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Technetium-99m-Labeled Liposomes to Image Experimental Colitis in Rabbits: Comparison with Technetium-99m-HMPAO-Granulocytes and Technetium-99m-HYNIC-IgG

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Scintigraphic techniques are routinely used for the evaluation of the extent and severity of inflammatory bowel disease. Currently, the radiopharmaceutical of choice is ^{99m}Tc-hexamethyl propyleneamine oxime (HMPAO)-leukocytes. We studied the imaging potential of two recently developed ^{99m}Tc-labeled agents, polyethylene glycol (PEG)-coated liposomes and hydrazinonicotinate (HYNIC) IgG, in a rabbit model of acute colitis, and compared them with that of ^{99m}Tc-labeled, granulocyte-enriched (>90%), white blood cells. **Methods:** Acute colitis was induced in rabbits by retrograde instillation of trinitrobenzene sulfonic acid. After 48 hr, 37 MBq of each radiopharmaceutical was administered intravenously. Gamma camera images were taken at 0, 1, 2, 4, 10 and 24 hr. At 4 and 24 hr postinjection, groups of rabbits were killed, and the uptake of the radiolabel in the dissected tissues was determined. For each affected 5-cm segment, the colitis index (CI, affected-to-normal-colon-uptake ratio) was calculated and correlated to the macroscopically scored severity of inflammation. **Results:** All three agents visualized the colitis lesions within 1 hr postinjection. The CI correlated with the severity of the abnormalities. With increasing severity, the CI at 4 hr postinjection for liposomes was 3.89 ± 0.73, 4.41 ± 0.47 and 5.76 ± 0.65; for IgG 1.67 ± 0.08, 3.92 ± 0.44 and 6.14 ± 0.65; and for granulocytes 2.90 ± 0.09, 6.15 ± 0.96 and 9.36 ± 3.35. For liposomes, the CI further increased during 24-hr postinjection to 6.56 ± 0.84, 8.50 ± 0.53 and 10.61 ± 1.34, respectively. The CI for the other two agents did not change significantly with time. **Conclusion:** In this rabbit model, ^{99m}Tc-labeled granulocytes, IgG and liposomes all rapidly visualized colonic inflammation. Granulocytes and liposomes showed the highest CI. Technetium-99m-labeled PEG-liposomes may be an attractive alternative for labeled leukocytes to image inflammatory bowel disease, because they can be prepared off the shelf and no handling of blood is required.

Key Words: colitis; liposomes; immunoglobulin; granulocytes; white blood cells; inflammation; inflammatory bowel disease; imaging; technetium-99m

J Nucl Med 1998; 39:2172–2178

Scintigraphic techniques have shown to be useful for evaluating inflammatory bowel disease, providing a rapid and effective method to assess extent and severity of the disease (*1,2*). Several radiopharmaceuticals are available, of which the labeled leukocytes are currently considered to be the most suitable agents (*3–5*). Imaging with ^{111}In-labeled and ^{99m}Tc-hexamethyl propylenamine oxime (HMPAO)-labeled leukocytes have both shown very good correlation with radiology, histology and endoscopy in patients with active inflammatory bowel disease (*1,2*). Compared with ^{111}In, ^{99m}Tc clearly has the advantage of a more favorable radiation dosimetry, better image

Received Jan. 1, 1998; revision accepted Apr. 12, 1998.

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quality and availability. The potential disadvantage of physiological bowel activity can be prevented by early imaging (*6*). However, the relatively complicated cell labeling procedure and the concern for possible cross-infection of patients or personnel with contaminated blood have stimulated the search for radiopharmaceuticals that are at least equally effective but easier to prepare.

Technetium-99m-labeled sterically stabilized liposomes could fulfill these demands. In the past, liposomes have been proposed as vehicles to image infection (*7*) and inflammation (*8*). However, these conventional liposomes have a short circulation time because of the rapid opsonization by cells of the mononuclear phagocytic system (MPS) (*9*). This hampers accumulation at the infection site and compromised image quality. Sterically stabilized liposomes have been modified by incorporation of polyethylene glycol (PEG) in the phospholipid bilayer (*9*). The hydrophilic PEG-tails cause the formation of a water mantle around the liposomes and thus the uptake by the MPS is reduced, resulting in prolonged circulation time and increased accumulation at sites of focal infection (*10–12*). We recently demonstrated that ^{111}In-labeled PEG-liposomes adequately delineated inflamed bowel tissue in a rabbit model of acute colitis (*13*). Because a ^{99m}Tc label is to be preferred over ^{111}In, we studied the performance of ^{99m}Tc-labeled PEG-liposomes in this rabbit model, and compared their in vivo characteristics with ^{99m}Tc-labeled white blood cells. Because rabbits have a relatively high lymphocyte count, a purified granulocyte mixture was used. Labeled polyclonal immunoglobulin G (IgG), an agent with high accuracy in the detection of clinical infection (*14*), was included in the study for comparison. For this purpose, IgG labeled to ^{99m}Tc by a nicotinyl hydrazine derivative (HYNIC) was used, a preparation that clinically appears to be equally as effective as ^{111}In-IgG (*15*).

