



The effect of hypertonic saline resuscitation on responses to severe hemorrhagic shock by the skeletal muscle, intestinal, and renal microcirculation systems: seeing is believing

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Abstract

Background: Decompensated hemorrhagic shock is often refractory to resuscitation, and we show here that it is associated with loss of vascular tone in skeletal muscle precapillary arterioles. We tested the hypothesis that microvascular derangements in the skeletal muscle, intestinal, and renal microcirculation systems would be reversed by initial hypertonic saline–dextran infusion.

Methods: Male Sprague-Dawley rats underwent precollicular brain stem transection without anesthesia for study. Parameters measured by in vivo videomicroscopy included cardiac output, mean arterial pressure, and microvascular responses in the skeletal muscle, ileum, and renal (i.e., the hydronephrotic kidney) microcirculation systems. Hemorrhaged was induced to a mean arterial pressure of 50 mm Hg until decompensation occurred. The rats were then initially resuscitated with (1) 4 mL/kg 7.5% NaCl in 6% dextran 70, (2) 33 mL/kg .9% NaCl in 6% dextran 70, or (3) 33 mL/kg .9% NaCl. Twenty minutes later they received shed blood plus 33 mL/kg .9% NaCl to maintain mean arterial pressure at baseline levels.

Results: Decompensated hemorrhagic shock decreased cardiac output to between 24% and 35% of baseline values and profoundly decreased microvascular blood flow to between 10% and 19% of baseline. At the completion of resuscitation cardiac output increased to greater than baseline in all groups. Microvascular blood flow increased toward baseline transiently but then progressively deteriorated to between 36% and 69% of baseline in the 3 tissues. There was no significant difference between the three resuscitative fluids.

Conclusions: Despite return of cardiac output to greater than baseline levels, muscle, intestinal, and renal microvascular blood flows remained significantly depressed. Hypertonic saline and/or dextran did not improve these deficits. © 2005 Excerpta Medica Inc. All rights reserved.

Keywords: Cardiac output; Decompensated hemorrhagic shock; Dextran; Hypertonic saline; Intestinal microcirculation; In vivo videomicroscopy; Renal microcirculation; Skeletal muscle microcirculation

It is a great honor to present at a meeting celebrating Dr. Polk's retirement the studies in the late 1980s that began our understanding of the microcirculatory responses to hemorrhagic shock. His influence and the opportunities he provided us are directly responsible for the ideas and studies I will present here.

When I asked to take 6 months off in the third year of my surgical residency in 1983, Dr. Polk encouraged me to work with Lewis Flint, who was taking a sabbatical with Pat Harris in the departments of Physiology and Biophysics at the University of Louisville. During that time we performed experi-

ments showing that the decompensatory phase of hemorrhagic shock was associated with dilation and loss of tone of the terminal precapillary arterioles in the skeletal muscle microcirculation system and that this loss of tone was not reversed with conventional resuscitation using blood and isotonic crystalloid resuscitation [1]. After I finished my residency in 1985, Dr. Polk made it possible for me to work with Pat Harris and Neal Garrison for 2 years, during which time I attained my doctorate and developed a state-of-the-art laboratory at the Louisville Veterans Administration Hospital (VAH) for studying microcirculation. After that Dr. Polk invited me to join the faculty at the University of Louisville. During that time I collaborated with a number of faculty and residents in our microvascular laboratory at the VAH. The studies that I present here were performed from 1987 through 1990 in col-

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laboration with John Gosche, Jeff Harbrecht, Greg Anigian, Bill Flynn, Neal Garrison, Pat Harris, Jean Jenkins, and Rebecca Galloway. They represent our initial attempts to restore microcirculation blood flow to the skeletal muscle, intestinal, and renal circulation systems after decompensated hemorrhagic shock with hypertonic saline. These studies led to the eventual successful restoration of microvascular blood flow with pentoxifylline [2]. They take on added relevance today with the resurging interest in hypertonic saline resuscitation for its anti-inflammatory properties and its potential in battlefield situations [3].

Prolonged hemorrhage and massive blood transfusion lead to decompensated hemorrhagic shock when normal compensatory peripheral vasoconstrictor responses fail and peripheral vasodilation markedly decreases systemic vascular resistance. This peripheral vasodilation is typically unresponsive to vasopressor agents and massive quantities of isotonic crystalloid solution and usually results in death even if bleeding has been controlled. In 1980, Velasco et al [4] demonstrated that small volumes of very hypertonic saline solutions were as effective as large volumes of isotonic crystalloid solutions for intravascular volume expansion after hemorrhagic shock. De Felippe et al [5] reported that small volumes of hypertonic saline could reverse clinically decompensated hemorrhagic shock in patients and prevent mortality. In 1986, Kramer et al [6] demonstrated that addition of dextran 70 plus hypertonic saline was more effective than hypertonic saline alone in resuscitation from compensated hemorrhagic shock, but no data existed regarding decompensated hemorrhagic shock. Rocha-e-Silva et al [7] reported that at the macroscopic level, hypertonic saline causes a differential interorgan vascular response after hemorrhage, thus allowing vasoconstriction to occur in skeletal muscle, although vasodilation occurs in the splanchnic and renal circulation. Finally, around that time we [8] found that restoration of cardiac output (CO) to greater-than-normal levels conferred a significant survival advantage in patients resuscitated from hemorrhagic shock. These observations led us to the working hypothesis that decompensated hemorrhagic shock leads to differential responses in different microvascular beds and that resuscitation with hypertonic saline may take advantage of this to allow successful restoration of microvascular flow and resuscitation. We developed a rat model that allowed the measurement of systemic CO and microvascular flow measurements in skeletal muscle, small intestine, kidney, and liver that was ideally suited to test this hypothesis.

