The cerebral microcirculation is protected during experimental hemorrhagic shock*

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Objective: Decreases in buccal microcirculation are indicative of the severity of hemorrhage, but incidental observations suggest that this may not apply to the cerebral microcirculation. We therefore hypothesized that the cerebral microcirculation may be preserved in hemorrhagic shock in which systemic and buccal microcirculatory flow are reduced. We propose to relate changes in the macrocirculation to the buccal and cerebral microcirculations during hemorrhage and after fluid resuscitation.

Design: Prospective, randomized, controlled animal study.

Setting: University-affiliated research laboratory.

Subjects: Sprague-Dawley rats.

Interventions: Fifteen male Sprague-Dawley rats were anesthetized and endotracheally intubated. Craniotomy exposed the parietal cortex for orthogonal polarization spectral imaging. Mean arterial pressure, cardiac output, arterial blood gases, and lactate were measured concurrently with determination of microcirculatory indices in buccal and cerebral areas. Animals were randomly assigned to bleeding either 35% or 25% of estimated total blood volume and compared with sham bled animals. Hypovolemia was maintained for 60 mins in test animals, after which saline in amounts to 2 times the blood loss, was administered over 30 mins. Cerebral and buccal microvascular indices were measured in vessels smaller than 20 μm, representing capillaries.

Measurements and Main Results: Mean arterial pressure and cardiac output were reduced and arterial blood lactate was increased in relationship to the magnitude of blood loss. Saline infusion increased mean arterial pressure and cardiac output. Buccal microcirculation decreased after bleeding but was restored after saline infusion. However, the cerebral microcirculation was essentially unaffected by hemorrhage and saline infusion.

Conclusion: In contrast to the systemic decreases in pressure and flow characteristics of hemorrhagic shock, including decreases in microcirculations of buccal mucosa, cerebral microvascular flow was preserved during moderate and severe blood losses. (Crit Care Med 2010; 38:928–932)

Key Words: buccal microcirculation; cerebrovascular circulation; hemorrhage; orthogonal polarization; rat

Decreases in the macrocirculation, including hypotension and critical reductions in cardiac output (CO), characterize the hemodynamic state of hemorrhagic shock, which are experimentally and clinically documented to result in inadequate end-organ perfusion, ischemic tissue, and organ injury with cell death (1–3). More recently, attention of the shock state has been extended to microcirculation. In settings of septic shock, dissociations between the macrocirculation with normal or even high CO, but selective decreases in microcirculatory flow have been identified (4, 5). With respect to hemorrhagic shock, however, there is a close parallel between the macrocirculatory hemodynamic measurements and the microcirculatory indices measured in sublingual or buccal mucosa that reflects the systemic microcirculation (6–9).

For >40 yrs clinicians have been aware that, even under the most severe conditions of hemorrhagic shock, survivors manifested no cerebral injury after resuscitation (1, 10). Accordingly, we were prompted to relate decreases in CO, arterial pressure, and microcirculatory flow in the buccal mucosa to concurrent microcirculatory changes in the cerebral cortex. If brain function is preserved even though CO and arterial perfusion pressures are critically reduced, then the question arises as to whether the microcirculatory flow to the brain is nevertheless maintained.

For study of microvascular blood flow to the cerebral cortex, both porcine and murine models have been evolved for visualization of pial microvessels after craniotomy (11–13). Because there was evidence indicating that pial microvessels respond like those of intraparenchymal microvessels (14), we measured changes in the pial vessels as representative of the cerebral cortex. With concurrent buccal measurements, which are practically accessible for noninvasive monitoring in rats, we anticipated that changes in the microvessels in buccal mucosa reflecting the systemic circulation might not be the same as those observed in the cerebral cortex. The “orthogonal polarization spectral” (OPS) and sidestream dark-field imaging techniques allow for noninvasive, direct in vivo visualization and quantification of the flow in microvessels <20 μm (15–17). In view of the unique neurologic outcomes of both patients and experimental animals who survived the most severe state of shock after bleeding, we hypothesized that the cerebral microcirculation may be selectively preserved.