MATERIALS AND METHODS

Animal Model

Acute colitis was induced in female New Zealand white rabbits (weight 2.3–2.7 kg) as described previously, with minor modifications (*16,17*). The experiments were performed in accordance with the guidelines of the local animal welfare committee. During the experiment, the rabbits were fasted but had water ad libitum. The animals were sedated with an intravenous injection of 0.7 ml Hypnorm (fentanyl 0.315 mg/ml + fluanisone 10 mg/ml; Janssen Pharmaceutical, Oxford, United Kingdom. A flexible silicone tube was introduced in the colon, and the tip was placed 20 cm from the anal sphincter. After flushing with 1 ml 50% ethanol, 1 ml of 30 mg trinitrobenzenesulfonic acid (TNBS; Sigma Chemicals, St. Louis, MO) in 40% ethanol was injected, followed by 3 ml 50% ethanol. As previously shown, this procedure induces acute colitis at the site of instillation, with variable extension into the distal colon (*13*). Histologically, transmural granulocyte infiltration and submucosal edema is seen within 24 hr after TNBS instillation. Forty-eight hours after colitis induction, the respective radiopharmaceuticals were injected through the ear vein.

Radiopharmaceuticals

Technetium-99m-labeled PEG-liposomes. Partially hydrogenated egg-phosphatidylcholine (PHEPC) with an iodine value of 35 was obtained from Lipoïd GmbH (Ludwigshafen, Germany). The PEG derivative of distearoylphosphatidyl-ethanolamine (PEG-DSPE) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Cholesterol and glutathione were from Sigma.

A lipid mixture in 100% ethanol was prepared in a molar ratio of 0.15:1.85:1 (PEG-DSPE:PHEPC:cholesterol) (*18*). A lipid film was formed by rotary evaporation under a high vacuum to remove residual organic solvent. The film was dispersed at room temperature in 50 m*M* glutathione in phosphate buffered saline (PBS), pH 7.4, at an initial phospholipid concentration of 120 m*M*. The resultant multilamellar vesicles were sized by multiple extrusion through two stacked polycarbonate membranes with a medium-pressure extruder (Avestin Inc., Ottawa, Ontario, Canada). Unentrapped glutathione was removed by gel filtration on a PD-10 column (Pharmacia, Uppsala, Sweden) eluted with PBS. The particle size distribution was determined by dynamic light scattering with a Malvern 4700 system using a 25 mW helium-neon laser (Malvern Instruments, Ltd., Malvern, United Kingdom). As a measure of particle size distribution of the dispersion, the polydispersity index was determined. This index ranges from 0.0 (entirely monodisperse dispersion) to 1.0 (completely polydisperse dispersion). The mean size of the liposome preparations was 100 nm with a polydispersity index of <0.1.

Preformed glutathione-containing liposomes were labeled with ^{99m}Tc essentially as previously described by Phillips et al. (*19*). Briefly, the liposomes (70 μmol phospholipid/ml) were incubated for 15 min at room temperature with freshly prepared ^{99m}Tc-HMPAO (10 MBq/μmol phospholipid). Labeling efficiency was between 70% and 80%. Removal of unencapsulated ^{99m}Tc-HMPAO was achieved by gel filtration on a PD-10 column with 5% glucose as the eluent. Injections of 37 MBq ^{99m}Tc-labeled liposomes were done intravenously.

Technetium-99m-HYNIC-IgG. HYNIC was synthesized and conjugated to human polyclonal IgG (Gammagard; Baxter/Hyland, Lessines, Belgium) according to the method described by Abrams et al. (*20*). Approximately one HYNIC group was coupled per IgG molecule, as determined spectrophotometrically. The purified HYNIC-conjugated IgG was diluted to 4 mg/ml in 0.15-*M* acetate (pH = 5.85), sterilized by membrane filtration and stored at −20°C in 0.5-ml aliquots. After 0.5 ml of the HYNIC-IgG conjugate was thawed, the conjugate was radiolabeled with ^{99m}Tc by adding 0.1 mg N-[Tris(hydroxymethyl)-methyl]glycine (Tricine; Sigma), 0.01 mg $SnSO_4$ and 400 MBq ^{99m}Tc-pertechnetate. The mixture was incubated for 15 min at room temperature. The radiochemical purity was determined by instant thin-layer chromatography on silica gel strips (Gelman Lab., Ann Arbor, MI) with 0.15-*M* acetate (pH = 5.85) as the mobile phase. Labeling efficiency was always higher than 95%. A dose of 0.2 mg IgG labeled with approximately 37 MBq ^{99m}Tc was injected intravenously.

Technetium-99m-HMPAO-granulocytes. Carotid artery canulation was performed in two anesthetized donor rabbits. A total amount of 200 ml blood was carefully drawn into acid citrate dextrose-coated tubes. The total leukocyte count of the donor rabbits was 6.0 and 7.4 $\times$ 10^9/liter, respectively, with approximately 50% granulocytes. Separation of granulocytes was performed according to the method described by Lillevang et al. (*21*) with minor modifications (*22*). Briefly, the blood was mixed with $\frac{1}{10}$ dextran (Dextran 500, Pharmacia) 6% solution in 0.9% NaCl and allowed to settle for 1 hr at room temperature. The leukocyte-rich supernatant was layered carefully on Nycoprep density medium (14.1% Nycodenz, 0.3% NaCl, 5 m*M* Tricine/NaOH pH 7.2, density = 1.077 g/ml, osmolality = 265 mOsm; Nycomed, Oslo, Norway) and centrifuged for 15 min at 600*g*. The plasma above the mononuclear cells, the mononuclear band and the density medium above the granulocyte pellet were carefully removed. The pellet was washed with 5 ml of Hank's balanced salt solution (HBSS) with 10% autologous plasma and centrifuged for 10 min at 50*g*. The cell pellet was resuspended in 1.5 ml HBSS, 10% plasma. Added to the cell suspension was 1 GBq freshly prepared ^{99m}Tc-HMPAO. The cells were incubated at room temperature for 30 min and centrifuged for 10 min at 50*g*. The cell pellet was resuspended