Materials and Methods

General animal preparation

Sprague-Dawley male rats weighing between 160 and 170 g were used for these studies. Rats were acclimated for 1 to 2 weeks in an Association for Assessment of Laboratory Animal Care–approved animal care center where they were fed standard Purina Rat Chow and water ad libitum

until 12 hours before each experiment when food but not water was withdrawn. All animals were handled according to Association for Assessment of Laboratory Animal Care guidelines for humane care and treatment. To avoid the effects of anesthesia on the microcirculatory response to hemorrhage, rats underwent precollicular brain stem transection according to the technique described in a previous publication [1]. This procedure renders animals cortically unconscious, thus allowing study without the potential confounding effects of drug anesthesia on microcirculation systems. The animals were then allowed 5 to 6 hours to completely recover from drug anesthesia before beginning the experiment. Rats then underwent tracheostomy for airway control. The left femoral artery was cannulated for blood pressure and heart rate measurements as well as withdrawal of blood for the hemorrhage protocol. The left femoral vein was cannulated to allow injection of resuscitative solutions. For measurement of CO by transpulmonary thermodilution, the right common carotid artery was cannulated with a thermistor, the tip of which was positioned in the aortic root just above the aortic valve. The right jugular vein was cannulated with PE-50 tubing, which was positioned in the superior vena cava just proximal to the right atrium. For generation of CO curves, a 40- μ L saline bolus was injected by way of this jugular cannula, and changes in arterial blood temperature were recorded as temperature time curves on a polygraph.

Skeletal muscle preparation

For skeletal muscle experiments, the rat cremaster muscle was prepared for in vivo videomicroscopy by a technique described in detail a previously publication [1]. Briefly, the cremaster muscle was dissected with its vascular and neural supply intact and spread over an optical port in a tissue bath filled with a modified Krebs solution. The animal and bath were then positioned on the stage of a trinocular microscope for transillumination. Nitrogen and carbon dioxide were continuously bubbled through the bath to maintain bath PO₂ at 15 to 25 mm Hg and bath carbon dioxide at 35 to 45 mm Hg. Bath temperature was maintained at normal body temperature by a heating coil in the bath. Rectal temperature was maintained in the normal range with a back heating pad.

The microvascular anatomy of the cremaster muscle was directly observed with a microscope that had 20 \times water immersion objectives and 10 \times eye pieces. Microvascular images were transmitted by way of a video camera to a television monitor and videocassette recorder using a lens system that produced a permanent videotape record of the vascular anatomy at a magnification of 1500 \times . Vascular anatomy of the cremaster muscle was defined by vessel branch order.

To calculate microvascular blood flow, red blood cell velocity was measured in first-order arterioles (A1) using an optical Doppler velocimeter. Blood flow through the A1

arteriole was calculated from the simultaneous measurements of red blood cell velocity and A1 diameter at each time interval during the experiment as described in a previous publication [1]. In each experiment, an A1 through A4 sequence of arterioles were selected so that the studied arteriolar branches arose from the A1 arteriole in which velocity was measured.

Small-intestine preparation

For small-intestine experiments, the small intestine was prepared for *in vivo* videomicroscopic observation as previously described [9]. Briefly, the abdomen was opened, and a short segment of small intestine was exteriorized and opened along its antimesenteric border using electrocautery. The opened intestinal loop was then suspended, with neurovascular connections intact and the serosal surface up, over an optical window in a specially designed tissue bath. The intestine was suffused with a modified Krebs solution as described previously and covered with washed cellophane. Intestinal microvascular anatomy was defined according to the vessel branch order similar to skeletal muscle.

Renal microvascular preparation

For experiments looking at the unilateral renal microvasculature, chronic hydronephrosis was induced in 100-g male Sprague-Dawley rats by ligation of the left ureter under sterile conditions as previously described [10]. Hydronephrosis was allowed to progress for 6 weeks when rats weighed between 350 and 450 g. At the time of acute experiments, the hydronephrotic kidney was exposed through a left-flank incision and dissected with its neurovascular pedicle intact. The kidney was bisected along the greater curvature in an avascular plane, and the optimal half was suspended over the optical port of a specially designed stage with the outside of the kidney facing upward. The kidney was suffused with warmed Krebs solution and sealed in cellophane wrap. The microcirculation of the kidney was positioned on the stage of a trinocular microscope for direct *in vivo* video microscopy as described earlier. The microvascular anatomy of the kidney flows from renal artery branches into interlobar arteries, which terminate in arcuate arteries and give rise to the afferent arterioles, which in turn enter individual glomeruli. Efferent arterioles exit each glomerulus.

