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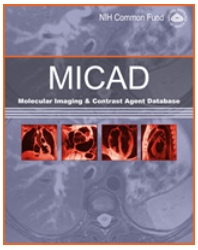

^{99m}Tc-Labeled succinimidyl-6-hydrazinonicotinate hydrochloride (SHNH)-conjugated visilizumab

^{99m}Tc-SHNH-visilizumab

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Chemical name:	^{99m}Tc-Labeled succinimidyl-6-hydrazinonicotinate hydrochloride (SHNH)-conjugated visilizumab	
Abbreviated name:	^{99m}Tc-SHNH-visilizumab	
Synonym:		
Agent Category:	Antibody	
Target:	CD3 antigen	
Target Category:	Antigen	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{99m}Tc	
Activation:		
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

CD3 antigen is a protein complex that is expressed on the T cell surface and is involved in the signal transduction of T cell activation. More than 95% of the circulating T cells in blood and activated T cells in inflamed tissues express CD3 antigen (1-3). Blocking CD3 antigen with monoclonal antibodies (mAbs) has been shown to be effective in suppressing the immunoreaction in inflammatory and graft-*versus*-host disease conditions. OKT3 is a mouse anti-human CD3 mAb that has been widely used to prevent and treat acute allograft rejection in clinical transplantation (4-6). However, the clinical utility of OKT3 therapy is limited by inducing a neutralizing anti-mouse antibody response and a cytokine release syndrome (7, 8). The syndrome has been thought to be derived from the enormous release of cytokines, particularly TNF-α from T cells in response to the antibody, and to be attributed to the cross-linking of T cells bearing CD3 molecules and Fc receptor (FcR)-bearing immune effector cells that bind to the Fc portion of the antibodies. The cross-linking activates both the T cells and the FcR-bearing cells, leading to the massive release of cytokines (9, 10).

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To reduce potential side effects and immunogenicity, Cole et al. generated a humanized version of the OKT3 mAb visilizumab (HuM291) (11). Visilizumab is characterized by decreased binding with FcRs due to the introduced mutations within the IgG_2 Fc at amino acid residues 234 and 237 (Val to Ala), and by decreased ability to activate human complements because of the human IgG_2 M3 isotype. Compared with OKT3 mAb, visilizumab has been shown to be much less mitogenic to T cells and to induce less toxicity from cytokine release *in vivo*, while retaining the ability to modulate the CD3 complex and inhibit the mixed lymphocyte reaction. The potential therapeutic effects of visilizumab have been examined in several immune system–related disorders, including ulcerative colitis, Crohn's disease, renal allograft rejection, and acute graft-*versus*-host disease (8, 12, 13)., However, visilizumab induces a rapid and severe lymphopenia after infusion in humans (12). The reason for this transient lymphopenia is unknown because the path of migration from blood after visilizumab infusion has not been clarified. Malviya et al. radiolabeled visilizumab with ^{99m}Tc and checked its *in vitro* and *in vivo* specificity for CD3-positive cells (2). The design of this study was based on their hypothesis that the use of a radiolabeled antibody against CD3 antigen might assist in the selective detection of resting and activated T cells and thus serve as a diagnostic tool for imaging T cell traffic and lymphocytic infiltration of tissues and organs affected in autoimmune diseases. Ultimately, the use of a radiolabeled antibody would provide a rationale for treatment with anti-CD3 mAb and would serve as a tool for therapy follow-up.

Relevant Resource Links

- Chapters in MICAD
- CD3 Gene information in NCBI
- Clinical trials (Visilizumab, OKT3)

Synthesis

[PubMed]

