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Pharmacokinetics and microbiodistribution of ^{64}Cu -labeled collagen binding peptides in chronic myocardial infarction

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Abstract

Objectives—To evaluate the pharmacokinetics and microbiodistribution of ^{64}Cu -labeled collagen binding peptides.

Method—The affinity constant (K_D), association (k_a) and dissociation rate constant (k_d) for the peptide collagelin or its analogue (named CRPA) binding to collagen were measured by bio-layer interferometric analysis. Rats ($n = 4-5$) with myocardial infarction or normal were injected IV with the ^{64}Cu -labeled peptides or ^{64}Cu -DOTA as a control. Dynamic PET imaging was performed for 60 min at 7- to 8-week post-infarct. [^{18}F]FDG PET imaging was performed to identify the viable myocardium. To validate the PET images, slices of heart samples from the base to the apex were analyzed using autoradiography and histology.

Result—The peptides bound to collagen with K_D of $\sim 0.9 \mu\text{M}$. The ^{64}Cu -peptides and ^{64}Cu -DOTA accumulated in the infarct area (confirmed by autoradiography and histology images) within 1 minute of injection and were excreted rapidly via the renal system. The blood clearance curves were bi-phasic with the elimination half-lives, 21.9 ± 2.4 , 26.2 ± 4.6 and 21.2 ± 2.1 min for ^{64}Cu -CRPA, ^{64}Cu -collagelin and the control ^{64}Cu -DOTA, respectively. The clearance half-lives from the focal fibrotic tissue (24.1 ± 1.5 , 25.6 ± 8.0 and 21.4 ± 1.3 min, respectively) and remote myocardium (20.8 ± 0.7 , 21.0 ± 5.5 and 19.1 ± 2.4 min, respectively) were not significantly different. The uptake ratios of infarct-to-remote myocardium (1.93 ± 0.18 , 2.15 ± 0.38 and 1.88 ± 0.08 , respectively) for ^{64}Cu -CRPA, ^{64}Cu -collagelin and ^{64}Cu -DOTA remained stable for the time period between 10 to 60 min.

Conclusion—The distribution of the ^{64}Cu -collagelin probes corresponds to the heterogeneous distribution of expanded extracellular space in the setting of myocardial infarction. The overall washout rate from the fibrous tissue was determined by the slow washout rate ($t_{1/2}$, 20 min) of

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the peptides from the extracellular space to the vasculature, not by the dissociation rate ($t_{1/2}$, 2 min) of the ^{64}Cu -peptides from collagen.

Keywords

PET imaging; Myocardial infarction; Fibrosis; Collagen-specific peptides; Extracellular space fraction

INTRODUCTION

Fibrosis is a common outcome in many chronic diseases of the heart, kidney, liver, lungs, or vasculature. It is increasingly recognized as an important feature of many chronic diseases [1]. Currently, the only method available for quantifying fibrosis is tissue biopsy, which is invasive and carries a risk of complications in a variety of organs and cannot be easily repeated [2]. Magnetic resonance imaging (MRI) and computed tomography (CT) can successfully identify myocardial fibrosis due to myocardial infarction using nonspecific, extracellular contrast agents [3–5]. However, these modalities have low sensitivity for early or diffuse fibrosis. Specific markers for fibrosis/collagen using noninvasive imaging would represent a substantial advance over currently available technologies.

Following acute myocardial infarction, necrotic myocytes are replaced by extracellular matrix components, mainly collagen type 1, eventually forming a fibrotic myocardial scar [6]. Collagen is present at relatively high concentrations (1–20 nmol/g) in many organs [7] and is entirely extracellular. During fibrosis development, collagen concentration can increase tenfold or more [6]. In addition, fibrosis forms a structural motif with a cross-linked helical structure of collagen [8] that exposes a higher number of peptide binding sites compared to constitutive collagen in organs [9]. Thus, it is anticipated that the increase in local collagen concentration and rearrangement of collagen structure in fibrotic tissue might result in a preferential uptake and retention of the collagen-specific binding probes in fibrous collagen as compared to constitutive collagen in organs.

A number of collagen-specific proteins [10–13] and peptides [9, 14, 15] have been investigated for *in vitro* and *in vivo* targeting of fibrous collagen. Collagelin is a cyclic 22 amino acid peptide (SGSGCPGRVMHGLHLGDDEGPC) that was reported to mimic the collagen binding site (underlined) of immunoadhesin glycoprotein VI (GPVI) and bind with high specificity and affinity to Type-I and Type-III collagens [9]. Muzard, et al. [9] labeled collagelin with $^{99\text{m}}\text{Tc}$ and used SPECT imaging to demonstrate the uptake of $^{99\text{m}}\text{Tc}$ -streptavidin-biotin-collagelin or $^{99\text{m}}\text{Tc}$ -biotin-collagelin in the cardiac area of rats with a myocardial infarction (MI) scar, but no uptake in normal myocardium of sham-operated rats. One disadvantage of the $^{99\text{m}}\text{Tc}$ -labeled collagelin was high background activity in abdomen due to high uptake in liver. Recently, Velikyan, et al. [16] modified collagelin (Col) with NOTA-PEG₂ and NODAGA-PEG₂ and investigated the distribution and clearance of these collagelin analogues labeled with ^{68}Ga from the blood and the major organs of normal rats. The pharmacokinetic studies demonstrated that ^{68}Ga -NO₂A-Col and ^{68}Ga -NODAGA-Col rapidly cleared from the blood and excreted via the renal system with low background radioactivity shown in the abdomen within 25 min post-injection.

