Radiopharmaceutical Evaluation of Tc-99m Hydroxymethylene Diphosphonate

Walter Majewski, A. Michael Zimmer, and Stewart M. Spies

Northwestern University Medical School, Chicago, Illinois

The radiopharmaceutical purity and stability of Tc-99m hydroxymethylene diphosphonate were studied over a ten-week period using miniaturized chromatography to evaluate levels of both free pertechnetate and hydrolyzed reduced Tc-99m. Results showed good preparation stability up to 4 hr postformulation even when 400 mCi of activity was added. After 4 hr postformulation, some inconsistent radiopharmaceutical degradation was observed. Both old and new formulations of Tc-99m hydroxymethylene diphosphonate were very sensitive to air introduction. The new formulation was less sensitive to air oxidation because of higher stannous chloride and gentisic acid levels. We stress careful technique when dispensing Tc-99m hydroxymethylene diphosphonate in order to minimize any air introduction.

The search for a more ideal radiopharmaceutical for bone imaging is still in progress. The newest bone imaging agent that has gained acceptance in nuclear medicine is hydroxymethylene diphosphonate (HDP) (Osteoscan, Procter and Gamble, Cincinnati, OH). We investigated the radiopharmaceutical purity and stability of this product under various clinical conditions.

Materials and Methods

While this study was in progress, commercial HDP was reformulated by the manufacturer; both "old" and "new" formulations for HDP are listed in Table 1. Various aspects of the study were performed with old and new formulations and results compared whenever appropriate.

Commercially available HDP (old formulation) was evaluated in a clinical setting over a five-week period. Approximately 150–300 mCi of generator pertechnetate was added to each vial daily. The same volume was maintained in each vial throughout the study by varying nonbacteriostatic normal saline. Levels of free pertechnetate and Tc-99m-hydrolyzed reduced (Tc-HR) were determined at 0.5, 2.5, 4.5, 6.5, and 7.5 hr after formulation using miniaturized chromatography (*1,2*). Whatman 31 ET chromatography paper with acetone was used to evaluate free pertechnetate and Gelman ITLC-SG chromatography paper with distilled water was used to evaluate Tc-HR. The chromatography procedure is described in Table 2.

The "loading" capacity of HDP was tested by adding increasing concentrations of pertechnetate, ranging from 100–400 mCi, to each of four HDP vials (old formulation). A constant 5-ml volume was maintained in each vial by varying nonbacteriostatic normal saline. Radiochemical evaluations were performed at 0.5, 2.5, 4.5, and 7.5 hr postformulation using the chromatography procedure described. Five repetitive determinations were made for each HDP prepara-

TABLE 1. Old and New HDP Formulations

Ingredients	Old formulation (mg/vial)	New formulation (mg/vial)
Stannous chloride	0.16	0.24
Oxidronate sodium	2.0	3.0
Gentisic acid	0.56	0.84

TABLE 2. Determining Free Pertechnetate and Tc-99m-HR in Commercial Tc-99m-Labeled HDP Agent

1. Place approximately 1 ml of acetone and 1 ml of distilled water in each 10-ml glass vial.
2. Spot radiopharmaceutical 1 cm from bottom of Whatman 31 ET and Gelman ITLC-SG chromatography strip (1 cm × 6 cm strips).
3. Develop 31 ET strip in acetone and SG strip in distilled water until solvent migrates to top of strip.
4. Cut 31 ET strip 2 cm from origin and SG strip 1 cm from origin.
5. Count all sections for activity (per unit time) using gamma counter and subtract background. Determine free pertechnetate and hydrolyzed reduced Tc-99m levels.

For reprints contact: A. Michael Zimmer, Dept. of Nuclear Medicine, Northwestern Memorial Hospital, Superior and Fairbanks Court, Chicago, IL 60611.

