Routine Application of Fractionated HMPAO Stored at −70°C for WBC Scintigraphy

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The radiochemical purity of ^{99m}Tc-HMPAO prepared from fractions of reconstituted HMPAO stored at −70°C and its application in the radiolabeling of human granulocytes was investigated. Upon reconstitution of a vial of HMPAO with 1 ml of saline and subsequent freezing at −70°C, small fractions were obtained on each of four consecutive days with the vial being refrozen after each dispensing. Following radiolabeling of the HMPAO fractions with pertechnetate, mean radiochemical purity results met or exceeded manufacturers' specifications for the radiopharmaceutical on each of the four days. Imaging with radiolabeled granulocytes using ^{99m}Tc-HMPAO prepared by this technique resulted in high quality clinical studies. These results demonstrate that a vial of HMPAO can be fractionated, after storage at −70°C with no loss of clinical utility for radiolabeling granulocytes and considerable cost savings.

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Hexamethylenepropyleneamine oxime (HMPAO) has allowed ^{99m}Tc to rival ^{111}In as the radionuclide of choice for white blood cell (WBC) labeling for a variety of inflammatory indications (*1–5*). The major advantages of ^{99m}Tc include availability, cost, reduced radiation exposure to patients, feasibility of portable WBC scintigraphy, increased information density and earlier diagnostic imaging.

Although ^{99m}Tc WBC scintigraphy grows in popularity, the constraints imposed by the use of HMPAO include the cost, the very short shelf life of the ^{99m}Tc-HMPAO and the need for high specific activity pertechnetate.

Many attempts have been made to improve the utilization of the labeled product (*6–10*). Investigations into the fractionation of HMPAO reconstituted with saline and subsequently frozen have demonstrated good results (*8, 10*) although we have found inconsistent reproducibility in our laboratory. Recently Hawkins et al. (*11*) have suggested that dispensed fractions of reconstituted HMPAO, stored at −66°C proved suitable for WBC radiolabeling although the radiochemical purity results appeared to be less than ideal.

We wished to utilize HMPAO in a more efficient manner and evaluated the long-term storage of reconstituted HMPAO at −70°C with subsequent thawing of frozen HMPAO, dispensing of small fractions and refreezing. The dispensed HMPAO fractions were radiolabeled with pertechnetate, and investigated for applicability in the radiolabeling of granulocytes.

MATERIALS AND METHODS

Technetium-99m-HMPAO Preparation

HMPAO (exametazine) was purchased commercially (Ceretec, Amersham). A vial of HMPAO was reconstituted with 1 ml of normal saline, and the day of initial reconstitution of a vial of HMPAO was considered as Day 0. The schedule of amounts dispensed is shown in Table 1. On Day 0, 0.18 ml of HMPAO (90 μg) was dispensed, and the vial then placed in a freezer at −70°C within 1 min. The dispensing of subsequent fractions of HMPAO was performed by thawing out the vial under warm tap water and withdrawing the fraction from the vial using a 1-cc syringe equipped with a 25 g × 16 mm needle. The vial was then replaced in the −70°C freezer within 1 min. Prior to dispensing the HMPAO the 'dead space' of the syringe and needle were filled with normal saline, thereby eliminating the presence of air.

Pertechnetate was obtained from an 80 GBq ^{99}Mo/^{99m}Tc dry generator (Nordion Int, Kanata, Ontario). This size of generator allows the pertechnetate volume to be maintained as small as possible. The pertechnetate (1000 MBq, 0.08–0.5 ml) was dispensed with a 1 cc syringe equipped with a 22 g × 40 mm needle. The "dead" space of the needle and syringe were first filled with normal saline thereby minimizing the introduction of air and allowing the most accurate dispensing of the volume of pertechnetate. Pertechnetate used in these preparations of ^{99m}Tc-HMPAO was less than 4 hr old and obtained from generators in which the in-growth time was 24 hr or less.

