Use of Stabilized Technetium-99m-Exametazime for Radiolabeling Leukocytes

Joseph C. Hung, Sushital Chowdhury and Brian P. Mullan
Nuclear Medicine, Department of Diagnostic Radiology, Mayo Clinic, Rochester, Minnesota

With a stabilizing agent (i.e., methylene blue and sodium phosphate buffer mixture), the in vitro stability of ^{99m}Tc-exametazime has been increased to 4–6 hr postreconstitution. However, it is not feasible to use the stabilized ^{99m}Tc-exametazime for leukocyte radiolabeling. This is due to the deep blue appearance of the mixture of stabilized ^{99m}Tc-exametazime and blood components, which makes it impossible to separate properly the supernatant from the leukocyte button. In our study, we have developed a practical methodology for overcoming this difficulty in order to use stabilized ^{99m}Tc-exametazime in leukocyte labeling. **Methods:** The stabilized ^{99m}Tc-exametazime preparation used in our method consisted of 2 ml 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc and 2 ml methylene blue/phosphate buffer solution. The separated leukocytes from 80-ml fresh venous blood were incubated with three different ages (i.e., 0-, 4-, or 6-hr postreconstitution) of stabilized ^{99m}Tc-exametazime (~925 MBq, ~25 mCi; 0.5–1 ml) at room temperature for 15 min. After incubation, 3 ml of 12.6% ACD/NS solution (anticoagulant citrate dextrose, solution A, USP mixed with 0.9% NaCl, v/v) was added to the tube and centrifuged at 160 g for 5 min. Three milliliters of the dark blue supernatant were carefully removed, and the bottom 1 ml portion was resuspended with 9 ml of 12.6% ACD/NS solution. After centrifugation (160 g for 5 min), the supernatant was clear enough to be drawn off without disturbing the radiolabeled leukocyte button. The white cell button was then resuspended in 4 ml of platelet-poor plasma. **Results:** The overall labeling efficiency (LE) of our new technique was 67.8%–91.9%, with the higher LE associated with fresher stabilized ^{99m}Tc-exametazime. During a 6-hr in vitro stability evaluation, radiolabeled leukocytes lost 1.2% ± 0.3% (n = 24), 1.3% ± 0.1% (n = 16) and 1.8% ± 0.1% (n = 16) each hour of the cell-bound 0-, 4-, and 6-hr-old ^{99m}Tc-exametazime, respectively. The ^{99m}Tc-exametazime-labeled leukocytes examined by the trypan blue staining technique at 6-hr postradiolabeling yielded nonstained cells indicating viable leukocytes. **Conclusion:** We concluded that with a small volume of ^{99m}Tc-exametazime and double dilution steps with 12.6% ACD/NS solution, stabilized ^{99m}Tc-exametazime can be used effectively for leukocyte radiolabeling with a high LE and long in vitro stability.

Key Words: stabilized technetium-99m-exametazime; leukocytes; viability; labeling efficiency; radiolabeled leukocytes; stability

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Technetium-99m-exametazime (^{99m}Tc-exametazime, commonly known as ^{99m}Tc-hexamethylpropylene amine oxime or ^{99m}Tc-HMPAO) was initially approved by the Food and Drug Administration (FDA) on December 30, 1988, to be used as an adjunct for detecting altered regional cerebral perfusion in stroke (*1*). Due to the rapid decomposition of ^{99m}Tc-exametazime, the shelf life of the reconstituted kit preparation was limited to 30-min postpreparation (*1*). Although Peter et al. (*2*) described the feasibility of radiolabeling leukocytes with ^{99m}Tc-exametazime in 1986, the clinical application of ^{99m}Tc-exametazime in labeling leukocytes as an aid for detecting infectious/inflammatory lesions was not listed in the 1988 package insert for Ceretec (kit to prepare ^{99m}Tc-exametazime, Amersham Corp., Arlington Heights, IL) (*1*).

Compared to ^{111}In-labeled leukocytes, the lipophilic property of ^{99m}Tc-exametazime, similar to ^{111}In-oxine, allows it to penetrate the cell membrane of the white blood cells (WBCs), and it is rapidly incorporated into the leukocytes to bind intracellularly (*3*). Unlike ^{111}In-oxine-labeled leukocytes, ^{99m}Tc-exametazime-labeled leukocytes offer all of the advantages of a ^{99m}Tc agent such as convenience, better imaging characteristics and reduced patient radiation absorbed dose (*4*). Additionally, ^{99m}Tc-exametazime-labeled leukocytes rapidly accumulate at sites of infection and inflammation, and this allows earlier imaging and more rapid results (*5–7*). However, these advantages are negated by the short useful life of unstabilized ^{99m}Tc-exametazime (i.e., 30 min postreconstitution) and the limited ^{99m}Tc activity that can be added to the Ceretec vial (i.e., 1.11 GBq, 30 mCi) (*1*).

