Published in final edited form as:
Surgery. 2005 August ; 138(2): 195–203.

Direct energy delivery improves tissue perfusion after resuscitated shock

El Rasheid Zakaria, MD, PhD[a,b], **William D. Ehringer, PhD**[a], **Nina Tsakadze, MD, PhD**[a], **Na Li, MD**[a], and **R. Neal Garrison, MD**[b,c]

a*From the Departments of Physiology and Biophysics, University of Louisville, Louisville, Ky*

b*From the Department of Surgery, University of Louisville, Louisville, Ky*

c*From the Department of Veterans Affairs Medical Center, University of Louisville, Louisville, Ky*

Abstract

Background. Conventional resuscitation (CR) from hemorrhagic shock (HS) does not restore intestinal blood flow. Indicators of anaerobic metabolism suggest that cellular energy production also is compromised. We hypothesize that the direct intravenous delivery of lipid-encapsulated high-energy phosphates to cells improves intestinal perfusion during HS and resuscitation (RES).

Methods. MAP (MAP) was monitored in male rats (200 g), terminal ileum microvessel diameters were measured by in vivo videomicroscopy, and blood flow (Doppler velocimetry) was calculated. Cellular energy delivery was accomplished by intravenous infusion during RES of fusogenic unilamellar lipid vesicles that contain adenosine triphosphate (ATP; VitaSol). Our protocol was HS to 50% baseline MAP for 60 minutes, 30 minutes of RES, and continued microscopy observation for 120 minutes. Experimental groups (n = 8 each) were HS+CR (group I); HS+CR+ VitaSol (group II); HS+CR+Vehicle, Vehicle is the phospholipid vesicles without magnesium ATP, (group III); HS + VitaSol (group IV); sham-operated control + VitaSol (group V); and a time-matched sham-operated control (group VI). The survival outcome and total tissue water from wet weight/dry weight ratio as a function of adjunct VitaSol resuscitation were evaluated in separate intact animal experiments.

Results. HS caused a selective vasoconstriction of the intestinal inflow arterioles (100 μm), which was not seen in the smaller intestinal premucosal arterioles (7-15 μm). CR, which restored baseline hemodynamics, resulted in an initial restoration of intestinal microvascular diameters at all arteriolar levels. However, this was followed by a progressive vasoconstriction and hypoperfusion in premucosal vessels at 120 minutes after RES (−20.48% ± 2.95% from baseline diameters). In contrast, VitaSol with CR caused enhanced premucosal dilation (+34.27% ± 4.62%) and augmented flow (+20.50% ± 10.70%) above prehemorrhage baseline. Vesicles alone had no effect, and VitaSol alone caused only a modest dilation. CR of moderate HS (40% of baseline MAP for 60 minutes, n = 10) caused 20% mortality, whereas adjunct VitaSol resuscitation had a 100% survival and less tissue water content.

Conclusions. Our data confirms that CR causes progressive intestinal hypoperfusion. Cellular resuscitation with direct intravenous energy delivery improves intestinal perfusion after CR and results in improved survival and less tissue edema.

Recognition of pathophysiologic events during traumatic shock and their relationship to local and remote tissue injuries, which culminate in multiple organ failure, has gradually evolved during the last few decades. Early studies have demonstrated the relationship between intracellular

Supported by a Veterans Affairs Merit Review grant and by a National Institutes of Health research grant #RO1 HL76163-01, funded by the National Heart, Lung, and Blood Institute and the United States Army Medical Resources and Material Command.

volume and subsequent alterations in cell membrane function during hemorrhagic shock.[1–3] These membrane alterations, including a decrease in transmembrane potential and an associated cellular swelling, are thought to be due to a decrease in adenosine triphosphate (ATP)-dependent sodium-potassium exchange and an increase in intracellular sodium, water, and calcium.[4] In addition, visceral organs experience a deterioration in mucosal blood flow in hemorrhage with conventional intravenous resuscitation, despite restoration of hemodynamics by aggressive fluid therapy.[5–7] The pathogenesis of such postresuscitation hypoperfusion is multifactorial in origin, but is closely related to an endothelial cell dysfunction largely the result of shock/resuscitation mediated cellular metabolic alterations. The sequence of tissue hypoperfusion is a decreased supply of oxygen, causing failure of ATP generation by oxidative phosphorylation, leading to a profound decrease in cellular levels of high energy phosphates.[8–10] Thus, all energy-dependent processes, including active membrane transport, are severely impaired. In addition, osmotic imbalances that result from failure to maintain ion gradients cause cellular edema. Similar abnormalities occur in membrane-bound organelles such as lysosomes and mitochondria. Alternative energy production by anaerobic glycolysis is stimulated from a low-energy charge with a resultant accumulation of lactic acid and a decrease in the pH of ischemic tissue. Few studies were designed to stimulate aerobic glycolysis and boost cellular energy by pharmacologic intervention.[10–12] However, in these studies, adjunct adenosine mono-phosphate precursors such as adenosine, adenine, ribose, hypoxanthine, and inosine proved ineffective as additives to resuscitation regimens because of many rate-limiting factors in the glycolysis pathway.[13]

Molecules such as ATP normally cannot pass through the cell membrane in quantities large enough to replenish and subsequently satisfy tissue metabolic requirements.[14–16] In a series of experiments, Chaudry[17–20] has shown that administration of ATP or $MgCl_2$ alone after ischemia was ineffective in improving cellular function, and tissue and mitochondrial magnesium levels. Furthermore, the half-life of free ATP in the blood is less than 40 seconds, thus limiting its efficacy as a bioenergetic substrate. In addition, intravascular ATP elicits a hypotensive response in a dose-dependent manner. Therefore, in the present study, we utilized a new compound of specially formulated, highly fusogenic, unilamellar lipid vesicles that contain ATP (VitaSol. This novel compound allows for delivery of high-energy phosphate directly into the cytosol of the endothelial cells of the intestinal microvasculature. We hypothesize that adjunct intravenous resuscitation with VitaSol restores splanchnic tissue perfusion after conventional resuscitation (CR) from hemorrhagic shock and thus improves survival.

