DOTA distribution.

Results. ^{11}C -acetate PET showed perfusion defects and histology showed myocardial necrosis in all animals after coronary ligation. Kinetic modeling of ^{68}Ga -DOTA showed significantly higher k_1 values in normal myocardium than in infarcted areas. There was a significant correlation ($r = 0.82$, $P = 0.001$) between k_1 values obtained with ^{68}Ga -DOTA and ^{11}C -acetate. After 10 minutes of tracer distribution, the ^{68}Ga -DOTA concentration was significantly higher in the infarcted than normal myocardium on PET imaging and autoradiography.

Conclusions. Our results indicate that acute MI can be detected as reduced perfusion, as well as increased late retention of ^{68}Ga -DOTA. (J Nucl Cardiol 2019)

Key Words: PET • myocardial perfusion • myocardial infarction • rat

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Reprint requests: Anne Roivainen, PhD, Turku PET Centre, University of Turku, Kiinamylynkatu 4-8, 20521 Turku, Finland; aroivan@utu.fi

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Abbreviations

⁶⁸ Ga-DOTA	Gallium-68-labeled 1,4,7,10-tetraazacyclododecane- <i>N',N'',N''',N''''</i> -tetraacetic acid
LCA	Left coronary artery
LV	Left ventricle
MBF	Myocardial blood flow
MI	Myocardial infarction
MRI	Magnetic resonance imaging
PET	Positron emission tomography
ROI	Region of interest
SUV	Standardized uptake value
1TCM	One-tissue compartmental model

INTRODUCTION

Gadolinium (Gd)-labeled chelates are widely used magnetic resonance imaging (MRI) contrast agents. Cardiac MRI with Gd chelates enables assessment of myocardial perfusion during the first-pass of the contrast agent and detection of myocardial infarction (MI) using the delayed-enhancement technique.¹⁻⁶ Delayed-enhancement imaging is based on the accumulation and retention of contrast agent in the infarcted myocardium due to the loss of cell membrane integrity, and hence reflects increased extracellular tissue volume.⁷

Positron emission tomography/computed tomography (PET/CT) perfusion imaging enables quantification of myocardial blood flow (MBF) and myocardial flow reserve (MFR). As it does not require an on-site cyclotron, rubidium-82 (⁸²Rb) chloride is the most widely used tracer for assessment of myocardial perfusion with PET. Quantification of myocardial perfusion on PET with the cyclotron products nitrogen-13-labeled ammonia (¹³N-ammonia) and oxygen-15-labeled water (¹⁵O-water) has been well validated.^{8,9} Another validated tracer for myocardial perfusion imaging is the ¹¹C-acetate used as a control in this study.¹⁰

Gallium-68-labeled 1,4,7,10-tetraazacyclododecane-*N',N'',N''',N''''*-tetraacetic acid (⁶⁸Ga-DOTA) chelate has similar kinetics to the Gd-DOTA used in MRI,¹¹ and is feasible for imaging of blood flow.^{12,13} Our research group found evidence that imaging of increased accumulation of ⁶⁸Ga-DOTA PET may be useful for detecting conditions associated with increased blood flow and vascular permeability, such as adenosine-induced increase in myocardial perfusion and inflammatory lesions.¹² This novel approach to PET perfusion imaging is generator-based and provides an opportunity for imaging centers distant to a radiochemistry unit to detect and quantify blood flow. ⁶⁸Ga is a

positron-emitting radionuclide that is readily available from a ⁶⁸Germanium (⁶⁸Ge)/⁶⁸Ga generator system possessing a 1 year life span (⁶⁸Ge $T_{1/2}$ = 270.8 days). In contrast to most positron-emitting radionuclides that require a cyclotron for production, the ⁶⁸Ge/⁶⁸Ga generator provides low-cost and easy access to a positron-emitting radionuclide. However, it is not known whether ⁶⁸Ga-DOTA PET can be used to detect abnormal myocardial perfusion and cell necrosis associated with MI.

