Acetyl-lys-lys-lys-lys-lys-cys-gly-cys-gly-gly-pro-leu-tyr-lys-lys-ile-ile-lys-lys-leu-leu-glu-ser-heparin-[^{99m}Tc]

P483H-^{99m}Tc

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Chemical name:	Acetyl-lys-lys-lys-lys-lys-cys-gly-cys-gly-gly-pro-leu-tyr-lys-lys-ile-ile-lys-lys-leu-leu-glu-ser-heparin-[^{99m}Tc]
Abbreviated name:	P483H-^{99m}Tc
Synonym:	
Agent Category:	Peptide
Target:	Leukocytes (unknown binding site)
Target Category:	Binding
Method of detection:	Single-photon emission computed tomography (SPECT) or gamma planar imaging
Source of signal:	^{99m}Tc
Activation:	No
Studies:	• *In vitro* • Non-primate non-rodent mammals • Humans

Background

[PubMed]

Because of their ability to accumulate at sites of infection and inflammation, the leukocyte and monocyte components of the white blood cell (WBC) have often been labeled under

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ex vivo conditions with radioisotopes such as indium (^{111}In), meta-stable technetium (^{99m}Tc), or radioactive compounds such as ^{99m}Tc-gluceptate, [^{67}Ga]gallium citrate, and ^{99m}Tc-labeled antibodies, etc (1). Radiolabeling of leukocytes or monocytes involves the isolation of these cells from the WBC pool, a process that is slow and expensive, involves the handling of blood, and can lead to contamination of the samples. In addition, the labeled cells have a slow clearance from circulation and may be imaged up to 24 h after administration to allow time for accumulation at the site of infection or inflammation. Also, these imaging agents usually have a low signal-to-noise ratio, especially during the early time points, and show a high intestinal uptake that interferes with diagnostic imaging of the abdominal area (2).

Radioactively labeled monoclonal antibodies have been used for the imaging of infections, but these radiopharmaceuticals have limited application because they also have a slow clearance from circulation and are not always sensitive enough to detect pulmonary or bone infections (3, 4). In addition, labeled antibodies have been shown to induce transient neutropenia and the formation of human anti-mouse antibodies (HAMA) (4, 5). The HAMA response can limit the repeat use of the antibodies because it neutralizes and alters the biodistribution of the agent (6). The use of peptides to detect sites of infection or inflammation is an attractive option because these compounds are cheap and easy to synthesize, can be modified to suit target requirements, and show rapid clearance from circulation (7, 8). Chemotactic peptides have been developed and evaluated for the imaging of infections and inflammation, but these compounds have limitations because the buffer components used to label the peptides may influence their biodistribution characteristics (8). Moyer et al. identified and developed a heparin-binding peptide, P483, for the imaging of infections (9). The sequence of this peptide was based on the platelet factor-4 heparin-binding region. The peptide comprises 23 amino acids, was modified to contain a lysine-rich region to allow rapid renal clearance, and could be labeled with ^{99m}Tc. The peptide was complexed with heparin and labeled with ^{99m}Tc to generate ^{99m}Tc-P483H, a peptide that targets leukocytes (9). Conjugation of the peptide with heparin enhanced the binding of the labeled peptide to WBC. The labeled peptide was evaluated for the imaging of infections in a rabbit model and also in humans (9, 10).

Synthesis

[PubMed]

The synthesis of ^{99m}Tc-P483H was described by Moyer et al. (9). The P483 peptide was synthesized with commercially available amino acids by use of solid-phase technology on a peptide synthesizer with N-α-Fmoc protection (11). The peptide was then purified by preparative reverse-phase high-performance liquid chromatography (RP-HPLC) and lyophilized as a trifluoroacetate (TFA) salt. Purity and composition of the peptide was confirmed by RP-HPLC and fast atom bombardment or electrospray mass spectrometry. Details of confirmation of the peptide composition with mass spectrometry were not provided by the investigators.

The peptide-heparin complex (P483H) was obtained by mixing 0.94 pmol of the peptide with 1 United States Pharmacopiea unit of heparin. Using peptide and heparin analysis, the optimal stochiometric ratio between the peptide and heparin for leukocyte labeling and *in vivo* localization at the infection site was determined to be 6.9 (9). Minimal accumulation of the labeled peptide at the site of infection was observed if the ratio was <3 or >60 (9).

For labeling with ^{99m}Tc, the TFA salt of P483 was reconstituted in saline and mixed with ^{99m}Tc-labeled glucoheptonate (9). The reaction was allowed to proceed at room temperature for 15 min. For quality control, the labeled peptide was assayed by silica gel instant thin-layer chromatography (ITLC) using two different solvent systems. With the first solvent system (i.e., saturated saline), ^{99m}Tc-P483 had an R_f between 0.0 and 0.7; ^{99m}Tc-pertechnetate and ^{99m}Tc-glucoheptonate had an R_f between 0.7 and 1.0. With the second solvent system (pyridine:acetic acid:water), the ^{99m}Tc-microcolloids had an R_f between 0.0 and 0.2, and the other labeled species had an R_f between 0.2 and 1.0. Criteria for acceptable labeling was defined as radiochemical purity (RCP) >90%. The reaction yield was not provided in the publication (9). Specific activity of the labeled peptide was determined to be 60–100 mCi/100 nmol (2,220–3,700 MBq/100 nmol).

