ORIGINAL ARTICLE

Basic Science

Visualization of Dynamic Expression of Myocardial Sigma-1 Receptor After Myocardial Ischemia and Reperfusion Using Radioiodine-Labeled 2-[4-(2-iodophenyl)piperidino]cyclopentanol (OI5V) Imaging

Hiroshi Wakabayashi, MD, PhD; Junichi Taki, MD, PhD; Hiroshi Mori, MD;
Tomo Hiromasa, MD, PhD; Norihito Akatani, MD; Anri Inaki, MD, PhD;
Takashi Kozaka, PhD; Kazuhiro Shiba, PhD;
Kazuma Ogawa, PhD; Seigo Kinuya, MD, PhD

Background: This study chronologically evaluated the expression of the intensity and distribution of the sigma-1 receptor (σ1R) demonstrated by radiolabeled 2-[4-(2-iodophenyl)piperidino]cyclopentanol (OI5V) in a rat model of myocardial ischemia and reperfusion.

Methods and Results: The left coronary artery was occluded for 30 min, followed by reperfusion. Dual-tracer autoradiography with ^{125}I-OI5V and ^{99m}Tc-MIBI was performed to assess the spatiotemporal changes in ^{125}I-OI5V uptake (n=5–6). Significant and peaked ^{125}I-OI5V uptake in the ischemic area was observed at 3 days after reperfusion, and the ^{125}I-OI5V uptake ratio of ischemic area to normally perfused left ventricular area decreased gradually from 3 to 28 days (mean value ± SD; 0.90±0.12 at 1 day, 1.89±0.19 at 3 days, 1.52±0.17 at 7 days, 1.34±0.13 at 14 days, and 1.16±0.14 at 28 days, respectively). Triple-tracer autoradiography with ^{125}I-OI5V, ^{99m}Tc-MIBI, and [201]TlCl was performed to evaluate ^{125}I-OI5V uptake in the ischemic area in relation to the residual perfusion at 7 days (n=4). The ^{125}I-OI5V uptake ratio of the non-salvaged area was higher compared to that of the salvaged area in the ischemic area. ^{123}I-OI5V and ^{99m}Tc-MIBI SPECT/CT was performed 3 days after reperfusion (n=3), and the in vivo images showed clear uptake of ^{123}I-OI5V in the perfusion defect area.

Conclusions: The present study confirmed the spatiotemporal expression pattern of σ1R expression. Non-invasive σ1R imaging with ^{123}I or ^{125}I-OI5V was feasible to monitor the expression of σ1R after myocardial ischemia and reperfusion.

Key Words: Memory imaging; Myocardial ischemia; OI5V; Sigma-1 receptor

Sigma receptor was first recognized as an opioid receptor subtype in the 1970s,[1] and was later reclassified as a unique receptor due to its different properties.[2] Two subtypes of sigma receptor (sigma-1 and -2 receptor) have been identified,[3] and the sigma-1 receptor (σ1R) was first cloned in 1996 from guinea pig liver.[4] The σ1R primarily resides at the endoplasmic reticulum (ER) membrane associated with mitochondria (MAM) and is also translocated to the plasma membrane under stress. It acts as a chaperone that regulates protein folding / degradation, ER / oxidative stress, and cell survival.[5] It is considered as a pluripotent modulator with resultant multiple functional manifestations in the living system.[6] It has been intensively studied in the central nervous system thus far and

Editorial p 2109

has been implicated in many diseases, including schizophrenia, clinical depression, Alzheimer's disease, Parkinson's disease, cocaine addiction, stroke/ischemia, myocardial hypertrophy, and cancer.[7,8]

In the heart, σ1R is also expressed in cardiomyocytes as a chaperon and regulates the response to ER stress, modulates calcium handling, and affects the function of voltage-gated-ion channels in abnormal states.[5] The σ1R knockout mouse model demonstrated cardiac dysfunction with mitochondrial dysfunction, abnormal mitochondrial architecture, and adverse cardiac remodeling.[9] The σ1R contributed to
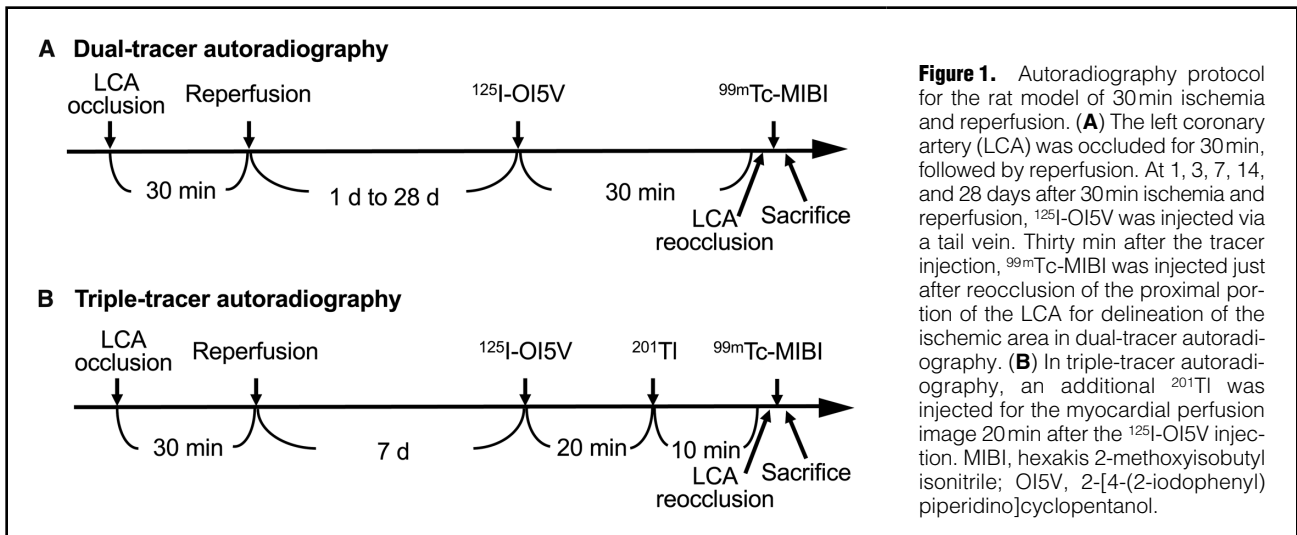
Figure 1. Autoradiography protocol for the rat model of 30 min ischemia and reperfusion. (**A**) The left coronary artery (LCA) was occluded for 30 min, followed by reperfusion. At 1, 3, 7, 14, and 28 days after 30 min ischemia and reperfusion, ^{125}I-OI5V was injected via a tail vein. Thirty min after the tracer injection, ^{99m}Tc-MIBI was injected just after reocclusion of the proximal portion of the LCA for delineation of the ischemic area in dual-tracer autoradiography. (**B**) In triple-tracer autoradiography, an additional ^{201}Tl was injected for the myocardial perfusion image 20 min after the ^{125}I-OI5V injection. MIBI, hexakis 2-methoxyisobutyl isonitrile; OI5V, 2-[4-(2-iodophenyl) piperidino]cyclopentanol.

