Kinetics of Copper-PTSM in Isolated Hearts: A Novel Tracer for Measuring Blood Flow with Positron Emission Tomography

Marc E. Shelton, Mark A. Green, Carla J. Mathias, Michael J. Welch, and Steven R. Bergmann

Cardiovascular Division, Department of Internal Medicine and Division of Radiation Sciences, Department of Radiology, Washington University School of Medicine, St. Louis, Missouri, and School of Pharmacy, Purdue University, West Lafayette, Indiana

Copper(II) pyruvaldehyde bis(N^4-methylthiosemicarbazone) ([Cu]PTSM) has shown potential as a flow tracer and can be labeled with the generator-produced positron emitting radionuclide ^{62}Cu as well as with other copper radioisotopes. To define the myocardial handling of [Cu]PTSM, the externally detected single pass extraction and retention of [^{67}Cu]PTSM was characterized after bolus administration in 12 isolated rabbit hearts perfused with erythrocyte-enriched modified Krebs-Henseleit buffer which permitted physiologic flow rates. The myocardial residual (extraction) fraction at control flow rates (~1.5 ml/g/min) was 45 ± 7(s.d.)% (n = 12), and was invariate with ischemia (flow = 0.15 ml/g/min, n = 4), hyperemia (flow = 3 ml/g/min, n = 4) or with hypoxia induced by perfusion at control flow rates with hypoxic buffer (n = 4) (residual fraction 45 ± 20, 43 ± 8, and 49 ± 8%, respectively, p = N.S.). Once extracted, the tracer was retained with a biologic $t_{½}$ of >3600 min in all groups. The high single-pass extraction, which is not influenced by flow, and the prolonged retention of this tracer under diverse conditions indicate that [Cu]PTSM could be a useful tracer for measuring blood flow with positron emission tomography.

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The feasibility of using positron emission tomography (PET) to accurately quantify regional myocardial blood flow noninvasively has been demonstrated using cyclotron-produced oxygen-15- (^{15}O) labeled water (*1–3*). However, many cardiac PET centers are being established without cyclotrons and are therefore limited to using rubidium-82 (^{82}Rb) for flow estimates. Although ^{82}Rb has proven useful in determining relative myocardial blood flow (*4,5*), the extraction and retention of rubidium is dependent upon and nonlinearly proportional to flow (*6*), and is independently influenced by the metabolic status of the heart (*7*) making quantification of myocardial blood flow in absolute terms difficult. In addition, because of the rapid physical $t_{½}$ of ^{82}Rb (75 sec), image quality is suboptimal. Accordingly, there is a need for a generator-produced imaging agent suitable for quantifying regional blood flow with PET at centers that do not have cyclotrons.

In biodistribution studies in rats the radiolabeled lipophilic compound copper(II) pyruvaldehyde bis(N^4-methylthiosemicarbazone) ([Cu]PTSM) has been shown to exhibit high tissue/blood ratios, prolonged tissue retention, and rapid blood-pool clearance (*8,9*); characteristics potentially favorable of a blood flow imaging agent. Copper PTSM can potentially be labeled with copper-62 (^{62}C) from the ^{62}Zn/^{62}Cu radionuclide generator (*10,11*). The 9.8-min half-life of the positron-emitting ^{62}Cu is well suited to perfusion imaging with PET. In addition, [Cu]PTSM can be labeled with any of the other copper radioisotopes: copper-67 ($t_{½}$ = 59 hr with gamma photons at 91 keV, (23% doublet) and 184 keV (40%)), potentially amenable to conventional single-photon scintigraphy; copper-64 (positron decay $t_{½}$ = 12.8 hr); copper-60 ($t_{½}$ = 24 min with positron decay); and copper-61 ($t_{½}$ = 3.3 hr with positron decay).

Preliminary studies have indicated that [Cu]PTSM accurately distributes in proportion to blood flow (*8,9*) although the myocardial kinetics of this compound by

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For reprints contact: Marc E. Shelton, MD, Cardiovascular Division, Washington University School of Medicine, 660 S. Euclid Ave., Box 8086, St. Louis, MO 63110.

the heart have not been defined. Accordingly, we characterized the extraction and retention of [Cu]PTSM (labeled with ^{67}Cu) in an isolated perfused rabbit heart preparation in which variables of tracer delivery, myocardial perfusion, and perfusate oxygenation could be controlled precisely. The results suggest that radiolabeled [Cu]PTSM is a potentially useful tracer of myocardial blood flow.

