Copper(II) Bis(thiosemicarbazone) Complexes as Potential Tracers for Evaluation of Cerebral and Myocardial Blood Flow with PET

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Wider application of positron emission tomography would be facilitated by the availability of positron-emitting radiopharmaceuticals labeled with nuclides, like ^{62}Cu, that are available from parent/daughter generator systems. Using a longer-lived copper isotope (^{67}Cu) we have examined three derivatives of copper(II) pyruvaldehyde bis(thiosemicarbazone) as potential tracers for evaluation of cerebral and myocardial blood flow: Cu(PTS), Cu(PTSM), and Cu(PTSM$_2$) (where PTS = pyruvaldehyde bis(thiosemicarbazone), PTSM = pyruvaldehyde bis(N^4-methylthiosemicarbazone), and PTSM$_2$ = pyruvaldehyde bis(N^4-dimethylthiosemicarbazone). All three lipophilic radiocopper complexes were obtained in high yield via a procedure that could be adapted to a "kit" formulation. In animal model systems Cu(PTSM) and Cu(PTSM$_2$) show excellent uptake in the brain and heart following i.v. injection. These tracers differ in that Cu(PTSM) exhibits microsphere-like retention in the brain and heart, whereas Cu(PTSM$_2$) substantially clears from these organs. The relative cerebral pharmacokinetics of [^{67}Cu]Cu(PTSM) and [^{67}Cu]Cu(PTSM$_2$) are consistent with their known reactivity towards intracellular sulfhydryl groups.

The zinc-62/copper-62 ($^{62}Zn/^{62}Cu$) radionuclide generator (*1–5*) is a possible source of radiopharmaceuticals for diagnostic imaging by positron emission tomography (PET) in locations that lack an in-hospital cyclotron for radionuclide production. However, despite a daughter half-life (10 min) well suited to perfusion imaging by PET, the potential of this generator to provide clinically useful ^{62}Cu radiopharmaceuticals has been largely unexplored (*2,6*). The principal disadvantage of the $^{62}Zn/^{62}Cu$ generator system is the rather short (9 hr) half-life of the cyclotron-produced parent. Nevertheless, this generator system might effectively support remote PET imaging centers, if a regional cyclotron facility could provide daily shipments of [^{18}F]-fluorodeoxyglucose (for study of cerebral and myocardial metabolism) and a $^{62}Zn/^{62}Cu$ generator (for preparation of tracers for measurement of blood flow and blood volume, along with previously reported (*2*) tracers for imaging the liver, kidneys, and lungs). Robinson

et al (*1*) estimated it would be possible to deliver an 80-mCi ^{62}Cu generator from the 160 mCi ^{62}Zn produced (EOB) by 1-hr irradiation of a natural copper target with 22-MeV protons at a beam current of 70 μA ($^{63}Cu(p,2n)^{62}Zn$). Such a generator would have a clinically useful life of a 1-2 days (*1*).

There are alternate radionuclide generator systems that could support PET imaging facilities (*7,8*), although none can currently deliver the armamentarium of radiopharmaceuticals that would be desirable for routine clinical PET. The $^{82}Sr/^{82}Rb$ generator may prove quite useful for assessment of myocardial perfusion (*9*); however, the limited chemistry of the Rb cation will severely hinder the development of cerebral blood flow (CBF) agents with this tracer. The germanium-68/gallium-68 ($^{68}Ge/^{68}Ga$) generator is commercially available and is attractive because of its long parent half-life. Numerous ^{68}Ga radiopharmaceuticals have been reported (*7,10*) and some are in routine use for human studies (*11,12*). Unfortunately, lipophilic ^{68}Ga tracers for perfusion imaging have proven somewhat elusive (*7,10,13,14*). There are tris(salicylaldimine) complexes of gallium that might be used for evaluation of myocardial perfusion (*13,14*), but no gallium tracers have

been developed that effectively cross the intact blood-brain barrier for CBF studies. The xenon-122/iodine-122 (^{122}Xe/^{122}I) generator can provide tracers for assessment of cerebral perfusion (15); however the high-energy (65 MeV) proton beam needed to produce the Xe-122 parent (16) makes this generator system less accessible than the ^{62}Zn/^{62}Cu generator, which can be delivered by a medium-energy cyclotron.

An investigation of lipophilic ^{67}Cu complexes has been initiated in order to screen potential tracers that could be used to evaluate regional cerebral and/or myocardial blood flow when labeled with ^{62}Cu. [Copper-67 decays with a half-life of 2.580 days producing gamma photons at 93 keV (16%) and 185 keV (48%) (17)]. Reported here are the results obtained with the copper(II) complexes of three derivatives of pyruvaldehyde bis(thiosemicarbazone) (Fig. 1).