understanding the pathophysiology of myocardial hypertrophy. In transverse aortic constriction (TAC) mice, σ1R is downregulated in cardiomyocytes following heart failure;[10] however, the spatiotemporal expression patterns of σ1R after myocardial ischemia and reperfusion have not been explored.

A radiotracer ^{125}I-2-[4-(2-iodophenyl)piperidino]cyclopentanol (OI5V), vesamicol analog with 5-membered ring structure, shows a high selective binding affinity for σ1R. ^{125}I-OI5V is a promising candidate for a σ1R imaging probe.[11] The use of hybrid imaging devices such as single-photon emission computed tomography (SPECT) with computed tomography (CT) enables non-invasive imaging of σ1R distribution in preclinical and human subjects. Here, we evaluated the spatiotemporal changes in the intensity of the expression of σ1R demonstrated by OI5V radiolabeled with ^{123}I or ^{125}I in a rat model of ischemia and reperfusion.

Methods

Animal Model of Acute Ischemia and Reperfusion

All experimental animal protocols were approved by the Institute for Animal Studies of Kanazawa University (AP-173846). All applicable Japanese and institutional guidelines for the care and use of animals were followed. Male Wistar rats, 8–9 weeks old, were anesthetized with intraperitoneal administration of secobarbital sodium, 40 mg/kg, and were ventilated mechanically with room air. After left thoracotomy and exposure of the heart, a 7-0 polypropylene suture on a small curved needle was passed through the myocardium beneath the proximal portion of the left coronary artery (LCA), and both ends of the suture were passed through a small vinyl tube to make a snare. The suture material was pulled tightly against the vinyl tube to occlude the LCA in 30 min. Myocardial ischemia was confirmed by ST-segment elevation on electrocardiography and by regional cyanosis of the myocardial surface. The snare was left loose on the surface of the heart for reocclusion of the LCA just before sacrificing the animals to identify the area at risk. For the sham operation group, only thread placement under LCA without ligation was conducted.

Radiolabeling of [125]I- and ^{123}I-OI5V

^{125}I-OI5V was prepared from 2-[4-(2-trimethylstannylphenyl)piperidino]cyclopentanol (OT5V) (50 μg/50 μL) and ^{125}I-NaI (37 MBq) using the iodo-destannylation reaction under no-carrier-added conditions.[11] After incubation for 10–20 min at room temperature, the prepared ^{125}I-OI5V was purified with HPLC [reverse phase HPLC column (Zorbax-ODS RX-C18, 9.6×250 mm), 40°C, 2.0 mL/min flow rate, 80:20:0.2 v/v/v acetonitrile/H₂O/monoethanolamine]. Radiolabeling of ^{123}I-OI5V was performed using the same method as for ^{125}I-OI5V, except for use of ^{123}I-NaI (111 MBq) instead of ^{125}I-NaI (37 MBq).

The radiochemical yield of [123]I- and ^{125}I-OI5V was 70–80%. After purification, [123]I- and ^{125}I-OI5V showed a radiochemical purity of over 99%.

Dual-Tracer Autoradiography

At 1, 3, 7, 14, and 28 days after 30 min ischemia and reperfusion, ^{125}I-OI5V (1.5 MBq) was injected via a tail vein (n=5 for 1 day and n=6 for 3, 7, 14, and 28 days). After 30 min of the tracer injection, 185 MBq of ^{99m}Tc-hexakis 2-methoxyisobutyl isonitrile (MIBI) was injected just after the reocclusion of the LCA for the delineation of ischemic area (area at risk) (**Figure 1A**). One minute later, the rat was euthanized, and the heart was removed for analysis. The heart was rinsed in saline, embedded in methyl cellulose, and cooled in a freezer. Serial short-axis heart sections of 20 μm thickness were obtained using a cryostat to create a series of rings for autoradiography. The first autoradiographic exposure on an imaging plate (BAS-MS; Fuji Film) was performed for 15–20 min to visualize ^{99m}Tc-MIBI distribution 1–2 h after sacrifice (area at risk). Three days later (12 half-lives of ^{99m}Tc), the second exposure was made for 4 days to image ^{125}I-OI5V distribution.