In this study, we aimed to validate ⁶⁸Ga-DOTA PET imaging measurement of myocardial perfusion, comparing it with ¹¹C-acetate, and comparing the kinetics of ⁶⁸Ga-DOTA between normal and infarcted myocardium in rats. Translation to a clinical setting should be feasible for ⁶⁸Ga-DOTA, as Gd-DOTA is already in clinical use (trade names Artirem®, Dotarem®).¹⁴

METHODS

Animal Model

The study protocol was approved by the national Animal Experiment Board in Finland and the Regional State Administrative Agency for Southern Finland, and carried out in compliance with the European Union directives relating to the conduct of animal experimentation. In total, 12 male Sprague-Dawley rats were used. MI was induced by permanent ligation of the left coronary artery (LCA), as previously described.¹⁵⁻¹⁸ Briefly, 0.2 mg·kg⁻¹ buprenorphine (Temgesic; Schering-Plough, Espoo, Finland) was administered intramuscularly for analgesia prior to operation. Anesthesia was induced by a combination of inhaled isoflurane and subcutaneous injection of 10 mg·kg⁻¹ xylazine (Rompun; Orion Pharma, Espoo, Finland) and 90 mg·kg⁻¹ ketamine (Ketaminol; Orion Pharma). Body temperature was maintained using a heating pad. The rats were intubated and connected to a rodent ventilator (TOPO dual mode ventilator; Kent Scientific, Torrington, CT, USA).¹⁹

The heart was exposed by a left lateral thoracotomy of the fourth intercostal space, the pericardium was opened, and the LCA was ligated near to its origin. Ligation was confirmed visually as a pale appearance of the myocardium at risk. The ribs, muscle layer, and skin were tightly sewn with a dissolving string. The anesthesia was reversed after the operation with an intramuscular injection of 1 mg·kg⁻¹ atipamezole (Antisedan; Orion Pharma). The sham operation consisted of all the same procedures except that the suture was not tightened around the LCA. Operative mortality in both coronary ligation and sham groups was ~ 25%, as previously described.¹⁵

Radiochemistry

⁶⁸Ga-DOTA synthesis was performed as previously described.¹² Briefly, ⁶⁸Ga was obtained from a ⁶⁸Ge/⁶⁸Ga

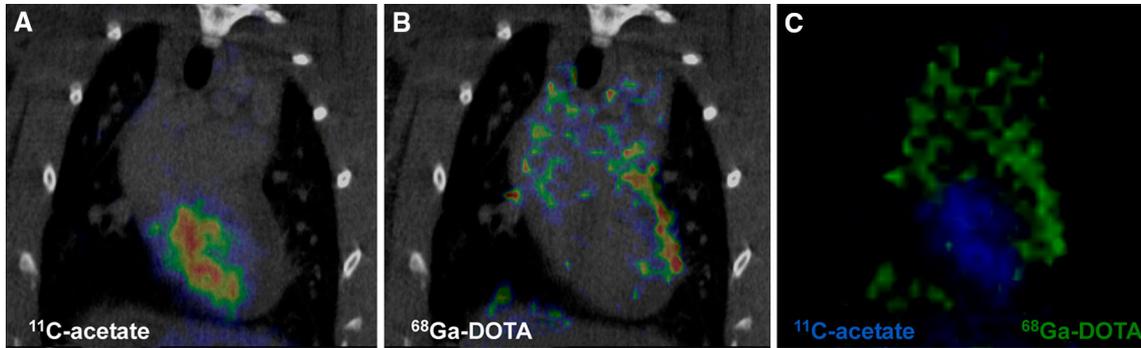


Figure 1. Representative coronal PET/CT images of (A) ¹¹C-acetate (sum image 30-90 seconds post-injection), (B) ⁶⁸Ga-DOTA (sum image 20-30 minutes post-injection), and (C) combined ¹¹C-acetate (blue) and ⁶⁸Ga-DOTA (green) in a rat with coronary ligation. Note the high ¹¹C-acetate uptake in the infero-posterior wall of the LV and defective uptake in the anterior wall subtended by the ligated coronary artery. ⁶⁸Ga-DOTA is present in the defect area at this time point.