METHODS

Animals were maintained in a facility approved by the American Association for the Accreditation of Laboratory Animal Care. The Institutional Animal Care and Use Committee and Biohazard Safety Committee at the Louisville Veterans Affairs Medical Center approved the research protocol. Sprague-Dawley rats (200 g) were used in the experiments. Animals were acclimated for 1 week before experimental use, during which time the animals received standard rat chow (15 g/day) and water ad libitum.

All animal and experimental interventions were performed under aseptic conditions. Anesthesia was induced and maintained with intraperitoneal pentobarbital (50 mg/kg) and supplemental sub-cutaneous injections (25% the original dose) as needed to maintain a surgical plane of anesthesia throughout the experimental protocol. Animals received a subcutaneous injection of 2 mL normal saline to maintain body fluid homeostasis during surgery and equilibration. Body temperature was maintained at 37° ± 0.5°C with a rectal probe and a servo-controlled heating pad. Surgery was carried out after loss of the blink and withdrawal reflexes.

The right femoral artery and vein were cannulated with PE-50 catheters for blood pressure measurement, blood withdrawal and resuscitation respectively.

Intestinal microvascular studies. The peritoneal cavity was exposed through a midline abdominal incision and a 2- to 3-cm segment of distal ileum was withdrawn gently from the peritoneal cavity with its neurovascular supply intact. The ileum was opened along the antimesenteric border with electrocautery. Enteric contents and mucus were gently washed from the mucosal surface. The animals were placed on a specially designed polyurethane board. The opened ileum was then suspended, serosal side up, over a viewing port with 4-0 silk sutures, while submerged in a tissue bath containing a nonvasoactive modified Krebs' solution that contained 6.92 g/L sodium chloride, 0.44 g/L potassium chloride, 0.37 g/L calcium chloride, and 2.1 g/L sodium bicarbonate at a pH of 7.4. The bathing solution was maintained at 37°C, and bubbled with nitrogen and carbon dioxide to maintain the pH throughout the experiment. Isoproterenol was added to the bathing solution in a very dilute concentration (0.01 μg/mL) to retard peristalsis. This dose of isoproterenol is below the threshold that alters vascular smooth muscle tone.[21] The animal was positioned on the stage of a trinocular microscope for direct in vivo intravital microscopy. Microvascular images were transmitted through the microscope to a photodiode array in an optical Doppler velocimetry (Microcirculation Research Institute, Texas A & M University, College Station, Tex) to measure centerline red blood cell velocity and the subsequent calculation of intestinal microvascular blood flow. The microvascular image was then transmitted to a digital camera (Hitachi Denshi, Model K-P- D50) and was stored as streamline video on a computer hard drive for later measurement of microvascular diameter with the use of video calipers. Criteria for an acceptable preparation of the intestine for intravital microscopy included a baseline MAP (MAP) > 90 mm Hg, a red blood cell velocity in a first order arteriole > 20 mm/s, and an active vasomotion in the arteriolar system.

The standard nomenclature for the intestinal microvessels as described originally by Bohlen and Gore[21] was utilized. Briefly, first-order arterioles (A1) arise from a mesenteric arcade artery, traverse the mesenteric border of the bowel wall, and penetrate through the muscle layers to the submucosal layer. In the submucosa, second-order arterioles (A2) arise from the A1 and run along the longitudinal axis of the bowel. First- and second-order venules parallel the A1 and A2 arterioles. Third-order arterioles (A3) branch at right angles from A2 arterioles and continue on to terminate in the mucosa as central villus arterioles. Along their course, A3 arterioles also give rise to smaller arterioles that supply the seromuscular layers of the bowel wall. Centerline red blood cell velocity in A1 arterioles was measured with an optical Doppler velocimetry. The maximum velocity signal, displayed digitally, was used to calculate blood flow according to the formula: $(V/1.6)$ $(R^2 \times 0.001)$, where V is the centerline flow velocity, 1.6 is a correction factor that converts centerline velocity to average cross-sectional velocity, R is the intraluminal microvascular radius in micrometers, and 0.001 is a conversion factor to express flow in nanoliters per second. This equation assumes a parabolic flow velocity and a circular conduit. Studies have identified 1.58 to 1.60 as the ideal correction factor for a wide range of microvessels.

Experimental protocol. After surgical preparation, 60 minutes was allowed for the animal to equilibrate and recover from the surgical stress. Blood pressure, heart rate, rectal and bath temperatures, and bath pH were monitored continuously (Digi-Med Signal Analyzers, Louisville, Ky) and recorded in real time every 5 minutes. Baseline microvascular measurements were taken during the last 10 minutes of the equilibration period when the variability in the measurements was less than 5%. Each time-point measurement, at 20-minute intervals, consisted of MAP, heart rate, rectal and bath temperatures, bath pH and diameters of the intestinal inflow A1 arteriole, premucosal pA3 and dA3 arterioles, and the centerline red cell velocity in the A1 arteriole. After 60 minutes of hemorrhagic shock, the animals were resuscitated with shed blood and normal saline according to the protocol. Microvascular and

hemodynamic data acquisition was continued at 20-minute intervals during shock and a subsequent 120 minutes after resuscitation.