As a blocking study, an excess of an unlabeled sigma ligand, (+/−)-pentazocine (10 μmol), was injected into the rat 20 min before the ^{125}I-OI5V injection at 3 days after ischemia and reperfusion (n=3).

Triple-Tracer Autoradiography

To compare the distribution of perfusion tracer [^{201}Tl chloride (^{201}Tl)] and ^{125}I-OI5V, triple-tracer autoradiography was performed using the rat model of 7 days after ischemia and
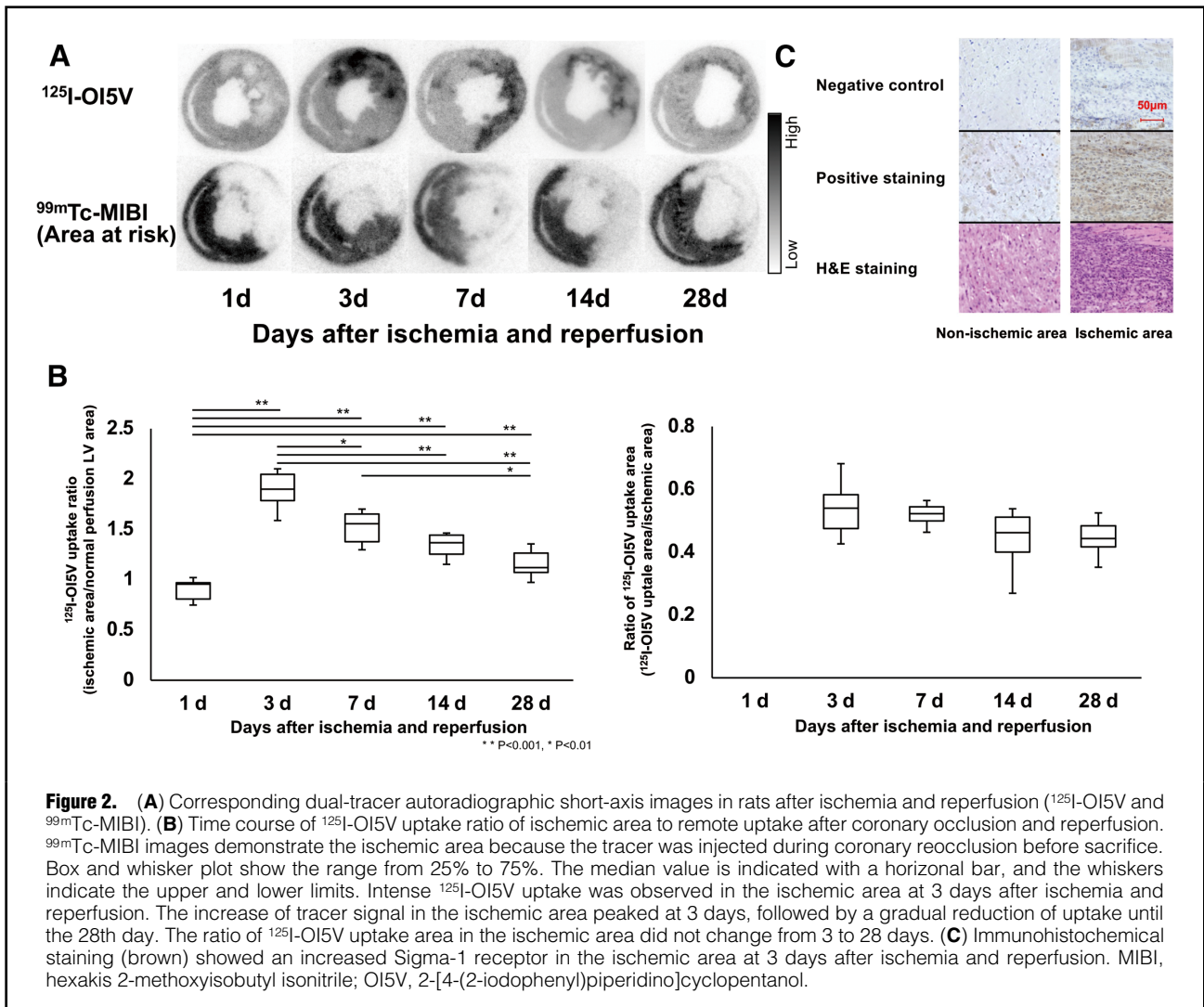
Figure 2. (**A**) Corresponding dual-tracer autoradiographic short-axis images in rats after ischemia and reperfusion (^{125}I-OI5V and ^{99m}Tc-MIBI). (**B**) Time course of ^{125}I-OI5V uptake ratio of ischemic area to remote uptake after coronary occlusion and reperfusion. ^{99m}Tc-MIBI images demonstrate the ischemic area because the tracer was injected during coronary reocclusion before sacrifice. Box and whisker plot show the range from 25% to 75%. The median value is indicated with a horizonal bar, and the whiskers indicate the upper and lower limits. Intense ^{125}I-OI5V uptake was observed in the ischemic area at 3 days after ischemia and reperfusion. The increase of tracer signal in the ischemic area peaked at 3 days, followed by a gradual reduction of uptake until the 28th day. The ratio of ^{125}I-OI5V uptake area in the ischemic area did not change from 3 to 28 days. (**C**) Immunohistochemical staining (brown) showed an increased Sigma-1 receptor in the ischemic area at 3 days after ischemia and reperfusion. MIBI, hexakis 2-methoxyisobutyl isonitrile; OI5V, 2-[4-(2-iodophenyl)piperidino]cyclopentanol.