Experimental protocol

After the baseline (BL) readings were obtained, the animals were heparinized (50 U), and hemorrhaged was induced by rapid withdrawal of blood for 3 to 5 minutes to a mean arterial pressure (MAP) of 50 mm Hg. Additional blood was withdrawn as required to maintain blood pressure at 50 mm Hg throughout the hypotensive period until decompensation occurred. Decompensation was defined as the

point at which blood pressure could no longer be maintained without reinfusion of shed blood, and it was frequently associated with decreased CO and changed respiratory pattern. Measured parameters were recorded at the time of decompensation, and the animals were then initially resuscitated with either (1) 4 mL/kg 7.5% NaCl, (2) 4 mL/kg 7.5% NaCl in 6% dextran 70 (HTSD), or equivalent solute loads as either (3) 33.3 mL/kg .9% NaCl in 6% dextran 70 (NSD) or (4) 33.3 mL/kg .9% NaCl (NS). Initial experiments with skeletal muscle were performed without additional blood or saline resuscitation, and observations were continued at 10-minute intervals until the animals died. In subsequent experiments, rats were further resuscitated by return of shed blood and additional crystalloid as .9% NaCl as required to maintain MAP at or near BL values. Observations were then continued for an additional hour or until death occurred. In the experiment with rats receiving 4 mL/kg 7.5% NaCl without dextran, the first 3 animals died <10 minutes before measurements could be made, and we aborted this protocol.

Statistical analysis

For statistical analysis, all data were expressed as percent change from BL values. Group means, SDs, and SEs were calculated for BL values and percent changes. Time points for analysis included BL values, decompensation, 10 minutes, and 20 minutes after initial resuscitation as well as after infusion of blood and NS. In addition, maximal compensation was defined as the point during the hypotensive period when MAP was maximally maintained despite decreasing cardiac output levels (generally this was closely associated with that point at which there was a decrease in requirement for further blood withdrawal to maintain MAP at 50 mm Hg).

Changes between and within treatment groups were analyzed initially using mixed-measures analysis of variance. Within-group changes from BL values were subsequently analyzed by analysis of variance for repeated measures with Dunnett's test. Differences in BL values, responses to resuscitation, and percentage changes at defined time points between groups were analyzed by one-way analysis of variance with Tukey Honestly Significant Difference follow-up test as indicated. Statistical correlations between variables were calculated by way of multiple regression analysis. Differences were accepted as significant $P = .05$. Unless otherwise noted, values are expressed as group means plus/minus SEM.

Skeletal muscle

Baseline data for general, hemodynamic, and microvascular variables are listed in Table 1. No important differences were noted between BL values in groups receiving NS versus HTSD. Hemorrhage caused similar systemic and

Table 1
Baseline values (mean \pm SEM): skeletal muscle

Variable	HTSD (n = 7)	NS (n = 5)
MAP (mm Hg)	112 \pm 6	111 \pm 4
CO (mL/min)	110 \pm 11	99 \pm 7
Flow (nL/s)	134 \pm 7	114 \pm 5
A1 diameter (μ m)	133 \pm 8	122 \pm 12
A2 diameter (μ m)	68 \pm 9	76 \pm 8
A3 diameter (μ m)	22 \pm 3	20 \pm 4
A4 diameter (μ m)	8 \pm 2	6 \pm 1

A1 = first-order arterioles; A2 = second-order arterioles; A3 = third-order arterioles; A4 = fourth-order arterioles; HTSD = 7.5% NaCl in 6% dextran 70; CO = cardiac output; MAP = mean arterial pressure; NS = 33.3 mL/kg .9% NaCl.

microvascular responses in both groups. Hemorrhage decreased MAP to 54% \pm 1% in HTSD and 52% \pm 1% in NS rats during the compensatory phase of hemorrhagic hypotension and 44% \pm 4% of BL in HTSD and 42% \pm 3% of BL in NS rats during the decompensatory phase of hemorrhagic shock. Hemorrhage decreased CO to 56% \pm 6% of BL in HTSD and 60% \pm 7% of BL in NS rats during the compensatory phase and decreased CO significantly further during the decompensatory phase to 37% \pm 6% BL in HTSD and 27% \pm 3% of BL in NS rats.

Resuscitation with 7.5% NaCl in 6% dextran 70 in HTSD and .9% NaCl in NS rats initially (10 minutes) increased CO and MAP to a similar degree. However, this effect was short lived in the NS group, and all animals died within 25 minutes of initiating resuscitation. In contrast, resuscitation with hypertonic saline–dextran HTSD allowed all animals to survive 40 minutes, 3 animals to survive for 50 minutes, and 2 animals to survive >1 hour.

Microvascular responses to hemorrhage were also similar in the 2 groups. Hemorrhage decreased skeletal muscle microvascular blood flow during the compensatory phase to 30% \pm 7% of BL values in HTSD and 33% \pm 6% in NS rats. During the decompensatory phase of hemorrhage, microvascular blood flow decreased significantly further to 17% \pm 4% of BL in HTSD and 15% \pm 4% of BL in NS rats. Normal saline resuscitation did not increase skeletal muscle microvascular blood flow in NS rats. However, hypertonic saline–dextran resuscitation significantly increased skeletal muscle microvascular blood flow in HTSD rats but not to BL levels, and the effect was transient.

