To develop an arterial injury model for testing hemostatic devices at well-defined high and low bleeding rates.

**Material and method.** A side-hole arterial injury was created in the carotid artery of sheep. Shed blood was collected in a jugular venous reservoir and bleeding rate at the site of arterial injury was controlled by regulating outflow resistance from the venous reservoir. Two models were studied: uncontrolled exsanguinating hemorrhage and bleeding at controlled rates with blood return to maintain hemodynamic stability. Transcutaneous Duplex ultrasound was used to characterize ultrasound signatures at various bleeding rates.

**Results.** A 2.5 mm arterial side-hole resulted in exsanguinating hemorrhage with an initial bleeding rate of 400 ml/min which, without resuscitation, decreased to below 100 ml/min in 5 minutes. After 17 minutes, bleeding from the injury site stopped and the animal had lost 60% of total blood volume. Reinfusion of shed blood maintained normal hemodynamics and both high and low bleeding rates could be maintained without hemorrhagic shock. Bleeding rate at the arterial injury site was held at 395±78 ml/min for 8 minutes, 110±11 ml/min for 15 minutes, and 12±1 ml/min for 12 minutes. Doppler flow signatures at the site of injury were characterized by high peak and end-diastolic flow velocities at the bleeding site which varied with the rate of hemorrhage.

**Conclusion.** We have developed a hemodynamically stable model of acute arterial injury which can be used to evaluate diagnostic and treatment methods focused on control of the arterial injury site.

**Key words:** arterial injury model, bleeding rate, arterial hemorrhage rate, Doppler ultrasound signature

Uncontrolled hemorrhage from battlefield trauma is the primary cause of death for soldiers (1, 2), and the second leading cause of civilian deaths from trauma (1, 3, 4, 5). Control of hemorrhage is of particular concern when evacuation of the injured patient is delayed, such as due to the tactical situations in recent conflicts in Desert Storm (6) and Somalia (7). Past studies have shown that prompt and effective control of hemorrhage would decrease mortality in combat casualties (8, 9). Historically, 20% of combat casualties were killed in action, with 90% of those dying before reaching a field hospital (2). A review of Vietnam War casualty statistics shows that nearly 40% of combat deaths from exsanguination could have been controlled using simple hemostatic techniques in the field (10).
Many animal models have been developed to study uncontrolled arterial hemorrhage, employing a variety of methods. Some studies have utilized localized incisions in peripheral or central arteries (11-15), while others have utilized uncontrolled blast injuries which include soft tissue as well as vascular injury (16, 17). A number of models include extensive liver lacerations (18, 19, 20), while others utilized side-hole injuries in the aorta (21, 22, 23). However, none of these studies assessed the injured artery bleeding rate separately and distinctly from the hemorrhagic shock response to the injury. Variations in vasoconstriction, blood pressure, and cardiac output cause bleeding rates in the injured artery to vary unpredictably with time. Arterial bleeding rates were very variable and could not be maintained for a sufficient length of time to assess the effectiveness of specific local hemostatic control measures at various rates of arterial hemorrhage. Major arterial injuries and hemorrhage may halt spontaneously as a result of vasoconstriction, hypovolemia and shock, and not as a result of a local hemostasis device, thus exposing the injured patient (soldier) to rebleeding when central hemodynamics are restored.

This investigation was undertaken to develop a test bed for the Deep Bleeder Acoustic Coagulator (DBAC) project, funded by the Defense Advanced Research Projects Agency (DARPA). The goal of the DBAC project was to utilize transcutaneous high intensity focused ultrasound (HIFU) to control acute arterial bleeding and achieve hemostasis in battlefield arterial injuries. The effectiveness of HIFU was to be evaluated in a test bed which had focal arterial injuries with well-defined high and low bleeding rates, uncomplicated by hemorrhagic shock responses. In this study we describe an experimental arterial injury model which is suitable for this purpose.