We sought to determine if fibrosis could be detected in a rat model of chronic myocardial infarction with collagelin and its analogue SGSGCGPRVMHGLHLGDDEGPC (CRPA) labeled with a positron emitter, ^{64}Cu using PET imaging with the aim of reduced background activity in liver. The peptide CRPA differs from collagelin in the peptide sequence arrangement with the position of proline at position 6 and glycine at position 7 exchanged. Herein, we report the ^{64}Cu labeling chemistry, *in vitro* collagen binding properties, and the uptake and clearance pharmacokinetics of these ^{64}Cu -labeled peptides in blood, cardiac fibrosis, normal myocardium and organs such as lung and liver by a dynamic PET imaging and compare their pharmacokinetic data with that of ^{64}Cu -DOTA. We also report their microdistribution in the cardiac fibrosis by autoradiography and compare with histology.

MATERIALS AND METHODS

Materials and Reagents

2-*S*-(4-isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (*p*-SCN-Bz-NOTA) was purchased from Macrocyclics, Inc. (Dallas, TX). Sep-Pak C18 cartridges were purchased from Waters (Milford, MA). $^{64}\text{CuCl}_2$ was prepared in high specific activity at the NIH Cyclotron facility by the method of Szajek et al. [17]. [^{18}F]FDG was purchased from Cardinal Health Nuclear Pharmacy (Cardinal Health, Inc., Beltsville, MD). Rat type-I collagen was from BD Biosciences (San Jose, CA). Masson's Trichrome staining kit (HT15) was purchased from Sigma-Aldrich (St. Louis, MO). All peptides were synthesized at Peptide 2.0 Inc. (Chantilly, VA) or PolyPeptide (Strasbourg, France). Streptavidin biosensors and sample diluent were supplied from ForteBio Inc. (Menlo Park, CA). All other reagents were of a reagent grade.

Peptide Synthesis

The collagen-specific peptides SGSGCGPRVMHGLHLGDDEGPC (CRPA) and SGSGCPGRVMHGLHLGDDEGPC (collagelin) were synthesized, and the peptides were or were not coupled to biotin at their N-terminus and made cyclic by disulfide bridging of two cysteine moieties (Cs). The carboxyl group at the C-terminus was substituted by an amide. These peptides were synthesized at PolyPeptide (Strasbourg, France) and Peptide 2.0 (Chantilly, VA), and the purity, amino acids composition, and molecular weight were assessed by high-performance liquid chromatography (HPLC) and Mass Spectrometry (MS). The purity of peptides was >95% at 220 nm by reversed phase-HPLC. The ESI-mass analysis of CRPA and collagelin detected $m/z = 1090.37$ for $[\text{M} + 2\text{H}]^{2+}$ with reconstituted molecular weight of 2178.43 and $m/z = 1090.81$ for $[\text{M} + 2\text{H}]^{2+}$ with reconstituted molecular weight of 2178.43, respectively.

Radiosynthesis

NOTA conjugation to collagelin and CRPA—Twenty μl of N-terminus of collagelin or CRPA solution (each 40 mM in dimethyl sulfoxide (DMSO)) was added with 20 μl of *p*-SCN-Bz-NOTA solution (4 mM in DMSO) to give the molar ratio of 10:1, and to this, 10 μl of 0.1 M sodium bicarbonate (pH 8.4) was added and incubated at 25°C overnight.

⁶⁴Cu-NOTA-collagelin and ⁶⁴Cu-NOTA-CRPA—Sixteen nmol of NOTA-Bz-SCN-collagelin (NOTA-collagelin) or NOTA-Bz-SCN-CRPA (NOTA-CRPA) was labeled with 1272 MBq of ⁶⁴CuCl₂ in 0.15 ml of 0.25 M acetate buffer, pH 4.2 at 25°C for 1 h, and 3.5 ml of 3 mM EDTA in PBS, pH 7.2 was then added to the reaction mixture to quench a potential free ⁶⁴Cu²⁺. The product solution was purified by Sep-Pak C18 cartridge (Waters, Milford, MA): the Sep-Pak C18 cartridge was preconditioned prior to the sample purification by washing with ethanol followed by distilled water and phosphate-buffered saline (PBS). The sample loaded cartridge was washed stepwise with PBS, 5% and 20% ethanol in PBS and eluted with 40% ethanol in PBS. The radiochemical purity was confirmed by the reverse phase (RP)-HPLC using Gilson Unipoint system (Middleton, WI) equipped with a ACE C18 column (100 x 4.6 μm, 5 μm; MAC-MOD Analytical Inc, Chadds Ford, PA), a UV monitor and an on-line flow radioactivity detector (BioScan, Washington DC) 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 65–0% eluent A, and 20–25 min with 100% eluent B; flow rate, 1.0 ml/min. The retention times of ⁶⁴Cu-NOTA-CRPA and ⁶⁴Cu-NOTA-collagelin were 10.4 and 11.1 min, respectively. In this system, the retention times (Rts) for cold CRPA, NOTA-conjugated CRPA, collagelin, and NOTA-conjugated collagelin were 9.5, 10.1, 10.3, and 10.8, respectively.

⁶⁴Cu-DOTA—⁶⁴Cu-DOTA radiosynthesis was described in our previous study [18]. Briefly, 30 μl of DOTA at 50 mM was incubated with 870 MBq of ⁶⁴CuCl₂ in 150 μl of 0.25 M acetate buffer, pH 4.2 at 40°C for 1 h. The product solution was then neutralized to pH 6.5, diluted with PBS, and sterile-filtered with a 0.22 μ sterile filter prior to IV injection to MI rats. RP-HPLC was used for the estimation of the radiolabeling yield and the radiochemical purity of ⁶⁴Cu-DOTA. The Rt of ⁶⁴Cu-DOTA was 3.9 min.

