Hyperfixation of Copper-62-PTSM in Rat Brain After Transient Global Ischemia

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We evaluated the regional distribution of ^{62}Cu-pyruvaldehyde bis(N^4-methylthiosemicarbazone) (^{62}Cu-PTSM), a potential PET perfusion agent, in the rat brain and observed hyperfixation in transient global ischemia in rats. **Methods:** The distribution of ^{62}Cu-PTSM was examined in comparison with that of ^{123}I-labeled p-iodophenyl-N-isopropylmethanphetamine (^{123}I-IMP) as a reference blood flow marker. Brain uptake of these two tracers was measured in Wistar rats subjected to 30-min four-vessel occlusion followed by recirculation for 10 min, 1 hr or 1, 3 or 5 days. Tracers were injected intravenously into rats 10 min before decapitation. The activities of Complex I and Complex I–III of mitochondria and the concentration of sulfhydryl (SH) groups were also measured. **Results:** Copper-62-PTSM showed accelerated accumulation in the brain at 1 hr and 1 day after reperfusion when compared with that of ^{123}I-IMP ($p < 0.01$), and this enhancement was considered to be due to hyperfixation. At these time points, SH concentration was significantly decreased ($p < 0.01$). On the other hand, the activity of Complex I was not influenced by ischemia/reperfusion, but that of Complex I–III was decreased to 65–70% of the control level ($p < 0.01$). **Conclusion:** Copper-62-PTSM showed hyperfixation most possibly as a result of increased NADH concentration, caused by disturbed electron transport in mitochondria.

Key Words: hyperfixation; cerebral ischemia; reperfusion injury; copper-62

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Generator-produced radiopharmaceuticals for PET have an important clinical role in PET imaging which does not require an in-house cyclotron. Copper-62-pyruvaldehyde bis(N^4-methylthiosemicarbazone) (Cu-PTSM) (Fig. 1) has been reported as a potentially useful perfusion imaging agent labeled with generator-produced ^{62}Cu, and its clinical applicability has been demonstrated in the diagnosis of brain as well as heart diseases (*1,2*). Copper-PTSM is believed to be retained by intracellular reductive decomposition (*3–5*), which is similar to the proposed retention mechanism of ^{99m}Tc-hexamethylpropyleneamine oxime (^{99m}Tc-HM-PAO) (*6*), a widely-used brain perfusion agent.

Recently, abnormal focal accumulation of ^{99m}Tc-HM-PAO in the vicinity of acute or subacute brain legions, so-called hyperfixation, has been reported (*7–9*). This phenomenon is not a desirable characteristic in an ideal perfusion agent, but is considered to be of diagnostic value in some brain diseases (*9*).

As a retention mechanism of ^{99m}Tc-HM-PAO, we hypothesized that in the damaged brain regions there might be elevated reductive conditions, and ^{62}Cu-PTSM may also show hyperfixation. In the present study, we evaluated the brain distribution of ^{62}Cu-PTSM and ^{123}I-labeled *p*-iodophenyl-N-isopropylmethanphetamine (^{123}I-IMP) in the four-vessel ischemia/reperfusion rat model of Pulsinelli and Brierly (*10*), in combination with enzymatic assays for mitochondrial activity and sulfhydryl (SH) groups.

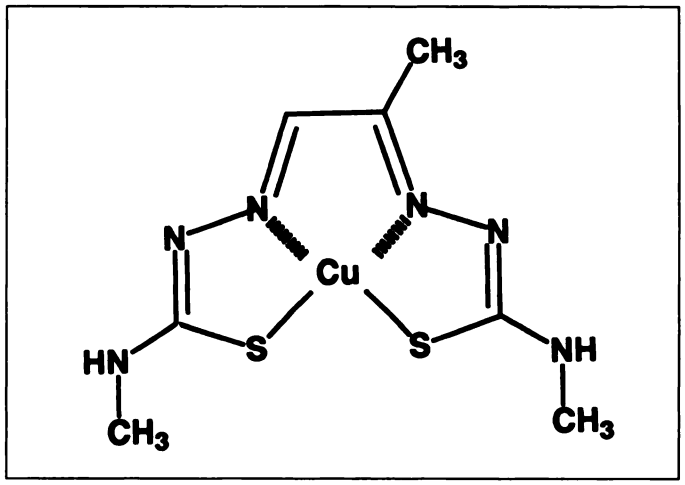

FIGURE 1. Chemical Structure of Cu-pyruvaldehyde bis(N^4-methylthiosemicarbazone) (Cu-PTSM).

