Comparison of Treatment Modalities for Hemorrhagic Shock

Anthony T. W. Cheung; Patricia L. To; Danielle M. Chan; Sahana Ramanujam; Michelle A. Barbosa; Peter C. Y. Chen; Bernd Driessen; Jonathan S. Jahr; Robert A. Gunther

* Department of Medical Pathology and Laboratory Medicine, University of California, Davis School of Medicine, Sacramento, CA, USA
* Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA
* Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA
* Department of Anesthesiology, University of California, Los Angeles David Geffen School of Medicine, Los Angeles, CA, USA
* Department of Anesthesiology, Charles Drew University of Medicine and Science, Los Angeles, CA, USA
* Department of Surgery, University of California, Davis School of Medicine, Sacramento, CA, USA

Online Publication Date: 01 March 2007
Comparison of Treatment Modalities for Hemorrhagic Shock

Anthony T. W. Cheung, Patricia L. (Duong) To, Danielle M. Chan, Sahana Ramanujam, and Michelle A. Barbosa
Department of Medical Pathology and Laboratory Medicine, University of California, Davis School of Medicine, Sacramento, CA, USA

Peter C. Y. Chen
Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA

Bernd Driessen
Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA, USA

The research was partially funded by NIH grant (HL67432) awarded to ATWC, a UC Davis Professional Development Award to ATWC, a UC Davis School of Medicine Faculty Research Award to ATWC, a UC Davis School of Medicine Robert Stowell (MD/PhD) Scholarship Award to PLDT, a UC Davis Hugh Edmondson Summer Research Fellowship to PLDT, and a Howard Hughes Medical Institute (S.H.A.R.P.) Research Fellowship to PLDT.

The subject materials in this manuscript have been presented, in parts, in a poster/discussion session in a workshop session lecture at the 9th International Symposium on Blood Substitutes (Tokyo, Japan), a lecture at the 23rd Meeting of the European Society for Microcirculation (Lisbon, Portugal), a lecture at the ’05 Asian Conference on the Microcirculation (Tokyo, Japan), and a lecture at the 10th International Symposium on Blood Substitutes (Providence, RI, USA). In addition, parts of this article have been published in the symposium proceedings of these international meetings.

The assistance of Cindy Her and Erin Lee, in the preparation of this manuscript, is very much appreciated.

Address correspondence to Dr. Anthony T. W. Cheung, Professor and Vice Chair, Department of Pathology and Laboratory Medicine, UC Davis School of Medicine, Research-III Building (Suite 3400), UC Davis Medical Center, 4645 Second Avenue, Sacramento, CA 95817, USA. E-mail: atcheung@ucdavis.edu
Abstract: Allogeneic blood resuscitation is the treatment of choice for hemorrhagic shock. When blood is unavailable, plasma expanders, including crystalloids, colloids, and blood substitutes, may be used. Another treatment modality is vasopressin, a vasoconstrictor administered to redistribute blood flow, increase venous return, and maintain adequate cardiac output. While much information exists on systemic function and oxygenation characteristics following treatment with these resuscitants, data on their effects on the microcirculation and correlation of real-time microvascular changes with changes in systemic function and oxygenation in the same animal are lacking. In this study, real-time microvascular changes during hemorrhagic shock treatment were correlated with systemic function and oxygenation changes in a canine hemorrhagic shock model (50–55% total blood loss with a MAP of 45–50 mmHg as a clinical criterion). Following splenectomy and hemorrhage, the dogs were assigned to five resuscitation groups: autologous/shed blood, hemoglobin-based oxygen carrier (Oxyglobin\textsuperscript{1}), crystalloid/saline, colloid/Hespan\textsuperscript{1} (6% hetastarch), and vasopressin. Systemic function and oxygenation changes were continuously monitored and periodically measured (during various phases of the study) using standard operating room protocols. Computer-assisted intravital video-microscopy was used to objectively analyze and quantify real-time microvascular changes (diameter, red-cell velocity) in the conjunctival microcirculation. Measurements were made during pre-hemorrhagic (baseline), post-hemorrhagic (pre-resuscitation), and post-resuscitation phases of the study. Pre-hemorrhagic microvascular variables were similar in all dogs (venular diameter = 42 ± 4 µm, red-cell velocity = 0.55 ± 0.5 mm/sec). All dogs showed significant (P < 0.05) post-hemorrhagic microvascular changes: ~20% decrease in venular diameter and ~30% increase in red-cell velocity, indicative of sympathetic effects arising from substantial blood loss. Microvascular changes correlated with post-hemorrhagic systemic function and oxygenation changes. All resuscitation modalities except vasopressin restored microvascular and systemic function changes close to pre-hemorrhagic values. However, only autologous blood restored oxygenation changes to pre-hemorrhagic levels. Vasopressin treatment resulted in further decreases in venular diameter (~50%) as well as red-cell velocity (~70%) without improving cardiac output. Our results suggested that volume replenishment—not oxygen-carrying capability—played...
an important role in pre-hospital/en route treatment for hemorrhagic shock. Vasopressin treatment resulted in inadvertent detrimental outcome without the intended benefit.