in 5 ml of cell-free autologous plasma. Granulocyte purity, as checked on Giemsa stained slides, was >90%. The labeling efficiency ranged between 75% and 85%. Functional integrity of the labeled granulocytes was assessed in vitro by trypan blue exclusion assay, showing cell viability of >98%. In addition, granulocyte function was evaluated by their in vivo performance, including transit through the lungs, hepatic uptake and recovery of labeled granulocytes in blood. A dose of 37 MBq ^{99m}Tc-labeled granulocytes was administered intravenously.

Study Design

Forty-eight hours after induction of colitis, three groups of five rabbits were injected through the ear vein with either ^{99m}Tc-labeled liposomes, ^{99m}Tc-labeled granulocytes or ^{99m}Tc-HYNIC-IgG. Each group comprised four rabbits with colitis and one normal control rabbit to assess physiological bowel excretion of the three radiopharmaceuticals. The animals were immobilized and placed prone on a gamma camera equipped with a parallel-hole, low-energy collimator (Orbiter; Siemens Inc., Hoffman Estate, IL). Each rabbit was imaged at 5 min, 1, 2, 4, 10 and 24 hr after injection. Images (300,000 counts/image, at 24 hr 100,000 counts/image) were obtained and stored in a 256 × 256 matrix.

The scintigraphic results were quantitatively analyzed by drawing regions of interest over the lungs, liver and spleen. Uptake in these organs was calculated.

After acquiring the last image at 24 hr, the rabbits were killed with an overdose of sodium phenobarbital and the biodistribution of the agents was determined. Blood was obtained by cardiac puncture. Tissue samples (muscle, lung, liver, spleen, kidney, normal [adjacent to the cecum] and affected colon) were dissected. The affected colon was washed with saline, divided into seven segments of 5 cm each and qualitatively scored for severity of macroscopic inflammation (0 = normal, 1 = inflammation, 2 = ulceration). Fecal contents of normal and inflamed colons were measured separately. All samples were weighed and the uptake of the radiolabel was measured in a shielded well scintillation gamma counter. To correct for physical decay and to calculate the uptake of the radiopharmaceuticals in each tissue sample as a fraction of the injected dose, aliquots of the injected dose were counted simultaneously. The results were expressed as percentage injected dose per gram (%ID/g). For each affected segment, the colitis index was calculated.

In a separate group of 14 rabbits, colitis was induced as previously described. After 48 hr groups of 4 rabbits were injected through the ear vein with either ^{99m}Tc-labeled liposomes, ^{99m}Tc-labeled granulocytes or ^{99m}Tc-HYNIC-IgG. At 4 hr postinjection, the rabbits were killed and dissected to determine the tissue distribution. To calculate granulocyte recovery, 2 rabbits were injected with ^{99m}Tc-labeled granulocytes, and serial blood samples were taken at 5, 15, 30 and 45 min, and 1, 2, 4, 10 and 24 hr after injection.

Statistical Analysis

All mean values are given as %ID/g or ratios ± 1 s.e.m. Statistical analysis of tissue distribution was performed using one-way analysis of variance (ANOVA). The Kruskal-Wallis test was used to evaluate differences in relative uptake of the ^{99m}Tc label. Both tests were corrected for multiple comparisons by the Bonferroni procedure. Correlations between relative uptake and degree of colitis were calculated using the Spearman rank correlation test. The level of significance was set at $p < 0.05$.

RESULTS

Forty-eight hours after colitis induction, all rabbits had diarrhea. Diffuse inflammation and patchy ulceration were seen at the site of the instillation, extending toward the rectum and transverse colon (Fig. 1).

The scintigraphic images of the three radiopharmaceuticals at 1, 2, 4, 10 and 24 hr postinjection are shown in Figure 2. With time, increasing accumulation was noted at the site of the colitis lesions. Technetium-99m-labeled PEG-liposomes, ^{99m}Tc-HYNIC-IgG and ^{99m}Tc-HMPAO-granulocytes all delineated the affected colon within 1 hr postinjection. From 4 hr postinjection onward, nonspecific bowel activity of labeled granulocytes became apparent in the normal control rabbit (image not shown). Figure 2B shows late focal accumulation of ^{99m}Tc-labeled granulocytes in a pretreated rabbit, probably due to nonspecific bowel excretion. Physiological bowel excretion was also observed with ^{99m}Tc-IgG but was only minimal with ^{99m}Tc-labeled liposomes (Figs. 2C and 2A).

