Biodistribution and Radiation Dosimetry of Stabilized ^{99m}Tc-Exametazime–Labeled Leukocytes in Normal Subjects

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Labeling leukocytes with ^{99m}Tc-exametazime is a validated technique for imaging infection and inflammation. A new radiolabeling technique has recently been described that enables leukocyte labeling with a more stable form of ^{99m}Tc-exametazime. A normal value study of stabilized ^{99m}Tc-exametazime–labeled leukocytes has been performed, including biodistribution and dosimetry estimates in normal subjects. **Methods:** Ten volunteers were injected with stabilized ^{99m}Tc-exametazime–labeled autologous leukocytes to study labeled leukocyte kinetics and dosimetry in normal subjects. Serial whole-body imaging and blood sampling were performed up to 24 h after injection. Cell-labeling efficiency and in vivo viability, organ dosimetry, and clearance calculations were obtained from the blood samples and imaging data as well as urine and stool collection up to 36 h after injection. **Results:** Cell-labeling efficiency of 87.5% ± 5.1% was achieved, which is similar to or better than that reported with the standard preparation of ^{99m}Tc-exametazime. In vivo stability of the radiolabeled leukocytes was also similar to in vitro results with stabilized ^{99m}Tc-exametazime and better than previously reported in vivo stability for nonstabilized ^{99m}Tc-exametazime–labeled leukocytes. Organ dosimetry and radiation absorbed doses were similar with a whole-body absorbed dose of 1.3×10^{-3} mGy/MBq. Urinary and fecal excretion of activity was minimal, and visual assessment of the images showed little renal parenchymal activity and no bowel activity up to 2 h after injection. **Conclusion:** Cell labeling and in vivo stability appear improved compared with the leukocytes labeled with the nonstabilized preparation of ^{99m}Tc-exametazime. There are advantages in more cost-effective preparation of the stabilized ^{99m}Tc-exametazime and an extended window for clinical usage, with good visualization of abdominal structures on early images. No significant increase in specific organ and whole-body dosimetry estimates was noted compared with previous estimates using nonstabilized ^{99m}Tc-exametazime–labeled leukocytes.

Key Words: stabilized ^{99m}Tc-exametazime; radiolabeled leukocytes; dosimetry; biodistribution; normal value study

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Studies were performed on 10 normal subjects using ^{99m}Tc-exametazime–labeled leukocytes prepared by a recently described "double-dilution" technique, which uses a methylene-blue/phosphate buffer solution to stabilize the ^{99m}Tc-exametazime preparation (*1*). This enabled evaluation of in vivo stability and demonstration of the normal biodistribution and pharmacokinetics of leukocytes labeled with stabilized ^{99m}Tc-exametazime in healthy individuals. Radiation dosimetry estimates for critical organs and the whole body have been calculated. Clinical evaluation of images was performed to determine any visual difference in the distribution of activity using the stabilized preparation of ^{99m}Tc-exametazime compared with nonstabilized, particularly in the gastrointestinal and renal tracts, because nonspecific bowel activity can be problematic on delayed images (*2*). It is possible that with stabilized ^{99m}Tc-exametazime there is less in vivo elution of secondary complexes from the granulocytes, resulting in reduced renal and bowel activity.

A practical advantage of using stabilized ^{99m}Tc-exametazime to label leukocytes, compared with the previous nonstabilized formulation, is increased in vitro stability (up to 4–6 h) and preparation with higher reconstituted activities is possible (up to 7400 MBq) (*1*). In addition, multiple doses of stabilized ^{99m}Tc-exametazime can be made from 1 kit preparation and used for leukocyte-labeling procedures as well as other uses, such as cerebral perfusion imaging, with associated cost savings (*1*).

MATERIALS AND METHODS

Subjects

Scans were performed on 10 healthy volunteers (6 men, 4 women; age range, 19–32 y; mean, 25 y) who gave negative histories for renal, hepatic, pulmonary, cardiovascular, and gastrointestinal pathology. Imaging was delayed in those with evidence of current inflammatory or infectious disease, including respiratory tract infections or antibiotic use in the preceding week. All subjects were interviewed and underwent physical examination. Each volunteer's medical records were reviewed to exclude a significant medical or surgical history. Pregnant or breast-feeding females were excluded, and females of childbearing potential ages required a negative serum pregnancy test within 48 h of the procedure. Nine volunteers were Caucasian and 1 was African-American. Two subjects had histories of moderate tobacco use. All subjects received compensation as determined by an internal committee,

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and the Mayo institutional review board approval of the study protocol was obtained before volunteer selection.

