Detection of Arterial Emboli Using Doppler Ultrasound in Rabbits

David Russell, MD, PhD; Kenneth P. Madden, MD, PhD; Wayne M. Clark, MD; Per Morten Sandset, MD, PhD; and Justin A. Zivin, MD, PhD

The purpose of this study was to develop an animal model that could be used to test the ability of Doppler ultrasound to detect arterial emboli composed of materials that are often involved in cerebral emboli. Emboli introduced into the rabbit aorta via the left renal artery consisted of clotted whole blood, platelets, atheromatous material, fat, or air. The ultrasound examination was carried out continuously during the studies using a multifrequency transcranial Doppler apparatus with a 2-MHz probe, a sample volume of 15 mm, at a depth of 15 mm. The intensity of the Doppler spectrum was measured and displayed as a 15-shade color scale, each shade representing a 3-dB difference. The diameter of the aorta at the site of the ultrasound examination was similar to the diameter of the middle cerebral artery in humans. All 125 emboli introduced were clearly detected because they caused a Doppler signal at least 15 dB greater than that of the surrounding blood. These results show that the potential for emboli detection using Doppler ultrasound in the clinical situation is now considerable. (Stroke 1991;22:253-258)

Although cerebral embolism is clearly one of the major causes of stroke, we do not have methods to detect emboli. Clinically, this results in uncertainty in the diagnosis and delays in the initiation of appropriate treatment to prevent further emboli from entering the cerebral circulation. The development of a method to detect emboli, especially during surgery, would therefore represent a significant advance in the management of patients.

Doppler ultrasound may theoretically be used to detect emboli. Normally, Doppler examination is used to measure blood flow velocity because the difference in frequency between transmitted and reflected ultrasound depends on the velocity of blood in the vessel. However, intensity of the reflected Doppler signal depends on other factors, including the size of particles in the blood and their acoustic impedance. An embolus may therefore cause Doppler signals of increased intensity if its size and acoustic impedance differ from those of normal blood components. Air has an acoustic impedance <1/4000 that of whole blood. The intensity of ultrasound reflected from an air bubble in a vessel is therefore much higher than that reflected from normal blood components. Doppler signals thought to be due to air or other gases have been reported in decompression sickness and during open heart surgery, hip arthroplasty, and carotid endarterectomy. Other reports have suggested the detection of emboli composed of elements other than gas. Kelly et al described Doppler signals in the femoral veins of patients following fractures of the tibia or femur that the authors proposed to be due to fat emboli. Similar findings were reported by Herndon et al during total hip replacement. Spencer et al performed transcranial Doppler examination of the middle cerebral artery in 91 patients undergoing carotid endarterectomy and reported that 35 (38%) had Doppler signals consistent with bubble emboli. Furthermore, 24 (26%) of the patients had similar signals observed when there was no invasion of the vasculature. The authors therefore proposed that these signals were due to elements other than air.

In clinical studies, however, it is extremely difficult to prove the origin of Doppler signals. The purpose of our study was to develop an animal model to test the ability of Doppler ultrasound to detect arterial emboli composed of materials that are often involved in cerebral embolism.

Materials and Methods

We used New Zealand White rabbits weighing 2.5–3.0 kg. The animals were anesthetized with...
halothane and, after a 10-cm midline vertical abdominal incision was made, the aorta and left renal artery were exposed. A transverse arteriotomy was performed on the distal end of the left renal artery, and a PE60 catheter filled with 0.2 ml heparinized saline was introduced and advanced to the junction of the renal artery and the aorta and secured with sutures. Emboli could then be slowly advanced through the catheter with an infusion pump at an infusion rate of 0.02 ml saline/min and introduced into the aorta.

The Doppler examination was carried out continuously during the studies using a TC 2000S multifrequency transcranial Doppler apparatus (Eden Medical Electronics Inc., Kent, Wash.). The apparatus had a 128 fast Fourier transform and was used with a 2-MHz probe, a sample volume of 15 mm, at a depth of 15 mm. The probe was clamped in position over the aorta at an angle of 45° 7 cm caudal to the left renal artery. The amplitude of frequencies in each vertical spectral line was given a relative digital value, which was then presented as a color scale with each of 15 sequential color shades representing a 3-dB difference. The data were also processed off-line to find the relative color scale that displayed the embolus signal most clearly. The internal diameter of the aorta at the site of the Doppler examination ranged from 2.0 to 2.5 mm, which is similar to the diameter of the middle cerebral artery in humans.9 Control saline infusions did not alter the Doppler signal.

For whole-blood emboli, blood from a donor rabbit was allowed to clot at 37°C for 2 or 36 hours. The clot was then sliced into small cubes with a razor blade, and each cube was weighed. The emboli were then suspended in 100 μl calcium-free Dulbecco’s phosphate-buffered saline and used within 2 hours. Thirty-eight emboli made from 2-hour-old clots were injected. They weighed 0.08–6.0 mg and had calculated widths of 0.4–1.8 mm. Twenty-two emboli made from 36-hour-old clots were injected. They weighed 0.07–3.08 mg and had calculated widths of 0.4–1.5 mm.