Hemorrhage and resuscitation model. Hemorrhagic shock was achieved with blood withdrawal (1 mL/min) from the femoral artery in a syringe prerinsed with 0.02 mL heparin (1000 U/mL). Blood withdrawal continued until 50% of baseline MAP was attained. The 50% MAP was maintained for 60 minutes with further blood withdrawal or reinfusion as required. On average, the total volume of blood withdrawn was 5.13 ± 0.18 mL. Conventional resuscitation was initiated with the intravenous return of shed blood over 5 minutes. Normal saline (0.9% NaCl) equal to 2 times the volume of shed blood was then infused over the next 25 minutes. Adjunct VitaSol resuscitation was initiated after return of the shed blood. The lipid vesicles containing ATP (2.5 mmol/L, VitaSol) were dissolved in the saline solution and infused intravenously over 25 minutes.

Experimental groups. Animals were randomized according to the after groups (n = 8 animals/group):

Group I: Hemorrhagic shock (HS) plus CR (HS+CR)

Group II: HS+CR plus VitaSol

Group III: HS+CR+Vehicle. Vehicle is the phospholipids vesicle without ATP enclosed

Group IV: HS+ VitaSol

Group V: Sham-operated control that received VitaSol but no HS

Group VI: Time-matched sham-operated control that did not receive any other intervention

Survival and tissue studies. Experiments were conducted in a model of moderate hemorrhagic shock to allow for reasonable assessment of treatment outcome before any pain and suffering became evident in the research animal. Using standard aseptic procedures, we inserted PE-50 polyethylene catheters into the femoral artery and vein through a 1-cm incision in the right groin of 20 animals. The animals were allowed to equilibrate until the variability in hemodynamics was less than 5%. After equilibration, the animals were bled to 40% of MAP. Hemorrhagic shock was maintained for 60 minutes with blood withdrawal or reinfusion, as required. After hemorrhagic shock, the animals were randomized for CR (n = 10) or CR plus adjunct VitaSol (n = 10). Upon completion of resuscitation, the femoral catheters were removed, vessels were ligated, the incision closed with an interrupted suture, and the animals allowed to recover from anesthesia. Each animal's body weight was determined before the animal was housed in an individual cage. All animals were allowed free access to food and water. Each animal was weighed daily and observed for survival during the subsequent 72 hours.

An additional experiment in which 2 groups of animals (n = 7 each) underwent CR or CR + Vita-Solwas performed and the total water content in tissues determined. Briefly, 2 hours after resuscitation was complete, the animals were euthanized by anesthetic overdose. Tissue samples from the anterior abdominal muscle, ileum, kidney, liver, lung, and heart were harvested, blotted, and weighed. Tissue samples were then placed in a 60°C oven until a constant weight was noted. Subsequently the wet weight to dry weight (WW/DW) ratio was calculated.

Statistical analysis. All data are presented as mean ± SEM unless stated otherwise. Differences among groups were compared by 2-way analysis of variance (ANOVA) or 1-way ANOVA and the Bonferroni post-test. A result was considered to be significant if the probability of a type-1 error was *P* less than *.05*.

RESULTS

Microvascular studies. There was no significant difference in baseline hemodynamics and intestinal microvascular diameter between the 6 experimental groups. As expected, hemorrhagic shock caused a significant reduction in MAP from baseline in all hemorrhage experimental groups (Fig 1). CR from hemorrhagic shock restored and maintained MAP during the entire 2 hours after resuscitation in the hemorrhage groups without subsequent additional fluid infusion (Fig 1). Hemorrhagic shock caused a differential response in the intestinal microvasculature (Figure 2). There was a marked constriction from baseline (−21.12 ± 1.34, $P < 0.01$) of the inflow A1 arteriole (100 μm diameter, upper panel) in the hemorrhage groups, which was not seen in the smaller premucosal A3 vessels (8-15 μm, middle and lower panels). CR initially restored A1 diameter to pre-hemorrhagic shock baseline level (Fig 2), which was followed by a progressive vasoconstriction from baseline of all intestinal arterioles in groups I and III (−18.02 ± 0.97, $P < .01$). In contrast, VitaSol treatment as an adjunct to CR in group II or VitaSol treatment alone in group IV completely prevented the post-resuscitation intestinal microvascular vasoconstriction and further enhanced premucosal arteriolar diameter by +31.53 ± 1.12 of baseline in group II. CR from hemorrhagic shock was associated with a progressive postresuscitation intestinal hypoperfusion despite restoration and maintenance of central hemodynamics by adequate resuscitation (Fig 3, group I). These results were similar to the postresuscitation intestinal hypoperfusion seen in group III animals that received vehicle alone without VitaSol. In contrast, adjunct VitaSol treatment in groups II and IV restored and maintained postresuscitation intestinal A1 blood flow to 14.41% ± 0.92% of baseline prehemorrhage value during the entire postresuscitation period (Fig 3). These blood flow data are similar to the 23.66% ± 3.65% observed in the control animals in group VI. Group V shams had stable microvascular parameters throughout the experimental period. VitaSol-treated shams in group VI showed a significant selective vasodilation in premucosal A3 arterioles (Fig 2).