METHODS

Isolated Heart Preparation

Male, New Zealand white rabbits weighing 1.5-2.0 kg were stunned by a blow to the head. Hearts were excised rapidly and perfused retrogradely at 37°C via the aorta with nonrecirculating, oxygenated (95% O_2/5% CO_2), modified Krebs-Henseleit containing 0.4 m*M* albumin, 5 m*M* glucose, and 70 munits/l insulin. Hearts were paced at 180 bpm with a right atrial bipolar electrode. Left ventricular pressure (LVP) was monitored continuously with a fluid-filled latex balloon inserted through the left atrium. Left ventricular end-diastolic pressure (LVEDP) was set at 5–10 mmHg by adding volume to the ventricular balloon. The first derivative of the LVP (dP/dt) and coronary perfusion pressure were also measured continuously. The pulmonary artery was cannulated for a collection of venous effluent. In order to study the myocardial kinetics of this tracer at physiologic flow levels, the modified Krebs-Henseleit buffer was enriched with washed sheep erythrocytes to a hematocrit of ~30%. The characteristics of this preparation have been described previously in detail (*12*). Oxygen content of the perfusate was measured with a cooximeter.

Detection and Analysis of Myocardial Time-Activity Curves

For external detection of radioactivity, paired NaI (Tl) gamma scintillation crystals were positioned at 180° apart, ~3 cm from surface of the heart at the midventricular level. Scintillation signals were recorded with Ortec 485 amplifiers and 488 single channel analyzers. The energy window of the single channel analyzer was centered around the 184 keV photopeak of the ^{67}Cu using a 20% window. Output was monitored on-line with a Digital Equipment Corporation RX-08 minicomputer. Data were stored on floppy disks for subsequent analysis.

The externally detected single pass extraction and retention of [^{67}Cu]-Cu-PTSM in hearts were evaluated following the injection of a 50–100 μCi bolus (in a volume of 0.1 ml) into the perfusate 1 cm distal to the aortic valve through a sidearm in the aortic cannulae. Time-activity curves were recorded for 20 min beginning with the time of the bolus injection. The fraction of ^{67}Cu counts retained by the heart after a single pass, referred to as residual fraction, was calculated by back extrapolation of the monoexponential terminal portion of the time-activity curve to obtain the ordinate value of the curve at the time of occurrence of peak counts (*13*). Myocardial clearance of ^{67}Cu was expressed in terms of biologic half-life calculated from the best-fit monoexponential function (e^{-kt}, where k represents the myocardial turnover rate constant) conforming to the terminal (5 to 20 min) portion of the time-activity curve.

Two tracer injections were made in each heart. To correct for residual radioactivity and clearance from the initial injection, after the experimental intervention was imposed, myocardial radioactivity was monitored prior to the second injection in order to obtain count data and clearance of residual (background) radioactivity. Data from the second injection were then corrected for residual activity and clearance (*13*). Sequential injections using this correction technique under control conditions demonstrated reproducibility of the data corrected in this manner.

Experimental Protocol

All hearts were perfused initially under control conditions with oxygenated KH-RBC at a control flow rate of 6 ml per min (~1.5 ml/g/min). After stabilization of left ventricular pressure, dP/dt, left ventricular end-diastolic pressure and perfusion pressure (at least 15 min), the kinetics of [^{67}Cu]PTSM were evaluated in all hearts under control (baseline) conditions and then hearts were randomized into three groups. To evaluate the effects of ischemia, after baseline data was obtained, four hearts were made ischemic by decreasing the pump flow to 10% of control. After hemodynamic stabilization at the ischemic flow rate and collection of radioactivity data (used to correct for residual radioactivity), a second bolus injection of [^{67}Cu]PTSM was administered. To characterize the effects of hyperemia, a second group of hearts (the hyperemic group, n = 4) were evaluated analogously. After baseline data collection, a second bolus of tracer was administered during high flow (12 ml/min, ~3 ml/g/min). A third group of hearts (the hypoxic group, n = 4) was studied to evaluate the effects of hypoxia. After initial baseline data had been collected, this group was subjected to hypoxia by switching the perfusion from erythrocyte enriched buffer to buffer without erythrocytes thereby reducing perfusate oxygen content by >80% while maintaining pH and pCO_2 at physiologic levels without altering flow. Tracer injection during hypoxic conditions followed a 20-min equilibration period with the hypoxic perfusate.