Preparation of Stabilized ^{99m}Tc-Exametazime–Labeled Leukocytes

Stabilized ^{99m}Tc-exametazime was prepared according to the manufacturer's instructions, using methylene-blue combined with a phosphate buffer solution and fresh eluate from a ^{99}Mo/^{99m}Tc generator that had been eluted within the previous 24 h (*3*). Each subject had a freshly reconstituted kit, because studies were performed on separate days. The Ceretec kit (Medi-Physics, Inc., Amersham Healthcare, Arlington Heights, IL) was reconstituted with 7400 MBq ^{99m}Tc activity in 2 mL, and the methylene-blue stabilizing solution was then immediately added to the reconstituted Ceretec vial. The radiochemical purity of the stabilized ^{99m}Tc-exametazime preparation was determined with a single-strip paper chromatography method (*4,5*).

A total of 80 mL whole blood was drawn from each volunteer and, after separation of a mixed leukocyte preparation, the cells were labeled with stabilized ^{99m}Tc-exametazime using the double-dilution technique recently described by Hung et al. (*1*). An average of 1058 MBq stabilized ^{99m}Tc-exametazime was initially incubated with the isolated mixed leukocytes for 15 min, and then the preparation went through the double-dilution process to isolate the final radiolabeled leukocytes (*1*). The final dose of stabilized ^{99m}Tc-exametazime–labeled leukocytes was measured to be 743.7 ± 40.7 MBq at re-injection. Quality control on each dose included in vitro assessment of cell viability by trypan-blue staining, determination of cell labeling efficiency, and complete blood count and cell differential.

Sample Collection

Blood samples (5 mL each) were obtained at 30 min and 1, 2, 4, 8, and 24 h after injection of stabilized ^{99m}Tc-exametazime–labeled leukocytes to determine leukocyte recovery and blood clearance. For each sample, 1 mL whole blood, plasma, and packed cells were each counted to obtain an estimate of the activity retained within the cells, which was then decay-corrected to give a percentage of the original injected dose. Stool and urine collections in 12-h aliquots were collected beginning immediately after injection and for 36 h (i.e., 12, 24, and 36 h) to enable estimation of excretion of unbound ^{99m}Tc-exametazime and free ^{99m}Tc-pertechnetate and, therefore, in vivo labeling stability. Representative samples of urine (5 mL each) and the entire stool sample were counted in a dose calibrator, and these values were then also decay-corrected to obtain a percentage of the injected dose.

Imaging Protocol

Volunteers were injected in the supine position under a γ camera equipped with a general-purpose, low-energy collimator (General Electric 500A; General Electric, Milwaukee, WI), and 60 30-s images over the chest were stored on a dedicated computer in 64 × 64 word matrix (Pinnacle System, Medasys, Ann Arbor, MI). Whole-body images at 1, 2, 4, 8, and 24 h after injection were performed using a whole-body, dual-head γ camera (Siemens Bodyscan, Des Plaines, IL) equipped with high-resolution collimators. The whole-body anterior and posterior images were acquired for 20 min per pass and stored on the computer in 1,024 × 256 word mode matrix. A known standard activity was included in the initial whole-body pass at 1 h to facilitate accurate dosimetry estimates from the images. Pulmonary transit times were calculated as well as determination of counts in regions of interest (ROIs) over liver, spleen, kidney, bone marrow, bladder, lungs, bowel, and brain. Three experienced nuclear medicine physicians evaluated the series of whole-body images, and the organ distribution of activity at each time-point was arbitrarily graded relative to the liver.

TABLE 1
Plasma and Whole Blood Activity as Percentage of Injected Dose over Time

Time after injection	Plasma activity (%)	Whole blood (%)
30 min	1.7 ± 0.5	17.6 ± 4.2
1 h	1.5 ± 0.4	16.3 ± 3.8
2 h	1.3 ± 0.3	14.2 ± 3.3
4 h	0.9 ± 0.4	11.4 ± 2.6
8 h	0.9 ± 0.1	8.9 ± 2.0
24 h	0.6 ± 0.2	5.3 ± 1.3

Dosimetry Calculation

The final injected dose was determined by subtracting the residual activity in the syringe after injection from the dispensed dose (both radioactivities were measured in a dose calibrator). We used this value to compare with the blood, urine, and stool sample activity, which was also measured in the dose calibrator.

The percentage distribution of activity in various organs was estimated from the whole-body images at different time points allowing for appropriate decay correction. The dosimetry program used for calculation allowed the selection of set organ ROIs, which were then applied to the series of whole-body images. The relative activity in the various organ systems compared with the injected dose was calculated at the specific imaging times. This technique was semiautomated and increased objectivity regarding ROI selection. The data were incorporated into the MIRDOSE 3 software program to determine residence times and relevant organ and whole-body absorbed dose estimates (*6*).

