Noninvasive Arterial Thrombus Imaging With $^{99m}$Tc Monoclonal Antifibrin Antibody

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Background. The T2G1s monoclonal antifibrin antibody binds specifically to fibrin but not to fibrinogen.

Methods and Results. In a canine model of acute arterial thrombosis, we determined the feasibility of imaging thrombi using a $^{99m}$Tc-labeled Fab' fragment. In 14 dogs, 10 carotid and 13 femoral artery thrombi were produced using 2-hour temporary occlusion, crush injury, and local thrombin injection methods. A sham-operated carotid artery served as control. Antifibrin antibody was injected intravenously at the end of temporary occlusion. Serial planar radionuclide images were obtained immediately and at 1 and 2 hours. Following killing the dogs at 2 hours, we measured antibody uptake ex vivo in 5-mm-long segments of thrombus, the adjacent injured artery, and a control artery. Antibody was cleared from the blood with a mean±SD τ½ of 121±23 minutes. The thrombi weighed 218±140 mg. Antibody uptake in the thrombi was patchy, and the thrombi were closely adherent to the injured arterial wall. In the segment with maximal ex vivo antibody uptake, the ratio of control artery to blood counts/g/sec was 0.65±0.46, the injured artery-to-blood ratio was 2.35±1.01 ($p<0.0001$ versus control), and the thrombus-to-blood ratio was 4.24±2.58 ($p<0.0001$ versus control). In three dogs, an isotype-matched ovarian tumor antibody labeled with $^{111}$In was injected with T2G1s but was not taken up in the thrombus or the adjacent arterial wall. Visual analysis of the in vivo carotid radionuclide images showed uptake by 2 hours in all 10 carotid thrombi. Quantitative image analysis, measured as the thrombus-to-opposite carotid artery ratio, showed increasing uptake over time with ratios of 1.1±0.3, 1.6±2.0, and 2.2±1.3 on the immediate, 1-hour, and 2-hour images, respectively. All quantitative ratios of 1.3 or greater were visually identified.

Conclusions. $^{99m}$Tc-labeled Fab' fragments of the T2G1s antibody are taken up specifically by acute arterial thrombi after intravenous injection. Uptake is progressive over a 2-hour period, and all thrombi are detected by radionuclide imaging at 2 hours. These results show that it is feasible to noninvasively detect arterial thrombi within 2 hours of formation. (Circulation 1992;85:298–304)

Arterial thrombosis is a causative factor in unstable angina, acute myocardial infarction, sudden cardiac death, transient ischemic attacks, and stroke.1–3 The ability to noninvasively localize thrombi could have widespread application in the diagnosis and treatment of these disorders. All existing noninvasive imaging techniques for thrombus detection have significant limitations. Anatomic methods, such as ultrasound and radiographic computed tomography, do not provide physiological information on the activity of a detected thrombus.

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Radionuclide methods using labeled platelets provide physiological information, but platelet labeling is time consuming and technically complex, and studies are usually not positive until 24–48 hours after isotope injection.4 A novel imaging approach is the use of specific antibodies directed at various thrombus components. One such antibody, T2G1s, reacts specifically with the first seven amino acids of the fibrin β-chain.5 A similar antifibrin antibody, 59D8, reacts with the same por-
tion of the β-chain.6 In humans and dogs, T2G1s binds to fibrin in areas of active thrombosis but not to the fibrinogen throughout the blood pool. The use of this antibody for imaging has been limited by a slow blood clearance and the poor imaging characteristics of 111In and 99mTc used for labeling.5–13 Recently, murine monoclonal Fab’ fragments of the T2G1s antifibrin antibody, which are cleared more rapidly from the blood than whole antibody, have been produced and labeled with 99mTc, a radioisotope with desirable imaging properties.14,15 Venous thrombi in human limbs have been imaged after intravenous administration of antifibrin antibodies.16,17 Unlike venous thrombi, which contain relatively large concentrations of fibrin, arterial thrombi consist mostly of platelets with some admixed fibrin.18 For this reason, it is unclear whether T2G1s will be useful for imaging arterial thrombi.

To determine the usefulness of 99mTc-labeled T2G1s Fab’ for the detection of acute arterial thrombi, we used a canine model of fresh arterial thrombosis to investigate the ex vivo binding characteristics and the in vivo detection and time course of uptake using radionuclide imaging.