The biodistribution data of the ^{99m}Tc label, 4 and 24 hr after injection of the radiopharmaceuticals, are give in Table 1. Uptake in the affected colon (all seven segments) at 4 hr postinjection was similar for the three radiopharmaceuticals. However, the ^{99m}Tc-labeled liposomes showed a marked increase of uptake in the lesions with time: As a result, uptake of ^{99m}Tc-labeled liposomes at 4 hr postinjection was twice as high as IgG uptake ($p < 0.01$) and three times higher than granulocytes uptake ($p < 0.001$). Consequently, and in view of the low uptake in normal colon, ^{99m}Tc-labeled liposomes showed significantly higher CIs at 24 hr postinjection than ^{99m}Tc-labeled granulocytes (Grades 0 and 1, $p < 0.05$) and ^{99m}Tc-IgG (all grades, $p < 0.01$) (Fig. 3). At 4 hr postinjection, the CIs of the three agents were similar for all grades of inflammation, except for the relatively low uptake of ^{99m}Tc-IgG in Grade 0 ($p < 0.05$). Although not significant, ^{99m}Tc-labeled granulocytes had somewhat higher values for Grades 1 and 2 than the other two agents. The CIs tended to increase with increasing severity of inflammation. At 24 hr postinjection, CIs of ^{99m}Tc-labeled granulocytes and ^{99m}Tc-labeled liposomes correlated best with the severity of the colitis ($rs = 0.53$, $p < 0.01$ and $rs = 0.50$, $p < 0.01$, respectively). At 4 hr postinjection, ^{99m}Tc-IgG showed the strongest correlation ($rs = 0.76$, $p < 0.0001$). Focal uptake of the labeled liposomes in normal and inflamed colonic tissues measured 24 hr postinjection was low compared with the

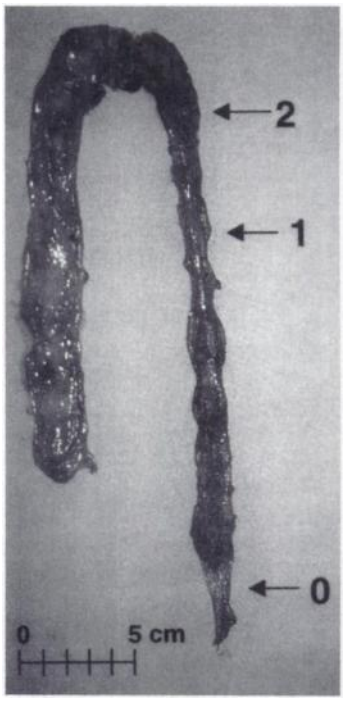

FIGURE 1. Macroscopic aspect of colonic mucosa in TNBS-induced acute colitis. Scoring of inflammation is illustrated: 0 = normal (rectum); 1 = inflammation; 2 = ulceration.