MATERIALS AND METHODS

Experimental Design

Fifty male Wistar rats (220–250 g) were studied. Transient global ischemia was achieved by 30-min occlusion followed by recirculation for 10 min, 1 hr and 1, 3 or 5 days. Sham-operated rats were treated under similar conditions but were not subjected to occlusion and used as controls. Three rats died after reperfusion and two rats that showed signs of fits were excluded.

Preparation of Global Ischemia Model

Transient global cerebral ischemia was induced in rats according to the method of Pulsinelli and Brierly (*10*). Briefly, on the day before the experiment, both common carotid arteries of rats anesthetized with sodium pentobarbital (10 mg, intraperitoneal administration) were surgically exposed through a ventral, midline cervical incision. An atraumatic arterial clasp (clasp pressure, 20–25 g) was placed loosely around each common carotid artery without interrupting the carotid blood flow, and the incision was closed with silk sutures. A second incision, 1 cm in length, was made posterior to the occipital bone overlying the first two cervical vertebrae. Under an operating microscope, the right and left alar foramina of the first cervical vertebra were exposed. An electrocautery needle 0.5 mm in diameter (Bovie Monopolar Electrocautery, Cincinnati, OH) was inserted through each alar foramen, and both vertebral arteries were subsequently occluded by electrocautery.

The rats recovered without sequelae 1–2 hr later. Animals were fasted overnight but were allowed free access to water. The next day, rats were anesthetized with diethyl ether, and ventral neck sutures were removed while the animals were held loosely. Both carotid clasps were then tightened to effect vascular occlusion. During the course of surgery to induce vascular occlusion, the

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animals remained unconscious and indicated no signs of pain or suffering. The behavior of the rats was observed during the period of occlusion.

During ischemia, the left femoral artery and vein were cannulated; arterial blood pressure was monitored with a pressure transducer and traced with a pen oscillograph recorder. Arterial blood was sampled anaerobically, and Pa_{O_2}, P_{CO_2} and pH were measured with a blood gas analyzer. The pH was corrected whenever necessary with intravenous infusion of sodium bicarbonate solution. The body temperature was maintained at 37°C with a thermostat-regulated warming device.

Successful four-vessel occlusion was confirmed by recorded electroencephalography that became isoelectric within a few seconds of clipping the carotid arteries. Clips were then removed after 30-min occlusion and patency of the carotid arteries was confirmed visually by observation of continuous blood flow.

Relative Regional Cerebral Blood Flow and Distribution of Copper-62-PTSM

The time course of brain accumulation of ^{62}Cu-PTSM was examined at 10 min after injection based on the previously reported distribution in rats (*3*). Accumulation of ^{123}I-IMP, a blood flow tracer clinically used for SPECT, was used for analysis of relative regional cerebral blood flow, as described previously (*11*).

Each animal received both intravenous ^{62}Cu-PTSM (60 μCi) and ^{123}I-IMP (3 μCi) concomitantly through the tail vein. Ten minutes after administration, arterial blood samples were collected, the rat was decapitated and the brain was promptly isolated. Pial vessel and choroid plexus were removed before dissecting the brain into the following five regions on an ice-cold cover glass: cerebellum, cortex, hippocampus, striatum and others. These regions were immediately weighed and put into their respective tubes. Radioactivity of ^{62}Cu in these samples was determined immediately with a well-type scintillation counter. Radioactivity of ^{123}I was measured after the decay of ^{62}Cu. Accumulation of ^{62}Cu-PTSM and ^{123}I-IMP was calculated as % dose/g brain. To normalize for the possible effects of differences in systemic metabolism of ^{62}Cu-PTSM and ^{123}I-IMP, the brain/blood ratio of each agent was calculated as cerebral accumulation ability. Flow-normalized ^{62}Cu-PTSM uptake was calculated as follows:

$$\text{Flow-normalized } ^{62}\text{Cu-PTSM uptake } (FNU_{Cu}) = \frac{\text{Brain/blood ratio of } ^{62}\text{Cu-PTSM}}{\text{Brain/blood ratio of } ^{123}\text{I-IMP}}$$

Preparation of Rat Brain Subcellular Particles (SMP)