Synthesis of [^{67}Cu]-Copper(II) pyruvaldehyde bis(N^4-methylthiosemicarbazone)

Copper-67 was obtained as copper(II) in a 2N HCl solution with high specific activity (~5×10^5 Ci/mol) from Los Alamos National Laboratory. The [^{67}Cu]-copper(II)-PTSM was prepared as described previously (*8*) and found to be 99.3 ± 0.5% radiochemically pure as determined by thin layer chromatography (with a Berthold LB285 automatic TLC analyzer using analytic silica 5 × 20 cm plates eluted with ethyl acetate) and by determining the octanol/saline (pH 6-7) partition coefficient (log P = 2.0 ± 0.1). The final [^{67}Cu]PTSM solution was diluted to 1 mCi/ml and to a 5% ethanol concentration (pH 5-6.)

Statistical Analysis

Data are expressed as mean ± s.d. Comparisons of independent or paired samples were subjected to analysis of variance followed by t-tests corrected for the number of comparisons by the Bonferroni method (*14*). Linear regression was calculated by the least squares method. Probabilities <0.05 were taken to indicate statistically significant differences.

RESULTS

Hemodynamics

Heart rate, perfusion pressure, left ventricular pressure, flow, and dP/dt during the control period were similar in all hearts. Hearts in the ischemic group exhibited >85% reduction of dP/dt and heart rate was diminished by ~⅓ because of atrio-ventricular block (Table 1). The hypoxic group exhibited 70% reduction of control dP/dt during perfusion with hypoxic buffer. The hyperemic group had slightly increased (~30%) dP/dt during perfusion at high flow rates (Table 1).

Myocardial Uptake and Clearance of [^{67}Cu]PTSM

Representative time-activity curves are shown in Figure 1. The myocardial residual fraction during control conditions was 45 ± 7% (n = 12), and was invariate with ischemia (45 ± 20%, n = 4), hyperemia (43 ± 8%, n = 4), or hypoxia (49 ± 8%, n = 4) (Fig. 2).

Once extracted, tracer was retained with an average biologic clearance $t_{½}$ of greater than 3600 min in all groups (Fig. 3), indicative of near irreversible binding of tracer within the myocardium under diverse flow conditions. Clearance was slightly more rapid at high flows, but the shortest $t_{½}$ observed was still greater than 600 min.

DISCUSSION

Copper(II)-PTSM is one of a group of lipophilic compounds (the copper(II) bis thiosemicarbazone complexes) that were studied in biologic systems initially for potential antineoplastic activity (*15*). The mechanism of copper entrapment in tumor cells has been shown to involve diffusion of the intact complex across the cell membrane [the octanol/water partition coefficient for [Cu]PTSM is 100/1 (*8,9*)], with subsequent reduction of copper(II) to copper(I) by ubiquitous intracellular sulfhydryl groups (*16*). In tumor cell models the reduced copper is believed to bind nonspecifically to intracellular macromolecules, thereby becoming trapped.

Green et al. have developed a simple, rapid preparation scheme for synthesis of [Cu]PTSM which may be adapted for use with any of the copper radionuclides (*8*). Recent tissue distribution studies of copper(II) bis (thiosemicarbazone) complexes in rats have demonstrated rapid blood-pool clearance, and high myocardial uptake with favorable heart-to-lung ratio following intravenous injection (*9*).