MATERIALS AND METHODS

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the
Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (Washington, DC, National Academy Press, 1996). The protocol was approved by the Institutional Animal Care and Use Committee of the Weil Institute of Critical Care Medicine. The animal laboratories of the Weil Institute are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The animals were obtained from Harlan Sprague-Dawley Laboratories.

Animal Preparation

Fifteen male Sprague-Dawley rats weighing 450 to 550 g were fasted overnight except for free access to water. The animals were anesthetized by intraperitoneal injection of pentobarbital (45 mg/kg). Anesthesia was maintained with additional doses of pentobarbital in amounts of 5 mg/kg that were administered at intervals of approximately 30 to 45 mins. The trachea was orally intubated with a 14-gauge cannula (Abbocath-T; Abbott Hospitals. The arterial in amounts of 5 mg/kg that were administered at intervals of approximately 30 to 45 mins. The trachea was orally intubated with a 14-gauge cannula (Abbocath-T; Abbott Hospital Division, North Chicago, IL) mounted on a blunt needle with a 145-degree angled tip as previously described (18). Animals breathed spontaneously. End-tidal CO₂ was continuously monitored with a sidestream infrared CO₂ analyzer (model 200; Instrumentation Laboratories, Lexington, MA). The upper and lower incisors were partially amputated for appropriate placement of the imaging probe onto the left buccal mucosa. Craniotomy was performed with excision of the right parietal bone (19). The dura was kept intact. For blood shedding, a 23-gauge polyethylene catheter (Intramedic PE-50; Becton Dickinson, Sparks, MD) was advanced through the left carotid artery into the descending aorta. Another PE-50 catheter was advanced through the left external jugular vein into the right atrium for central venous pressure monitoring. A PE-50 catheter was advanced through the femoral artery into the thoracic aorta for measurement of aortic pressure with a high-sensitivity pressure transducer (model 42584-01; Abbott Critical Care Systems, North Chicago, IL). This catheter also provided a source for arterial blood sampling for blood gas analyses. A PE-50 catheter was advanced through the femoral vein into the inferior vena cava for injection of additional doses of pentobarbital to maintain anesthesia, sampling venous blood, transfusion of donor blood, and fluid resuscitation utilizing a commercial infusion pump. A rectal thermistor (Yellow Springs Instruments Laboratory, Yellow Springs, OH) was inserted into the rat rectum for measurement of body temperature, which was maintained at 37°C ± 0.5°C with the aid of a heating lamp and a heating pad. All catheters were flushed intermittently with saline solution containing 5 IU/mL crystalline bovine heparin.

Experimental Procedures

The animals were randomly assigned to three groups. The first group represented severe blood loss after removal of 35% of estimated blood volume. Estimated blood volume was calculated to be 6.12% of rat body weight (8). The second group represented moderate blood loss of 25% of estimated blood volume. The third group was a sham control that underwent identical procedures except no blood was withdrawn and no fluid was infused. Blood was withdrawn with a syringe in six equal amounts at 10-min intervals for a total of 60 mins. After an additional 60 mins of untreated hemorrhagic shock, animals in both hemorrhage groups were resuscitated over a 30-min interval by continuous infusion of normal saline in amounts corresponding to twice the volume of blood loss.

Two hours after the completion of fluid infusion, the animals were euthanized with an intravenous injection of pentobarbital (150 mg/kg). Autopsy was performed for gross examination to identify potential injuries caused by surgical interventions.