Methods

Thrombosis Model

All animal and experimental and surgical procedures were approved by the Animal Care Committee of the Seattle Department of Veterans Affairs Medical Center. In 14 dogs sedated with sodium thiamyl and anesthetized with 1–2% halothane, arterial clot was induced in a 4–5-cm segment of a carotid artery (n = 1), a femoral artery (n = 4), or both (n = 9). Blood gases, arterial pressure, and heart rate were monitored and maintained in the physiological range. The artery was temporarily ligated and subjected to external crush injury using forceps, and 100–200 units thrombin was injected locally. The proximal ligation was released, and the artery was allowed to fill with blood. After the ligation was retyed, the occlusion was maintained for 2 hours. This method has been used by us extensively and produces occlusive thrombi in more than 90% of arteries.19,20 In three dogs, Doppler flow probes were applied distal to the thrombus, and there was no evidence of blood flow throughout the course of the experiment. Thirty seconds before releasing both the proximal and distal ligatures, 99mTc T2G1s was injected intravenously. In three dogs, a non–fibrin-specific, isotype-matched monoclonal antibody (OV-TL3, specific for ovarian tumor–associated antigen; donated by Centocor, Inc, Malvern, Pa.) was labeled with 111In and coinjected with the antifibrin antibody.21 A surgically exposed but uninjured carotid artery served as a control vessel. Dogs were killed upon completion of the protocol by injection of potassium chloride.

T2G1s Radiolabeling With 99mTc

T2G1s Fab’ was prepared from immunoglobulin G produced by murine hybridoma methods.14 Radiola-

veling was performed by adding 30–40 mCi 99mTc to a lyophilized mixture of D-glucaric acid (ligand used to chelate the reduced 99mTc), stannous chloride, concentrated hydrochloric acid buffered with sodium bicarbonate to a final pH 7.0, and 0.5 mg T2G1s.15 The solution was allowed to react at room temperature for 15 minutes. Labeling efficiency was determined by impregnated thin-layer chromatography, and the total injected dose was assayed in a dose calibrator just before injection. The average labeling efficiency was 87.4±4.4%, and the average injected dose was 21±3.6 mCi. Prior studies in a dog model have shown that for 2 hours after intravenous injection, 83% of 99mTc T2G1s Fab’ labeled by this method remains intact and is capable of binding to thrombi.15 Serial blood samples for well counting to monitor the clearance of labeled T2G1s were drawn at 5, 10, 15, 30, 60, and 90 minutes and before death at 120 minutes.

OV-TL3 Radiolabeling With 111In

Labeling was performed by mixing 125 μl of OV-TL3 Fab-DTPA (concentration, 1.7 mg/ml), 125 μl of 0.2 M citrate (pH 5.0), and 125 μl of 111In chloride (5 mCi/ml). This was incubated at room temperature for 30 minutes, and the percent incorporated was measured by impregnated thin-layer chromatography using 0.2 M citrate. The average labeling efficiency was 93±1.8%, and the average injected dose was 1.32±0.05 mCi.

Radionuclide Imaging

Studies of the femoral and carotid regions were acquired using a large-field-of-view gamma camera equipped with a general, all-purpose parallel hole collimator and interfaced to a dedicated computer system. Immediately before injection of 99mTc T2G1s, 57Co markers were placed at the side of the proximal and distal ligatures, and images acquired to identify the area of thrombus and the dog and camera were stationary throughout the remainder of the experiment. 99mTc or 111In images were acquired immediately and at 1 and 2 hours after intravenous antibody injection. All studies were acquired in the anterior projection using a 64×64 image matrix with ±20% energy windows centered on 122 keV for 57Co, 140 keV for 99mTc, and the 247- and 173-keV peaks for 111In. Total counts over the entire field of view for the 2-minute 99mTc acquisitions ranged from 700,000 to 2 million.

Ex Vivo Processing

The injured artery containing the thrombus and an equal-sized segment of the control carotid were excised, the proximal and distal ends were marked, the vessel was opened longitudinally, and the entire specimen was fixed overnight in formalin. Thrombus was separated from the arterial wall, and the specimens were sliced into numbered 5-mm-long segments, individually weighed, and well counted. Segments and whole blood were well counted for 1 minute on a Packard Minax Autogamma-5000 gamma counter with correction for background activity. Appropriate corrections for 111In backscatter
into the $^{99m}$Tc window were made in specimens into which $^{111}$In and $^{99m}$Tc antibodies were injected. The thrombus and vessel wall were closely adherent, and upon separation, portions of the thrombi remained attached to the wall. Uptake of labeled antibody was expressed in counts per gram per second of tissue and normalized to whole blood at the time of death.