**Keywords:** Blood substitutes; Hemorrhagic shock; Oxygenation; Real-time microcirculation; Systemic function

**INTRODUCTION**

Hemorrhagic shock, when not properly managed or treated in a timely manner, can lead to irreversible damage and fatality. Therefore, appropriate management and treatment of hemorrhagic shock are crucial modalities that can serve to prevent deleterious outcomes. Pre-hospital—en route—treatment, which on the average is conducted within 1–2 hours after blood loss, normally consists of resuscitations using non-oxygen carrying plasma expanders (crystalloid or colloid solution) or catecholamines, including epinephrine and norepinephrine [1–5]. These treatments are designed to increase cardiac output so as to maintain mean arterial pressure (MAP), by redistributing blood flow and increasing venous return, until arrival at the hospital for allogeneic blood resuscitation or other definitive intervention.

In the past few years, hemoglobin-based oxygen carriers (HBOC) have been used as resuscitants in experimental trials to treat hemorrhagic shock [1,6–8]. The HBOC were designed to be very similar to allogeneic blood in oxygen-carrying and hemodynamic properties, with the intent that they could serve literally as oxygen-carrying blood substitutes. A few HBOC have been tested in animal models and are now in clinical trials in South Africa and Europe. One of the HBOC, Oxyglobin®, has been approved by the Food and Drug Administration (FDA) for canine use in the United States.

Recently, it has been suggested that treatment using vasopressin—the most potent naturally occurring vasoconstrictor known—could adequately and alternatively serve to redistribute blood flow and, therefore, may be a more effective treatment modality than conventional plasma expanders or catecholamines [3–5,9]. Clinical reports have indicated that in cases where fluid (plasma expander) or catecholamine resuscitations were futile, alternative treatment with vasopressin led to successful outcomes [10–12]. Several animal studies have shown that vasopressin treatment provided hemodynamic stabilization (monitored via MAP) during the pre-hospital phase of shock studies, while fluid- or catecholamine-treated animals showed no significant improvement and did not survive the studies [5,9,12]. It was expected, but not proven, that vasopressin resuscitation may serve as an effective treatment modality during the
critical pre-hospital phase of hemorrhagic shock treatment by redistributing blood flow to improve cardiac output and maintain MAP.

HBOC and vasopressin resuscitations, therefore, are modalities that have great potential to manage and treat hemorrhagic shock in a pre-hospital setting, in addition to the availability of crystalloid(s) and colloid(s), which have been used for decades, albeit with variable outcomes.

The goal of this study was to compare the different treatment modalities available for pre-hospital use, including an oxygen-carrying blood substitute/HBOC (Oxyglobin\textsuperscript{W}), a non-oxygen-carrying crystalloid (saline), a non-oxygen-carrying colloid (Hespan\textsuperscript{H}), and a potent vasoconstrictor (vasopressin), using autologous/shed blood—the gold standard for the treatment of hemorrhagic shock—as positive control. The experimental protocol was designed to longitudinally and simultaneously measure systemic functions, oxygenation characteristics, and microvascular activities in pre-hemorrhagic (baseline), post-hemorrhagic (pre-resuscitation) and post-resuscitation phases of the study for data correlation (in the same animal), efficacy comparison, and critical outcome evaluation.