Hemorrhage constricted large A1 arterioles to 72% \pm 5% of BL in HTSD and 67% \pm 5% of BL in NS rats during the compensatory phase and constricted these vessels further to 66% \pm 4% of BL in HTSD and 62% \pm 5% of BL in NS rats during the decompensatory phase. In contrast, hemorrhage dilated third-order (A3) arterioles during the compensatory phase to 129% \pm 16% of BL in HTSD and 134% \pm 11% of BL in NS rats and dilated A3 arterioles further during the decompensatory phase to 139% \pm 10% of BL in HTSD and 147 \pm 14% of BL in NS rats. In a similar fashion, hemorrhage dilated fourth-order (A4) arterioles to

200% \pm 19% of BL in HTSD and 173% \pm 29% of BL in NS rats during the compensatory phase and dilated those A4 arterioles further during the decompensatory phase to 223% \pm 32% of BL in HTSD and 212% \pm 17% of BL in NS rats. All A3 and A4 vessels studied exhibited a normal rhythmic, spontaneous vasomotion (constriction alternating with dilation at a rate of approximately 12/m) during BL. Vasomotion persisted in A3 and A4 vessels during the compensatory phase of hemorrhage, but it ceased during the decompensatory phase such that A3 and A4 vessels were essentially maximally dilated because of a complete loss of vascular smooth muscle tone.

Resuscitation with both normal saline and hypertonic saline–dextran initially dilated A1 arterioles toward BL values (77% \pm 4% in HTSD and 75% \pm 7% in NS rats) for 10 minutes, but these A1 vessels then constricted back to decompensated shock levels (62% \pm 3%) within 20 minutes in both groups. A3 arterioles, which had dilated maximally and lost vasomotion during the decompensatory phase of hemorrhage, responded to normal saline resuscitation with a short-lived return of vasomotion and constriction back to 120% \pm 23% of BL for 10 minutes, but they quickly dilated back to 142% \pm 12% of BL and lost vasomotion again by 20 minutes. Hypertonic saline–dextran resuscitation restored vasomotion and constricted A3 arterioles to 86% \pm 5% of BL for 40 to 60 minutes. Similarly, A4 arterioles, which had maximally dilated and lost vasomotion during decompensated hemorrhagic shock, regained vasomotion and constricted toward BL (127% \pm 13% in HTSD and 154% \pm 20% in NS) 10 minutes after resuscitation. However, in the normal saline–resuscitated group, vasomotion was lost, and A4 vessels dilated maximally again (219% \pm 14% of BL) by 20 minutes, whereas A4 vasomotion and constriction (96% \pm 10% of BL) was restored to near BL levels for 40 to 60 minutes with hypertonic saline–dextran resuscitation. All HTS rats (n = 3) died 3 to 8 minutes after 7.5% NaCl infusion before data could be obtained. These data demonstrated that although hypertonic saline–dextran was better than NS, neither fluid was sufficient to return CO to normal. However, the fact that HTSD restored tone to A3 and A4 arterioles was encouraging. Therefore, in subsequent experiments we included blood and NS in the resuscitation protocol.

Small intestine

Baseline values for CO, intestinal microvascular blood flow, and vascular diameters were similar in the 3 groups (Table 2). During hemorrhage, vasoconstriction of A1 vessels occurred to 71% \pm 3%, 83% \pm 4%, and 76% \pm 8% in the NS, NSD, and HTSD rats, respectively, at decompensation. A2 arterioles constricted to 69% \pm 5%, 85% \pm 3%, and 90% \pm 9% in the NS, NSD, and HTSD rats, respectively, at decompensation. A3 responses were variable, without a significant diameter change, in all 3 groups.

Table 2
Baseline values: intestine

Variable	NS (n = 5)	NSD (n = 5)	HTSD (n = 5)
MAP (mm Hg)	104 ± 6	98 ± 7	101 ± 2
CO (mL/min)	65 ± 4	69 ± 6	75 ± 7
A1 flow (nL/s)	10 ± 2	11 ± 2	15 ± 5
A1 diameter (μm)	96 ± 5	90 ± 3	106 ± 11
A2 diameter (μm)	74 ± 4	73 ± 4	70 ± 7
A3 diameter (μm)	27 ± 3	26 ± 2	30 ± 4

A1 = first-order arterioles; A2 = second-order arterioles; A3 = third-order arterioles; A4 = fourth-order arterioles; HTSD = 7.5% NaCl in 6% dextran 70; CO = cardiac output; MAP = mean arterial pressure; NS = 33.3 mL/kg .9% NaCl; NSD = 33.3 mL/kg .9% NaCl in 6% dextran 70.

Cardiac output decreased to 30% ± 2%, 24% ± 3%, and 30% ± 1% and intestinal microvascular blood flow to 10% ± 2%, 11% ± 2%, and 19% ± 2% in NS, NSD, and HTSD rats, respectively, at decompensation (Figs. 1 and 2). At the time of decompensation, the intestinal microcirculation could be characterized as intense generalized vasoconstriction with virtual cessation of flow. There was evidence of capillary occlusion in some capillaries and margination of polymorphonuclear (PMN) cells in both small arterioles and venules.

Resuscitation resulted in an initial increase in CO, MAP, and intestinal microvascular blood flow along with dilation of arterioles and venules back toward BL with all three fluids. The initial increase in CO was to 105% ± 7% NSD, 92% ± 6% , and 77% ± 5% (Fig. 1). Intestinal microvascular blood flow initially increased to 48% ± 14%, 42% ± 3%, and 51% ± 11% in the NS, NSD, and HTSD groups, respectively (Fig. 2). Subsequent addition of blood and NS to the resuscitation resulted in an increase in CO to greater than BL in all 3 groups (NS 140% ± 10%, NSD 138% ± 12%, and HTSD 124% ± 5% of BL) (Fig. 1). Intestinal microvascular blood flow increased toward BL after return of shed blood in the NS

