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⁶⁴Cu-DOTA as a surrogate positron analog of Gd-DOTA for cardiac fibrosis detection with PET: Pharmacokinetic study in a rat model of chronic MI

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Abstract

Objectives—To investigate the pharmacokinetics of ⁶⁴Cu-DOTA, a positron surrogate analog of late Gd-enhancement cardiac magnetic resonance agent, Gd-DOTA in a rat model of chronic myocardial infarction (MI) and its microdistribution in the cardiac fibrosis by autoradiography.

Methods—DOTA was labeled with ⁶⁴Cu-acetate. CD rats (n = 5) with MI by LAD ligation and normal rats (n = 6) were injected *iv* with the ⁶⁴Cu-DOTA (18.5 MBq, 0.02 mmol DOTA/Kg). Dynamic PET imaging was performed for 60 min after injection. [¹⁸F]-FDG PET imaging was performed to identify the viable myocardium. For the ROI analysis, the ⁶⁴Cu PET image was co-registered to the [¹⁸F]-FDG PET image. To validate the PET images, slices of heart samples from the base to the apex were analyzed using autoradiography and histological staining with Masson's trichrome.

Results—⁶⁴Cu-DOTA was rapidly taken up in the infarct area. The time-activity curves demonstrated that the ⁶⁴Cu-DOTA concentrations in blood, the fibrotic tissue and perfusion-rich organs peaked within a minute of post injection and thereafter rapidly washed out in parallel with the blood clearance and excreted via the renal system. The blood clearance curve was bi-phasic, with the distribution half-life of < 3 min and the elimination half-life of ~ 21.8 min. The elimination half-life of ⁶⁴Cu-DOTA from the focal fibrotic tissue (~ 22.4 min) and the remote myocardium (~ 20.1 min) was similar to the blood elimination half-life. Consequently, the uptake ratios of focal fibrosis-to-blood and remote myocardium-to-blood remained stable for the time period between 10 to 60 min. The corresponding ratios obtained from images acquired from 30 to 60 min were 1.09 and 0.59, respectively, indicating that the concentration of ⁶⁴Cu-DOTA in the focal fibrosis was 1.85 (1.09/0.59) times greater than in remote myocardium. Thus, this finding indicates that the extracellular volume fraction was 1.85 times greater in the focal fibrosis than in

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remote myocardium. The accumulation of ^{64}Cu -DOTA in fibrotic tissue was further supported by autoradiography and histology images. The autoradiography images of ^{64}Cu -DOTA in fibrotic tissues were qualitatively superimposed over the histology images of the fibrotic tissues. The histology images of the infarct areas were characterized by a heterogeneous distribution of thin bands of fibrotic-collagen, myocytes, and expanded extracellular space.

Conclusion— ^{64}Cu -DOTA is a useful surrogate positron analog of Gd-DOTA, enabling to quantitatively measure the uptake values in fibrotic tissues by the dynamic PET imaging and calculate the extracellular volume fractions of the fibrotic tissues. At a microscopic level, the distribution of ^{64}Cu -DOTA is non-uniform, corresponding to the heterogeneous distribution of expanded extracellular space in the setting of myocardial infarction.

Keywords

^{64}Cu -DOTA; PET imaging; Myocardial infarction; Extracellular volume; Pharmacokinetics

Introduction

Gadolinium (Gd)-metal chelates with 1, 4, 7, 10-azacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA) has been widely used as a late Gd-enhancement (LGE) magnetic resonance agent. This contrast agent is a small polar molecule that is distributed to the extracellular space but is not able to passively diffuse into intact cellular compartments. Its tissue uptake kinetics reflects blood flow, perfusion and extracellular space volume [1-3]. Thus, this LGE agent has been used as a gold standard for infarct sizing and assessment of viability after myocardial infarction (MI) by measuring the elevation of the extracellular space volume created by damaged or dead cells due to MI [4, 5]. The increase in gadolinium concentration within fibrotic tissue causes T1 shortening which appears as bright signal intensity in cardiac magnetic resonance (CMR) images. The discrimination between scarred/fibrotic and normal myocardium relies on differences in the contrast concentration combined with the chosen setting of the inversion time (TI) manually to “null” the normal myocardial signal that will appear dark in the final image relative to the bright signal of the scarred/fibrotic myocardium [6, 7]. The LGE technique is suitable for focal myocardial scar, but not for diffuse myocardial fibrosis since absolute uptake and clearance pharmacokinetic are not obtained using routine LGE techniques [8, 9]. We have undertaken this study using a rat model of chronic MI to 1) measure the absolute uptake values of ^{64}Cu -DOTA, a surrogate positron analog of Gd-DOTA to gain information on the uptake and clearance pharmacokinetics of ^{64}Cu -DOTA in blood, cardiac fibrosis, normal myocardium and organs such as lung and liver by a dynamic PET imaging method and 2) to investigate its microdistribution in the cardiac fibrosis by autoradiography and histology.

