Technetium-99m-*meso*-HMPAO as a Potential Agent to Image Cerebral Glutathione Content

Toru Sasaki and Michio Senda
Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

To clarify whether the content of glutathione (GSH) in the brain can be estimated by the uptake of ^{99m}Tc-*meso*-HMPAO, we conducted the following in vivo and in vitro experiments. **Methods:** We investigated the effect of diethyl maleate (DEM) and buthionine sulfoximine (BSO) administration on the brain uptake of ^{99m}Tc-*meso*-HMPAO in the mouse, rat and rabbit, and the chemical specificity of in vitro interaction of ^{99m}Tc-HMPAO to GSH using measurements of octanol-extractable radioactivity as an index of remaining intact tracer. **Results:** The uptake of ^{99m}Tc-*meso*-HMPAO in the mouse and rat brain were reduced together with decreased content of GSH by preloading of DEM, a GSH depletor that acts through glutathione S-transferase. Neither ^{99m}Tc-*meso*-HMPAO uptake nor GSH content was affected in the rabbit brain. Similarly, the uptake of ^{99m}Tc-*meso*-HMPAO and GSH content in the mouse brain was reduced by preinjection of BSO, a GSH depletor that acts through γ-glutamylcysteine synthetase. In an in vitro study, ^{99m}Tc-HMPAO showed reactivity to the molecules possessing a -SH group, but were not specific to GSH. The order of ^{99m}Tc-*meso*-HMPAO reactivity to the mouse brain homogenate agreed with the order of GSH concentration: normal > BSO > DEM. GSH was a major contributor to the conversion reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex in mouse brain homogenate. **Conclusion:** GSH may have a major responsibility for trapping ^{99m}Tc-HMPAO in the brain, suggesting the possibility of in vivo measurement of brain GSH with ^{99m}Tc-*meso*-HMPAO.

Key Words: glutathione; localization; technetium-99m-*meso*-hexamethyl propyleneamine oxime; brain

J Nucl Med 1997; 38:1125–1129

In our previous study (*1*), a decrease in the glutathione (GSH) content in diethyl maleate-treated (DEM) mouse brain accompanied a decrease in ^{99m}Tc-*meso*-HMPAO uptake but no decrease in ^{99m}Tc-*d,l*-HMPAO uptake by the brain. We studied scintigraphic images of DEM-treated rats using ^{99m}Tc-*meso*-HMPAO and found a reduction in the uptake of ^{99m}Tc-*meso*-HMPAO in the brain. However, ^{99m}Tc-*meso*-HMPAO uptake in the rabbit brain was not reduced by DEM treatment (data not shown).

The exact retention mechanism of ^{99m}Tc-HMPAO has not been clarified, but the following mechanism is proposed. As a lipophilic compound, ^{99m}Tc-HMPAO diffuses across the blood brain barrier and is rapidly converted to a hydrophilic form retainable within the brain tissue. GSH is supposed to be responsible for the hydrophilic conversion and retention of ^{99m}Tc-*d,l*-HMPAO in the brain (*2–4*). However, these experiments have only been conducted in vitro, and the relationship of GSH to the retention of ^{99m}Tc has yet to be clarified. In this study, the relationship between the tissue GSH content and the uptake of ^{99m}Tc-*meso*-HMPAO was further studied in rats, rabbits and mice treated with DEM or buthionine sulfoximine (BSO), which are GSH depleting agents. We also studied the in vitro interaction of ^{99m}Tc-*meso*- and *d,l*-HMPAO with GSH, other thiols, ascorbate and oxidized glutathione (GSSG) using measurements octanol-extractable radioactivity, which remains intact in ^{99m}Tc-HMPAO, as an indicator of the reactivity. The interaction of GSH with ^{99m}Tc-*meso*-HMPAO was also studied in vitro using DEM-, BSO- and nontreated mouse brain homogenates.

Received May 17, 1996; revision accepted Jul. 31, 1996.
For correspondence or reprints contact: Toru Sasaki, PhD, Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1-1 Naka-cho, Itabashi, Tokyo, Japan.