Myocardial Kinetics of Copper-PTSM

In isolated perfused hearts, the single-pass extraction of [Cu]PTSM was >40% and invariant over a range of conditions including normal physiologic flow, hyperemia, ischemia, and hypoxia. The extraction of [Cu] PTSM observed was similar to the single-pass extraction of the cyclotron-produced flow marker, $^{13}NH_3$, using the same experimental model in our laboratory (*17*). The extraction fraction of $^{13}NH_3$, unlike that of [Cu] PTSM, varied widely from control (55%) to hypoxia (18%) and ischemia (48%) indicating that quantification of myocardial flow with $^{13}NH_3$ requires correction for extraction and clearance (*17–20*). Similar studies have shown analogous results with other cationic tracers used for estimation of flow such as ^{201}Tl (*13,21–23*) and ^{82}Rb (*24,25*). In addition, the extraction and retention of cationic tracers are sensitive to the metabolic status of the heart as induced by drugs or disease processes (*6,7,13,17–19,21–25*). It is unknown whether certain drugs or pathophysiologic processes affect the extraction and retention of [Cu]PTSM by the heart. The result of the studies reported here appear to indicate that under the conditions studied, the extraction and retention of this tracer are not affected by wide disparities in flow or oxygenation and the metabolic sequelae of these interventions.

Once extracted, the ^{67}Cu was essentially irreversibly trapped under all experimental conditions, with a biologic clearance of $t_{½}$ of >3600 min in all groups. There was a slight (not statistically significant) increase in clearance at hyperemic flows. However, the fastest clearance was still markedly prolonged ($t_{½}$ = 600 min) and would not influence flow estimates judging from initial studies in vivo in experimental animals with imaging times of 15 min (unpublished observations).

Such prolonged, microsphere-like, retention should be useful clinically. Recent studies have demonstrated that high-quality images in intact dogs after i.v. administration of tracer can be obtained since blood-pool clearance is rapid and, once extracted, tissue radioactivity essentially constant (*26*). Should results of biodistribution studies in rats be corroborated in other species, it would appear that multiple organ flow could be obtained after one administration of tracer since all tissues studied so far appear to have prolonged tissue retention of tracer (*26–27*). If extraction fraction can

TABLE 1
Hemodynamic Variables During Single Pass Extraction and Retention Studies of [^{67}Cu]PTSM

	HR (bpm)	Flow (ml/min)	dP/dt (mmHg/sec)
Control (n = 12)	176 ± 8	5.7 ± 0.3	1100 ± 300
Ischemia (n = 4)	124 ± 52	0.6 ± 0.1	150 ± 135
Hypoxia (n = 4)	181 ± 10	5.7 ± 0.1	330 ± 40
Hyperemia (n = 4)	173 ± 2	12 ± 0.1	1450 ± 200

Values are mean ± s.d.
HR = heart rate; dP/dt = first derivative of left ventricular pressure.