Preparation of the ^{99m}Tc-HMPAO used for clinical studies was performed in a sterile Laminar flow hood. The pertechnetate was added to the syringe containing the HMPAO fraction through the hub via the 22 g × 40 mm needle. The length of the 22 g × 40 mm needle enabled the pertechnetate to be added throughout the entire volume of HMPAO to ensure thorough mixing. The needle was then replaced on the 1 cc syringe now containing the ^{99m}Tc-HMPAO. The mixture was left to incubate for 3 min.

Radiochemical purity (RCP) was assessed by instant thin-layer chromatography using short strips of solvent absorption pads (Gelman Sciences, Ann Arbor, MI) with ethyl acetate as the

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TABLE 1
HMPAO Fraction Dispensing Schedule

Day	0	1	2	3
Volume	0.18 μl	0.20 μl	0.22 μl	0.24 μl
HMPAO	90 μg	100 μg	110 μg	120 μg

solvent. This technique has been shown to give similar results to the manufacturer recommended technique (*12*). Percentage primary bound ^{99m}Tc-HMPAO was calculated according to:

$$\frac{^{99m}\text{Tc counts top half}}{^{99m}\text{Tc counts top half} + ^{99m}\text{Tc activity bottom half}}.$$

Separation and Radiolabeling of Granulocytes

Eighty milliliters of whole blood was collected using ACD (Acid Citrate Dextrose, Solution A, University Hospital, London, Ontario) anticoagulant in a ratio of 1:7. Heta-starch red blood cell sedimentation was followed by separation of the granulocytes from leukocyte-rich plasma using a discontinuous percoll/plasma gradient (*13*). Purified granulocytes were resuspended in 1 ml of Hanks Balanced Salt Solution for radiolabeling. The ^{99m}Tc-HMPAO was prepared at this time. A total of 1000 MBq (0.26–0.66 ml) of ^{99m}Tc-HMPAO was added to the cells. The mixture was incubated for 15 min with occasional mixing by inversion. After incubation the granulocytes were washed once with 5 ml of autologous plasma, the labeling efficiency determined and the cells resuspended in 2 ml of autologous plasma for injection into the patient. Cell viability was assessed by Trypan Blue exclusion.

Technetium-99m-Granulocyte Imaging

Technetium-99m-granulocytes labeled as above were utilized in clinical studies in patients with suspected focal infections. Approximately 370 MBq were slowly infused intravenously. Images of the abdomen and pelvis were obtained within the first hour and subsequent images of the entire body or images localized to the site of questionable infection were obtained 4–6 hr after infusion. In general, images of the torso were obtained to 500,000 counts while images of the extremities were acquired to the same time as the torso images. At the authors' institution there is over 5 yr experience with ^{99m}Tc-granulocyte imaging. Since there was no reason to suspect that ^{99m}Tc-granulocytes labeled using the current fractionated HMPAO technique should behave differently from ^{99m}Tc granulocytes labeled using fresh ^{99m}Tc-HMPAO, a strict evaluation of sensitivity and specificity was not felt necessary. However, all images of 15 studies utilizing ^{99m}Tc-granulocytes labeled using the fractionated HMPAO technique were reviewed for overall image quality, both in areas of normal distribution and areas of known inflammation or infection. Comparison was made to the authors' historic experience with ^{99m}Tc-granulocyte imaging.

Retrospective sterility and pyrogen testing were performed to evaluate the reconstituted contents of vials of HMPAO handled by these techniques. A series of nine spent HMPAO vials were tested for sterility by routine aerobic culture. Each vial had been entered four times for HMPAO sampling and stored at −70°C from 2 to 12 days.

Also a series of six spent HMPAO vials were pyrogen tested retrospectively by standard limulus testing. Each vial had been entered four times for HMPAO sampling and stored at −70°C from 3 to 5 days.