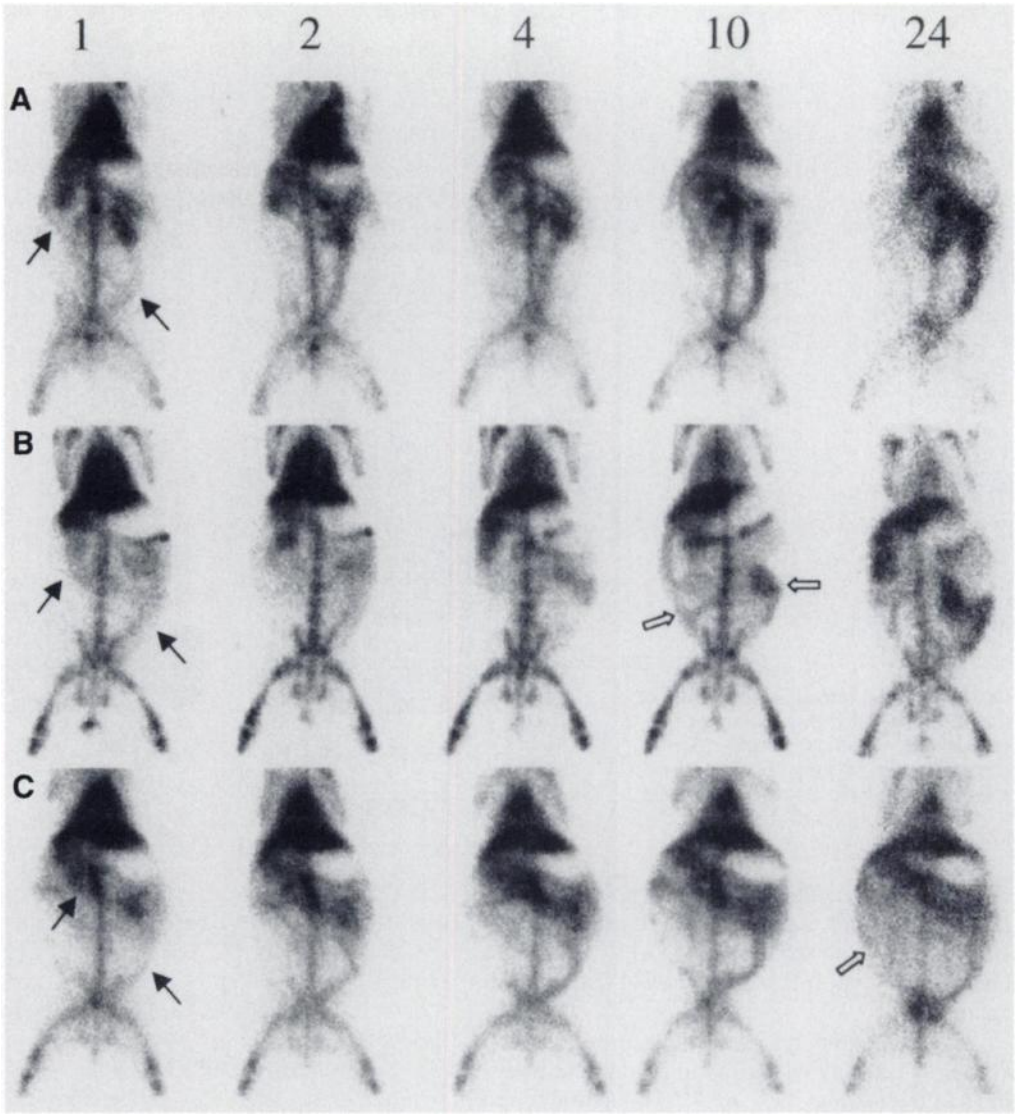

FIGURE 2. Scintigraphic images of rabbits with experimental colitis, imaged at 1, 2, 4, 10 and 24 hr after injection of (A) ^{99m}Tc-labeled PEG-liposomes, (B) ^{99m}Tc-HMPAO-granulocytes and (C) ^{99m}Tc-HYNIC-IgG. Small arrows denote colitis lesions. Physiological bowel excretion is illustrated in 10-hr image of ^{99m}Tc-HMPAO-granulocytes and 24-hr image of ^{99m}Tc-HYNIC-IgG (B and C; open arrow).

other two agents. Labeled IgG showed the highest focal uptake in the affected colon ($p < 0.01$). Technetium-99m-labeled granulocytes showed significantly lower blood levels than the other two agents, resulting in a lower uptake in background tissues (muscle, small intestine). Localization in most other tissue samples was remarkably similar. Splenic uptake of ^{99m}Tc-labeled granulocytes initially was higher than that of ^{99m}Tc-labeled liposomes and of ^{99m}Tc-IgG ($p < 0.01$ at 4 hr postinjection) but decreased to similar levels at 24 hr postinjection. In contrast, an over time increase in renal ^{99m}Tc activity

TABLE 1
Biodistribution of ^{99m}Tc-labeled Polyethylene Glycol-Liposomes, ^{99m}Tc-HMPAO-Granulocytes and ^{99m}Tc-HYNIC-IgG in Rabbits with Experimental Colitis (%ID/g; mean ± s.e.m.)

	4 hr postinjection						24 hr postinjection					
Tissue	Lipsomes	p*	Granulocytes	p†	IgG	p‡	Liposomes	p*	Granulocytes	p†	IgG	p‡
Blood	0.52 ± 0.03	§	0.09 ± 0.004	§	0.43 ± 0.03		0.30 ± 0.02	§	0.06 ± 0.01	§	0.24 ± 0.02	
Muscle	0.006 ± 0.001	‖	0.001 ± 0.0001	#	0.007 ± 0.001		0.004 ± 0.001	‖	0.001 ± 0.0002	#	0.006 ± 0.001	
Liver	0.12 ± 0.02		0.24 ± 0.05		0.19 ± 0.03		0.10 ± 0.01		0.17 ± 0.02		0.23 ± 0.03	‖
Lung	0.15 ± 0.02		0.17 ± 0.02		0.14 ± 0.02		0.09 ± 0.004	‖	0.04 ± 0.01	‖	0.10 ± 0.02	
Spleen	0.25 ± 0.03	#	1.34 ± 0.19	§	0.12 ± 0.03		0.23 ± 0.02		0.51 ± 0.14		0.14 ± 0.01	
Kidney	0.22 ± 0.06		0.06 ± 0.004		0.12 ± 0.01		0.15 ± 0.01		0.24 ± 0.05		0.12 ± 0.01	
Small intestine	0.06 ± 0.01		0.03 ± 0.01		0.05 ± 0.004		0.07 ± 0.01		0.02 ± 0.003		0.08 ± 0.03	
Feces colon	n.d.		n.d.		n.d.		0.02 ± 0.01		0.05 ± 0.01		0.02 ± 0.01	
Feces affected	n.d.		n.d.		n.d.		0.02 ± 0.01		0.07 ± 0.01		0.14 ± 0.02	#
Colon	0.03 ± 0.01		0.03 ± 0.01		0.03 ± 0.002		0.03 ± 0.003		0.02 ± 0.01		0.05 ± 0.01	
Affected colon	0.16 ± 0.02		0.18 ± 0.05		0.11 ± 0.02		0.27 ± 0.02	§	0.09 ± 0.02		0.12 ± 0.01	#

* = ^{99m}Tc-labeled liposomes versus ^{99m}Tc-labeled granulocytes; † = ^{99m}Tc-labeled granulocytes versus ^{99m}Tc-IgG; ‡ = ^{99m}Tc-IgG versus ^{99m}Tc-labeled liposomes; ‖ $p < 0.05$; # $p < 0.01$; § $p < 0.001$; n.d. = not determined.