reperfusion. After 20 min of ^{125}I-OI5V (1.5 MBq) injection (n=4), [201]Tl (10 MBq) was injected (**Figure 1B**). Ten minutes later, 185 MBq of ^{99m}Tc-MIBI was injected just after reocclusion of the proximal portion of the LCA for delineation of the ischemic area. One minute later, the rat was euthanized and the heart was removed for analysis. The heart was rinsed in saline, embedded in methyl cellulose, and cooled in a freezer. Serial short-axis heart sections of 20 μm thickness were obtained using a cryostat to create a series of rings for autoradiography. The first autoradiographic exposure on an imaging plate was performed for 15–20 min to visualize ^{99m}Tc-MIBI distribution 1–2 h after sacrifice. Three days later (12 half-lives of ^{99m}Tc), the second exposure was made for 5–6 h to image the myocardial perfusion expressed by ^{201}Tl distribution. One month (10 half-lives of ^{201}Tl) or more later, the third exposure was made for 7 days to image the σ1R expression by ^{125}I-OI5V distribution.

Data Analysis

^{125}I-OI5V accumulation was evaluated in 3 myocardial slices (20-μm thickness) at the mid-ventricular level spaced 1-mm apart from one another. The distribution of the tracers was determined by analysis of the digitized autoradiographs. The photostimulated luminescence in each pixel (50×50 μm) was determined using a bioimaging analyzer (BAS-5000; Fuji Film).

For quantitative analysis, the uptake values for each region of interest (ROI) were expressed as the background-corrected photostimulated luminescence per unit area (0.25 mm^2). A background ROI was set adjacent to the left ventricle. Ischemic and normally perfused areas were defined manually from the ^{99m}Tc-MIBI image, and these ROIs were applied to the ^{125}I-OI5V images to evaluate the uptake of ^{125}I-OI5V. The ^{125}I-OI5V uptake ratio was calculated by dividing the uptake value of an ischemic area by that of a normally perfused area in the dual-autoradiography.

Area of preserved perfusion area (salvaged area) and decreased perfusion area (non-salvaged area) in the ischemic area were defined from the ^{201}Tl image. The salvaged and non-salvaged areas were defined as ≥60% uptake and <60% uptake of a normally perfused area, respectively.[12] These ROIs were applied to the ^{125}I-OI5V images to evaluate the uptake of ^{125}I-OI5V. The ^{125}I-OI5V uptake ratio was calculated by dividing the uptake values of a non-salvaged area and salvage area by that of a normally perfused area.
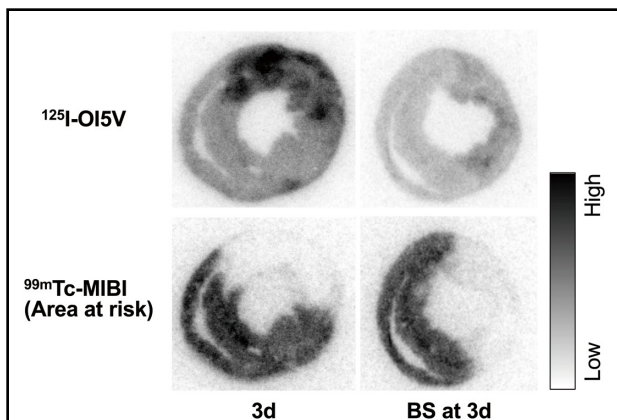
Figure 3. Dual-tracer autoradiographic short-axis images in the blocking study. The blocking study with (+/−)-pentazocine showed a significant reduction of ^{125}I-OI5V concentration at 3 days after ischemia and reperfusion. BS, blocking study; MIBI, hexakis 2-methoxyisobutyl isonitrile; OI5V, 2-[4-(2-iodophenyl)piperidino]cyclopentanol.

Figure 4. Corresponding triple-tracer autoradiographic short-axis images in rats after ischemia and reperfusion (^{125}I-OI5V, ^{99m}Tc-MIBI, and ^{201}Tl). ^{99m}Tc-MIBI and ^{201}Tl images demonstrate ischemic area (area at risk) and the hypoperfusion area, respectively. Intense ^{125}I-OI5V uptake was observed predominantly in the non-salvaged ischemic myocardium, but mild uptake was also observed in the salvaged area at 7 days after ischemia and reperfusion. MIBI, hexakis 2-methoxyisobutyl isonitrile; OI5V, 2-[4-(2-iodophenyl)piperidino]cyclopentanol.

Immunohistochemical Staining

Tissues were fixed in 4% buffered formalin and processed for paraffin embedding. Left ventricular short axial sections (5-μm thickness) were cut from the middle area. Short-axis heart sections were stained with anti-σ1R antibody (ab151288, abcam) using a direct immunoperoxidase technique (ab64261, abcam). Counterstaining was performed to visualize nuclei and tissue architecture with hematoxylin. Hematoxylin-eosin staining was performed by using standard procedures. The site and distribution of the expression of σ1R were examined by immunofluorescence microscopy (400×).