Materials and methods

Materials and Reagents

1, 4, 7, 10-Tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) was purchased from Macrocyclics, Inc. (Dallas, TX). $^{64}\text{CuCl}_2$ was prepared in high specific activity at the NIH Cyclotron facility by the method of Szajek et al. [10]. ^{18}F -FDG was purchased from

Cardinal Health Nuclear Pharmacy (Cardinal Health, Inc., Beltsville, MD). Tissue-Tek® OCT compound was purchased from Sakura® Finetek USA Inc. (Torrance, CA, USA). Masson's trichrome staining kit (HT15) was purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of a reagent grade.

Radiosynthesis

Thirty μl of DOTA (50 mM) was incubated with 870 MBq of $^{64}\text{CuCl}_2$ in 150 μl of 0.25 M acetate buffer, pH 4.2 at 40°C for 1 h. The radiolabeling yield was determined by the reverse-phase high-performance liquid chromatography (RP-HPLC). The product solution was then neutralized to pH 6.5, diluted with PBS, and sterile-filtered with a 0.22 μm sterile filter prior to *in vitro* and *in vivo* studies. The radiochemical purity of ^{64}Cu -DOTA used for *in vitro* and *in vivo* studies was confirmed by RP-HPLC.

In vitro Serum Stability Test

^{64}Cu -DOTA (specific activity, 0.54 MBq/nmol; 11.1 MBq, 15 - 35 μl) was added to 235 - 200 μl of rat serum with 0.04% sodium azide. The solution (pH 7.2) was incubated at 37 °C for 2 h. Twenty microliter of incubated serum mixture was taken out at 0, 1, and 2 h, diluted to 20 μl with PBS, and centrifuged at 12,000 g $\times$ 10 min at 4 °C (Centrifuge 5424R, Eppendorf). The supernatant was analyzed by instant thin layer chromatography with silica gel impregnated on glass fiber (ITLC; VARIAN, Lake Forest, CA) and size-exclusion high-performance liquid chromatography (SE-HPLC).

After 1 hour dynamic PET imaging of normal rats injected with ^{64}Cu -DOTA, urine samples ($n = 3$) were collected from bladder and centrifuged at 12,000 rpm $\times$ 10 min at 4 °C. The supernatant was analyzed with SE-HPLC, and RP-HPLC to determine ^{64}Cu -DOTA and its metabolites.

Analytical Methods

ITLC—An aliquot (1-5 μl) was spotted on an ITLC strip (VARIAN, Lake Forest, CA) and was allowed to air-dry. The ITLC was developed with a solvent mixture, methanol: 10% ammonium acetate in water: 0.5 M citric acid (70:20:10 v/v/v%) as the mobile phase. On ITLC, proteins or colloids associated with ^{64}Cu remain at the origin of sample application and ^{64}Cu -DOTA moves with the solvent front. The radioactivity peak areas on the ITLC strips were integrated with a Bioscan AR-2000 radiochromatogram scanner (Bioscan Inc, Washington, DC).

RP-HPLC—RP-HPLC analysis was carried out on Gilson Unipoint system (Middleton, WI) equipped with a ACE C18 column (100 $\times$ 4.6 mm, 5 μm ; MAC-MOD Analytical Inc, Chadds Ford, PA), a UV monitor and an on-line flow radioactivity detector (BioScan, Washington DC). The HPLC system was operated under the following gradient elution conditions: eluent A, 50 mM triethylammonium phosphate (pH 2.25) in water and eluent B, 100% acetonitrile; gradient, 0–2 min with 100% eluent A, 2–5 min with 100% to 80% eluent A, 5–12 min with 80% to 65% eluent A, 12–20 min with 65% to –0% eluent A, and 20-25 min with 100% eluent B; flow rate, 1.0 ml/min. The retention time of ^{64}Cu -DOTA and free ^{64}Cu ion was 3.9, and 1.6 min, respectively.