The injury is an arterial side-hole, similar to clinically encountered arterial injuries in soldiers and patients (fig. 1). Activation of the coagulation cascade was minimized by creation of an endothelial lined venous collection reservoir which also served to evacuate blood and prevent compression by hematoma. Hemorrhagic shock was prevented in this model by returning shed blood via the venous system and bleeding rate at the site of the injury was controlled by regulating the outflow resistance of the collecting reservoir. Since ultrasound localization and characterization of the bleeding site is used to guide the application of HIFU in the DBAC system, ultrasound signal characteristics of defined bleeding rates were studied in this model.

MATERIAL AND METHODS

All animals received humane care in compliance with the “Guide for Care and Use of Laboratory Animals”, prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85-23, Revised 1996). This study was approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AALAC) certified Stanford University Administrative Panel on Laboratory Animal Care (APLAC) and conducted in accordance with Stanford University animal care and use program.

Procedure

Sheep were chosen as the experimental model because of similarities in cardiovascular characteristics to humans. Mean arterial pressure, cardiac output, pulse and stroke volume in sheep are comparable to humans, and equivalent-sized arterial injuries should bleed at similar rates (24-28). Animals were sedated, intubated and anesthetized with 0.5 mg/kg of intramuscular atropine, 4 mg/kg of intravenous ketamine hydrochloride, 0.5 mg/kg of intravenous diazepam, and inhalation of 1-3% isoflurane. Femoral arterial and venous sheaths were placed and maintenance lactated Ringers solution was administered at a rate of 10 mg/kg/hr.

The right common carotid artery (CCA) and right jugular vein (JV) were exposed through a midline neck incision and animals were anticoagulated with 10,000 units of aqueous heparin. The CCA and JV were cross-clamped, and a side-hole was created in the CCA using a 2.5 mm aortic punch to control the size of the arterial injury. Additional punches in a longitudinal direction could be used to increase the length of the opening to 5-6 mm. An opening was then made in the adjacent jugular vein (fig. 1) and the artery and vein were sewn together in a side-to-side fashion with 6-0 polypropylene suture. When cross-clamps were
removed, the large caliber jugular vein served as a collection reservoir for shed blood exiting the side-hole in the carotid artery (fig. 2). Two experimental models were used: in the first, the proximal jugular vein was ligated and the distal jugular vein was cannulated with a large bore tube to collect shed blood from the venous reservoir. This provided information on bleeding rates in uncontrolled, exsanguinating hemorrhage from arterial side holes of varying dimensions. In the second model, the distal jugular vein was ligated and the proximal jugular vein was utilized to allow reinfusion of shed blood from the arterial injury. Flow rates at the site of the arterial injury were controlled by constricting the proximal jugular vein to provide outflow resistance and the rate of blood flow exiting the arterial injury site was monitored using an ultrasonic flowmeter on the jugular vein returning blood to the heart. The sternocleidomastoid muscle was mobilized to cover the carotid artery and the midline skin incision was closed. The site of arterial injury could thus be imaged and accessed through covering tissue, and intact skin and bleeding rate could be controlled with a pinch clamp on the jugular vein remote from the arterial injury site.

Duplex ultrasound imaging was used to document flow characteristics in the CCA before and after creation of the arterial injury, to locate the bleeding site, and to characterize variation in ultrasound signatures around the injury site at various flow rates. Flow signatures were obtained in the CCA and arterial injury site with bleeding rates ranging from 5 to 400 ml/min. A comparison of known bleeding rate to peak systolic velocity, end diastolic velocity, and resistive index was made from the reinfusion model.

Shed blood and hemodynamic parameters were monitored during the course of the experimental procedure and animals were eu-

Fig. 1. On the left, a patient with a side-hole injury of the superficial femoral artery, caused by a gunshot wound. On the right, a 2.5 x 6 mm side-hole in the sheep carotid artery. This is anastomosed to a similar size hole in the jugular vein which acts as a reservoir to collect shed blood exiting the side-hole in the carotid artery

Fig. 2. On top: the carotid artery side-hole, anastomosed to the jugular vein which is ligated on one end. On the bottom: a duplex ultrasound image of blood flow exiting the carotid artery side-hole into the jugular venous reservoir
thanized with 100 mg/kg of intravenous pentobarbital at the completion of the procedure.