RESULTS

Radiochemical purity results for ^{99m}Tc-HMPAO prepared from various fractions dispensed from vials of HMPAO frozen at −70°C are shown in Table 2. All of the ^{99m}Tc-HMPAO preparations yielded RCP results greater than or equal to 80% (range 80%–95%). It is apparent from results on Day 3 that even after three freeze/thaw cycles the mean percent bound primary ^{99m}Tc-HMPAO (92.0% ± 2.7%) using 4 hr old pertechnetate, still meets manufacturers' RCP specifications (>80%). Mean labeling efficiency for granulocytes was 41% (n = 46, range 28%–90%). This compares well with a mean labeling efficiency of 51% (n = 272) observed over the previous 12 mo period at our institution when the radiolabeling protocol used one vial of HMPAO per WBC study. Routine trypan blue results showed greater than 95% viability.

Clinical radiolabeled WBC studies average approximately one study per day per annum in our institution. Therefore, a vial fractionated by this technique rarely lasts more than four days. However, on occasion vials have been stored frozen up to 12 days. The RCP results of ^{99m}Tc-HMPAO prepared from fractions stored this length of time averaged 88.3% (n = 3, range 86%–90%). Longer term storage conditions have not been evaluated.

Excellent quality images were obtained in all 15 patients studied using ^{99m}Tc granulocytes labeled with the fractionated HMPAO technique outlined. No disturbances of normal distribution were noted and known sites of inflammation or infection had excellent uptake (Figs. 1–3). In all respects the images of both normal areas of distribution and sites of inflammation or infection were thought to be comparable to previous ^{99m}Tc-granulocyte imaging performed at our institution.

Sterility testing from the series of nine spent HMPAO vials was consistently reported as negative. No pyrogens were detected in the six spent vials examined by Limulus testing at a 1/40 dilution with a sensitivity controlled at 0.25 endotoxin units per milliliter.

DISCUSSION

Since the introduction of ^{99m}Tc-HMPAO as a lipophilic radiopharmaceutical for regional cerebral blood flow scintigraphy and its application to radiolabeling WBC, researchers have attempted to maximize the utilization of a vial of HMPAO (*6–11*). Most of the work towards this goal has been aimed at stretching the shelf life of the radioactive product (*6,9*). Recently, fractionating a reconstituted vial into single doses and storing the individual doses at cold temperatures (−10°C to −66°C) has been evaluated with modest results (*8,10,11*). A common theme to most of this work has been the delicate manipulation of this radiopharmaceutical in light of the very low concentration of stannous ion present in the kit. We specu-

TABLE 2
Radiochemical Chemical Purity of ^{99m}Tc-HMPAO After 0–3 Days of Storage of Reconstituted HMPAO at −70°C

Age of TcO_4^-	Day 0	Day 1	Day 2	Day 3
2 hr	91.5% ± 1.7% (n = 4)	90.3% ± 1.0% (n = 4)	90.8% ± 1.5% (n = 4)	92.3% ± 3.9% (n = 4)
3 hr	89.8% ± 1.3% (n = 4)	84.4% ± 4.3% (n = 5)	90.6% ± 1.5% (n = 5)	91.3% ± 1.5% (n = 4)
4 hr	88.3% ± 3.2% (n = 3)	90.5% ± 1.9% (n = 4)	93.0% (n = 1)	92.0% ± 2.7% (n = 4)

lated that if the number of manipulations to the chemical contents of the vial of HMPAO were minimized, then the integrity of the final ^{99m}Tc-radiopharmaceutical may yield more consistent and reproducible RCP results and therefore maximize clinical usefulness.

With the contents of a vial of HMPAO reconstituted with saline, the chemical is then accessible with a needle and syringe. The volume of reconstitution was kept to 1.0 ml of normal saline and subsequent small volumes of HMPAO used to radiolabel with pertechnetate. Thus, high concentration and small volume maximized radiolabeling efficiency (*14*). The initial amount dispensed on Day 0 (Table 1) was extrapolated from the work of Sampson et al. (*14*). The subsequent fractions dispensed on Days 1 through 4 were increased 10% each day based on the assumption that even at a storage temperature of −70°C a small rate of decomposition of HMPAO could necessitate a slightly greater amount of chemical to be used each day to maintain RCP and ultimately granulocyte radiolabeling efficiency.