To label visilizumab and the control antibody, Malviya et al. tested both direct and indirect labeling methods (2). The control mAb was a humanized IgG_2 antibody without FcR and without CD3 binding. The direct labeling with ^{99m}Tc was achieved with the 2-mercapthoethanol method. The indirect labeling was completed first by conjugating the heterobifunctional linker succinimidyl-6-hydrazinonicotinate hydrochloride (SHNH) with the mAb to obtain a SHNH-mAb conjugate, and then by coupling the SHNH-mAb complex with ^{99m}Tc to obtain the desired product, ^{99m}Tc-SHNH–mAb. With the direct method, a high labeling efficiency (~99%) with a high specific activity (≥2,600 MBq/mg (70.3 mCi/mg)) was achieved for the control mAb but not for visilizumab. The directly labeled control mAb was then used in all experiments. With the indirect method, a high labeling efficiency (≥90%) with a high specific activity (10,360–11,100 MBq/mg (280–300 mCi/mg) was obtained for visilizumab but not for the control mAb (labeling efficiency: <40%; specific activity: <4,440 MBq/mg (<120 mCi/mg)). An average of 4–10 hydrazino groups were conjugated to each molecule of visilizumab. The indirect labeling method was then used to label visilizumab, and the labeled product ^{99m}Tc-SHNH–visilizumab was used in all experiments. The reason for the differences of labeling efficiency and specific activity between the two antibodies was not given. Both ^{99m}Tc-SHNH–visilizumab and the ^{99m}Tc-labeled control mAb were stable in human serum for up to 24 h at 37°C (87% for visilizumab and 89% for control Ab). The cysteine challenge assay also demonstrated high stability of the radiolabeled mAbs up to a 500:1 ratio between cysteine and the radiolabeled mAb. Sodium dodecyl sulphate polyacrylamide gel electrophoresis confirmed that visilizumab (in molecular weight) was not modified by the labeling procedure, as shown by a single band at 150 kDa.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Malviya et al. first analyzed the CD3 expression on human peripheral blood mononuclear cells (hPBMCs), human lymphoma cells (HuT78), T cell–derived human acute lymphoblastic leukemia cells (CEM), and immortalized T lymphocytes (Jurkat cell line) with fluorescence-activated cell sorter analysis after staining cells with a fluorescein isothiocyanate–conjugated anti-CD3 antibody (2). The investigators observed a mean fluorescence intensity of 300 for hPBMCs (80% positive cells), 38 for CEM (50% positive cells), 18 for HuT78 (>95% positive cells), and 30 for Jurkat cells (60% positive cells), indicating different CD3 expression levels among the cell lines. hPBMCs and HuT78 were then used for experiments. Specific binding saturation on HuT78 cells was obtained at ^{99m}Tc-SHNH–visilizumab concentrations of more than 5×10^{-10} mol, and ^{99m}Tc-SHNH–visilizumab binding was displaced by a 100-fold molar excess of unlabeled antibody, indicating that ^{99m}Tc-SHNH–visilizumab retained its specific binding activity with CD3 antigens expressed on HuT78 cells. The immunoreactive fraction of ^{99m}Tc-SHNH–visilizumab on hPBMCs in two experiments was 68% and 75%, respectively.

Animal Studies

Rodents

[PubMed]

To evaluate the binding ability of ^{99m}Tc-SHNH–visilizumab with HuT78 cells *in vivo*, experiments were performed in athymic nude BALB/c *nu/nu* mice ($n = 9$) bearing subcutaneously implanted HuT78 cells in one thigh and CD3-negative tumor cells (TPC1) in the other thigh (control) (2). At 2 h after cell implantation, mice were injected in the tail vein with ^{99m}Tc-SHNH–visilizumab. High-resolution γ-camera imaging showed that the uptake level in the HuT78 cells was significantly higher and visible than in CD3-negative control cells ($P < 0.05$). The target/background ratio increased significantly with increased numbers of HuT78 cells (0.98 ± 0.03 for 5×10^6 cells, 1.17 ± 0.03 for 10×10^6 cells, and 1.34 ± 0.12 for 20×10^6 cells at 6 h; and 1.08 ± 0.06 for 5×10^6 cells, 1.30 ± 0.17 for 10×10^6 cells, and 1.56 ± 0.13 for 20×10^6 cells at 24 h) ($P < 0.05$). A small but statistically nonsignificant increase of the cell uptake from 6 h to 24 h was observed, indicating fast binding kinetics of the ^{99m}Tc-SHNH–visilizumab to target cells. In vivo competition studies with hPBMCs (2×10^7 cells) showed that the uptake of ^{99m}Tc-SHNH–visilizumab decreased significantly with pretreatment with an excess of unlabeled anti-CD3 mAb (96% at 6 h and 87% at 24 h). The target/background ratios in mice without and with pre-injected cold visilizumab were 1.91 ± 0.6 and 1.04 ± 0.2 at 6 h, and 3.06 ± 0.8 and 1.27 ± 0.3 at 24 h, respectively (all $P < 0.05$).

Biodistributions of the ^{99m}Tc-labeled visilizumab and control mAb were investigated in female SCID mice reconstituted with hPBMCs (2). Immediately after intravenous reconstitution (8×10^6 hPBMCs per mouse), mice were injected with visilizumab or control mAb ($n = 3$ mice/group) (10% injected antibodies were labeled with ^{99m}Tc). With sequential imaging, ^{99m}Tc-labeled visilizumab and control mAb presented different migration patterns of the lymphocytes and different biodistributions of the radioactivity. ^{99m}Tc-SHNH–visilizumab, by binding to lymphocytes in blood, induced cells to accumulate in the liver (>400%) spleen (>720%), and small bowel (>230%) at levels higher than those induced by the control mAb (all $P < 0.05$). By contrast, ^{99m}Tc-labeled control mAb showed a long resident time and high radioactivity (>140% at 3 h, $P < 0.05$) in the blood, with little accumulation of radioactivity in the liver and small intestine. Minor and statistically nonsignificant differences were observed in other organs, including the lung, kidney, and large bowel, with the counts being higher for ^{99m}Tc-SHNH–visilizumab than for ^{99m}Tc-labeled control mAb (data not shown). These results indicated that, after intravenous injection of a pharmacological dose of visilizumab, lymphocytes migrated from the blood to the liver, spleen, and small bowel. Histological examination of the organs confirmed the difference in the presence of human lymphocytes between visilizumab and control mAb–injected mice for the spleen and small bowel, but not for the liver, lungs, or large bowel. Lymph nodes and intestinal mucosa–associated lymphoid tissues showed the same behavior as the spleen in mice injected with visilizumab. These