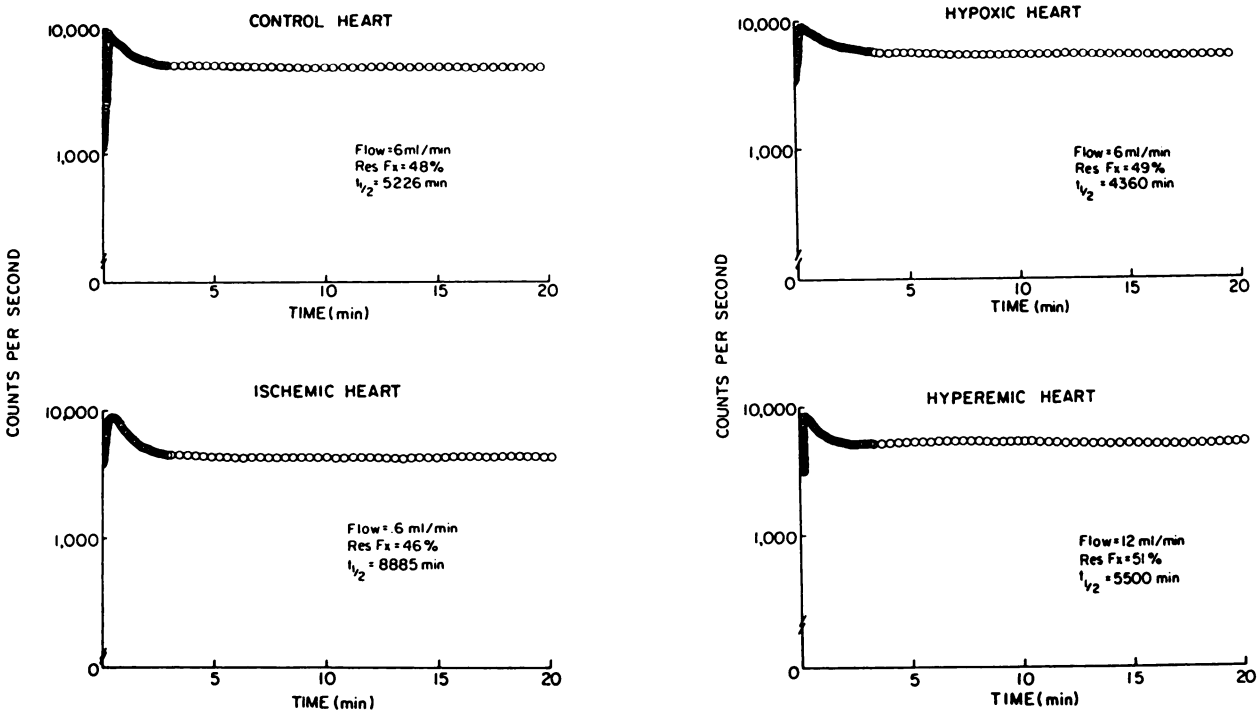

FIGURE 1
Myocardial time-activity curves from one heart in each group. Single-pass extraction of [^{67}Cu]-PTSM was invariate and retention of extracted activity essentially irreversible under all conditions studied. Res Fx = residual fraction. $t_{½}$ = biologic half-time.

be established or arterial input function is known or can be measured, absolute blood flow (ml/g/min) can be obtained.

Conclusions

The results of the present study indicate that the lipophilic compound [Cu]PTSM is highly extracted by heart and essentially irreversibly bound under diverse conditions. These characteristics coupled with the ability to rapidly label this agent with either gamma or positron emitting copper radionuclides appear to make this compound particularly promising for noninvasive quantitative evaluation of blood flow.

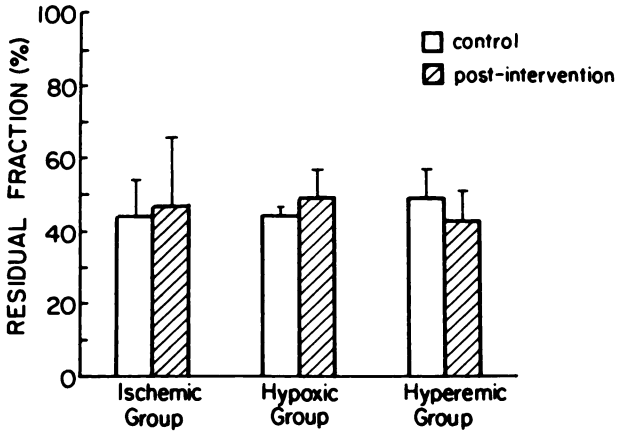

FIGURE 2
Histogram summarizing the residual fraction of [^{67}Cu] PTSM following bolus injection during control (open bars) and postintervention (hatched bars) conditions (n = four hearts in each group). Residual fraction was similar under all conditions.

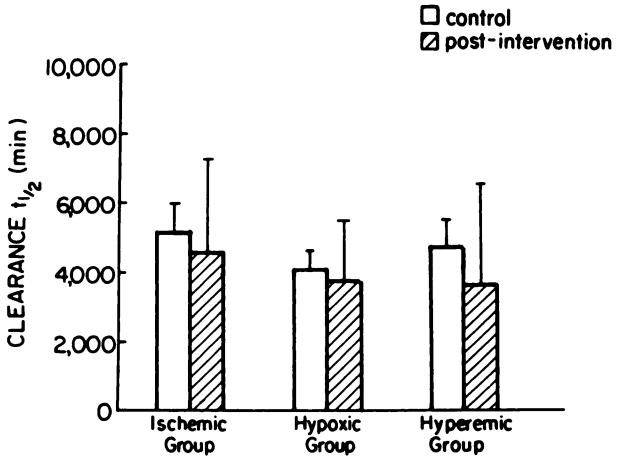

FIGURE 3
Biologic clearance $t_{½}$ (min) of ^{67}Cu radioactivity during control (open bars) and postintervention (hatched bars) studies (n = four hearts in each group). Average clearance $t_{½}$ was greater than 3600 min under all conditions consistent with essentially irreversible binding of tracer within the myocardium.

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