Filling the dead space of the needle and syringe used to dispense the HMPAO serves a number of functions. First, it reduces the amount of air (oxygen) the HMPAO and stannous ion are exposed to. This is thought to be important due to the very low levels of stannous ion present in the kit. Second, with the dead space filled, the accuracy of dispensing with a 1 cc syringe is maximized. Initial results (not shown) of this technique, without having the dead space filled, gave inconsistent RCP results. Similarly, filling the dead space of the pertechnetate syringe and needle allows accurate dispensing of the pertechnetate and minimizes exposure to air when the pertechnetate is added to the HMPAO.

In the early stages of testing this technique, the pertechnetate was delivered into the HMPAO syringe via a 1 cc syringe equipped with a 25 g × 16 mm needle. The length

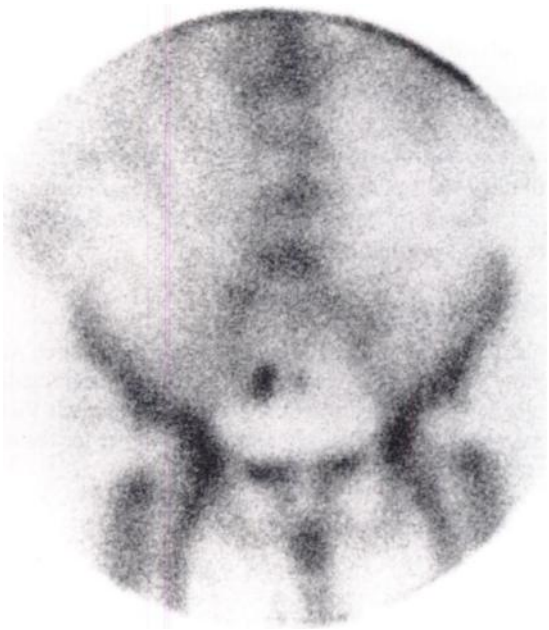

FIGURE 1. Anterior image of the pelvis obtained 30 min after the infusion of ^{99m}Tc-granulocytes in a 32-yr-old male with a small inflammatory mass in the pelvis. The discrete and clear uptake at 30 min was even more pronounced in subsequent images at 4 hr.

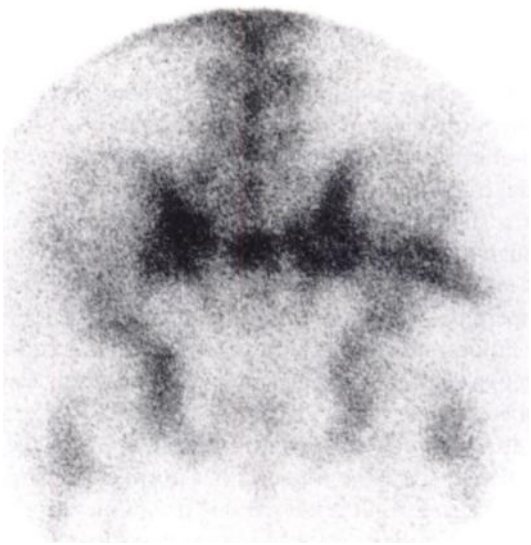

FIGURE 2. Posterior pelvic image of ^{99m}Tc-granulocytes obtained 4.5 hr after reinfusion in a 70-yr-old male patient. Moderately intense and abnormal uptake is noted in the right buttock, which corresponded exactly to a very tender, hardened inflammatory mass secondary to multiple intramuscular injections.