MATERIALS AND METHODS

Radiotracer Labeling

Technetium-99m-sodium pertechnetate was obtained from a ^{99}Mo/^{99m}Tc generator. Technetium-99m-*meso*-HMPAO was prepared by mixing 5 ml of fresh eluent (less than 2-hr old) ^{99m}Tc sodium pertechnetate with 0.5 mg of *meso*-HMPAO and 7.6 mg of stannous chloride, in a sealed 10 ml glass vial under the atmosphere of N_2. The ^{99m}Tc-*d,l*-HMPAO was prepared by adding 5 ml of fresh eluent of ^{99m}Tc-sodium pertechnetate to a kit containing 0.5 mg of freeze-dried *d,l*-HMPAO and 7.6 μg of stannous chloride according to the manufacture's instructions. Radiochemical purity, which was checked by three different chromatographic systems (*5*) was >94% for ^{99m}Tc-*meso*-HMPAO and >90% for ^{99m}Tc-*d,l*-HMPAO, respectively.

Treatment of Animals with DEM and BSO

DEM was dissolved in corn oil and injected intraperitoneally into five ddY mice weighing 29–31 g, five Wistar rats weighing 180–220 g and three Japanese White rabbits weighing about 2.4 kg, with a dose of 550 mg/kg body weight. Control animals were injected with only corn oil. One hour after the DEM treatment, 1.85, 1.85 and 37 MBq of ^{99m}Tc-*meso*-HMPAO were injected intravenously into the mouse, rat and rabbit, respectively.

BSO was dissolved in distilled water with pH adjusted to 7.4 by sodium bicarbonate. Five ddY mice were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and administered with BSO (0.5 mg) in a volume of 20 μl into bilaterally cerebral hemispheres. Control animals were injected with the same volume of 0.9% NaCl. Two and three days after the BSO injection, 1.85 MBq of either ^{99m}Tc-*meso*- or *d,l*-HMPAO were injected intravenously into the mice. Thirty minutes after the tracer injection, the animals were killed, and radioactivity in the brain measured by an auto well scintillation counter.

Determination of GSH and Nonprotein SH Content

GSH and thiol content were measured as follows. The tissues were homogenized with 10 volumes of 1 *M* PCA or 50 m*M* Hepes buffer (pH 7.4). The GSH content in the PCA soluble fraction was measured using the high-performance liquid chromatographic-electrochemical detection method (HPLC-ECD) with a glassy carbon electrode at 1.1V (cation-exchange column; Shodex SP-825; 8 mm i.d. × 75 mm; elution with 10 m*M* citric acid and 10 m*M* disodium hydrogen phosphate solution adjusted to pH2.1 with metaphosphoric acid; at the flow rate of 1 ml/min) (*6*). The thiol content in the whole homogenate (Hepes buffer, pH7.4) (total thiols) and as well as that in the PCA soluble fraction (nonprotein thiols) was determined by the 5,5′-dithiobis-2-nitrobenzoic acid (DTNB)-method (*7*). The thiol content in the perchloric acid

TABLE 1
Effect of Diethyl Maleate Administration on the Uptake of Technetium-99m-*meso*-HMPAO, Content of GSH and Nonprotein Thiols in the Mouse, Rat and Rabbit Brain

			Uptake of ^{99m}Tc-HMPAO (% dose/g tissue)	GSH (m*M*)	Acid-soluble-thiols (m*M*)
Mouse	Control	(n = 5)	2.343 ± 0.063 ((100)	2.025 ± 0.137 (100)	2.035 ± 0.099 (100)
	DEM	(n = 5)	0.818 ± 0.163 (34.9)†	0.684 ± 0.071 (33.8)*	0.935 ± 0.029 (45.9)*
Rat	Control	(n = 5)	0.281 ± 0.024 (100)	1.972 ± 0.017 (100)	2.065 ± 0.052 (100)
	DEM	(n = 5)	0.153 ± 0.009 (54.6)†	1.138 ± 0.106 (57.7)*	1.375 ± 0.052 (66.6)†
Rabbit	Control	(n = 3)	0.0336 ± 0.0018 (100)	2.149 ± 0.075 (100)	2.179 ± 0.101 (100)
	DEM	(n = 3)	0.0348 ± 0.0185 (104)	2.163 ± 0.147 (101)	2.247 ± 0.145 (103)

*$p < 0.05$.
†$p < 0.01$.
Parenthesis indicates percentage of control level. DEM = diethyl maleate administration.

precipitated fraction (protein thiols) was calculated by subtracting nonprotein thiols from total thiols.