In Vitro Stability Test in Serum

For the evaluation of *in vitro* stability, ⁶⁴Cu-NOTA-collagelin or ⁶⁴Cu-NOTA-CRPA (11.1 MBq/ 0.3 nmol in 50 μl of 40% ethanol in PBS buffer) was added to 200 μl of rat serum, and the solution was incubated at 37°C for 1 h. Twenty microliter of incubated serum mixture was taken at 0.33 and 1 h and analyzed by size exclusion (SE)-HPLC equipped with a TSK G2000SWXL or TSK G3000SWXL column (TosoHaas, Philadelphia, PA), a UV monitor and an on-line flow radioactivity detector (BioScan, Washington DC). The HPLC column was eluted with a solvent mixture composed of 0.067 M PBS and 0.1 M KCl (pH 6.8) at a flow rate of 1 ml/min. Paper chromatography also was performed using 3 MM Whatman CHR paper strip (GE Health Life Sciences, Pittsburg, PA) pretreated with or without 5% human serum albumin and saline as the developing solvent in order to detect colloidal formation and ⁶⁴Cu bound to serum proteins. In this system with the albumin pretreatment, only radio-colloids remain on the origin of sample application, and radiolabeled peptides and protein-bound radioactivity move with the solvent front. In contrast, the system without the albumin pretreatment results in protein-bound radioactivity (by non-specific binding to the paper) and radio-colloids both remaining on the origin of sample application and only radiolabeled peptide moving with the solvent front.

Bio-layer Interferometry Assay

The affinity constant (K_D) for CRPA or collagelin binding to collagen was assessed by bio-layer interferometry assays using the Blitz instrument (ForteBio Inc., Menlo Park, CA) and following the company instruction. Briefly, we first optimized the ligand (biotin-CRPA or -collagelin) concentration to immobilize onto Streptavidin biosensor based on two criteria, the signal sensitivity and the signal leveling-off effect due to steric hindrance when a very high concentration of the ligand is immobilized on the biosensor. The analyte (collagen) concentration was then optimized to satisfy a guideline that a concentration of the analyte should be within a range between about 10–20 times K_D down to about 0.1 times K_D , where is K_D is dissociation constant ($K_D = 1/K_A$; K_A is affinity constant) to ensure for reliable binding kinetic data (ForteBio Inc., Biomolecular Binding Kinetics Assays on the Octet Platform by Renee Tobias, renee_tobias@pall.com; Sriram Kumaraswamy, sriram_kumaraswamy@pall.com): The optimal ligand concentration was found to be 1.7 μM , and the optimal concentration range of analyte to be 0–8 μM . In our binding kinetics studies, streptavidin biosensors (ForteBio Inc.) were pre-hydrated in sample diluent (ForteBio Inc.) and loaded with biotin-CRPA or -collagelin at 1.7 μM in PBS as a ligand prior to kinetic analysis. Collagen (analyte) at several different concentrations (0–8 μM in sample diluent at pH 7.4) was then added to the biosensor at room temperature to measure the association rate constant that was followed by dilution in the sample diluent solution to measure the dissociation rate constant. The affinity constant ($K_D = 1/\text{association constant}$), association rate constant (k_a) and dissociation rate constant (k_d) were calculated by Blitz software (ver 1.1.0.8). The kinetic constant data were calculated after subtracting the background signal (using sample diluent without collagen).

Animal 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 7 or 8 week post-MI surgery to allow myocardial scar formation (body weight: 462.8 ± 37.8 g, $n = 4$ to 5). 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 at 7- or 8-week post-MI ($n = 4$ to 5) were performed using a Siemens Inveon micro-PET scanner (Siemens Medical Solutions USA, Inc. Knoxville, TN) in the prone position. The rats were placed in general anesthesia chamber (Siemens Medical Solution Inc., Knoxville, TN) with 5% isoflurane in oxygen at 2 L/min. Once anesthetized, rats were IV injected through the tail vein with 18.5 MBq of [^{18}F]FDG in 0.5 ml of normal saline and maintained anesthesia with 3% isoflurane plus 100% oxygen at 2 L/min. Static images were obtained in rats 1 h after the injection for 10 min to define the anatomic reference of viable myocardium. One day after [^{18}F]FDG imaging, the same animals were intravenously injected with ^{64}Cu -NOTA-CRPA (18.5 MBq with 6 nmol CRPA), ^{64}Cu -

NOTA-Collagelin (18.5 MBq with 5 nmol collagelin) or ^{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. To obtain reference uptake values in normal myocardium, normal rats were injected with [^{18}F]FDG and a day later injected with ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagelin, or ^{64}Cu -DOTA for PET imaging as described above.

PET data analysis

List mode 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 filter was applied to the reconstructed image to enhance signal to noise ratio. Dynamic PET data were binned into time frames of $10 \times 30 \text{ s}$; $10 \times 60 \text{ s}$; $3 \times 300 \text{ s}$; $3 \times 600 \text{ s}$. In this study, we used [^{18}F]FDG images as an anatomical reference for viable myocardium; the higher accumulation of ^{64}Cu -collagen tracers in skin, blood pool and the adjacent area of the decreased [^{18}F]FDG accumulation enabled us to reliably overlay ^{64}Cu -tracer images over the [^{18}F]FDG images. ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagelin and ^{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 labeled peptides and ^{64}Cu -DOTA were selected for the ROIs of infarct lesion, remote myocardium, and LVBP. The ROIs of infarct area were drawn around the focal high uptake area in the myocardium on a transverse image, corresponding to the decreased [^{18}F]FDG uptake. Remote myocardium ROIs were selected on the contralateral myocardium near the septum, not including the septum in order to avoid potential spill-over activity from the right ventricle (RV) and LV blood pool. ROI for LVBP were selected from a mid-upper myocardial section. Myocardial ROIs in normal rats were defined in anterior wall corresponding to infarcted area and the contralateral myocardium near the septum for comparison with remote myocardium in MI. These ROIs were projected onto the dynamic PET images to generate time-activity curves (TACs) from all time frames. The ROI pixel values were automatically expressed as radioactivity per cubic centimeter (Bq/cc) by applying an experimentally-determined calibration factor to the reconstructed images. The average of the three mean uptake values obtained from three representative transverse slices was used to calculate the standard uptake value (SUV). Based on the assumption that tissue density is 1 g/mL, the SUV was calculated using the formula: $\text{SUV} = [\text{voxel activity concentration (Bq/cc)}] / [\text{injected activity per body weight (Bq/g)}]$. Both activities, injected and measured from the image, were 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.