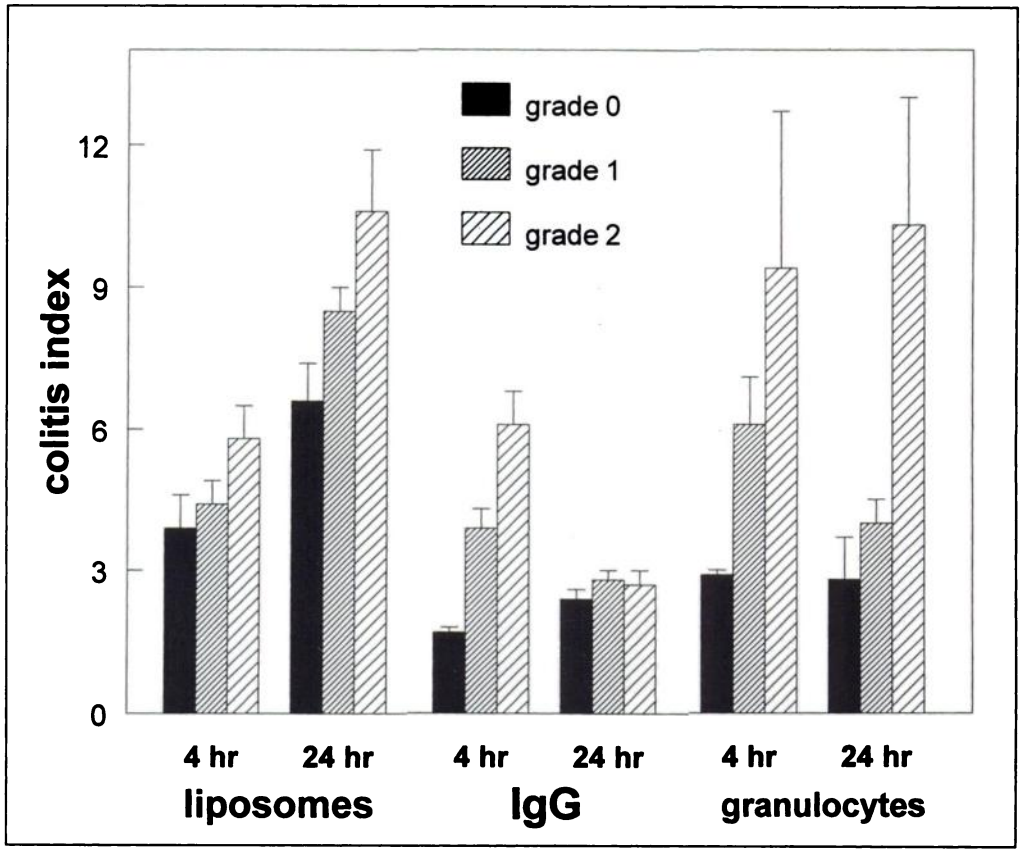

FIGURE 3. Colitis index (affected-to-normal-colon-uptake ratio) of radiopharmaceuticals at 4 and 24 hr postinjection, compared with severity of macroscopic inflammation (Grade 0 = normal, Grade 1 = inflammation, Grade 2 = ulceration). Error bars represent s.e.m.

with the labeled granulocytes was noted (from 0.06% to 0.24% ID/g, $p < 0.05$), reflecting the excretion of ^{99m}Tc-HMPAO complexes eluted from labeled cells. Absolute kidney uptake, however, did not differ significantly from that of the other two agents.

Quantitative analysis of the scintigrams of ^{99m}Tc-labeled granulocytes showed a rapid initial lung transit and low hepatic uptake, indicating that the ^{99m}Tc-labeling procedure did not affect granulocyte function (Fig. 4). In addition, granulocyte recovery at 30–45 min was >40% (Fig. 4), which confirms preserved granulocyte integrity (*23*). Whole-body clearance of ^{99m}Tc-labeled granulocytes was significantly faster than that of both ^{99m}Tc-IgG and ^{99m}Tc-labeled liposomes (from 1 hr onward, $p < 0.05$; data not shown). Whole-body clearance of ^{99m}Tc-labeled liposomes was similar to that of ^{99m}Tc-IgG.

DISCUSSION

Radiolabeled leukocytes are currently considered the agent of choice for the scintigraphic evaluation of inflammatory bowel disease. We recently showed that ^{111}In-labeled PEG-liposomes