RESULTS

The change in plasma and whole-blood activity over time as a percentage of the injected dose is presented in Table 1. The mean cell-labeling efficiency was 87.5% ± 5.1%, and the distribution of cellular elements in the final preparation of mixed leukocytes is given in Table 2. The lung half-

TABLE 2
Normal Value Study of 10 Patients with Stabilized ^{99m}Tc-Exametazime–Labeled Leukocytes

Study	Normal value
Labeling efficiency (%)	87.5 ± 5.1
Injected dose (MBq)	743.7 ± 40.7
Total WBCs in injected dose (×10^6)	179.1 ± 48.9
Total PMNs in injected dose (×10^6)	106.6 ± 42.6
Cell % in injected dose	
WBCs (%)	13.6 ± 3.3
RBCs (%)	64.7 ± 6.8
PLTs (%)	21.7 ± 6.0

WBCs = white blood cells; PMNs = polymorphonuclear leukocytes; RBCs = red blood cells; PLTs = platelets.

TABLE 3
Percentage ^{99m}Tc-Exametazime–Labeled Leukocytes in Various Body Compartments over Time

Organ	Percentage distribution of injected cells over time (%)				
	1 h	2 h	4 h	8 h	24 h
Liver	24.3 ± 6.3	20.1 ± 5.4	17.6 ± 3.7	16.0 ± 3.6	15.2 ± 2.9
Spleen	18.5 ± 5.0	18.2 ± 3.8	15.4 ± 4.2	12.5 ± 2.3	9.3 ± 4.3
Spine	3.6 ± 1.1	4.3 ± 1.1	4.5 ± 0.9	4.1 ± 0.9	3.8 ± 1.1
Bladder	3.2 ± 0.8	4.3 ± 1.6	4.1 ± 3.2	3.4 ± 2.7	2.2 ± 0.8
SI	0	0.1 ± 0.2	0.1 ± 0.3	0	0
ULI	0.7 ± 0.9	0.7 ± 1.0	0.8 ± 1.1	1.4 ± 1.1	2.3 ± 2.0
LLI	0.2 ± 0.4	0.2 ± 0.5	0.3 ± 0.5	0.2 ± 0.3	0.5 ± 0.5
Brain	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
Kidneys	0	0	0	0.1 ± 0.1	0.1 ± 0.2
Lungs	7.6 ± 1.1	5.8 ± 0.7	5.1 ± 0.6	4.3 ± 0.5	3.3 ± 0.5
Blood	16.3 ± 3.8	14.2 ± 3.3	11.4 ± 2.6	8.9 ± 2.0	5.3 ± 1.3
Remainder	26.1 ± 0.8	30.8 ± 2.5	35.5 ± 3.6	32.4 ± 3.0	33.1 ± 7.6

SI = small intestine; ULI = upper large intestine; LLI = lower large intestine.

clearance time was performed on the initial dynamic image series of the chest to assess cell integrity, and the mean half-clearance time was 16.0 ± 6.0 min.

Urine activity collected during the 36 h after injection ranged from 17%–26% of the injected dose. Stool activity was minimal, and only 2 volunteers had detectable excreted activity in the 36 h after injection. In 1 volunteer this was 2.6% of injected activity at 24 h, and in the other volunteer this was 1.5% at 36 h.

The percentage of administered activity of stabilized ^{99m}Tc-exametazime–labeled leukocytes in various body compartments over time is presented in Table 3. This confirms minimal activity in the renal and gastrointestinal tracts. The relative activity in the liver and spleen progressively decreases over time, whereas activity in the urinary bladder and bowel remains stable up to 4–8 h. Low-grade activity over the region of the brain likely represents blood-pool activity in the scalp. Table 4 lists the residence times for various organs used in dosimetry calculations with the MIRDOSE 3 program. The residence time can be thought of as the "average" or "effective" life of administered activity in a source organ and accounts for physical decay as well as biological accumulation and removal. In most situations this cannot be represented by a single exponential, and the residence time is not the retention half-time of activity in a source organ. The residence time is longest in the red marrow, followed by liver and spleen. The radiation absorbed dose estimates for critical organs are listed in Table 5. The highest dose is to the spleen, followed by red marrow, liver, bone, and urinary bladder wall. The estimated whole-body absorbed dose is 0.0013 mGy/MBq, and the calculated effective dose is 0.007 mSv/MBq (*6,7*).

On clinical evaluation of the whole-body images, the spleen was the organ of most intense uptake visually,

TABLE 4
Residence Times of Activity in Source Organs

Organ	Residence time (h)
Red marrow	4.19 ± 0.95
Liver	1.96 ± 0.47
Spleen	1.56 ± 0.36
Lungs	0.55 ± 0.13
Urinary bladder	0.36 ± 0.13
SI	0
ULI	0.13 ± 0.11
LLI	0.03 ± 0.04
Kidneys	0.01 ± 0.01
Brain	0.01 ± 0.01
Remainder	0.80 ± 1.01

SI = small intestine; ULI = upper large intestine; LLI = lower large intestine.