Post-Mortem Tissue Counting

In the model of 30 min ischemia and reperfusion and sham-operated rats, ^{125}I-OI5V (0.25 MBq) was injected through a tail vein catheter at 3 days after 30 min ischemia and reperfusion (n=5 for each group). The rats were euthanized at 30 min after tracer administration. Then, organs of interest (blood, lung, heart, liver, kidney, spleen, pancreas, stomach, intestine, muscle, bone) were collected, weighed, and counted for radioactivity in an automated gamma counter. For the ischemia and reperfusion rats, the heart was divided into ischemic and non-ischemic areas. In the sham-operated rats, the heart was arbitrarily divided into ischemic (LCA territory) and non-ischemic area (lower septal area). Tissue radioactivity concentrations were measured and expressed as injected dose per gram (%ID/g) of each organ sample.

In Vivo SPECT/CT Imaging

Three rats were imaged using a small animal SPECT system (versatile emission computed tomography, VECTor) equipped with a general-purpose rat collimator (MI-labs, The Netherlands). At 3 days after ischemia and reperfusion, the rats received 74 MBq of ^{123}I-OI5V via a tail vein 30 min before SPECT. The animals were scanned for 10 min under 1–2% isoflurane anesthesia. Then, 185 MBq of ^{99m}Tc-MIBI was injected via a tail vein 60 min before SPECT, in order to evaluate the decreased perfusion area. Again, 10 min gated SPECT imaging was performed.
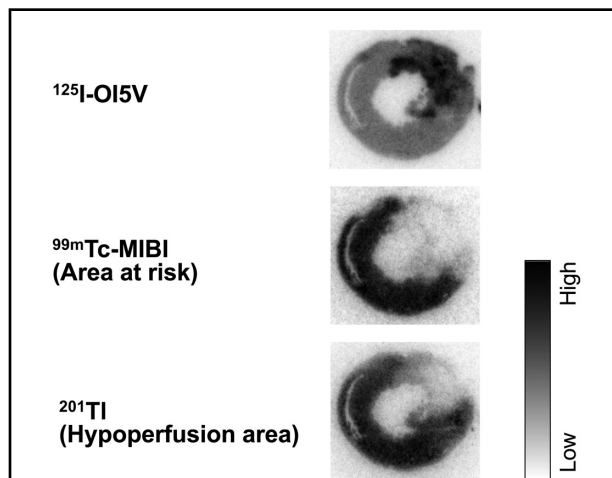
SPECT Data Reconstruction

Data were acquired in list mode and photopeak windows were set after the acquisition. Triple energy window scatter correction was employed (a 20% photopeak window centered at 159 and 140 keV for ^{123}I and ^{99m}Tc, respectively, abutting the upper and lower scatter windows 4.5% in width).

Data were reconstructed using pixel-based order-subsets expectation maximization, without correction for attenuation on CT, in 13 subsets and 6 iterations.[13,14] The voxel size was 0.8×0.8×0.8 mm.

The obtained SPECT images were analyzed using an image-processing application (AMIDE Imaging software, version 1.0.1).[15] Three-dimensional regions of interest were manually drawn over the area of decreased perfusion and normally perfused area in serial 15 slices. We set the ROI at the mid-ventricular level to avoid the physiological liver uptake of ^{123}I-OI5V. The mean concentration of radioactivity within the regions of interest was calculated. The count ratio was calculated as the ratio of the average counts per voxel in the area of decreased perfusion divided by that in the normally perfused area.

Statistical Analysis

For all statistical analyses, we used a statistical software package (JMP® SAS Institute, Inc., Cary, NC, USA.). All results were expressed as the mean value±standard deviation (SD). Group comparisons were performed using analysis of variance. Statistical significance was determined using analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test for comparing the ^{125}I-OI5V uptake ratio and the ratio of ^{125}I-OI5V uptake area in the ischemic area in the dual-tracer autoradiography. The paired-t analysis was performed to compare the ^{125}I-OI5V uptake ratio in the area of non-salvage and the salvage area in the triple-tracer autoradiography. The t-test was performed to compare the tracer signals between the model

Table. Postmortem Tissue Counting in Rats at 30 min After Injection of ^{125}I-OI5V

Organ	Uptake (%ID/g)†		P value
	Sham-operated rats	Rats with ischemia and reperfusion	
Blood	0.08±0.01	0.07±0.01	NS
Lung	3.53±0.80	3.74±0.80	NS
Heart			
Non-ischemic area	0.53±0.06	0.62±0.03	NS
Ischemic area (operated area)	0.53±0.03	1.76±0.37	<0.0001
Liver	1.38±0.15	1.18±0.15	NS
Kidney	1.96±0.14	2.10±0.14	NS
Spleen	1.62±0.11	1.67±0.11	NS
Pancreas	2.66±0.39	2.44±0.32	NS
Stomach	1.03±0.25	0.67±0.25	NS
Intestine	2.18±0.66	2.02±0.66	NS
Muscle	0.10±0.01	0.12±0.01	0.03
Bone	0.33±0.01	0.36±0.01	NS