MATERIALS AND METHODS

Animals

Fifteen dogs (healthy adult, male or female, 30–35 kg) were studied in a 12-month period. They were prepared, instrumented, splenectomized, hemorrhaged (to attain a MAP of 45–50 mmHg as a clinical criterion; equivalent to 50–55% blood loss), and resuscitated as performed in previously reported studies [13–15]. The dogs were randomly assigned to five resuscitation groups (autologous/shed blood, n = 3; Oxyglobin\textsuperscript{W}, n = 3; saline, n = 3; Hespan\textsuperscript{H}, n = 3; vasopressin, n = 3) as briefly described in Methods. A schematic flow-chart of the study is shown in Figure 1.

Resuscitants

Autologous/shed blood was anti-coagulated blood from the same dog collected during hemorrhaging for use as positive resuscitation control.

Oxyglobin\textsuperscript{W} (Hemoglobin Glutamer-200 [Bovine]; Biopure Corporation, Cambridge, MA) was an oxygen-carrying blood substitute that has been approved by the FDA for canine use in the United States.

Saline (0.9% NaCl injection USP; Baxter Healthcare Corporation, Deerfield, IL) was used as a non-oxygen-carrying crystalloid solution that has been commonly used as a fluid replenisher/plasma expander.
Hespan® (6% hetastarch; Abbott Laboratories, Chicago, IL) was a non-oxygen-carrying colloid solution that has been commonly used as a plasma expander.

Arginine Vasopressin (Vasopressin injection USP; AVP; American Regent Laboratories, Shirley, NY) was a potent vasoconstrictor commonly used in the hospital with the intent to redistribute blood flow.

**Animal Preparation and Instrumentation**

A total of 15 healthy, adult mongrel dogs were used within a 12-month period. Approval (Animal Welfare Assurance #A-3433–01) by the University of California, Davis Animal Care and Use Committee was obtained for this study. The experimental protocol used in this study was in compliance with the Guide for the Care of Laboratory Animals (National Institutes of Health Publication #86–23, revised 1985).

Each dog was premedicated with IM oxymorphone (0.02 mg/kg) and atropine (0.02 mg/kg), followed by percutaneous catheterization of the cephalic vein for continuous infusion of lactated Ringer’s solution (LRS) at a rate of 10 mL/kg/hr throughout the initial preparation and
instrumentation period and during the administration of drugs. Anesthesia was induced with IV propofol (2–4 mg/kg) and diazepam (0.5 mg/kg), followed by orotracheal intubation. Anesthesia was maintained following a balanced protocol, using isoflurane and fentanyl to minimize potential confounding hemodynamic effects [16,17]. During animal preparation and instrumentation, isoflurane in oxygen was delivered at an end-tidal concentration of 0.8–1.2%, and fentanyl infused at a rate of 0.7 μg/kg/min following an initial bolus of fentanyl (10 μg/kg). The dog was mechanically ventilated with an anesthesia ventilator (Model 2000; Hallowell EMC, Pittsfield, MA) using tidal volumes (VT) of 15–20 mL/kg and a respiratory rate of 9–12 breaths per minute to ensure an arterial partial pressure of carbon dioxide (P_aCO_2) in the range of 35–45 torr (4.6–6.0 kPa). End-tidal partial pressure of CO_2 (P_ETCO_2), end-tidal concentration of isoflurane (ISOET), and inspired O_2 concentration (F_iO_2) were continuously monitored using a Datex airway gas monitor (Datex 254; Helsinki, Finland).

Each dog was instrumented initially in dorsal recumbency and then placed on its side. Further monitoring included continuous recording of the electrocardiogram (ECG; Monitor Model 78353B, Hewlett Packard, Andover, MA) and arterial O_2 saturation (SaO_2; Model N-180 pulse oximeter, Nellcor Inc., Hayward, CA). Further instrumentation included placement of catheters into the dog’s femoral artery for arterial blood withdrawal, and determinations of systemic arterial pressures using membrane transducers (Model 1290A, Hewlett Packard, Watham, MA). An 8-fr balloon-tipped flow-directed thermodilution pulmonary arterial catheter (OptiQ®, Abbott Laboratories, Chicago, IL) was also inserted via the jugular vein and floated into the pulmonary artery under direct monitoring of pressure traces for measurements of central venous pressure (CVP), pulmonary occlusion pressure (POP), mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean pulmonary arterial pressure (MPAP), core body temperature and cardiac output (CO). The pulmonary arterial catheter was connected to a cardiac output computer (Critical Care System QVUE, Oximetrix 3, Abbott Laboratories, Chicago, IL) for continuous CO monitoring. Cardiac output was also assessed by thermodilution in triplicate using 10 mL of saline at room temperature. Body temperature was maintained between 38–39°C by means of a heating pad and circulating warm air blanket (Bair Hugger®, Model 505, Augustine Medical Inc., Eden, MN).

