EDITORIAL

Will a Radiolabeled Antibody Replace Indium-111-Platelets to Detect Active Thrombus?

The article by Stratton et al. (*1*) in this issue of the *Journal* calls attention to a persistent challenge facing nuclear medicine: the quest for a reliable imaging agent to detect active and mature thrombi that will contribute to clinical and therapeutic decisions. Since the late 1970s, ^{111}In-labeled platelets have provided physiologic data of platelet survival and sequestration, affording useful data for therapeutic decisions in patients with low platelet counts (*2–5*). Indium-111 platelets have also provided a physiologic imaging technique to assess platelet deposition at atherosclerotic plaques (*6,7*), at angioplasty sites (*8,9*), and in vascular grafts (*9,10*), as well as to detect active arterial and venous thrombi (*11–16*).

Nevertheless, a less cumbersome, noninvasive imaging test that can diagnose active thrombi amenable to anticoagulant and/or thrombolytic therapy is needed. Currently, the use of labeled platelets is the only FDA-approved functional imaging technique for detection of active thrombus formation. The labeling procedure requires up to 2 hr, and imaging delays of 6–24 hr for venous thrombi and 3–4 days for arterial thrombi are required to achieve diagnostic results without using background subtraction (*9,15,16*). Therefore, a rapid and specific imaging technique is needed to provide a more practical clinical test.

There are several labeled monoclonal antibodies (Mabs) specific for platelets and fibrin that have been evaluated as thrombus imaging agents, and some have undergone clinical trials (*17–31*). Other articles have discussed some of these thrombus-specific antibodies (*21,32,33*). The type of investigative model, species specificity, target-to-background ratios and absolute binding are all key factors in the development of clinically useful radiopharmaceuticals.

To date, labeled T2G1s Fab' antifibrin antibody has undergone the most extensive clinical testing and has shown a relatively low human antimouse antibody (HAMA) response (*23,24*). Animal studies have shown localization of this antifibrin antibody in both acute venous and arterial thrombi when there is active fibrin deposition (*18,23*). However, this antibody does not bind to fibrin degradative products, which are formed within hours of acute thrombus formation.

In addition, Mabs specific for plasmin-digested fibrin, including GC4, 15C5, and 3B6/22, have shown promise as thrombus imaging agents (*22,25–27*). Bini et al. (*28,29*) conducted an immunohistochemical study comparing the binding and specificity of T2G1s and GC4 for fibrin in various disease states, including atherosclerosis, renal disease and neoplastic growth. Plaques of all ages showed considerable binding of both of these Mabs. The T2G1s antibody bound to areas of acute thrombosis, whereas GC4 bound to the more advanced thrombotic regions. These findings make it unlikely that the T2G1s antibody will achieve significant localization in older thrombi, which are of clinical importance.

There are several potentially important clinical uses for a noninvasive imaging technique which could detect active arterial and venous thrombi. Cerqueira et al. (*30*) have shown that acute arterial thrombi can be visualized in dogs within 2 hr of injection of ^{99m}Tc-labeled T2G1s antifibrin antibody. Stratton and associates (*10–12,34*) have had additional experience with the use of platelet imaging in various clinical situations. The current clinical article compares the results of T2G1s antifibrin imaging to those of ^{111}In platelet scintigraphy in patients with large vessel "chronic" arterial thrombi: 13 of the 18 were in vascular grafts, three in aneurysms and two in the left ventricle (*1*).

This study is one of a few that compares a potential clinical radiopharmaceutical with a proven functional radiotracer technique, as well as with a structural imaging technique, in the same patient. Most clinical scintigraphic studies of active thrombus formation have compared a radiotracer with a structural test such as contrast venography and/or ultrasonography. The results of this clinical trial show that T2G1s antibody scintigraphy was less likely to detect "chronic" thrombi than ^{111}In platelet scintigraphy. It would be useful to know if the patients with a positive T2G1 and a positive platelet scan had a higher incidence of complications compared with those with only a positive platelet scan. Patients with a positive T2G1 scan were likely to have active thrombus propagation and thus would be more likely to have embolic and/or other complications.