Reactivity of Technetium-99m-*meso*- and *d,l*-HMPAO with GSH, Thiols, Ascorbate and GSSG In Vitro

Solutions of ^{99m}Tc-*meso*- and *d,l*-HMPAO were prepared as described above. Solutions of GSH, L-cysteine, Gly-Cys-Glu (synthesized by peptide synthesizer; chemical purity 91%), GSSG and L(+)-ascorbate were prepared in 1 ml of Hepes buffer (50 m*M*, pH 7.4) at a concentration between 500 n*M* and 9 m*M* for meso-isomer experiment and between 5 n*M* and 0.45 m*M* for d,l-isomer experiments. Fifty micro liters of freshly prepared ^{99m}Tc-*meso*- or *d,l*-HMPAO were added to test tubes containing 1 ml of various solutions as described above. The tubes were capped and mixed on a vortex mixer, then incubated at 37°C for 60 min. After incubation, 1 ml of octanol was added and the test tubes were shaken and centrifuged. The radioactivity in the organic and water phase was measured by an auto well scintillation counter. Data were expressed as the percentage of extractable radioactivity in the organic phase. Each concentration point for GSH, cysteine, Gly-Cys-Glu, GSSG and ascorbate was determined twice and their mean value plotted.

Effect of GSH Depletion on Technetium-99m-*meso*-HMPAO Reaction in Mouse Brain Homogenate

The brains of non-, DEM- and BSO-treated mice, were homogenized with 30 volumes of ice-cold 0.9% NaCl solution. The DEM and BSO had been administered in the same manner as that described above. Six kinds of brain homogenates were examined: control mouse brain homogenate alone, control brain homogenate plus N-ethylmaleimide (1 m*M*), control brain homogenate plus p-chloromercuribenzoic acid (1 m*M*), DEM-treated mouse brain homogenate, BSO-treated brain homogenate and heat inactivated (80°C for 3 min) control brain homogenate. The homogenates were prepared in a volume of 1 ml, and 50 μl of freshly prepared ^{99m}Tc-*meso*-HMPAO was added. The tubes were capped and mixed in a vortex mixer, then incubated at 37°C for 5, 10, 30 and 60 min. After the incubation, aliquots of 50 μl of homogenate were mixed with 1 ml of octanol and 1 ml of 0.9% NaCl solution and were shaken for 1 min. Then, the tubes were centrifuged and the radioactivity in the organic and in water phase measured. Data were expressed as the percentage of radioactivity extracted in the organic phase, which corresponds to intact ^{99m}Tc-*meso*-HMPAO. Each point was expressed as the mean ± s.d. of four determinations. The first-order rate constant (min^{-1}) for the conversion of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex was calculated from the rate of degradation of intact ^{99m}Tc-*meso*-HMPAO in each sample. The rate constant for the saline (0.9% NaCl) was also calculated. The relationship between the conversion rate constant (min^{-1}) and GSH concentration in non-, DEM- and BSO-treated mouse brain homogenates was plotted.

RESULTS

Effect of DEM and BSO on Technetium-99m-HMPAO Uptake

The uptake of ^{99m}Tc-*meso*-HMPAO in the mouse brain was reduced to 34.9% of the control by the DEM treatment. Accordingly, GSH content in DEM-treated mouse brain was reduced to 33.8% of the control. The DEM-treated rats showed a similar tendency to the mice, i.e., the uptake of ^{99m}Tc-*meso*-HMPAO and the GSH content were both reduced by DEM treatment to 54.6% and 55.7% of the control values, respectively. On the other hand, neither the ^{99m}Tc-*meso*-HMPAO uptake nor GSH content in the rabbit brain was reduced by DEM treatment (Table 1).

The results of ^{99m}Tc-*meso*- and *d,l*-HMPAO uptake and the GSH content in BSO-administered mouse brain are shown in Table 2. The uptake of ^{99m}Tc-*meso*-HMPAO in the brain decreased to 74% of the control value on the second day after BSO administration, and returned to the control levels on the third day. The GSH content in BSO-administered mouse brain also decreased to 62.2% of the control value on the second day after BSO administration. However, the uptake of ^{99m}Tc-*d,l*-HMPAO was not significantly decreased by BSO administration on the second day after BSO administration.