Rats subjected to ischemia-reperfusion under the conditions described above were used. After decapitation, the whole brain was promptly isolated, weighed and homogenized in an isolation medium (70 mmol/L sucrose, 220 mmol/L mannitol, 1 mmol/L EDTA buffered to pH 7.4 with 2.5 mmol/L HEPES) to 10% (W/V). Brain submitochondrial particles were prepared by sonication as previously reported (*5*). The mitochondrial precipitate after centrifugation of the homogenate was digested with digitonin solution (12 mg/ml-isolation medium) for 20 min at 4°C. After digestion, a three-fold isolation medium was added, and the mixture was centrifuged at 10,000 × g for 10 min at 4°C. The precipitate was resuspended in distilled water (about 2 mg-protein/ml) and centrifuged at 10,000 × g for 15 min. After centrifugation, the precipitate was resuspended and sonicated for 90 sec (15 sec × 6 times) at 4°C. The resulting SMP suspension was adjusted to the initial volume of the homogenate.

Biochemical Assay

NADH dehydrogenase (Complex I) activity was determined spectrophotometrically by measuring NADH oxidation at 340 nm (*12*). Rotenone-sensitive NADH cytochrome c reductase (Complex I–III) activity was followed spectrophotometrically at 550 nm by measuring the reduction of cytochrome c in the presence and absence of rotenone (5 μg) (*13*).

Brain homogenate samples were diluted with 1% sodium dodecyl sulfate (SDS), and then SH concentration was determined by the DTNB method (*14*).

Each sample was diluted with 1% SDS, and the protein concentration was then measured using a BCA protein assay reagent kit.

Zinc-62/Copper-62 Generator and Preparation of Copper-62-PTSM

Zinc-62/Copper-62 generator was prepared by the method reported by Fujibayashi et al. (*15*). Approximately 4 ml of ^{62}Cu was eluted and mixed with 0.4 ml of PTSM solution (0.5 mg/ml-dimethylsulfoxide, DMSO) (*5*).

Reagents

PTSM was prepared as described previously (*4*). ^{123}I-IMP was a kind gift from Nihon Mediphysics (Japan). Male Wistar rats (220–250 g body weight) were fed a commercial diet and tap water ad libitum.

RESULTS

Distribution of Iodine-123-IMP and Copper-62-PTSM after Reperfusion

Biodistributions of ^{123}I-IMP and ^{62}Cu-PTSM in rats with occlusion-reperfusion injury are shown in Tables 1 and 2, respectively. Both agents showed complicated changes during the experiment. In addition, blood levels of the agents also varied independently, suggesting differences in systemic metabolism. Brain/blood ratio was used as input-normalized accumulation in the brain.

The brain/blood ratio of ^{123}I-IMP accumulation was significantly ($p < 0.05$ to $p < 0.01$) elevated at 10 min after reperfusion in all brain regions. However, brain/blood ratio at 1 hr after reperfusion fell to about 60% of control ($p < 0.01$, in the cortex, hippocampus and striatum). In all brain sites, recovery to control level was observed at 1 day after reperfusion before a significant increase ($p < 0.05$ to $p < 0.01$) after reperfusion for 3 days (in all brain regions) to 5 days (in the cerebellum, hippocampus and striatum).

Brain/blood ratio of ^{62}Cu-PTSM at 10 min after reperfusion was significantly ($p < 0.01$) increased similarly to ^{123}I-IMP uptake. After an abrupt initial increase, ^{62}Cu-PTSM recovered to control level at 1 hr after reperfusion, earlier than the second increase of ^{123}I-IMP, except in the cerebellum (significant increase, $p < 0.01$). Moreover, a significant increase in the brain/blood ratio of ^{62}Cu-PTSM ($p < 0.05$ to $p < 0.01$) was observed in all brain regions after reperfusion for 1 to 3 days. In the cerebellum and hippocampus, brain/blood ratio at 5 days after reperfusion was significantly higher than in controls ($p < 0.05$). No significant increase was observed in the other brain sites.