MATERIALS AND METHODS

General

Literature methods were used for the preparation of the pyruvaldehyde bis(thiosemicarbazone) ligands (18). Copper-67 was obtained as copper(II) in a 2N HCl solution with high specific activity (~ 5 × 10⁵ Ci/mol) from Los Alamos National Laboratory. Iodine-125-labeled iodoantipyrine was obtained commercially (DuPont Company, No. Billerica, MA) in >97% radiochemical purity. It was further purified by CHCl₃ extraction to remove contaminating [^{125}I]iodide immediately prior to use (13). Procedures for partition coefficient measure-
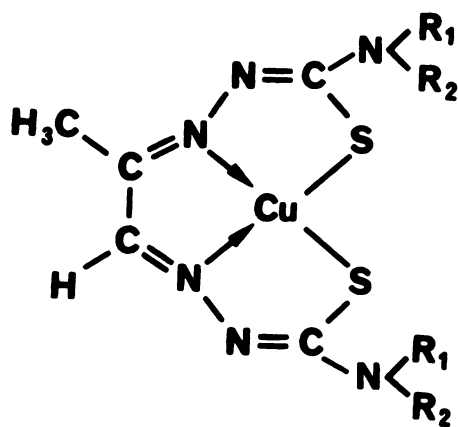
Complex	R_1	R_2	M.W.
Cu-PTS	H	H	280
Cu-PTSM	H	CH₃	308
Cu-PTSM₂	CH₃	CH₃	336

FIGURE 1
Derivatives of copper(II) pyruvaldehyde bis(thiosemicarbazone).

ment were described previously (6), as were the thin layer chromatography conditions for determination of the radiochemical purity of the ^{67}Cu chelate complexes (6,19). Rat biodistribution studies were also conducted as described previously (6), following 0.15-ml femoral vein injections of 1–3 μCi of each of the ^{67}Cu pyruvaldehyde bis(thiosemicarbazone) complexes.

Preparation of [^{67}Cu]-Copper(II) Bis(thiosemicarbazone) Complexes

The ^{67}Cu complexes of the bis(thiosemicarbazone) ligands studied (Fig. 1) were prepared by addition of a 1N NaOH solution of the appropriate ligand to an acetate-buffered ethanol solution of the ionic ^{67}Cu. The solution would then be diluted with saline or distilled water (maintaining a minimum ethanol concentration of 5% to ensure solubility of the lipophilic complexes) and filtered through a 0.2-μm PTFE filter prior to use. In a typical preparation 0.1 mCi ^{67}Cu in 0.020 ml 2N HCl was diluted with 0.5 ml ethanol and 0.5 ml 1N acetate buffer (pH 4.6), followed by addition of 5 × 10⁻⁴ g H₂(PTSM) dissolved in 0.040 ml 1N NaOH. This general procedure consistently provided the desired product in >95% radiochemical yield. Solutions used in animal studies generally contained 5% ethanol at the time of injection.

Electrophoresis of [^{67}Cu]Complexes

The copper bis(thiosemicarbazone) complexes, [^{67}Cu]Cu(PTS), [^{67}Cu]Cu(PTSM), and [^{67}Cu]Cu(PTSM₂), were studied by electrophoresis on Seprapore III cellulose acetate strips (2.5 × 17.1 cm), along with [^{99m}Tc]pertechnetate and [^{201}Tl] thallium chloride. The five samples were run simultaneously in a pH 7.5 phosphate buffer (ionic strength 0.28) (20) and 5% ethanol (to insure solubility of the lipophilic complexes). A total current of 4 mA was applied to the five strips for a period of 45 min. The strips were analyzed by cutting them into 1-cm sections (centered about the origin) and counting in a well counter.

Brain Uptake Index

The brain uptake index (BUI) (21,22) was measured in Sprague-Dawley rats anesthetized with ketamine (100 mg/kg i.m.) and acepromazine (1 mg/kg i.m.). A mixture of [^{125}I] iodoantipyrine (0.5 μCi) and the [^{67}Cu]bisthiosemicarbazone (1.0 μCi) in 0.15 ml 5% ethanol: 95% saline was rapidly injected into the right common carotid artery. The animal was killed 15 sec postinjection and the brain quickly removed. Copper-67 levels in the right anterior brain and a sample of the injectate were immediately measured in a well-counter. After a 2-wk delay to allow for decay of the ^{67}Cu, the samples were again counted to determine ^{125}I concentrations. The BUI value was calculated as described in the literature (21,22) using the [^{125}I]iodoantipyrine (rather than [^{3}H]water) as the freely diffusible reference tracer (23,24). The BUI values reported in the text for [^{67}Cu]Cu(PTS), [^{67}Cu]Cu(PTSM), and [^{67}Cu]Cu(PTSM₂) represent the means of five, seven, and six rats, respectively.