The nonprotein thiol content in animal brain, determined by the DTNB-method (*7*), is shown in Table 1. The nonprotein thiol content in the brain was reduced by the treatment with

TABLE 2
Effect of Buthionine Sulfoximine Administration on Biodistribution of Technetium-99m-*meso*- and *d,l*-HMPAO and GSH Content in Mouse Brain

	Uptake of ^{99m}Tc-HMPAO (% dose/g tissue)	GSH (m*M*)
[^{99m}Tc]*meso*-HMPAO		
Control	1.970 ± 0.118 (100)	2.082 ± 0.100 (100)
BSO (2nd day)	1.458 ± 0.106 (74.0)†	1.296 ± 0.233 (62.2)*
BSO (3rd day)	1.914 ± 0.020 (97.2)	1.728 ± 0.118 (83.0)
[^{99m}Tc]*d,l*-HMPAO		
Control	3.565 ± 0.258 (100)	
BSO (2nd day)	3.264 ± 0.157 (91.6)	

*$p < 0.05$.
†$p < 0.01$.
Numbers in parentheses indicate percentage of control level. BSO = buthionine sulfoximine administration.

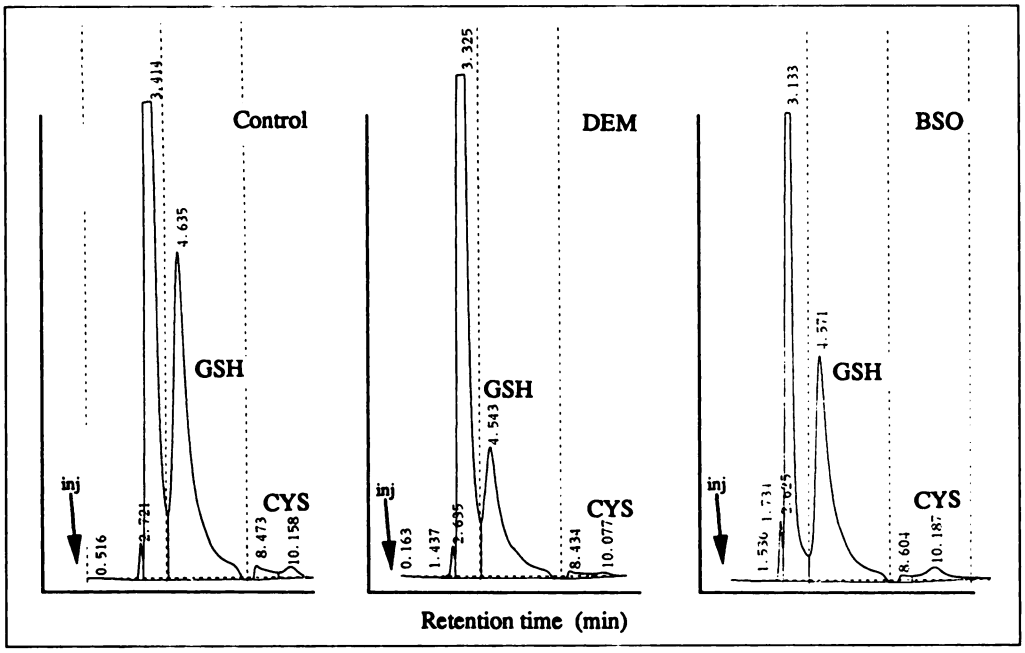

FIGURE 1. High-performance liquid chromatographic electrochemical detection chromatograms of GSH and cysteine (CYS) in samples prepared from control mouse brain diethyl maleate-treated (DEM) mouse and buthionine sulfoximine-treated (BSO) mouse brain.

DEM in mice and rats, but this was not the case in the rabbits. A major portion (>95%) of nonprotein thiols was accounted for GSH in the control mouse, rat and rabbit brain, as well as DEM-treated rabbit brain. However, the percentage of GSH/total nonprotein thiols in the DEM-treated mice and rats was 73.2% and 82.8%, respectively. Typical HPLC-ECD chromatogram pattern from samples of control, DEM- and BSO-treated mouse brain are shown in Figure 1. In all samples, the major portion of nonprotein thiols was GSH, and the cysteine content in the brain ranged from 0.02–0.03 m*M*.