To quantify uptake of the labeled antibody on the well-counted specimens, we calculated the following ratio:

\[
\text{Artery or thrombus-to-blood ratio} = \frac{\text{counts/g/sec of artery or thrombus}}{\text{counts/g/sec of whole blood at death}}
\]

Because whole blood is always contiguous to thrombus in vivo and isotope is present in blood for a variable time period, this is an important index for determining feasibility of detection by external imaging.

Because antibody uptake was not uniform throughout the length of the large preformed thrombus or arterial wall, we calculated a maximal uptake ratio for the single artery or thrombus segment with the highest uptake as well as the average ratio, which was the mean value of all of the 5-mm-long segments of artery or thrombus.

**Histological Tissue Preparation**

Eight injured arteries (four femoral and four carotid) and 10 thrombi (five femoral and five carotid) were processed for histological sectioning. The 5-mm-long segments of wall and thrombi were embedded in paraffin, and a representative 6-mm-thick section, oriented perpendicular to the long axis of the vessel, was stained with hematoxylin and eosin. Each was reviewed by two independent observers under light microscopy, and the proportion of red blood cells and platelets present in the thrombus was visually estimated. The average of the two observers was used.

**Quantitative Image Analysis**

Each of the carotid radionuclide images was read independently by two experienced observers. The thrombosed carotid artery was compared with the opposite normal vessel and scored using the following system: 0, less than the normal vessel; 1, same as the normal vessel; 2, slight increase relative to the normal vessel; 2, moderate increase relative to the normal vessel; and 3, marked increase relative to the normal vessel. The mean value of the two scores was used for analysis. The femoral thrombi had visually increased uptake relative to the adjacent areas of normal vessel, but analysis was not possible due to increased uptake of antifibrin at the insertion site of an arterial line in the opposite femoral artery. In addition, blood pool activity in the penis and urine activity in the urethra and bladder made visual interpretation difficult. Thus, only well counting and histology results are reported for the femoral thrombi.

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Plots of blood clearance curves (counts/g/sec) in three representative dogs plotted on a semilogarithmic scale during 120 minutes of observation. Blood clearance of T2G1s in 11 dogs averaged 121±23 (mean±SD) minutes.

**Qualitative Image Analysis**

Using the cobalt marker images, the length of the injured artery on the $^{99m}$Tc antibody images was identified visually, and the area containing the maximal activity was marked. Next, a 1-pixel-thick line was drawn through the area of maximal activity and extended to the opposite normal carotid. An activity profile for counts per pixel was generated, the peaks corresponding to the thrombosed and normal carotid arteries were identified, and the ratio of maximal uptake in the damaged to normal carotid was calculated.

**Statistical Analysis**

All values are expressed as the mean±1 SD value in the text and plotted as the mean±SEM value in the figures. Ex vivo uptake in the injured artery and thrombus were compared with the control artery, and differences were determined using two-tailed, unpaired t tests. p<0.05 was considered statistically significant.

**Results**

T2G1s was cleared from the blood with a calculated half-life of 121±23 minutes, with a range of 87–168 minutes (n=11). Three representative dogs are shown in Figure 1. Between the initial blood sample at 5 minutes and the final predeath blood sample at 2 hours, there was a 54% reduction in total counts.

**Histology**

The average weight of the 23 arterial thrombi was 218±140 mg and ranged from 36 to 553 mg. The 10 carotid thrombi weighed 269±59 mg (range, 87–553 mg), and the 13 femoral thrombi weighed 179±41 mg (range, 36–439 mg). The weight difference between the carotid and femoral thrombi was not statistically significant (p=0.13).

The thrombi were predominately of the red cell type. The majority of samples consisted of 90–95% red cell thrombi; the remainder were platelet thrombi. In a few samples, 30–50% of the thrombus...
mass consisted of platelet aggregates. Shown in Figure 2 is an example of a typical thrombus. The darker areas are red cell thrombus, and the paler lines of Zahn represent platelets with fibrin. The arterial walls showed hemorrhage into cleftlike spaces of the media and thin layers of red cell thrombi adherent to the intima. These changes were most likely caused by the crush injury.

Ex Vivo Well Counting

The variability in T2G1s uptake shown in the histological sections is reflected in the well-counting data. Figure 3 shows the results from a single dog of a well-counted femoral thrombus sectioned into 5-mm-long segments. The proximal segment has the greatest uptake, the four central segments have uniformly low uptakes, and the distal segment shows a slight increase. In general, for all thrombi examined, uptake was greatest at the proximal and distal ends of the thrombus, but there was considerable variability.