On April 7, 1995, the FDA approved a stabilized formulation of ^{99m}Tc-exametazime (*8*). The extended stability of ^{99m}Tc-exametazime (from the previous 30 min to 4–6 hr postpreparation) (*1,8*) is achieved by adding methylene blue stabilizer (i.e., 0.5 ml methylene blue injection USP 1% and 4.5 ml 3 m*M* monobasic sodium phosphate USP and diabasic sodium phosphate USP) to the reconstituted Ceretec vial (Amersham Healthcare, Arlington Heights, IL) (*8*).

With approval of the new formulation of ^{99m}Tc-exametazime, a new clinical usage and indication other than the brain imaging study has been added to the Ceretec package insert. Technetium-99m-exametazime is the first radiopharmaceutical approved for radiolabeling WBCs with ^{99m}Tc (*8*), and ^{99m}Tc-exametazime-labeled leukocytes are indicated for the localization of intra-abdominal infection and inflammatory bowel disease (IBD) (*6,9–11*). However, only ^{99m}Tc-exametazime without methylene blue stabilizer (i.e., unstabilized ^{99m}Tc-exametazime) can be used for radiolabeling leukocytes as indicated in the Ceretec package insert (*8*). This is due to the dark blue appearance of the mixture of stabilized ^{99m}Tc-exametazime/methylene blue stabilizer and isolated leukocytes, which makes it impossible to properly pipette out the supernatant without disturbing the radiolabeled WBC button.

With methylene blue stabilizer, the stabilized ^{99m}Tc-exametazime has a longer shelf life (i.e., 4–6 hr postpreparation) and a higher reconstituted ^{99m}Tc activity (up to ~2.0 GBq, 54 mCi) (*8*). To use stabilized ^{99m}Tc-exametazime in a more cost-effective and diversified way (i.e., multidose usage in either SPECT brain imaging or radiolabeling leukocytes), the purpose of our study was to develop an effective and practical method for radiolabeling leukocytes with stabilized ^{99m}Tc-exametazime.

MATERIALS AND METHODS

Cold Kit Formulation of Ceretec

The stabilized Ceretec kit formulation consists of three nonradioactive vials: (a) Ceretec reaction vial; (b) methylene blue vial; and (c) phosphate buffer vial (*8*).

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For correspondence or reprints contact: Joseph C. Hung, PhD, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

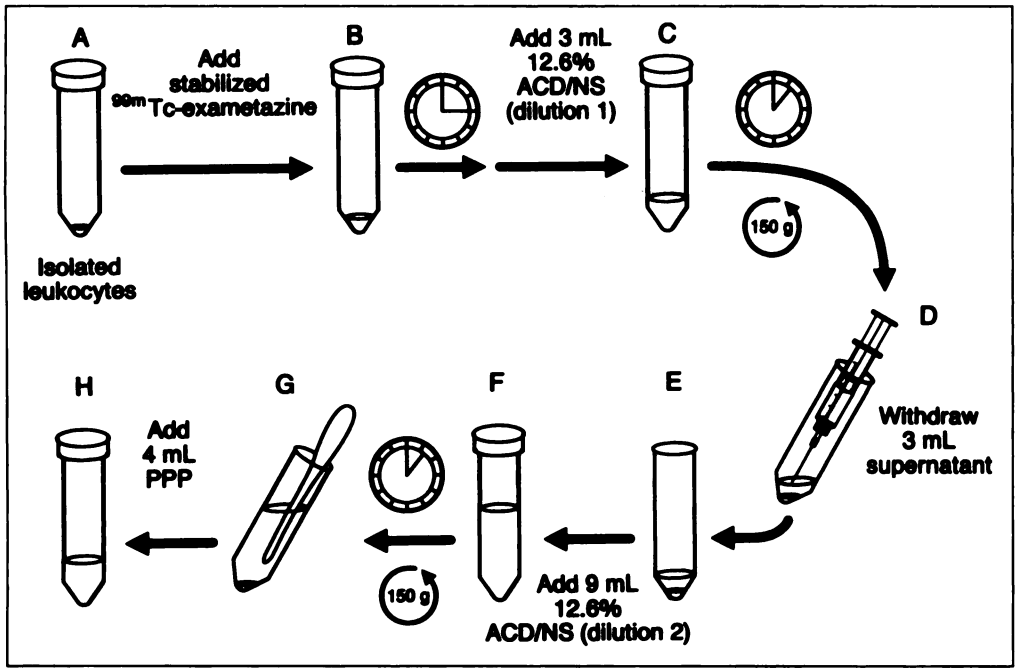

FIGURE 1. Schematic diagram of various steps (A–H) of stabilized ^{99m}Tc-exametazime-labeling leukocyte procedure with double dilution technique.

The Ceretec reaction vial contains a sterile, nonpyrogenic, lyophilized mixture of 500 μg exametazime ([RR, SS]−4,8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime), 7.6 μg stannous chloride dihydrate (minimum Sn^{2+} 0.6 μg; maximum total Sn^{2+} and Sn^{4+} 4.0 μg), and 4.5 mg NaCl (*8*). Nitrogen is added to the contents of the reaction vial to prevent oxidation of Sn^{2+} ions.