†Uptake values are presented as mean percentage of the injection dose per gram of tissue (%ID/g)±standard deviation.
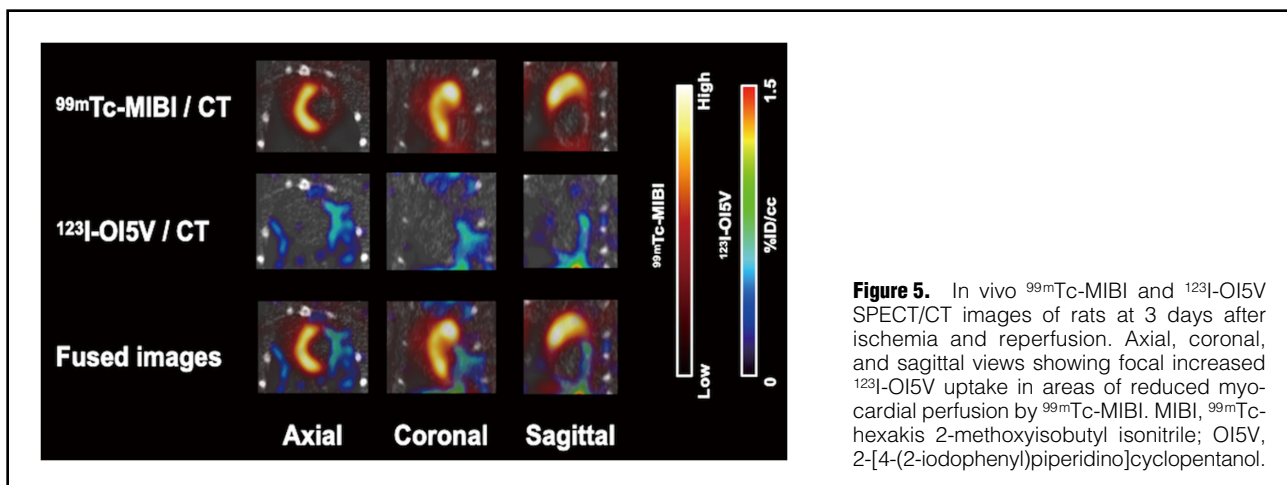NS, not significant.

Figure 5. In vivo ^{99m}Tc-MIBI and ^{123}I-OI5V SPECT/CT images of rats at 3 days after ischemia and reperfusion. Axial, coronal, and sagittal views showing focal increased ^{123}I-OI5V uptake in areas of reduced myocardial perfusion by ^{99m}Tc-MIBI. MIBI, ^{99m}Tc-hexakis 2-methoxyisobutyl isonitrile; OI5V, 2-[4-(2-iodophenyl)piperidino]cyclopentanol.

and sham-operated rats in the post mortem tissue counting study and the mean ^{123}I-OI5V uptake ratio of the decreased perfusion area to the preserved perfusion area in the in vivo SPECT imaging. A value of P<0.05 was considered statistically significant.

Results

Dual-Tracer Autoradiography
Autoradiography showed homogeneous ^{125}I-OI5V uptake in the myocardium in healthy rat hearts (n=2). Perfusion defects and focal ^{125}I-OI5V uptake were not observed in the sham-operated rats (n=4).

The area of increased ^{125}I-OI5V uptake was localized in the area of ischemia (area at risk), as shown by ^{99m}Tc-MIBI uptake defect. No significant elevation of ^{125}I-OI5V uptake was found in the non-ischemic area, identified by ^{99m}Tc-MIBI. At 1 day after ischemia and reperfusion, a ^{125}I-OI5V image demonstrated reduced tracer activity in the area at risk compared to the normally perfused area; however, ^{125}I-OI5V uptake increased significantly and

peaked at 3 days after ischemia and reperfusion followed by gradual reduction until 28 days (**Figure 2A**). The ^{125}I-OI5V uptake ratios of ischemic area to normal perfusion LV area were 0.90±0.12 at 1 day, 1.89±0.19 at 3 days, 1.52±0.17 at 7 days, 1.34±0.13 at 14 days, and 1.16±0.14 at 28 days (**Figure 2B**). The ratio of ^{125}I-OI5V uptake area to the ischemic area was 0±0 at 1 day, 0.54±0.09 at 3 days, 0.52±0.04 at 7 days, 0.44±0.10 at 14 days, and 0.44±0.06 at 28 days. The ratio of ^{125}I-OI5V uptake area in the ischemic area did not change from 3 to 28 days. The blocking study with (+/−)-pentazocine showed a marked reduction of ^{125}I-OI5V uptake at 3 days from 1.89±0.20 to 1.13±0.18 (P<0.05, **Figure 3**).

Immunohistochemical Analysis
Significant immunohistochemical staining showed an increased σ1R on the myocardium in the ischemic area (area at risk) at 3 days after ischemia and reperfusion (**Figure 2C**).

Triple-Tracer Autoradiography
^{125}I-OI5V uptake was observed in both the salvaged and

non-salvaged areas of the ischemic area at 7 days after ischemia and reperfusion (**Figure 4**), whereas no significant regional elevation of ^{125}I-OI5V uptake was found in the non-ischemic area. The salvaged area was observed in 67±13% of the ischemic area (area at risk). The ^{125}I-OI5V uptake ratio of the non-salvaged area was higher compared to that of the salvaged area (2.11±0.30 vs. 1.46±0.14, P<0.01).

Systemic ^{125}I-OI5V Distribution

In vivo biodistribution was evaluated at 30min after ^{125}I-OI5V administration. The lung, kidney, pancreas, and intestine exhibited high tracer concentration, and tracer uptake in the blood remained very low. Tracer uptake in the cardiac ischemic area was higher in rats with an ischemia and reperfusion model compared with that of sham-operated rats. In the non-ischemic area, tracer uptake tended to be higher after the ischemia and reperfusion. The results of the post mortem tissue counting study are summarized in **Table**.