Monkey Imaging Study

A pentobarbital anesthetized male cynomologus monkey (5 kg) was provided by an ophthalmology laboratory following surgical removal of the eyes. The animal was positioned with its head and chest above the crystal of a Pho 5 gamma camera and sequential 1-min images (posterior view) obtained for 45

min, commencing with a femoral vein injection of 170 μCi [⁶⁷Cu]Cu(PTSM). Anesthesia was maintained with ketamine and acepromazine (i.m.). At the conclusion of this dynamic study, a single anterior image of the chest and abdomen was obtained. Ninety minutes postinjection a venous blood sample was drawn and the monkey killed by rapid i.v. injection of 10 ml saturated KCl solution. The animal was dissected and organ samples were weighed and counted. An aliquot of a weighed sample of the injectate was also counted and the biodistribution then calculated as percent injected dose per gram of wet tissue and percent injected dose per organ. Time-activity curves for the brain and heart were generated from specified regions of interest using the series of dynamic images.

Gerbil Stroke Model

Male mongolian gerbils (80-100 g) were anesthetized with ketamine (150 mg/kg i.m.) and acepromazine (1.7 mg/kg i.m.). The right common carotid artery was exposed and ligated to create a flow deficit in the right brain. After a delay of 1 to 5 min, the animal would receive a femoral vein injection of 0.1 ml 10% ethanol: 90% saline containing a mixture of 2.1 μCi [⁶⁷Cu]Cu(PTSM) and 1.1 μCi [¹²⁵I]iodo-antipyrine. Animals were killed at either 15 or 30 sec postin-jection and the brain removed and cut into quarters. The samples were weighed and counted immediately to determine regional [⁶⁷Cu] uptake. Tissue samples were stored frozen in sealed vials and recounted two weeks later to determine regional [¹²⁵I]iodoantipyrine uptake.

This study was later repeated with separate injections of [⁶⁷Cu]Cu(PTSM) and [¹²⁵I]iodoantipyrine. In this repeat study the stroked animal received the [⁶⁷Cu]Cu(PTSM) injection via the left femoral vein. After a 1 to 3-min delay, the animal received the [¹²⁵I]iodoantipyrine via the right femoral vein as a second injection. At 15 sec following the [¹²⁵I] injection the gerbil was killed and the brain removed, quartered, and counted as described above.

RESULTS AND DISCUSSION

Radiochemistry

Bis(thiosemicarbazone) ligands have a high affinity for the copper(II) ion, providing a square-planar N_2S_2 metal coordination sphere (log K = 17.8 (where K = formation constant) for both Cu(PTS) and Cu(PTSM) at physiological pH (25)). The current study involves three derivatives of pyruvaldehyde bis(thiosemicarba-zone) (Fig. 1) that differ simply in the extent of methyl substitution about the terminal amino groups: pyruval-dehyde bis(thiosemicarbazone), PTS; pyruvaldehyde bis(N⁴-methylthiosemicarbazone), PTSM; and pyruval-dehyde bis(N⁴-dimethylthiosemicarbazone), PTSM₂.

The ⁶⁷Cu complexes of these ligands are stable in vitro for >12 hr and were readily prepared in high yield by a procedure that could be adapted to a "kit" formulation for use with the ionic ⁶²Cu eluent of the Robinson generator (1). The reported procedure pro-duced the desired lipophilic products in >95% radi-ochemical yield, as assessed by thin layer chromatog-raphy. Electrophoresis studies suggest that all three

radiocopper bis(thiosemicarbazone) complexes are un-charged at physiological pH. None migrated on cellu-lose acetate electrophoresis under conditions that pro-duced marked anodic migration of [⁹⁹ᵐTc]pertechnetate and cathodic migration of the ²⁰¹Tl cation.

The octanol/water partition coefficients measured for this series of copper bis(thiosemicarbazone) com-plexes (Table 1) span the range (0.9 <log P <2.5) where Dischino and Welch (26) observed quantitative cerebral extraction of [¹¹C]-labeled alcohols and ethers at cere-bral blood flow values up to 1 ml/min · g in a baboon model. This suggests that nonspecific binding of these lipophilic copper complexes by plasma proteins should not affect their ability to freely diffuse across the blood-brain barrier. For this series of copper complexes the observed incremental increase in log P by ~0.5 for each added methyl substituent corresponds well with the known effect of methyl-substitution on the lipophilicity of organic molecules (27). The log P value measured for [¹²⁵I]iodoantipyrine (Table 1) agrees with the value recently reported by Kung et al. (28).