Survival studies. In the CR group, 2 animals died at 6 and 72 hours postresuscitation. Postmortem examination did not reveal an apparent cause of death in the first animal, but the second animal had a progressive loss of weight of −6%, −10%, and −13% of the prehemorrhage body weight at 24, 48, and 72 hours, respectively. Postmortem examination revealed gut ischemia and necrosis at the antimesenteric border of the lower third of the jejunum. In contrast, all 10 animals that received adjunct VitaSol resuscitation survived the 72 hours of observation without morbidity or mortality (Table I). CR animals maintained a slightly negative body weight percentage from baseline over 72 hours. This contrasts with the positive body weight percentage from baseline observed in the VitaSol treated group (Table I). Tissue water content at 2 hours after resuscitation was lower quantitatively in the skeletal muscle and solid organs of animals treated with adjunct VitaSol resuscitation (Fig 4). Although there was a clear trend of a decreased tissue edema in the VitaSol-treated group, the difference between the means did not reach statistical significance for any of the tissues investigated.

DISCUSSION

After (CR) from hemorrhagic shock, splanchnic microvessels progressively constrict leading to impairment of blood flow. This occurs despite restoration and maintenance of central hemodynamics by adequate intravenous fluid therapy[5,6]. In this study, we demonstrated that delivery of high-energy phosphates directly into the endothelial cells of the intestinal microvasculature resulted in prevention of the postresuscitation splanchnic vasoconstriction and hypoperfusion, and improved survival. However, adjunct VitaSol therapy produced a preferential vascular dilation in the smaller premucosal intestinal arterioles, with a lesser vasodilation in the larger intestinal inflow arterioles (A1). Although the A1 blood flow was enhanced and maintained at 20% of baseline prehemorrhage level during the entire 2 hours

after CR and VitaSol treatment, a major fraction of such blood flow enhancement likely is attributed to the significant vasodilation and subsequent reduction in outflow resistance noted in the downstream A3 arterioles. This observation suggests a significant enhancement in local blood flow, while whole organ blood flow might have increased only marginally. This suggestion is supported by our recent data that premucosal A3 arterioles possess greater adenosine-related vascular control mechanisms and, therefore, would be more sensitive to the end products of energy utilization.[22]

In in vitro protocols, fusion of the lipid vesicles with the cell membrane and the rate of delivery of high-energy phosphate directly into the cytosol of cells have been verified and validated from incubation of the vesicles with human umbilical endothelial cells, rat hepatocytes, and rat cardiomyocytes (unpublished data). In these experiments, microscopic examination of fluorescent distribution within the human umbilical endothelial cells suggested that carboxyfluorescein delivery was diffuse rather than punctuated, indicating that vesicles simply were not aggregating on the cell surface but rather fusing with cell membranes. The amount of ATP that accumulated in the endothelial cells was demonstrated to be dependent on the mole fraction of the fusogenic lipid of the vesicle, the concentration of ATP-lipid vesicle used, the time of vesicle exposure, and the ATP concentration in the vesicles. Therefore we assume that the mechanism for the effect of VitaSol in our work is direct ATP delivery to the energy-dependent vascular membrane and cytosol functions.

It has been known for more than 2 decades that hemorrhagic shock results in changes in resting membrane potential and membrane fluidity, which paradoxically causes cellular edema, and profoundly lowers cellular energy stores in the liver, kidney, lung and gut. Pharmacologic agents such as glutamine, a metabolic substrate and a precursor of ATP synthesis, or crocetin, an oxygen diffusion enhancer, have been shown to enhance cellular ATP levels and to prevent the severity of apoptosis seen at 24 and 48 hours after shock.[23,24] However, the effect of these agents on the splanchnic derangements after CR and the overall patho-physiology of shock are not clear. While the confounding local tissue hypoxia plays a major role in organ injury attributed to shock-induced hypoperfusion, much of this organ injury is sustained during reperfusion. In fact, degradation of ATP to its purine bases, hypoxanthine and xanthine, occurs during ischemia. With reperfusion, oxygen is added, and the xanthine oxidase reaction is activated rapidly to produce a burst of superoxide and oxygen-free radicals that can cause injury directly or through upregulation of endothelial surface adhesion molecules. These changes could account for the intestinal microvascular dysfunction noted in our model after CR. However, it is interesting to note that in group IV, which received only blood resuscitation and VitaSol treatment, no postresuscitation microvascular derangements were noted. Instead, there was restoration of the A1 diameter to prehemorrhage baseline, a preferential A3 vasodilation, and a concomitant enhancement of A1 blood flow. These data suggest that VitaSol treatment has a protective effect against reperfusion injury. In addition, adjunct VitaSol therapy appears to possess an inotropic effect since the MAP in group IV was restored and maintained at its baseline prehemorrhage level, despite a considerably lower resuscitation volume.

Theoretically, replacement of the fluid deficit with CR from hemorrhagic shock should improve cardiac filling and cardiac output, and lessen the need for increasing the peripheral resistance to sustain and maintain an effective arterial pressure. Two separate clinical studies document that, despite normalization of blood pressure, heart rate, and urine output, tissue hypoperfusion persists in 80% to 85% of patients, as evidenced by lactic acidemia and decreased mixed venous oxygen saturation.[25,26] Other clinical studies have shown that the level and rate of normalization of serum lactate (an index of tissue oxygen utilization) correlates with mortality, both in the degree of elevation and in the time-dependent rate of normalization.[27,28] Systemic base deficit (an index of tissue perfusion) also shows a similar predictive pattern of mortality.[29] However, interventions that focus on correction of this oxygen

debt by driving oxygen transport variables such as cardiac index or oxygen delivery index after CR to supernormal levels fail to reduce mortality in severely injured patients.[30,31] Similarly, in animal models of shock/resuscitation in which splanchnic vasculature and blood flow were monitored directly, progressive vasoconstriction and hypoperfusion were observed even with adequate CR that restores and maintains central hemodynamics.[5,6,32] Therefore, any therapy as an adjunct to CR from hemorrhagic shock should possess a favorable effect on tissue perfusion to reverse the overall pathophysiology of shock.[33]