TABLE 5
Radiation Absorbed Dose Estimates for Critical Organs

Organ	Radiation absorbed dose (mGy/MBq)
Spleen	0.0851 ± 0.0170
Red marrow	0.0203 ± 0.0044
Liver	0.0111 ± 0.0038
Bone surfaces	0.0099 ± 0.0021
Urinary bladder	0.0090 ± 0.0036
Lungs	0.0056 ± 0.0014
ULI wall	0.0027 ± 0.0022
LLI wall	0.0010 ± 0.0014
Small intestine	0.0002 ± 0.0002
Kidneys	0.0003 ± 0.0004
Breasts	0.0001 ± 0.0001
Thyroid	0.0007 ± 0.0017
Ovaries	0.0001 ± 0.0001
Testes	0.0001 ± 0.0002
Total body	0.0013 ± 0.0002
Effective dose (body)	0.0070 ± 0.0009 mSv/MBq

ULI = upper large intestine; LLI = lower large intestine.

followed by the liver and urinary bladder. Bone marrow was seen well in all images as expected. The brain and thyroid gland were never visualized. The renal parenchyma was faintly evident on all images, although urinary activity within the renal pelvis and, especially, the bladder was seen in most subjects. Lung activity was most evident in the first 2 h, consistent with blood-pool activity, and decreased significantly thereafter, with virtually absent lung activity at 24 h in 6 of 10 volunteers and minimal activity in the remainder. The 2 volunteers who smoked did not show elevation of their lung half-clearance time or significant delayed lung activity.

Two volunteers had no visualized small bowel activity at any stage. No bowel activity was seen on the 30-min or 1-h images in any volunteer (Fig. 1). A trace of small bowel activity was first seen in 4 volunteers at 2 h and in 4 others at 4 h. This activity appeared to pass through into the large bowel in the majority of volunteers, with persistent small-bowel activity in only 3 subjects at 8 h after injection. The large bowel was first seen at 4 h in half the volunteers and the remainder at 8 h after injection (Fig. 2). All volunteers had some activity in the large bowel at 24 h. The gallbladder was seen in only 1 volunteer on the 1- to 8-h images.

DISCUSSION

There are many reported studies using ^{111}In-oxine– and ^{99m}Tc-exametazime–labeled leukocytes to image inflammation and infection. Initial studies using ^{99m}Tc-exametazime–labeled leukocytes were reported by Peters et al. (*8*) in 1986, and ^{111}In-oxine labeling techniques in humans were described as early as 1977 (*9*). The neutral and lipid soluble ^{111}In-oxine penetrates cell membranes, and indium is then displaced from oxine by intracellular components with higher binding affinities for the indium (*10*). The high labeling efficiency and stability of ^{111}In-oxine–labeled leukocytes make it a useful imaging agent that has been used clinically with good diagnostic accuracy for various skeletal and soft tissue pathologies. The imaging characteristics of ^{111}In, however, are not ideal, and a ^{99m}Tc-based labeling agent was sought.

The labeling efficiency of radiolabeled leukocytes that we used in our study was determined to be 87.5% ± 5.1%. This labeling yield is comparable with the ones obtained in our previous study (*1*) and by Sampson et al. (*11*) and Ozker et al. (*12,13*). Although the yield is not as high as the labeling efficiency of ^{111}In-oxine–labeled leukocytes, it certainly is a significant improvement compared with the 50%–60% yield range associated with leukocytes radiolabeled with nonstabilized ^{99m}Tc-exametazime (*8,14–16*). The kinetics and biodistribution of ^{99m}Tc-exametazime–labeled leukocytes are similar to those of ^{111}In-labeled granulocytes with similar lung transit times and splenic, bone marrow, and hepatic uptakes as described in several studies (*17–19*). Reticuloendothelial activity up to 24 h is similar in both labeled-cell preparations. With nonstabilized ^{99m}Tc-exametazime, however, significantly more nonspecific activity is seen in the kidneys, urine, gallbladder, and bowel. The bowel is often seen as early as 1–2 h after injection and is seen consistently in the