Biodistribution Study in Rat

The biodistribution of each of these copper tracers: [⁶⁷Cu]Cu(PTS), [⁶⁷Cu]Cu(PTSM), and [⁶⁷Cu]Cu (PTSM₂), was determined following intravenous injec-tion into rats. Tables 2-4 report the results of these studies as percent injected dose per gram of tissue for a series of time points postinjection. [⁶⁷Cu]Cu(PTSM) and [⁶⁷Cu]Cu(PTSM₂) were both rapidly cleared from the blood and showed excellent uptake in the brain. At 1 min postinjection, ~3.2% of the injected dose was found in the brain with either of these two tracers. They differ in that the brain level of [⁶⁷Cu]Cu(PTSM₂) drops to 1.0% of the injected dose at 15 min postinjection, while the brain level of [⁶⁷Cu]Cu(PTSM) remains con-stant over the period of 1 min to 2 hr postinjection. Indeed, [⁶⁷Cu]Cu(PTSM) afforded "microsphere-like" retention of ⁶⁷Cu in all the major organs examined, commencing with the shortest time point of the study (1 min postinjection). It should also be noted that [⁶⁷Cu] Cu(PTSM) shows relatively high myocardial uptake, while the myocardial uptake of [⁶⁷Cu]Cu(PTSM₂) was found to be significantly lower.

In contrast to the bis(monomethyl)- and bis(dimethyl)-complexes, [⁶⁷Cu]Cu(PTS) penetrates the blood-brain barrier much less efficiently (0.5% of the injected dose in the brain at 1 min). However, the

TABLE 1
Octanol/Saline Partition Coefficients (Measured)

Compound	log P
[⁶⁷Cu]Cu(PTS)	0.75
[⁶⁷Cu]CU(PTSM)	1.95
[⁶⁷Cu]Cu(PTSM2)	2.7
[¹²⁵I]iodoantipyrine	1.27

TABLE 2
Biodistribution of [^{67}Cu]Cu(PTS) in Rats*

Organ	% Injected dose per gram		
	1 min	5 min	15 min
Blood	1.5 ± 0.3	0.76 ± 0.08	0.63 ± 0.06
Heart	4.7 ± 0.4	3.9 ± 0.5	4.3 ± 0.8
Lungs	14.5 ± 2.5	8.7 ± 1.5	7.7 ± 0.7
Liver	1.3 ± 0.4	1.8 ± 0.2	2.0 ± 0.2
Spleen	1.0 ± 0.9	1.2 ± 0.9	0.7 ± 0.1
Kidney	5.6 ± 2.2	5.8 ± 2.2	7.4 ± 1.5
Brain	0.27 ± 0.09	0.18 ± 0.03	0.21 ± 0.05

* Values shown represent the mean of four rats (166-191g).

TABLE 4
Biodistribution of [^{67}Cu]Cu(PTSM$_2$) in Rats*

Organ	% Injected dose per gram			
	1 min	5 min	15 min†	30 Min†
Blood	0.43 ± 0.05	0.37 ± 0.03	0.48 ± 0.05	0.42 ± 0.03
Heart	1.5 ± 0.2	0.65 ± 0.21	0.45 ± 0.05	0.47 ± 0.04
Lungs	4.6 ± 1.1	1.6 ± 0.3	1.3 ± 0.3	1.3 ± 0.2
Liver	1.3 ± 0.3	2.7 ± 0.2	3.0 ± 0.4	2.6 ± 0.2
Spleen	0.7 ± 0.3	0.65 ± 0.11	0.61 ± 0.13	0.60 ± 0.03
Kidney	2.2 ± 0.6	1.4 ± 0.2	2.9 ± 0.5	3.1 ± 0.4
Brain	1.9 ± 0.3	1.3 ± 0.2	0.58 ± 0.02	0.53 ± 0.02

* Values shown represent the mean of five (four†) rats (169-226g).

myocardial uptake of this complex is comparable to that of [^{67}Cu]Cu(PTSM), suggesting that the low brain uptake is not the result of tight binding by blood macromolecules.

Of these three bis(thiosemicarbazone) complexes, Cu(PTSM) appears in these studies to have the most potential for clinical utility, showing excellent uptake and microsphere-like retention in both the heart and brain. The brain/blood ratio obtained with [^{67}Cu]Cu(PTSM) exceeds that reported for [^{99m}Tc]Tc(d,l-HM-PAO) (29,30). As indicated by Neirinckx, et al (30), at these brain/blood levels the tracer remaining in the blood will have little affect on the quality of the resulting brain images, due to the comparatively small amount of blood-borne brain activity.