To date, there has been little clinical success in imaging arterial thrombi with a radiotracer technique. The optimal technique should have good thrombus affinity, low background activity and a stable radiolabel. It should also be easy and rapid to perform and have high thrombus (embolus) specificity. Current research includes: investigating the use of smaller antibody-based segments with single-chain antibodies (*35*) or molecular recognition units (MRUs), which are synthetic amino acid replications of antibody binding regions (*36*), and two-step approaches, for example, utilizing the high binding affinity of streptavidin for the vitamin biotin (*37,38*).

Received May 27, 1994; accepted Jun. 13, 1994.
For correspondence or reprints contact: James E. Seabold, MD, Division of Nuclear Medicine, Dept. of Radiology, The University of Iowa Hospitals and Clinics, 200 Hawkins Dr., Iowa City, IA 52242.

These new techniques are designed to obtain optimal target-to-nontarget activity ratios by fast renal clearance of the radioactive agent and to help reduce patient radiation exposure by reducing background levels commonly found with radiolabeled antibodies and their fragments. Radiolabeled synthetic amino acid replications of the active binding region of an antibody or an analog have been used in animals to image deep vein thrombi and pulmonary emboli (*39–41*).

Radiolabeled thrombus-binding peptides have shown potential to detect active thrombi (*36,39–43*). Peptides have the theoretical advantage of rapid blood clearance and are less immunogenic than murine antibodies. In general, however, peptides have had low thrombus affinity and rapid renal clearance, resulting in low absolute thrombus accumulation. In animal studies, ^{123}I-bitistatin has shown good absolute binding to thrombus and rapid blood clearance, allowing detection of deep vein thrombi and pulmonary emboli (*40*).

In summary, antifibrin antibodies have shown the greatest clinical utility to detect active venous thrombus formation. Antiplatelet antibodies hold promise for detection of both active arterial and venous thrombus formation. They may also be of value to monitor thrombus dissolution, but further clinical testing is needed. Peptides have also shown promising results, but have not yet undergone extensive clinical trials.