Equipment

The bleeding rate was measured using a Transonic TS410 Flowmeter Module with a Transonic 5PXL Sensor (Transonic Systems, Inc, Ithaca, NY). The flow rate data was recorded at 20 Hz using a National Instruments DAQPad 6020E (National Instruments Corporation, Austin, TX). Arterial and venous blood pressures were recorded via catheters in the femoral artery and vein, connected to a Medex MX 860 blood pressure transducer (Medex Inc., Hillard, OH). The heart rate was recorded using 3M ECG electrode pads (3M, St Paul, MN). Philips iU22 diagnostic imaging ultrasound equipment (Philips Medical Systems, Bothell, WA) was used to externally scan the neck prior to and during the procedures. A 9 MHz linear transducer (L9-3) was used to obtain both grayscale and color Doppler images along with pulsed wave Doppler spectral analysis. Ultrasound recorded the peak systolic velocity, the end diastolic velocity, resistive index and heart rate. Resistive index is equal to (peak systolic velocity – end diastolic velocity) / peak systolic velocity. A Medtronic Aortic Punch 2.5 mm in diameter created the vascular injury (Medtronic, Inc, Minneapolis, MN). Pinch valves with fine thread screw were chosen to give finer control over bleeding rate.

RESULTS

In the first model of uncontrolled exsanguinating hemorrhage, the initial bleeding rate through the 2.5 mm diameter arterial side-hole was 400 ml/min. The rate of bleeding through the side-hole decreased rapidly as the animal became hypotensive. After 2 minutes, mean blood pressure dropped from a baseline of 100 mm Hg to 50 mm Hg and bleeding rate decreased to 150 ml/min. After 5 minutes, bleeding rate had decreased to a rate of less than 100 ml/min and the animal had lost approximately 50% of total blood volume (fig. 3). After 6 minutes, mean blood pressure was 40 mm Hg and the animal had lost 53% of total blood volume. After 7 minutes, the animal had lost 60% of blood volume. Bleeding rate dropped to 10 ml/min at 10 min and ceased entirely after 17 minutes with 63% total blood volume loss. Heart rate rose from 100 to 150 bpm during this period. There was no difference in uncontrolled bleeding rate between a 2.5x2.5 mm carotid hole and a 2.5x6 mm carotid hole.

Once bleeding from the arterial injury had stopped, normal saline was administered into both the brachial and femoral veins at a rate of 100 ml per minute for 30 minutes. This resulted in a 15 mm Hg rise in mean arterial pressure and a 30 bpm decrease in pulse rate with resumption of bleeding from the arterial injury site at a rate of 30-40 ml/min for the 30 minute period of fluid resuscitation time (fig. 3).

In the second model, reinfusion of blood from the jugular venous reservoir prevented blood loss and hypovolemic shock. Flow rates through the arterial injury could be adjusted and controlled by the pinch valve and were maintained at the desired level while central hemodynamics remained stable throughout the experiment. As shown in fig. 4, a bleeding rate of 395±78 ml/min was maintained for 8 minutes, a bleeding rate of 110±11 ml/min was held steady for 15 minutes, and a bleeding rate of 12±1 ml/min was held for 12 minutes. The animals were hemodynamically stable and longer periods of bleed flow at each desired level could be maintained as needed.
Localized control of exsanguinating arterial hemorrhage: an experimental model

Duplex ultrasound successfully located the arterial injury site in each model. Blood velocity at the bleeding site was confirmed and differentiated from arterial and venous flow. Doppler flow characteristics of the common carotid artery prior to creation of the arterial side-hole included a peak systolic velocity of 229 cm/sec, an end-diastolic velocity of 54 cm/sec (fig. 5). After creation of the side-hole arterial injury, peak systolic flow velocity in the common carotid artery proximal to the injury decreased to 153 cm/sec with an end-diastolic velocity of 63 cm/sec. Distal to the site of injury, common carotid artery peak systolic velocity decreased to 101 cm/sec with a fluctuating end-diastolic velocity of 27 cm/sec (fig. 6).