After preparation and instrumentation, the dog was splenectomized following a midline laparotomy to prevent release of sequestered blood during sympathetic stimulation [13]. After splenectomy, the dog went through a stabilizing period of 60 min, after which the dog was ready for hemorrhaging.
Measurement of Systemic Variables

A bioengineering station, which was designed to monitor and study hemorrhagic shock and treatment modalities in large animals in an operation room setting, was established for this study in our laboratory at UC Davis. The bioengineering station was capable of measuring numerous systemic function and oxygenation variables. Of interest to this study, the measured variables included ECG, MAP, SAP, DAP, MPAP, POP, CVP, heart rate (HR), stroke volume index (SVI), and CO. Arterial and mixed-venous heparinized blood samples were collected intermittently (whenever appropriate) from the femoral artery and the right atrium, respectively. Immediately after collection, blood samples were sealed and stored on ice. Subsequently, arterial and mixed-venous total hemoglobin (aHb_total; vHb_total), arterial plasma hemoglobin (aHb_plasma) and methemoglobin (Met-Hb) concentrations, and arterial and mixed-venous O₂ saturation (SaO₂; SvO₂) were measured, using a co-oximeter (Model 482, Instrumentation Laboratories, Lexington, MA). Arterial and mixed-venous O₂ content (CaO₂; CvO₂) were directly measured in triplicate, using an oxygen-specific electrode (LEXO₂CON-K, Hospex Fiberoptics, Chestnut Hill, MA). Mean tissue oxygenation (MtO₂) was measured by an oxygen-measuring microprobe inserted percutaneously into the quadriceps femoris muscle of the thigh, using the OxyFlo™/OxyLite® microprobe technology (Oxford Instruments; Oxford, UK). Arterial and mixed-venous lactate (lactate_a; lactate_v) concentrations were determined in duplicate by means of a lactate analyzer (Model 1500, YSI Inc., Yellow Springs, OH). Arterial and mixed-venous pH (pH_a; pH_v) and partial pressures of O₂ (P_aO₂; P_vO₂) and CO₂ (P_aCO₂; P_vCO₂) were analyzed with a blood gas analyzer (Rapidlab Model 248, Bayer Corporation Diagnostics Division, Norwood, MA). Blood gas values were corrected for the body temperature of the animal at the time of sampling. Arterial and mixed venous standard base excesses (SBE_a; SBE_v) and bicarbonate levels (aHCO₃; vHCO₃) were computed by the blood gas analyzer. Other variables, including body surface area (BSA in m²), systemic vascular resistance (SVR), etc., were calculated using standard equations.

Computer-Assisted Intravital Microscope (CAIM)

The CAIM system was anchored along the front edge of the operating table for non-invasive videotaping of the real-time conjunctival microcirculation in the eye of the dog. CAIM was originally designed to study real-time microvascular changes in vivo in diabetic and sickle cell anemia patients [18–22]. To incorporate CAIM as part of the instrumentation in
the bioengineering station, it has been substantially redesigned and modified to study the microcirculation in the bulbar conjunctiva of the dog so that real-time microvascular changes in the conjunctival microcirculation could be videotaped simultaneously with the measurements of systemic functions and oxygenation in the same animal for critical correlation (see Figure 2). CAIM was macro-optic based and has an optical magnification of 4.5× and an on-screen magnification of 125×. The

![Image](image-url)

