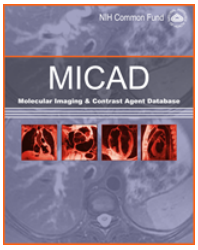
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Chopra A. ^{99m}Tc-labeled anti-tumor necrosis factor-alpha monoclonal antibody. 2007 Sep 25 [Updated 2007 Oct 22]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



^{99m}Tc-labeled anti-tumor necrosis factor-alpha monoclonal antibody

^{99m}Tc-anti-TNF-α mAb

Arvind Chopra[1]

Created: September 25, 2007; Updated: October 22, 2007.

Chemical name:	^{99m}Tc-labeled anti-tumor necrosis factor-α monoclonal antibody	
Abbreviated name:	^{99m}Tc-anti-TNF-α mAb	
Synonym:	Remicade®	
Agent Category:	Monoclonal antibody	
Target:	Tumor necrosis factor-α	
Target Category:	Antibody-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 • Humans	Click here for the protein and nucleotide sequence of TNF α.

Background

[PubMed]

The tumor necrosis factor alpha (TNF-α) is synthesized as a 26-kDa membrane-bound protein on certain cell types, e.g., T cells. The membrane-bound protein is cleaved to release a 17-kDa soluble form that exists as a homotrimer. It has been implicated in the pathogenesis of different diseases including cancer and inflammatory diseases such as rheumatoid arthritis (RA), Crohn's disease (CD), and ulcerative colitis (1, 2). The stimulation of TNF-α induction is known to activate several pro-inflammatory cytokines, chemokines, matrix metalloproteinases, and endothelial adhesion molecules that attract cells known to promote inflammation (3). Because of its role in the pathogenesis of different inflammatory diseases, a variety of anti–TNF-α agents have been developed and investigated for the treatment of these conditions (4). Several anti–TNF-α antibodies such

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

as infliximab, adalimumab, and certolizumab are now available for the treatment of TNF-α–mediated inflammatory diseases (2).

Infliximab is an anti–TNF-α, chimeric mouse-human IgG1 monoclonal antibody (mAb) that is commercially available and is approved by the United States Food and Drug Administration for the treatment of a variety of inflammatory diseases (2, 4). This mAb is known to bind TNF-α in the serum or on the cell membranes and to inhibit its biological activity mediated through the TNF-α receptor. To evaluate its *in vivo* use for the detection of TNF-α in the various inflammatory diseases, infliximab was labeled with radioactive meta-stable technetium (^{99m}Tc) and used as detailed below (2, 5-7).

Synthesis

[PubMed]

The ^{99m}Tc labeling of infliximab was initiated by first reducing the mAb using the 2-mercaptoethanol (2-Me) method as described elsewhere (8). In brief, commercially available infliximab was dissolved in phosphate-buffered saline (PBS) and 2-Me was added to it in a 2-Me:infliximab ratio of 2,000:1 (5). The reaction was allowed to proceed for 30 min at room temperature, and the reduced antibody was purified on a Sephadex G25 column. The reduced antibody was aliquoted and stored in liquid nitrogen until required.

The methylene diphosphonate (MDP) bone scan kit (containing MDP, tin chloride, and ascorbic acid) was used for the labeling of infliximab (5). The MDP kit was reconstituted in saline, aliquoted, and stored at -20°C until necessary. The reconstituted MDP was added to the reduced infliximab and ^{99m}Tc-labeled pertechnetate was added to the mixture. The solution was incubated at room temperature for 10 min and the preparation was analyzed by instant thin-layer chromatography to determine the labeling efficiency. The radiolabeled antibody had an Rf of 0, whereas pertechnetate and ^{99m}Tc-labeled MDP had an Rf of 0.9–1.0. The labeling efficiency of the reaction was determined to be 98.2 ± 0.8%. The labeled antibody had a radiochemical purity of >95% as determined by high-performance liquid chromatography on a gel-filtration column. The specific activity of the labeled antibody was 2,775 ± 555 kBq/6.6 pmol (75 ± 15 μCi/6.6 pmol), and the radiotracer was determined to be stable for up to 8 h in human serum or saline.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding characteristics of labeled infliximab were determined with a competitive binding assay using human lymphocytes (5). Induction of membrane-bound TNF-α was carried out by exposure of the cells to phytohemagglutinin. The cells expressing TNF-α were then exposed either to an increasing concentration of ^{99m}Tc-labeled infliximab alone or [^{99m}Tc]infliximab in the presence of a molar excess of unlabeled antibody. A competitive, dose-dependent inhibition of [^{99m}Tc]-infliximab by the unlabeled antibody was observed. The labeled antibody had a dissociation constant (K_d) of 4.67×10^{-10} M for TNF-α on the stimulated lymphocytes.