Figure 4 shows the ex vivo thrombus- or artery-to-whole blood uptake ratios of the average and maximum segments for 13 control arteries, 23 injured arteries, and 23 thrombi. The control artery-to-whole blood ratio was 0.41±0.29 for the average of all segments and 0.65±0.46 for the segment with

![Figure 3. Bar graphs of T2G1s uptake (counts/g/sec) in a femoral thrombus from a single dog shown for each of six 5-mm-long segments. Uptake is heterogeneous and highest in proximal segment, with distal segment having a slight increase relative to four middle segments.](image)

![Figure 4. Bar graphs of artery- or thrombus-to-blood ratios (mean±SEM) shown for control artery (n=13), injured artery (n=23), and thrombus (n=23), using average uptake for all 5-mm segments or segment with maximal uptake of T2G1s. Uptake was significantly higher in injured artery and thrombus relative to control artery using either average or maximum counts, but maximum counts gave highest ratios.](image)

![Figure 2. Photomicrograph of hematoxylin and eosin-stained sections of a recently formed femoral artery thrombus. Darker areas consist mainly of red blood cell aggregates, and lighter areas (lines of Zahn) consist predominantly of platelets and strands of fibrin. We estimated that 90% of this thrombus consists of erythrocytes. Magnification, ×60.](image)
maximal uptake, indicating less activity in the control artery than in whole blood at the time of death. The injured arterial wall took up more $^{99m}$Tc T2G1s than the control artery as reflected in the artery-to-whole blood ratio for the average segment (1.42±0.62, p<0.002) and for the maximal segment (2.35±1.01, p<0.0001).

The thrombus-to-whole blood ratio was 2.13±1.80 for the average segment (p<0.0001 versus control artery) and 4.24±2.58 for the maximal segment (p<0.0001 versus control artery).

Artery- or thrombus-to-blood ratios for the $^{111}$In-labeled OV-TL3 antibody were low using the average or maximal counts; for the control artery, injured artery, and thrombus, ratios were 0.25±0.12 (n=3), 0.55±0.06 (n=5), and 0.42±0.17 (n=5) using average and 0.35±0.09, 0.70±0.11, and 0.56±0.27 using maximal uptake, respectively.

**Imaging Data (Carotid Arteries)**

Figure 5 shows the mean visual and quantitative ratios for the two observers on the immediate and 1-hour and 2-hour carotid images. There was exact scoring agreement between the two observers in 22 of 30 vessels scored and disagreement by only one grade in the remaining eight. There was slight uptake in three dogs immediately after injection. By 1 hour, all 10 thrombosed vessels had slightly-to-markedly increased uptake (mean score, 1.7±0.9), and at 2 hours, the mean uptake score was 2.4±0.7. At 2 hours, a score of 2 (moderate uptake) or 3 (marked uptake) was present in nine of 10 dogs. The dog in Figure 6 had little T2G1s uptake on the immediate images but had marked uptake at 1 and 2 hours.

As shown in Figure 5, the uptake of T2G1s into the carotid thrombi, as measured by a quantitative ratio that compared the thrombosed artery with the contralateral control artery, was time dependent. The mean quantitative thrombus-to-normal vessel ratios were 1.1±0.3, 1.6±0.5, and 2.2±1.3 on the immediate, 1-hour, and 2-hour images. Ratios of 1.3 or greater were associated with visually positive images, and this value was achieved by 2 hours in every dog.

**Discussion**

In a canine model of acute arterial thrombosis, we have shown that $^{99m}$Tc T2G1s Fab' administered intravenously after thrombus formation is cleared from blood and taken up specifically in sufficient quantity to allow noninvasive radionuclide imaging 2 hours after injection.

Prior studies involving imaging thrombi with anti-fibrin antibody have been limited by using polyclonal...
or whole antibodies and labeling the antibody with
131I or 111In. Polyclonal antibodies react nonspecifically
with fibrin and fibrinogen, and whole antibody is
cloned very slowly from blood due to minimal renal
filtration.7,8 This results in persistently high back-
ground activity due to both free and fibrinogen-
associated labeled antibody. Under these circum-
stances, noninvasive radionuclide imaging was pos-
sible only at 24–48 hours to allow adequate
background clearance and uptake by thrombi. This
required the use of long half-life isotopes such as
131I or 111In. Although antibody labeling with these radio-
isotopes is technically simple, the high-energy, low-
photon release results in poor image quality.