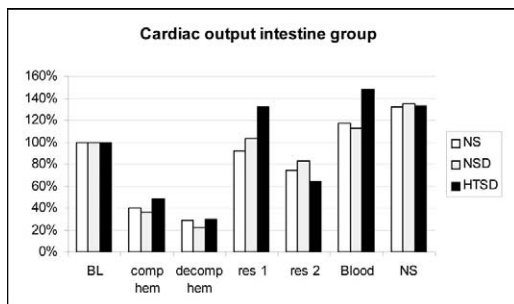


Fig. 1. Intestinal experiment cardiac output responses to compensated and decompensated hemorrhagic shock followed by resuscitation with NS, NSD, or HTSD. Data are expressed as percent of BL values. There were no significant differences between groups. BL = baseline; comp = compensated; decomp = decompensated; hem = hemorrhagic; HTSD = 7.5% NaCl/6% dextran 70; NS = .09% NaCl; NSD = 0.9% NaCl/6% dextran 70; res 1 = 10 minutes; res 2 = 20 minutes.

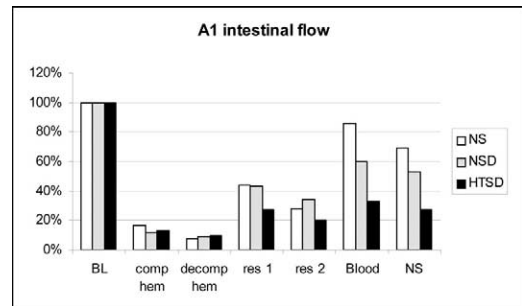


Fig. 2. A1 intestinal blood flow responses to compensated and decompensated hemorrhagic shock followed by resuscitation with NS, NSD, or HTSD. Data are expressed as percent of BL values. There were no significant differences between groups. BL = baseline; comp = compensated; decomp = decompensated; hem = hemorrhagic; HTSD = 7.5% NaCl/6% dextran 70; NS = .09% NaCl; NSD = 0.9% NaCl/6% dextran 70; res 1 = 10 minutes; res 2 = 20 minutes.

(93% ± 33% of BL) and the NSD groups, but it decreased to 61% ± 9% and 46% ± 7% of BL in the NSD and HTSD groups, respectively, although CO was greater than BL (Fig. 2). It is of note that in the NS group, microvascular flow returned to above normal in 2 but remained low in 3 animals. This variability precluded a statistical significance between groups.

Renal microcirculation

Baseline values are listed in Table 3. MAP showed a progressive decrease that did not vary significantly among groups during hemorrhage at maximum compensation and at decompensation. MAP returned to BL after normal saline resuscitation (109% ± 6%) but not after hypertonic saline dextran (71% ± 14%) or normal saline dextran (81% ± 9%), and this difference among groups was statistically significant. After return of shed blood and NS, MAP returned to nearly BL levels. CO was consistently depressed across all groups during hemorrhage at maximum compensation and at decompensation (Fig. 3). At decompensation, CO was 30% ± 2%, 31% ± 5%, and 34% ± 3% in the NS, NSD, and HTSD groups, respectively (Fig. 3). CO transiently increased to 136% ±

Table 3
Baseline values: kidney

Variable	NS (n = 6)	NSD (n = 5)	HTSD (n = 7)
MAP (mm Hg)	101 ± 7	99 ± 6	97 ± 2
CO (mL/min)	128 ± 4	96 ± 9	97 ± 2
IL flow (nL/s)	17 ± 11	19 ± 8	16 ± 5
IL diameter (μm)	39 ± 1	32 ± 6	29 ± 6
AFF diameter (μm)	11 ± 1	12 ± 1	10 ± 1
EFF diameter (μm)	12 ± 1	14 ± 2	12 ± 1

AFF = afferent arterial; CO = cardiac output; EFF = efferent arterial; HTSD = 7.5% NaCl in 6% dextran 70; IL = intralobular; MAP = mean arterial pressure; NS = 33.3 mL/kg .9% NaCl.

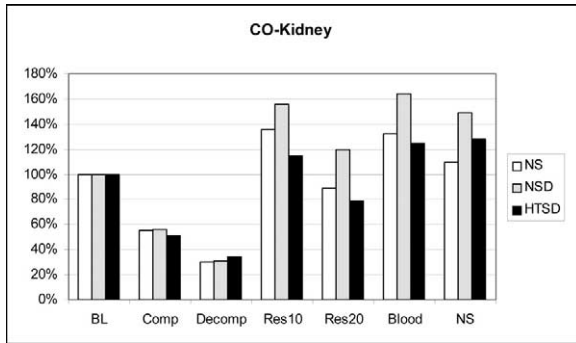


Fig. 3. Kidney experiment cardiac output responses to compensated and decompensated hemorrhagic shock followed by resuscitation with NS, NSD, or HTSD. Data are expressed as percent of BL values. There were no significant differences between groups. BL = baseline; comp = compensated; decomp = decompensated; hem = hemorrhagic; HTSD = 7.5% NaCl/6% dextran 70; NS = .09% NaCl; NSD = 0.9% NaCl/6% dextran 70; res 1 = 10 minutes; res 2 = 20 minutes.