The 1-ml methylene blue vial is a sterile and nonpyrogenic methylene blue injection USP, 1% w/v containing 10 mg phenothiazin-5-ium,3,7-bis (dimethylamino)-chloride, trihydrate q.s. The final pH of the methylene blue solution is adjusted with NaOH and/or HCl.

The 4.5-ml vial of 3 m*M* phosphate buffer solution is sterile and nonpyrogenic. Each milliliter of the phosphate buffer solution contains 0.276 mg monobasic sodium phosphate monohydrate, 0.142 mg dibasic sodium phosphate anhydrous and 9 mg NaCl in water for injection q.s. The total calculated osmolarity of the 3 m*M* phosphate buffer solution is 317 mOsmol/l. Each ml provides 0.285 mg (3 m*M*) of phosphate, 0.157 mEq of Na^+ and 0.154 mEq of Cl^-.

Preparation of Stabilized Technetium-99m-Exametazime

According to the manufacturer's instructions for preparing stabilized ^{99m}Tc-exametazime (*8*), 2 ml of methylene blue stabilizing solution was drawn from a mixture [that must be used within 30 min of preparation (*8*)] of 0.5-ml methylene blue and 4.5-ml 3 m*M* phosphate buffer solution. Using a fresh ^{99m}Tc eluate from a ^{99}Mo/^{99m}Tc generator previously eluted within 24 hr, the Ceretec kit was reconstituted with 2 ml of 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc activity. The methylene blue stabilizing solution was added immediately to the reconstituted Ceretec vial (*8*).

Radiochemical Purity Determination of Stabilized Technetium-99m-Exametazime

The radiochemical purity (RCP) of the stabilized ^{99m}Tc-exametazime preparation was determined with the single-strip paper chromatography method, which was developed initially by Jurisson et al. (*13*) in 1986 for RCP determination of Tc(V)-oxo-tetradentate amine oxime complexes. This RCP method involves using single-strip miniaturized Gelman solvent saturation pads (1 cm × 8.5 cm) as the stationary phase with ether used as the mobile phase. Only the primary, lipophilic ^{99m}Tc-exametazime complex migrates to the top of the strip. Based on our previous comparison study (*14*), the single-strip Gelman paper with ether method not only offers the quickest RCP analysis of ^{99m}Tc-exametazime, but it also produces RCP results comparable to the recommended three-strip paper chromatography method (*7*).

Separation of Mixed Leukocytes

Using the aseptic technique in a vertical laminar flow hood, donor blood samples of 40 ml (total: 80 ml whole blood) were drawn into each of two 60-ml syringes containing 10 ml of anticoagulant citrate dextrose solution (ACD), USP, solution A. Twenty-five milliliters of anticoagulated blood was then placed into four 50-ml conical tubes each containing 10 ml hydroxyethyl starch [6% hetastarch in 0.9% NaCl solution (Volex, American Critical Care, McGaw Park, IL; Hespan, DuPont Pharmaceuticals, Wilmington, DE)]. These tubes were placed in a rack at a 40° angle and allowed to gravity sediment at room temperature for 30–45 min.

After sedimentation, the leukocyte-rich-platelet-rich-plasma (LRPRP) supernatant was removed and placed into two 50-ml conical tubes. The LRPRP supernatant tubes were centrifuged at 100 g for 5 min to remove additional erythrocytes. Next, the supernatant was again removed and placed into two additional 50-ml conical tubes. These tubes were then centrifuged for 10 min at 160 g to create pellet-rich plasma (PRP) and a button of mixed cells including the leukocytes. Twelve millimeters of the supernatant were pipetted into two 15-ml tubes. The tubes were centrifuged at 3200 g for 10 min to remove all cellular matter and create the platelet-pool plasma (PPP). The PPP was used for the final resuspension of the separated leukocytes. The remainder of the supernatant was discarded and 5 ml of 12.6% ACD/NS (ACD mixed with 0.9% NaCl, v/v) was added to the leukocyte button. This suspension was centrifuged at 100 g for 5 min to remove additional platelets that remained in the supernatant. After this centrifugation, the supernatant was removed and discarded to leave the bottom pellet in the tube (Figs. 1A and 2A).