SE-HPLC—SE-HPLC was equipped with a TSK gel G3000SWXL column (7.8×300 mm, $5 \mu\text{m}$, TOSOH Bioscience, Japan) with mobile phase 0.067 M sodium phosphate/0.15 M sodium chloride (pH 6.8; 1.0 ml/min), a UV monitor and an on-line flow radioactivity detector. Flow rate was 1.0 ml/min.

Myocardial infarction model

CD rats (150 - 250 g) with myocardial infarction (MI) were purchased from Charles River Laboratories (Wilmington, MA, USA). Briefly, when rats were 6 weeks old, an ischemic lesion was induced using ligation of left anterior descending coronary artery (LAD) (myocardial infarct in the CD rats, www.criver.com) which produced the ischemic lesion comprising 20-30% of the total cardiac tissue. PET imaging was performed at 14 week (body weight: 569.8 ± 58.5 g, $n = 5$) post-myocardial infarction surgery. As a control, normal CD rats (17 week old, body weight = 468.8 ± 21.1 g, $n = 6$) were used. All experiments were performed according to a protocol approved by National Institutes of Health (NIH) Animal Care and Use Committee.

PET imaging

Micro PET scans for MI rats ($n = 5$) were performed using a Siemens Inveon micro PET scanner (Siemens Medical Solutions USA, Inc.) at 14 week post-LAD ligation. The axial field of view of the scanner was 12.7 cm with a ring diameter of 16.1 cm (transaxial field of view: 10 cm). The spatial resolution in transverse plane was < 1.8 mm full-width half maximum (FWHM) at the center. PET data were acquired within an energy window of 350-650 keV and 3.432 ns of coincidence window. Isoflurane anesthesia of the animals was induced by placing the animals in a plastic chamber flushed continuously with 5% isoflurane in oxygen at 2 L/min using an isoflurane rodent anesthesia system (Siemens Medical Solution Inc., Knoxville, TN). Isoflurane vaporizer was adjusted to 3% isoflurane for maintenance of the anesthesia. The animals were injected intravenously (*iv*) through the tail vein with 18.5 MBq of [^{18}F]-FDG in 0.5 ml of normal saline to define the anatomic reference of viable myocardium. After 1 h post-injection, 10 min static PET images were acquired. One day after [^{18}F]-FDG injection, the same animals were injected *iv* with ^{64}Cu -DOTA (18.5 MBq with 0.02 mmol DOTA/Kg) in 0.5 ml of PBS and the dynamic PET imaging was performed for 60 min under anesthesia. Imaging was performed in the prone position. To obtain reference uptake values in normal myocardium, normal rats were injected with [^{18}F]-FDG and a day later injected with ^{64}Cu -DOTA for PET imaging as described above.

PET data analysis

Listmode data were sorted into sinograms and reconstructed using ordered subset expectation maximization (OSEM) method with 4 iterations. Image matrix was $128 \times 128 \times 159$, pixel size was $0.077 \times 0.077 \text{ cm}^2$, and slice thickness was 0.079 cm. Two mm FWHM Gaussian kernel was applied to the reconstructed image to enhance signal to noise ratio. Dynamic PET data were binned into time frames of 10×30 s; 10×60 s; 3×300 s; 3×600 s. ^{64}Cu -DOTA images were co-registered to [^{18}F]-FDG images. The region of interest (ROI) analysis was performed using ASIPro (provided by Siemens, v6.8.6.2) on decay-