Reactivity of Technetium-99m-*meso*- and *d,l*-HMPAO with GSH, Thiols, Ascorbate and GSSG In Vitro

Figure 2 shows the hydrophilic conversion of ^{99m}Tc-*meso*- and *d,l*-HMPAO by various concentration of GSH and other agents. The conversion of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex depend on GSH concentration. The conversion-concentration curve for GSH was similar to that for Gly-Cys-Glu. Cysteine was four times as potent as GSH, but GSSG and ascorbate had no effect below 9 m*M*. Qualitatively, the conversion-concentration curves for ^{99m}Tc-*d,l*-HMPAO showed the same pattern as that of ^{99m}Tc-*meso*-HMPAO. However, the conversion of ^{99m}Tc-*d,l*-HMPAO to hydrophilic complex occurred with far lower concentration (1/37) of GSH, Gly-Cys-Glu or cysteine than that of ^{99m}Tc-*meso*-HMPAO.

Effect of GSH Depletion on Technetium-99m-*meso*-HMPAO Reaction

Figure 3A illustrates the time course of hydrophilic conversion of ^{99m}Tc-*meso*-HMPAO by various brain homogenates. The estimated conversion rate constant for the control brain homogenate was 0.00452 min^{-1}, with the GSH content 0.0667 m*M*. The rate constant for homogenate of DEM- and BSO-treated mouse brain, was 0.00240 and 0.00311 min^{-1}, and their GSH content was 0.0210 m*M* and 0.0355 m*M*, respectively (Fig. 3A). The rate constant for the 0.9% NaCl solution was very small (0.000273 min^{-1}). There was good correlation between the rate constant (min^{-1}) and the GSH concentration (m*M*) among non-, DEM- and BSO-treated mouse brain homogenates. The relationship between the conversion rate constant (y: min^{-1}) and the GSH concentration (x: m*M*) in non-, DEM- and BSO-treated-mouse brain homogenates was plotted in Figure 3B. The regression line was calculated as y = 0.00142 + 0.0465x (r2 = 0.99), and was extrapolated to the point of GSH = 0 as 0.00142 (min^{-1}). This value corresponds by 31.4% to the rate constant for the control brain homogenate (0.00452 min^{-1}). Therefore, contribution of GSH to the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex in the mouse brain homogenate was estimated to be 68.6%. If the

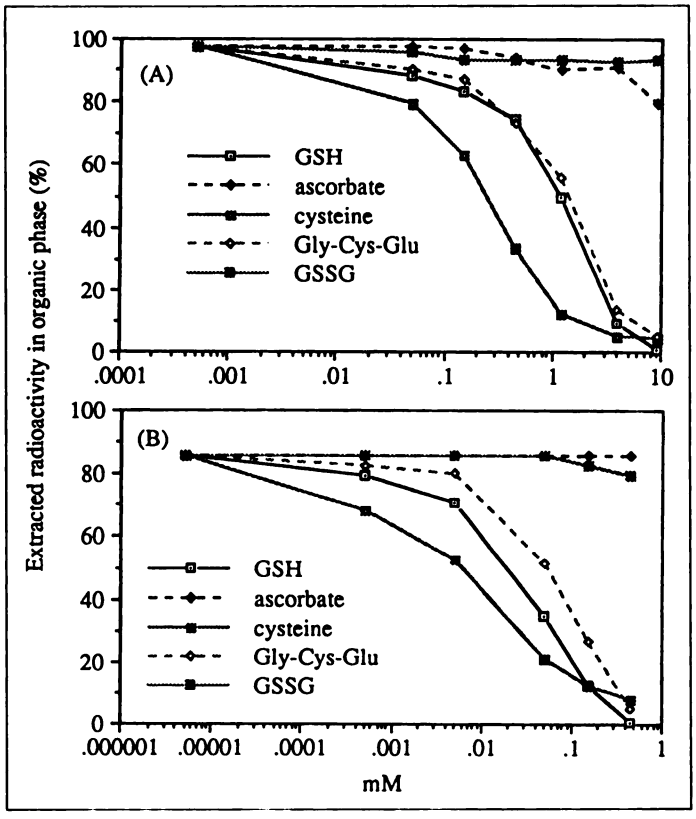

FIGURE 2. Effect of GSH, various thiols and reductant on the remaining intact fraction of ^{99m}Tc-*meso*- (A) and *d,l*-HMPAO (B) after 60 min incubation at 37°C, as expressed by extracted radioactivity in the octanol phase.