Ex vivo autoradiography

After 1 h of dynamic PET scanning of MI rats with ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagenin or ^{64}Cu -DOTA, the rat hearts were excised, embedded and frozen in Tissue-Tek[®] CRYO-OCT compound (Sakura[®] Finetek USA Inc., Torrance, CA, USA) at 20°C for 3 h. Serial 20 μm thick short axis sections were cut in 400 μm intervals covering the entire heart 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 the Typhoon FLA 7000 (GE Healthcare Life Sciences, Pittsburgh, PA, USA) with 25 μm pixel resolution and analyzed with Image Quant TL8.1 software.

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 $\times$ 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 methods

Data are expressed as mean $\pm$ SD. Statistical analysis used the unpaired Student's *t* test with unequal variance for comparison between control and MI animals. A *p* value <0.05 was considered statistically significant.

RESULTS

Chemistry and Radiochemistry

The conjugation yield of *p*-SCN-Bz-NOTA (4 mM) to CRPA (40 mM) or collagenin (40 mM) was quantitative based on *p*-SCN-Bz-NOTA. Thus, the NOTA-conjugated peptides were labeled with ^{64}Cu chloride in acetate buffer without further purification. Radiolabeling yields were $74.1 \pm 1.5\%$ (72.4 – 75.0 %, *n*=3) for ^{64}Cu -NOTA-CRPA and $87.4 \pm 10.9\%$ (81.1 – 100 %, *n*=3) for ^{64}Cu -NOTA-collagenin. The specific activities of both radio-peptides varied between 48.8 and 92.9 MBq/nmol. The radiochemical purity was >95% after the purification with the solid phase extraction using a Sep Pak C18 column. The chemical structure and radiochemical purity of ^{64}Cu -NOTA-CRPA and ^{64}Cu -NOTA-collagenin used for the *in vitro* and the *in vivo* studies are shown in Fig. 1. The radiolabeling of ^{64}Cu -DOTA was quantitative based on RP-HPLC showing only one peak representing ^{64}Cu -DOTA with a retention time of 3.9 min. On this RP-HPLC, free ^{64}Cu acetate showed a retention time of 1.6 min. The specific activity of ^{64}Cu -DOTA was 423.3 ± 175.8 MBq/ μmol (*n*=3) at the end of the radiolabeling experiment.

In vitro Stability Tests

The retention time (R_t) of ^{64}Cu -NOTA-CRPA or ^{64}Cu -NOTA-collagelin was 12.1 min on SE-HPLC. After the incubation in rat serum at 37°C for 1 h, >90 % of ^{64}Cu -NOTA-CRPA or ^{64}Cu -NOTA-collagelin existed as the intact form (R_t , 12.1 min), and 3.3 % protein-bound radioactivity peak was shown at a retention time identical to that of rat serum albumin (R_t , 8.4 min) when analyzed by SE-HPLC (Fig. S1). A shoulder peak (~ 7 %) was eluted out immediately before the peak corresponding to ^{64}Cu -NOTA-peptides at time 0 of the serum incubation. We think that this shoulder peak was formed by a radiation induced fragmentation of the peptides during the storage of the ^{64}Cu -NOTA-peptides because we performed the serum stability studies several hours after the PET imaging studies were done. However, the intensity of this peak did not increase 1 h after incubation with serum possibly because serum protein (albumin) is a good inhibitor against the radiation induced fragmentation of peptides. There was no radioactivity associated with colloids that would show at the origin of the sample application on paper chromatography.

In Vitro Binding to Collagen

Fig. 2 shows the representative binding kinetic profile, K_D , k_a , and k_d obtained by the biolayer interferometry analysis when the streptavidin biosensors loaded with biotin-CRPA at 1.7 μM as ligands and collagen at 0–8 μM was used as an analyte. The K_D , k_a and k_d obtained were ($0.9 \pm 0.4 \mu\text{M}$, $n = 5$), $(11.4 \pm 13.3) \times 10^3 \text{ M}^{-1}\text{second}^{-1}$, and $(6.8 \pm 2.8) \times 10^{-3} \text{ second}^{-1}$, respectively based on the collagen concentrations estimated by its molecular weight of 100 kDa.

In vivo PET imaging analysis

The maximum intensity projection images for the initial 120 seconds after injection of ^{64}Cu -NOTA-CRPA and ^{64}Cu -NOTA-collagelin show that the ^{64}Cu -labeled peptides rapidly accumulated in the region of myocardial infarction and perfusion-rich normal organs including remote myocardium, lung and liver. The ^{64}Cu -peptides were then washed out of these organs in parallel with their clearance from blood and were rapidly excreted via the renal system without appreciable ^{64}Cu activity remaining in liver (Fig. 3A and Table 1). We used [^{18}F]FDG images as anatomical references for viable myocardium: The infarcted myocardium shows decreased [^{18}F]FDG uptake compared to normal myocardium (Figs. 3B and S2) [19, 20]. ^{64}Cu -CRPA or -collagelin images were co-registered to [^{18}F]FDG images to define the accumulation of ^{64}Cu -peptides in the infarct area. Fig. 3B shows representative transverse, coronal, and their fusion images of a MI heart injected with ^{64}Cu -peptides and [^{18}F]FDG, and the fusion images of the transverse and coronal images of ^{64}Cu -peptides overlaid on the corresponding CT images. The crosshair shows the fibrotic lesion in the myocardium. We found that the area of ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagelin or ^{64}Cu -DOTA accumulation in the fibrous lesion of the myocardium was well matched with the decreased uptake area of [^{18}F]FDG. Fig. S3 shows dynamic images for ^{64}Cu -NOTA peptides and ^{64}Cu -DOTA in MI heart. The time-activity curves (TAC) obtained from the ROI analysis of the dynamic images demonstrated that the concentrations of these ^{64}Cu -probes peaked within a minute of post injection in the infarcted myocardium, remote myocardium and blood, and thereafter rapidly washed out in parallel with the blood