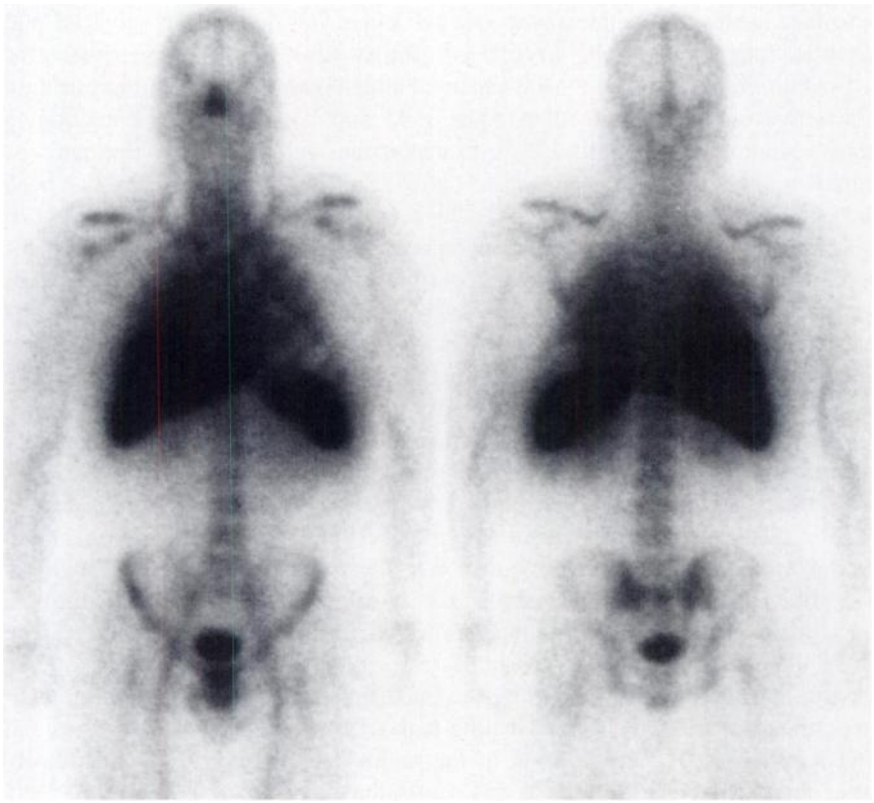

FIGURE 1. No significant bowel activity was present at 1 h after injection. Prominent blood-pool activity persists in heart and large vessels and to lesser extent the lungs with marrow uptake visible. Urinary activity is present in bladder and right renal pelvis.

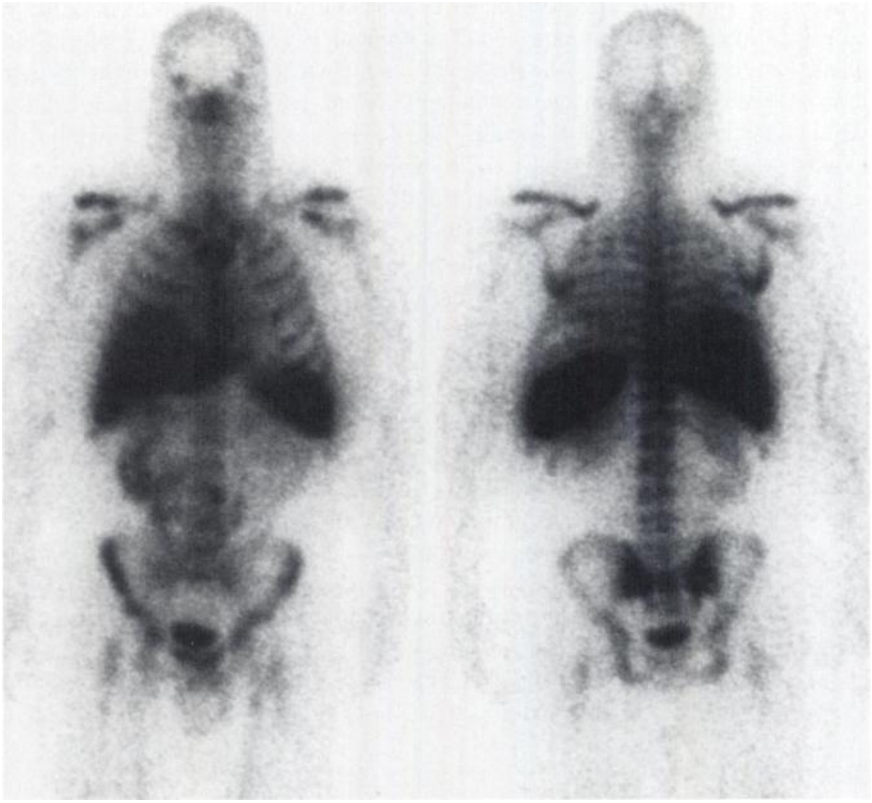

FIGURE 2. Background and blood-pool activity has decreased at 8 h. Prominent large-bowel activity is present in ascending colon, and some small bowel activity is present in midabdomen. Lung activity has decreased, whereas normal leukocyte activity in liver, spleen, and marrow persists.

colon by 4 h after injection (*15,17,19*). This nonspecific activity is a disadvantage of leukocytes radiolabeled with nonstabilized ^{99m}Tc-exametazime in interpretation of abdominal images.

The lipophilic character of ^{99m}Tc-exametazime enables it to diffuse through the cell membrane, where it is thought to form a hydrophilic complex that is trapped within the cell (*20*). There is, however, variable elution of activity from the labeled cells with subsequent renal and biliary excretion of secondary complexes, but this process remains poorly understood. Unbound lipophilic ^{99m}Tc-exametazime or ^{99m}Tc-pertechnetate is minimal, because no significant thyroid or brain uptake is usually identified (*17*). Details of the kidneys, collecting system, bladder, and gastrointestinal tract are often obscured by this excreted activity, which is a significant disadvantage in certain abdominal infectious or inflammatory disease processes when using nonstabilized ^{99m}Tc-exametazime instead of ^{111}In-oxine.