James E. Seabold
The University of Iowa College of Medicine
Iowa City, Iowa

Scott F. Rosebrough
University of Rochester Medical Center
Rochester, New York

REFERENCES

1. Stratton JR, Cerqueira MD, Dewhurst TA, Kohler TR. Imaging arterial thrombosis in humans: comparison of technetium-99m-labeled monoclonal antifibrin antibodies and indium-111-labeled platelets. *J Nucl Med* 1994;35:1731–1737.
2. Thakur ML, Welch MJ, Joist JH, Coleman RE. Indium-111-labeled platelets: studies on preparation and evaluation of in vitro and in vivo functions. *Thromb Res* 1976;9:345–357.
3. Heyns A duP, Lotter MG, Badenhorst PN, et al. Kinetics, distribution and sites of destruction of ^{111}In-labeled human platelets. *Br J Haematol* 1980;44:269–280.
4. Najean Y, Dufour Y, Rain JD, Toubert ME. The site of platelet destruction in thrombocytopenic purpura as a predictive index of the efficacy of splenectomy. *Br J Haematol* 1991;79: 271–276.
5. Lamy T, Moisan A, Dauriac C, Ghandour C, Morice P, Le Prise PY. Splenectomy in idiopathic thrombocytopenic purpura: its correlation with the sequestration of autologous indium-111-labeled platelets. *J Nucl Med* 1993;33: 182–186.
6. Davis HH II, Siegel BA, Sherman LA, et al. Scintigraphic detection of carotid atherosclerosis with indium-111-labeled autologous platelets. *Circulation* 1980;61:982–988.
7. Powers WJ, Siegel BA, Davis HH II, Mathias CJ, Clark HB, Welch MJ. Indium-111 platelet scintigraphy in cerebrovascular disease. *Neurology* 1982;32:938–943.
8. Pope CF, Ezekowitz MD, Smith EO, et al. Detection of platelet deposition at the site of peripheral balloon angioplasty using indium-111 platelet scintigraphy. *Am J Cardiol* 1985;55: 495–497.
9. Dewanjee MK. Cardiac and vascular imaging with labeled platelets and leukocytes. *Semin Nucl Med* 1984;14:154–187.
10. Stratton JR, Thiele BL, Ritchie JL. Natural history of platelet deposition on Dacron aortic bifurcation grafts in the first year after implantation. *Am J Cardiol* 1983;52:371–374.
11. Stratton JR, Ritchie JL, Hamilton GW, Hammermeister KE, Harker LA. Left ventricular thrombi: in vivo detection by indium-111 platelet imaging and two dimensional echocardiography. *Am J Cardiol* 1981;47:874–881.
12. Stratton JR, Ritchie JL. The effects of antithrombotic drugs in patients with left ventricular thrombi: assessment with indium-111 platelet imaging and two-dimensional echocardiography. *Circulation* 1984;69:561–568.
13. Ezekowitz MD, Wilson DA, Smith EO, et al. Comparison of indium-111 platelet scintigraphy and two-dimensional echocardiography in the diagnosis of left ventricular thrombi. *N Engl J Med* 1982;306:1509–1513.
14. Ezekowitz MD, Pope CF, Sostman HD, et al. Indium-111 platelet scintigraphy for the diagnosis of acute venous thrombosis. *Circulation* 1986;73:668–674.
15. Seabold JE, Conrad GR, Kimball DA, Ponto JA, Bricker JA. Pitfalls in establishing the diagnosis of deep venous thrombophlebitis by indium-111 platelet scintigraphy. *J Nucl Med* 1988;29:1169–1180.
16. Honkanen T, Jauhola S, Karppinen K, Paul R, Sakki S, Vorne M. Venous thrombosis: a controlled study on the performance of scintigraphy with ^{99m}Tc-HMPAO-labelled platelets versus venography. *Nucl Med Commun* 1992;13: 88–94.
17. Oster ZH, Srivastava SC, Som P, et al. Thrombus radioimmunoscintigraphy: an approach using monoclonal antiplatelet antibody. *Proc Natl Acad Sci USA* 1985;82:3465–3468.
18. Rosebrough SF, Kudryk BJ, Grossman ZD, et al. Radioimmunoimaging of venous thrombi using ^{131}I-labeled fibrin-specific monoclonal antibody. *Radiology* 1985;156:515–517.
19. Som P, Oster ZH, Zamora PO, et al. Radioimmunoimaging of experimental thrombi in dogs using technetium-99m-labeled monoclonal antibody fragments reactive with human platelets. *J Nucl Med* 1986;27:1315–1320.
20. Stuttle AWJ, Klosok J, Peters AM, Lavender JP. Sequential imaging of post-operative thrombus using the ^{111}In-labelled platelet-specific monoclonal antibody P256. *Br J Radiol* 1989;62: 963–969.
21. Knight LC. Radiopharmaceuticals for thrombus detection. *Semin Nucl Med* 1990;20:52–67.
22. Rosebrough SF, McAfee JG, Grossman ZD, et al. Thrombus imaging: a comparison of radiolabeled GC4 and T2G1s fibrin-specific monoclonal antibodies. *J Nucl Med* 1990;31:1048–1054.
23. Manspeaker P, Weisman HF, Schaible TF. Cardiovascular applications: current status of immunoscintigraphy in the detection of myocardial necrosis using antimyosin (R11D10) and deep venous thrombosis using antifibrin (T2G1s). *Semin Nucl Med* 1993;23:133–147.
24. Schaible T, DeWoody K, Weisman H, et al. Accurate diagnosis of acute deep venous thrombosis with technetium-99m antifibrin scintigraphy: final phase III trial results [Abstract]. *J Nucl Med* 1992;33:848–849.
25. Hashimoto Y, Stassen JM, Brigitte L, et al. Thrombus imaging with an ^{123}I-labeled F(ab')2 fragment of an anti-human fibrin monoclonal antibody in a rabbit model. *Radiology* 1989;171: 223–226.
26. Lee FT, Milner LJ, Boniface GR, et al. Evaluation of thrombus detection in a rabbit model using a technetium-99m-labelled anti-fibrin monoclonal antibody. *Immunol Cell Biol* 1992; 70:173–179.
27. Bautovich G, Angelides S, Lee F-T, et al. Detection of deep venous thrombi and pulmonary embolus with technetium-99m-DD-3B6/22 antifibrin monoclonal antibody Fab' fragment. *J Nucl Med* 1994;35:195–202.
28. Bini A, Fenoglio JJ Jr, Mesa-Tejada R, Kudryk B, Kaplan KL. Identification and distribution of fibrinogen, fibrin and fibrin(ogen) degradation products in atherosclerosis. Use of monoclonal antibodies. *Arteriosclerosis* 1989;9:109–121.
29. Bini A, Fenoglio JJ Jr., Mesa-Tejada R, Kudryk B, Kaplan KL. Immunohistochemical characterization of fibrin(ogen)-related antigens in human tissues using monoclonal antibodies. *Lab Invest* 1989;60:814–821.
30. Cerqueira MD, Stratton JR, Vracko R, Schaible TF, Ritchie JL. Noninvasive arterial thrombus imaging with ^{99m}Tc monoclonal antifibrin antibody. *Circulation* 1992;85:298–304.
31. Civelek AC, Gargan PE, Bell WR. Diagnostic capacity of ^{99m}Tc-MH1-Fab' for the detection of thrombi in humans. A new thrombus specific monoclonal antibody fragment [Abstract]. *J Nucl Med* 1994;35:80P.
32. Oster ZH, Som P. Of monoclonal antibodies and thrombus-specific imaging [Editorial]. *J Nucl Med* 1990;31:1055–1058.
33. Som P, Oster ZH. Thrombus-specific imaging: approaching the elusive goal [Editorial]. *J Nucl Med* 1994;35:202–203.
34. Stratton JR, Ritchie JL. 111-In platelet imaging of left ventricular thrombi. Predictive value for systemic emboli. *Circulation* 1990;81:1182–1189.
35. Laroche Y, Demaeyer M, Stassen J, et al. Characterization of a recombinant single-chain molecule comprising the variable domains of a monoclonal antibody specific for human fibrin