Comparison Between Iodine-123-IMP and Copper-62-PTSM (FNU_{Cu})

The ratios of ^{62}Cu-PTSM to ^{123}I-IMP accumulation (brain/blood ratio) were calculated as FNU_{Cu} for all time point (Fig. 2). As compared with controls, FNU_{Cu} at 10 min after reperfusion showed a tendency to increase in all brain sites, and the hippocampus showed a significant increase ($p < 0.05$). After reperfusion for 1 hr to 1 day, FNU_{Cu}s were significantly higher ($p < 0.05$ to $p < 0.01$) than in controls; the former being mainly due to the decrement of ^{123}I-IMP accumulation, and the latter

TABLE 1
Iodine-123-IMP Accumulation in Rat Brain after 30 Min of Transient Global Ischemia

Tissue % dose/g	Control (8)	Time after reperfusion (number of rats studied)				
		10 min (4)	1 hr (5)	1 day (5)	3 days (4)	5 days (4)
Cerebellum	0.89 ± 0.19	0.85 ± 0.17	0.72 ± 0.07	1.08 ± 0.14	0.77 ± 0.13	0.87 ± 0.04
Cortex	0.88 ± 0.21	1.08 ± 0.35	0.48 ± 0.07	0.94 ± 0.12	0.80 ± 0.11	0.67 ± 0.04
Hippocampus	0.80 ± 0.16	1.12 ± 0.40	0.36 ± 0.04*	0.70 ± 0.12	0.71 ± 0.13	0.90 ± 0.50
Striatum	1.00 ± 0.26	1.63 ± 0.48†	0.43 ± 0.09*	1.08 ± 0.22	0.63 ± 0.09	0.92 ± 0.38
Others	0.94 ± 0.19	0.90 ± 0.16	0.73 ± 0.09	1.17 ± 0.17	0.82 ± 0.11	0.82 ± 0.05
Blood	0.12 ± 0.03	0.10 ± 0.01	0.12 ± 0.08	0.16 ± 0.04	0.08 ± 0.01	0.10 ± 0.04
Tissue/blood						
Cerebellum	6.69 ± 0.74	8.63 ± 1.54†	5.91 ± 0.59	6.87 ± 1.58	9.14 ± 1.22‡	8.43 ± 0.66†
Cortex	6.54 ± 0.98	16.51 ± 4.01‡	3.96 ± 0.61*	6.11 ± 2.04	9.52 ± 0.44‡	6.52 ± 0.60
Hippocampus	4.94 ± 0.33	10.87 ± 3.11‡	2.94 ± 0.25*	4.58 ± 1.54	8.45 ± 1.57‡	8.57 ± 4.48†
Striatum	5.59 ± 1.03	11.41 ± 3.76‡	3.52 ± 0.83*	6.88 ± 1.88	7.52 ± 0.58†	8.86 ± 3.39†
Others	6.88 ± 1.34	9.17 ± 1.42†	5.98 ± 0.71	7.46 ± 1.91	9.75 ± 0.71‡	7.95 ± 0.72

Values are means ± s.d.; ^{123}I-labeled p-iodophenyl-N-isopropylmetanphetamine (^{123}I-IMP) was injected intravenously 10 min before decapitation.
*$p < 0.01$ decrease
†$p < 0.05$ increase
‡$p < 0.01$ increase vs. sham-operated controls

by the increment of ^{62}Cu-PTSM. However, FNU_{Cu} recovered to the control value at 3 and 5 days after reperfusion.

Effects of Ischemia Reperfusion on Enzyme Activity

The effects of ischemia reperfusion on enzyme activity are shown in Table 3. The experiments were performed at 1 hr and 1 day after reperfusion. The activity of Complex I was almost the same as in controls. On the other hand, the activity of Complex I–III was reduced to 65–70% of that in controls ($p < 0.01$).

Effects of Ischemia Reperfusion on SH Concentration

SH concentrations after ischemia-reperfusion are shown in Table 3. At 1 hr and 1 day after reperfusion, significant decreases were observed in SH concentration ($p < 0.01$).

DISCUSSION

Copper-62-PTSM has been developed as a clinical PET perfusion tracer for use without an inhouse cyclotron and excellent perfusion images in human subjects have already been demonstrated with this method (*1,2*). Okazawa et al. reported that the relative distribution of ^{62}Cu-PTSM is correlated well with the regional cerebral blood flow obtained by ^{15}O-water PET. Copper-62-PTSM shows a slight underestimation in the high-flow range, but this is not as severe as that of ^{99m}Tc-HM-PAO (*2*).

