Assessment of Regional Myocardial and Renal Blood Flow With Copper-PTSM and Positron Emission Tomography

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We recently demonstrated in isolated, perfused hearts that radiolabeled pyruvaldehyde bis(N4-methylthiosemicarbazonato)copper(II) (Cu-PTSM) is well extracted throughout a range of conditions including ischemia, hypoxia, and hyperemia. Once extracted, binding of radioactivity by the isolated heart was essentially irreversible, giving this tracer microspherelike qualities. Because Cu-PTSM can be readily prepared with the generator-produced positron-emitting copper 62 and other gamma- or positron-emitting copper radionuclides, we evaluated its usefulness for measuring regional myocardial and renal blood flow in vivo in intact dogs at rest, after ischemia, or after coronary hyperemia was induced by intravenous administration of dipyridamole. After intravenous administration of radiolabeled Cu-PTSM, the tracer cleared rapidly from the blood. Myocardial uptake of single photon-emitting 62Cu-labeled Cu-PTSM was measured directly in myocardial samples 15 minutes after tracer administration, and it increased proportionally with blood flow throughout the flow range (estimated concomitantly with radiolabeled microspheres) of 0.0–6.0 ml/g/min (n=340 samples from 17 dogs, r=0.99, Ycopper radioactivity=85Xmicrosphere flow−7x^{2}+17). Renal uptake of radiolabeled Cu-PTSM was also proportional to blood flow. Positron emission tomography was performed in four intact dogs after intravenous administration of 64Cu-labeled Cu-PTSM (19% positron decay, t1/2=12.8 hours). High-quality images of heart and kidney were obtained. Accordingly, radiolabeled Cu-PTSM should be a useful, generator-produced tracer for estimating regional myocardial and renal blood flow with positron emission tomography. (Circulation 1990;82:990–997)

Estimation of myocardial blood flow constitutes the cornerstone of the diagnosis and assessment of efficacy of treatment of coronary artery disease. We and others have previously demonstrated the feasibility of using positron emission tomography (PET) to accurately quantify regional myocardial blood flow noninvasively with cyclotron-produced oxygen 15–labeled water.1–5 Tomography with cyclotron-produced nitrogen 13–labeled ammonia has also been used extensively for qualitative and quantitative estimates of blood flow.6,7 However, many cardiac PET centers are being established without cyclotrons.

Currently, rubidium 82–labeled chloride is the only generator-produced positron-emitting radiotracer available for estimating myocardial perfusion with PET. Although 82Rb has proven clinically useful for determining relative myocardial blood flow,8–11 the extraction and retention of rubidium are dependent on, and nonlinearly proportional to flow,12–14 and are independently influenced by the metabolic status of the heart,13–16 making absolute quantification of myocardial blood flow difficult. In addition, because of the extremely short half-life of 82Rb (t1/2=75 seconds), imaging must be completed within a relatively short period of time after tracer administration, which can limit image quality dependent on
the dose of tracer administered, the tracer infusion protocol used, and the efficiency of tomography. For these reasons, among others, there is interest in alternative generator-produced tracers for estimating perfusion with PET.

We recently demonstrated in isolated, perfused hearts that the lipophilic compound pyruvaldehyde bis(N4-methylthiosemicarbazonato)copper(II) (Cu-PTSM) has a first-pass extraction fraction of 45±7% in the heart and that this extraction fraction was only modestly affected by changes in blood flow over the flow ranges studied (between 10% of normal and two times normal in a preparation perfused at physiological flow rates) or by hypoxia. Once extracted, the tracer was essentially irreversibly bound with a biological half-time in the heart of more than 60 hours. Those characteristics indicated that this compound might be a promising tracer for assessing myocardial perfusion.

Green et al. have developed a simple, rapid preparation scheme (synthesis time of 5 minutes) for Cu-PTSM that may be adapted for use with any of the five available copper isotopes: copper 67 (t1/2=59 hours with gamma photons at 91 [23%] and 184 keV [40%]), potentially amenable to conventional single-photon scintigraphy; copper 64 (19% positron decay, t1/2=12.8 hours); copper 60 (93% positron decay, t1/2=24 minutes); copper 61 (62% positron decay, t1/2=3.3 hours); and the generator-produced positron-emitting copper 62 isotope (100% positron decay, t1/2=9.7 minutes). Accordingly, we performed this preliminary study in intact dogs to evaluate, over a range of myocardial blood flows, the correlation of retained copper radioactivity with blood flow (as estimated concomitantly with retained radiolabeled microspheres). In addition, because Cu-PTSM is extracted by many organs by virtue of its lipophilicity (the octanol:water partition coefficient is 100:118,19), we evaluated the ability of this agent in assessing renal blood flow.