The essence of the pathogenesis of circulatory collapse attributed to blood loss stems from a disparity between central and peripheral regulation mechanisms in the face of a progressive tissue hypoperfusion. Hemorrhagic shock sets off a series of physiologic compensatory adjustments aimed at preservation of vital heart and brain perfusion, such that neurohumoral reflexes mediated by catecholamines, vasopressin, and angiotensin II promote vasoconstriction in certain vascular beds to ensure that an adequate fraction of the cardiac output supplies oxygen and nutrients to vital organs.[34,35] This preferential redistribution of cardiac output occurs at the expense of other vascular beds such as the gut.[36] With continued peripheral circulatory insufficiency, tissue hypoperfusion becomes self-destructive when blood flow falls below the metabolic needs of parenchymal cells, and the vital organs that curtailed much of the cardiac output will develop signs of irreversible tissue damage. Hemorrhagic shock affects all segments of the splanchnic vasculature, including the gut. Superior mesenteric artery blood flow drops to −74% of the prehemorrhage control value when arterial pressure is reduced and maintained at 50 mm Hg for 4.5 hours.[37] Blood flow in the hindquarters followed a pattern qualitatively similar to that of the superior mesenteric artery.[37] Our current work has confirmed that vessel diameter of smaller premucosal arterioles of the gut (7-20 μm)—but not that of the inflow arterioles (100 μm in diameter)—is usually preserved during hemorrhagic hypotension. Thus, in the arterial segment, there is a selective vasoconstriction of arteries and inflow arterioles.[5] This adaptive constriction of large vessels is mediated by neurohormonal reflexes and aimed at redistribution of the cardiac output to support vital organs. A number of studies have shown that the selective hemorrhage-induced vasoconstriction can be attenuated by blocking the renin-angiotensin axis or by administering local intrarterial phenoxybenzamine.[37-39] In the venous segment, stasis and low blood flow are associated with a slight decrease in vessel diameter. The same applies to the postcapillary venules, where there is a progressive resistance to flow and an inflammatory response characterized by enhancement of leukocyte-endothelial interaction, oxidant production, and protein extravasation. In capillaries, leukocyte plugging, a stop-flow phenomenon, and disruption of the permselectivity of the capillary wall are accompanied by abnormal fluid shifts. Thus, the obvious cause of hemorrhage-induced tissue hypoperfusion is the intense constriction of the arterial segment, and unless it can be modified, adequate tissue perfusion cannot be achieved. On the other hand, this arterial constriction is an essential adaptive response of the central control of blood pressure. Unless peripheral resistance is restored, the falling cardiac output during shock will be unable to sustain an effective systemic blood pressure. Our current and previous studies have demonstrated that the hemorrhage-induced arteriolar vasoconstriction and hypoperfusion can be reversed but only for a short time by volume replacement therapy.[5-7,40] However, adequate volume replacement therapy does not correct the ongoing splanchnic hypoperfusion. Therefore, adequate resuscitation should be redefined by the ability of the resuscitation regimen to restore cell membrane function and tissue perfusion, and to minimize the reperfusion injury. A key to this endpoint-resuscitation definition is the restoration, maintenance, and enhancement of splanchnic tissue perfusion. Our previous studies have demonstrated that splanchnic tissue perfusion can be restored by pentoxifylline, heparan sulphate, complement inhibition, or glucose or glutamine suffusion.[41-44] Certainly, the mechanisms by which these adjunctive therapies restore splanchnic blood flow are diverse, and their clinical application is limited. Adjunct VitaSol therapy delivers a high-energy charge directly into the cytosol of cells. This provides energy for the cellular energy–dependent processes, such as active membrane

transport, that maintain the normal ion gradient and hence restore cell volume. The trend of quantitatively lower water content in the animals treated with VitaSol suggests the importance of energy charge in cell volume regulation.

CONCLUSION

Our data confirm that CR from hemorrhagic shock causes a progressive intestinal hypoperfusion. Cellular resuscitation with direct intravenous energy delivery augments intestinal perfusion after shock resuscitation and results in improved survival.