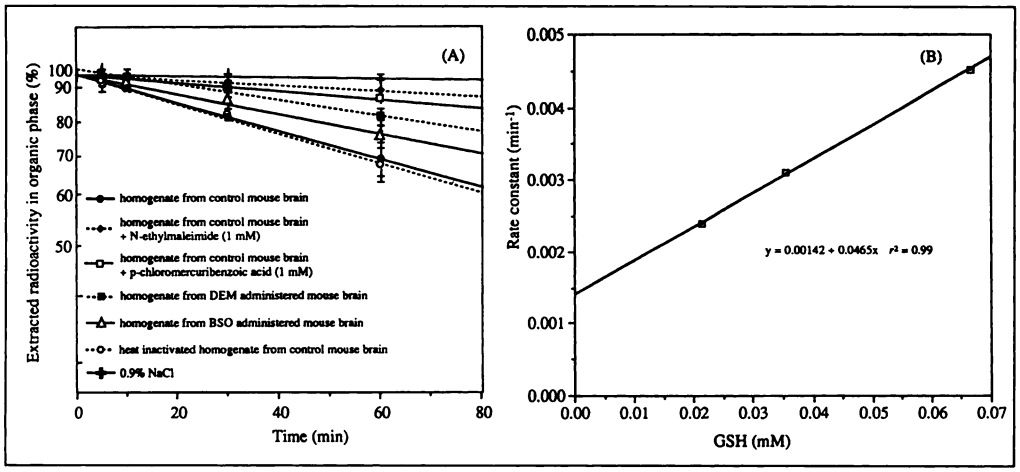

FIGURE 3. Time course of in vitro hydrophilic conversion of ^{99m}Tc-*meso*-HMPAO by various mouse brain homogenates as expressed by extractable radioactivity in organic phase (A). Each point is mean ± s.d. for four determinants. The relationship between the conversion rate constant (y: min-1) and the GSH concentration (x: m*M*) in non-DEM and BSO-treated-mouse brain homogenates (B).

automatic degradation was corrected for using the rate constant value for the saline solution, the GSH contribution was 73.0%. The hydrophilic conversion of ^{99m}Tc-*meso*-HMPAO in non-treated mouse brain homogenate was markedly reduced by the addition of N-ethylmaleimide (1 m*M*) or p-chloromercuribenzoic acid (1 m*M*) to 19.2 and 29.5% that of the control, respectively. Heating the homogenate at 80°C for 3 min to inactivate enzymes did not affect the rate constant of ^{99m}Tc-*meso*-HMPAO conversion to hydrophilic complex.

DISCUSSION

Specie differences were observed in the effect of DEM-treatment on the reduction of ^{99m}Tc-*meso*-HMPAO uptake by the brain and on the GSH depletion in the brain (Table 1). Nevertheless, the order of the reduction of ^{99m}Tc-*meso*-HMPAO uptake agreed with the order of GSH depletion: mouse > rat ≫ rabbit (not changed). α,β-unsaturated carbonyl compounds, such as DEM, react with GSH by glutathione transferase (*8*). When DEM is administered in vivo, it consumes GSH (*8*). While DEM depresses hepatic GSH levels in mice and rats, however, rabbits are resistant due to low glutathione transferase activity (*8–10*). The order in level of glutathione transferase activity between species is known to be: mouse > rat > rabbit. In this study, the reduction in GSH levels in the brain also corresponded to this order.

We also examined the effect that the administration of BSO, a GSH depletor (*11*) with a different depleting mechanism, had to the GSH from DEM (Table 2). BSO inhibits γ-glutamylcysteine synthetase, which is an enzyme for the synthesis of the GSH precursor (*11,12*). Since BSO does not cross the blood-brain barrier, it is administered through intracerebral injection (*12,13*). When BSO was administered intracerebrally into mice, the uptake of ^{99m}Tc-*meso*-HMPAO was depressed along with GSH content.