clearance (Fig. 4). The blood clearance curves for ^{64}Cu -CRPA, ^{64}Cu -collagelin, and ^{64}Cu -DOTA were bi-phasic: distribution and elimination phases. The blood distribution half-lives were < 3 min for all three probes estimated from the TAC between 0 to 5 min. The blood elimination half-lives calculated from the elimination rate constants between 10 to 60 min were 21.9 ± 2.4 , 26.2 ± 4.6 and 21.2 ± 2.1 min, respectively for ^{64}Cu -CRPA, ^{64}Cu -collagelin, and ^{64}Cu -DOTA. The corresponding elimination half-lives from the infarct myocardium and the remote myocardium were 24.1 ± 1.5 , 25.6 ± 8.0 and 21.4 ± 1.3 min, respectively and 20.8 ± 0.7 , 21.0 ± 5.5 and 19.1 ± 2.4 min, respectively (Table 2). The clearance half-lives of the ^{64}Cu -peptides from the infarct myocardium and remote myocardium were somewhat slower than that of ^{64}Cu -DOTA, probably due to the effect of slightly slower clearance of the ^{64}Cu -peptides from the blood. Consequently, the corresponding uptake ratios of infarct myocardium-to-blood, remote myocardium-to-blood and infarct myocardium-to-remote myocardium remained stable for the time period between 10 to 60 min (Table 1). The corresponding uptake ratios of infarct myocardium-to-remote myocardium were 1.93 ± 0.18 , 2.15 ± 0.38 and 1.88 ± 0.08 , respectively, calculated based on the ROI analysis of the images acquired from 30 to 60 min (Table 1).

Autoradiography and histochemical analysis

Autoradiography and histological analysis of tissue slices from the apex (left) of the heart to the base (right) were compared (Fig. 5). The autoradiography images indicate that the ^{64}Cu -peptides and ^{64}Cu -DOTA were not uniformly distributed in infarcted myocardium. This finding was also supported by the Masson's Trichrome staining of fibrotic collagen in extracellular space of the infarcted myocardium showing non-uniform distribution of fibrotic collagen bands. The autoradiography images of the three ^{64}Cu -probes in infarcted myocardium were qualitatively superimposed over the corresponding histology images. 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. The magnified histology images showed that myocytes were co-mingled with the bands of fibrotic collagen.

DISCUSSION

A common endpoint of myocardial injury is myocardial fibrosis [21]. Fibrosis is the formation of excess fibrous connective tissue largely composed of type I collagen as a result of a reparative process. The molecular targeting of the intermediates in fibrosis pathways were well reviewed by Sinusas, et al. [22, 23]. In this research, we used collagen-specific ^{64}Cu -labeled NOTA-peptides to investigate the deposition of collagen following myocardial infarction and the biotin-labeled peptides to determine the affinity constant (K_D), association constant (k_a) and dissociation constant (k_d) for the binding to collagen in vitro. We anticipated that the conjugation of biotin or NOTA to the N-terminus which is a 7 amino acid spacer away from the collagen-binding amino acid sequence would not appreciably affect the binding of the peptide to collagen. This notion was supported by Velikyan, et al. [16] who reported that ^{68}Ga -labeled collagelin derivatives, $[^{68}\text{Ga}]\text{Ga-NOTA-PEG}_2\text{-c}[\text{CPGRVMHGLHLGDDEGPC}]$ and $[^{68}\text{Ga}]\text{Ga-NODAGA-PEG}_2\text{-c}[\text{CPGRVMHGLHLGDDEGPC}]$ bound to cryosections of the heart tissue with the equilibrium K_D of $2.3 \pm 0.8 \mu\text{M}$ and $2.1 \pm 0.9 \mu\text{M}$, respectively. These dissociation constants

(K_D) are similar to that (K_D , $0.9 \pm 0.4 \mu\text{M}$, $n = 5$) we determined by the interferometry assay using the biotin-labeled peptides. Variations among the K_D values may result from different methodologies used. We, therefore, think that the conjugation of biotin or *p*-SCN-Bz-NOTA to the peptide at the 7 amino acid spacer away from the collagen-binding amino acid sequence would not substantially affect the affinity constant of the peptide binding to collagen.

We next assessed *in vivo* activity of the collagen binding peptides in the rat model of myocardial infarction by micro-PET imaging. The time-activity curves obtained from dynamic PET imaging demonstrated that the ^{64}Cu -peptides rapidly accumulated in fibrotic tissues and perfusion-rich organs such as remote myocardium, lung and liver with peak uptakes within a min of injection (Fig. 4). The ^{64}Cu -peptides washed out of these organs in parallel with their clearance from blood and were rapidly excreted via the renal system without appreciable radioactivity remaining in liver (Table 1 and Fig. 3A). The *in vivo* washout kinetics of the ^{64}Cu -peptides from fibrous tissues was similar to that of ^{64}Cu -DOTA, which does not have specific binding to collagen. This suggests that washout rate of the ^{64}Cu -peptides from infarct tissue was determined by the diffusion rate from the extracellular space to the vasculature, similar to that of ^{64}Cu -DOTA, rather than by the dissociation rate of the peptides from the fibrous collagen.

The uptake of these three ^{64}Cu -probes in fibrotic tissue was confirmed by *ex vivo* autoradiography and histology images stained by Masson's Trichrome. The distribution of the radioactivity on autoradiography images was qualitatively superimposed over the histology images of the infarct area. The histological myocardial infarct area was characterized by a heterogeneous distribution of thin bands of fibrotic-collagen, myocytes, and expanded extracellular space. At a microscopic level, the distributions of the ^{64}Cu -probes were non-uniform, corresponding to the heterogeneous distribution of expanded extracellular space in the setting of myocardial infarction.