6%, $156\% \pm 22\%$, and $115\% \pm 17\%$ after resuscitation with NS, NSD, and HTSD, respectively (Fig. 3). After further resuscitation with blood and NS, CO remained greater than BL values in all 3 groups (Fig 3). The difference among groups was not statistically significant. Afferent arteriolar diameters showed an initial dilation during shock, then a vasoconstriction at the time of decompensation. On resuscitation, afferent vessels in each group vasodilated progressively with each resuscitative fluid to $117\% \pm 6\%$, $124\% \pm 2\%$, and $135\% \pm 7\%$ in the NS, NSD, and HTSD groups, respectively. The difference was not significant between groups. Efferent arterioles vasoconstricted modestly to $80\% \pm 4\%$, $87\% \pm 7\%$, and $85\% \pm 10\%$ in the NS, NSD, and HTSD groups, respectively, at decompensation and then dilated back to BL after resuscitation. Interlobular arteries vasoconstricted to $56\% \pm 4\%$, $56\% \pm 3\%$, and $57\% \pm 6\%$ in the NS, NSD, and HTSD groups, respectively, at decompensation (Fig. 4). On resuscitation with the experimental fluids, interlobular arteries dilated back to $78\% \pm 4\%$, $69\% \pm 13\%$, and $75\% \pm 3\%$ in the NS, NSD, and HTSD groups, respectively. Successive resuscitative steps led to small degrees of vasodilation, but the vessels remained vasoconstricted relative to BL diameters. There was no statistically significant difference between groups. Interlobular blood flow showed a highly significant decrease to $14\% \pm 2\%$, $15\% \pm 1\%$, and $16\% \pm 5\%$ in the NS, NSD, and HTSD groups, respectively, at decompensation. Flow initially increased to $55\% \pm 7\%$, $64\% \pm 23\%$, and $73\% \pm 18\%$ with bolus in the NS, NSD and HTSD groups, respectively, but it then decreased to $51\% \pm 7\%$, $36\% \pm 22\%$, and $38\% \pm 7\%$ in the NS, NSD, and HTSD groups, respectively, by the end of resuscitation (Fig. 4). These effects were not statistically significant between groups. Flow remained statistically significantly less than BL in all groups despite CO greater than BL values.

Discussion

Cardiovascular failure during prolonged hemorrhagic hypotension appears to involve a loss of active vascular smooth muscle tone predominantly in skeletal muscle [1]. In Wigger's shock model, the onset of cardiovascular decompensation is indicated by the spontaneous uptake of shed blood from the reservoir. We previously showed [1] that loss of normal vasomotion and maximal dilation of skeletal muscle A3 and A4 arterioles coincides with uptake of blood and appears to be the principal site of peripheral vascular failure in skeletal muscle during decompensated hemorrhagic shock.

In the present study, we compared the ability of hypertonic saline–dextran, or an equivalent sodium chloride load in isotonic saline, to restore microvascular blood flow and small arteriolar tone after decompensated hemorrhagic shock. Because decompensation is associated with return of shed blood, potential artifactual changes in vessel diameter and flow may not represent the shock state. Therefore, we altered the traditional decompensated hemorrhagic shock protocol to avoid reinfusion of shed blood so that resuscitative fluids alone caused the observed responses. Instead of reinfusing shed blood to maintain MAP at the preselected level, when reinfusion of blood would have been necessary to maintain MAP, we allowed MAP to decrease while making the decompensatory phase measurements and then immediately administered resuscitative fluids. Because Bond et al [11] has already shown that skeletal muscle decompensation actually begins a few minutes before uptake of blood occurs, we believe that our approach is justified. As further evidence for this approach, skeletal muscle A3 and A4 vessels maximally dilated and lost vasomotion during this phase as they did after 30% return of shed blood in our previous study [1] using standard Wigger's protocol.

During decompensation there was a marked vasoconstriction of larger arterioles and a severe decrease in micro-

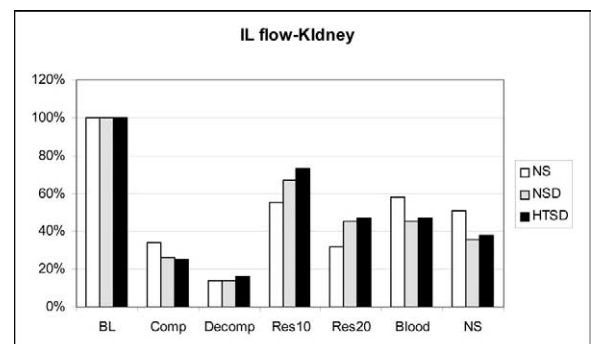


Fig. 4. Kidney IL blood flow responses to compensated and decompensated hemorrhagic shock followed by resuscitation with NS, NSD, or HTSD) Data are expressed as percent of BL values. There were no significant differences between groups. BL = baseline; comp = compensated; decomp = decompensated; hem = hemorrhagic; HTSD = 7.5% NaCl/6% dextran 70; IL = ; NS = .09% NaCl; NSD = 0.9% NaCl/6% dextran 70; res 1 = 10 minutes; res 2 = 20 minutes.

vascular blood flow in all 3 vascular beds. Precapillary arterioles dilated in skeletal muscle during decompensation. Although there was a variable dilation of A3 intestinal arterioles and afferent renal arterioles during the compensatory phase of hemorrhage, these vessels tended to constrict during decompensation. These data support the idea that the decrease in vascular tone associated with decompensation occurs primarily in skeletal muscle, at least early on in the process. During the decompensatory phase of hemorrhage, skeletal muscle large A1 and A2 arteriolar constriction did not decrease, but the small A3 and A4 arterioles dilated maximally and lost all signs of vasomotion, suggesting that they had lost vascular smooth muscle tone. This loss of A3 and A4 tone is thought to be the reason for decompensation in skeletal muscle and is similar to our previous findings using a standard Wigger's shock model [1].