corrected whole-body images. The ROIs were drawn manually on individual infarct lesion, and remote myocardium, left ventricle blood pool (LVBP), lung, and liver. Three representative transverse slices showing the highest uptake of ^{64}Cu -DOTA in the infarct lesion were selected for the ROI of infarct lesion, remote myocardium, and LVBP on a mid-upper myocardial section. The ROIs of infarct area ($14.7 \pm 5.6 \text{ mm}^3$) were drawn around the focal ^{64}Cu -DOTA uptake area in the myocardium on a transverse image, corresponding to [^{18}F]-FDG uptake decrease. Remote myocardium ROIs ($11.9 \pm 0.4 \text{ mm}^3$) were selected on the contralateral myocardium near the septum, not including the septum in order to avoid for potential spill-over activity from the right ventricle (RV) and LV blood pool. Myocardial ROIs in normal rats were defined in anterior wall for comparison with infarcted area and in the contralateral myocardium near the septum for comparison with remote myocardium. These ROIs were projected onto the dynamic PET images to generate time-activity curves (TACs) from all time frames. The radioactivity concentration (nCi/cc) was obtained from the mean uptake values within the ROI. The average of the three mean uptake values obtained from three representative transverse slices was used to calculate the standard uptake value (SUV). The SUV was calculated using the formula: $\text{SUV} = [\text{voxel activity concentration (nCi/cc)}] / [\text{injected activity per body weight (nCi/g)}]$. Activities, injected and measured from the image, were both decay-corrected to the same time reference. Injected activity was measured with a dose calibrator previously cross-calibrated to the scanner. The uptake ratios (MI lesion/blood, remote myocardium/blood, and MI lesion/remote myocardium) were also calculated for MI rats.

Pharmacokinetic analysis

Blood and perfusion rich organs' TACs for ^{64}Cu -DOTA were obtained from dynamic micro PET images after single *iv* injection. The plots of SUV of ^{64}Cu -DOTA versus time showed biphasic curves: it was distributed rapidly in blood and other organs including focal fibrotic tissue with the peak uptakes reached within a minute and then eliminated according to the law of first-order kinetics. The elimination rate constant (k_{el}) was calculated from the first-order kinetic equation. The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln(2)/k_{el}$. The distribution half-life of ^{64}Cu -DOTA was calculated from the time-activity curve between 0 (immediately after injection) to 5 min.

Autoradiography of ^{64}Cu -DOTA in myocardial infarction sections

After 1 h dynamic PET scanning of myocardial infarcted rats with ^{64}Cu -DOTA, the rats were euthanized by exsanguination under isoflurane anesthesia, and the hearts ($n = 3$) were removed and frozen with Tissue-Tek® OCT compound (Sakura® Finetek, Torrance, CA, USA) for 3 h. The 20 μm thick myocardial sections were cut from the base of the heart to the apex using a Leica CM1850 cryostat (Leica Microsystems Inc., Buffalo Grove, IL, USA), and exposed in the phosphor screen for 16 h. Signals were obtained by the use of a Cyclone phosphorimager (Perkin Elmer) with 50 μm resolution and analyzed with the LUT (lookup table) by Image J software (NIH).

Masson's trichrome staining of collagen in myocardial infarction sections

Masson's trichrome staining was performed according to the procedure from Sigma-Aldrich (St. Louis, MO) kit (HT15). Briefly, frozen slides that were used for autoradiography were fixed in Bouin's solution. After incubation in Weigert's Iron Hematoxylin solution, the slides were stained with Biebrich Scarlet-Acid Fuchsin and Aniline Blue and dehydrated in ethanol and xylene. Extensive washes with water were performed between each staining steps. The stained slides were mounted with Permount™ Mounting Medium (Thermo Fisher Scientific Inc.) and digitally imaged at an apparent 20× magnification (0.50 μm/pixel) using a whole-slide scanner, Aperio ScanScope CS (Aperio Technologies, Vista, CA). The collagen fibers were stained blue, the nuclei were stained black and myocardium was stained dark-red.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical analysis used the unpaired Student's t test with unequal variance for comparison between normal and MI animals. A *p* value <0.05 was considered statistically significant.

Results

Radiolabeling and Stability Studies

The radiolabeling of ⁶⁴Cu-DOTA was quantitative based on RP-HPLC showing only one peak representing ⁶⁴Cu-DOTA with a retention time of 3.9 min (Fig. 1). On this RP-HPLC, free ⁶⁴Cu acetate showed a retention time of 1.6 min. ⁶⁴Cu-DOTA was stable against a transchelation to serum proteins such as transferrin and albumin when incubated with serum for 2 h at 37 °C. No ⁶⁴Cu transchelated to proteins was detected on SE-HPLC in which it shows a single peak with its retention time (13.5 min) identical to that of ⁶⁴Cu-DOTA was detected. The analysis of urine samples collected at 1 h post injection showed a single peak representing ⁶⁴Cu-DOTA and no radiocatabolite formed *in vivo* when tested on both RP-HPLC and SE-HPLC.