Radiolabeling of Mixed Leukocytes with Stabilized Technetium-99m-Exametazime

Approximately 925 MBq (25 mCi) stabilized ^{99m}Tc-exametazime was added to the tube containing the isolated leukocyte button (Figs. 1B and 2B). Depending on the age of the stabilized ^{99m}Tc-exametazime used for incubation with the leukocyte button,

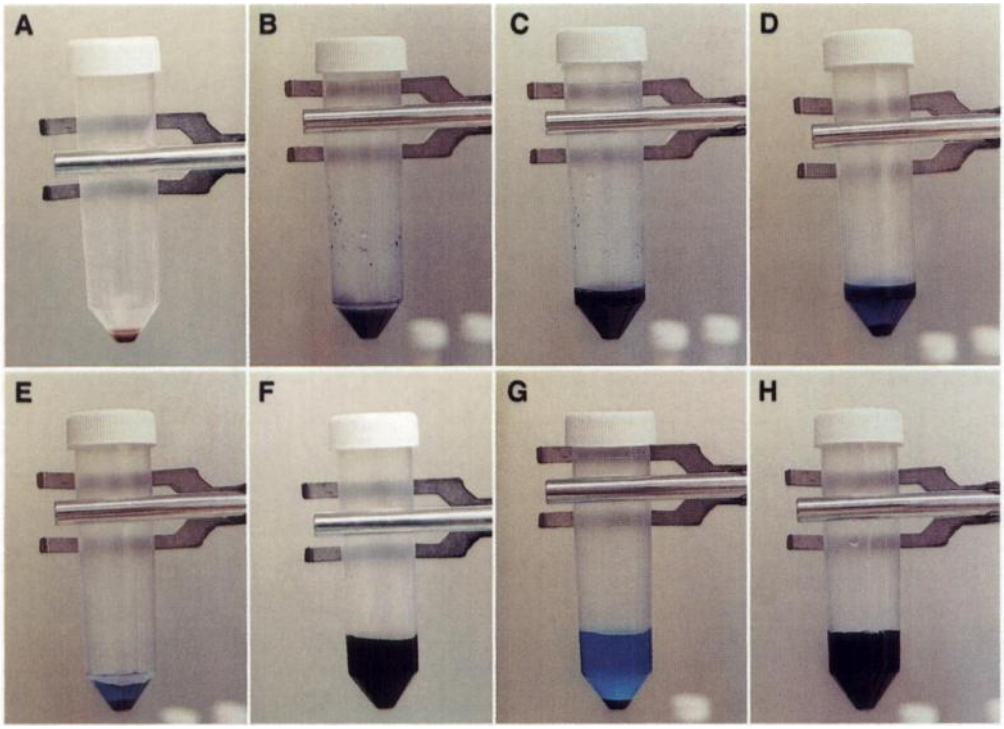

FIGURE 2. Step-by-step (A–H) depiction of color/appearance of each tube as shown in Figure 1 (with 1 ml stabilized ^{99m}Tc-exametazime).

the volumes of the stabilized ^{99m}Tc-exametazime withdrawn were as follows: (a) 0.5 ml (62.5 μg exametazime); (b) 0.8 ml (100 μg exametazime); and (c) 1 ml (125 μg exametazime) for 0-, 4-, and 6-hr-old stabilized ^{99m}Tc-exametazime, respectively, in order to obtain an initial incubated activity of 925 MBq (25 mCi).

Double Dilution Technique

First Dilution Process. After a 15-min incubation at room temperature, the first dilution process was conducted by adding 3 ml of the 12.6% ACD/NS solution to the tube (Figs. 1C and 2C) to lighten the deeply colored appearance of the mixture of stabilized ^{99m}Tc-exametazime and mixed cells (Fig. 2B). Even after the tube was centrifuged at 150 g for 5 min, the supernatant and the white cell button were still indistinguishable (Fig. 2D). The previous example (Fig. 2) demonstrates a darker color or appearance of each tube when 1 ml stabilized ^{99m}Tc-exametazime (i.e., the largest volume tested in our study) was used for WBC radiolabeling. If only 0.5 ml stabilized ^{99m}Tc-exametazime was incubated with the leukocytes, the supernatant and the WBC button would be more recognizable (Fig. 3, left). To avoid disturbance of the leukocyte button, only 3 ml of the dark supernatant was withdrawn with < 1 ml leukocyte button and supernatant remaining in the tube (Figs. 1E and 2E).

Second Dilution Process. To further dilute the dark color of the material in the tube, another 9 ml 12.6% ACD/NS solution was added to the tube (Figs. 1F and 2F). The capped tube was then centrifuged at 150 g for 5 min (Fig. 1). After centrifugation, the demarcation between the white cell button and the lighter blue supernatant (Fig. 2G) was clearly seen. The demarcation was even clearer when only 0.5 ml stabilized ^{99m}Tc-exametazime was used for radiolabeling the isolated leukocytes (Fig. 3, right). The supernatant in the tube was then carefully pipetted out, and the labeled leukocytes remained in the tube (Figs. 1G and 2G). The labeled leukocytes were then resuspended in ~4 ml PPP (Figs. 1H and 2H).

