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Copper-62 ATSM as a hypoxic tissue tracer in myocardial ischemia

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Copper-62 labeled diacetyl-bis(N^4-methylthiosemicarbazone) (^{62}Cu-ATSM) has been proposed as a generator produced positron-emitting tracer for hypoxic tissue imaging. To clarify the usefulness of ^{62}Cu-ATSM for myocardial ischemia, ^{62}Cu-ATSM PET was performed in 7 patients with coronary artery disease. Increased myocardial uptake of ^{62}Cu-ATSM was observed (myocardium/blood ratio: 3.09) in one patient with unstable angina, who had increased ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake under the fasting condition. The other 6 patients, who were clinically stable, did not have increased ^{62}Cu-ATSM uptake, although abnormal ^{18}F-FDG uptake was seen in 4 patients. This preliminary study suggests that ^{62}Cu-ATSM is a promising PET tracer for hypoxic imaging in acute ischemia.

Key words: copper-62 ATSM, hypoxia, coronary artery disease, fluorine-18 FDG, PET

INTRODUCTION

VISUALIZATION OF HYPOXIC TISSUE is important for the evaluation of ischemic change in the brain and heart, and for the characterization of tumors. Nitroimidazole compounds are of great interest because of their selective accumulation in hypoxic tumors[1] as well as ischemic tissues.[2] Various groups have attempted to design nitroimidazole-based drugs labeled with ^{18}F,[3] ^{123}I,[4] ^{131}I,[5] or ^{99m}Tc[6] for imaging hypoxia but these tracers had low target accumulation due to slow blood clearance and low membrane permeability.[7]

^{62}Cu labeled diacetyl-bis(N^4-methylthiosemicarbazone) (^{62}Cu-ATSM) has been proposed as a generator-based positron-emitting tracer for imaging hypoxia.[8] ^{62}Cu-PTSM, developed as a perfusion tracer, is easily reduced by the electron transport system in mitochondria, which can explain its retention.[9] On the other hand, ^{62}Cu-ATSM, an analogue of ^{62}Cu-PTSM, cannot be reduced by normal mitochondria due to its low redox potential. Therefore, ^{62}Cu-ATSM is not retained in the brain and heart, although it has high membrane permeability. The hypoxia-selective retention of ^{62}Cu-ATSM requires an abnormally high NADH concentration caused by oxygen depletion, and also intact mitochondria. It was reported that ^{62}Cu-ATSM has shown sign of high myocardial accumulation in a perfused rat heart model under hypoxic conditions,[8] as well as in an *in vivo* rat model immediately after LAD occlusion.[10] It was also reported that as the blood flow decreased the ^{62}Cu-ATSM accumulation increased; but at flow rates that were approximately 40% of normal, the uptake began to decrease.[10]

To clarify the usefulness of ^{62}Cu-ATSM in myocardial ischemia, ^{62}Cu-ATSM PET was performed in 7 patients with coronary artery disease.

MATERIALS AND METHODS

Copper-62 was obtained with a ^{62}Zn/^{62}Cu generator system from [^{62}Zn]$ZnCl_2$ solution.[11] Cu-ATSM was synthesized according to the method of Gingas et al.,[12] and confirmed by elemental analysis and mass spectrometry. ^{62}Cu-ATSM was prepared as follows[8]: Briefly, 4 m*l* of

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Table 1 Clinical data of 7 patients

Patient no.	Age (yr)	Sex	Clinical diagnosis	Interval* (days)	Stenosis on CAG (%)		
					RCA	LAD	LCX
1	71	F	Inferior MI	721	100		
2	80	F	Anterior MI	30		99	
3	72	M	Anterior MI	1656		99	99
4	63	M	Anterior MI	878		90	
5	75	M	Lateral MI	48			100
6	64	M	Anterior MI	51		99	
7	65	F	Unstable angina				99

*Interval from the most recent onset of infarction to time of the study.
CAG = coronary angiography; RCA = right coronary artery; LAD = left anterior descending artery; LCX = left circumflex artery; MI = myocardial infarction

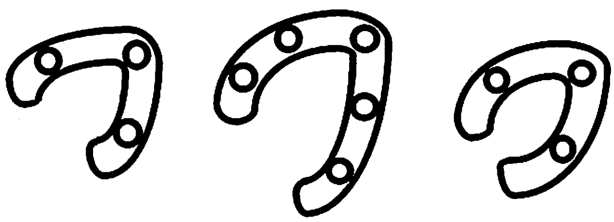

Fig. 1 Three transaxial slices illustrate 11 regions of interest definition.

^{62}Cu-glycine (non-carrier added ^{62}Cu) solution obtained from the generator was mixed with 0.2 m*l* of ATSM solution (0.4 mM in dimethyl sulfoxide). The radiochemical purity of ^{62}Cu-ATSM was confirmed by HPLC in combination with authentic Cu-ATSM.

The study involved 7 patients with coronary artery disease (4 male, 3 female, age range 63–80 yr). Six patients had prior myocardial infarction and 1 patient was diagnosed with unstable angina because she had a few angina pectoris attacks per day, which were refractory to medical treatment (Table 1). The study was approved by the Ethical Committee of Fukui Medical University and written informed consent was obtained from all the subjects before the PET study.