In the present study, we showed that occlusion-reperfusion injury induced complicated changes in the biodistributions of ^{62}Cu-PTSM and ^{123}I-IMP, changes being found not only in cerebral accumulation but also in blood levels. Moreover, the changes in cerebral accumulation were not always in the same direction as those of the blood levels, indicating that the brain was not the major organ affecting blood levels of these agents. Under these conditions, cerebral accumulation should be normalized. It has been reported that single blood sampling can allow quantitative measurement of cerebral perfusion using

TABLE 2
Copper-62-PTSM Accumulation in Rat Brain after 30 Min of Transient Global Ischemia

Tissue % dose/g	Control (8)	Time after reperfusion (number of rats studied)				
		10 min (4)	1 hr (5)	1 day (5)	3 days (4)	5 days (4)
Cerebellum	1.52 ± 0.20	2.31 ± 0.30‡	1.31 ± 0.21	1.90 ± 0.35†	0.97 ± 0.08*	1.29 ± 0.04
Cortex	1.25 ± 0.24	2.53 ± 0.56‡	0.79 ± 0.15*	1.34 ± 0.28	0.95 ± 0.09*	0.91 ± 0.04*
Hippocampus	1.21 ± 0.33	2.64 ± 0.56‡	0.61 ± 0.14*	1.19 ± 0.32	0.86 ± 0.04	1.17 ± 0.67
Striatum	1.59 ± 0.51	3.60 ± 0.69‡	0.70 ± 0.15*	1.66 ± 0.44	0.77 ± 0.04	1.22 ± 0.65
Others	1.45 ± 0.26	2.16 ± 0.19‡	1.12 ± 0.18	1.81 ± 0.43	0.97 ± 0.04*	1.10 ± 0.03
Blood	1.28 ± 0.31	1.35 ± 0.09	0.94 ± 0.22	1.07 ± 0.11	0.66 ± 0.04*	0.90 ± 0.04
Tissue/blood						
Cerebellum	1.07 ± 0.19	1.73 ± 0.35‡	1.43 ± 0.14‡	1.76 ± 0.17‡	1.48 ± 0.20†	1.44 ± 0.11†
Cortex	0.92 ± 0.13	2.69 ± 0.60‡	0.86 ± 0.08	1.25 ± 0.23‡	1.44 ± 0.10‡	1.01 ± 0.10
Hippocampus	0.70 ± 0.17	1.90 ± 0.50‡	0.65 ± 0.02	1.11 ± 0.25‡	1.31 ± 0.07‡	1.28 ± 0.68†
Striatum	0.86 ± 0.17	1.98 ± 0.56‡	0.76 ± 0.14	1.56 ± 0.42‡	1.18 ± 0.10†	1.34 ± 0.66
Others	1.02 ± 0.15	1.61 ± 0.26‡	1.21 ± 0.13	1.68 ± 0.29‡	1.48 ± 0.12‡	1.22 ± 0.03

Values are means ± s.d.; ^{62}Cu-labeled copper(II)-pyruvaldehyde bis(N^4-methylthiosemicarbazone) (^{62}Cu-PTSM) was injected intravenously 10 min before decapitation.
*$p < 0.01$ decrease
†$p < 0.05$ increase
‡$p < 0.01$ increase vs. sham-operated controls