Measurements

Cerebral and buccal microcirculations were visualized with the aid of OPS and sidestream dark-field imaging video microscope (MicroScan; MicroVision Medical, Amsterdam, The Netherlands). For OPS techniques, polarized light illuminates the area of interest and is absorbed by hemoglobin, which produces high-contrast reflected light images (20). For the sidestream dark-field technique, a light wavelength that is absorbed equally by oxyhemoglobin and deoxyhemoglobin is delivered by light-emitting diodes surrounding the light guide to achieve optimal imaging of the microcirculation (20). The objective encompassed a 940 μm × 750 μm field. The 5× optical probe was applied manually by the lead author to the saline-moistened translucent dura and the buccal mucosa. Three discrete fields were examined. Measurements were made at baseline, immediately on completion of bleeding, before infusion of saline, after infusion, and 1 hr and 2 hrs later. Microvascular images were recorded on a video cassette with a conventional video cassette recorder (CT-330; Samsung Electronics America, Secaucus, NJ). Video clips are analyzed offline for quantitating microcirculatory blood flow by the method adapted from Spronk et al (21). This provided semiquantitative scores as microvessel flow indices in which 0 represented “no flow,” 1 represented “markedly reduced flow,” 2 represented “reduced flow,” and 3 represented “normal flow” in vessels <20 μm. The images were also analyzed for vascular density by the method of De Backer et al (22). Three equidistant horizontal and three equidistant vertical lines were drawn. The microvascular density of vessels <20 μm was calculated as the number of microvessels crossing these lines divided by the total length of the lines. The type of flow was defined as continuous, intermittent, or absent. The proportion of perfused microvessels (%) may be calculated as follows: 100 × [(total number of microvessels – [no flow + intermittent flow]) / total number of microvessels]. Perfused microvessel density, an estimate of functional capillary density, may be calculated by multiplying vessel density by the proportion of perfused vessels. The data of the three areas were averaged in each rat. Vessel size was measured with a micrometer scale superimposed on the video display. Video recordings were analyzed by two independent observers. Agreement between observers was >95%.

End-tidal CO₂, aortic, and right atrial pressures were continuously recorded with the aid of PC-compatible data-acquisition system (DATAQ Instruments, Akron, OH). CO was measured with the aid of a Philips echocardiographic system utilizing a 12.5-Hz transducer (HD 11 XF; Philips Ultrasound, Bothell, WA). Venous and arterial blood samples were collected for measurement of hemoglobin, blood lactate, O₂, and CO₂ tension and saturation. The 0.5-mL aliquots of blood that were withdrawn from the arterial and venous sites were replaced with an equal amount of blood from an anesthetized donor rat as previously described (8, 18). Measurements of the blood samples were performed with a multianalyzer (Stat Profile pHox Analyzer; Nova Biomedical Corporation, Waltham, MA), with the exception that blood lactate concentrations were measured with a Lactate Analyzer (model 2300 Stat Plus; Yellow Springs Instruments).

Statistical Analysis

Normal distribution of the measurements was confirmed with the Kolmogorov-Smirnov test. All variables were compared by use of either parametric tests (analysis of variance [ANOVA]) or nonparametric tests (Mann-Whitney U test). For measurements among groups, ANOVA and Scheffe multiple-comparison techniques were used. Comparisons between time-based measurements within each group were performed with a paired sample Student t test. Linear correlations were calculated using Spearman correlation coefficient. The ordinal data of microcirculations were reported as median and 25% and 75% quartile ranges. The other data were reported as mean ± SD; p < .05 was regarded as statistically significant.

RESULTS

No differences in baseline hemodynamic, arterial blood gas, and microcirculatory flows were observed among the three
groups. Mean arterial pressure and CO decreased during bleeding, and more so after 35% blood loss, but partially recovered over the following untreated hour in both hemorrhagic groups. After saline infusion, mean arterial pressure was further increased but failed to return to baseline levels. This contrasted with a CO exceeding baseline values after saline infusion (Fig. 1).

Microvessel density and microvessel flow indices measured in the buccal mucosa were strikingly reduced and corresponded to the magnitude of blood loss, as shown in Figures 2 and 3. Fluid infusion partially restored buccal microcirculatory indices to normal or near-normal levels in both hemorrhage groups. However, this contrasted strikingly with the absence of any significant changes in cerebral microvessel density and microvessel flow (Fig. 4). Furthermore, the dynamic changes of buccal microcirculation were significantly correlated with time coincident values of mean arterial pressure ($r = .741; p < .01$) and also correlated with CO ($r = .674; p < .01$) and end-tidal CO$_2$ ($r = .592; p < .01$).