The uptake of ^{99m}Tc-*d,l*-HMPAO, a clinically used blood flow imaging agent (*5*) was not significantly affected by the BSO treatment. We have shown a similar result in an in vivo study (*1*) using DEM-administered mice. As for ^{99m}Tc-*meso*-HMPAO in the brain, k3 (rate constant for the conversion to the retainable form) is much smaller than k2 (rate constant for the washout of diffusible form), and the tissue uptake is determined by k3. On the other hand, for ^{99m}Tc-*d,l*-HMPAO in brain, k3 is so large that the tissue uptake is determined by the blood flow (*3*). El-Shirbiny et al. (*14*) reported that in spite of GSH depletion by DEM, uptake of ^{99m}Tc-*d,l*-HMPAO in rat brain was not affected. We found that a decrease in GSH content in DEM treated mouse brain accompanied a decrease in ^{99m}Tc-*meso*-HMPAO uptake (*1*). They did not examine using a ^{99m}Tc-*meso*-HMPAO. The result of the present work with BSO-administered mice was consistent with the previous results of DEM-treated mice.

The rate of conversion of ^{99m}Tc-*d,l*-HMPAO to hydrophilic complex by GSH was much faster than that of ^{99m}Tc-*meso*-HMPAO, occurring at 1/37 GSH concentration (Fig. 2). This result is consistent with those obtained in previous reports (*2,3*). There was no difference between *meso*- and *d,l*-isomers in the spectrum of in vitro reactivity with GSH, other thiols, ascorbate and oxidized glutathione (GSSG) (Fig. 2). We synthesized Gly-Cys-Glu, an analog of reduced GSH (gglutamylcysteinylglycine), and compared its reactivity to ^{99m}Tc-HMPAO with that to GSH (Fig. 2). The reactivity of Gly-Cys-Glu was not different from that of GSH. This result indicates that ^{99m}Tc-HMPAO does not specifically recognize GSH. However, ^{99m}Tc-HMPAO did not have reactivity to GSSG or ascorbate. We found that cysteine, as a simple molecule, reacts with ^{99m}Tc-HMPAO at four-times lower concentration as GSH. Nonprotein thiols, such as GSH and cysteine, react faster with DTNB than with protein thiols (*15*). On oxidation of thiols by chemicals, GSH and cysteine are oxidized faster than are protein thiols (*8*). These findings indicate that the conformation around the -SH group of the compound determines the reactivity with ^{99m}Tc-HMPAO. From this point of view, cysteine has a more reactive -SH group than GSH. However, Lang et al. (*16*) showed that ^{99m}Tc-HMPAO interacts with cysteine as well as GSH, and that the interation rate with cysteine is not different from that with GSH. They indicated that difference in pH between GSH and cysteine affects their reactivity with ^{99m}Tc-HMPAO. We have examined the interaction of ^{99m}Tc-HMPAO with GSH and other thiols in buffered solution (50 m*M* of Hepes buffer pH 7.4). Since the solution is well-buffered (50 m*M*), the different pH effects between GSH and cysteine is not considered to contribute to different interaction of ^{99m}Tc-HMPAO with GSH and cysteine.

This study indicates that ^{99m}Tc-HMPAO does not react specifically to GSH, but does react nonspecifically to thiols (Fig. 2). In previous studies, we determined the thiols in the nonprotein and protein-fractions of DEM-treated mouse brain. The nonprotein fraction was responsible for ^{99m}Tc-*meso*-HMPAO retention while GSH accounted for almost all nonpro-

tein thiols. These findings indicate that GSH is the major determinant of ^{99m}Tc-*meso*-HMPAO uptake in vivo.

In the in vitro experiment using mouse brain homogenates, SH-blocking agents (*15,17,18*), such as N-ethylmaleimide and p-chloromercuribenzoic acid, significantly inhibited the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex. The SH-blocking agents block either nonprotein or protein thiols, including enzymes (*15,17,18*). When the homogenate was heated at 80°C for 3 min to inactivate enzymes, but the conversion-reaction was not inhibited by this treatment (Fig. 3A). These results indicate that thiols in the homogenates are responsible for the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex, but enzymatic reactions are not.