In this study, the uptake and clearance kinetics of ^{64}Cu -CRPA and -collagelin in perfusion rich organs and blood were similar to those of ^{64}Cu -DOTA [18] and Gd-DOTA [24]. Autoradiography slices from the base to the apex of heart after the PET imaging studies indicate that the microdistribution patterns of the ^{64}Cu -peptides in the entire fibrotic tissues were similar to that of ^{64}Cu -DOTA and that the autoradiography images were well correlated with histology images. Although CRPA and collagelin specifically bind to collagen *in vitro*, collagen-specific binding was not reflected in the *in vivo* washout kinetics because the dissociation rates of the peptides from collagen with its half-life (< 2 min) (Fig. 4) were much faster than the diffusion rates of the peptides from the extracellular space of the fibrous tissues into the vasculatures with its half-life (21.4 – 25.6 min) (Table 2).

A feasible mechanism to explain the uptake and clearance pharmacokinetics of these peptides from the infarct area is as follows: The peptides in the blood compartment readily cross the vasculature into the extracellular space of the fibrotic tissue; they then rapidly associate and dissociate with collagen with rate constants that are much faster than the rate of diffusion between the extracellular space and the vasculature. The peptides that diffuse out to the vasculature are rapidly eliminated via renal excretion (Fig. 3A). The observed

clearance rate from fibrotic tissue is determined by the slowest (rate limiting) step which is the wash-out (diffusion out) rate of the ^{64}Cu probes from the extracellular space out to vasculature.

The standard uptake values of the ^{64}Cu -peptides and ^{64}Cu -DOTA in infarcted myocardium are about 2 times greater than those in remote myocardium (Table 1). The extracellular space fraction of normal tissue is 25% of the total tissue volume [25], while that of myocardial infarction is approximately 50% [26]. Thus, the SUV values of the ^{64}Cu -peptides are consistent with that of distribution to the extracellular space, similar to ^{64}Cu -DOTA [18]. We conclude that further improvement of the affinity of the collagen-binding ligand is needed in order to provide physiologic utility for identification of collagen.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Caravan P, Das B, Dumas S, Epstein FH, Helm PA, Jacques V, et al. Collagen-targeted MRI contrast agent for molecular imaging of fibrosis. *Angew Chem Int Ed Engl*. 2007; 46:8171–8173. [PubMed: 17893943]
2. Helm PA, Caravan P, French BA, Jacques V, Shen L, Xu Y, et al. Postinfarction myocardial scarring in mice: molecular MR imaging with use of a collagen-targeting contrast agent. *Radiology*. 2008; 247:788–796. [PubMed: 18403626]
3. El Aidi H, Adams A, Moons KG, Den Ruijter HM, Mali WP, Doevendans PA, et al. Cardiac magnetic resonance imaging findings and the risk of cardiovascular events in patients with recent myocardial infarction or suspected or known coronary artery disease: a systematic review of prognostic studies. *J Am Coll Cardiol*. 2014; 63:1031–1045. [PubMed: 24486280]
4. Wichmann JL, Arbaciauskaite R, Kerl JM, Frellesen C, Bodelle B, Lehnert T, et al. Evaluation of monoenergetic late iodine enhancement dual-energy computed tomography for imaging of chronic myocardial infarction. *European radiology*. 2014
5. Wichmann JL, Bauer RW, Doss M, Stock W, Lehnert T, Bodelle B, et al. Diagnostic accuracy of late iodine-enhancement dual-energy computed tomography for the detection of chronic myocardial infarction compared with late gadolinium-enhancement 3-T magnetic resonance imaging. *Invest Radiol*. 2013; 48:851–856. [PubMed: 23907104]
6. Jugdutt BI, Joljart MJ, Khan MI. Rate of collagen deposition during healing and ventricular remodeling after myocardial infarction in rat and dog models. *Circulation*. 1996; 94:94–101. [PubMed: 8964124]
7. Neuman RE, Logan MA. The determination of collagen and elastin in tissues. *J Biol Chem*. 1950; 186:549–556. [PubMed: 14794650]
8. Caravan P, Das B, Deng Q, Dumas S, Jacques V, Koerner SK, et al. A lysine walk to high relaxivity collagen-targeted MRI contrast agents. *Chem Commun (Camb)*. 2009:430–432.