Quality Control of the Technetium-99m-Exametazime-Labeled Leukocytes

Labeling Efficiency. The labeling efficiency (LE) of the radiolabeled leukocytes with stabilized ^{99m}Tc-exametazime was calculated by the equation:

$$\text{LE}(\%) = \frac{\text{Final radiolabeled activity}}{\text{Total activity after 15-min incubation}} \times 100$$

In Vitro Stability. The in vitro stability of ^{99m}Tc-exametazime-labeled leukocytes was determined by measuring the amount of unbound ^{99m}Tc activity eluted from the radiolabeled white cells. The suspension of the mixed leukocytes labeled with stabilized ^{99m}Tc-exametazime and the 4 ml PPP was stored at room temperature for 6 hr. During the 6-hr evaluation, the radioactivity in the supernatant and radiolabeled leukocyte button was measured separately to determine the amount of ^{99m}Tc activity released from the

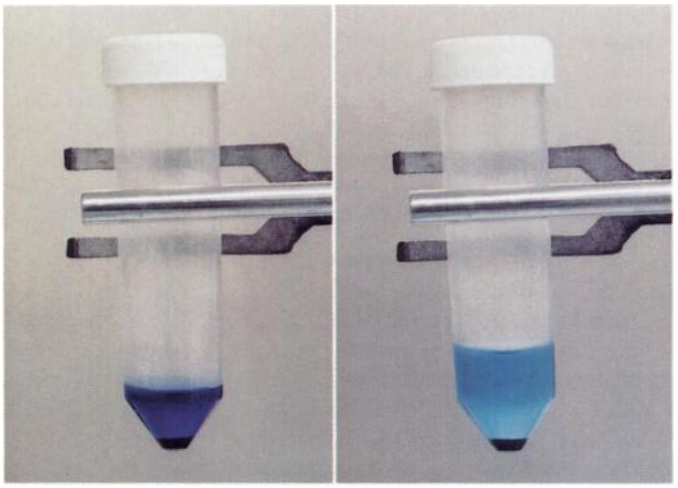

FIGURE 3. The color and appearance of two tubes (left: step D and right: step G) when 0.5 ml stabilized ^{99m}Tc-exametazime was used to incubate with the leukocytes.

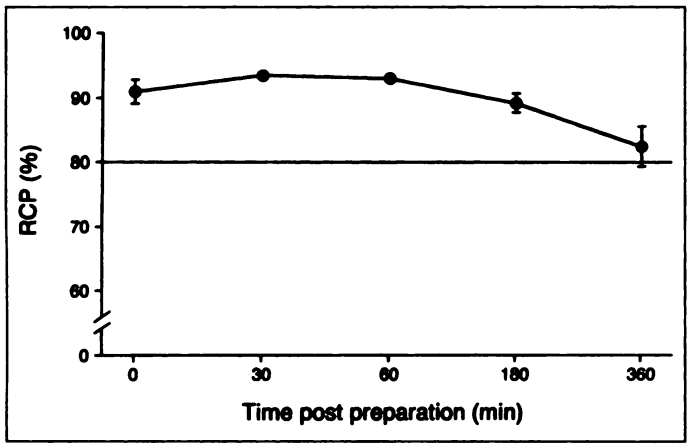

FIGURE 4. LE plot stabilized ^{99m}Tc-exametazime prepared with 2 ml of 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc and 2 ml methylene blue stabilizer. Each point is mean ± s.d. for six kit preparations. The horizontal solid line represents minimum acceptance level of RCP (i.e., 80%) (*8*).

labeled mixed leukocytes. This evaluation process was conducted at 0 min, 3 hr and 6 hr postleukocyte radiolabeling.

In Vitro Viability Test. All radiolabeled leukocyte suspensions in our study were stained with trypan blue (Allied Chemicals, Morristown, NJ) to evaluate the viability of the mixed leukocytes. Twenty-five microliters of trypan blue were added to a test tube, and an equal volume of mixed leukocyte suspension was added to the tube. This combination was gently mixed, and a drop of this solution was placed on a hemacytometer, which was then placed under a light microscope for observation. Any uptake of trypan blue by the leukocytes was an indication of nonviability of cells.

Complete Blood Count. Aliquots of all radiolabeled leukocyte preparations were analyzed to obtain the distribution and morphology of neutrophils, nongranulocytes, lymphocytes, monocytes, erythrocytes and platelets.

RESULTS

Radiochemical Purity Values of Stabilized Technetium-99m-Exametazime Preparations

According to the package insert for the stabilized formulation of Ceretec (*8*), an RCP value of > 80% is necessary for product acceptance. As depicted in Figure 4, the stabilized ^{99m}Tc-exametazime preparations used for the leukocyte radiolabeling maintained an average RCP value above the RCP acceptance limit (i.e., 80%) (*8*). The significance of this is that ^{99m}Tc-exametazime samples maintained at an acceptable RCP value up to 6 hr even after the cold kit was reconstituted with approximately four to five times the recommended ^{99m}Tc activity (i.e., 7.4–8.0 GBq, 200–215 mCi versus 2.0 GBq, 54 mCi) (*8*). During the first hour postreconstitution of the kit, the RCP of the stabilized ^{99m}Tc-exametazime held at the 91%–94% range and gradually descended to ~87% and ~83% at 4 hr and 6 hr postpreparation, respectively (Fig. 4). There was one preparation of stabilized ^{99m}Tc-exametazime that showed an RCP value of 77.3% at 6 hr postreconstitution (Fig. 4).