generator (Eckert&Ziegler, Valencia, CA, USA) by elution with 0.1 M HCl. DOTA chelate (35 nmol in aqua; Macrocyclics, Dallas, TX, USA) was added, and the mixture was incubated at + 100°C for 15 minutes. Radiochemical purity of the final product was analyzed by high-performance liquid chromatography (HPLC) using μ Bondapak column and trifluoroacetic acid/acetonitrile/H₃PO₄ gradient. Retention time for ⁶⁸Ga-DOTA was 3.9 ± 0.1 minutes and for unbound ⁶⁸Ga 14.6 ± 0.2 minutes.

¹¹C-Acetate was synthesized as previously described.²⁰

For quality assessment, the final product was analyzed by HPLC.

PET/CT

The rats that survived surgery were imaged using small-animal PET/CT (Inveon Multimodality; Siemens Medical Solutions, Knoxville, TN, USA) at 24 ± 4 hours ($n = 6$) after coronary ligation or at 24 ± 4 hours ($n = 6$) after the sham operation. The rats were anesthetized using 1.5% isoflurane, and their body temperature was maintained with a heating pad throughout the imaging. First, a 10-minute ¹¹C-acetate PET (27 ± 12 MBq) was performed to evaluate myocardial perfusion and to localize the infarcted area. Then, after five half-lives of ¹¹C, ⁶⁸Ga-DOTA imaging was performed. To evaluate the myocardial kinetics of the tracer, the rats in the ligation group were injected with a bolus of 29 ± 3 MBq of ⁶⁸Ga-DOTA and PET imaged for 30 minutes. Both ¹¹C-acetate and ⁶⁸Ga-DOTA PET acquisitions were started prior to injection to catch the first-pass peak of the imaging tracer. Images were collected according to the following time frames: 30×3 seconds, 9×10 seconds, 4×30 seconds, 5×60 seconds, and 4×300 seconds. Immediately after PET, 200 μ L of intravascular iodinated eXIATM160XL contrast agent (Binitio Biomedical Inc, Ottawa, ON, Canada) was injected and high-resolution CT was acquired to delineate the myocardial borders. Sham-operated rats were injected with 14 ± 1 MBq of ⁶⁸Ga-DOTA and PET/CT imaged as described above.

Alignment of PET and CT images was automatically performed and confirmed visually according to anatomical landmarks. Image analysis was performed using Carimas v.2.8 software (Turku PET Centre, Turku, Finland). Regions of interest (ROIs) were defined on the high-resolution CT image to delineate the infarcted myocardium (verified by ¹¹C-acetate perfusion images) and remote myocardium. Results are reported as standardized uptake values (SUVs).

The kinetic parameters for both ⁶⁸Ga-DOTA and ¹¹C-acetate were determined using the Carimas 2.8 heart plugin modeling option. For ⁶⁸Ga-DOTA, an input function was obtained from the left ventricle (LV), which was identified using high-resolution contrast-enhanced CT. The uptake rate (k_1) was estimated assuming a one-tissue compartmental model (1TCM), as we previously validated.¹² Modeling for ¹¹C-acetate was performed using a tracer-specific mode readily available in the Carimas software.¹⁵ An ¹¹C-acetate k_1 polar map was used to determine specific segments in the normal areas in the septum and infarcted area in the anterior or anterolateral LV wall, with these same segments being used to compare the ⁶⁸Ga-DOTA kinetics. In addition to perfusion, the myocardial infarct area was determined as the percent of non-viable tissue according to the k_1 polar maps. The polar maps were normalized to the highest k_1 value in the viable part of the myocardium, which was set as the 100% k_1 value, and a cutoff of < 60% of the k_1 value was used to determine the infarcted area.²¹