^{99m}Tc-exametazime has advantages over ^{111}In-oxine, however, with respect to availability, radiation dosimetry, and γ energy. A larger dose can be given using ^{99m}Tc-exametazime, and earlier imaging is performed with shorter acquisition times, resulting in improved image quality. ^{99m}Tc-exametazime–labeled leukocytes have become the investigation material of choice in certain instances, such as inflammatory bowel disease and acute bacterial soft tissue infection. Unlike ^{111}In-labeled leukocyte studies for abdominal pathology, however, when using ^{99m}Tc-exametazime it is necessary to perform early images (within 1–2 h after injection) to minimize confusion with normal gallbladder and bowel activity (*17*).

Previous studies have been performed to determine the normal biodistribution and dosimetry of nonstabilized ^{99m}Tc-exametazime–labeled leukocytes in healthy subjects, and results have been similar (*21*). The package insert for Ceretec was changed after Food and Drug Administration approval in April 1995 and now describes a method to stabilize ^{99m}Tc-exametazime with methylene-blue and sodium phosphate buffer (*3*). The in vitro stability has been increased from 30 min to 4–6 h after preparation (*3*). However, the manufacturer currently recommends the stabilized compound only for brain imaging and not for leukocyte labeling, because of the difficulty in separating out the leukocyte button in a dark blue medium (*3*). A recent study from our laboratory describes a double-dilution technique that enables a simple and safe leukocyte label that shows a higher labeling efficiency and much greater in vitro stability of radiolabeled leukocytes (*1*).

Our previous study also demonstrated that Ceretec can be reconstituted with a higher ^{99m}Tc activity and still maintain acceptable purity 6 h after preparation, allowing multiple doses of stabilized ^{99m}Tc-exametazime to be obtained from a single kit preparation (*1*). This results in cost-effective usage of cold kits of Ceretec. With the combined usage of the stabilized ^{99m}Tc-exametazime and the double-dilution technique for labeling leukocytes, a higher yield and lower in vitro elution of the radiolabeled leukocytes can be easily achieved. If this is also the case in vivo, one would expect

less unbound background activity and reduced renal and biliary excretion of eluted activity. Other stabilizing techniques have been described, including cobalt chloride hexahydrate, but the labeling efficiency and radiochemical purity appear unsatisfactory. None have been recognized by the manufacturer or listed in the package insert (*22,23*).

The results in Table 1 suggest that the release of unbound activity eluted from the cells is very low. Average cell-labeling efficiency of 87.5% is higher than the previous studies with nonstabilized ^{99m}Tc-exametazime (47%–71%) and similar to results with ^{111}In-oxine–labeled granulocytes in normal volunteers (*8,15,17,21,24*). The in vivo stability of leukocytes labeled with the stabilized ^{99m}Tc-exametazime, as assessed by the free plasma activity, and excreted urinary activity was similar to the results obtained in vitro (*1*). The distribution of cellular elements in the final preparation (Table 2) is also similar to the results with the nonstabilized ^{99m}Tc-exametazime–labeled leukocyte preparation (22).

The half-clearance time from the lungs ranged from 8.0–28.5 min, with a mean value of 16.0 min, compared with the results of Becker et al. (*17*) with 7 min and Brown et al. (*21*) with 9.8 min. There is, however, no clear cutoff value for lung clearance at which cellular damage is indicated. We did not have any visual evidence of prolonged lung retention, and, in fact, residence times and dosimetry values for the lungs are less compared with those reported previously with nonstabilized ^{99m}Tc-exametazime–labeled leukocytes (Tables 3, 4, and 5) (*21*). There was no appreciable activity within the lungs at 24 h in any of our subjects on visual assessment.

The whole-blood activity levels measured at various time points and listed in Table 1 represent the granulocyte recovery from the circulating granulocyte pool over time. Because we have used a mixed leukocyte preparation, there will be a very small contribution from red cell and platelet activity. Our value of 17.6% ± 4.2% (range, 12.3%–28.1%) at 30 min appears less than values previously obtained for ^{99m}Tc-exametazime–labeled leukocytes in humans of 29%–37% (*15,21*). The cause for this is not clear but may indicate some increased sequestration from the circulating blood pool. This may explain the slightly greater liver activity in our study (Table 4) compared with previous values obtained in our department (24.3% versus 17.4% at 1 h, respectively) using nonstabilized ^{99m}Tc-exametazime–labeled leukocytes (*21*). One author of an editorial review suggested that the circulating granulocyte-associated activity after equilibration (at 30–40 min) was a sensitive variable for in vitro activation (*25*). Therefore, the slightly lower recovery may indicate some labeling-induced changes in the leukocytes, although this is difficult to prove. Even if this were the case, there is no reason to suggest that this would significantly compromise the sensitivity of the test.