PET was performed with a high-resolution, whole-body PET scanner with an 18-ring detector arrangement (Advance, GE Medical Systems, Milwaukee, WI, USA). The physical characteristics of this scanner have been described in detail by DeGrado et al.[13] Briefly, the system permits the simultaneous acquisition of 35 transaxial images with an interslice spacing of 4.25 mm. Both axial and transaxial resolution are 4.2 mm, allowing multidirectional reconstruction of the images without loss of resolution. The FOV and the pixel size of the reconstructed images were 256 and 2 mm, respectively. A 10-min transmission scan was acquired with a ^{68}Ge/^{68}Ga source for attenuation correction, followed by intravenous injection of 370 to 740 MBq of ^{62}Cu-ATSM over 30 sec. Static scan was performed for 10 min (10–20 min post injection). In order to compare ^{62}Cu-ATSM images with blood flow and glucose metabolism of the myocardium, nitrogen-13 ammonia ($^{13}NH_3$) and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET was performed after overnight fast within a week. Static PET images were acquired over 10 min beginning 10 min after an intravenous injection of $^{13}NH_3$ (740 MBq). ^{18}F-FDG (370 MBq) was then injected intravenously, and static images acquired over 10 min beginning 60 min after the injection.

Eleven circular regions of interest (ROI) were placed on the ^{62}Cu-ATSM PET images of the left myocardium (Fig. 1), and left atrium (0.6 cm^2 and 1.8 cm^2, respectively, in area). The myocardial activity of ^{62}Cu-ATSM was normalized by the arterial blood activity, which was derived from the ROI placed over the left atrium of the PET image (uptake ratio). The upper limit of ^{62}Cu-ATSM was defined as 2.6, which was the mean + 2SD in the 4 normal subjects in our previous report.[14]

The standardized uptake value (SUV) images of ^{18}F-FDG were calculated with the following formula:

$$SUV = \text{radioactivity concentration (Bq/m}l\text{)}/\{\text{injected dose (Bq)/body weight (g)}\}$$

The same ROIs as used in the ^{62}Cu-ATSM PET images of the left ventricle were placed on both the $^{13}NH_3$ and ^{18}F-FDG images. In the study of myocardial perfusion, the myocardial uptake percent was calculated after normalization to each peak value in the study. The uptake ratio of ^{62}Cu-ATSM was compared with myocardial blood flow (%) and glucose metabolism (SUV) under the fasting condition. The normal range of ^{18}F-FDG uptake was defined as < 3.5 mg/m*l* (SUV), which was previously reported.[15]

RESULTS

Increased ^{62}Cu-ATSM uptake was observed (3.09) in one segment of a patient with unstable angina, who had

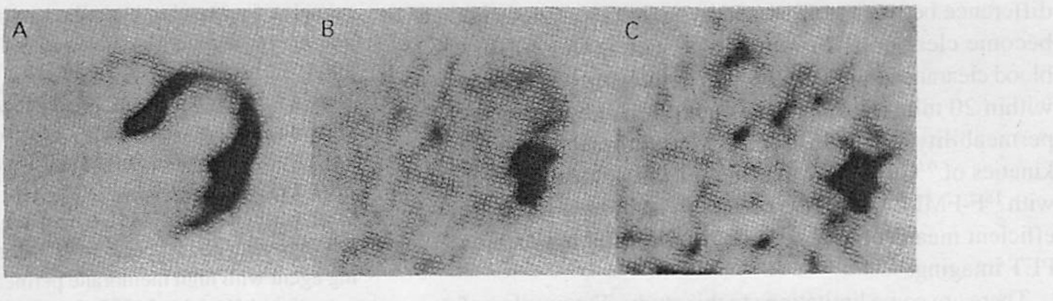

Fig. 2 A 65-yr-old woman with unstable angina. She had frequent attacks, which were refractory to medical treatment. The transaxial images of $^{13}NH_3$ (A), ^{18}F-FDG (B), ^{62}Cu-ATSM (C) are shown. Coronary angiography showed 99% stenosis in circumflex artery. Increased both ^{18}F-FDG and ^{62}Cu-ATSM accumulation in areas with moderately reduced myocardial blood flow was observed in the lateral wall.

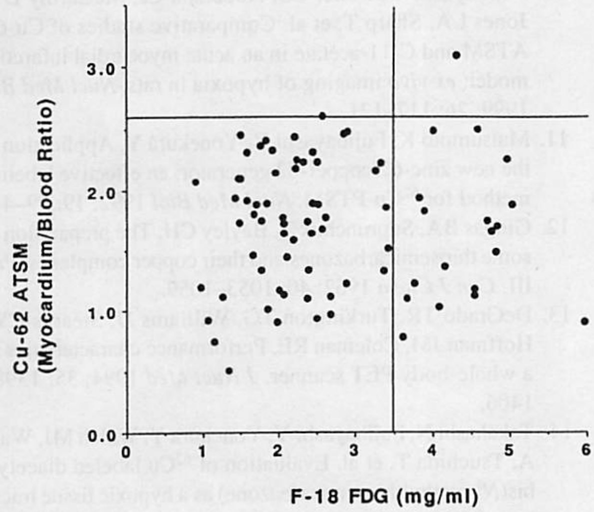

Fig. 3 The ^{18}F-FDG uptake (SUV) was plotted against its ^{62}Cu-ATSM uptake ratio for myocardial segments in each patient with ischemic heart disease. No correlation was observed between ^{62}Cu-ATSM uptake ratio and ^{18}F-FDG uptake.