At the site of the 2.5 mm arterial injury, at high flow rates of 150 ml/min, peak systolic flow velocity was 321 cm/sec with a high and continuous end-diastolic velocity of 201 cm/sec. At low flow rates of 7 ml/min, peak systolic velocity was only 62 cm/sec with end-diastolic flow velocity of zero cm/sec (fig. 5).

A summary of ultrasound flow data at the site of the arterial injury with representative data at low and high bleeding rates are shown in fig. 5. Peak systolic velocity and end-diastolic velocity increased as the rate of bleeding increased from 5 to 150 ml/min. At a bleeding rate of 12 ml/min, peak systolic velocity was

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Fig. 4. Bleeding rates at site of arterial injury in reinfusion model. The rate of bleeding was controlled by regulating outflow resistance from the jugular venous reservoir. Flow was maintained at 395±78 ml/min for 8 minutes, at 110±11 ml/min for 15 minutes and at 12±1 ml/min for 12 minutes.

Fig. 5. Summary of Doppler ultrasound data at arterial injury. On the left: a comparison of ultrasound flow signatures. Normal blood flow in the common carotid artery, high rate bleeding at the injury site, and low rate bleeding at the injury site. On the right: peak systolic velocity, end-diastolic velocity with increasing flow at site of injury. Note that resistive index decreased with increasing flow at site of injury.
69 cm/sec and end-diastolic velocity was 0 cm/sec. At a bleeding rate of 110 ml/min peak systolic velocity was 319 cm/sec with end-diastolic velocity of 123 cm/sec. Resistive index in the common carotid artery at baseline was 0.76, where resistive index = (peak systolic velocity – end diastolic velocity) / peak systolic velocity. At the site of arterial injury, resistive index decreased as bleeding rate increased. At a bleeding rate of 10 ml/min, resistive index was 1.0 and at a bleeding rate of 150 ml/min resistive index was 0.37. At clinically significant bleeding rates above 30 ml/min, resistive index was less than 0.55. Heart rate was 99±8 bpm during these measurements.

**DISCUSSION**

This experimental model is unique as it is the first model of acute arterial injury designed to study the arterial bleeding point separately and distinctly from the hemorrhagic shock response to injury. This was motivated by a DARPA project focused on integrating high intensity therapeutic ultrasound technology with diagnostic duplex ultrasound into a single device which can be used to control battlefield hemorrhage. The requirements of the model to test these new devices included focal arterial injuries with defined rates of bleeding. Previously described models of arterial injury were unsuitable because of hemodynamic instability or lack of information on bleeding rate at the site of injury (11-23). This model provides a stable test-bed with variable and controllable rates of arterial hemorrhage which can be used to study the characteristics of the bleeding site and the effectiveness of measures directed at the bleeding site.

The first issue in developing this model was to establish the clinical significance of the arterial injury. In the uncontrolled exsanguination experiment the sheep lost 53% of total blood volume through the 2.5 mm diameter carotid artery side-hole in the first 6 minutes of bleeding (fig. 3), thus demonstrating the lethality of the model. The 2001 Military Medicine Workshop on Animal Models in Hemorrhage and the Combat Fluid Resuscitation noted that a 50% blood loss in most species results in a 50% mortality rate (27, 29). We also demonstrated the well known fact that bleeding stops with severe hypotension and that bleeding resumes when blood pressure is increased with fluid resuscitation. The DBAC device being developed by DARPA is designed to deal with this issue with continuous ultrasound monitoring and detection of bleeding sites, coupled with HIFU treatment, if necessary.

