Interaction of Technetium-99m-HM-PAO with Glutathione

TO THE EDITOR: A number of publications have recently appeared in *The Journal of Nuclear Medicine* which dealt with the decomposition of the stereoisomers of technetium-99m-hexamethyl-propyleneamine oxime (^{99m}Tc-HM-PAO), with the lipophilic-hydrophilic transformation, with kinetic analyses of the decomposition, and with the role of glutathione (GSH) in these processes (*1–3*).

During the development of an HM-PAO kit destined for use in our own laboratory, we have already encountered a considerable proportion of the problems mentioned in the publications referred to above. The bulk of our experimental results are in accord with those described in the above publications. In the present communication, I should like to mention briefly those of our results which supplement the published data or which put them in a somewhat different light.

It was reported by Ballinger et al. (*3*) that the stereoisomers of HM-PAO interact to different extents with GSH, and they suggest that this might be the reason for the differences in cerebral retention. We have repeated the experiments they describe and have found that they were perfectly reproducible (effects of GSH concentration and incubation time on the extractability of d,l and meso-HM-PAO from an aqueous GSH phase), but we do not agree completely with the conclusions drawn from the experiment. Ballinger et al. appear to have worked with a non-buffered aqueous medium (at least their article makes no mention of a buffer). Under the experimental conditions they applied, interval 0-10 mg/ml the pH varied between 6.5 and 2.75. We have performed an experiment with Tc-d, l-HM-PAO in which the Ballinger method was used and the pH of the aqueous medium was varied between 6.5 and 2.9 with 0.05 *M* HCl. After a mixing time of 4 min, the following results were obtained:

At pH 6.5, the aqueous phase contained 3.4% ± 0.82% of the radiopharmacon. At pH 2.9, the aqueous phase contained 12.72% ± 1.61% of the radiopharmacon.

In these experiments, n = 5. This result demonstrates that in their experiment Ballinger et al. measured the resultant of the effects of GSH and pH. However, there is no doubt that the pH has only a negligible effect on the lipophilic-hydrophilic transformation. For at a GSH concentration of 10 mg/ml, where the pH was 2.75, the aqueous phase contained 84.6% ± 2.8% of the test substance.

Acknowledging the fact that the pH is approximately neutral under physiologic conditions, we carried out experiments under the previous conditions, using an aqueous medium containing various GSH concentrations, but neutralized with $NaHCO_3$. We obtained the following results:

At a GSH concentration of 0.1 mg/ml and pH 4.4, the aqueous phase contained 78.4% ± 1.9% of the radiopharmacon and contained 4.29% ± r0.627% of the radiopharmacon at pH 7.59 (buffered).

These results demonstrated that in neutral or mildly basic medium GSH had no effect at all on the lipophilic-hydrophilic transformation.

When GSH was replaced by L-cysteine HCl under otherwise identical conditions, we obtained the following results:

At an L-cysteine HCl concentration of 0.1 mg/ml and pH 3, the aqueous phase contained 68.2% ± 2.3% of the radiopharmacon and at pH 7.4 (buffered), the aqueous phase contained 5.87% ± 1.1% of the radiopharmacon.

Since GSH and cysteine exert similar effects, there is a high probability that GSH does not have a specific effect on the lipophilic-hydrophilic transformation.

In our view, these experimental results strongly suggest that, under in vitro conditions, GSH acts on the lipophilic-hydrophilic transformation merely as a reductant, and that this effect can be suspended by neutralization of the medium. This view is supported by our experimental results which (similarly to those of Hung et al.) indicated that increase of the quantity of Sn^{2+} in the HM-PAO kit accelerates transformation of the lipophilic Tc-d, l-HM-PAO complex to the hydrophilic complex (*4,5*).

We consider that the foregoing in vitro experiments demonstrate only that the lipophilic-hydrophilic transformation of Tc-d, l-HM-PAO is greatly accelerated by reductants. Accordingly, any redox system present physiologically in the organism and operating at physiologic pH can be "suspected" for the lipophilic-hydrophilic transformation of Tc-d, l-HM-PAO.

REFERENCES

1. Hung JC, Corlija M, Volkert WA, Holmes RA. Kinetic analysis of technetium-99m d,l-HM-PAO decomposition in aqueous media. *J Nucl Med* 1988; 29:1568–1576.
2. Neirinckx RD, Harrison RC, Forster AM, Burke JF, Andersen AR, Lasser NA. A model for in-vivo behaviour of Tc-99m d,l-HM-PAO in man [Abstract]. *J Nucl Med* 1987; 28:559.
3. Ballinger JR, Reid RH, Gulenchyn KY. Technetium-99m HM-PAO stereoisomers: differences in interaction with glutathione. *J Nucl Med* 1988; 29:1998–2000.

Jenö J. Láng
Department of Nuclear Medicine
Albert Szent-Györgyi Medical University
Szeged, Hungary

REPLY: We thank Dr. Lang for his interest in our work (*1*) and welcome this opportunity to respond to his letter (*2*).