Animal Studies

Rodents

[PubMed]

The use of [^{99m}Tc]-infliximab as an inflammation imaging agent was evaluated and compared to ^{99m}Tc-tin colloid–labeled leukocytes in a rat colitis model (6). Uptake ratios of 2.7 ± 1.0 and 2.6 ± 0.3 were observed in the inflamed colon at 1 and 4 h, respectively, after the administration of [^{99m}Tc]-infliximab. The labeled leukocytes yielded ratios of 19.5 ± 9.9 and 41.2 ± 16.1 at 1 and 4 h, respectively. The investigators suggested that although

the uptake was lower for [^{99m}Tc]-infliximab, the labeled antibody can be used for imaging inflammatory bowel disease in humans because it is easy to prepare compared to the labeled leukocytes (6).

The biodistribution of [^{99m}Tc]-infliximab was investigated in mice (5). The labeled antibody showed a slow clearance from the blood and had a mixed hepatic/renal metabolism with little accumulation over 24 h in the gut of the animals. Similar pharmacokinetics have also been observed with other labeled antibodies (9).

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

Imaging studies of CD patients treated with ^{99m}Tc-infliximab showed renal accumulation of the label immediately after the treatment, and the radioactivity was cleared from blood circulation by ~6 h (5). For comparison the patients were also treated with ^{99m}Tc-labeled hexamethylpropyleneamine oxime (HMPAO) autologous white blood cells. Bowel uptake of the radioactivity was reported in 6 of the 10 treated patients. With the [^{99m}Tc]-HMPAO-labeled leukocytes, 8 of 10 patients were positive for the label. The investigators observed that the 6 patients positive for [^{99m}Tc]-infliximab showed a lower accumulation of the label compared to the accumulation of labeled leukocytes.

An increased level of TNF-α has been reported in the inflamed joints of individuals suffering from synovial inflammation, and the use of intra-articular infliximab was reported to be beneficial for the treatment of RA, ankylosing spondylitis, and Behçet's disease (10-12). Conti et al. used an *in vivo* injection of [^{99m}Tc]-infliximab to assess the expression of TNF-α in the arthritic knee of an individual (13). The investigators observed an accumulation of the label in the affected knee, indicating that an increased level of TNF-α was present in the joint. They removed the synovial fluid from the knee and injected a single bolus of cold infliximab, which resulted in complete remission of the arthritis. After complete remission the patient was given another injection of labeled infliximab and evaluated by scintigraphy for the accumulation of the label. No radioactivity was detected in the knee joint, indicating the absence of TNF-α . From these observations the investigators suggested this approach could be used to screen patients for intra-articular infliximab therapy (13).

In another study, van der Laken et al. investigated the development of anti-infliximab antibodies in patients with RA who did not respond to infliximab therapy (7). For this the investigators infused infliximab responders and infliximab non-responders with [^{99m}Tc]-infliximab. By planar gamma imaging they observed lower whole-body retention of the label (76% of injected dose) in the non-responders compared to the responders (98–100% of the injected dose) at 24 h after the injection. Compared to responders, the label was observed to accumulate in the liver, spleen, and inflamed joints of the non-responding individuals. Using a sucrose density gradient, the serum of non-responders was observed to possibly contain infliximab-IgG complexes that were absent in the serum obtained from the responding individuals (7). The investigators suggested that the non-responders had probably developed anti-infliximab antibodies that bound to infliximab and were taken up by the liver.