We did not detect thyroid or stomach activity on any images, indicating there was no significant circulating free ^{99m}Tc-pertechnetate. Likewise, the brain was never visualized, indicating absence of unbound lipophilic ^{99m}Tc-exametazime. Visually there was minimal renal parenchymal activity in all subjects, and this corresponds with the low renal dosimetry values seen with previous studies using nonstabilized ^{99m}Tc-exametazime (*21*). Urinary activity within the bladder was seen in all subjects on the earliest images. Excreted urinary activity represented approximately 22% of injected dose after 36 h. This is significantly less than the 54% reported in the previous study from our department using nonstabilized ^{99m}Tc-exametazime–labeled leukocytes but is similar to the 17% reported by Peters et al. (*19,21*).

The gallbladder was seen in only 1 of our subjects, which is comparable with the 10% gallbladder visualization described by other authors such as Roddie et al. (15) and lower than the rate of gallbladder visualization reported by Becker (*17*). Visualization of bowel, which can often hinder diagnosis of intra-abdominal pathology, also appears similar or possibly reduced compared with previous studies. Becker et al. (*17*) detected activity in small and large bowel at 1 h in all patients; Costa et al. (*18*) detected activity in small bowel at 30–60 min in 16% of their studies; and Brown et al. (*21*) detected bowel activity at 2 h in all but 1 patient. One study by Peters et al. (*19*) reported that significant bowel activity was never seen before 4 h after injection. In our study group no bowel activity was seen before 2 h. In 4 volunteers a trace of small bowel activity was first seen at 2 h, with an additional 4 volunteers showing activity at 4 h and beyond. Small bowel activity was never visualized in 2 volunteers. Activity in small bowel in our study appears less than that encountered in several of the studies cited above, and the minimal activity seen at 2 h in 4 subjects was considered unlikely to present significant diagnostic confusion for the reviewing physicians (*17,18,21*). The appearance of nonspecific large bowel activity followed that of small bowel activity and was present in all subjects at 24 h. No subjects, however, had activity in the colon before 4 h after injection, which is similar to the experience of Peters et al. (*17*) and better than results reported by Becker et al. (*19*).

The measured fecal excretion of activity also appears similar to other studies, although only 2 volunteers returned stool samples that contained activity during 36 h. Our result of 2.3% administered activity excreted in stool suffers from a very small sample size but is comparable with the 1.5% and 6% reported in other studies (*19,21*).

Radiation absorbed doses and residence times will differ slightly depending on the technique used to calculate them. We have used a semiautomated technique, which improves reproducibility of ROI selection and objectivity, and we have used the published MIRDOSE data to enable individual organ and whole-body dosimetry estimation (*6*). The individual organ dosimetry results obtained and whole-body absorbed dose estimate of 0.0013 mGy/MBq were slightly less than those reported from our department using nonstabilized ^{99m}Tc-exametazime–labeled leukocytes and less than those listed in the package insert for Ceretec (*3,21*). The whole-body absorbed dose per study was therefore approxi-

mately 1.0 mGy. There was no evidence that there was an increased radiation dose using the new preparation of labeled cells, despite the improved stability and retention of ^{99m}Tc-exametazime within the leukocytes.

CONCLUSION

Proposed benefits of using this technique include more cost-effective preparation of stabilized ^{99m}Tc-exametazime in the nuclear pharmacy with greater flexibility in the clinical use of stabilized ^{99m}Tc-exametazime because of prolonged (4–6 h) in vitro stability. Our findings also suggest there is less in vivo elution from the leukocytes, which may improve imaging characteristics compared with the use of nonstabilized ^{99m}Tc-exametazime–labeled leukocytes. This may be advantageous in imaging the kidney and gastrointestinal tract and may expand the clinical applications of ^{99m}Tc-labeled leukocytes. There is no increase in patient absorbed dose using the stabilized preparation compared with the nonstabilized preparation of ^{99m}Tc-exametazime–labeled leukocytes. These results confirm that clinical trials using stabilized ^{99m}Tc-exametazime–labeled leukocytes in select patient populations can be undertaken safely and cost efficiently.