9. Muzard J, Sarda-Mantel L, Loyau S, Meulemans A, Louedec L, Bantsimba-Malanda C, et al. Non-invasive molecular imaging of fibrosis using a collagen-targeted peptidomimetic of the platelet collagen receptor glycoprotein VI. *PLoS One*. 2009; 4:e5585. [PubMed: 19440310]
10. Gawaz M, Konrad I, Hauser AI, Sauer S, Li Z, Wester HJ, et al. Non-invasive imaging of glycoprotein VI binding to injured arterial lesions. *Thromb Haemost*. 2005; 93:910–913. [PubMed: 15886808]
11. Viaene A, Crab A, Meiring M, Pritchard D, Deckmyn H. Identification of a collagen-binding protein from *Necator americanus* by using a cDNA-expression phage display library. *J Parasitol*. 2001; 87:619–625. [PubMed: 11426727]
12. Krahn KN, Bouten CV, van Tuijl S, van Zandvoort MA, Merckx M. Fluorescently labeled collagen binding proteins allow specific visualization of collagen in tissues and live cell culture. *Anal Biochem*. 2006; 350:177–185. [PubMed: 16476406]
13. Chen J, Lee SK, Abd-Elgalil WR, Liang L, Galende EY, Hajjar RJ, et al. Assessment of cardiovascular fibrosis using novel fluorescent probes. *PLoS One*. 2011; 6:e19097. [PubMed: 21533060]
14. Depraetere H, Viaene A, Deroo S, Vauterin S, Deckmyn H. Identification of peptides, selected by phage display technology, that inhibit von Willebrand factor binding to collagen. *Blood*. 1998; 92:4207–4211. [PubMed: 9834225]
15. Kolodziej AF, Zhang Z, Overoye-Chan K, Jacques V, Caravan P. Peptide optimization and conjugation strategies in the development of molecularly targeted magnetic resonance imaging contrast agents. *Methods Mol Biol*. 2014; 1088:185–211. [PubMed: 24146405]
16. Velikyan I, Rosenstrom U, Estrada S, Ljungvall I, Haggstrom J, Eriksson O, et al. Synthesis and preclinical evaluation of ⁶⁸Ga-labeled collagen analogs for imaging and quantification of fibrosis. *Nucl Med Biol*. 2014; 41:728–736. [PubMed: 25023066]
17. Szajek LPM, Plascjak W, Eckelman P, WC. Semi-remote production of [⁶⁴Cu]CuCl₂ and preparation of high specific activity [⁶⁴Cu]Cu-ATSM for PET studies. *Radiochimica Acta*. 2005; 93:239–244.
18. Kim H, Lee SJ, Davies-Venn C, Kim JS, Yang BY, Yao Z, et al. 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. *Nucl Med Commun*. 2016; 37:188–196. [PubMed: 26488428]
19. Sun X, Yan Y, Liu S, Cao Q, Yang M, Neamati N, et al. ¹⁸F-FPPRGD2 and ¹⁸F-FDG PET of response to Abraxane therapy. *J Nucl Med*. 2011; 52:140–146. [PubMed: 21149494]
20. Gao H, Lang L, Guo N, Cao F, Quan Q, Hu S, et al. PET imaging of angiogenesis after myocardial infarction/reperfusion using a one-step labeled integrin-targeted tracer ¹⁸F-AIF-NOTA-PRGD2. *Eur J Nucl Med Mol Imaging*. 2012; 39:683–692. [PubMed: 22274731]
21. Mewton N, Liu CY, Croisille P, Bluemke D, Lima JA. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. *J Am Coll Cardiol*. 2011; 57:891–903. [PubMed: 21329834]
22. Dobrucki LW, Sinusas AJ. PET and SPECT in cardiovascular molecular imaging. *Nat Rev Cardiol*. 2010; 7:38–47. [PubMed: 19935740]
23. Sinusas AJ, Thomas JD, Mills G. The future of molecular imaging. *JACC Cardiovasc Imaging*. 2011; 4:799–806. [PubMed: 21757172]
24. Bousquet JC, Saini S, Stark DD, Hahn PF, Nigam M, Wittenberg J, et al. Gd-DOTA: characterization of a new paramagnetic complex. *Radiology*. 1988; 166:693–698. [PubMed: 3340763]
25. Kellman P, Wilson JR, Xue H, Bandettini WP, Shanbhag SM, Druey KM, et al. Extracellular volume fraction mapping in the myocardium, part 2: initial clinical experience. *J Cardiovasc Magn Reson*. 2012; 14:64. [PubMed: 22967246]
26. Ugander M, Oki AJ, Hsu LY, Kellman P, Greiser A, Aletras AH, et al. Extracellular volume imaging by magnetic resonance imaging provides insights into overt and sub-clinical myocardial pathology. *Eur Heart J*. 2012; 33:1268–1278. [PubMed: 22279111]

Figure 1. Chemical structures and RP-HPLC profiles of ^{64}Cu -NOTA-CRPA and ^{64}Cu -NOTA-collagenin with their retention times. RP-HPLC conditions are described in Materials and Methods section.

Figure 2.

Representative specific peptide binding to collagen: Kinetic profiles of biotin-CRPA binding to collagen by bio-layer interferometry on a Blitz system. For the binding kinetic assay, biotin-CRPA or biotin-collagelin was first immobilized on a streptavidin biosensor, the association reaction was performed at the concentrations of collagen at 2, 5 and 8 μM , followed by the dissociation reaction in the sample diluent. The kinetic profiles show collagen binding with CRPA immobilized on the biosensor. The mean value ($n = 5$) and SD of K_D , k_a and k_d calculated by the interferometry analysis were 0.9 ± 0.4 μM , $(11.4 \pm 13.3) \times 10^3 \text{M}^{-1}\text{second}^{-1}$ and $(6.8 \pm 2.8) \times 10^{-3} \text{second}^{-1}$, respectively.

Figure 3.

A) Representative maximum intensity projection images for the initial 120 seconds after injection of ^{64}Cu -NOTA-CRPA and ^{64}Cu -NOTA-collagelin. The images show that the ^{64}Cu -labeled peptides rapidly accumulated in the region of myocardial infarction and perfusion-rich normal organs including remote myocardium, lung and liver. The ^{64}Cu -peptides were then washed out of these organs in parallel with their clearance from blood and were rapidly excreted via the renal system without appreciable ^{64}Cu activity remaining in liver. **B)** Representative transverse and coronal images of ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagelin, ^{64}Cu -DOTA and $[^{18}\text{F}]\text{FDG}$, and their fusion views, and the fusion images of ^{64}Cu PET overlaid on CT in MI rats. The images were obtained at 30–60 min. The crosshair shows the fibrotic lesion in the myocardium. $[^{18}\text{F}]\text{FDG}$: Black/White scale; ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagelin, and ^{64}Cu -DOTA: Blue/Green/Red/Yellow scale.

Figure 4.

Time-activity curves for ^{64}Cu -NOTA-CRPA, ^{64}Cu -NOTA-collagelin, and ^{64}Cu -DOTA generated from dynamic micro-PET images. The standard uptake values (SUVs) were obtained from region of interest (ROI) analysis drawn on micro-PET images. Distribution phase is shown between the time for the peak uptake to 5 min. Elimination phase is shown between 5 and 60 min. The ^{64}Cu -probes were eliminated according to the laws of first-order reaction kinetics ($A_t = A_0 e^{-kt}$) where A_0 is initial concentration of A , A_t is concentration of A at time t , and k is rate constant.