findings support the hypothesis that visilizumab induces lymphocyte migration from blood into primary and secondary lymphoid organs.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Guy C.S., Vignali D.A. *Organization of proximal signal initiation at the TCR:CD3 complex.* . Immunol Rev. 2009;232(1):7–21. PubMed PMID: 19909352.
2. Malviya G., D'Alessandria C., Bonanno E., Vexler V., Massari R., Trotta C., Scopinaro F., Dierckx R., Signore A. *Radiolabeled humanized anti-CD3 monoclonal antibody visilizumab for imaging human T-lymphocytes.* . J Nucl Med. 2009;50(10):1683–91. PubMed PMID: 19759100.
3. Portoles P., Rojo J.M. *The TCR/CD3 complex: opening the gate to successful vaccination.* . Curr Pharm Des. 2009;15(28):3290–300. PubMed PMID: 19860678.
4. Ilan, Y., E. Zigmond, G. Lalazar, A. Dembinsky, A. Ben Ya'acov, N. Hemed, I. Kasis, E. Axelrod, L. Zolotarov, A. Klein, M. El Haj, R. Gandhi, C. Baecher-Allan, H. Wu, G. Murugaiyan, P. Kivisakk, M.F. Farez, F.J. Quintana, S.J. Khoury, and H.L. Weiner, *Oral Administration of OKT3 Monoclonal Antibody to Human Subjects Induces a Dose-Dependent Immunologic Effect in T Cells and Dendritic Cells.* J Clin Immunol, 2009
5. Kung P., Goldstein G., Reinherz E.L., Schlossman S.F. *Monoclonal antibodies defining distinctive human T cell surface antigens.* . Science. 1979;206(4416):347–9. PubMed PMID: 314668.
6. Ochi H., Abraham M., Ishikawa H., Frenkel D., Yang K., Basso A., Wu H., Chen M.L., Gandhi R., Miller A., Maron R., Weiner H.L. *New immunosuppressive approaches: oral administration of CD3-specific antibody to treat autoimmunity.* . J Neurol Sci. 2008;274(1-2):9–12. PubMed PMID: 18804221.
7. Baumgart D.C., Lowder J.N., Targan S.R., Sandborn W.J., Frankel M.B. *Transient cytokine-induced liver injury following administration of the humanized anti-CD3 antibody visilizumab (HuM291) in Crohn's disease.* . Am J Gastroenterol. 2009;104(4):868–76. PubMed PMID: 19240707.
8. Baumgart, D.C., S.R. Targan, A.U. Dignass, L. Mayer, G.V. Assche, D.W. Hommes, S.B. Hanauer, U. Mahadevan, W. Reinisch, S.E. Plevy, B.A. Salzberg, A.L. Buchman, G.M. Mechkov, Z.A. Krastev, J.N. Lowder, M.B. Frankel, and W.J. Sandborn, *Prospective randomized open-label multicenter phase I/II dose escalation trial of visilizumab (HuM291) in severe steroid-refractory ulcerative colitis.* Inflamm Bowel Dis, 2009
9. Wing M. *Monoclonal antibody first dose cytokine release syndromes-mechanisms and prediction.* . J Immunotoxicol. 2008;5(1):11–5. PubMed PMID: 18382853.
10. Breslin S. *Cytokine-release syndrome: overview and nursing implications.* . Clin J Oncol Nurs. 2007;11 Suppl(1):37–42. PubMed PMID: 17471824.

11. Cole M.S., Stellrecht K.E., Shi J.D., Homola M., Hsu D.H., Anasetti C., Vasquez M., Tso J.Y. *HuM291, a humanized anti-CD3 antibody, is immunosuppressive to T cells while exhibiting reduced mitogenicity in vitro.* . Transplantation. 1999;68(4):563–71. PubMed PMID: 10480417.
12. Carpenter P.A., Appelbaum F.R., Corey L., Deeg H.J., Doney K., Gooley T., Krueger J., Martin P., Pavlovic S., Sanders J., Slattery J., Levitt D., Storb R., Woolfrey A., Anasetti C. *A humanized non-FcR-binding anti-CD3 antibody, visilizumab, for treatment of steroid-refractory acute graft-versus-host disease.* . Blood. 2002;99(8):2712–9. PubMed PMID: 11929757.
13. Kaufman A., Herold K.C. *Anti-CD3 mAbs for treatment of type 1 diabetes.* . Diabetes Metab Res Rev. 2009;25(4):302–6. PubMed PMID: 19319985.