The first of the present experiments demonstrates that infusion of 4 mL/kg hypertonic saline–dextran restored skeletal muscle A3 and A4 microvascular tone for 40 to 60 minutes, whereas an equivalent sodium chloride load given as an isotonic solution had similar effects for only 10 minutes. Furthermore, hypertonic saline–dextran resuscitation transiently increased skeletal muscle microvascular blood flow, whereas normal saline infusion did not. Our data indicate that addition of dextran to the hypertonic saline solution is necessary for this effect because all animals given hypertonic saline alone died within the first 10 minutes after infusion. Hypertonic saline infusion is thought to cause a sudden hypertonic state in plasma. As a result of this sudden hypertonic state, fluid shifts from the intracellular space into the extracellular compartment and thus will move into the intravascular space and dilute plasma until osmotic balance is achieved [12]. Meanwhile, sodium moves rapidly down its concentration gradient from the intravascular space into the interstitium, taking water with it. Once the sodium has equilibrated, very little fluid is retained in the intravascular space [12]. This would explain our finding that hypertonic saline alone had little effect in our model of decompensated hemorrhagic shock. However, when dextran is added to the solution, the increased intravascular oncotic pressure (70 mm Hg/100 mL) counterbalances the effects of sodium diffusion across the capillaries so that a larger proportion of the removed intracellular fluid remains in the intravascular compartment [12]. Mazzoni et al [12] modeled the fluid shifts associated with administration of 7.5% sodium chloride plus 6% dextran 70. Experimental values from our study were entered into this model (Fig. 5). When CO and skeletal muscle microvascular blood flow responses from our study were plotted with the predicted blood volume changes from this model (Fig. 5), the resulting data indicated that the changes in CO and skeletal muscle microvascular blood flow we observed after infusion of hypertonic saline–dextran infusion were probably caused by the increase in intravascular volume because the curves were essentially parallel.

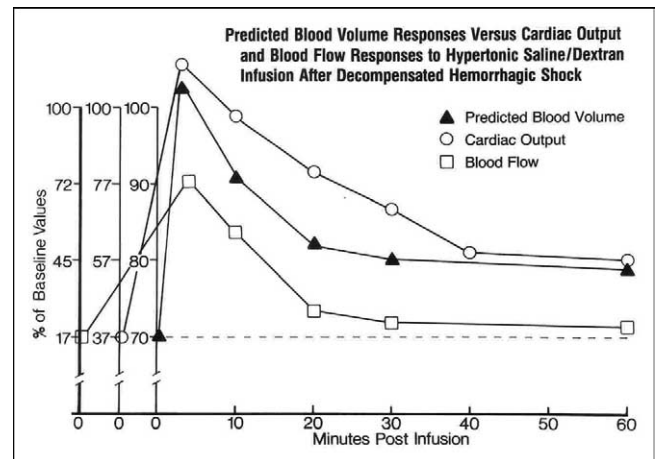


Fig. 5. Shock followed by resuscitation with HTSD. Data are expressed as percent of BL values. Predicted blood volume responses (as determined by M.C. Mazzoni, personal communication) versus cardiac output and blood flow responses to hypertonic saline–dextran infusion after decompensated hemorrhagic shock. Data were normalized according to the percent change from BL to the value at the end of decompensated hemorrhagic shock (indicated by dashed line) such that the scale is different for each variable on this graph. HTSD = 7.5% NaCl/6% dextran 70.

Clearly, hypertonic saline–dextran alone did not increase MAP and CO to a sufficient degree in our study to completely resuscitate the animal from shock. This fact undoubtedly contributed to the decreased skeletal microvascular blood flow. However, the fact that hypertonic saline–dextran infusion restored small arteriolar tone and reactivity in the skeletal muscle microcirculation after decompensated hemorrhagic shock was encouraging.

Clinical decompensated hemorrhagic shock is typically refractory to resuscitation and is almost uniformly fatal. The cause is believed to be nonreactive maximally dilated peripheral arterioles increasing the capacitance of the intravascular space to such a degree that the heart cannot generate enough output to maintain MAP at a level sufficient to maintain perfusion. De Felipe et al [5] anecdotally reported that 11 of 12 patients in terminal (decompensated) hypovolemic shock who had not responded to volume and inotropic support responded to administration of 100 to 400 ml 7.5% sodium chloride with an immediate increase in MAP, resumption of urine flow, and a decrease in iso-osmotic fluid requirements by 90%. In this remarkable report, 9 of 12 patients ultimately survived. Data from our initial study demonstrate that infusion of 4 mL/kg hypertonic saline plus 6% dextran 70 can restore skeletal muscle microvascular tone, skeletal muscle microvascular blood flow, CO, and MAP to greater-than-compensatory shock levels for 40 to 60 minutes in a rat model of decompensated hemorrhagic shock.

Based on this idea, we attempted to produce a fully resuscitative protocol in a rat model of decompensated hemorrhagic shock to determine if hypertonic saline dextran might convert decompensated shock to compensated shock from which standard resuscitation with blood and isotonic crystalloid might be possible. In subsequent studies, we

were able to increase CO to levels greater than BL and return blood pressure to near BL for extended periods of time, but this required continuous fluid replacement. Hypertonic saline dextran did not appear to have any advantage over normal saline or normal saline dextran.