With respect to the rate of blood loss, we identified three clinically relevant bleeding
rates: 400 ml/min, 100 ml/min, and 10 ml/min. The bleeding rate of 400 ml/min would be experienced at the time of the injury, but would likely be measured only under experimental conditions. The bleeding rate would have slowed to approximately 100-150 ml/min within 5 minutes after injury, which represents the likely amount of time required for a combat medic to reach the casualty and apply a hemostatic device. Since humans have approximately twice the blood volume of sheep, an injured patient would have lost a smaller percent of total blood volume at this time point than we found in this experiment (25-28). The bleeding rate of 10 ml/min would be expected from a major arterial injury in patients who are hypovolemic and in shock. However, this bleeding rate could rise with resuscitation and a useful battlefield hemorrhage control device should be able to detect such low volume bleeding points in a soldier who is in shock.

Stable bleeding rates from 10 ml/min to 400 ml/min at the site of arterial injury were readily achieved in the second model with precise control of the rate of bleeding by the pinch valve on the proximal jugular vein. Blood return via the jugular vein prevented hypotension and maintained stable hemodynamics, thus allowing sufficient time to apply and test the hemostatic control device at both high and low flow conditions. Since the arterial side-hole empties into the jugular vein, shed blood does not enter the surrounding tissue, where it would initiate the coagulation cascade and create a hematoma which may compress the artery and alter its flow. The proposed DBAC hemostatic device is applied externally in the battlefield, with multiple ultrasound detectors to localize the bleeding site and guidance systems to cauterize and coagulate the bleeding artery with high intensity focused ultrasound (HIFU). Additional holes of varying sizes can be made in the carotid artery, each emptying into the jugular pouch, to test the ability of the DBAC device to localize and treat more complex wounds. This model could also be used to assess other external methods of hemorrhage control, such as a smart tourniquet, which inflates to occlude the artery flow proximal to the injury site only if bleeding is detected. The effectiveness of internal methods to control bleeding, such as endovascular stents or a transvascular balloon, either at the site of injury or in the proximal artery, could also be assessed using this model. Since bleeding in this model is contained and not exposed to surrounding tissues, it will not be a useful model to test prothrombotic substances or bandages applied to open hemorrhagic wounds.

Vaezy et al. at the University of Washington have demonstrated the validity of using HIFU to achieve hemostasis in acute arterial injuries in experimental models (14, 15). These investigators created arterial injuries with bleeding rates varying between 27.5 ml/min and 240 ml/min in their models. However, the rate and duration of hemorrhage was difficult to control and replicate due to hemodynamic instability, thus allowing little time to prepare and evaluate the HIFU treatment. In addition, it was difficult to differentiate between hemostasis due to successful HIFU application and bleeding cessation due to hemorrhagic shock. Our model avoids these difficulties and can provide a stable experimental model to evaluate the effectiveness of HIFU in controlling bleeding sites.

Ultrasound localization and characterization of the bleeding site is an important component of the DBAC system, and resistive index may be a key factor in such an algorithm. Luo et al. made a correlation between resistive index and the differentiation of an arterial, side-hole injury site from the normal rabbit femoral artery (30). They observed a resistive index of 1.0 in the uninjured artery, compared to a lower resistive index of 0.47 at the injury site. We identified a similar trend in our studies. In both the exsanguinating hemorrhage and reinfusion models, the resistive index of normal CCA flow was approximately 0.75. The resistive index at the site of injury is generally less than that of normal flow at clinically significant bleeding rates. More study is required to characterize the precise relationship between resistive index and bleeding rates.

In this study, we used heparin to prevent coagulation during the creation of the experimental arterial side-hole. However, this model could also be used without heparin, since the arterial hemorrhage enters the venous reservoir of the jugular vein and thus does not initiate the coagulation cascade. It is important to note that during their experiments, Vaezy et al. showed little difficulty halting bleeding with HIFU in heparinized animals (15).
CONCLUSIONS

We have developed an experimental model of acute arterial injury that can maintain both high and low bleeding rates without inducing hemorrhagic shock. This hemodynamically stable model can create variable bleeding rates at the site of injury from 10 to 400 ml/min and can be used as a test-bed to evaluate diagnostic and therapeutic methods designed focused on the control of arterial bleeding sites.

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Address correspondence: Christopher K. Zarins, MD; 318 W Campus Dr, Stanford University, Stanford, CA 94305-5431; email: zarins@stanford.edu