**Figure 2.** An overall view of the bioengineering station followed by a close-up view of the intravital microscope.
optical magnification of CAIM was fixed because of its macro design; this non-changeable magnification was important as it assured that all microvascular measurements made in various phases of the experimentation were quantified on the same basis without a magnification variable. An anti-red (#58 Wratten green filter) fiber-optic light source was used to illuminate the conjunctival microcirculation and to enhance vessel visualization. The angle and level of the front element of CAIM were adjusted to provide the flattest possible surface for focusing. A COHU video camera (Model 2622-100; 1/2-inch monochrome CCD-format, San Diego, CA) was used to non-invasively videotape the real-time conjunctival microcirculation via a high-resolution video-recorder. Focus of CAIM was centered on the microcirculation in the bulbar conjunctiva of the left eye of the dog. The microvascular activities were videotaped and subsequently analyzed via computer-assisted image analysis for microvascular morphometry and dynamics using in-house developed imaging software VASCAN and VASVEL [13–15,18–22]. The imaging software can objectively quantify over 20 parameters of microvascular characteristics. However, only venular diameter (morphometric) and red-cell velocity (dynamic) characteristics, which we consider relevant based on previous investigations, were objectively quantified and reported in this study [13–15]. Most conjunctival vessels have unique shapes and forms and were easily recognizable. In this study, the vessels of interest were identified and relocated for time-dependent videotaping. During various phases of the experimentation, the same vessels were relocated and restudied so that relevant measurements (e.g., vessel diameter and red-cell velocity) were made for longitudinal comparison, with each vessel serving as its own reference control (see Figures 3 and 4).

**Figure 3.** A series of three images showing the same location in the conjunctival microcirculation of a dog during pre-hemorrhagic (baseline), post-hemorrhagic and post-resuscitation phases of the experiment in the autologous/shed blood study. Because of the unique shape and form of the vessels under observation, the vessels in the same location could be identified and relocated for longitudinal evaluations, using the baseline measurements of each vessel as control. Magnification = 125× on-screen.
Canine Hemorrhagic Shock Model

Each dog was allowed to stabilize after the splenectomy. The amount of sequestered blood in the spleen from each dog was measured and included in the computation of total blood loss (50–55%). At the end of a 60-min stabilization period, pre-hemorrhagic (baseline) measurements on systemic function and oxygenation were made (pre-hemorrhagic phase). In addition, videotape sequences were made via CAIM on the conjunctival microcirculation simultaneously. Immediately after the baseline measurements were made, 50–55% of the blood volume of the dog (based on body weight and the amount of sequestered blood in the spleen measured after splenectomy) was withdrawn from the lateral saphenous and femoral veins—at an overall hemorrhage rate of 32–36 mL/kg/hr—until a MAP of 45–50 mmHg was achieved (normally ~45 min). The method of attaining a MAP of 45–50 mmHg as a clinical criterion was used to ensure the induction of acute but non-lethal hypovolemia and hemorrhagic shock. Within the post-hemorrhagic 1-hr acclimatization period to induce hemorrhagic shock, small amounts of blood were withdrawn if needed (whenever MAP increased to over 50 mmHg) to maintain a MAP of ≤50 mmHg (within a 45–50 mmHg range). Shed blood was collected, anticoagulated and used in autologous blood resuscitation to serve as control. In similar manner to pre-hemorrhagic (baseline) measurements, post-hemorrhagic measurements and videotape sequences were made (post-hemorrhagic phase). At the end of post-hemorrhagic measurements, the dogs were randomly assigned to one of the five resuscitation groups: autologous/shed blood (resuscitated at 30 mL/kg/hr for one hour), Oxyglobin® (resuscitated at 10 mL/kg/hr for one hour), saline (resuscitated at 30 mL/kg/hr for one hour), Hespan® (resuscitated at 30 mL/kg/hr for one hour), and

Figure 4. A series of three images showing the same location in the conjunctival microcirculation of a dog during pre-hemorrhagic (basline), post-hemorrhagic and post-resuscitation phases of the experiment in the crystalloid/saline study. Again, the same vessels were identified and relocated for longitudinal evaluations. Magnification = 125× on-screen.

Canine Hemorrhagic Shock Model
vasopressin (resuscitated at a loading bolus dose of 0.4 IU/kg followed by 4.8 IU/kg/hr for one hour). Systemic function and oxygenation measurements were again made at the completion of the resuscitation process (post-resuscitation phase). Videotape sequences were also made simultaneously.

Statistics

All results were averaged and reported as mean ± SD. Analysis of variance, student’s t-test and post hoc Bonferroni corrections were used whenever appropriate. A 0.05 significance level was used in this study. P values smaller than 0.01 (e.g., P = 0.000045 or P = 5.793 × 10⁻⁴) were presented as P < 0.01 for simplicity.