TABLE 2
In Vitro Stability of ^{99m}Tc-Exametazime-Labeled Leukocytes

	Elution of unlabeled ^{99m}Tc activity (%)		
	Age of stabilized ^{99m}Tc-exametazime		
Time post preparation (hr)	0-hr-old (n=6)	4-hr-old (n=4)	6-hr-old (n=4)
0	1.1 ± 0.2	1.7 ± 0.1	1.9 ± 0.1
1	1.4 ± 0.4	1.8 ± 0.1	1.9 ± 0.1
3	2.1 ± 1.0	1.8 ± 0.2	2.6 ± 0.3
6	2.3 ± 0.5	2.9 ± 0.4	4.8 ± 0.2

Labeling Efficiency of Radiolabeled Leukocytes with Stabilized Technetium-99m-Exametazime

With different ages of stabilized ^{99m}Tc-exametazime preparations, the LE of the ^{99m}Tc-exametazime-labeled leukocytes with 0-, 4- and 6-hr-old ^{99m}Tc-exametazime were 89.1% ± 2.8% (n = 6), 77.7% ± 0.8% (n = 4), and 69.7% ± 1.5% (n = 4), respectively. The age of the stabilized ^{99m}Tc-exametazime had a detrimental effect on the final LE of the radiolabeled leukocytes. This was due to the higher percentage of unlabeled ^{99m}Tc-exametazime components, i.e., free ^{99m}Tc and hydrophilic, secondary ^{99m}Tc-exametazime complex, which increased as the time postreconstitution of stabilized ^{99m}Tc-exametazime increased (Fig. 4).

Washed-off Radioactivity During Two Dilution Steps

Table 1 shows that when freshly stabilized ^{99m}Tc-exametazime (i.e., 0-hr-old) was incubated with the leukocytes, the percentage of the washed off activity (i.e., ~10%) during the two dilution steps corresponded to the percentage of the radiochemical impurities (i.e., free ^{99m}Tc and hydrophilic, secondary ^{99m}Tc-exametazime complex). However, the percentages of the radioactivity washed off the radiolabeled leukocytes with 4- and 6-hr-old ^{99m}Tc-exametazime were higher than the average values of radiochemical impurities for 4- and 6-hr-old stabilized ^{99m}Tc-exametazime (i.e., ~9%–10% and ~13% differences for 4- and 6-hr preparations, respectively) (Table 1 and Fig. 4).

Quality Control of Technetium-99m-Exametazime-Labeled Leukocytes

In Vitro Stability of Technetium-99m-Exametazime-Labeled Leukocytes. As shown in Table 2, the leukocytes labeled with stabilized ^{99m}Tc-exametazime demonstrated a high in vitro stability over the entire 6-hr incubation at room temperature. The overall elutions of the unlabeled ^{99m}Tc activity from the radiolabeled leukocytes each hour with 0-, 4- and 6-hr-old stabilized ^{99m}Tc-exametazime were 1.2% ± 0.3% (n = 24), 1.3% ± 0.1% (n = 16) and 1.8% ± 0.1% (n = 16), respectively. The mixed leukocytes labeled with 6-hr-old sta-

TABLE 1
Washed-off Radioactivity During Double Dilution Steps

Age of stabilized ^{99m}Tc-exametazime (hr)	Sample size	Percentage of washed off activity from dilution 1 (%)*	Percentage of washed off activity from dilution 2 (%)*
0	6	7.7 ± 2.3	3.2 ± 0.6
4	4	16.5 ± 1.2	6.5 ± 1.2
6	4	23.2 ± 1.2	6.5 ± 0.4

*The washed off activity from the radiolabeled leukocyte preparation was initially decay-corrected and then expressed as a percentage of the total incubated radioactivity.

bilized ^{99m}Tc-exametazime showed a slightly higher released ^{99m}Tc activity at 6-hr postincubation in vitro (i.e., ~2% higher than the 0- and 4-hr-old stabilized ^{99m}Tc-exametazime groups) (Table 2).

Trypan Blue Staining. Using visual assessment, all of the mixed leukocyte labeled with the stabilized ^{99m}Tc-exametazime for all three different age groups (i.e., 0-, 4- and 6-hr-old) did not concentrate the dye, which indicates a viable mixed leukocyte population.

Complete Blood Count. A complete blood count was done on the final leukocyte samples labeled with three different ages of stabilized ^{99m}Tc-exametazime (i.e., 0-, 4- and 6-hr-old). Leukocytes counts isolated from the initial 80-ml whole blood were in the range of 212.2–296.3 × 10^6 cells with 72.6%–77.4% neutrophils. The counts of the other cell components were comparable among the three age groups except the platelet amount from the 0-hr-old preparation (i.e., 278.9 ± 170.9 × 10^6) was smaller than from the other two groups (i.e., 555.0 ± 44.3 × 10^6 and 583.0 ± 98.7 × 10^6 for 4- and 6-hr-old preparations, respectively).