Figure 5.

Comparison of ex vivo autoradiography and histology of chronic MI from the apex (left) of the heart to the base (right). Immediately after the completion of the PET imaging with ^{64}Cu -NOTA-CRPA (A), ^{64}Cu -NOTA-collagelin (B), and ^{64}Cu -DOTA (C), the rats were euthanized. The hearts were dissected and processed for autoradiography and Masson's Trichrome staining. Top row shows autoradiography images from Typhoon FLA 7000. Middle row shows the corresponding histological sections stained with Masson's Trichrome for collagen-rich areas of fibrosis (scale bar, 3 mm). Lower row shows the enlarged portion of infarcted heart from whole images of middle row (scale bar, 0.5 mm). The collagen fibers were stained blue and myocardium was stained dark-red.

Uptake ratios of ^{64}Cu -probes in myocardial infarction 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.

Table 1

Time (min)	Fibrosis to Remote myocardium	Fibrosis to LVBP	Remote myocardium to LVBP	Lung to LVBP	Liver to LVBP
CRPA					
0-10	1.69 ± 0.26	0.88 ± 0.09	0.53 ± 0.06	0.52 ± 0.12	0.40 ± 0.07
10-20	1.70 ± 0.22	0.99 ± 0.11	0.59 ± 0.09	0.55 ± 0.13	0.53 ± 0.08
20-30	1.89 ± 0.23	1.05 ± 0.12	0.56 ± 0.06	0.51 ± 0.07	0.59 ± 0.08
30-40	1.91 ± 0.25	1.05 ± 0.18	0.55 ± 0.08	0.52 ± 0.12	0.66 ± 0.12
40-50	1.92 ± 0.22	1.00 ± 0.22	0.52 ± 0.08	0.49 ± 0.14	0.69 ± 0.16
50-60	1.89 ± 0.26	0.93 ± 0.21	0.50 ± 0.10	0.50 ± 0.14	0.79 ± 0.18
30-60	1.93 ± 0.18	1.00 ± 0.19	0.52 ± 0.08	0.50 ± 0.13	0.70 ± 0.15
Collagenin					
0-10	1.77 ± 0.36	0.70 ± 0.16	0.42 ± 0.15	0.41 ± 0.18	0.32 ± 0.09
10-20	1.93 ± 0.43	0.83 ± 0.27	0.44 ± 0.16	0.43 ± 0.17	0.42 ± 0.12
20-30	1.88 ± 0.23	0.87 ± 0.22	0.46 ± 0.11	0.41 ± 0.16	0.51 ± 0.14
30-40	2.05 ± 0.44	0.88 ± 0.20	0.45 ± 0.15	0.44 ± 0.18	0.55 ± 0.14
40-50	2.18 ± 0.39	0.87 ± 0.26	0.41 ± 0.15	0.41 ± 0.18	0.59 ± 0.16
50-60	2.33 ± 0.48	0.95 ± 0.35	0.43 ± 0.20	0.47 ± 0.17	0.75 ± 0.24
30-60	2.15 ± 0.38	0.89 ± 0.26	0.43 ± 0.16	0.44 ± 0.18	0.62 ± 0.17
DOTA					
0-10	1.70 ± 0.14	0.85 ± 0.06	0.50 ± 0.03	0.43 ± 0.03	0.35 ± 0.03
10-20	1.78 ± 0.22	1.03 ± 0.10	0.59 ± 0.09	0.47 ± 0.03	0.52 ± 0.03
20-30	1.82 ± 0.21	1.02 ± 0.14	0.57 ± 0.07	0.46 ± 0.04	0.67 ± 0.05
30-40	1.88 ± 0.16	1.02 ± 0.08	0.54 ± 0.05	0.45 ± 0.05	0.80 ± 0.04
40-50	1.82 ± 0.11	0.94 ± 0.21	0.51 ± 0.09	0.42 ± 0.10	0.97 ± 0.15
50-60	1.80 ± 0.15	0.87 ± 0.17	0.49 ± 0.12	0.43 ± 0.12	1.14 ± 0.16
30-60	1.88 ± 0.08	0.95 ± 0.14	0.51 ± 0.06	0.43 ± 0.06	0.94 ± 0.09

The data are expressed as mean ± SD (n = 4 to 5).

Table 2

Half-lives calculated from first-order kinetic equation ($A_t = A_0e^{-kt}$).

Time (min)	$t_{1/2}$ (LVBP)			$t_{1/2}$ (Fibrosis)			$t_{1/2}$ (Remote myocardium)		
	CRPA	Collagelin	DOTA	CRPA	Collagelin	DOTA	CRPA	Collagelin	DOTA
0–5	2.2 ± 0.1	2.1 ± 0.2	2.5 ± 0.4	2.6 ± 0.4	2.6 ± 0.2	3.2 ± 0.7	2.6 ± 0.2	2.4 ± 0.1	2.6 ± 0.3
5–60	19.4 ± 1.5	22.8 ± 4.2	19.5 ± 1.7	21.5 ± 1.4	22.9 ± 5.5	20.2 ± 1.4	19.3 ± 0.9	19.8 ± 6.3	18.5 ± 1.9
10–60	21.9 ± 2.4	26.2 ± 4.6	21.2 ± 2.1	24.1 ± 1.5	25.6 ± 8.0	21.4 ± 1.3	20.8 ± 0.7	21.0 ± 5.5	19.1 ± 2.4
15–60	24.3 ± 1.5	27.5 ± 2.3	22.4 ± 2.4	25.0 ± 2.5	29.3 ± 5.8	24.2 ± 1.0	24.3 ± 2.4	24.0 ± 8.4	20.1 ± 3.8

The data are expressed as mean ± SD (n = 4 to 5).