Heparin was added to the radiolabeled peptide once it passed the quality-control test to generate ^{99m}Tc-P483H. The heparin-conjugated peptide was then evaluated with thin-layer chromatography using solvent saturation pads with acetonitrile:acetic acid:water as the solvent system. With this solvent system the heparin conjugate had an R_f between 0.0 and 0.5 (9).

Formulated kit vials were also prepared by adding 0.015–0.025 μM P483 as a TFA salt with or without heparin (9). These formulations also contained sufficient glucoheptonate, tin chloride, and buffers to produce an RCP >95% after the addition of ^{99m}Tc-pertechnetate. The P483 kits without heparin were either reconstituted with ^{99m}Tc-pertechnetate and then heparin was added to the labeled peptide, or *vice versa* (9). The ^{99m}Tc-labeled peptides generated with the formulated kits were evaluated for quality, as detailed above, before use.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

To determine the *in vitro* association of ^{99m}Tc-P483H, human whole blood was diluted and exposed to the labeled peptide-heparin conjugate (9). The labeled blood samples were then fractionated by density gradient centrifugation over Ficoll-Hypaque in neutrophil isolation medium. As a control, the blood samples were labeled with ^{111}In and ^{99m}Tc-gluceptate alone. On average 76.2% of the applied radioactivity was determined to be associated with the WBC; of the cell-associated activity, the polymorphonuclear cells had only 36%, whereas the leukocytes/monocytes had the remaining 64% of the label. Among these cells, 54% of the total label was associated with the monocytes. The investigators reported that labeling of the whole blood at room temperature for >8 h produced the

same radioactivity distribution, indicating that the radiolabeled peptide was stable in whole blood for prolonged periods (9).

The *in vitro* labeling of purified WBC with ^{111}In-oxine, ^{99m}Tc-gluceptate, and ^{99m}Tc-P483H was also compared by the investigators (9). Among these, ~73% of ^{111}In and ^{99m}Tc-P483H were associated with the WBC, but <15% ^{99m}Tc-gluceptate was found with these cells. These results indicated that ^{99m}Tc-P483H associated quantitatively with the WBC. Further isolation of monocytes from the lymphocytes indicated that ^{99m}Tc-P483H was associated primarily with the monocytes (9). Such a distribution of radioactivity was not investigated for the ^{111}In-labeled WBC.

Animal Studies

Rodents

[PubMed]

No references are currently available.

Other Non-Primate Mammals

[PubMed]

The biodistribution of ^{99m}Tc-P483H was investigated in rabbits and compared to the distribution of ^{67}Ga-citrate, ^{99m}Tc-gluceptate, ^{131}I-albumin, ^{99m}Tc-nanocolloid, ^{111}In-WBC, and ^{111}In-diethylenetriamene pentaacetic acid (9). New Zealand White rabbits were injected with the respective labeled compounds 18 to 24 h after infection with *Escherichia coli* in the left calf muscle. Imaging of the animals was performed 4 h after administration of the labeled compounds. ^{99m}Tc-P483H was evaluated in 48 animals with the use of the various formulations. For the other radiochemicals the number of animals used varied from 1 to 5 (9). On average, 0.062 ± 0.029% of the injected dose (ID)/g of ^{99m}Tc-P483H was detected in the infected tissue, and the infected/control tissue ratio was 26.8 ± 16.8, which was the highest among all the labeled compounds (9). A study of the time course of accumulation of label at the site of infection showed that ~1.8 to 2.0% ID/g was detected at the site 10–12 h after administration of the label. Detailed distribution of the compounds is provided in Table 2 of the publication by Moyer et al. (9). Similarly the infected tissue/blood ratio for ^{99m}Tc-P483H was 3.1, which was higher than any other radiolabeled compound in the study.

In another study, the WBC were labeled with ^{99m}Tc-P483H, washed of excess radioactivity, and administered to rabbits ($n = 2$) with *E. coli*–infected calf muscles (9). For comparison, another group of *E. coli*–infected rabbits were injected with ^{111}In-WBC. The two compounds behaved similarly; during the early period both labels were detected in the lungs, but by 4 h after injection the contrast was evident at the infected site. On average, 0.0381 ± 0.022% ID/g tissue of ^{99m}Tc-P483H–labeled WBC were detected at the infection site. No blocking studies were carried out.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

The safety and efficacy of ^{99m}Tc-P483H for the detection of infection in humans was evaluated by Palestro et al. (10). In this study, the effects of peptide dose on performance were also determined, and results obtained with the peptide were compared to those obtained with *in vitro*–labeled leukocytes. Thirty patients were given three different doses of the labeled peptide (2.9 nmol (n = 11), 14.5 nmol (n = 10), 29.0 nmol (n = 9)) and imaged at 15 min and 90–120 min after the injection. Scintigraphy was also performed on 20 patients after the administration of autologous ^{111}In-labeled leukocyte. Among these patients, 7 had osteomyelitis, 5 had vascular grafts, 2 had abscess, 1 had joint replacement, 1 had a surgical wound, and 1 had pneumonia. The sensitivity, specificity, and accuracy were determined to be 0.86, 0.81, and 0.83, respectively, for both imaging time points. From this study no correlation between the peptide dose and accuracy was apparent. Patients who underwent both procedures, labeled peptide and autologous *in vitro*–labeled leukocyte administration, followed by imaging had an identical accuracy of 80%. From this study the investigators concluded that ^{99m}Tc-P483H was safe and able to detect infections rapidly and accurately. Imaging results obtained with the labeled peptide were comparable to those obtained with *in vitro*–labeled leukocytes.

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