Platelet-rich thrombi were prepared from whole blood from two donor rabbits (nine volumes) drawn into citrate anticoagulant (one volume, 0.1 mol/l). The citrated whole blood was centrifuged at 100g for 15 minutes. Platelet-rich plasma was decanted and contained 250–550×10^3 platelets/μl as determined in a hemocytometer. Platelet-rich thrombi were prepared by adding 2 ml of 10 units/ml bovine thrombin (Parke-Davis, Ann Arbor, Mich.) to 1 ml of platelet-rich plasma. After 30 minutes at 20°C, the platelet-rich thrombus was washed twice with and stored in Tris-buffered saline. Light microscopy showed that the thrombi contained >99% platelets. The thrombi were sliced into small cubes and weighed. Fourteen platelet emboli were used within 6 hours. They weighed 0.22–1.24 mg and had calculated widths of 0.6–1.1 mm.

Human atheromatous material from a patient who underwent carotid endarterectomy was sliced into small cubes, which were weighed. Eight atheromatous emboli were used <6 hours after surgical dissection. They weighed 0.17–1.34 mg and had approximate widths of 0.5–1.1 mm.

Subcutaneous rabbit fat was sliced into small cubes using a razor blade, and each cube was weighed. Thirty fat emboli were used <4 hours after dissection. They weighed 0.11–3.51 mg and had calculated widths of 0.5–1.5 mm.

Small volumes of air were measured using a 1-μl Hamilton syringe (Hamilton Co., Reno, Nev.). Thirteen air emboli were used. Their volumes were 0.1–6.0 μl, and they had approximate diameters of 0.3–1.1 mm.

The approximate widths of the emboli were calculated to give an indication of the size vessels they might occlude. For this calculation we assumed that the emboli were approximately cube-shaped and that all elements other than air had a specific gravity of 1.0 g/mm^3. We calculated the diameters of the air emboli assuming that they became spherical when they passed into the aorta.

**Results**

A total of 125 emboli were introduced into the aorta, and all were readily detected both visually in the Doppler spectrum and by their characteristic “chirping” sound as they passed the Doppler probe. Examples are shown in Figures 1–5. These Doppler recordings show the velocity spectrum of particles in the blood on the vertical axis in centimeters per second. The relative intensities of the signals reflected back to the transducer are represented by a color scale (Figure 1). The emboli caused Doppler signals (orange) of a much greater intensity than those reflected from erythrocytes (blue). Signals due to emboli were seen during systole, diastole, or both, and in some cases the signals persisted during several cardiac cycles (Figure 5). All emboli caused a Doppler signal that was at least 15 dB greater than that of the surrounding blood. Emboli composed of air or fat (Figures 4 and 5, respectively) produced stronger signals than those of clotted whole blood, platelets, or atheromatous material (Figures 1, 2, and 3, respectively). Our observations also suggest that the intensity of the reflected signal depends on the size of the embolus. Although we were unable to quantify signal magnitude absolutely, large emboli appeared to cause greater Doppler signals than small emboli of the same type.

**Discussion**

This study clearly shows that Doppler ultrasound may be used to detect arterial emboli. Using this method, we readily detected all types of emboli injected. These represent the most common cerebral embolic materials that are encountered clinically.10 The high sensitivity of Doppler ultrasound in detecting emboli is emphasized by our ability to detect emboli with approximate diameters or widths of 0.3–0.5 mm. This was not the detection limit of the instrumentation but simply the minimum size emboli that we managed to introduce into the aorta. To put these findings into perspective, the middle cerebral
FIGURE 1. Doppler recording after introduction of embolus composed of 2-hour-old clotted whole blood weighing 0.1 mg. Embolus caused Doppler signal (orange) with intensity 15 dB greater than that of surrounding blood (blue). Each shade from left to right on color scale (top) represents increase in signal intensity of 3 dB. Blood flow velocities are shown in centimeters per second on vertical axis, and time base is 2.5 seconds.

FIGURE 2. Doppler signal (orange) caused by 0.87-mg platelet embolus. Time base is 1.25 seconds, and color scale for signal intensity is shown in Figure 1.

FIGURE 3. Doppler signal (orange) caused by embolus comprised of atheromatous material weighing 0.24 mg. Time base is 1.25 seconds, and color scale for signal intensity is shown in Figure 1.
FIGURE 4. Doppler signal (orange) caused by 0.85-mg fat embolus that overloaded the instrumentation. Time base is 1.25 seconds, and color scale for signal intensity is shown in Figure 1.