REFERENCES

1. Shires GT, Cunningham JN, Backer CR, et al. Alterations in cellular membrane function during hemorrhagic shock in primates. Ann Surg 1972;176:288–95. [PubMed: 4627396]
2. Shires GT, Williams J, Brown F. Simultaneous measurement of plasma volume, extracellular fluid volume, and red cell mass in man utilizing I^{131}, $S^{25}O_4$, and Cr^{51}. J Lab Clin Med 1960;55:776–83. [PubMed: 14446054]
3. Sayeed MM, Adler RJ, Chaudry IH, Baue AE. Effect of hemorrhagic shock on hepatic transmembrane potentials and intracellular electrolytes, in vivo. Am J Physiol 1981;240:R211–9. [PubMed: 7212093]
4. Trunkey DD, Illner H, Wagner IY, Shires GT. The effect of hemorrhagic shock on intracellular muscle action potentials in the primate. Surgery 1973;74:241–50. [PubMed: 4197935]
5. Fruchterman TM, Spain DA, Wilson MA, Harris PD, Garrison RN. Selective microvascular endothelial cell dysfunction in the small intestine after resuscitated hemorrhagic shock. Shock 1998;10:417–22. [PubMed: 9872681]
6. Zakaria ER, Spain DA, Harris PD, Garrison RN. Resuscitation regimens for hemorrhagic shock must contain blood. Shock 2002;18:567–73. [PubMed: 12462567]
7. Zakaria ER, Garrison RN, Spain DA, Matheson PJ, Harris PD, Richardson JD. Intraperitoneal resuscitation improves intestinal blood flow after hemorrhagic shock. Ann Surg 2003;237:704–13. [PubMed: 12724637]
8. Chaudry IH, Baue A. Alterations in adenosine 3′,5′–mono-phosphate levels in hemorrhagic shock. Surg Forum 1976;27:51–3. [PubMed: 190724]
9. Chaudry IH, Sayeed MM, Baue AE. Alterations in high-energy phosphates in hemorrhagic shock as related to tissue and organ function. Surgery 1976;79:666–8. [PubMed: 1273752]
10. Van WC III, Dhar A, Morrison DC, Longorio MA, Maxfield DM. Cellular energetics in hemorrhagic shock: restoring adenosine triphosphate to the cells. J Trauma 2003;54:S169–76. [PubMed: 12768121]
11. Fink MP. Ringer's ethyl pyruvate solution: a novel resuscitation fluid for the treatment of hemorrhagic shock and sepsis. J Trauma 2003;54:S141–3. [PubMed: 12768116]
12. Mongan PD, Capacchione J, West S, et al. Pyruvate improves redox status and decreases indicators of hepatic apoptosis during hemorrhagic shock in swine. Am J Physiol Heart Circ Physiol 2002;283:H1634–44. [PubMed: 12234818]
13. Pasque MK, Wechsler AS. Metabolic intervention to affect myocardial recovery after ischemia. Ann Surg 1984;200:1–12. [PubMed: 6428332]
14. Ehringer WD, Niu W, Chiang B, Wang OL, Gordon L, Chien S. Membrane permeability of fructose-1,6-diphosphate in lipid vesicles and endothelial cells. Mol Cell Biochem 2000;210:35–45. [PubMed: 10976756]
15. Ehringer WD, Chiang B, Chien S. The uptake and metabolism of fructose-1,6-diphosphate in rat cardiomyocytes. Mol Cell Biochem 2001;221:33–40. [PubMed: 11506184]
16. Ehringer WD, Su S, Chiangb B, Stillwell W, Chien S. Destabilizing effects of fructose-1,6-bisphosphate on membrane bilayers. Lipids 2002;37:885–92. [PubMed: 12458624]
17. Chaudry IH. ATP-MgCl2 and liver blood flow after shock and ischemia. Prog Clin Biol Res 1989;299:19–31. [PubMed: 2657790]
18. Chaudry IH, Stephan RN, Dean RE, Clemens MG, Baue AE. Use of magnesium-ATP after liver ischemia. Magnesium 1988;7:68–77. [PubMed: 3294518]

19. Chaudry IH, Clemens MG, Baue AE. The role of ATP-magnesium in ischemia and shock. Magnesium 1986;5:211–20. [PubMed: 3523059]
20. Chaudry IH. The effect of ATP on survival in intestinal ischemia shock, hemorrhagic shock, and endotoxin shock in rats. Circ Shock 1982;9:629–31. [PubMed: 6983934]
21. Bohlen HG, Gore RW. Preparation of rat intestinal muscle and mucosa for quantitative microcirculatory studies. Microvasc Res 1976;11:103–10. [PubMed: 1263858]
22. Li N, Harris PD, Zakaria ER, Matheson PJ, Garrison RN. Microvascular responses to adenosine help explain functional and pathologic differences between intestinal segments. Am J Surg 2004;188:526–31. [PubMed: 15546564]
23. Kamiike W, Watanabe F, Hashimoto T, et al. Changes in cellular levels of ATP and its catabolites in ischemic rat liver. J Biochem (Tokyo) 1982;91:1349–56. [PubMed: 7096291]
24. Van Way CW, Dhar A, Reddy R, Evans L, Wogahn B, Helling TS. Changes in adenine nucleotides during hemorrhagic shock and reperfusion. J Surg Res 1996;66:159–66. [PubMed: 9024829]
25. Scalea TM, Maltz S, Yelon J, Trooskin SZ, Duncan AO, Sclafani SJ. Resuscitation of multiple trauma and head injury: role of crystalloid fluids and inotropes. Crit Care Med 1994;22:1610–5. [PubMed: 7924373]
26. Abou-Khalil B, Scalea TM, Trooskin SZ, Henry SM, Hitchcock R. Hemodynamic responses to shock in young trauma patients: need for invasive monitoring. Crit Care Med 1994;22:633–9. [PubMed: 8143473]
27. Abramson D, Scalea TM, Hitchcock R, Trooskin SZ, Henry SM, Greenspan J. Lactate clearance and survival after injury. J Trauma 1993;35:584–8. [PubMed: 8411283]
28. Broder G, Weil MH. Excess lactate: An index of reversibility of shock in human patients. Science 1964;143:1457–9. [PubMed: 14107454]
29. Rutherford EJ, Morris JA Jr, Reed GW, Hall KS. Base deficit stratifies mortality and determines therapy. J Trauma 1992;33:417–23. [PubMed: 1404512]
30. Dunham CM, Siegel JH, Weireter L, et al. Oxygen debt and metabolic acidemia as quantitative predictors of mortality and the severity of the ischemic insult in hemorrhagic shock. Crit Care Med 1991;19:231–43. [PubMed: 1989759]
31. Heyland DK, Cook DJ, King D, Kernerman P, Brun-Buisson C. Maximizing oxygen delivery in critically ill patients: a methodologic appraisal of the evidence. Crit Care Med 1996;24:517–24. [PubMed: 8625644]
32. Scalia SV, Taheri PA, Force S, et al. Mesenteric microcirculatory changes in nonlethal hemorrhagic shock: the role of resuscitation with balanced electrolyte or hypertonic saline/dextran. J Trauma 1992;33:321–5. [PubMed: 1380565]
33. Garrison RN, Conn AA, Harris PD, Zakaria ER. Direct peritoneal resuscitation as adjunct to conventional resuscitation from hemorrhagic shock: a better outcome. Surgery 2004;136:900–8. [PubMed: 15467677]
34. Neutze JM, Wyler F, Rudolph AM. Changes in distribution of cardiac output after hemorrhage in rabbits. Am J Physiol 1968;215:857–64. [PubMed: 4877655]
35. Kovach AG, Rosell S, Sandor P, Koltay E, Kovach E, Tomka N. Blood flow, oxygen consumption, and free fatty acid release in subcutaneous adipose tissue during hemorrhagic shock in control and phenoxybenzamine-treated dogs. Circ Res 1970;26:733–41. [PubMed: 4393107]
36. Reilly PM, Wilkins KB, Fuh KC, Haglund U, Bulkley GB. The mesenteric hemodynamic response to circulatory shock: an overview. Shock 2001;15:329–43. [PubMed: 11336191]
37. Hollenberg NK, Nickerson M. Changes in pre- and post-capillary resistance in pathogenesis of hemorrhagic shock. Am J Physiol 1970;219:1483–9. [PubMed: 4394382]
38. Reilly PM, Bulkley GB. Vasoactive mediators and splanchnic perfusion. Crit Care Med 1993;21:55–68.
39. Toung T, Reilly PM, Fuh KC, Ferris R, Bulkley GB. Mesenteric vasoconstriction in response to hemorrhagic shock. Shock 2000;13:267–73. [PubMed: 10774614]
40. Zakaria ER, Garrison RN, Spain DA, Harris PD. Impairment of endothelium-dependent dilation response after resuscitation from hemorrhagic shock involved postreceptor mechanisms. Shock 2004;21:175–81. [PubMed: 14752293]