Methods

Experimental Protocol

Seventeen closed-chest dogs weighing between 18 and 25 kg were premedicated with morphine sulfate (1 mg/kg s.c.) before induction of anesthesia with sodium thiopental (12.5 mg/kg i.v.) and α-chloralose (72 mg/kg i.v.). The left femoral artery and vein were cannulated for measuring arterial pressure and for administering drugs. A left atrial catheter was positioned under fluoroscopic guidance in the right femoral artery for administering radiolabeled microspheres.

Three groups of dogs were studied to evaluate the myocardial kinetics of Cu-PTSM over a wide range of blood flow. Four dogs without any further mechanical interventions were studied under control conditions (one dog) or with β-receptor stimulation with 15 μg/kg/min dobutamine (three dogs), a dose selected to raise myocardial and kidney perfusion. To produce regional ischemia, nine animals were subjected to coronary artery thrombosis induced by placement of a copper coil into the left anterior descending coronary artery as described previously. Coronary occlusion was documented angiographically. Coronary artery stenosis was induced in four dogs by the insertion of a Teflon stenosis into the left anterior descending coronary artery as previously described. In these dogs, hyperemia was induced with an intravenous infusion of dipyridamole (0.14 mg/kg/min for 4 minutes), and radiolabeled microspheres and Cu-PTSM were administered 4 minutes after the end of the dipyridamole infusion.

Four dogs (one from the control group, two from the ischemic group, and one from the stenosis-dipyridamole group) were studied with positron emission tomography when we were able to obtain the positron-emitting 64Cu isotope (t1/2=12.8 hours, 19% positron emission). All other dogs (13) were studied after intravenous administration of 0.5–1.5 mCi 67Cu-labeled Cu-PTSM (t1/2=59 hours with gamma photons at 91 and 184 keV). Radiolabeled Cu-PTSM was administered intravenously concomitantly with strontium 85-labeled microspheres (3M Corp., Minneapolis, Minn.) (injected into the left atrium) while arterial blood was withdrawn from the femoral artery at a rate of 10 ml/min with the use of a constant withdrawal pump. In some studies, arterial blood samples for copper activity determination were also obtained serially throughout the study interval. Fifteen minutes after the infusion of tracer, dogs were killed with an overdose of thiopental and saturated potassium chloride. The heart and kidneys were excised. Eight to 12 samples (approximately 1.0 g each) were obtained from both the normal postero-lateral myocardium and from regions distal to the coronary occlusion or stenosis and placed in preweighed vials. Six samples of kidney (approximately 2 g) were obtained and divided into cortex and medulla. Radioactivity levels in tissue and blood samples were assayed in a gamma well-counter. Blood flow according to radiolabeled microspheres was calculated by the standard reference technique.

Positron Emission Tomography

Animals studied with 64Cu-labeled Cu-PTSM were secured in a Plexiglas shell and positioned in the PETT VI tomograph. To independently assess myocardial blood flow before administering 64Cu, we administered 25–30 mCi H215O i.v., and data were collected for 90 seconds. To delineate the blood pool after decay of H215O to background, animals inhaled 40–50 mCi C18O. After allowing 1 minute for radioactivity to clear the lungs, we collected data for 5 minutes. After decay of C18O, 5–8 mCi i.v. 64Cu-labeled Cu-PTSM was administered, and three sequential 5-minute static acquisitions of the heart were taken starting at the time of tracer administration. Typically, 300,000–500,000 counts/slice for each 5-minute scan were obtained. One animal was repositioned for renal imaging after the myocardial study was complete. In this dog, the kidneys were imaged.
with a 5-minute static collection that started approximately 20 minutes after \(^{64}\text{Cu}\)-labeled Cu-PTSM administration. In this study, 250,000 counts/slice/min scan were obtained.