PET Imaging

We used [¹⁸F]-FDG images as anatomical references for viable myocardium because it was previously reported that normal myocardium showed very high FDG uptake due to the high glucose metabolism, whereas ischemic myocardium showed decreased [¹⁸F]-FDG uptake [11, 12]. ⁶⁴Cu-DOTA images were co-registered to [¹⁸F]-FDG images to define the accumulation of ⁶⁴Cu-DOTA in the infarct area. The representative PET images of MI heart are shown in Fig. 2. The fusion images show that ⁶⁴Cu-DOTA was taken up in the infarct area. The time-activity curves obtained from the ROI analysis of the dynamic images demonstrated that the ⁶⁴Cu-DOTA concentrations in the fibrotic tissue and perfusion-rich organs peaked within a minute of post injection and thereafter rapidly washed out in parallel with the blood clearance (Fig. 3). The dynamic images also showed that the radioactivity in blood was rapidly excreted via the renal system. The blood clearance curve was a bi-phasic with the distribution half-life of < 3 min and the elimination half-life of 21.8 min as the distribution half-life was estimated from the time-activity curve between 0 to 5 min, and the

elimination half-life was estimated from the curve between 10 to 60 min. The elimination half-life of ^{64}Cu -DOTA from the focal fibrotic tissue and the remote myocardium was 22.4 and 20.1 min, respectively, similar ($p > 0.1$) to the elimination half-life of ^{64}Cu -DOTA from the blood when calculated from the time-activity curves between 10 and 60 min (Table 2). Consequently, the uptake ratios of focal fibrosis-to-blood and remote myocardium-to-blood remain stable for the time period between 10 to 60 min (Table 1). The corresponding ratios obtained from images acquired from 30 to 60 min were 1.09 and 0.59, respectively (Table 1), indicating that the concentration of ^{64}Cu -DOTA in the focal fibrosis was almost twice ($1.09/0.59 = 1.85$) of the concentration in remote myocardium. The remote myocardium (0.59)-, lung (0.50)- and liver (1.16)-to-blood ratio from the MI rats were similar to the myocardium (0.57)-, lung (0.53)- and liver (1.18)-to-blood ratio from the normal rats. The myocardium-to-blood and lung-to-blood ratios remained unchanged over time, indicating that there was no preferential retention of ^{64}Cu -DOTA in myocardium and lung. However, the liver-to-blood ratios increased over time, consistent with that reported for Gd-DOTA [13].

Autoradiography and histology

Autoradiography and histology of tissue slices from the base of the heart (left) to the apex (right) were compared (Fig. 4). The autoradiography images indicate that ^{64}Cu -DOTA was not uniformly distributed in fibrotic tissues in MI heart. This finding was also supported by the Masson's trichrome staining of fibrotic collagens in extracellular space of the fibrotic tissues showing non-uniform distribution of fibrotic collagen bands. The autoradiography images of ^{64}Cu -DOTA in fibrotic tissues were qualitatively superimposed over the histology images of the fibrotic tissues. In Masson's trichrome staining images, the infarct areas were characterized by a combination of extracellular space, the thin bands of fibrotic-collagen, and myocytes. Two times magnification of histology images showed that myocytes were commingled with the bands of fibrotic collagen network in a number of different areas within the fibrotic tissue slices and its distribution was not uniform throughout the entire fibrotic tissues.

Discussion

Cardiac magnetic resonance (CMR) imaging with a late gadolinium enhancement (LGE) agent such as Gd-DOTA or Gd-DTPA has been widely used for infarct sizing and assessment of viability after myocardial [3, 14]. While CMR imaging offers greater spatial and temporal resolution, absolute uptake values are difficult to obtain so that the uptake and clearance of agents is not typically assessed. T1 mapping techniques have more recently been developed for CMR, allowing better quantitation of uptake, although the method remains indirect, based on relaxivity values. In contrast, the nuclear techniques do not offer as great resolution as CMR, but enable to measure the absolute uptakes and determine the pharmacokinetics by a continuous dynamic PET imaging. Thus, the CMR measurements of infarction scar can be complemented by the corresponding PET measurement with ^{64}Cu -DOTA, a surrogate positron analog of Gd-DOTA, for the analysis of the cardiac fibrosis in a rat model of chronic MI.