41. Flynn WJ, Cryer HG, Garrison RN. Pentoxifylline restores intestinal microvascular blood flow during resuscitated hemorrhagic shock. Surgery 1991;110:350–6. [PubMed: 1858043]

42. Flynn WJ Jr, Gosche JR, Garrison RN. Intestinal blood flow is restored with glutamine or glucose suffusion after hemorrhage. J Surg Res 1992;52:499–504. [PubMed: 1619919]

43. Fruchterman TM, Spain DA, Wilson MA, Harris PD, Garrison RN. Complement inhibition prevents gut ischemia and endothelial cell dysfunction after hemorrhage/resuscitation. Surgery 1998;124:782–91. [PubMed: 9781002]

44. Watkins JM, Spain DA, Krysztopik RJ, Downard PJ, Wilson MA, Garrison RN. Heparan preserves intestinal perfusion after hemorrhage and resuscitation. J Surg Res 1996;66:154–8. [PubMed: 9024828]

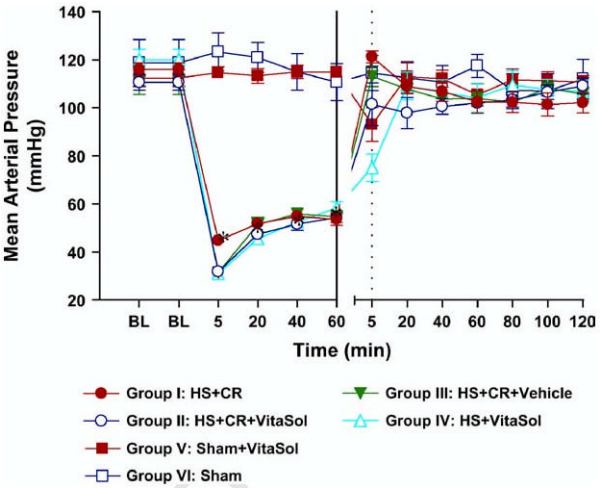

Fig 1.

MAP data. *$P < .01$ versus corresponding baseline by repeated measures 2-way ANOVA and Bonferroni post-test. *BL*, Baseline; *HS + CR*, hemorrhagic shock (HS) and conventional resuscitation (CR); *HS + CR + VitaSol*, HS + CR and adjunct VitaSol resuscitation; *HS + CR + Vehicle*, HS + CR and adjunct lipid vesicles without ATP; *HS + VitaSol*, HS and adjunct VitaSol resuscitation; *Sham + VitaSol*, sham operation but no hemorrhagic shock and VitaSol infusion; *Sham*, time-matched control without further intervention.