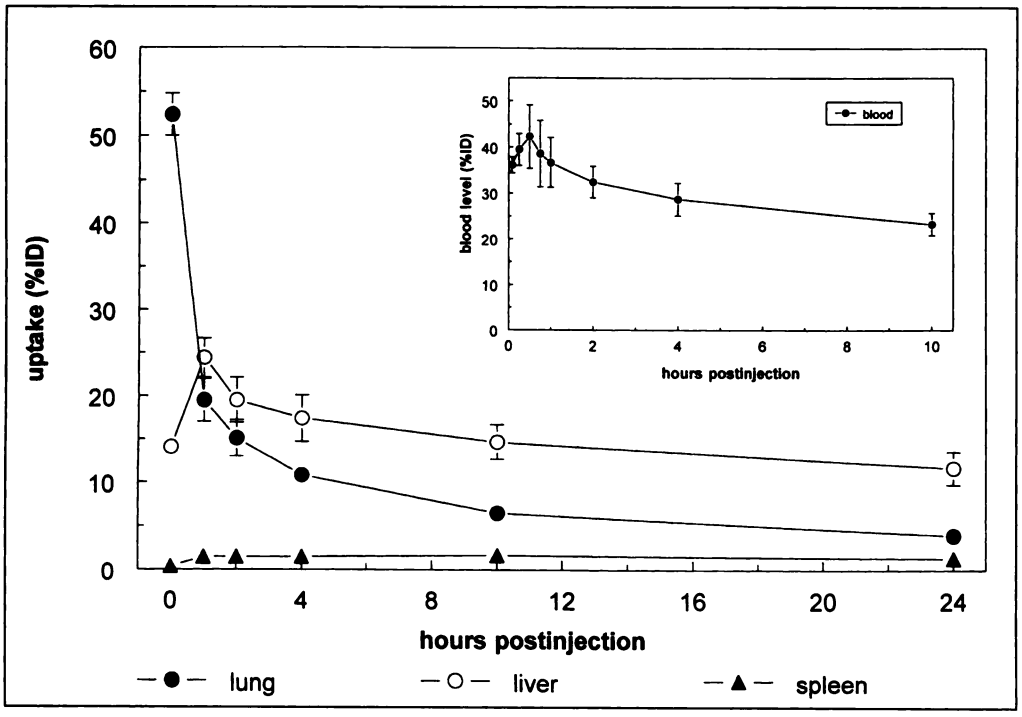

FIGURE 4. Quantitative analysis (mean ± s.e.m.) of scintigraphic images of rabbits injected with ^{99m}Tc-HMPAO-granulocytes, showing clearance from lungs (●), liver (○) and spleen (▲). Whole-body activity measured 5 min postinjection was set at 100% Error bars represent s.e.m. Inset: Blood clearance of ^{99m}Tc-HMPAO-granulocytes, as calculated from serial blood samples.

compared favorably with ^{111}In-labeled leukocytes in experimental colitis (*13*). However, several reports have indicated the superiority of ^{99m}Tc-HMPAO-leukocytes over ^{111}In-labeled leukocytes for the evaluation of colitis (*24–26*). In addition, the relatively high lymphocyte count in the mixed ^{111}In-labeled leukocyte preparation could have adversely affected its performance in the colitis model. Therefore the performance of ^{99m}Tc-labeled PEG-liposomes and ^{99m}Tc-HYNIC-IgG in experimental colitis was compared with a ^{99m}Tc-labeled purified granulocyte mixture.

Our results show that, up to 4 hr postinjection, both ^{99m}Tc-labeled liposomes and ^{99m}Tc-IgG performed at least as well as ^{99m}Tc-labeled granulocytes. All agents visualized the abnormalities within 1 hr and showed similar absolute and relative uptake in the affected colon. At 24 hr postinjection, ^{99m}Tc-labeled liposomes were the superior agents: Absolute uptake in the affected colon increased to a level two to three times higher than with the other two agents, while only minimal physiological bowel excretion occurred. Relative uptake in mild inflammation was remarkably high, indicating the potential to image mild disease as well. Although the absolute uptake of ^{99m}Tc-labeled granulocytes in the affected colon decreased over time, the CI remained high, suggesting adequate delineation of inflamed tissue at 24 hr postinjection. However, biodistribution in the colon segments was measured without fecal contamination, and thus does not represent the in vivo situation, as depicted by the gamma camera images (Fig. 2B). The biodistribution data also show relatively high focal uptake compared with uptake in normal and affected colons at later time points. Indeed, most authors recommend early imaging, not exceeding 4 hr postinjection, to avoid nonspecific bowel activity (*5,6,24*). According to Lantto et al. (*6*), abnormal images can be obtained as early as 2 min after injection of ^{99m}Tc-labeled leukocytes, with optimal disease localization at 2 hr postinjection. This study was not designed to determine the earliest diagnostic image of each agent but to compare their efficacy and optimal image time in experimental colitis. While at 4 hr postinjection, no difference among the three agents was noted, the images of ^{99m}Tc-labeled liposomes further improved from 4 to 24 hr postinjection because of the increasing fecal accumulation in colitis lesions and the persistent low uptake in normal colon and feces. In addition, compared with radiolabeled granulocytes, labeled liposomes have the important advantage of an easier preparation without the need for blood in vitro manipulation.

The later images with ^{99m}Tc-HYNIC-IgG provided little additional information because of the low relative uptake in colitis lesions, the lack of correlation between uptake and degree of colitis and mild physiological bowel excretion. Although the relatively high focal uptake in inflamed tissue could aid in the identification of affected parts, it also hampers their accurate localization. The moderate performance of ^{99m}Tc-HYNIC-IgG compared with ^{99m}Tc-labeled liposomes is remarkable, as both agents are thought to extravasate in inflammatory areas by virtue of increased vascular permeability (*27*) and have similar slow blood clearance. This could be due to washout of the smaller IgG molecule in advanced colitis or to additional specific uptake of liposomes in inflammatory cells. Still, the delineation of the affected colon on the early ^{99m}Tc-HYNIC-IgG images is promising, compared with the performance of ^{99m}Tc-iminothiolane-IgG (HIG) in patients with inflammatory bowel disease. The latter agent yielded low sensitivity and specificity at 4 hr postinjection (*28–30*). This is probably related to the relatively fast blood clearance of ^{99m}Tc-HIG, limiting IgG diffusion into colitis lesions, the marked kidney accumulation and physiological bowel excretion, all compromising adequate evaluation of colitis. Whether ^{99m}Tc-HYNIC-IgG shows a better performance in localizing inflammatory bowel disease remains to be assessed in clinical studies.

In conclusion, the performance of ^{99m}Tc-labeled liposomes and ^{99m}Tc-IgG was very similar compared with ^{99m}Tc-labeled granulocytes in the scintigraphic evaluation of experimental colitis up to 4 hr postinjection. At later time points, ^{99m}Tc-labeled liposomes were superior to the other two agents, providing additional diagnostic information because of the high and increasing focal uptake, correlating to the severity of inflammation, in combination with minimal nonspecific bowel excretion. Given the simple and safe procedure of preparation, ^{99m}Tc-labeled liposomes could be an attractive alternative to ^{99m}Tc-labeled leukocytes in the evaluation of inflammatory bowel disease.