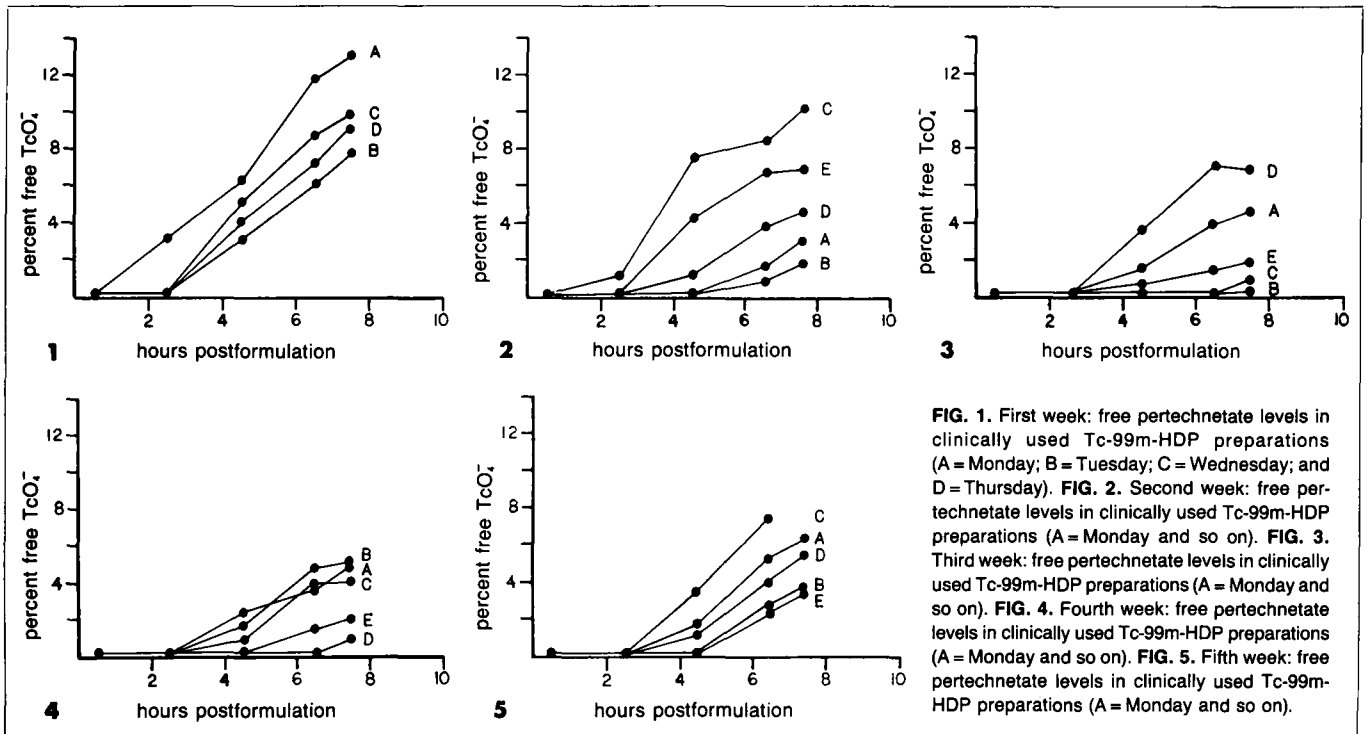

FIG. 1. First week: free pertechnetate levels in clinically used Tc-99m-HDP preparations (A = Monday; B = Tuesday; C = Wednesday; and D = Thursday). **FIG. 2.** Second week: free pertechnetate levels in clinically used Tc-99m-HDP preparations (A = Monday and so on). **FIG. 3.** Third week: free pertechnetate levels in clinically used Tc-99m-HDP preparations (A = Monday and so on). **FIG. 4.** Fourth week: free pertechnetate levels in clinically used Tc-99m-HDP preparations (A = Monday and so on). **FIG. 5.** Fifth week: free pertechnetate levels in clinically used Tc-99m-HDP preparations (A = Monday and so on).

tion at each specific time period and the data statistically summarized.

We compared the stability of the old and new Tc-99m-HDP formulations by adding approximately 300 mCi of pertechnetate to each preparation (total volume: 5 ml) and evaluating free pertechnetate and Tc-HR levels as outlined above.

We tested the sensitivity of HDP preparations (both old and new formulations) to air oxidation. For each formulation, 10 ml of air was introduced into one of two identical HDP preparations, each of which was previously reconstituted by adding approximately 250 mCi of pertechnetate activity. Chromatographic analysis was performed at 0.5, 2.5, 4.5, and 8.5 hr after formulation as previously described. For each preparation, five repetitive determinations were performed at each time interval and the data statistically summarized.

Results and Discussion

Results of the radiochemical evaluation of Tc-99m-HDP preparations (old formulation) are shown in Figs. 1–5; each graph corresponds to one week. In general, good preparation stability was observed up to 4 hr postformulation as shown by the relatively low free pertechnetate levels in the preparations. After 4 hr, free pertechnetate levels increased, reaching levels up to 13% at 7.5 hr after preparation. Technetium-HR levels remained at less than 3% throughout the study. No apparent correlation was observed between day of generator technetium elution and stability of radiopharmaceutical preparation. The data indicate that once initial chromatography is performed, Tc-99m-HDP preparations may be used within 4 hr after preparation. However, for longer time periods, additional quality control should be performed to confirm radiopharmaceutical quality.

The results of the effects of increasing pertechnetate on the stability of Tc-99m-HDP preparations (old formulation) are shown in Table 3. Greater instability was observed with higher activity pertechnetate preparations. The 300-mCi Tc-99m-HDP preparation showed higher levels of free pertechnetate than other preparations, including the 400-mCi activity preparation. Free pertechnetate levels of 6.2% were observed at 7.5 hr postformulation for the 300-mCi radiopharmaceutical

TABLE 3. Stability of Tc-99m-HDP (Old Formulation) with Increasing Pertechnetate Activity