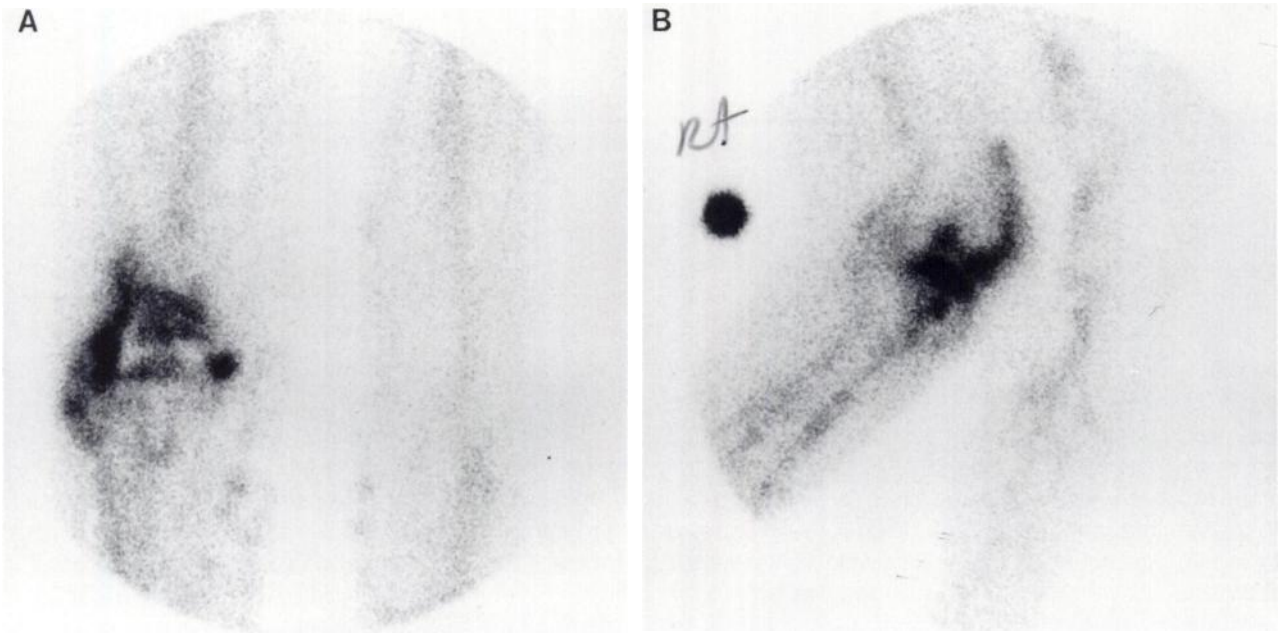

FIGURE 3. Anterior image (A) and right lateral image (B) of the right knee obtained 3.5 hr after the reinfusion of ^{99m}Tc-granulocytes in a 65-yr-old woman with a previous right total knee arthroplasty. The intense focal accumulation of granulocytes within the knee joint is clearly demonstrated consistent with a septic arthritis subsequently confirmed by aspiration.

of this needle only allowed the pertechnetate to be layered on top of the HMPAO solution, with mixing being performed by inversion of the syringe. This resulted in inconsistent RCP results (not shown). By using a 22 g × 40 mm needle, the pertechnetate can be delivered throughout the entire volume of the HMPAO solution, thereby improving mixing and reducing the introduction of air. This technique results in a greater reproducibility of the RCP results (Table 2).

The use of a bench top liquid nitrogen dewar to store a reconstituted vial of HMPAO seemed to be a reasonable, cost-effective way of bringing this technique on line. An initial trial showed promising RCP results. However, there was a substantial positive pressure inside the vial, great enough to force the plunger out the top of the syringe, and this technique was abandoned. It is speculated that at the temperature of liquid nitrogen (−270°C), contraction of the liquid and atmosphere inside the vial resulting in a negative pressure possibly followed by contraction of the rubber stopper to a greater degree than the glass vial resulting in nitrogen entering the vial. When the vial is warmed to thaw the liquid, a net positive pressure develops. The end result rendered this technique impractical.

Retrospective sterility and pyrogen testing on spent vials of HMPAO that had been stored at −70°C from 2 to 12 days were negative. These results demonstrate that this fractionating technique of reconstituted vials of HMPAO can result in a sterile, pyrogen-free pharmaceutical even after entry into the vial four times with storage at −70°C. However, each institution would have to verify their own technique and confirm sterility and apyrogenicity.