fragment D-dimer. *J Biol Chem* 1991;266: 16343–16349.
36. Knight LC, Radcliffe R, Maurer AH, Rodwell JD, Alvarez VL. Thrombus imaging with technetium-99m synthetic peptides based upon the binding domain of a monoclonal antibody to activated platelets. *J Nucl Med* 1994;35:282–288.
37. Rosebrough SF. Pharmacokinetics and biodistribution of radiolabeled avidin, streptavidin, and biotin. *Nucl Med Biol* 1993;20:663–668.
38. Rosebrough SF. Plasma stability and pharmacokinetic of radiolabeled deferoxamine-biotin derivatives. *J Pharmacol Exp Ther* 1993;265: 408–415.
39. Lister-James J, Moyer BR, Buttram S, Knight LC, Vallabhajosula S, Dean RT. A structure-activity-relationship (SAR) study of GPIIb/IIIa receptor-binding peptides radiolabeled with ^{99m}Tc for imaging thromboembolism. *J Nucl Med* 1994;35:257P.
40. Knight LC, Maurer AH, Romano J, Buczala S. Preliminary evaluation of labeled disintegrins (snake venom peptides) for thrombus imaging [Abstract]. *J Nucl Med* 1993;34:66P.
41. Muto P, Lastoria S, Varella P, et al. Detection of deep venous thrombosis by Tc-99m-labelled synthetic peptides P-280: preliminary results [Abstract]. *J Nucl Med* 1994;35:80P.
42. Knight LC, Abrams MJ, Schwartz DA, et al. Preparation and preliminary evaluation of technetium-99m-labeled fragment E1 for thrombus imaging. *J Nucl Med* 1992;33:710–715.
43. Knight LC, Lister-James J, Dean RT, Maurer AH. Evaluation of ^{99m}Tc-labeled cyclic peptides for thrombus imaging [Abstract]. *J Nucl Med* 1993;34:17P.

ERRATUM

Due to a production error, a formula in the article "Evaluation of Thrombocytopenia in Patients Treated with Rhenium-186-HEDP: Guidelines for Individual Dosage Recommendations" by J.M.H. de Klerk et al. (*J Nucl Med* 1994;35:1426–1428) was printed incorrectly. The formula should be:

$$\text{Dosage (MBq)} = \frac{\text{BSA} * [(1 - 75/\text{baseline count}) * 100 - 0.714 * \text{BSI} + 0.008 * \text{BSI}^2 - 2.994]}{0.018 * 1.73},$$