Brain Uptake Index

For each of the [^{67}Cu]-labeled tracers (Fig. 1) the brain uptake index (BUI) (21, 22) was measured in rats using [^{125}I]iodoantipyrine as a diffusible standard (23, 24). Animals were killed 15 sec following co-injection of the test and reference tracers into the right common carotid artery and BUI calculated as the [^{67}Cu/^{125}I] ratio in the right anterior brain divided by the [^{67}Cu/^{125}I] ratio in the injectate. BUI values of 0.16 ± 0.04; 1.5 ± 0.2; and 1.5 ± 0.2 were obtained for [^{67}Cu]Cu(PTS); [^{67}Cu]Cu(PTSM); and [^{67}Cu]Cu(PTSM$_2$), respectively. The BUI values of Cu(PTSM) and Cu(PTSM$_2$) indicate

TABLE 3
Biodistribution of [^{67}Cu]Cu(PTSM) in Rats*

Organ	% Injected dose per gram			
	1 Min	5 Min	15 Min	2 hr†
Blood	0.68 ± 0.09	0.51 ± 0.04	0.49 ± 0.01	0.51 ± 0.17
Heart	3.8 ± 0.8	5.5 ± 1.6	3.8 ± 0.4	4.6 ± 1.2
Lung	6.9 ± 2.7	5.8 ± 1.2	5.9 ± 0.3	3.9 ± 1.9
Liver	1.4 ± 0.3	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.2
Spleen	1.4 ± 0.5	0.76 ± 0.04	0.69 ± 0.15	0.54 ± 0.16
Kidney	4.4 ± 0.7	3.4 ± 0.5	4.6 ± 0.3	4.7 ± 0.5
Brain	2.0 ± 0.3	1.9 ± 0.3	1.9 ± 0.1	1.9 ± 0.2

* Values shown represent the mean of three (four†) rats (195-225g).

that these complexes undergo very high cerebral extraction in a single pass through the brain. The magnitude of these BUI values (BUI > 1.0) indicates that some clearance of extracted [^{125}I]iodoantipyrine must occur during the 15 sec-time span of the experiment (23,24). (Active transport of Cu(PTSM) and Cu(PTSM$_2$) across the blood-brain barrier might also elevate the measured BUI; however, this explanation seems unlikely).

The low BUI value obtained for [^{67}Cu]Cu(PTS) is consistent with the low brain uptake of this tracer observed following intravenous injection (above). While binding of tracer to plasma proteins might depress the brain uptake of [^{67}Cu]Cu(PTS) following intravenous injection, protein-binding effects are expected to be much reduced in the BUI experiment (31). This suggests that the diminished brain uptake of Cu(PTS) relative to Cu(PTSM) and Cu(PTSM$_2$) results from intrinsic differences that affect their ability to freely diffuse across the blood-brain barrier.

Monkey Imaging Study

Sequential 1-min gamma images were obtained from a cynomolgus monkey following i.v. injection of [^{67}Cu]Cu(PTSM). Figure 2 shows a representative image (posterior view) from this dynamic study in which the brain, heart, and liver are readily visualized. Computer analysis of the dynamic study (Fig. 3) demonstrates microsphere-like retention of tracer in the brain and heart from 1 to 45 min postinjection, consistent with the results obtained in the rat. An anterior image of the abdomen at 50 min postinjection (Fig. 4) provides distinct visualization of the heart, liver, and kidneys. There is no evidence for clearance of the renal activity into the bladder, again consistent with microsphere-like retention of ^{67}Cu in the major organs.

The monkey was killed 90 min postinjection and tissue levels of ^{67}Cu quantitated (Table 5). Dissection and counting of cerebral gray and white matter reveals differential uptake of ^{67}Cu in these two brain regions, with a gray/white ratio of 3.0. The magnitude of this ratio approximates the relative perfusion of these tissues (23,24,32,33). However, definitive conclusions regard-
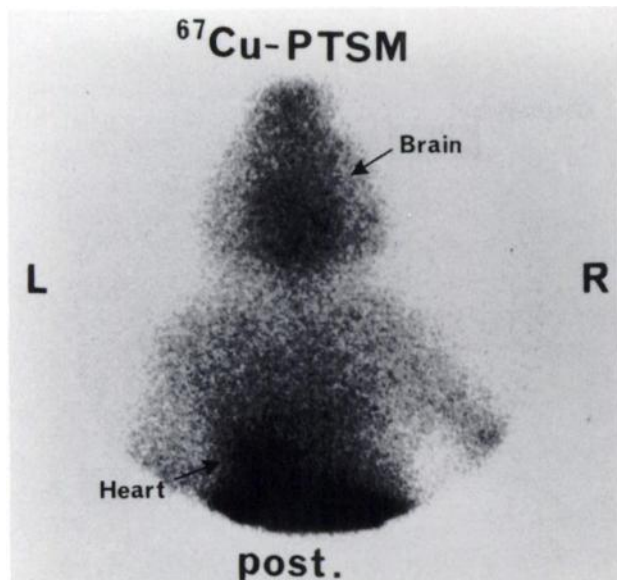
FIGURE 2
Gamma image (posterior view) of monkey following intravenous injection of [^{67}Cu]Cu(PTSM). Note tracer uptake in brain, heart, and liver (the nose of the animal is at the top of the image).