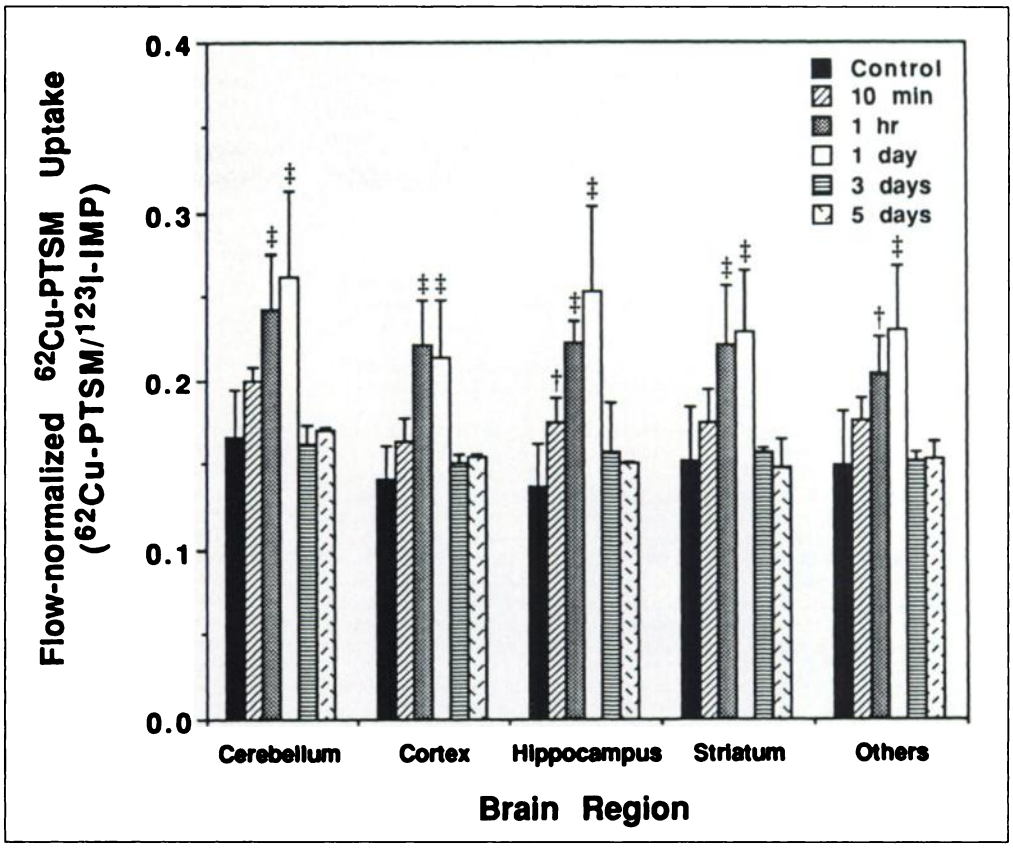

FIGURE 2. Bar graph showing flow-normalized ^{62}Cu-pyruvaldehyde bis(N^4-methylthiosemicarbazone) (^{62}Cu-PTSM) uptake after 30 min of transient global ischemia in the brain, which was calculated by dividing each brain-site/blood ratio of ^{62}Cu-PTSM by that of ^{123}I-IMP. †$p < 0.05$ significant increase, ‡$p < 0.01$ significant increase compared with control (Student's t-test).

^{123}I-IMP (*16*). In the case of ^{62}Cu-PTSM, radioactivity in the blood was rapidly fixed which renders it unavailable, but can be considered a result of integrated input levels of ^{62}Cu-PTSM. Thus, in this study, the brain/blood ratio 10 min after injection was selected as a practical parameter of cerebral accumulation normalized with input levels.

Copper-62-PTSM showed increased accumulation in the brain, detected as elevated brain/blood ratio, 1 hr and 1 day after reperfusion, when compared with that of ^{123}I-IMP. The uptake of ^{123}I-IMP after ischemia followed by reperfusion bore a close resemblance to that of iodoantipyrine in a four-vessel occlusion rat model as reported by Pulsinelli et al. (*17*), and ^{123}I-IMP uptake was considered to reflect regional cerebral blood flow. Thus, the accelerated accumulation of ^{62}Cu-PTSM shown as increased FNU_{Cu} was taken to be so-called "hyperfixation."

TABLE 3
Enzyme Activities and SH Concentration in Rat Brain after 30 Min of Transient Global Ischemia

		Time after reperfusion (number of rats)	
	Control (5)	1 hr (5)	1 day (5)
Complex I	105.8 ± 10.5	101.4 ± 7.6	105.4 ± 14.1
Complex I–III	925.2 ± 78.6	643.9 ± 70.2*	611.1 ± 60.5*
SH groups	102.7 ± 6.5	71.2 ± 2.3*	85.1 ± 2.9*

Values are means ± s.d. of 5 rats. Enzymatic activities of Complex I and Complex I–III, and concentration of SH groups were measured in each rat. Units of activity are Complex I, nmole NADH oxidized/min/mg protein; Complex I–III, nmole cytochrome c reduced/min/mg protein. Concentration of SH groups is nmole/mg-protein.

*$p < 0.01$ decrease vs. sham-operated controls.

Two reduction mechanisms have been proposed for the intracellular retention of ^{62}Cu-PTSM: (a) reduction by ubiquitous intracellular sulfhydryl (SH) groups, such as glutathione (GSH) (*1,3,6*); and (b) NADH-dependent reduction by Complex I in the mitochondrial electron transport chain (ETC) (*4,5*).