Hours after formulation	Fraction	Activity (mCi) in each vial			
		100	200	300	400
0.5	Tc-pertechnetate	0.2 ± 0.05*	0.1 ± 0.05	0.07 ± 0.01	0.08 ± 0.01
	Tc-HR	0.5 ± 0.05	0.5 ± 0.1	0.4 ± 0.05	0.4 ± 0.2
2.5	Tc-pertechnetate	0.1 ± 0.05	0.08 ± 0.02	0.001 ± 0.01	0.08 ± 0.01
	Tc-HR	0.9 ± 0.5	0.9 ± 0.4	0.9 ± 0.5	0.65 ± 0.1
4.5	Tc-pertechnetate	0.1 ± 0.05	0.1 ± 0.05	2.4 ± 0.05	0.3 ± 0.05
	Tc-HR	1.0 ± 0.8	0.8 ± 0.4	0.9 ± 0.1	0.7 ± 0.1
7.5	Tc-pertechnetate	0.3 ± 0.05	0.2 ± 0.05	6.2 ± 0.1	3.3 ± 0.1
	Tc-HR	2.0 ± 1.8	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1

* mean percent ± standard deviation; N = 5.

preparation. Technetium-HR levels remained at less than 2% throughout the study.

The higher free pertechnetate levels obtained with the 300-mCi activity preparation were further examined and it was discovered that multiple syringe entries were made into this particular preparation. The experiment was repeated for the 300-mCi activity Tc-99m-HDP preparation (old and new formulations) with minimal syringe entries and the results are shown in Table 4. Results demonstrate minimal radiopharmaceutical oxidation as shown by the low pertechnetate levels even up to 7.5 hr postpreparation for both old and new formulations (0.1% free pertechnetate). Technetium-HR levels again remained at or less than 1%. The significant differences between the 300-mCi activity preparations of Tables 3 and 4 prompted the hypothesis that air introduction during syringe entry was responsible for radiopharmaceutical oxidation and subsequent free pertechnetate production.

TABLE 4. Stability of 300-mCi Tc-99m-HDP Preparations: Old Versus New Formulation

Hours after formulation	Fraction	Old formulation	New formulation
0.5	Tc-pertechnetate	0.1 ± 0.06*	0.1 ± 0.05
	Tc-HR	0.8 ± 0.2	0.6 ± 0.2
2.5	Tc-pertechnetate	0.1 ± 0.05	0.1 ± 0.05
	Tc-HR	0.9 ± 0.2	0.6 ± 0.1
4.5	Tc-pertechnetate	0.1 ± 0.05	0.1 ± 0.05
	Tc-HR	1.0 ± 0.1	0.6 ± 0.2
7.5	Tc-pertechnetate	0.1 ± 0.05	0.1 ± 0.05
	Tc-HR	0.8 ± 0.2	0.7 ± 0.3

* mean percent ± standard deviation; N = 5.

The effect of air introduction on free pertechnetate production in Tc-99m-Sn-HDP preparations is shown in Table 5. Free pertechnetate levels increased dramatically from 0.1% at 0.5 hr to 11.3% at 8.5 hr after preparation in the old formulation. Air introduction also increased free pertechnetate levels in the new formulation; however, the increases were not as dramatic (5.2% free pertechnetate after 8.5 hr). With no air introduction, free pertechnetate in both old and new formulations remained low even after 8.5 hr (0.4% or less free pertechnetate).

Although both types of formulations were sensitive to air oxidation, the newer formulation appeared to be less sensitive than the older formulation. This may be due to higher stannous chloride and gentisic acid levels in the newer formulation (Table 1). Excess tin (stannous) and gentisic acid (antioxidant) could maintain Tc-99m in the reduced complexed form even in the presence of additional oxidizing agents (air). The sensitivity of Tc-99m-Sn-HDP to air oxidation indicates that multidose dispensing must be carefully controlled in order to minimize air introduction, which can result in radiopharmaceutical degradation.

TABLE 5. Effect of Air on Old and New Tc-99m-HDP Formulations

Hours after formulation	Fraction	Old formulation		New formulation	
		Air added	No air	Air added	No air
0.5	Tc-pertechnetate	0.1 ± 0.05*	0.02 ± 0.01	0.1 ± 0.05	0.05 ± 0.01
	Tc-HR	0.6 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.7 ± 0.5
2.5	Tc-pertechnetate	0.5 ± 0.1	0.04 ± 0.02	0.2 ± 0.05	0.1 ± 0.05
	Tc-HR	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.05	0.4 ± 0.05
4.5	Tc-pertechnetate	5.3 ± 0.1	0.03 ± 0.01	0.2 ± 0.05	0.3 ± 0.1
	Tc-HR	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
6.5	Tc-pertechnetate	8.7 ± 0.2	0.05 ± 0.01	2.2 ± 0.05	0.5 ± 0.1
	Tc-HR	0.9 ± 0.3	0.6 ± 0.1	0.5 ± 0.1	2.2 ± 0.1
8.5	Tc-pertechnetate	11.3 ± 0.1	0.1 ± 0.01	5.2 ± 0.1	0.4 ± 0.1
	Tc-HR	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.0 ± 0.4

*mean percent ± standard deviation; N = 5.

References

1. Zimmer AM, Pavel DG. Rapid miniaturized chromatographic quality control procedures for Tc-99m radiopharmaceuticals. *J Nucl Med* 1977;18:1230-33.

2. Taukulis RA, Zimmer AM, Pavel DG, et al. Technical parameters associated with miniaturized chromatography systems. *J Nucl Med Technol* 1979;7:19-22.