The percentage of GSH contribution to the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex in mouse brain homogenate was calculated from the plots of relation between the rate constant of conversion-reaction and the GSH concentration among in non-, DEM- and BSO-treated-mouse brain homogenates (Fig. 3B). The result indicated that 68.6% (73.0% if corrected for autodegradation) of the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex in mouse brain homogenate was caused by GSH. The remaining 27.0% may be attributed to cysteine, other nonprotein thiols and unknown factors. In the study of determination of nonprotein thiols in animal brain, GSH accounted for almost all nonprotein thiols, and cysteine was found only at 1/100 of GSH concentration (Table 1, Fig. 1). Although cysteine is more potent (about 4 times) than GSH on the conversion of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex. However, we have not estimated the contribution of cysteine and protein-thiols to the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex in mouse brain homogenate. These results indicate that GSH is a major contributor to the conversion-reaction of ^{99m}Tc-*meso*-HMPAO to hydrophilic complex in mouse brain homogenate, but more complex factors as well as GSH may be involved.

CONCLUSION

This study confirmed results from an earlier report (*1*) and further suggested that GSH is the major determinant of brain uptake of ^{99m}Tc-*meso*-HMPAO in rodents.

ACKNOWLEDGMENTS

We thank Dr. J.D. Kelly, Amersham International, United Kingdom, for the kind gift of *meso*-HMPAO.

REFERENCES

1. Sasaki T, Toyama H, Oda K, et al. Assessment of antioxidative ability in brain: technetium-99m-*meso*-hexamethyl propyleneamine oxime as an imaging agent for glutathione localization. *J Nucl Med* 1996;37: 1698–1701.
2. Ballinger JR, Reid RH, Gulenchyn KY. Technetium-99m-HMPAO stereoisomers: differences in interaction with glutathione. *J Nucl Med* 1988;29:1998–2000.
3. Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen NA. The retention mechanism of technetium-99m-HMPAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metabol* 1988;8:S4–S12.
4. Andersen AR, Friberg H, Lassen NA, Kristensen K, Neirinckx RD. Assessment of the arterial input curve for ^{99m}Tc-*d,l*-HMPAO by rapid octanol extraction. *ibid* 1988;8: S23–S30.
5. Sharp PF, Smith FW, Gemmell HG, et al. Technetium-99m-HMPAO stereoisomers as potential agents for imaging regional cerebral blood flow: human volunteer studies. *J Nucl Med* 1987;27:171–177.
6. Demaster EG, Shirota FN, Redfern B, Goon DJW, Nagasawa HT. Analysis of hepatic reduced glutathione, cysteine and homocysteine by cationе-exchange high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 1984;308:83–91.
7. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70–77.
8. Plummer JL, Smith BR, Sies H, Bend JR. Chemical depletion of glutathione in vivo. *Methods Enzymol* 1981;77:50–59.
9. Boyland E, Chasseaud LF. Enzyme-catalysed conjugations of glutathione with unsaturated compounds. *Biochem J* 1967;104:95–102.
10. Grover PL, Sims P. Conjugations with glutathione. *Biochem J* 1964;90:603–606.
11. Griffith OW, Meisster A. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (S-n-butyl homocysteine sulfoximine). *J Biol Chem* 1979;254: 7558–7560.
12. Griffith OW, Meister A. Differential inhibition of glutamine and γ-glutamylcysteine synthetases by α-alkyl analogs of methionine sulfoximine that induce convulsions. *J Biol Chem* 1978;253:2333–2338.
13. Mizui T, Kinouchi H, Chan PH. Depletion of brain glutathione by buthionine sulfoximine enhances cerebral ischemic injury in rats. *Am J Physiol* 1992;262:H313–H317.
14. El-Shirbiny AM, Sadek S, Owunwanne A, Yacoub T, Suresh L, Abrel-Dayem HM. Is ^{99m}Tc-hexamethyl-propyleneamine oxime uptake in the tissues related to glutathione cellular content? *Nucl Med Commun* 1989;10:905–911.
15. Boyne AF. A methodology for analysis of tissue sulfhydryl components. *Anal Biochem* 1972;46:639–653.
16. Lang JJ. Interaction of technetium-99m-HMPAO with glutathione [Letter to the Editor]. *J Nucl Med* 1990;31:1115.
17. Beppu M, Takahashi T, Hayashi T, Kikugawa K. Mechanism of macrophage recognition of SH-oxidized erythrocytes: recognition of glycophorin A on erythrocytes by a macrophage receptor for sialosaccharides. *Biochim Biophys Acta* 1994;1223:47–56.
18. Yamaguchi T, Komoda Y, Nakajima H. Biliverdin-IX a reductase and biliverdin-IX b reductase from human liver. Purification and characterization. *J Biol Chem* 1994;269: 24343–24348.