Green et al. (*1,3,6*) postulated that ^{62}Cu-PTSM is reduced by ubiquitous SH groups in cells. If this is the case, hyperfixation can be considered to be a result of increased SH concentration in the damaged brain. However, GSH can serve as a radical scavenger and decreases in levels of GSH have been reported (*18,19*). This is consistent with the results reported here that showed a significant decrease in SH level. Therefore, SH cannot play a crucial role in the mechanism of hyperfixation of ^{62}Cu-PTSM.

Previously, we showed that Cu-PTSM can be reduced by Complex I in mitochondria, and this effect was NADH-dependent (*5*). In this case, two possible mechanisms can be proposed to explain the hyperfixation of ^{62}Cu-PTSM: (a) an increase in reduced forms of the electron transport chain substrates, especially NADH; and (b) an increase in the activity of Complex I. In the present study, the activity of Complex I, the engine for the reductive decomposition of ^{62}Cu-PTSM in mitochondria, was hardly influenced by ischemia-reperfusion. On the other hand, Complex I–III activities 1 hr and 1 day after reperfusion decreased to 65–70% of the control values. This decline in enzyme activity was supported by the observation that NADH cytochrome c reductase shows a tendency to be damaged by reactive oxygen species (*20,21*). It is reasonable to assume that NADH concentration was increased by reperfusion-induced damage, and that the reductive retention of ^{62}Cu-PTSM was accelerated under these conditions. It has also been reported that NADH concentration in the postischemic brain, especially 30–60 min after recirculation, is higher than that in the preischemic brain (*22,23*).

CONCLUSION

This study suggests that the hyperfixation of ^{62}Cu-PTSM may be a result of increased NADH concentration caused by disturbed electron transport in mitochondria. As mentioned above, hyperfixation is considered valuable for detecting brain damage and, from this viewpoint, ^{62}Cu-PTSM might be a useful diagnostic agent for detection of hyperfixation.

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FIRST IMPRESSIONS

Linear Soft-Tissue Accumulation of Bone Seeking Tracer

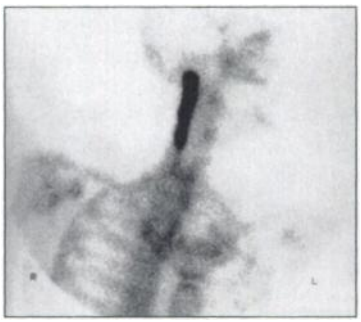

Figure 1.

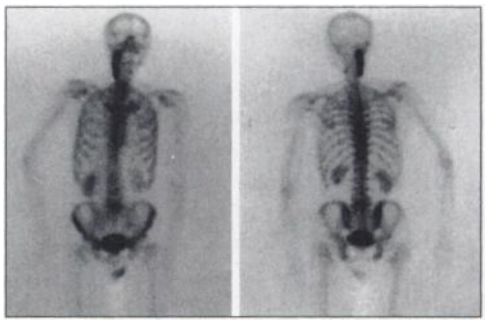

Figure 2.

PURPOSE

A 78-yr-old man had surgery for esophageal cancer 4 yr ago. He complained of recent abdominal pain and vomiting and was diagnosed as having intestinal ileus. An intravenous catheter was introduced through the right subclavian vein, through which antibiotics and fluid were given but no chemeotherapeutic agent was administered. Bone scintigraphy was performed for metastatic work-up. He complained of slight pain at the right neck when ^{99m}Tc-hedroxymethylene diphosphonate (HMDP) was administered through the indwelling catheter. Physical examination revealed a band-like tender area with a red hot spot along the right neck. The intravenous catheter was removed after administration of ^{99m}Tc-HMDP. Linear, intense accumulation of ^{99m}Tc-HMDP was observed along the right neck (Figs. 1, 2). The swelling and tenderness subsided shortly after removal of the catheter. It was concluded that the catheter in the right subclavian vein was incidentally introduced into the right internal jugular vein. The findings are consistent with the catheter having perforated the venous intima and the tracer dissecting through the media, which accounts for the sudden onset of pain and radionuclide retention.

TRACER

Technetium-99m-HMDP, 740 MBq

ROUTE OF ADMINISTRATION

Intravenous

TIME AFTER INJECTION

Four hours

NSTRUMENTATION

Shimadzu Scintipac 700 gamma camera with a low-energy, high-resolution collimator

CONTRIBUTORS

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