Autoradiography

A subgroup of MI rats ($n = 3$) were further analyzed to determine the microscopic distribution of the ⁶⁸Ga-DOTA at 30 minutes post-injection of the tracer. The LV was frozen in cooled isopentane and sliced into serial transverse cryosections of 8 and 20 μ m at 1 mm intervals (five to six intervals per heart) from apex to base. Distribution of ⁶⁸Ga-DOTA in the infarcted and remote myocardium was analyzed by autoradiography of tissue sections. Air-dried sections were

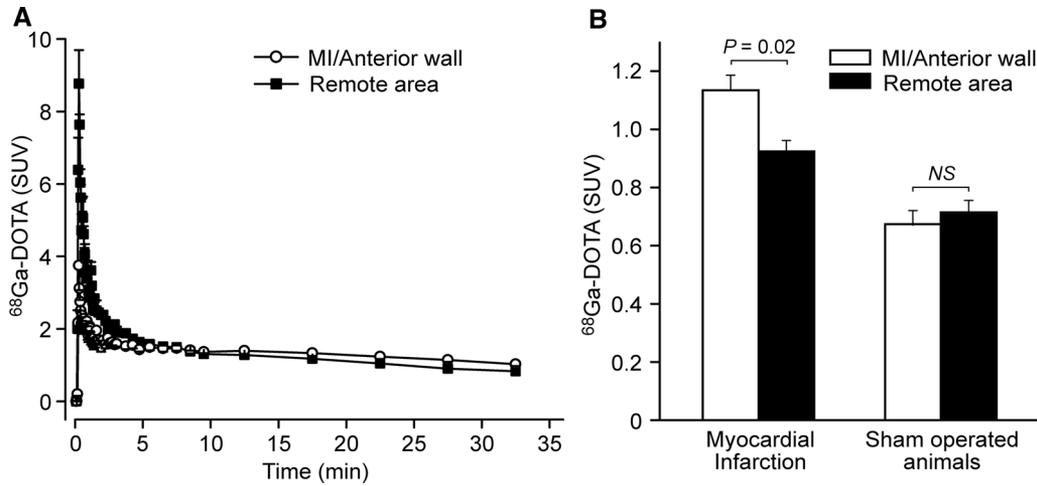


Figure 2. (A) Radioactivity concentration as a function of time (time-activity curves) for myocardial ⁶⁸Ga-DOTA uptake in rats with coronary ligation (mean, *n* = 6). Radioactivity peaks were higher in the remote myocardium than in the infarcted area in the initial distribution phase of the ⁶⁸Ga-DOTA. (B) Tracer uptake in the heart at the end of the 30 minutes imaging study in MI and sham-operated animals demonstrates late phase contrast enhancement in the infarcted myocardium.

apposed to an imaging plate (BAS-TR2025; Fuji Photo Film Co. Tokyo, Japan), and after more than two radionuclide half-lives, the plates were scanned with a Fuji BAS-5000 analyzer (internal resolution of 25 μ m). Tracer accumulation was measured as counts per area (photostimulated luminescence per square millimeter, PSL/mm²) with TINA software v.2.1 (Raytest Isotopenmessgeräte, GmbH, Straubenhardt, Germany).

After co-registration of autoradiography and histological images, ROIs were defined in the infarcted and remote areas of the LV. The background area count densities were subtracted from the image data. After autoradiography, the serial LV cryosections were stained with hematoxylin-eosin (HE) for histology.

Statistical Analysis

All results are expressed as mean \pm standard deviation (SD). Correlation coefficients were computed using linear regression analysis. Student's *t*-test was used for comparison between two groups. A *P* value less than 0.05 was considered statistically significant. All statistical analyses were conducted using Origin 7.5 software (Microcal Software, Inc., Northampton, MA, USA).

RESULTS

Radiochemistry

The radiochemical purity of ⁶⁸Ga-DOTA was 99% \pm 0.3% (*n* = 4), and the molar activity was

9.1 \pm 3.5 MBq·nmol⁻¹ (*n* = 4). The radiochemical purity of ¹¹C-acetate was consistently > 99%.

