^{99m}Tc-succinimidyl-6-hydrazinopyridine-3-carboxylate-interleukin 12

^{99m}Tc-IL-12

Arvind Chopra, PhD[1]

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Chemical name:	^{99m}Tc-succinimidyl-6-hydrazinopyridine-3-carboxylate-interleukin 12	
Abbreviated name:	^{99m}Tc-IL-12	
Synonym:	^{99m}Tc-labeled IL-12	
Agent Category:	IL-12	
Target:	IL-12 receptor	
Target Category:	Receptor-ligand binding	
Method of detection:	Single-photon emission computed tomography (SPECT) or gamma planar imaging	
Source of signal:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click here for the nucleotide (Alpha and Beta subunits) and protein precursor (Alpha and Beta subunits) sequence of IL-12.

Background

[PubMed]

Interleukin 12 (IL-12) is a 75-kDa, heterodimeric, glycosylated protein that is a key cytokine for the differentiation of T helper 1 (Th1) cells, and it is also involved in the activation of natural killer (NK) cells (1). Although IL-12 is secreted mainly by monocytes, B cells, and cells positive for major histocompatibility complex II, the IL-12

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

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receptor is expressed only on the activated T or NK cells (2). The Th1 cells are characterized by the production of interferon-γ and are believed to have a role in the development of chronic inflammatory disorders because of their function of clearing intracellular pathogens (3). Currently only a few non-invasive techniques are available for the diagnosis or monitoring of chronic inflammation and infection. Radioactive fluorine-labeled fluorodeoxyglucose (^{18}F-FDG) has been used with positron emission tomography (PET) to study chronic inflammation, but it lacks specificity to distinguish inflammation from infection because of its cellular uptake mechanism (4-6). The labeling of lymphocytes with radioactive metastable technetium (^{99m}Tc)-labeled hexamethyl propyleneamine oxime to study inflammation was also attempted, but the low labeling efficiency of the cells yielded poor scintigraphic images and the investigators concluded that this was not a suitable method to investigate inflammation (7). Labeled peptides have also been used to detect inflammation, but these radiopharmaceuticals did not always give optimal results and have not found much clinical application (8). Interleukin-2 (IL-2) radiolabeled with radioactive iodine (^{123}I) or ^{99m}Tc has been used successfully for the *in vivo* detection of chronic inflammation, but it was reported to be tedious and expensive to label this cytokine because it has a low solubility and stability (9, 10). Because IL-12 binds to cells that mediate inflammation, Annovazzi et al. decided to label recombinant human IL-12 (rhIL-12) with ^{99m}Tc to obtain ^{99m}Tc-IL-12 and evaluated the labeled cytokine to detect inflammation in a mouse model (11). Non-radioactive rhIL-12 is approved by the United States Food and Drug Administration for use in clinical trials to treat a variety of conditions.

Synthesis

[PubMed]

Details for expression and purification of the rhIL-12 used to produce ^{99m}Tc-IL-12 are given elsewhere (12). Briefly, the recombinant protein was produced from the culture supernatant of mammalian cells using an appropriate expression vector. The protein was purified by a multi-step purification procedure as described by Leonard et al. (12). The cytokine has also been produced as a freeze-dried preparation (13). A previously frozen solution of IL-12 was used for the preparation of ^{99m}Tc-IL-12 (11).

Succinimidyl-6-hydrazinopyridine-3-carboxylate (HYNIC-NHS) was synthesized and conjugated to IL-12 using the modified method of Abrams et al. (14). The conjugated IL-12 was generated by mixing IL-12 with HYNIC-NHS dissolved in dimethyl sulphoxide at a molar ratio of 6:1 HYNIC-NHS:IL-12 for 2 h (11). An average of 1.9 mol HYNIC-NHS was conjugated to IL-12. Free HYNIC-NHS was subsequently removed from HYNIC-IL-12 on a G50 microspin column. The purity of HYNIC-IL-12 was checked by high-performance liquid chromatography (HPLC) with a size-exclusion column. Aliquots of HYNIC-IL-12 were stored at –20^{o} C until required.