DISCUSSION

Technetium-99m-exametazime-labeled leukocytes have been recognized as the radiopharmaceutical of choice for the detection of IBD and for most forms of acute sepsis, especially intra-abdominal infection (*6,9–11*). The superior physical characteristics of ^{99m}Tc (i.e., optimal imaging energy, higher photon flux and better resolution) have allowed shorter imaging time, which is crucial in minimizing any potential artifact associated with the rapid small bowel movement during imaging acquisition. Compared to ^{111}In, the lower gamma energy and shorter physical half life of ^{99m}Tc allow a much higher injected dosage without significantly increasing the radiation dose to the patient. Technetium-99m-exametazime-labeled leukocytes rapidly accumulate at sites of infection and inflammation, which make it possible to detect and diagnose acute infectious/inflammatory sites in a timely manner. Allan et al. (*7*) have demonstrated that the diagnostic information obtained from the 1-hr ^{99m}Tc-exametazime-labeled leukocyte images for IBD is comparable to the 3-hr ^{111}In-labeled WBC images.

Although the manufacturer has developed a stabilized formulation for ^{99m}Tc-exametazime and has obtained an approved clinical indication/usage for WBC radiolabeling and imaging from the FDA, the radiolabeling of leukocytes is limited only to the less stable and more expensive unstabilized ^{99m}Tc-exametazime (due to single use of the entire kit preparation for radiolabeling leukocytes) (*8*).

With the stabilized formulation of Ceretec, we have demonstrated that up to 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc can be used effectively to prepare the stabilized ^{99m}Tc-exametazime, which maintained an > 80% RCP value for 6 hr postreconstitution. This is nearly four to five times the recommended maximum activity (i.e., 2 GBq, 54 mCi) (*8*) that can be added to the stabilized Ceretec preparation. Although lower LEs of ^{99m}Tc-exametazime-labeled leukocytes were obtained with the older ^{99m}Tc-exametazime (i.e., ~78% and ~70% LE for 4-hr and 6-hr-old ^{99m}Tc-exametazime, respectively), these yields are comparable to (*15–18*) or in some cases higher than (*2,19–24*) the published LE results. The higher reconstituted activity and the 6-hr useful life result in a more cost-effective use of stabilized ^{99m}Tc-exametazime for WBC labeling and brain SPECT imaging procedures.

It has been found that the LE of ^{99m}Tc-exametazime-labeled leukocytes decreases as the incubated ^{99m}Tc-exametazime volume (0.25–12.5 ml) increases (*15,18*). On the other hand, LE decreases as the amount (25–200 μg) of incubated exametazime decreases (*15*). To obtain an optimal LE of ^{99m}Tc-exametazime-labeled leukocytes and yet maintain a practical concentration of ^{99m}Tc-exametazime for routine clinical applications, we have reconstituted the Ceretec kit with 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc in a total volume of 4 ml (i.e., 2 ml ^{99m}Tc and 2 ml methylene blue stabilizer). With 925 MBq (25 mCi) ^{99m}Tc activity to incubate with the isolated mixed leukocytes, the specific concentration (i.e., 1.9–2.0 GBq/ml, 50–54 mCi/ml) from a 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc-exametazime kit preparation allowed the use of only ~0.5 ml of the freshly reconstituted ^{99m}Tc-exametazime preparation (~62.5 μg exametazime) to obtain an average LE of 89.4% ± 2.8% (n = 6) for ^{99m}Tc-exametazime-labeled leukocytes.

The high starting concentration of ^{99m}Tc-exametazime preparation (i.e., 1.9–2.0 GBq/ml, 50–54 mCi/ml) also allowed a small volume of ^{99m}Tc-exametazime at 4 hr (~0.8 ml) and 6 hr (~1 ml) postreconstitution to be used for leukocyte incubation. Leukocytes were incubated with a higher amount of exametazime when a larger volume of ^{99m}Tc-exametazime was used (e.g., 100 μg for 0.8 ml and 125 μg for 1.0 ml).

With the combined use of a higher volume of ^{99m}Tc-exametazime (decreased LE) and a larger amount of exametazime (increased LE) (*15,18*), with regard to LE, the effects of both volume and amount of exametazime should cancel each other out. However, this hypothesis did not work with the final LE of radiolabeled leukocytes with either 4-hr or 6-hr-old ^{99m}Tc-exametazime. Although the final LE of leukocytes labeled with the 0-hr-old ^{99m}Tc-exametazime reflected the ~10% decomposition of the RCP value (i.e., RCP: ~90% and LE: ~90%), final radiolabeled leukocyte preparations were ~9%–10% (4 hr) and ~13% (6 hr) lower than anticipated LE values after correction of decreased RCP at 4 hr and 6 hr postreconstitution (i.e., RCP: ~87% and LE: ~78% for the 4-hr-old preparation; RCP: ~83% and LE: ~70% for the 6-hr-old preparation). The volume of incubated ^{99m}Tc-exametazime may have a more detrimental effect on the LE of ^{99m}Tc-exametazime-labeled leukocytes.