Tracer Kinetics in the Infarcted vs. Normal Myocardium

⁶⁸Ga-DOTA radioactivity concentration declined rapidly after initial peak in the remote and infarcted areas, but some remaining radioactivity was visible in the infarcted area 10 minutes after injection of the ⁶⁸Ga-DOTA tracer. Infarct area was also visualized as a defect area with ¹¹C-acetate in every MI animal (Fig. 1A). An example PET/CT image of the remaining radioactivity in the infarcted rat myocardium 30 minutes post-injection of ⁶⁸Ga-DOTA is shown in Figure 1B.

A peak was detected in the myocardial uptake of ⁶⁸Ga-DOTA within 20 seconds post-injection (Fig. 2B). At this time point, the peak in the MI rat group was higher in the remote myocardium (SUV: 8.8 \pm 0.93) than in the infarcted area (SUV: 3.8 \pm 0.67, *P* = 0.0014). At the end of the 30 minutes imaging study (25-30 minutes post-injection), the infarcted area (SUV: 1.13 \pm 0.13) showed significantly higher uptake than the remote myocardium (SUV: 0.93 \pm 0.087, *P* = 0.023; Fig. 2B). There was no difference between the anterior wall (SUV: 0.67 \pm 0.13) and the septum (SUV: 0.72 \pm 0.10) in the sham-operated animals (*P* = 0.11). The MI/remote ratio (1.2 \pm 0.18) in the MI group was significantly higher than the anterior wall/

