Technetium-99m HM-PAO Stereoisomers: Differences in Interaction with Glutathione

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[^{99m}Tc]HM-PAO exists as two stereoisomers, d,l and meso, only one of which is retained in the brain. It has been suggested recently that the biodistribution of [^{99m}Tc]d,l-HM-PAO can be explained by its interaction with glutathione (GSH) in the tissues. We studied the interactions of the d,l and meso isomers with GSH in vitro by measuring the partitioning of activity between ethyl acetate and aqueous GSH solutions at various concentrations. Partitioning of both isomers demonstrated a sigmoidal relationship with GSH concentration, but the d,l isomer showed eightfold greater reactivity than the meso isomer. In a separate experiment, the d,l isomer showed a sevenfold greater interaction rate with GSH than the meso isomer. These results suggest that the stereoisomers of [^{99m}Tc]HM-PAO show differences in their interaction rate with GSH which may explain their different retention in the brain.

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Technetium-99m hexamethyl propyleneamine oxime ([^{99m}Tc]HM-PAO) was developed as a radiopharmaceutical for imaging regional cerebral perfusion (*1*). HM-PAO exists as two stereoisomers, d,l and meso, which show different in vivo behavior. Although both isomers are extracted into the brain, only the d,l isomer is retained while the meso isomer washes out (*1*). The commercial radiopharmaceutical kit contains only the d,l isomer (Ceretec, Amersham Canada Limited, Oakville, ON).

Addition of pertechnetate to an HM-PAO kit produces a mixture of radiochemical species that changes with time. In addition to the desired lipophilic [^{99m}Tc] HM-PAO complex, there are free pertechnetate, reduced-hydrolyzed technetium, and an unidentified secondary complex which is less lipophilic than [^{99m}Tc] HM-PAO (*2*).

It has been suggested recently that the biodistribution of [^{99m}Tc]d,l-HM-PAO can be explained by an interaction of the lipophilic complex with glutathione (GSH) in the tissues (*3*). We investigated the hypothesis that the difference in retention of the two isomers in the brain might be explained by a difference in interaction with GSH.

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MATERIALS AND METHODS

Solutions of d,l- and meso-[^{99m}Tc]HM-PAO were prepared as reported previously (*1,2*) by adding 1,500 MBq [^{99m}Tc] pertechnetate in 2.5 ml saline to kits containing 0.5 mg of the ligand. The radiochemical purities were determined by a three-system chromatographic technique (*1,2*) and by a simple extraction procedure validated in our laboratory (*4*).

Solutions of GSH (reduced form, Aldrich Chemical Co., Milwaukee, NJ) were freshly prepared at concentrations between 0.01 and 10 mg/ml in distilled water. Solutions of d,l- or meso-[^{99m}Tc]HM-PAO were freshly prepared and the lipophilic complex was extracted into ethyl acetate. Aliquots of 0.05 ml of the ethyl acetate solutions were added to test tubes which contained 3 ml ethyl acetate and 3 ml GSH solutions at each concentration or 3 ml distilled water as blank. The tubes were immediately capped and mixed on a vortex mixer for 1 min, then centrifuged briefly to separate the phases. The top phase was transferred to a fresh tube, and the activity in each phase was measured in a dose calibrator, to allow calculation of the % activity extracted into the aqueous GSH phase.

A second series of experiments examined the time course of the interaction. Aliquots of 0.1 ml freshly prepared d,l- or meso-[^{99m}Tc]HM-PAO were added to test tubes which contained 3 ml ethyl acetate and 3 ml GSH solution (0.1 mg/ml) or 3 ml distilled water as blank. The tubes were mixed for 0.5, 1, 2, or 4 min and analyzed as above.

RESULTS

The radiochemical purities of all preparations of d,l- and meso-[^{99m}Tc]HM-PAO were >89% at the time of

use. The results in Figure 1 show the effect of GSH concentration on extraction of the two isomers into the aqueous phase. In the absence of GSH, <5% of the activity from each isomer was present in the aqueous phase as hydrophilic impurities. Conversely, at high concentrations of GSH, >98% of the activity from each isomer was present in the aqueous phase. However, at intermediate concentrations of GSH, the d,l isomer partitioned into the aqueous phase to a greater extent than the meso isomer and there was a sigmoidal relationship between GSH concentration and % extracted.

This experiment used as starting materials the lipophilic complex of [^{99m}Tc]HM-PAO purified by extraction into ethyl acetate. Results obtained when the kit solutions were used directly (without purification) were similar after correction for the contribution due to ~10% hydrophilic impurities (data not shown).

Table 1 shows the effect of the mixing time on partitioning of the two isomers at GSH concentrations of 0 or 0.1 mg/ml. In the absence of GSH, the % extracted did not increase with mixing time for either isomer. In the presence of GSH, the % extracted increased with time, but the d,l isomer was extracted to a greater extent than the meso isomer.

The blank values were used to correct the results obtained in the presence of GSH for the contribution due to hydrophilic impurities. These corrected values were subtracted from 100% to obtain the % remaining in the organic phase. Although the mixing time was carefully controlled, interaction between [^{99m}Tc]HM-PAO and GSH continued to an unknown degree until centrifugation and separation were complete. Therefore, the data for the two isomers were extrapolated back to their point of intersection to estimate the effective reaction time, which turned out to be 1.2 min longer than the mixing time. This is a reasonable value given the manner in which the experiment was actually performed. Figure 2 presents a semi-log plot of % remaining in the organic phase as a function of the effective reaction time. The rates of disappearance (slopes) were 0.24 and 0.035 %/min for the d,l and meso isomers, respectively.