**Synthesis of Radiolabeled Cu-PTSM**

\(^{64}\text{Cu}\) was obtained as copper(II) in a 2N HCl solution with high-specific activity (approximately \(10^3\) Ci/mol) from Los Alamos or Brookhaven National Laboratories. \(^{64}\text{Cu}\) was obtained as copper(II) in 0.6N HCl solution with a specific activity of approximately \(5 \times 10^3\) Ci/mmol or more from the University of Missouri Research Reactor. Radiolabeled Cu-PTSM was prepared with either \(^{67}\text{Cu}\) or \(^{64}\text{Cu}\) (depending on availability) as described previously.

**Statistical Analysis**

Data are expressed as mean±SD. Flow estimates were compared by a t test. Regressions were calculated by the least-squares fit to first- or second-order polynomial functions. Probabilities less than 0.05 were considered significant.

**Results**

**Blood Pool Clearance**

Blood pool clearance of radioactivity after administration of radiolabeled Cu-PTSM was rapid (Figure 1). By 2 minutes, arterial blood radioactivity decreased to less than 10% of peak in all animals.

**Myocardial Cu-PTSM Uptake**

Myocardial copper uptake correlated closely with blood flow estimated from radiolabeled microspheres in all of the groups studied. An example of the correlation between myocardial copper radioactivity and microsphere-determined blood flow is shown in Figure 2A from one dog with induced myocardial ischemia. Myocardial \(^{64}\text{Cu}\) radioactivity increased linearly with microsphere-determined blood flow over the range from 0 to 1.0 ml/g/min (\(r=0.95\)) in 24 samples of myocardium. Dogs with induced coronary stenoses and with intravenous dipyridamole administration allowed comparison of copper uptake in the myocardium at higher flow ranges. Figure 2B shows the correlation between myocardial \(^{67}\text{Cu}\) radioactivity and microsphere-assessed blood flow in one such dog and shows a linear correlation in the flow range from 1.5 to 5.0 ml/g/min (\(r=0.97\)) based on 24 samples. The dogs studied without mechanical interventions allowed comparison of retained copper activity with blood flow in the flow range from 0.8 to 2.5 ml/g/min.

To correlate myocardial copper uptake with blood flow, copper radioactivity in each of the 340 myocardial samples from all 17 dogs was normalized to correct for differences in administered radioactivity. The best fit line of the relation between tissue copper radioactivity and microsphere-estimated blood flow was computed in each animal and normalized so that a copper radioactivity of 100 counts/g/min corresponded to a microsphere-determined blood flow of 1.0 ml/g/min. The normalized myocardial copper radioactivity for the range of flows (grouped into 0.25-ml/g/min intervals at flows less than 1.0 ml/g/min and into 0.5-ml/g/min intervals at flows more than 1.0 ml/g/min) in each dog was then averaged, and the results are depicted in Figure 3. Normalized copper radioactivity increased monotonically in the flow range studied (from 0 to 6.0 ml/g/min). The increase of copper radioactivity was linear up to a flow of approximately 2.5 ml/g/min and then increased more gradually at higher flows. The data conformed best to a second-order polynomial (\(Y_{\text{copper radioactivity}} = 85X_{\text{microsphere flow}} - 7x^2 + 17, \ r=0.99\)) (Figure 3).

**Correlation of Copper Radioactivity With Blood Flow in the Kidney**

Renal copper radioactivity was compared with microsphere-assessed blood flow in the same manner as described for myocardial copper radioactivity. Two ranges of flows estimated with microspheres were evident corresponding to the renal cortical samples with flows in the 3–5.5-ml/g/min range and to the renal medullary samples with microsphere-determined blood flow.
Figure 2. Panel A: Correlation between microsphere-assessed blood flow and myocardial $^{64}$Cu radioactivity for one of the dogs with coronary occlusion. In this dog, myocardial $^{64}$Cu activity increased linearly with microsphere-assessed blood flow with a correlation coefficient of 0.95 in the flow range from 0 to 1.0 ml/g/min. Cts., counts. Panel B: Correlation between microsphere-assessed blood flow and myocardial $^{64}$Cu radioactivity in one dog with left anterior descending coronary artery stenosis after hyperemia was induced with intravenous dipyridamole. Myocardial $^{67}$Cu activity increased linearly with microsphere-assessed blood flow with a correlation coefficient of 0.97 in the flow range from 1.0 to 5.0 ml/g/min.

flows in the 0–0.5-ml/g/min range. Shown in Figure 4 are data compiled from all dogs correlating renal flow estimated with microspheres (grouped into 0.5-ml/g/min intervals) compared with the normalized renal copper activity. The relation between normalized copper activity in the renal cortex for the blood flow range of 3–5.5 ml/g/min was similar to the relation observed in the myocardium for the corresponding range. Normalized copper radioactivity in the renal medulla with microsphere-determined blood flows of 0–0.5 ml/g/min was slightly higher than that observed in the heart for the same flow range.