FIGURE 4
Anterior view of monkey abdomen 50 min following intravenous injection of [^{67}Cu]Cu(PTSM), illustrating tracer uptake and retention in the heart, liver, and kidneys.

ing the relationship between regional perfusion and regional cerebral ^{67}Cu uptake can not be drawn, as a validated reference flow tracer was not available for comparison. Nevertheless, these results suggest that there will be a correlation of [^{67}Cu]Cu(PTSM) uptake with regional cerebral blood flow.

Gerbil Stroke Model

To further assess[^{67}Cu]Cu(PTSM) as a potential radiopharmaceutical for evaluation of cerebral blood flow, it has been studied in a dual tracer experiment employing the gerbil stroke model (34–38). Gerbils
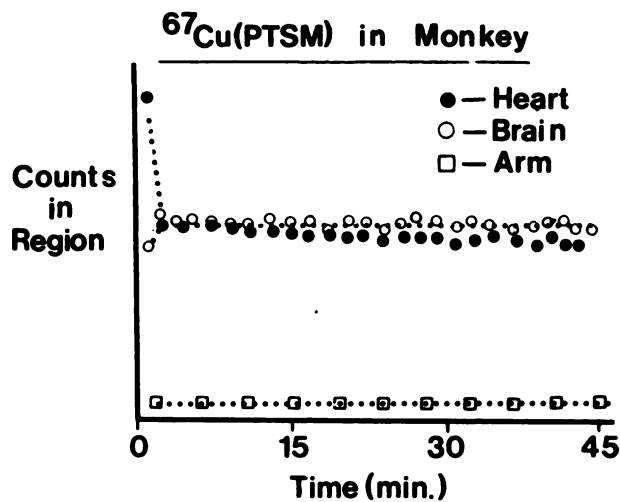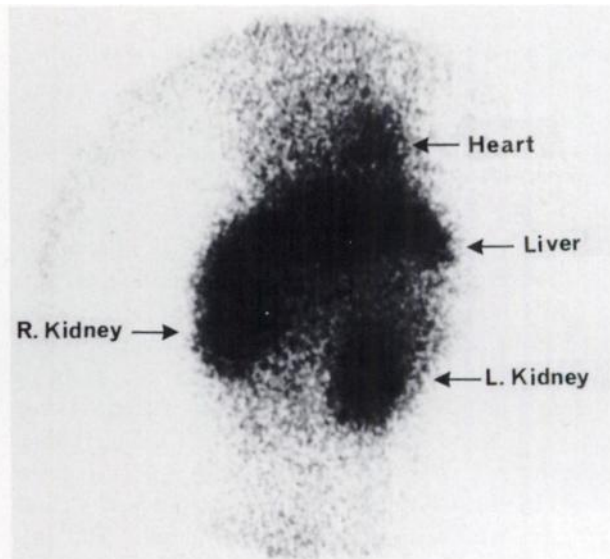
provide a suitable model for stroke because they possess essentially no posterior communicating arteries connecting the basilar artery circulation to internal carotid circulation (35). The stroked animals received a femoral vein injection of a mixture of [^{125}I]iodoantipyrine and [^{67}Cu]Cu(PTSM). Upon death at either 15 sec or 30 sec postinjection the brain was removed and cut into quarters (left anterior; right anterior; left posterior; and right posterior). Relative regional blood flow in each quarter of the brain should be reflected by the relative [^{125}I] uptake (23,24). Thus, using one high-flow brain quarter as a reference region (generally the left posterior quarter), each animal provides three data points in the plot correlating regional ^{67}Cu uptake with blood flow

FIGURE 3
Time-activity curves derived for regions of interest in the dynamic imaging study that produced Figure 2.

TABLE 5
Distribution of [^{67}Cu]Cu(PTSM) in the Monkey*

Tissue	% Injected dose/ organ	% Injected dose/gram
Brain	3.7	0.055
Cerebral gray matter	—	0.077
Cerebral white matter	—	0.026
Heart	2.2	0.18
Lungs	7.5	0.33
Liver	30.6	0.34
Gall bladder and contents	0.21	0.052
Spleen	1.2	0.14
Kidney (each)	6.0	0.71
Blood	—	0.021
Muscle (skeletal)	—	0.012
Fat (mesenteric)	—	0.029

* 90 min postinjection.

(Fig. 5). The correlation between regional [^{67}Cu] uptake and regional [^{125}I] uptake is excellent (Fig. 5; death at 15 and 30 sec produced virtually identical plots, so data at both time points have been combined).