The dynamic imaging studies demonstrated that the in vivo distribution of ^{64}Cu -DOTA reflected the blood pool signal with high levels of radioactivity in perfusion-rich tissues including cardiac fibrosis, normal myocardium, and lungs with the peak concentration of ^{64}Cu -DOTA reached within 1 min after i.v. injection. Thereafter, ^{64}Cu -DOTA rapidly cleared from these perfusion-rich tissues in parallel with the blood clearance and was excreted via the renal system (Fig. 3), similar to the reports for the LGE agent [15, 16]. ^{64}Cu -DOTA was washed out of the fibrotic tissue and remote myocardium with their elimination rates similar to the elimination rate from blood. These findings indicate that there was rapid free diffusion of the radiolabel between the blood and the extracellular space and no appreciable differences in wash-in and wash-out rates of ^{64}Cu -DOTA in the extracellular space of fibrotic lesion and the extracellular space of remote myocardium. Thus, the results of our study support a hypothesis that the difference in the ^{64}Cu -DOTA concentration between the fibrotic tissue and remote myocardium was primarily determined by the fraction of the extracellular space volume (ECV) of fibrotic tissue and remote myocardium. The fibrosis-to-remote myocardium ratio of 1.85 in Table 1 appears to indicate that the ECV fraction of infarct lesion we defined for the ROI analysis was 1.85 fold larger than that of the remote myocardium because ^{64}Cu -DOTA accumulates only in the extracellular space, but not in viable myocytes. This ratio of 1.85 also indicates that the ECV fraction of the infarct lesion we defined is 46 % of the total volume and the rest (54 %) of the volume is occupied by viable myocytes because the ECV fraction of normal tissue has been reported to be 25% of the total volume of the tissue [14, 17]. We investigated the uptake and pharmacokinetics of ^{64}Cu -DOTA in the myocardium of normal rats as the reference values for a viable myocardium and compared with those from the remote myocardium of the chronic MI rats. We found that the normal myocardium-to-blood ratio of ^{64}Cu -DOTA was identical to that of the remote myocardium-to-blood ratio in the chronic MI rats (Table 1), indicating that the remote myocardium we defined for the ROI analysis was viable. The histology images of the heart slices also showed no staining with Masson's trichrome in the remote myocardium of the MI rats and normal myocardium of the normal rats.

The blood elimination half-life of ^{64}Cu -DOTA (Table 2) was consistent with that of Gd-DOTA (23 min) [13] and ^{68}Ga -DOTA (22.3 ± 10.6 min) [1]. We also evaluated the elimination half-lives from blood, fibrosis and remote myocardium from the first order elimination constants obtained from the TAC from 5 to 60 min, 10 to 60 min, and 16 to 60 min (Table 2). We found that the half-lives obtained from the end phase of the TAC (16 to 60 min in this case) were the longer with the best coefficient of determination ($R^2 = \sim 0.99$), indicating that this end phase of the TAC best fits the elimination phase. However, in all cases, the elimination half-lives from fibrosis and remote myocardium were similar to the blood elimination half-life. Thus, these findings also support a hypothesis that the differences in the ^{64}Cu -DOTA concentrations found in fibrosis and normal myocardium for a time period between 10 to 60 min was primarily determined by differences in the extracellular volume fractions between fibrotic tissue and normal tissue [18]

Conclusion

The uptake and clearance kinetics of ^{64}Cu -DOTA in perfusion rich organs and blood was similar to that of Gd-DOTA reported previously. Thus, ^{64}Cu -DOTA appears to be a useful surrogate positron analog of Gd-DOTA. ^{64}Cu -DOTA enables to quantitatively measure the absolute uptake values in fibrotic tissues by the dynamic PET imaging and calculate the extracellular volume fractions of the fibrotic tissues. ^{64}Cu with its decay half-life of 12.7 h also is a suitable positron emitter providing time required for making autoradiography slices from the base to the apex of heart to visualize the microdistribution of the radiolabel in the entire fibrotic tissues and correlate the autoradiography images with histology images. This is the first study, to the best of our knowledge, to report the uptake, the clearance pharmacokinetics, the extracellular volume fraction estimation, the microdistribution of ^{64}Cu -DOTA in cardiac fibrosis by autoradiography and compare autoradiography images with histology images.

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Figure 1.
RP-HPLC of ^{64}Cu -DOTA. RP-HPLC conditions are described in *Materials and Method*.

Figure 2.