The radiolabeling of HYNIC-IL-12 with ^{99m}Tc to generate ^{99m}Tc-IL-12 was performed in acetate buffer (pH 5.5) (11). The conjugate was incubated with ^{99m}Tc for 30 min in

presence of stannous chloride. The labeling efficiency was determined by instant thin-layer chromatography, and the unbound ^{99m}Tc was separated from the radiolabeled compound on a microspin G50 column. The ^{99m}Tc-IL-12 was analyzed by size-exclusion chromatography on HPLC (11). The labeling efficiency was determined to be between 75% and 85%, with a maximum specific activity of ~70.3 MBq/1.33 pmol (1.9 mCi/1.33 pmol). The radiochemical purity of the labeled conjugate was >95% and colloidal content was <5% in all preparations. Stability studies indicated that the labeled IL-12 lost only ~10% purity at 6 h when stored in either serum or saline (11).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The receptor binding of ^{99m}Tc-IL-12 was investigated with Kit225 cells (a human IL-2–dependent cell line) that constitutively express the IL-12 receptor (15). Increasing concentrations of ^{99m}Tc-IL-12 were incubated with the cells at 4°C for 60 min. The cells were then harvested by centrifugation and counted for bound radioactivity (11). The mean affinity constant (K_d) of ^{99m}Tc-IL-12 was determined to be 2.09×10^{-9} M ($P < 0.05$).

Animal Studies

Rodents

[PubMed]

The biodistribution of ^{99m}Tc-IL-12 was studied in normal mice (11). The labeled conjugate was detected primarily in the liver with significant uptake in the spleen and thymus up to 6 h after injection. The investigators suggest that the accumulation of ^{99m}Tc-IL-12 in these organs was probably the result of the presence of resident T lymphocytes.

The specificity and targeting of ^{99m}Tc-IL-12 was investigated in mice bearing Kit225 cell tumors (injected in a Matrigel vehicle) in the left flank. For control, mice were injected with Matrigel alone in the right flank (11). After injection of ^{99m}Tc-IL-12, the mice ($n = 3$ per time point) were killed at different time points (1.5, 3 and 6 h) and the Matrigel pellets were removed, weighed, and counted for radioactivity. Uptake of the label was determined as percent of injected activity per gram tissue weight (% IA/g). At any given time point, the amount of radioactivity detected in the Matrigel that contained Kit225 cells was approximately twice that detected in the Matrigel alone. This indicated that the labeled IL-12 would accumulate at sites of inflammation where lymphocytes are attracted because of an immune response. No competition data with cold IL-12 was presented by the investigators (11).

The binding of ^{99m}Tc-IL-12 was also investigated in mice with trinitrobenzene sulphonic acid–induced chronic colitis (11). For this study, control mice were treated with 50%

ethanol in 50% phosphate buffered saline . Six mice with chronic colitis were injected with ^{99m}Tc-IL-12 and killed 3 h later. The large bowel was removed, weighed, counted, and imaged with a gamma camera. The percent of secreted activity was determined by separating the stool and the intestinal wall from the inflamed tissue (11). The affected colon was divided into consecutive segments and each segment was examined histologically, weighed, and counted for radioactivity to determine uptake of the label (% IA/g). The label was observed to have accumulated primarily in the inflamed sections of the colon, and little uptake was noted in the non-inflamed sections or in the colon from the control mice. The level of label detected in the inflamed sections correlated with the degree of bowel lymphatic infiltration observed during histological evaluation. The stool was observed to contribute 10.4 ± 7.5% and 9.2 ± 3.2% of the label in affected and non-affected areas of the colon, respectively (11). Blocking studies were not reported by the investigators.

The investigators suggested that high doses of the radiochemical could be used in mice without side effects because the IL-12 receptors in these animals bind the cytokine but do not trigger biological activity (11). From these results they concluded that labeled IL-12 could potentially be used in humans only after specific activity of the radiochemical was improved and the dosage was carefully evaluated.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

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