In Vivo SPECT/CT Imaging

Focally increased ^{123}I-OI5V uptake in the myocardium was clearly observed in the hypoperfused area showing reduced ^{99m}Tc-MIBI uptake at 3 days after ischemia and reperfusion (**Figure 5**). The mean count ratio of the decreased perfusion area to the preserved perfusion area was 2.0±0.2 at 3 days after ischemia and reperfusion (n=3).

Discussion

The present study demonstrated the feasibility of imaging with OI5V targeted at the σ1R, and documented, for the first time, spatiotemporal profiling of σ1R expression after myocardial ischemia and reperfusion. We surmise that in the model of ischemia and reperfusion, ischemia is the major factor determining the degree of σ1R expression, in that the ^{125}I-OI5V uptake was higher in the non-salvaged area than the salvaged area. Furthermore, ^{125}I-OI5V imaging revealed that the σ1R expression changes dynamically after ischemia and reperfusion.

σ1R has been found in cardiomyocytes, where it was documented to play a role in contractility and beating frequency.[16,17] σ1R ligands, agonists or antagonists have been applied to observe the downstream effects to elucidate the mechanism of its action in the heart. σ1Rs as chaperones have been shown to be involved in ER stress, calcium handling, voltage-gated ion channeling, cellular toxicity/apoptosis, and hypertrophy.[5,18] Here, the modulation of the σ1R signal with ligands was implicated in cardioprotection. However, the possible involvement of σ1R expression in the pathophysiological process has not yet been examined in ischemic heart disease, though ischemia and reperfusion injury trigger calcium imbalance, ER stress, and apoptosis.[19] The current study confirms that σ1R overexpression occurs shortly after ischemia and reperfusion.

σ1R has been found to act as a pluripotent modulator in various conditions, including ischemia.[18] Our imaging study is in line with prior studies showing the presence of σ1R under ischemia in the brain of mice with permanent middle cerebral artery occlusion,[20] in which σ1R expression increased in the peri-infarcted area at 1 week after infarction. However, its chronological changes and distribution in the brain are unknown. We here clarified the spatiotemporal σ1R expression in the damaged heart after ischemia

and reperfusion.

In the autoradiographic study, significant σ1R expression was observed in the myocardium within the ischemic area, with the expression peaking at 3 days, followed by a gradual reduction to 28 days. At 3 days after ischemia and reperfusion, we also confirmed the presence of σ1R in the ischemic area by immunostaining, a high tracer signal in the ischemic area by postmortem tissue counting, and the significant reduction of the tracer signal by a blocking study. σ1R is an ER protein that primarily resides specifically in the mitochondria-associated ER membrane; however, it can be translocated to the plasma membrane, mitochondria, nucleus envelope and cytosol, and functions as the chaperone during various increasing cellular stresses.[5,6,17] Based on the presence of σ1R under stress, the ^{125}I-OI5V uptake demonstrated by autoradiography may correspond to the upregulated σ1R in the cardiomyocytes after ischemia and reperfusion.

The current study demonstrated the inhomogeneous expression of ^{125}I-OI5V in the ischemic area regardless of the salvage status. The triple autoradiography showed higher ^{125}I-OI5V uptake in the non-salvage area compared with the salvaged area in the ischemic region. The discordant ^{125}I-OI5V uptake in the decreased perfusion area may reflect the presence of residual myocardium jeopardized by more severe ischemia. In response to severe ischemia, cardiomyocytes increase the expression of various chaperones and activities as a salvage mechanism to manage emergency conditions.[21] If a more intense expression of σ1R reflects more severe ischemic damage in viable myocardium, σ1R imaging could embody the concept of the stress imaging, reflecting prior damage or current stress.

ER stress plays an essential role in developing and progressing cardiovascular disease.[22,23] We speculated that σ1R imaging also might be beneficial in non-ischemic heart disease such as myocardial hypertrophy due to TAC; the σ1R was gradually downregulated along with ventricular dysfunction.[10,18,24] In such a condition, the tracer uptake in the heart might decrease. For the evaluation of σ1R downregulation, absolute quantification of the tracer uptake should be important.

The OI5V was radiolabeled with radioiodine so that the radioactivity would reveal the in vivo dynamics of σ1R expression. The average ^{123}I-OI5V count ratio in in vivo SPECT/CT is similar to the average ratio of the ischemic area to normal LV area obtained from the ^{125}I-OI5V autoradiography study. For future clinical application as a SPECT, ^{123}I-OI5V would be beneficial to monitor and clarify how σ1R is involved pathophysiologically in the period of post myocardial infarction induced by ischemia and reperfusion. However, in the interpretation of inferior myocardial uptake, we must take into consideration the effect of relatively high liver uptake.

Conclusions

The present study demonstrated the feasibility of targeted imaging for serial assessment of regional expression of σ1R after myocardial ischemia and reperfusion. It also demonstrated the expression pattern in relation to myocardium perfusion status after 30 min of ischemia and reperfusion. The data from this study implied that, by allowing visualization of σ1R expression, radiolabeled OI5V imaging may be a non-invasive way to monitor myocardial injury and its ER stress after ischemia and reperfusion.

Disclosures

The authors declare that they have no conflict of interests.