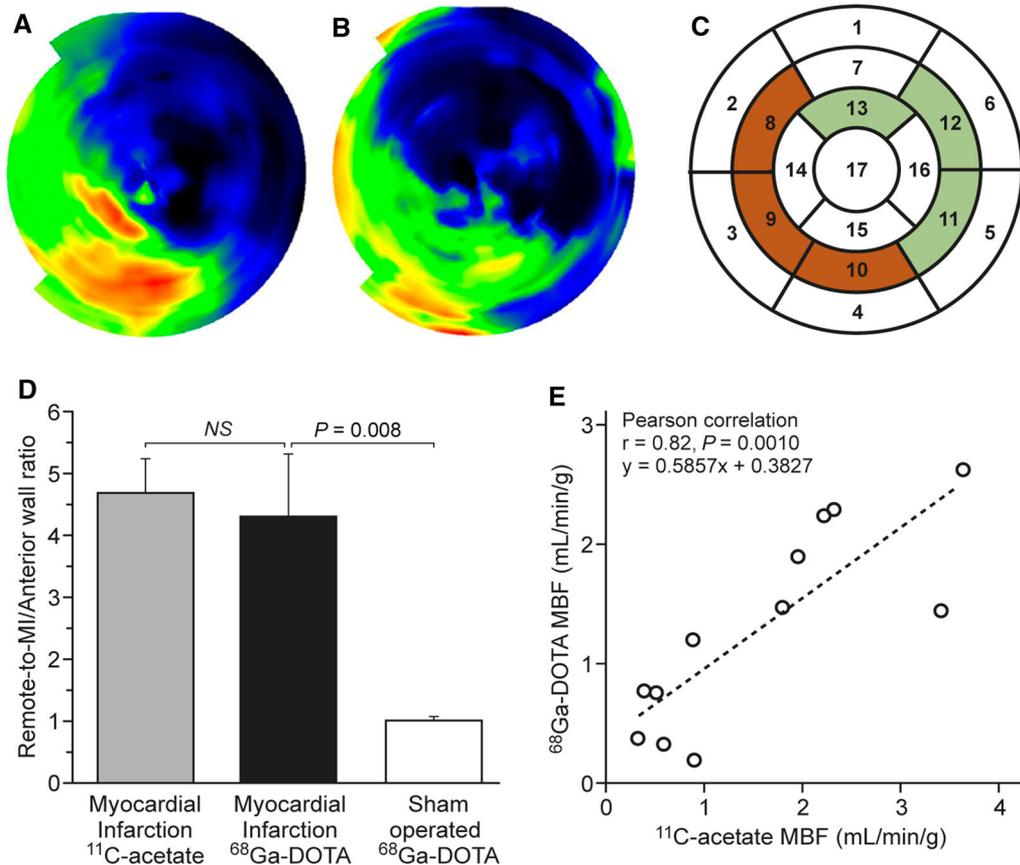


Figure 3. Polar maps of k_1 values obtained with (A) ¹¹C-acetate and (B) ⁶⁸Ga-DOTA in a rat with coronary ligation. (C) Representation of segments used in MBF quantification. Segments marked red were used for quantification of MBF on infarcted area whereas areas in green were used for quantification of remote area. Selected segment varied from animal to animal according localization of infarcted area. Same segment was used for both ¹¹C-acetate and ⁶⁸Ga-DOTA MBF quantification per animal. (D) Quantification of the remote/MI ratio of k_1 values in MI rats imaged with ¹¹C-acetate and ⁶⁸Ga-DOTA, and a sham-operated animal imaged with ⁶⁸Ga-DOTA, reveals comparable kinetics. (E) Correlation between ⁶⁸Ga-DOTA and ¹¹C-acetate k_1 values is statistically significant. $y = ax + b$ is the linear regression formula used to fit the k_1 data of ¹¹C-acetate and ⁶⁸Ga-DOTA.

septum ratio (0.93 ± 0.087) in the sham-operated control group ($P = 0.0049$).

Determination of MBF

⁶⁸Ga-DOTA k_1 values reflecting myocardial perfusion were calculated from the PET image data by application of a 1TCM. Polar maps demonstrated higher k_1 values in the normal myocardium than infarcted myocardium using either ⁶⁸Ga-DOTA or ¹¹C-acetate (Fig 3A, 3B). Remote/MI ratios of k_1 values were 4.7 ± 0.6 ($P < 0.001$) for ¹¹C-acetate and 4.3 ± 1.0 ($P < 0.001$) for ⁶⁸Ga-DOTA (Fig. 3D). Regression analysis showed that the ⁶⁸Ga-DOTA values were

closely comparable to the ¹¹C-acetate values ($y = 0.59x + 0.38, r = 0.82, P = 0.0010$, Fig. 3D). The ratios of the remote to MI k_1 values showed no significant differences between the ¹¹C-acetate and ⁶⁸Ga-DOTA k_1 . In the sham-operated animals, the ratio of k_1 values for ⁶⁸Ga-DOTA in the corresponding areas in the septum and anterior wall was 1.0 ± 0.06 ($P = 0.8$, Fig. 3d). When the threshold of 60% of maximum flow was used to define viable myocardium, the perfusion defect size was slightly overestimated with ⁶⁸Ga-DOTA ($64\% \pm 3.9\%$) in comparison with ¹¹C-acetate ($57\% \pm 1.8\%$), but this difference was not significant ($P = 0.13$).