A baboon study of the cerebral kinetics of [^{67}Cu] Cu(PTSM) following intracarotid injection (Green MA, Mathias CJ, and Welch MJ, to be reported separately) revealed tracer clearance during the initial 50-sec time span that preceded microsphere-like retention of ^{67}Cu beyond 1 min. The gerbil stroke study was, therefore, repeated to determine the relationship between blood flow and regional brain levels of ^{67}Cu at a time of microsphere-like [^{67}Cu] retention. Stroked gerbils were given a femoral vein injection of [^{67}Cu]Cu(PTSM). Following a 1 to 3 min delay the animals were given the [^{125}I]iodoantipyrine reference tracer via a second injection into the contralateral femoral vein. The animals were killed 15 sec following the ^{125}I injection and the regional brain levels of these two tracers were compared as before (Fig. 6). For reasons that are unclear,
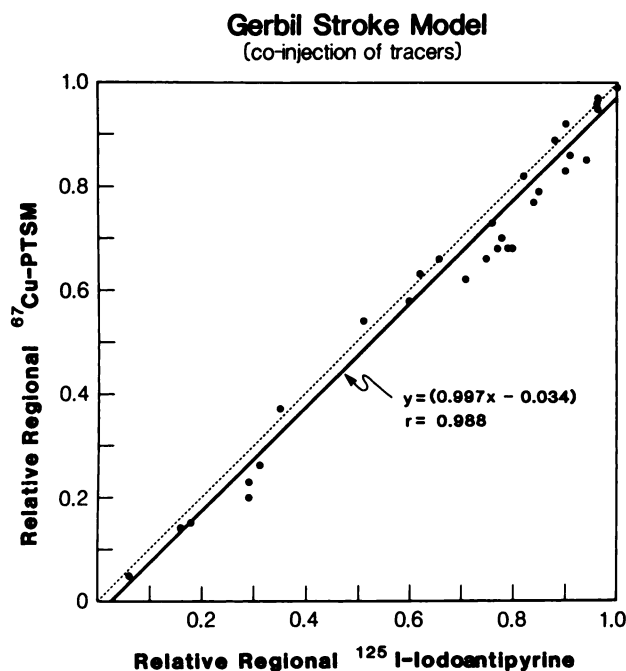
Gerbil Stroke Model
(co-injection of tracers)

FIGURE 5
Comparison of regional cerebral [^{67}Cu]Cu(PTSM) uptake with relative regional cerebral blood flow assessed by [^{125}I] iodoantipyrine in mongolian gerbils with ligated right common carotid arteries. For each tracer the plotted data represents the ratio:

$$\frac{[\text{counts per gram}]_{\text{region } x}}{[\text{counts per gram}]_{\text{reference region}}}.$$

The solid line is the least squares fit of the data from ten animals (30 data points). The dotted line represents the "idealized" line passing through the origin with a slope equal to 1. Tracers were co-injected as an intravenous bolus and animals sacrificed at either 15 or 30 sec postinjection.

Gerbil Stroke Model
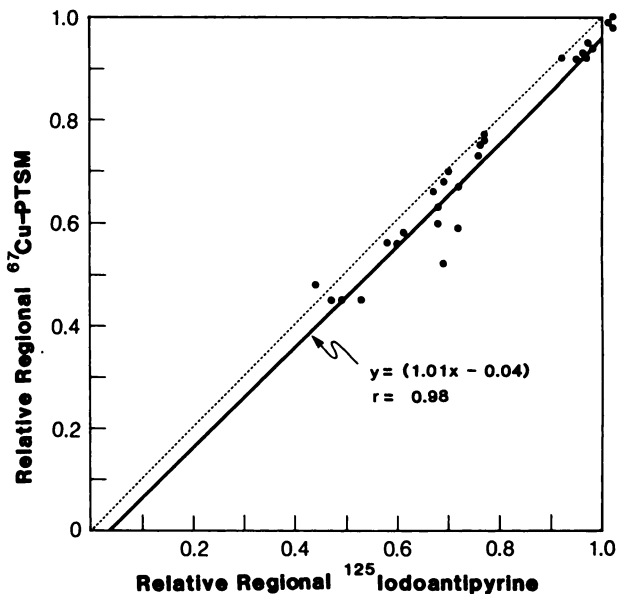(sequential injection of tracers)

FIGURE 6
Comparison of regional cerebral ^{67}Cu-67 levels (1-3 min postinjection) with relative regional cerebral blood flow (assessed using [^{125}I]iodoantipyrine) in mongolian gerbils with ligated right common carotid arteries. Data is plotted as in Fig. 5. In this experiment the animal was given an intravenous injection of [^{67}Cu]Cu(PTSM) followed by an intravenous injection of [^{125}I]iodoantipyrine after a delay of 1 to 3 min. Animals were killed at 15 sec following the [^{125}I]injection.

the flow gradients achieved in this experiment were not as extreme as those obtained in the study that produced Figure 5. However, Figure 6 again shows a good correlation between regional ^{67}Cu uptake and relative regional blood flow measured by [^{125}I]iodoantipyrine. Thus while some ^{67}Cu may clear from the brain at times shorter than 1 min postinjection, this may not significantly alter the relationship between ^{67}Cu levels and relative perfusion.