TABLE 1
Effect of Mixing Time on Extraction of d,1- and meso-[^{99m}Tc]HM-PAO into Aqueous Solutions

Mixing time (min)	% Extracted into aqueous solution*			
	[GSH] = 0		[GSH] = 0.1 mg/ml	
	d,l	meso	d,l	meso
0.5	7.7 ± 0.2	9.4 ± 0.8	39.1 ± 4.4	17.9 ± 1.5
1	7.9 ± 1.0	8.9 ± 0.7	50.3 ± 11.9	19.1 ± 2.4
2	7.4 ± 0.5	8.2 ± 0.3	65.0 ± 10.0	20.5 ± 1.4
4	6.6 ± 1.0	7.6 ± 0.3	76.3 ± 0.7	24.9 ± 2.0
	n = 3	n = 3	n = 4	n = 4

* Mean ± s.d. for n determinations.

DISCUSSION

It was originally believed that retention of [^{99m}Tc]HM-PAO in the brain and other tissues involved rapid conversion to the secondary complex (*2*). We previously have demonstrated that this conversion does not

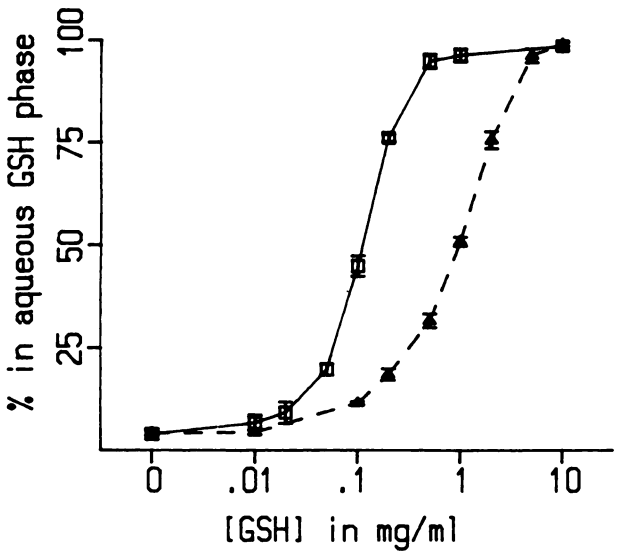

FIGURE 1
Effect of GSH concentration on extraction of d,1- and meso-[^{99m}Tc]HM-PAO into aqueous GSH solutions. Each point is mean ± s.d. for five determinations. d,1-[^{99m}Tc]HM-PAO: □——□, meso-[^{99m}Tc]HM-PAO: Δ- - - -Δ.

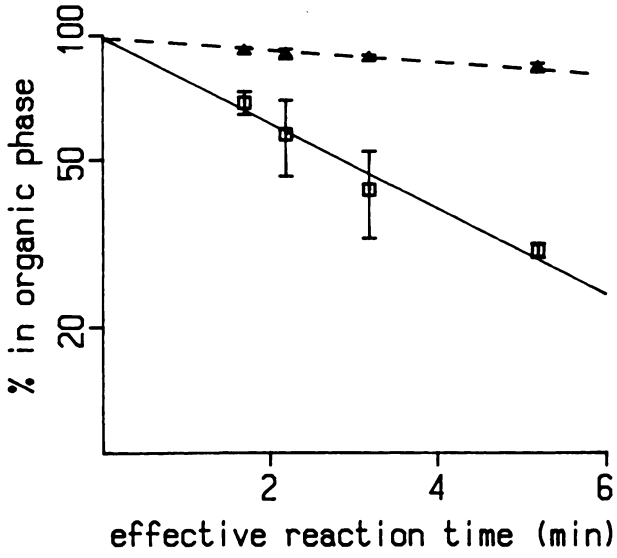

FIGURE 2
Semi-log plot of corrected % activity remaining in organic phase as a function of effective reaction time. Each point is mean ± s.d. for four determinations. d,1-[^{99m}Tc]HM-PAO: □——□, meso-[^{99m}Tc]HM-PAO: Δ- - - -Δ.

occur spontaneously in a lipophilic environment as the conversion of thallium-201 diethyldithiocarbamate ([^{201}Tl]DDC) to a polar species does (*5*). It recently has been suggested that the retention of [^{99m}Tc]d,l-HM-PAO in the brain involves interaction with GSH (*3*), but the possible reaction of the meso isomer with GSH has not to our knowledge been reported.

In the absence of GSH, <5% of the activity from each isomer was present in the aqueous phase, as hydrophilic impurities, while lipophilic HM-PAO partitioned into the organic phase. The quantitative extraction of both isomers by high concentrations of GSH shows that both isomers can interact with GSH. However, the differences in partitioning of the isomers at intermediate GSH concentrations suggest that the d,l isomer shows a more rapid interaction with GSH and/or a greater affinity for GSH. The shift between the sigmoidal curves for each isomer represents an approximately eightfold difference in reactivity.

In the second experiment, the % extracted into the aqueous GSH phase increases with reaction time and the d,l isomer is extracted to a greater extent than the meso isomer. These effects are not seen in the absence of GSH and therefore are not due to nonspecific decomposition. When account has been taken for the contribution due to hydrophilic impurities and the effective reaction time, the difference in slopes in Figure 2 suggests that the rates of interaction of the two isomers are different, and this seven-fold difference may explain the eightfold difference determined from the data in Figure 1.

The chemical form of the activity in the GSH solution was examined with the three-system chromatographic technique (*2*), but this method was too crude to identify the species present with any certainty. Gradient high-pressure liquid chromatography (HPLC) will be required to differentiate between such possibilities as a complex between GSH and HM-PAO or catalysis by GSH of the conversion of lipophilic HM-PAO to the secondary complex.

In conclusion, these results suggest that the stereoisomers of [^{99m}Tc]HM-PAO show differences in their interaction with GSH, which may explain their different retention in the brain.

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