ACKNOWLEDGMENTS

We thank Gerrie Grutters, Geert Poelen, Albert Peters and Mart Faassen for the expert assistance in the animal experiments and Peter Mast for his assistance in the hematological measurements. The study was supported by a grant no. NGN 55.3665 from the Technology Foundation (Technologiestichting, STW), The Netherlands.

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Distribution of Glutathione and Technetium-99m-*meso*-HMPAO in Normal and Diethyl Maleate-Treated Mouse Brain Mitochondria

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The aim of this study was to explain the contribution of mitochondria to the accumulation of ^{99m}Tc-*meso*-hexamethyl propyleneamine oxime (HMPAO) in the brain, after examinations were performed. **Methods:** We studied subcellular distribution of ^{99m}Tc-*meso*-HMPAO and glutathione (GSH) in normal and diethyl maleate (DEM)-administered mice. **Results:** In normal brain, major radioactivity was found in the mitochondrial (49.0%) and cytosolic fractions (33.0%), while the GSH content was high in the cytosol (63.2%) and mitochondria (30.6%). The radioactivity in mitochondrial, cytosolic, microsomal and nuclear fractions was decreased in a dose-dependent manner by DEM, a GSH depleting agent, to 32.2% (mitochondrial) and 24.7% (cytosolic) of the control by a dose of 550 mg/kg. The GSH content in mitochondrial and cytosolic fractions also decreased in a dose-dependent manner on DEM treatment to 29.3% (mitochondrial) and 30.0% (cytosolic) of the control by 550 mg/kg of DEM. A good correlation was found between the uptake of ^{99m}Tc-*meso*-HMPAO and GSH content in mitochondrial, cytosolic and nuclear fractions, with a correlation coefficient (r) of 0.814, 0.834 and 0.784, respectively. **Conclusion:** Mitochondria are a major subcellular fraction for the uptake of ^{99m}Tc-*meso*-HMPAO by the brain, and GSH in mitochondria contributes to the accumulation of ^{99m}Tc-*meso*-HMPAO.

Key Words: subcellular distribution; glutathione; mitochondria; technetium-99m-*meso*-hexamethyl propyleneamine oxime; brain

J Nucl Med 1998; 39:2178–2183

Technetium-99m-*d,l*-hexamethyl propyleneamine oxime (HMPAO) has been used widely as a blood flow imaging agent for the brain. In our previous study (*1*), we found that the uptake of *meso*-isomer of ^{99m}Tc-HMPAO was decreased in diethyl maleate (DEM, a glutathione depletor that acts through glutathione S-transferase)-treated mouse brain accompanying a decrease in glutathione (GSH) content, but that ^{99m}Tc-*d,l*-HMPAO uptake was not affected by the same treatment. In another experiment (*2*), we found similar observations in DEM-treated rat brain and buthionine sulfoximine (BSO, a GSH depletor that acts through γ-glutamylcysteine)-treated mouse brain. Based on these observations, we suggested the *meso*-isomer of ^{99m}Tc-HMPAO be used as a GSH imaging agent for the brain.

The exact mechanism of the retention of ^{99m}Tc-HMPAO, either *d,l*- or *meso*-, has not been clarified, although the following is proposed. As a lipophilic compound, ^{99m}Tc-HMPAO diffuses across the blood-brain barrier and is rapidly converted to a hydrophilic form retainable within the brain tissue. GSH is supposed to be responsible for the hydrophilic conversion and for the retention of ^{99m}Tc-HMPAO in the brain (*3,4*). We found that ^{99m}Tc-HMPAO showed reactivity not only to GSH but also to other molecules possessing a −SH group, such as GSH analog (Gly-Cys-Glu) and cysteine. However, ^{99m}Tc-HMPAO did not react with oxidized GSH or ascorbic acid. In a previous study (*2*), we determined the thiols in the nonprotein and protein-fractions of DEM-treated mouse and indicated that the nonprotein thiols were responsible for the retention of ^{99m}Tc-HMPAO in the brain. GSH accounted for almost all of nonprotein thiols in the brain. Another experiment in mouse brain homogenates indicated that GSH is a major contributor to the retention of ^{99m}Tc-HMPAO in the brain (*2*).

The rate of conversion of ^{99m}Tc-*d,l*-HMPAO to hydrophilic complex by GSH was much higher than that of ^{99m}Tc-*meso*-HMPAO: the same rate is achieved at only 1/37 the GSH concentration (*2*). Therefore, the kinetics of ^{99m}Tc-*d,l*-HMPAO are virtually unaffected by the GSH content, while uptake is determined mainly by blood flow. On the other hand, the conversion of ^{99m}Tc-*meso*-HMPAO to the retainable form by GSH is slower than the washout of the diffusible form from brain to blood. Accordingly, the uptake of *meso*-isomer reflects GSH content more than does blood flow.

In an in vitro study, Fujibayashi et al. (*5*) demonstrated that the conversion of ^{99m}Tc-*d,l*-HMPAO to hydrophilic complex in brain homogenates was enhanced when mitochondiral mem-

Received Mar. 2, 1998; accepted Apr. 12, 1998.

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