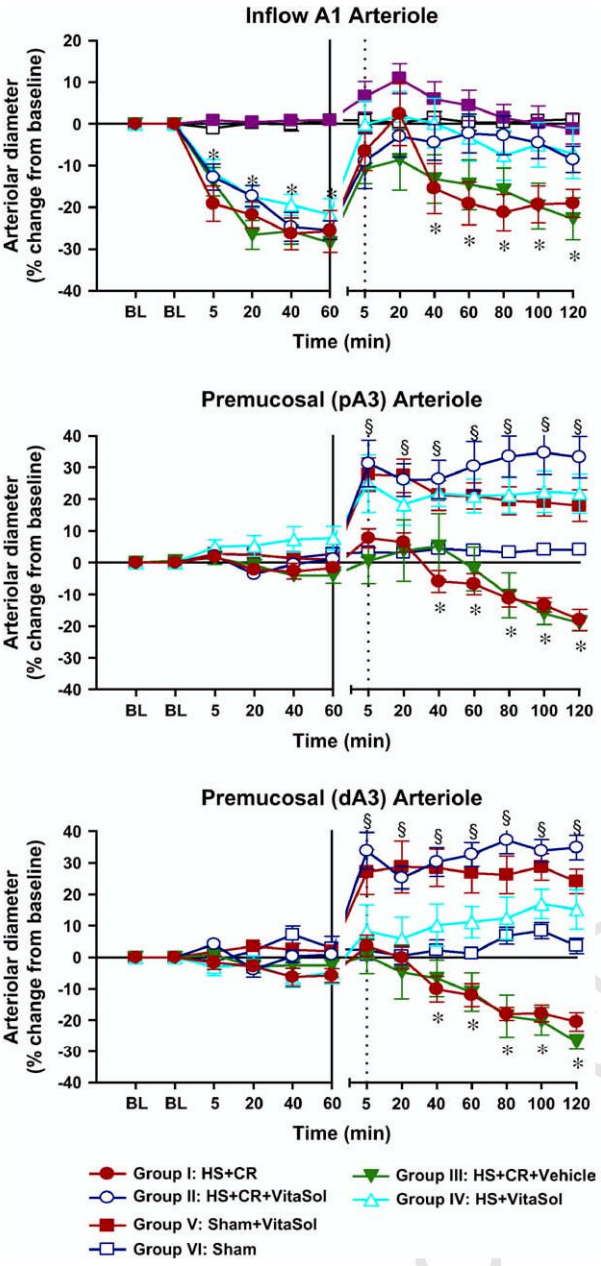

Fig 2.

Intestinal microvascular diameter. *$P < .01$ versus corresponding baseline by repeated measures 2-way ANOVA and Bonferroni post-test. §$P < .01$ versus CR or corresponding baseline by repeated measures 2-way ANOVA and Bonferroni post-test. *A1*, inflow intestinal arteriole; *pA3*, proximal premucosal A3 arteriole; *dA3*, distal premucosal A3 arteriole. (See Fig 1 legend for explanation of other abbreviations.)

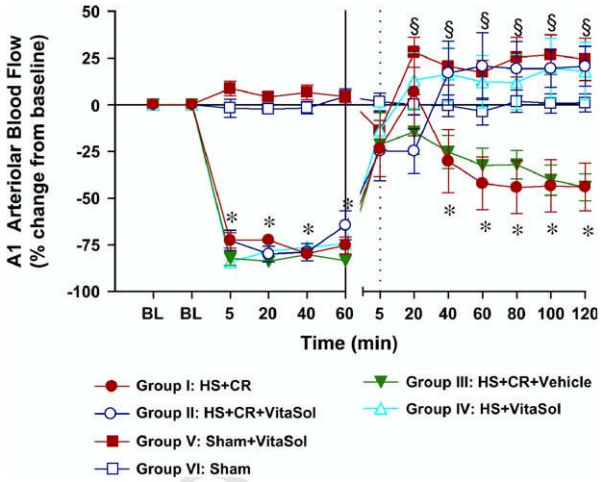

Fig 3.

Intestinal A1 blood flow. *$P < .01$ versus corresponding baseline by repeated measures 2-way ANOVA and Bonferroni post-test. §$P < .01$ versus CR or corresponding baseline by repeated measures two-way ANOVA and Bonferroni post-test. (See Fig 1 legend for explanation of abbreviations.)

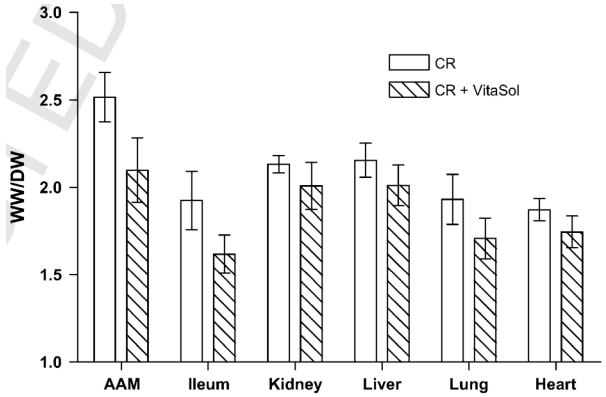

Fig 4.

Total tissue water content. *WW/DW*, tissue wet weight to dry weight ratio; *AAM*, anterior abdominal muscle (skeletal); *CR*, conventional resuscitation; *CR + VitaSol*, CR + adjunct VitaSol treatment.

Table 1

Parameters of treatment outcome

Group	BW_0 (g)	24-h BW (% From BW_0)	48-h BW (% From BW_0)	72-h BW (% From BW_0)	Mortality rate
CR	202 ± 1	0.61 ± 1.19	−1.70 ± 1.16	−1.43 ± 1.41	2/10
VitaSol	200 ± 1	1.36 ± 0.47	1.51 ± 0.60	1.05 ± 0.57	0/10

BW_0, Prehemorrhage body weight; *24-h BW*, *48-h BW*, *72-h BW*, body weight at 24, 48, and 72 hours after completion of the experiment;

CR, hemorrhagic shock and CR; *VitaSol*, hemorrhagic shock plus CR and adjunct VitaSol resuscitation.