Representative *In vivo* PET images acquired from 30 to 60 min. (A) Transverse and coronal images of ^{64}Cu -DOTA and $[^{18}\text{F}]$ -FDG, and their fusion in transaxial and coronal views at 14-wk post MI. The yellow arrow pointed myocardial infarction lesion. The region of ^{64}Cu -DOTA uptake coincides with the area of the decreased $[^{18}\text{F}]$ -FDG uptake. (B) Transverse and coronal images in normal rats of ^{64}Cu -DOTA and $[^{18}\text{F}]$ -FDG, and their fusion in transaxial and coronal views. ($[^{18}\text{F}]$ -FDG: Black-White scale; ^{64}Cu -DOTA: Blue/Green/Red/Yellow scale)

Figure 3.

Time-activity curves generated from dynamic small-animal PET images. (A) Distribution phase in MI rats (up to 5 min). (B) Elimination phase in MI rats between 5 and 60min. ^{64}Cu -DOTA was eliminated according to the laws of first-order reaction kinetics. The equation is $A_t = A_0 e^{-kt}$. A_0 is initial concentration of A, A_t is concentration of A at time t, k is rate constant and t is time

Figure 4.

Comparison of autoradiography and histology of chronic MI from the base (left) of the heart to the apex (right). Immediately after the completion of the PET imaging, the rats were euthanized. The hearts were dissected and processed for autoradiography and histology staining. Top row shows autoradiography images from Cyclone phosphorimager. Middle row shows the corresponding histological sections stained with Masson's trichrome for collagen-rich areas of fibrosis (scale bar, 3mm). The collagen fibers were stained blue and myocardium was stained dark-red. Lower row shows the enlarged portion of infarcted heart from whole images of row (inset magnification, $\times 2$)

Table 1

Uptake ratios in myocardial infarction and normal rats between 0 to 60 min. The uptake ratios for focal fibrosis-to-blood and remote myocardium-to-blood remain stable for the time period.

Time (min)	Myocardial infarction rats				Normal rats		
	Fibrosis to Remote myocardium	Fibrosis to LVBP	Remote myocardium to LVBP	Lung to LVBP	Liver to VBP	Normal myocardium to LVBP	Liver to LVBP
0-10	1.71 ± 0.25	0.93 ± 0.05	0.55 ± 0.07	0.45 ± 0.07	0.34 ± 0.01	0.59 ± 0.05	0.39 ± 0.03
10-20	1.75 ± 0.27	1.03 ± 0.09	0.60 ± 0.12	0.46 ± 0.05	0.53 ± 0.04	0.63 ± 0.07	0.59 ± 0.08
20-30	1.70 ± 0.29	1.04 ± 0.05	0.62 ± 0.09	0.48 ± 0.06	0.74 ± 0.05	0.62 ± 0.06	0.78 ± 0.09
30-40	1.89 ± 0.30	1.11 ± 0.09	0.60 ± 0.15	0.51 ± 0.07	0.92 ± 0.13	0.60 ± 0.03	0.92 ± 0.08
40-50	1.81 ± 0.38	1.05 ± 0.05	0.60 ± 0.16	0.49 ± 0.06	1.10 ± 0.09	0.56 ± 0.04	1.15 ± 0.17
50-60	2.00 ± 0.26	1.11 ± 0.15	0.56 ± 0.04	0.51 ± 0.16	1.44 ± 0.20	0.56 ± 0.08	1.49 ± 0.23
30-60	1.90 ± 0.09	1.09 ± 0.03	0.59 ± 0.03	0.50 ± 0.01	1.16 ± 0.27	0.57 ± 0.02	1.18 ± 0.29

Table 2

Half-lives calculated from first-order kinetic equation.

Time (min)	$t_{1/2}$ LVBP (R^2)	$t_{1/2}$ Fibrosis (R^2)	$t_{1/2}$ Remote myocardium (R^2)
0-5	2.1 ± 0.1 (0.7977)	2.4 ± 0.2 (0.8148)	2.6 ± 0.3 (0.8123)
5-60	19.3 ± 1.1 (0.9561)	20.2 ± 0.7 (0.9566)	18.5 ± 1.2 (0.9641)
10-60	21.8 ± 1.9 (0.9803)	22.4 ± 0.7 (0.9717)	20.1 ± 1.4 (0.9791)
16-60	24.4 ± 3.0 (0.9882)	26.7 ± 1.0 (0.9942)	23.1 ± 2.9 (0.9960)

R^2 is coefficient of determination.