The double dilution steps with 3 and 9 ml of 12.6% ACD/NS solution have enabled a clear separation of the radiolabeled leukocytes without disturbing the white cell button (Fig. 2G). The total washed-off activity was related to unbound activity of fresh ^{99m}Tc-exametazime (i.e., ~11% washed-off activity and ~10% impure activity for 0-hr old ^{99m}Tc-exametazime), whereas ~9%–10% and ~13%, more activities were washed off than the percentages of the radiochemical impurities when 4- and 6-hr-old ^{99m}Tc-exametazime, respectively, were used (Table 1). This was probably due to the lower LEs associated with usage of larger volumes of 4- and 6-hr-old stabilized ^{99m}Tc-exametazime during the 15-min incubation period. The higher washed-off activity of the 6-hr-old preparation (Table 1) may also be caused by a faster decomposition rate for ^{99m}Tc-exametazime that had reached the end of its useful life (i.e., 6 hr) (Fig. 4) (*8*).

The release of unbound activity from the mixed leukocytes radiolabeled with unstabilized ^{99m}Tc-exametazime has been found to be ~12%–14% in the first 2–4 hr incubation in vitro (*19,20,24*). The high elution of ^{99m}Tc-exametazime-labeled white cells, which severely limit quantification and kinetic studies, may be the reason for the higher renal, biliary and bowel activity (*2,3*). With stabilized ^{99m}Tc-exametazime to label the leukocytes, our study demonstrated that the in vitro stability of the mixed leukocytes was quite high (Table 2). The age of the stabilized ^{99m}Tc-exametazime had no effect on the in vitro stability of ^{99m}Tc-exametazime-labeled leukocytes (1.2%

± 0.3% [n = 24], 1.3% ± 0.1% [n = 16], and 1.8% ± 0.1% [n = 16]) of the released activity during the 6-hr incubation in vitro at 25°C for 0-hr, 4-hr and 6-hr-old ^{99m}Tc-exametazime, respectively). It appeared that the methylene blue stabilizer used for stabilizing the ^{99m}Tc-exametazime was also taken inside the leukocytes. The methylene blue and phosphate buffer might also play an important role in entrapping the ^{99m}Tc-exametazime inside the leukocytes. The more stable ^{99m}Tc-exametazime-labeled leukocytes help eliminate the problem associated with the nonspecific bowel, biliary and urinary activity encountered with unstabilized ^{99m}Tc-exametazime (*2,3*).

Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) was also used to stabilize ^{99m}Tc-exametazime (*25*). The addition of this stabilized solution (i.e., 200 μg cobalt chloride hexahydrate in 2 ml of water) showed that the shelf life of the reconstituted ^{99m}Tc-exametazime preparation has been extended to at least 5 hr postreconstitution (*25*). In a recent study using this stabilized ^{99m}Tc-exametazime to radiolabel leukocytes (*26*), even with $CoCl_2 \cdot 6H_2O$ as a stabilizer in the Jones et al. study, the initial RCP of stabilized ^{99m}Tc-exametazime was quite low (i.e., 81%), and the RCP dropped to 77% at 3 hr postpreparation (*26*). Additionally, the LEs for ^{99m}Tc-exametazime-labeled leukocytes with $CoCl_2 \cdot 6H_2O$ as a stabilizer were 49%, 51% and 52% for 0-hr, 1-hr and 3-hr-old stabilized ^{99m}Tc-exametazime, respectively (*26*). The elution of unbound activity from ^{99m}Tc-exametazime-labeled mixed cells at 1 hr postpreparation was 9% for each of the labeled leukocyte preparations using different ages of stabilized ^{99m}Tc-exametazine (*26*). Although the $CoCl_2 \cdot 6H_2O$ stabilized ^{99m}Tc-exametazime can be used to label leukocytes, it is only comparable to using unstabilized ^{99m}Tc-exametazime for leukocyte labeling with regard to LE and eluted activity (i.e., LE = 43% and elution activity = 12%) (*26*). Our study with the addition of methylene blue stabilizer to the ^{99m}Tc-exametazime preparation for radiolabeling leukocytes has shown that a much higher LE and lower elution of unbound activity can be used up to 6 hr postreconstitution.

CONCLUSION

The addition of methylene blue stabilizer has not only extended the shelf life of the ^{99m}Tc-exametazime preparation to 4–6 hr postreconstitution, but we have also found that up to 7.4–8.0 GBq (200–215 mCi) ^{99m}Tc can be added effectively to the kit preparation. With the double dilution technique, stabilized ^{99m}Tc-exametazime can be used effectively to radiolabel the leukocytes. This has allowed stabilized ^{99m}Tc-exametazime not only to be useful in various brain imaging procedures [e.g., stroke (*27*), epilepsy (*28*) and dementia (*29*)] but also in radiolabeling leukocytes.

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