Refinements in the production of monoclonal an-
tifibrin antibody and the use of antibody fragments
rather than whole antibody optimize specificity,
blood clearance, and penetration into the target area
and make it practical to consider imaging thrombi
within several hours of their formation. Two mono-
clonal antifibrin antibodies, 59D8 developed by Hui
et al5 and T2G1s developed by Kudryk et al,6 have
high specific affinity for the NH2 terminal portion of
the β-chain of fibrin II. Because these antibodies
have similar high-affinity constants for the same
epitope, they are considered comparable.15 They do
cross-react much more directly with fibrinogen and over-
come the specificity problem encountered with the use of
poly

clonal antibodies. F(ab′)2 and Fab′ fragments, which
have higher renal filtration and better penetration
into the site of thrombus formation, have been pro-
duced to facilitate blood clearance and further
improve thrombus-to-blood ratios. However, the
12-hour half-life is still long, and more rapidly clearing
platelet- or fibrin-specific targeting agents are
needed. With these two antifibrin antibodies in ani-
mal models, venous thrombi created in vivo can be
noninvasively imaged at 24–48 hours, and ex vivo
thrombus-to-blood ratios of 7–10:1 are present.9–12
However, in these studies, imaging was still per-
formed at 24–48 hours using 131I or 111In to obtain
optimal detection of thrombi. Human studies using
111In labeling have also shown that imaging of venous
thrombosis was possible.16,17 Studies in the lower
extremities frequently became positive within the
first few hours after intravenous injection due to
minimal surrounding blood pool activity. Because
venous thrombi differ from arterial thrombi in the
relative percentages of erythrocytes, platelets, and
fibrin, the imaging results from these studies may not
be applicable to arterial thrombi.18,22

Radiolabeling the T2G1s Fab′ fragment with 99mTc
provides optimal imaging energy and count rate using
this widely available isotope. Although 99mTc labeling
of proteins has been technically difficult, recent ad-
ances have made it possible, and all of our studies
were performed using an instant kit.15 We used
99mTc-labeled Fab′ fragments of the antifibrin anti-
body to image thrombi in an animal model that
mimics the clinical setting of acute arterial thrombo-
sis.23,24 Imaging was performed only over the first 2

hours to reflect a realistic time frame for the identi-
fication of and/or possible monitoring of therapy for
clinical arterial thromboses. Our data show that in
this model, identification of thrombus within 2 hours
was easily accomplished. The thrombi in our model
were created and present in arteries with an initially
high flow rate; however, the necessity of temporary
occlusion to reliably produce thrombi presumably
makes them somewhat different from arterial thrombi
formed in pathological states.23–25 Histologically, these thrombi were mixed platelet and red cell
thrombi, with red cell thrombi predominating. In
occlusive coronary thrombi in humans, the proximal
portion is predominately platelets, with the majority
of downstream material of the red cell type. Alter-
atively, in one animal model of coronary reocclusion,
platelet thrombi predominate.24 In clinical syn-
dromes of arterial thrombosis, it is uncertain whether
sufficient red cell fibrin thrombi might be present to
be detected by antifibrin imaging.

In the present study, we created thrombi in periph-
ereal arteries. Radionuclide imaging is optimized in
these arteries in that with total occlusions, blood
activity is present only at each end of the occlusion,
with the contrast with surrounding tissue relatively
high. In the coronary arteries, a smaller-diameter clot
is formed, and it always overlaps or is very close to
the central cardiac blood pool activity. Even with the
relatively short (121±23 minutes) half-life of T2G1s,
this blood pool activity may limit identification of
thrombi formed in the coronary or pulmonary arter-
ies. It is possible that late imaging, when blood pool
activity has diminished, may allow imaging of these
central thrombi. In a pilot study of coronary occlusive
thrombosis in two dogs, we failed to detect thrombus
by external imaging after intravenous injection of
antifibrin antibody despite thrombus-to-blood ratios
of 13:1 and 22:1 (unpublished observations). Our
model produces total occlusions in which antibody
was predominantly taken up only at the active ends.
In clinical situations, incomplete occlusion and/or
reperfusion might expose a greater surface of throm-
bus to antibody, producing relatively greater uptake.

In summary, in an animal model of acute arterial
thrombosis, we have demonstrated avid uptake of
labeled antifibrin antibody. This uptake is seen
within a sufficiently rapid time frame to be approp-
arate for the diagnosis and/or possible monitoring of
interventions in the clinical setting of acute arterial
thrombosis. Whether these findings can be applied to
the clinical setting now appears to deserve study, with
thrombi in peripheral arteries being those most likely
to be easily identified.

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