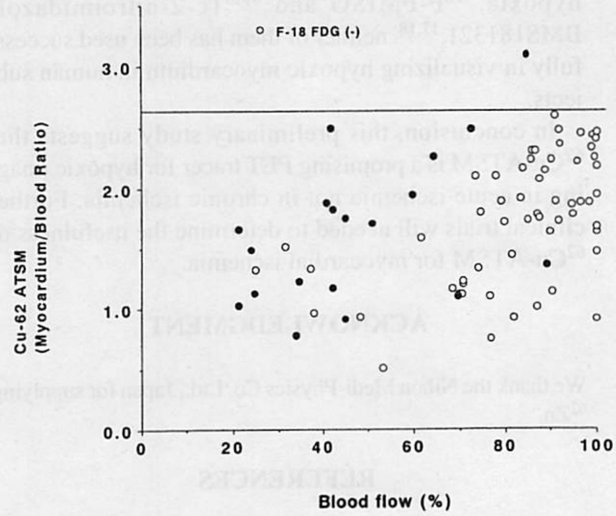

Fig. 4 The relative blood flow was plotted against its ^{62}Cu-ATSM uptake ratio for myocardial segments in each patient with ischemic heart disease. No correlation was found between ^{62}Cu-ATSM uptake ratio and relative blood flow.

increased ^{18}F-FDG uptake under the fasting condition (Fig. 2). The other 6 patients, who were clinically stable, did not have increased ^{62}Cu-ATSM uptake, although abnormal ^{18}F-FDG uptake was seen in 18 segments, in 4 of the patients (Fig. 3). Enhanced uptake of ^{62}Cu-ATSM was not seen in the moderately low flow area (Fig. 4).

DISCUSSION

Although increased glucose metabolism was seen in 5 of 7 patients, only 1 patient with unstable angina had enhanced myocardial uptake of ^{62}Cu-ATSM. In addition, no increase in ^{62}Cu-ATSM uptake was observed in the moderately low flow area, which was apparent in the rat acute ischemia model.[8,10] As the impairment of contractile function reduces the oxygen demand of hypoperfused myocardium in hibernating myocardium,[16] lowered oxygen demand may reduce electron transport in the mitochondria. Accordingly, retention of ^{62}Cu-ATSM was not increased in the chronically ischemic myocardium. After long duration of ischemia, the myocardium is irreversibly injured, and the leakage of intramitochondrial enzymes occurs, which is necessary for ^{62}Cu-ATSM retention. Accordingly, ^{62}Cu-ATSM is considered to be a PET tracer for hypoxic imaging in acute ischemia, highly sensitive to the intactness of mitochondria. ^{18}F-FDG uptake might indicate abnormality of myocardial metabolism, but not intactness of the energy production system.

Although we have not compared ^{62}Cu-ATSM and ^{18}F-fluoromisonidazole (^{18}F-FMISO) in this study, ^{62}Cu-ATSM has two advantages. First, ^{62}Cu can be obtained by a generator system from ^{62}Zn, which has a 9 hr half-life and could be delivered for long distances. The second advantage is that the faster myocardial uptake of ^{62}Cu-

ATSM than ^{18}F-FMISO allows more rapid imaging of ischemic but viable myocardium. In ^{18}F-FMISO PET, the difference between normal and hypoxic tissues does not become clear until 2 hours post injection due to slow blood clearance.[17] ^{62}Cu-ATSM PET imaging can be done within 20 min after injection due to its high membrane permeability.[10] Therefore, the more efficient washout kinetics of ^{62}Cu-ATSM in acute ischemia in comparison with ^{18}F-FMISO offers the possibility of a faster and more efficient means of evaluating of myocardial hypoxia by PET imaging.

There are some limitations to this study. The number of patients was small and the results are preliminary but enhanced uptake of ^{62}Cu-ATSM was observed in a patient with unstable angina, and imaging was completed only 20 minutes after the injection. Although experimental studies have already supported the possibility of identifying myocardial hypoxia with the other agents for imaging hypoxia, ^{18}F-FMISO and ^{99m}Tc-2-nitroimidazole BMS181321,[17,18] neither of them has been used successfully in visualizing hypoxic myocardium in human subjects.

In conclusion, this preliminary study suggests that ^{62}Cu-ATSM is a promising PET tracer for hypoxic imaging in acute ischemia not in chronic ischemia. Further clinical trials will needed to determine the usefulness of ^{62}Cu-ATSM for myocardial ischemia.

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