

## SURVIVAL IN A RAT MODEL OF LETHAL HEMORRHAGIC SHOCK IS PROLONGED FOLLOWING RESUSCITATION WITH A SMALL VOLUME OF A SOLUTION CONTAINING A DRAG-REDUCING POLYMER DERIVED FROM ALOE VERA

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**ABSTRACT**—Drag-reducing polymers (DRP) increase tissue perfusion at constant driving pressure. We sought to evaluate the effects of small-volume resuscitation with a solution containing a DRP in a rat model of hemorrhage. Anesthetized rats were hemorrhaged at a constant rate over 25 min. In protocol A, total blood loss was 2.45 mL/100 g, whereas in protocol B, total blood loss was 3.15 mL/100 g. Five minutes after hemorrhage, the animals were resuscitated with 7 mL/kg of either normal saline (NS) or NS containing 50 µg/mL of an aloe vera-derived DRP. In protocol B, a third group (CON) was not resuscitated. Whole-body O<sub>2</sub> consumption (V<sub>O<sub>2</sub></sub>) and CO<sub>2</sub> production (V<sub>CO<sub>2</sub></sub>) were measured using indirect calorimetry. In protocol A, 5/10 rats in the NS group and 8/10 rats in the DRP group survived for 4 h (*P* = 0.14). Mean arterial pressure was higher in the DRP-treated group than in the NS-treated group 45 min after resuscitation (89 ± 8 vs. 68 ± 5 mmHg, respectively; *P* < 0.05). In protocol B, survival rates over 2 h in the DRP, NS, and CON groups were 5/15, 1/14, and 0/7, respectively (*P* < 0.05). Compared with NS-treated rats, those resuscitated with DRP achieved a higher peak V<sub>O<sub>2</sub></sub> (9.0 ± 1.0 vs. 6.3 ± 1.0 mL/kg/min) and V<sub>CO<sub>2</sub></sub> (9.0 ± 1.1 vs. 6.0 ± 1.0 mL/kg/min) after resuscitation. We conclude that resuscitation with a small volume of DRP prolongs survival in rats with lethal hemorrhagic shock.

**KEYWORDS**—Hemorrhagic shock, small-volume resuscitation, drag-reducing polymers, oxygen consumption, lactate/pyruvate ratio

### INTRODUCTION

Trauma is the leading cause of death among civilians younger than 40 years old (1). In the United States, traumatic injuries result in approximately 150,000 deaths per year (1). Early deaths are secondary to exsanguination or overwhelming central nervous system injuries, whereas late deaths are secondary to sepsis and multiple organ system dysfunction syndrome (2, 3). Severe hypovolemia from hemorrhage is a major causative factor in almost half of these deaths, especially during the acute period (<2 h) after injury (2, 3).

Currently, the primary strategy for treating hemorrhagic shock is to control ongoing bleeding and restore intravascular volume by infusing an asanguineous fluid (e.g., Ringer's lactate solution) and packed red blood cells. Interestingly, if intravascular volume expansion successfully restores cardiac output and arterial blood pressure before definitive hemostasis has been achieved, then, paradoxically, resuscitation can promote bleeding and shorten survival (4–8). Additionally, conventional approaches toward resuscitation require administration of large volumes of fluids that are intrinsically heavy and bulky. Thus, logistic considerations limit the capacity of first responders to provide adequate conventional resuscitation on the battlefield and even in some cases of civilian trauma. In

view of these considerations, an ideal initial resuscitation fluid for the management of hemorrhagic shock would require administration of only a small volume to improve tissue perfusion and oxygen utilization without increasing blood pressure to such an extent that endogenous hemostatic mechanisms (soft platelet-fibrin plugs) are disrupted.

Blood-soluble high-molecular-weight polymers have been tested previously as additives to resuscitation fluids in a pressure-controlled model of hemorrhagic shock in rats (9). Polymers with a molecular mass >10<sup>6</sup> Da and a relatively linear structure belong to the class of the so-called drag-reducing polymers (DRP) that have been shown to reduce resistance to turbulent flow in pipes, thereby increasing flow rate at constant pressure (Toms effect) (10). The flow conditions associated with the Toms effect probably do not occur when blood flows through arteries, arterioles, capillaries, venules, and veins. Nevertheless, a number of studies have shown that intravenous administration of DRP to experimental animals increases blood flow rate and decreases blood pressure and calculated peripheral vascular resistance without affecting blood viscosity or vascular smooth muscle tone (9, 11–14). Prompted by these considerations, we hypothesized that intravenous administration of a small volume of a solution of an aloe vera-based DRP might improve oxygen consumption and extend survival time in rats subjected to otherwise lethal hemorrhage.

### MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and followed the guidelines for the use of experimental animals of the US National Institutes of Health.

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### Preparation of aloe-based polymer

The aloe vera-derived DRP used for these studies is a complex of polysaccharides with average molecular mass of  $\sim 4 \times 10^6$  Da (9). To prepare the DRP, mucilage was obtained by gentle extraction from fresh leaves of aloe vera plant. Purification of the DRP was performed using standard methods of precipitation with 100% ethanol and further dissolving the precipitate in saline containing 0.25 mg/mL of gentamicin (GentaMax™ 100, Phoenix, St. Joseph, MO). The preparation was dialyzed against saline with 0.25 mg/mL of gentamicin for 48 h using a dialysis membrane with a 50,000-Da molecular mass cutoff (Spectra/Por membrane, MWCO:50,000, Spectrum Laboratories, Rancho Dominguez, CA). Size-exclusion chromatography (GPC-Triple Detector, Viscotek, Houston, TX) and hydrodynamic and rheological characterizations were used to assure the polymer's physicochemical properties. Before each experiment, the polymer was dissolved in pyrogen-free sterile normal saline solution (50  $\mu$ g/mL). The final solution (DRP) contained 0.05 mg/mL of gentamicin. The control solution (NS) consisted of normal saline containing the same concentration of gentamicin.