**Positron Emission Tomography**

Tomographic images of good quality were obtained after intravenous administration of $^{64}$Cu-labeled Cu-PTSM. Two tomograms from one dog subjected to coronary occlusion are shown in Figure 5. On the left panel is the PET reconstruction obtained after administration of 20 mCi $^{15}$O$_2$, corrected for vascular radioactivity as described previously. On the right panel is the same slice from a 5-minute static collection started 10 minutes after intravenous administration of 5.3 mCi $^{64}$Cu-labeled Cu-PTSM. The regional uptake of $^{64}$Cu in the heart corresponds closely with perfusion delineated by $^{15}$O$_2$. Once extracted, no significant loss of tracer from the myocardium was evident in the scanning interval (Figure 6). Corroborating results obtained in isolated hearts.

A tomographic reconstruction obtained at the level of the midleft kidney after administration of $^{64}$Cu-labeled Cu-PTSM is shown in Figure 7. This data reconstruction was obtained with a 5-minute collection beginning 5 minutes after completion of the myocardial imaging or approximately 20 minutes after intravenous administration of tracer. Definition of the normal kidney was evident.

**Discussion**

Copper-PTSM is one of a group of lipophilic compounds [the copper(II) bis(thiosemicarbazone) complexes] that were studied in biological systems initially for potential antineoplastic activity. The mechanism of copper entrapment in tumor cells has been shown to involve diffusion of the intact complex across the cell membrane, with subsequent reduction of copper(II) to copper(I) by ubiquitous intercellular sulfhydryl groups. In tumor cell models, the reduced copper is believed to bind nonspecifically to intracellular macromolecules, thereby becoming trapped.

Initial tissue distribution studies after intravenous injections of copper(II) bis(thiosemicarbazone) complexes in rats demonstrated rapid blood pool clearance of the tracer, and high myocardial uptake with favorable heart-to-lung ratios. We recently showed in isolated, perfused hearts that Cu-PTSM is avidly extracted by the myocardium and essentially irreversibly bound throughout a wide range of conditions including normal physiological flow, hyperemia, ischemia, and hypoxia. The extraction fraction of Cu-PTSM observed in those studies (45%) was similar to the single-pass extraction fraction of the cyclotron-produced flow marker $^{11}$NH$_3$ (55%), with the same experimental preparation in our laboratory. In isolated hearts, the extraction fraction of Cu-PTSM was constant throughout the range of blood flows studied (from 10% to 200% of normal). These observations in isolated, perfused hearts indicated that the extraction and retention of Cu-PTSM was not affected by moderate disparities of flow or oxygenation and the metabolic sequelae of those interventions, suggesting that Cu-PTSM was a promising candidate for further
evaluation as a tracer of blood flow, prompting this initial study in intact dogs.

Myocardial Cu-PTSM Uptake In Vivo

Normalized copper radioactivity increased monotonically throughout the blood flow ranges of 0–6.0 ml/g/min in this study. The increase of copper radioactivity was linear up to a flow of approximately 2.5 and then increased more gradually at higher flows. Because net extraction of tracer plateaued at flows greater than 2.5 ml/g/min in some studies, the extraction mechanism for Cu-PTSM by the heart appears to be saturated at blood flows greater than those initially studied in isolated, perfused hearts or the permeability-surface area product appears not to increase in proportion to increases in blood flow. Based on our observations in isolated, perfused hearts, we had hoped that absolute myocardial blood flow could be calculated with Cu-PTSM with an approach analogous to that used for microspheres. However, studies performed concurrently demonstrated that, within 1 minute after intravenous administration in intact animals, Cu-PTSM was completely metabolized so that radioactivity was no longer associated with PTSM.26 Preliminary studies have demonstrated that these metabolites are not extracted by the myocardium (unpublished observations from our laboratory). These factors precluded our use of total copper radioactivity in the pump-withdrawal syringe (used for determining the reference flow with microspheres) for measuring the integral of arterial Cu-PTSM radioactivity and for calculating myocardial blood flow in absolute terms with Cu-PTSM in a manner analogous to that used for microspheres. Accordingly, the normalization scheme corrected for differences in administered radioactivity and for correlations of net tissue copper radioactivity with blood flows estimated with microspheres. Thus, for absolute quantification of myocardial perfusion with Cu-PTSM, the decrease in extraction fraction at hyperemic flows and the influence of metabolites in arterial blood will need to be considered.26,27 Nonetheless, preliminary PET studies demonstrated that high-quality images are obtainable with short scan periods because of the rapid blood pool clearance of tracer, the avid myocardial