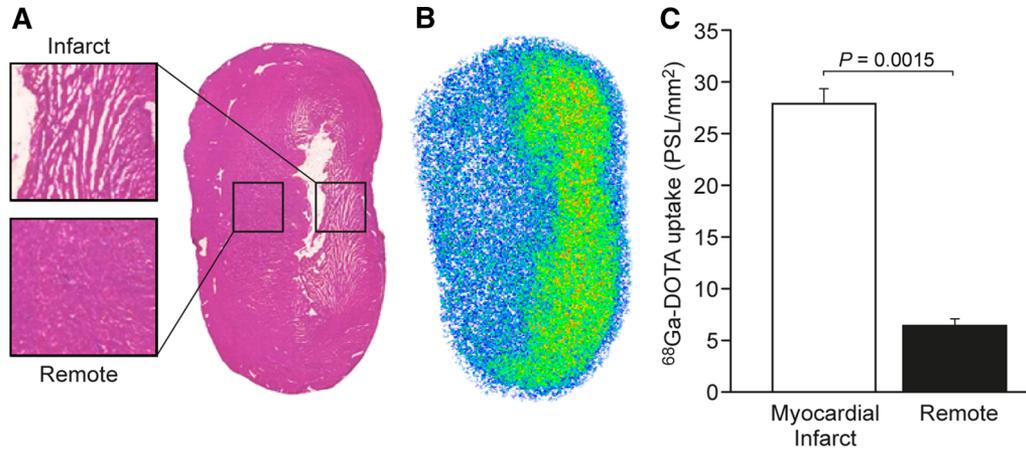


Figure 4. (A) Hematoxylin-eosin staining of the left ventricle shows defective myocardium that co-localizes with higher radioactivity concentration. (B) Autoradiography of the left ventricle myocardium 30 minutes post-injection of ⁶⁸Ga-DOTA in a rat with myocardial infarction due to coronary ligation shows higher retention of ⁶⁸Ga-DOTA than in remote myocardium. (C) Quantification of autoradiography analysis shows significantly higher radioactivity concentration in the ligated area in comparison with remote myocardium.

Autoradiography

A representative autoradiograph of myocardial ⁶⁸Ga-DOTA uptake at 30 minutes post-injection is shown in Figure 4. Infarcted area, which was confirmed by histology (Fig. 4A), demonstrated a necrotic core surrounded by granulated tissue that was seen in the anterior or anterolateral LV wall in all animals that underwent surgical coronary ligation. There was a local increase in ⁶⁸Ga-DOTA tracer accumulation in the anterolateral wall of the LV, which was co-localized with the histologically infarcted area in the rats with coronary ligation. In the rats with coronary ligation, the average ⁶⁸Ga-DOTA radioactivity concentration in the infarcted area was 4.4-fold higher than in the remote myocardium (28 ± 1.5 vs. 6.5 ± 0.7 PSL·mm², $P = 0.0015$).

DISCUSSION

Gd based MRI is safe, accurate, and practical for the imaging of patients with cardiac events.⁵⁻⁷ Both first-pass perfusion imaging and delayed contrast enhancement have been utilized for this chelate in cardiac MRI. In the current study, our investigations provide evidence that ⁶⁸Ga-DOTA has similar potential for the measurement of MBF, as well as showing a delayed washout in acutely infarcted myocardium. The ⁶⁸Ga-DOTA works in this setting as a dual-function tracer. In the first-pass and early time frames it can be used to model blood perfusion in the remote myocardium. It is only at later time points when the tracer is accumulated in the defect

area due to increased extracellular space. The PET perfusion imaging using tracer kinetic modeling allowed quantitative measurements of absolute MBF. This method is suitable for several applications in research and clinical diagnostics. In rat models, the ⁶⁸Ga-DOTA chelate is comparable to two gold-standards of perfusion imaging, ¹⁵O-water, as we have previously shown,¹² and now with ¹¹C-acetate. This novel tool would offer the opportunity to in vivo image blood perfusion at imaging sites more distant from cyclotron facility.

Elevated radioactivity concentration was seen in the infarcted areas in the later time points of the ⁶⁸Ga-DOTA imaging, with this probably reflecting the delayed washout kinetics of the tracer distributed in the increased extracellular space, i.e., delayed contrast enhancement. This finding is analogous to the late-enhancement imaging used in contrast-enhanced cardiac MRI, which allows visualization of the infarcted myocardium where cells have lost their membrane integrity or myocardium has been replaced by fibrosis. Higher radioactivity concentration in the infarcted areas was confirmed by autoradiography of the LV tissue sections.