References

1. Maisey N. Antitumor Necrosis Factor (TNF-a) Antibodies in the Treatment of Renal Cell Cancer. Cancer Invest. 2007.:1–5. PubMed PMID: 17852116.
2. Tilg H., Moschen A., Kaser A. Mode of function of biological anti-TNF agents in the treatment of inflammatory bowel diseases. Expert Opin Biol Ther. 2007; **7** (7):1051–9. PubMed PMID: 17665993.
3. Locksley R.M., Killeen N., Lenardo M.J. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell. 2001; **104** (4):487–501. PubMed PMID: 11239407.
4. Kooloos W.M., de Jong D.J., Huizinga T.W., Guchelaar H.J. Potential role of pharmacogenetics in anti-TNF treatment of rheumatoid arthritis and Crohn's disease. Drug Discov Today. 2007; **12** (3-4):125–31. PubMed PMID: 17275732.
5. D'Alessandria, C., G. Malviya, A. Viscido, A. Aratari, F. Maccioni, A. Amato, F. Scopinaro, R. Caprilli, and A. Signore, Use of a (99m)Tc labeled anti-TNFalpha monoclonal antibody in Crohn's disease: in vitro and in vivo studies. Q J Nucl Med Mol Imaging, 2007.
6. Tsopelas C., Penglis S., Ruskiewicz A., Bartholomeusz D.L. Scintigraphic imaging of experimental colitis with technetium-99m-infliximab in the rat. Hell J Nucl Med. 2006; **9** (2):85–9. PubMed PMID: 16894410.
7. van der Laken C.J., Voskuyl A.E., Roos J.C., Stigter van Walsum M., de Groot E.R., Wolbink G., Dijkmans B.A., Aarden L.A. Imaging and serum analysis of immune complex formation of radiolabelled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. Ann Rheum Dis. 2007; **66** (2):253–6. PubMed PMID: 16793840.
8. Mather S.J., Ellison D. Reduction-mediated technetium-99m labeling of monoclonal antibodies. J Nucl Med. 1990; **31** (5):692–7. PubMed PMID: 2341907.
9. Chianelli M., Parisella M.G., D'Alessandria C., Corsetti F., Scopinaro F., Signore A. The developing role of peptide radiopharmaceuticals in the study of chronic inflammation: new techniques for novel therapeutic options. Q J Nucl Med. 2003; **47** (4):256–69. PubMed PMID: 14973418.
10. Partsch G., Wagner E., Leeb B.F., Dunky A., Steiner G., Smolen J.S. Upregulation of cytokine receptors sTNF-R55, sTNF-R75, and sIL-2R in psoriatic arthritis synovial fluid. J Rheumatol. 1998; **25** (1):105–10. PubMed PMID: 9458212.
11. Andonopoulos A.P., Meimaris N., Daoussis D., Bounas A., Yiannopoulos G. Intra-articular anti-tumor necrosis factor alpha antibody in recalcitrant arthritis of Behcet's disease. Clin Exp Rheumatol. 2003;**21** Suppl 30 (4):S57–8. PubMed PMID: 14727465.
12. Nikas S.N., Temekonidis T.I., Zikou A.K., Argyropoulou M.I., Efremidis S., Drosos A.A. Treatment of resistant rheumatoid arthritis by intra-articular infliximab injections: a pilot study. Ann Rheum Dis. 2004; **63** (1):102–3. PubMed PMID: 14672902.
13. Conti F., Priori R., Chimenti M.S., Coari G., Annovazzi A., Valesini G., Signore A. Successful treatment with intraarticular infliximab for resistant knee monarthritis in a patient with spondylarthropathy: a role for scintigraphy with 99mTc-infliximab. Arthritis Rheum. 2005; **52** (4):1224–6. PubMed PMID: 15818709.