RESULTS

Pre-hemorrhagic systemic function, oxygenation, and microvascular variables were similar in all 15 dogs with no significant statistical difference between the five resuscitation groups.

Immediately after hemorrhaging, all dogs remained stable under anesthesia with no significant changes in body temperature, heart rhythm (EKG), respiratory rate, cardiopulmonary characteristics, ventilatory parameters and end-tidal inhalant anesthetic concentration over time. MAP was reduced to 45–50 mmHg. In addition, all 15 dogs showed similar significant (P < 0.01) post-hemorrhagic changes in systemic function and oxygenation characteristics, including decreases in Hct, Hb_total, Hb_plasma, MPAP, SAP, DAP, CO, CaO₂, and CvO₂, and increases in HR and lactic acidosis. In all 15 dogs, significant (P < 0.01) post-hemorrhagic changes also occurred simultaneously in microvascular characteristics, including ~20% decrease in venular diameter and ~30% increase in red-cell velocity, compensatory changes which were indicative of sympathetic effects arising from substantial blood loss (see Figures 5 and 6).

Immediately following resuscitation, which was initiated 60 minutes after the completion of hemorrhaging to ensure the establishment of hemorrhagic shock, shed blood restored systemic function changes to pre-hemorrhagic (baseline) values. Oxyglobin®, saline, Hespan®, and vasopressin resuscitations restored most systemic function changes, except CO and HR, to pre-hemorrhagic values in similar manner to shed blood. However, Oxyglobin® and vasopressin failed to restore CO to pre-hemorrhagic values. HR values decreased after Oxyglobin®, saline, Hespan®, and vasopressin resuscitations—but the decreases were to
different extents and did not return to pre-hemorrhagic values, with Hespan\textsuperscript{\textregistered} being most effective and Oxyglobin\textsuperscript{\textregistered} being least effective.

Figure 5. Post-hemorrhagic and post-resuscitation changes in venular diameters induced by the five resuscitation treatment modalities.

Shed blood resuscitation restored post-hemorrhagic microvascular changes to pre-hemorrhagic values. Oxyglobin\textsuperscript{\textregistered}, saline, and Hespan\textsuperscript{\textregistered}

Figure 6. Post-hemorrhagic and post-resuscitation changes in red-cell velocity induced by the five resuscitation treatment modalities.
resuscitations, similar to the effect of shed blood, also restored post-hemorrhagic microvascular changes close to pre-hemorrhagic values (see Figures 5 and 6). However, vasopressin treatment resulted in further decreases in venular diameter (~50%) as well as red-cell velocity (~70%) (see Figures 5–7).

Shed blood resuscitation restored post-hemorrhagic oxygenation (CaO₂ and CvO₂) changes to pre-hemorrhagic (baseline) values. However, Oxyglobin®, saline, Hespan®, and vasopressin resuscitations did

**Figure 7.** A series of two images showing the same location in the conjunctival microcirculation of a dog during post-hemorrhagic and post-resuscitation phases of the experiment in the vasopressin study. Again, the same vessels were identified and relocated for longitudinal evaluations. Note the extensive vasoconstriction arising from the vasopressin infusion. Magnification = 125× on-screen.

**Figure 8.** Post-hemorrhagic and post-resuscitation changes in arterial oxygen content (CaO₂).
not have any effect in restoring these post-hemorrhagic oxygenation changes (see Figures 8 and 9). The unexpected result that Oxyglobin\textsuperscript{1}, an oxygen carrier, did not restore oxygenation changes led to the suspicion that standard blood sample based oxygenation measurements may not adequately measure tissue oxygenation levels. To investigate this.

**Figure 9.** Post-hemorrhagic and post-resuscitation changes in venous oxygen content (CvO\textsubscript{2}).

**Figure 10.** Post-hemorrhagic and post-resuscitation changes in mean tissue oxygenation (MtO\textsubscript{2}).
installing, the Oxyflo™/Oxylite™ microprobe technology was used to measure tissue level oxygenation levels in the same animals. The results confirmed that, of all the resuscitants used in this study, only shed blood restored oxygenation at the tissue level to pre-hemorrhagic values. In addition, it was also shown that vasopressin resuscitation led to a further decrease in tissue oxygenation (see Figure 10).