We previously showed the feasibility of ⁶⁸Ga-DOTA chelate for the detection of increased perfusion in inflammation foci,¹² detecting higher ⁶⁸Ga-DOTA signal at the site of inflamed skin/muscle in comparison with healthy muscle, thereby suggesting that inflammation-induced changes in blood flow and vascular permeability can be detected with ⁶⁸Ga-DOTA. In addition, we were able to detect increased perfusion in healthy rat heart following induction of pharmacological

hyperemia caused by intravenous infusion of adenosine. The MFR measured with ⁶⁸Ga-DOTA was comparable to that measured with ¹⁵O-water, which is a tracer used for PET imaging of MBF.^{22–24}

The in vivo imaging of MBF is difficult in small animals due to the limited spatial and temporal resolution of PET scanners, partial-volume effects (PVEs), spill over, and the high positron energy of ⁶⁸Ga. However, studies have indicated the possibility of imaging myocardial perfusion in rat heart with several different tracers.^{10,23–25} Tracers that have been successfully used for the measurement of myocardial infarct size in small animals include 2-deoxy-2-¹⁸F-fluoro-*D*-glucose (¹⁸F-FDG), which is a marker of viable myocardium.^{25–27} Here, we demonstrated that ⁶⁸Ga-DOTA has potential for the measurement of myocardial perfusion in small animals. The results were validated using ¹¹C-acetate, which is well established for the imaging of myocardial perfusion.¹⁰ The quantitative analysis method of ¹¹C-acetate is based on fitting 1TCM parameters K_1 , k_2 , and V_b to the tissue curves covering the whole 10-minute scan, with input function derived from the LV. The model parameter K_1 represents perfusion, k_2 represents rate of oxidative metabolism, and V_b is the fractional volume of blood in the tissue. The model is essentially the same as is applied for the analysis of ¹⁵O-water and ⁶⁸Ga-DOTA. Usage of K_1 from ¹¹C-acetate for assessing myocardial perfusion (in addition to oxidative metabolism) has been previously validated against gold-standard ¹⁵O-water method in rats¹⁰ and in humans,^{28,29} and also against ¹³N-ammonia. In addition to ¹¹C-acetate, it would have been possible to use ¹⁵O-water as a control. However, due to 2-minute half-life of ¹⁵O, the transfer of ¹⁵O-water from the cyclotron/radiochemistry unit to the animal imaging facility was not feasible due to the long distance between sites. During this study ¹³N-ammonia was not available at our center.

In this study, we obtained the input function from the LV cavity on dynamic PET images. A direct comparison of the ⁶⁸Ga-DOTA radioactivity concentration obtained from blood samples and PET images would be a valuable approach in a larger animal model (e.g., pigs), which would allow multiple blood samples to be taken from the same animal throughout the PET imaging. A dedicated small-animal PET/CT scanner was used to image the animals in this study, and even though it provided good resolution and we had the anatomical CT references to aid in ROI drawing, the structures being imaged were quite small (less than 10 mm in diameter). The in vivo imaging of small structures such as rat myocardium may therefore be affected by PVE, which might have influenced the in vivo quantitative PET data. When PVE is present, the apparent pixel values in PET images may be influenced by surrounding

high pixel values. Other factors, such as the reconstruction algorithm and filter, scanner sensitivity, and scan duration, may also affect the accuracy of imaging data, and require evaluation.

CONCLUSION

Our results indicate that ⁶⁸Ga-DOTA is a potential tracer for evaluating myocardial resting perfusion in small animals. Acute MI can be detected as both reduced perfusion and increased late retention of the tracer. The myocardial kinetics of ⁶⁸Ga-DOTA assessed from the early distribution of the chelate reflected myocardial perfusion, while delayed contrast enhancement was demonstrated at later time points, showing delayed washout in the infarcted area with an increase in the extracellular space. This approach may provide an opportunity to conduct quantitative PET perfusion imaging studies in centers distant from the cyclotron unit. Further analyses will be needed to evaluate the potential of ⁶⁸Ga-DOTA kinetic analysis for differentiating viable and non-viable myocardial areas in a clinical setting.

NEW KNOWLEDGE GAINED

This study demonstrates that ⁶⁸Ga-DOTA PET has potential for in vivo quantification of MBF.

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Disclosure

The authors declare that they have no conflicts of interest.

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