Routine application of fractionated HMPAO to ^{99m}Tc radiolabeling of granulocytes has resulted in significant cost savings. Previously, we usually utilized one vial of HMPAO for each WBC study. It is estimated that the utilization of this technique for WBC scintigraphy will reduce HMPAO costs by 50%–75% in our department.

CONCLUSION

Storage of a reconstituted vial of HMPAO at −70°C, with subsequent dispensing of small fractions as needed to radiolabel granulocytes results in a consistent reproducible high quality radiopharmaceutical. Technetium-99m-granulocytes labeled by this technique yield excellent clinical studies with considerable cost savings.

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REFERENCES

1. Costa DC, Lui D, Ell PJ. White cells radiolabeled with ^{111}In and ^{99m}Tc—a study of relative sensitivity and in-vivo reliability. *Nucl Med Commun* 1988;9:725–731.
2. Becker W, Schomann E, Fishback W, et al. Comparison of ^{99m}Tc-HMPAO and ^{111}In-oxime labeled granulocytes in man: first clinical results. *Nucl Med Commun* 1988;9:435–447.
3. Peters AM, Roddie ME, Danpure HJ, et al. ^{99m}Tc labeled leukocytes: comparison with ^{111}In-tropolonate labelled granulocytes. *Nucl Med Commun* 1988;9:449–463.
4. Roddie ME, Peters AM, Danpure HJ, et al. Inflammation: imaging with

^{99m}Tc-HMPAO labeled leukocytes. *Radiology* 1988;166:767–772.
5. Peters AM. Imaging inflammation: current role of labeled autologous leukocytes [Editorial]. *J Nucl Med* 1992;33:65–67.
6. Hung JC, Volkert WA, Holmes RA. Stabilization of technetium-99m-D,L-hexamethylenepropylene oxime (^{99m}Tc-D,L-HMPAO) using gentisic acid. *Nucl Med Biol* 1989;16:675–680.
7. Bayne VJ, Forster AM, Tyrell DA. Use of sodium iodide to overcome the eluate age restriction for Ceretec® reconstitution. *Nucl Med Commun* 1989; 10:29–33.
8. Ballinger I. Preparation of ^{99m}Tc-HMPAO [Letter]. *J Nucl Med* 1990;31: 1892.
9. Billinghurst MW, Abrams DN, Lawson MS. Stabilization of ^{99m}Tc-HMPAO-1 ethanolic preparation. Stabilization of ^{99m}Tc-HMPAO-1 ethanolic preparation. *Appl Radiat Isot* 1991;42:607–612.
10. Piera C, Pavia A, Bassa P. Preparation of ^{99m}Tc-HMPAO [Letter]. *J Nucl Med* 1990;31:127–128.
11. Hawkins T, Reeder A, Keavey PM, et al. The long term stability of reconstituted exametazine: a clinical study and laboratory evaluation. *Nucl Med Commun* 1991;12:1045–1055.
12. Tubergen K, Corlija M, Volkert WA, et al. Sensitivity of ^{99m}Tc-d,l-HMPAO to radiolysis in aqueous solution. *J Nucl Med* 1991;32:111–115.
13. Dooley DC, Simpson JF, Meryman HT. Isolation of large number of fully viable human neutrophils: a preparative technique using Percoll density gradient centrifugation. *Exp Haematol* 1982;10:591–599.
14. Sampson CB, Solanski C, Barber RW. ^{99m}Tc-exametazine-labeled leucocytes: effect of volume and concentration of exametazine on labeling efficiency, and clinical protocol for high efficiency multi-dose radiolabeling. *Nucl Med Commun* 1991;12:719–723.

CORRECTION

In the table of contents of the December issue of the *Journal*, page numbers were listed incorrectly for two articles. "Editorial: PET Imaging of Carbon-11-S-Adenosylhomocysteine: A Measure of Myocardial Energy Balance" by Gary V. Martin begins on page 2144. "Noninvasive Detection of Hypoxic Myocardium Using Fluorine-18-Fluoromisonidazole and Positron Emission Tomography" by Gary V. Martin et al. begins on page 2202.