References

1. Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE. The effects of morphine- and nalorphine- like drugs in the non-dependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther* 1976; **197:** 517–532.
2. Quirion R, Bowen WD, Itzhak Y, Junien JL, Musacchio JM, Rothman RB, et al. A proposal for the classification of sigma binding sites. *Trends Pharmacol Sci* 1992; **13:** 85–86.
3. Hellewell SB, Bruce A, Feinstein G, Orringer J, Williams W, Bowen WD. Rat liver and kidney contain high densities of sigma 1 and sigma 2 receptors: Characterization by ligand binding and photoaffinity labeling. *Eur J Pharmacol* 1994; **268:** 9–18.
4. Hanner M, Moebius FF, Flandorfer A, Knaus HG, Striessnig J, Kempner E, et al. Purification, molecular cloning, and expression of the mammalian sigma1-binding site. *Proc Natl Acad Sci USA* 1996; **93:** 8072–8077.
5. Stracina T, Novakova M. Cardiac sigma receptors: An update. *Physiol Res* 2018; **67:** S561–S576.
6. Su TP, Su TC, Nakamura Y, Tsai SY. The Sigma-1 receptor as a pluripotent modulator in living systems. *Trends Pharmacol Sci* 2016; **37:** 262–278.
7. Penke B, Fulop L, Szucs M, Frecska E. The role of Sigma-1 receptor, an intracellular chaperone in neurodegenerative diseases. *Curr Neuropharmacol* 2018; **16:** 97–116.
8. Vilner BJ, John CS, Bowen WD. Sigma-1 and sigma-2 receptors are expressed in a wide variety of human and rodent tumor cell lines. *Cancer Res* 1995; **55:** 408–413.
9. Abdullah CS, Alam S, Aishwarya R, Miriyala S, Panchatcharam M, Bhuiyan MAN, et al. Cardiac dysfunction in the Sigma 1 receptor knockout mouse associated with impaired mitochondrial dynamics and bioenergetics. *J Am Heart Assoc* 2018; **7:** e009775.
10. Tagashira H, Bhuiyan S, Shioda N, Hasegawa H, Kanai H, Fukunaga K. Sigma1-receptor stimulation with fluvoxamine ameliorates transverse aortic constriction-induced myocardial hypertrophy and dysfunction in mice. *Am J Physiol Heart Circ Physiol* 2010; **299:** H1535–H1545.
11. Shigeno T, Kozaka T, Kitamura Y, Ogawa K, Taki J, Kinuya S, et al. In vitro and in vivo evaluation of [(125/123)I]-2-[4-(2-iodo-phenyl)piperidino]cyclopentanol([(125/123)I]-OI5V) as a potential sigma-1 receptor ligand for SPECT. *Ann Nucl Med* 2021; **35:** 167–175.
12. Sahul ZH, Mukherjee R, Song J, McAteer J, Stroud RE, Dione DP, et al. Targeted imaging of the spatial and temporal variation of matrix metalloproteinase activity in a porcine model of postinfarct remodeling: Relationship to myocardial dysfunction. *Circ Cardiovasc Imaging* 2011; **4:** 381–391.
13. Branderhorst W, Vastenhouw B, Beekman FJ. Pixel-based subsets for rapid multi-pinhole SPECT reconstruction. *Phys Med Biol* 2010; **55:** 2023–2034.
14. Miwa K, Inubushi M, Takeuchi Y, Katafuchi T, Koizumi M, Saga T, et al. Performance characteristics of a novel clustered multi-pinhole technology for simultaneous high-resolution SPECT/PET. *Ann Nucl Med* 2015; **29:** 460–466.
15. Loening AM, Gambhir SS. AMIDE: A free software tool for multimodality medical image analysis. *Mol Imaging* 2003; **2:** 131–137.
16. Ela C, Barg J, Vogel Z, Hasin Y, Eilam Y. Sigma receptor ligands modulate contractility, Ca++ influx and beating rate in cultured cardiac myocytes. *J Pharmacol Exp Ther* 1994; **269:** 1300–1309.
17. Novakova M, Ela C, Barg J, Vogel Z, Hasin Y, Eilam Y. Inotropic action of sigma receptor ligands in isolated cardiac myocytes from adult rats. *Eur J Pharmacol* 1995; **286:** 19–30.
18. Lewis R, Li J, McCormick PJ, Huang CLH, Jeevaratnam K. Is the sigma-1 receptor a potential pharmacological target for cardiac pathologies?: A systematic review. *Int J Cardiol Heart Vasc* 2019; **26:** 100449.
19. Zhou H, Wang S, Hu S, Chen Y, Ren J. ER-Mitochondria microdomains in cardiac ischemia-reperfusion injury: A fresh perspective. *Front Physiol* 2018; **9:** 755.
20. Ruscher K, Shamloo M, Rickhag M, Ladunga I, Soriano L, Gisselsson L, et al. The sigma-1 receptor enhances brain plasticity and functional recovery after experimental stroke. *Brain* 2011; **134:** 732–746.
21. Tarone G, Brancaccio M. Keep your heart in shape: Molecular chaperone networks for treating heart disease. *Cardiovasc Res* 2014; **102:** 346–361.
22. Wang S, Binder P, Fang Q, Wang Z, Xiao W, Liu W, et al. Endoplasmic reticulum stress in the heart: Insights into mechanisms and drug targets. *Br J Pharmacol* 2018; **175:** 1293–1304.
23. Liu MQ, Chen Z, Chen LX. Endoplasmic reticulum stress: A novel mechanism and therapeutic target for cardiovascular diseases. *Acta Pharmacol Sin* 2016; **37:** 425–443.
24. Bhuiyan MS, Tagashira H, Shioda N, Fukunaga K. Targeting sigma-1 receptor with fluvoxamine ameliorates pressure-overload-induced hypertrophy and dysfunctions. *Expert Opin Ther Targets* 2010; **14:** 1009–1022.