Changes in blood values are shown in the Table. As anticipated, hemoglobin was significantly reduced after blood shedding, and further reduced after infusion of saline. In these spontaneously breathing animals, hyperventilation was associated with blood loss and accounted for decreases in PCO$_2$ and end-tidal CO$_2$. Increases in arterial blood lactate concentrations were characteristic of the anaerobic metabolism that followed circulatory shock and corresponded to the severity of the blood loss. After saline infusion, lactate significantly decreased.

**DISCUSSION**

The present study demonstrated that, in contrast to the effects of hemorrhage on the macrocirculation with sharp declines in CO and arterial pressure together with reduced buccal microcirculatory flow, the cerebral microcirculations were consistently preserved. When CO is decreased during hypovolemia, a series of compensatory responses follow to maintain arterial perfusion pressure and, to a lesser extent, augment blood flow to crit-
Evidence that the cerebral blood flow is redirected from peripheral organs to the brain has been previously documented by Slater et al (27). They found that cerebral blood flow calculated as a percentage of CO is itself increased during the early stages of hemorrhage. In addition to global compensation, it is believed that the brain itself has an effective autoregulation system to maintain blood flow despite systemic hypoperfusion (28, 29). However, this belief is based on the alterations of arterioles of approximately 100–μm diameter (29, 30), or on the observations of cerebral blood flow, or on the average of blood flow in all the vessels of any diameter in the sampling volume (31). All of these represent the macrocirculation rather than microcirculation of the brain attributable to the limitations of measuring methods. These limitations have constrained our understanding of anatomical sites and functional mechanisms by which the true microcirculation is controlled during shock state.

The new OPS and sidestream dark-field imaging methods have now presented opportunity for relating major hemodynamic crises to the role of the microcirculation. Inconsistencies between flows in the microvessels <20 μm and in the conventional large vessels have been reported in gastric and buccal areas (32). Our data further expose the striking differences relating specifically to the microcirculations of various compartments. The findings provide additional evidence of dissociation between the macrocirculation and microcirculation during hemorrhagic shock. The consistent perfused microvessel density in the brain indicates unaltered functional capillary density in the shock state; the absence of change in cerebral microvessel flow indices provides an estimate of continuous microcirculatory flow during decreased macrocirculation; and the constancy of cerebral proportion of perfused microvessels implicates little blood flow heterogeneity in the brain after shock compared with the buccal area. These data identified that there was no microvessel rarefaction or heterogeneity in cerebral perfusion and confirmed that in our experimental setting, the cerebral microcirculation, independent of global hemodynamics, is protected by systemic and cerebral autoregulatory mechanisms. To our best knowledge, this study provides direct visual evidence confirming for the first time that the previously believed autoregulatory effects extend to cerebral microcirculation in hemorrhagic shock and assures a selective role of microcirculation discordant with macrocirculation. This may contribute to the preserved postresuscitation brain function after hemorrhagic shock.

We recognize important limitations in the experimental method and the interpretation of our findings. It is possible that the window created in the parietal bone during craniotomy would alter intracranial pressure and, therefore, cerebral perfusion. Yet, cerebral perfusion was, in fact, preserved and there was no overt loss of cerebrospinal fluid. The effects of changes of arterial PCO2 (33) and PO2 (34) on the cerebral microvascular response were not independently evaluated. There is also no assurance that OPS measurement in one visual field is representative of the entire brain. Regional heterogeneity in the microcirculation therefore is not excluded (35). Furthermore, OPS technology currently limits visualization to microvessels 1 mm below the dura. The velocity of red blood cells in the microvessels was not analyzed. Although cerebral microvascular hemodynamics were unaffected, during severe hemorrhagic hypotension cellular and/or molecular functions (36) independent of the microcirculation were not assessed. In addition, potential obfuscating effects of anesthesia were not separately controlled. Finally, the extension of our findings on cerebral vascular dynamics in this rodent model after a relatively short duration of circulatory shock cannot be applied directly to the usually longer clinical course in human patients during and after hemorrhagic shock.

**CONCLUSIONS**

Within these limitations, we conclude that in the experimental setting of this rodent model, cerebral microcirculation, independent of the systemic macrocirculation, is remarkably preserved during hemorrhagic shock and after fluid resuscitation. These findings potentially explain unimpaired cerebral function after...
REFERENCES