**Figure 3.** Plot of data compiled from all 340 myocardial samples obtained from all 17 dogs correlating microsphere-assessed blood flows with normalized myocardial copper activity. Number of dogs with blood flows in each flow range are indicated in the parentheses. Normalized copper activity increased monotonically over the flow range from 0 to 6.0 ml/g/min. Increase of copper activity is linear up to a flow of approximately 2.5 and then more gradual at higher flows.

**Figure 4.** Plot of data compiled from all dogs that correlates renal flow estimated with microspheres with normalized renal copper activity. Two ranges of flows were evident corresponding to cortical samples with flows ranging from 3 to 5.5 ml/g/min and medullary samples with flows ranging from 0 to 0.5 ml/g/min. No data are plotted in the flow range from 0.5 to 3.0 ml/g/min because no samples had values of flow in this range.
FIGURE 5. Color tomosgrams from one dog subjected to left anterior descending coronary artery occlusion. On the left is a positron emission tomogram reconstruction obtained after administration of 20 mCi \( H_2^{15}O \), corrected for vascular radioactivity. Posterolateral wall is to the left, the septum is to the right, and the ischemic anterior wall to the top. On the right is a reconstruction obtained in the same transaxial slice from a 5-minute static collection started 10 minutes after intravenous injection of \( ^{64}Cu \)-labeled Cu-PTSM. Distribution of \( ^{64}Cu \) radioactivity in the heart correlates closely with that of \( H_2^{15}O \).

FIGURE 6. Myocardial and arterial time-activity curves obtained from four normal myocardial regions of interest and from the left ventricular blood pool in one dog after administration of \( ^{64}Cu \)-labeled Cu-PTSM and after dynamic position emission tomography. Once extracted, myocardial radioactivity remains constant.

extraction, and the protracted myocardial retention of extracted tracer. The distribution of \( ^{64}Cu \)-labeled Cu-PTSM correlated closely with the distribution of radiolabeled water.

Renal Cu-PTSM Uptake

Because Cu-PTSM is extracted by many organs by virtue of its lipophilicity, we also assessed copper uptake in the kidney. Good definition of the normal kidney was evident with PET after intravenous administration of \( ^{64}Cu \)-labeled Cu-PTSM. Two ranges of renal blood flows were evident. Cortical samples had flows in the 3–5.5-ml/g/min range, and medullary samples had microsphere-determined blood flows in the 0–0.5-ml/g/min range. Because the normalized copper radioactivity in the renal cortex for the blood flow ranging from 3 to 5.5 ml/g/min was similar to that in the myocardium for the corresponding range, the compound may be handled similarly by the heart and the renal cortex. Normalized copper radioactivity in the renal medulla was modestly higher than that observed in the heart throughout the same blood flow range. Although microspheres accu-
rately measure total renal cortical flow, they tend to underestimate renal medullary flow by as much as 20–50%, perhaps because of the preferential distribution to high-flow regions. Accordingly, the disparity between normalized copper radioactivity and microsphere-assessed blood flow in the renal medulla may be due to underestimation of medullary flow by microspheres.

**Clinical Implications**

The results of the present investigation indicate that Cu-PTSM activity correlates closely with microsphere-assessed blood flow in the heart and the kidney. High-quality images can be obtained because blood pool clearance of the tracer is rapid, and once extracted, tissue radioactivity remains constant throughout the scanning interval. Recent studies also showed that Cu-PTSM uptake in the brain is closely correlated with cerebral blood flow. Thus, multiple organ flow may be obtained after a single administration of this tracer. If the relation between flow and myocardial extraction fraction at hyperemic flows can be established and if the arterial input function is known and corrected for metabolites, absolute blood flow in milliliters per gram per minute should be obtainable.

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**References**


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