Dr. Lang is quite correct in assuming that our glutathione (GSH) solutions were not buffered and therefore varying pH

TABLE 1
Effect of Choice of Acid on Extraction of d,1- and meso-^{99m}Tc-HM-PAO into Aqueous Solutions

Aqueous phase	pH	% Extracted into aqueous solution*	
		d,1	meso
Water	6.4	14.0 ± 1.2	10.6 ± 0.8
HCl	3.0	37.0 ± 2.9	25.2 ± 1.2
Acetic acid	3.0	28.7 ± 3.0	20.1 ± 1.5

* Mean ± s.d. for 4 determinations.

did contribute to the effects observed, although this contribution is relatively small, as he demonstrated (*2*). However, in his experiments pH was adjusted with HCl and there may be an effect of chloride ion concentration as well as hydrogen ion concentration. The results in Table 1 show that when acetic acid rather than HCl was used to adjust the pH to 3, there was less transformation of either isomer of ^{99m}Tc-HM-PAO into hydrophilic species.

The observation that there was no interaction with GSH in neutral or mildly basic medium is puzzling. In attempting to reproduce these results, we found that interaction was slower, but not stopped. Furthermore, the experiments in which Neirinckx et al. determined interaction rates of d,1- and meso-^{99m}Tc-HM-PAO with GSH were carried out at pH 7.4 (*3*).

We, too, have noted that ^{99m}Tc-HM-PAO interacts with L-cysteine (Cys), and also with ascorbic acid, both of which we were evaluating as potential antioxidant stabilizers. The interaction with Cys should not be surprising, since Cys is a component of GSH (Glu-Cys-Gly). We have now repeated the concentration-effect and reaction-rate experiments reported previously (*1*) with Cys in place of GSH. The results can be summarized as follows:

1. The interaction of ^{99m}Tc-HM-PAO with Cys shows a sigmoidal relationship similar to that with GSH (see Fig. 1 in ref. *1*).
2. The disappearance rates for the isomers of ^{99m}Tc-HM-PAO in the presence of 0.05 mg/ml Cys HCl (approximately equimolar to 0.1 mg/ml GSH) were: d,1 0.052 min^{-1}; meso 0.017 min^{-1}. These values for interaction with Cys differ by a factor of 3 between the isomers, compared to 7-8 with GSH (*1*).

Thus, ^{99m}Tc-HM-PAO interacts with Cys but this interaction appears to be less stereoselective than that with GSH and may not be sufficient to explain the differential retention of the isomers in the brain. Furthermore, as discussed by Neirinckx et al. (*3*), GSH constitutes the bulk of all free thiols in mammalian tissue and is present in millimolar concentrations similar to those used in the in vitro experiments. We feel that the evidence still supports GSH as the main but not the only biologic target of ^{99m}Tc-HM-PAO.

REFERENCES

1. Ballinger JR, Reid RH, Gulenchyn KY. Technetium-99m HM-PAO stereoisomers: differences in interaction with glutathione. *J Nucl Med* 1988; 29:1998–2000.
2. Lang JJ. Interaction of ^{99m}Tc-HM-PAO with glutathione [Letter]. *J Nucl Med* 1990; 31:1115.
3. Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen NA. The retention mechanism of technetium-99m-HM-PAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metab* 1988; 8:S4–S12.

James R. Ballinger
Ottawa Civic Hospital
Ottawa, Ontario, Canada

PET Cardiac Viability

TO THE EDITOR: The clinical pathologic conference (CPC) discussed by Abraham (*1*) is timely, important, and elegant. Owing to my own particular research with this subject (*2–4*), I have reviewed clinical PET data on cardiac viability.

In the established evolution of acute infarction, recently reviewed (*5*) acute infarction without reperfusion has no viability. Reperfused myocardium may evolve into subendocardial, midmyocardial, and transmural necrosis, or fractions thereof. Salvaged myocardium is viable.

Hibernation is an attractive clinical state without sound experimental proof. Specifically, a recent conference on viability so concluded (*6*), since no investigation has shown that an area of myocardium can be akinetic, hypoperfused, and metabolically active. The clinical measurement of regional blood flow has only been reported by one group (*7*) and the data is preliminary.

As the comment in the CPC states, a paper by Tillisch et al. (*8*) indicated that the detection of myocardial viability by PET would have predictive value before revascularization. I must say that I was less convinced by the data. Seventeen consecutive patients with coronary disease and resting wall motion (WM) abnormalities had: a PET study (nitrogen-13-ammonia and fluorodeoxyglucose (FDG), a WM study before surgery, and a WM study after surgery. Of the 17 patients, 15 had prior infraction and EKG Q-waves. Patients had two- or three-vessel disease and a preoperative ejection fraction of 32% ± 14%. Before operation, 73 segments had abnormal WM. Postoperatively, WM improved in 37 (51%) of the segments. Most of the septal (77%) abnormalities did not improve. The inferior wall of the heart could not have been assessed since only axial PET slices were obtained. Of 16 revascularized areas that had preoperative PET viability (higher FDG uptake than ^{13}N-ammonia hypoperfusion), 13 had improved WM after operation. There was no correlation between preoperative WM and degree of postoperative improvement of WM, or lack thereof.

The following questions need answering:

1. The PET device used had a resolution of 1.8 cm or greater. Was this a factor in results?
2. The evaluation of WM was nonquantitative. Did this bias the comparison?
3. The PET planes and the WM planes could not have been accurately matched. Was this important?
4. If the septal regions with abnormalities did not improve and since the inferior wall was not evaluated (there were no short-axis or long-axis images used), what exactly did the data reflect?