DISCUSSION

Allogeneic blood resuscitation is the treatment of choice for hemorrhagic shock. However, in situations when blood is not available (e.g., battlefield or rural area injury), crystalloids, colloids, artificial blood substitutes, or vasoactive agents have been used. The goal of this study was to evaluate the efficacy of these resuscitants in the pre-hospital treatment of hemorrhagic shock, with special emphasis on studying their effects on the microcirculation, the raison d’etre of the vasculature [1]. Few studies have focused on the real-time microcirculation in large animals due to a lack of relevant technologies and appropriate non-invasive research sites. In addition, rarely have real-time microvascular changes been simultaneously studied and correlated with systemic function and oxygenation changes in the same animal. The availability of CAIM provided a means for non-invasive evaluation of the effects of different resuscitants on the real-time microcirculation. In addition, the large size of the dog allowed for extensive measurements of systemic function, blood chemistry, and oxygenation changes to correlate with changes in microvascular parameters. This study represents the first of its kind in which systemic functions, oxygenation, blood chemistry, and microvascular characteristics were measured in the same animal simultaneously. In addition, the results can serve as a benchmark for testing treatment modalities for hemorrhagic shock, and in the future development of artificial blood substitutes.

In order to adequately evaluate the efficacy of different resuscitants, a suitable hemorrhagic shock model (substantially modified from the Wiggers model [23]) was adapted in our laboratory in which the dogs were maintained for a 1-hr acclimatization period following hemorrhaging to ensure the development of hemorrhagic shock for this study. This model may serve as a testing platform for future hemorrhagic shock and resuscitation studies.

In this study, only shed blood resuscitation restored post-hemorrhagic systemic function and oxygenation changes to pre-hemorrhagic levels. To different degrees, Oxyglobin®, saline, Hespan®, and vasopressin resuscitations restored systemic function changes. Standard blood transfusion protocols were used for shed blood, saline, and Hespan® resuscitations.
at a rate of 30 mL/kg/hr. However, Oxyglobin\textsuperscript{1} was administered at a slower rate (10 mL/kg/hr) as recommended by the manufacturer to prevent excessive circulatory hemoglobin overload. This significantly slower rate of administration, which replenished less blood volume compared with the standard protocol, may account for the failure of Oxyglobin\textsuperscript{1} to restore CO and HR to baseline values. While it was expected that saline, Hespan\textsuperscript{1}, and vasopressin resuscitations would not restore oxygenation characteristics due to their non-oxygen carrying capability, it was surprising that oxygen-carrying Oxyglobin\textsuperscript{1} did not restore oxygenation (CaO\textsubscript{2} and CvO\textsubscript{2}) characteristics. To address the possibility that blood-sample based measurements of oxygenation characteristics may not be reflective of tissue level oxygenation, the OxyFlo\textsuperscript{TM}/OxyLite\textsuperscript{TM} microprobe technology was used to obtain measurements of oxygenation at the tissue level. The results indicated that only shed blood resuscitation restored tissue oxygenation to pre-hemorrhagic values and that, indeed, the oxygen-carrying Oxyglobin\textsuperscript{1} was not effective in improving blood oxygenation. In addition, it was also convincingly shown that vasopressin treatment resulted in a further decrease in tissue oxygenation (see Figure 10).

In this longitudinal study when efficacy studies were made to mimic pre-hospital treatment of hemorrhagic shock, autologous/shed blood, Oxyglobin\textsuperscript{1}, crystalloid (saline), and colloid (Hespan\textsuperscript{1}) resuscitations were effective in restoring MAP from a post-hemorrhagic value of 45–50 mmHg to a close-to-baseline (pre-hemorrhagic) level of 95–100 mmHg immediately after completion of resuscitation. However, follow-up studies to evaluate the effects of these resuscitants 3–4 hrs after resuscitation are needed to show their long-term efficacies in the treatment of hemorrhagic shock.

Vasopressin treatment, instead of achieving the intended goal of redistributing blood flow to improve CO and maintain MAP, resulted in detrimental effects, including extensive peripheral vasoconstriction, which led to a cessation of blood flow, significant reduction in red-cell velocity, significant disappearance of capillaries and arterioles, and significant decreases in tissue oxygenation. These results indicate that vasopressin may not be an appropriate choice as a resuscitant for the treatment of hemorrhagic shock.

REFERENCES