FIGURE 5. Doppler signals (orange) caused by 0.5 µl air, which overloaded the instrumentation and persisted during several cardiac cycles. Time base is 2.5 seconds, and color scale for signal intensity is shown in Figure 1.
artery of humans has a diameter at its origin of 2.4–4.6 mm,9 which is roughly twice that of the anterior and posterior cerebral arteries.11,12 The smallest emboli that we used would pass easily through these vessels but could block the small penetrating arteries in the human brain (diameters 0.1–0.4 mm), potentially producing small cortical or subcortical infarcts.10,13 The clinical effects of such small infarcts in humans depend on anatomic features, and the patient may not experience symptoms if the infarct is located in a clinically silent area of the brain.14–19 This suggests that Doppler ultrasound may be used to detect cerebral emboli that are clinically silent.

The instrumentation used in our study provided a relative and not an absolute assessment of signal intensity in the Doppler spectrum. A more detailed analysis of the amplitude of the reflected signal may therefore help to define the ultrasonic characteristics of emboli. We were unable to correlate the absolute signal intensity caused by an embolus and its type or size. However, we observed that air and fat emboli caused signals of greater intensities than emboli composed of clotted whole blood, atheromatous material, or platelets. In addition, our observations suggest that large emboli produce stronger Doppler signals than small emboli of the same type.

The potential for emboli detection with Doppler ultrasound in the clinical situation is considerable. This method is noninvasive and has no known side effects and may therefore be used to monitor blood flow in both intracranial and extracranial arteries over long periods. An immediate clinical application is monitoring during invasive cardiovascular surgery, such as open heart surgery and carotid endarterectomy, where cerebral macroemboli and microemboli are a major cause of neurologic and neuropsychological dysfunction.20–25 Continuous transcranial Doppler monitoring of the middle cerebral arteries in this situation may alert the surgical team that emboli are entering the cerebral circulation so that immediate preventive or therapeutic measures may be undertaken.

The ability to detect cerebral emboli may also be of help in resolving the controversy regarding the pathogenesis of transient ischemic attacks (TIAs), especially if long-term Doppler recordings become possible using small portable tape recorders. It remains a matter of opinion as to what proportion of patients with TIA experience hemodynamic versus thromboembolic events. This information has important implications for both the management of individual patients and the assessment of new therapies since a treatment that improves blood flow would not be expected to have a beneficial effect on patients with thromboembolic events.

In conclusion, our study has shown that advances in Doppler technology have made possible the detection of arterial emboli composed of elements that are frequently involved in cerebral embolism. Further studies are now being carried out to define the Doppler characteristics of arterial emboli in more detail so that this instrumentation can be optimally adapted for clinical use.

Acknowledgment

We would like to thank Eden Medical Electronics Inc., Kent, Wash., for providing the Doppler instrumentation for this study.

References

7. Sasaki W, Yanagisawa S, Maki K, Onodera A, Awaji T, The instrumentation used in our study provided a relative and not an absolute assessment of signal intensity in the Doppler spectrum. A more detailed analysis of the amplitude of the reflected signal may therefore help to define the ultrasonic characteristics of emboli. We were unable to correlate the absolute signal intensity caused by an embolus and its type or size. However, we observed that air and fat emboli caused signals of greater intensities than emboli composed of clotted whole blood, atheromatous material, or platelets. In addition, our observations suggest that large emboli produce stronger Doppler signals than small emboli of the same type.

The potential for emboli detection with Doppler ultrasound in the clinical situation is considerable. This method is noninvasive and has no known side effects and may therefore be used to monitor blood flow in both intracranial and extracranial arteries over long periods. An immediate clinical application is monitoring during invasive cardiovascular surgery, such as open heart surgery and carotid endarterectomy, where cerebral macroemboli and microemboli are a major cause of neurologic and neuropsychological dysfunction.20–25 Continuous transcranial Doppler monitoring of the middle cerebral arteries in this situation may alert the surgical team that emboli are entering the cerebral circulation so that immediate preventive or therapeutic measures may be undertaken.

The ability to detect cerebral emboli may also be of help in resolving the controversy regarding the pathogenesis of transient ischemic attacks (TIAs), especially if long-term Doppler recordings become possible using small portable tape recorders. It remains a matter of opinion as to what proportion of patients with TIA experience hemodynamic versus thromboembolic events. This information has important implications for both the management of individual patients and the assessment of new therapies since a treatment that improves blood flow would not be expected to have a beneficial effect on patients with thromboembolic events.

In conclusion, our study has shown that advances in Doppler technology have made possible the detection of arterial emboli composed of elements that are frequently involved in cerebral embolism. Further studies are now being carried out to define the


KEY WORDS • cerebrovascular disorders • embolism • ultrasonics • rabbits
Detection of arterial emboli using Doppler ultrasound in rabbits.
D Russell, K P Madden, W M Clark, P M Sandset and J A Zivin

Stroke. 1991;22:253-258
doi: 10.1161/01.STR.22.2.253

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/22/2/253

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/