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REFERENCES

1. Hung JC, Chowdhury S, Mullan BP. The use of stabilized technetium-99m exametazime for radiolabeling leukocytes. *J Nucl Med.* 1998;39:912–917.
2. Peters AM. Imaging inflammation and infection with Tc-99m HMPAO labeled leukocytes. In: Henkin RE, Boles MA, Dillehay GL, et al. *Nuclear Medicine.* Vol. II. St. Louis, MO: Mosby; 1996:1636–1655.
3. Amersham Healthcare. Package insert. Ceretec® [kit for preparation of ^{99m}Tc exametazime injection]. Arlington Heights, IL: Amersham Healthcare; April 1995.
4. Jurisson S, Schlemper EO, Troutner DE, Canning LR, Nowotnik DP, Neirinckx RD. Synthesis, characterization and X-ray structural determinations of technetium (V)-oxotetradentate amine oxime complexes. *Inorg Chem.* 1986;25:543–549.
5. Hung JC, Taggart TR, Wilson ME, Owens TP. Radiochemical purity testing for ^{99}Tc^{m}-exametazime: a comparison study for three-paper chromatography. *Nucl Med Commun.* 1994;15:569–574.
6. Stabin MG. MIRDOSE: personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med.* 1996;37:538–546.
7. International Commission on Radiological Protection. *1990 Recommendations of the International Commission on Radiological Protection.* ICRP Publication 60. New York, NY: Pergamon Press; 1991.
8. Peters AM, Danpure HJ, Osman S, et al. Clinical experience with ^{99m}Tc HMPAO for labeling leukocytes and imaging inflammation. *Lancet.* 1986;ii:946–949.
9. Thakur ML, Lavender JP, Arnot RN, Silvester DJ, Segal AW. Indium-111 labeled autologous leukocytes in man. *J Nucl Med.* 1977;18:1014–1016.
10. Thakur ML, Segal AW, Louis L, et al. Indium-111-labeled cellular blood components: mechanism of labeling and intracellular location in human neutrophils. *J Nucl Med.* 1977;18:1022–1026.
11. Sampson CB, Solanki C, Barber RW. ^{99}Tc^{m}-exametazime-labelled leucocytes: effect of volume and concentration of exametazime on labelling efficiency, and clinical protocol for high efficiency multi-dose radiolabelling. *Nucl Med Commun.* 1991;12:719–723.
12. Ozker K, Collier BD, Hellman RS, Istman AT, Uzum F, Steffel FG. Technetium-99m exametazime (^{99m}Tc-HMPAO)-labeled white blood cells: a high efficiency multidose procedure. *Eur J Nucl Med.* 1995;22:182–183.
13. Ozker K, Collier BD, Hellman RS, et al. A high-efficiency, rapid and multi-dose procedure for white blood cell labelling with ^{99}Tc^{m}-HMPAO. *Nucl Med Commun.* 1996;17:342–345.
14. Danpure HJ, Osman S, Carroll MJ. The development of a clinical protocol for the radiolabelling of mixed leucocytes with ^{99}Tc^{m}-hexamethylpropyleneamine oxime. *Nucl Med Commun.* 1988;9:465–475.
15. Roddie ME, Peters AM, Danpure HJ, et al. Inflammation imaging with Tc-99m HMPAO-labeled leukocytes. *Radiology.* 1988;166:767–772.
16. Solanki KK, Mather SJ, Janabi MA, Britton KE. A rapid method for the preparation of ^{99}Tc^{m} hexametazime-labelled leucocytes. *Nucl Med Commun.* 1988;9:753–761.
17. Becker W, Schomann E, Fischbach W, Börner W, Gruner KR. Comparison of ^{99m}Tc HMPAO and ^{111}In oxine labelled granulocytes in man: first clinical results. *Nucl Med Commun.* 1988,9:435–447.
18. Costa DC, Lui D, Ell PJ. White cells radiolabelled with ^{111}In and ^{99}Tc^{m}: a study of relative sensitivity and in vivo viability. *Nucl Med Commun.* 1988;9:725–731.
19. Peters AM, Roddie ME, Danpure HJ, et al. ^{99}Tc^{m} HMPAO labelled leukocytes: comparison with ^{111}In tropolonate labelled granulocytes. *Nucl Med Commun.* 1988;9:449–463.
20. Ell PJ, Hocknell JML, Jarritt PH, et al. A ^{99}Tc^{m} labelled radiotracer for the investigation of cerebral vascular disease. *Nucl Med Commun.* 1985;6:437–441.
21. Brown ML, Hung JC, Vetter RJ, O'Connor MK, Chowdhury S, Forstrom LA. The radiation dosimetry and normal value study of ^{99m}Tc HMPAO labeled leukocytes. *Invest Radiol.* 1994;29:443–447.
22. Papos M, Lang J, Rajtar M, Csernay L. Leukocytes labelling with ^{99m}Tc HMPAO. The role of the in vitro stability of HMPAO on the labelling efficacy and image quality. *Nucl Med Biol.* 1994;21:893–895.
23. Jones JM, Pearse JH, Evans WD, Richards AR. Stabilized ^{99m}Tc HMPAO for white cell labeling [abstract]. *Eur J Nucl Med* 1996; 23:1263.
24. Forstrom LA, Dunn WL, Rowe FA, Camilleri M. ^{111}In oxine-labeled granulocyte dosimetry in normal subjects. *Nucl Med Commun.* 1995; 16:349–356.
25. Peters AM. Just how big is the pulmonary granulocyte pool? *Clin Sci (Colch).* 1998;94:7–19.