Definitive conclusions regarding the relationship between regional blood flow and regional uptake of tracer Cu(PTSM) will require further studies in which *absolute flow* can be determined. However, these preliminary studies are most encouraging and clearly indicate the merit of the more elaborate studies required to measure both copper uptake and absolute blood flow in an animal model system.

Rationalization of Pharmacokinetics

Copper(II) bis(thiosemicarbazone) complexes have been studied extensively in biological systems because of the antineoplastic activity exhibited by some derivatives (*18,19,39–43*). Those studies have shown that the intact copper(II) bis(thiosemicarbazone) complexes diffuse across tumor cell membranes, where reduction by cellular sulfhydryl groups liberates the copper to be

nonspecifically bound by intracellular macromolecules (40–43). Such an intracellular redox process in other body tissues would explain the microsphere-like retention of ^{67}Cu following intravenous injection of [^{67}Cu] Cu(PTSM). The observed difference between the cerebral pharmacokinetics of [^{67}Cu]Cu(PTSM) and [^{67}Cu] Cu(PTSM$_2$) is consistent with the report (41) that Cu(PTM$_2$) undergoes this intracellular redox process at a rate 400 times slower than Cu(PTSM). Thus, while both complexes penetrate the intact blood-brain barrier, the kinetically inert complex is substantially cleared while the more reactive complex is chemically trapped to afford microsphere-like retention.

Petering et al, have shown that titration of Ehrlich cells with a related copper(II) bis(thiosemicarbazone) complex, Cu(KTS) (KTS = 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone)), leads to specific binding of copper to metallothionein by 1:1 displacement of zinc (44). Under conditions of complete metallothionein titration (1.25-2.5 nmol Cu(KTS) per 10^7 cells) cellular DNA synthesis was temporarily inhibited. However, new zinc metallothionein was subsequently produced and no long-term effects on cell proliferation were observed at these Cu(KTS) levels (44).

The current results and the existing literature point to a discrete chemical trapping mechanism that might be more generally exploited in the design of transition metal radiopharmaceuticals exhibiting nonspecific and prolonged trapping in tissue, as is needed in many SPECT applications. For example, it may be possible to methodically design lipophilic ^{99m}Tc radiopharmaceuticals that will be susceptible to inner-sphere reduction and trapping by cellular sulfhydryl groups. Redox processes have been previously implicated in the trapping of some SPECT CBF tracers (45–48), notably [^{99m}Tc]Tc(HM-PAO). Trapping by an inner-sphere redox process should be kinetically very sensitive to any alteration in ligand conformation that might block or restrict access to the metal coordination sphere and could thus account for unexpected variations in pharmacokinetics [e.g., compare the d,l- and meso- isomers of Tc(HM-PAO) (49,50)]. Reaction of Tc(HM-PAO) with glutathione has recently been proposed to account for its cerebral retention (46), although other mechanisms have also been posited (47,48).

CONCLUSIONS

The reported studies clearly demonstrate the technical feasibility of imaging the brain and heart with easily prepared copper-labeled radiopharmaceuticals and support our belief that the ^{62}Zn/^{62}Cu generator could be a versatile source of radiopharmaceuticals for PET imaging in hospitals remote from a cyclotron facility. Copper-labeled Cu(PTSM) shows excellent brain up-

take and retention in animal model systems and shows promise as a perfusion agent. Myocardial uptake and kinetics are similarly encouraging. These results indicate [^{67}Cu]Cu(PTSM) could provide the fixed radioisotope distribution needed for use of the relatively inexpensive HIDAC positron camera (51). Additional studies are clearly needed, but the existing chemical literature does allow the relative cerebral pharmacokinetics of [^{67}Cu]Cu(PTSM) and [^{67}Cu]Cu(PTSM$_2$) to be rationalized at the molecular level.

Only limited data are available on the systemic toxicity of α-ketoaldehyde bis(thiosemicarbazone)s (18,52), although their cytotoxicity has been well-studied in isolated cell systems (18,19,39–43). Pyruvaldehyde bis ([N^4]-methylthiosemicarbazone), H$_2$(PTSM), is reported to have a single-dose LD$_{50}$ of greater than 4000 mg/kg (i.p.) in Swiss mice (18).

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