We noted a discrepancy between systemic hemodynamic response to the experimental resuscitation fluid and local microcirculatory blood flow response. Resuscitation improved CO to levels greater than BL and improved MAP to BL or greater. However, renal and intestinal microvascular blood flow improved only transiently and never achieved BL levels. Therefore, the improvement in MAP and CO to BL values did not reflect the permanent dysfunction in the intestinal and renal microcirculatory systems. We noted that the decompensatory phase of hemorrhagic shock was characterized by severe vasoconstriction and, ultimately, microcirculatory shutdown in renal microcirculation. This was even more pronounced in intestinal microcirculation. In addition, there was evidence of capillary and even small arteriolar and venular occlusion during the decompensated shock phase that appeared to be from PMN or aggregated blood cells sticking to the endothelium. This was most evident in renal microcirculation where cessation of flow occurred in glomeruli to varying degrees, which is usually coincident with PMN cells sticking to the wall of the afferent arteriole. Initial resuscitation often resulted in restoration of flow in these occluded glomeruli, but inevitably they occluded again as microvascular blood flow decreased. This phenomenon was not prevented by hypertonic saline–dextran resuscitation and was similar in all 3 of our experimental groups. Haljamae [13] described a similar decompensatory microcirculatory arrest in skeletal muscle. He found that only 30% to 50% of capillaries were reperfused with resuscitation and held white blood cell plugging of the microvessels accountable for this vascular stasis. Our findings are consistent with a similar reperfusion injury that is not alleviated by hypertonic saline or dextran. Our data are consistent with activation of PMNs and endothelial cells during both the decompensated shock phase and after resuscitation. The near shut-down of flow in the microcirculation could lead to endothelial cell ischemia. Kanimiua and Pinsky [14] have shown that endothelial cell hypoxia is a potent stimulus for inflammation. Our data are also consistent with more recent findings that large-volume isotonic saline resuscitation can be deleterious and result in edema formation, neutrophil activation and sequestration, and overall initiation of the inflammatory cascade [15].

Hypertonic saline has been shown to attenuate several receptor-mediated cytotoxic responses of PMNs and decrease PMN-mediated tissue damage in several models of hemorrhagic shock [16,17]. Although our data do not support such a protective action of hypertonic saline, our experiments were not designed to measure PMN activity directly. Another mechanism that has been shown to contribute to plugging and decreased capillary flow during hemorrhagic shock is capillary lumen narrowing from en-

dothelial swelling [12]. This is thought to occur from impaired Na^+/K^+ pump function allowing entry of Na^+ and water into the cell. Hypertonic saline–dextran has been shown to improve capillary flow by decreasing endothelial cell swelling and capillary narrowing after hemorrhagic shock [12]. We cannot say if capillary narrowing or PMN rolling and sticking were decreased by HSTD in our study, but if they were it was not enough to restore microvascular blood flow in our model of decompensated hemorrhagic shock.

It is possible that something about our tissue bath conditions led to deterioration of microcirculation. However, we found similar results in hepatic circulation using flow probes and galactose clearance, a completely different method for measuring microvascular flow without using tissue bath at all [18]. Furthermore, we were able to restore intestinal microvascular flow by adding pentoxifylline to the initial resuscitation regimen in our model [2]. These findings argue against a model problem and suggest that hypertonic saline and dextran do not allow restoration of microvascular blood flow after decompensated hemorrhagic shock.

More recent studies looking at A3 venular flow in rat cremaster muscle [17] and spermatic fascia [16] showed a return of venular flow to normal after resuscitation from hemorrhagic shock with both HTSD and blood and Ringers lactate (RL) plus blood resuscitation. In these studies, RL-resuscitated animals had a marked increase of PMNs rolling and sticking to the venular endothelium, which was prevented in HTSD-resuscitated animals. The 3 major differences between these studies and ours are that the model was compensated rather than decompensated hemorrhagic shock, blood was given immediately with RL or HTSD at the initiation of resuscitation, and dextran was not added to the resuscitation.

It is possible that dextran masked the potential benefits of hypertonic saline in our model. Dextran is known to stimulate rat MAST cells to undergo histamine secretion, which may cause dilation of arterioles and decreased peripheral vascular resistance. Although numerous hemorrhagic shock experiments have been performed with HTSD in the rat, and this effect is thought to be minimal in the Sprague-Dawley rats we used, this is still of potential concern [19]. Importantly, we did not observe increased vasodilation in dextran-resuscitated animals compared with NS controls, but MAP did not increase to the same levels as in NS control animals. Despite this limitation, there is no other animal model allowing both the sequential measurement of CO and microvascular blood flow in multiple organ systems.

Comments

Our studies demonstrated that decompensated hemorrhagic shock results in a profound shut-down in the skeletal muscle, intestinal, and renal microcirculations that is characterized by intense vasoconstriction, severe blood flow deficits, and plugging of capillaries with white blood cells

and blood cell aggregates. Resuscitation is characterized by an initial restoration of flow toward BL values followed by deterioration in microvascular blood flow as resuscitation progresses. This occurred in all 3 microvascular beds despite return of CO to values great than BL. Microvascular deterioration is characterized by decreased flow and margination of white blood cells and variable capillary plugging, which is consistent with a reperfusion-type injury that is not prevented by initiating resuscitation with hypertonic saline–dextran.

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