### Surgical procedures

Seventy-three male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighting 160–250 g were used. Free access to food and water was allowed until the day of the experiment. The animals were anesthetized with an intramuscular injection of ketamine (200 mg/kg; Abbot Laboratories, North Chicago, IL). Lidocaine 1% (Abbot Laboratories) was locally applied before performing surgical cutdowns for vascular access and tracheotomy. Animals were kept in a supine position during the experiment.

A cervical incision was made, and a tracheotomy was performed to secure the airway. Polyethylene tubing (PE 240, Becton Dickinson, Sparks, MD) was introduced into the trachea, and the animals were allowed to breathe spontaneously. The right jugular vein was exposed, ligated distally, and cannulated with polyethylene tubing (PE 10) for infusion of resuscitation solutions. A cutdown was performed in the right groin area. The femoral artery was isolated and ligated distally. A silicon catheter (Chronic-Cath, Norfolk Medical, Skokie, IL) was inserted for blood withdrawal. This catheter was attached to a pressure transducer that allowed instantaneous measurement of mean arterial blood pressure before and after blood withdrawal.

Finally, a short (<0.5 cm) midline abdominal incision was made, the peritoneum was opened, and a microdialysis catheter (CMA 20, CMA Microdialysis, Acton, MA) was placed inside the abdominal cavity in contact with the serosa of intestines. The catheter outlet tubes were fixed to the skin with a suture to minimize the risk of being pulled out. At the end of the surgical procedures, all of the animals received heparin (500 units/kg; APP, Schaumburg, IL) through the arterial catheter.

All the animals were instrumented in less than 30 min. The positioning of the different devices described above was checked postmortem.

### Protocol A: Severe volume-controlled hemorrhage

Following surgical preparation and a 30-min stabilization period to obtain baseline readings, 23 anesthetized rats were subjected to a 25-min period of blood withdrawal from the femoral artery. Steady withdrawal of blood (total volume: 2.45 mL/100 g) was achieved with a syringe pump (KD100, KD Scientific, New Hope, PA) at a constant rate for each animal. After hemorrhage (T0) and a 5-min shock period, the animals were randomized into two groups. Those in the NS group ( $n = 10$ ) were resuscitated with 7 mL/100 g body weight of the control solution; those in the DRP group ( $n = 10$ ) were resuscitated with 7 mL/100 g body weight of DRP solution. The resuscitation solutions were infused via the jugular vein cannula using a syringe pump (KD100, KD Scientific) at a constant rate over a 5-min period.

Microdialysis samples were collected every 5 min. Blood samples (0.3 mL each) were withdrawn through the arterial catheter at the beginning of hemorrhage (T–25), at the end (T0), and 60 min after resuscitation ended (T70) to determine blood pH, hemoglobin concentration [Hgb], base excess, PO<sub>2</sub>, PCO<sub>2</sub>, SpO<sub>2</sub>, lactate concentration, and glucose concentration using a commercial autoanalyzer (Model ABL 725, Radiometer Copenhagen, Westlake, OH). Blood pressure was recorded continuously using a commercial strain-gauge transducer, amplifier, and monitor (S90603a, SpaceLabs, Redmond, WA).

At this point (T70), the cannulas were withdrawn, the vessels tied, and the incisions closed with 3-0 silk sutures. The rats were followed for 3 h or until expiration (defined by apnea for >1 min). At the end of the experiment, the animals that were still alive were euthanized with an overdose of KCl.

### Protocol B: Lethal volume-controlled hemorrhage

Fifty animals were used for this experiment. After surgical instrumentation, the animals were placed inside a sealed transparent chamber to measure whole-body O<sub>2</sub> consumption (Vo<sub>2</sub>) and CO<sub>2</sub> production (Vco<sub>2</sub>). Catheters and tubing were brought out to allow adequate blood withdrawal and infusion of the resuscitation solutions during the experiment. Microdialysis tubing was also exteriorized to allow continuous collection of samples during the whole experiment.

Baseline recordings of Vo<sub>2</sub>, Vco<sub>2</sub>, and microdialysis samples were obtained during a stabilization period of 30 min. Thereafter, blood was withdrawn at 3.15 mL/100 g from the arterial catheter over 25 min. Hemorrhage was finished at T0, and, after 5 min of shock, the animals were randomized into three groups. One group (CON) received no resuscitation at all. The other two groups (NS and DRP) were resuscitated with either normal saline solution or DRP solution, exactly as in Protocol A. Those animals that died before receiving the full treatment were excluded from data analysis.

Vo<sub>2</sub> and Vco<sub>2</sub> were monitored continuously at 30-s intervals. Intraperitoneal microdialysis samples were collected at 5-min intervals during the entire experiment.

The animals were observed for 120 min after resuscitation ended (i.e., until T130) or until expiration as defined before. At the end of the 3-h experiment, survivors were euthanized with a supersaturated KCl bolus through the jugular catheter.

### Indirect calorimetry

Vo<sub>2</sub> and Vco<sub>2</sub> were measured by indirect calorimetry using an OxyMax system (Columbus Instruments, Columbus, OH). This equipment was housed in a thermoneutral environment (22–24°C). This system is an indirect, open-circuit calorimeter that monitors O<sub>2</sub> and CO<sub>2</sub> concentrations at the inlet and outlet of a gas-tight chamber through which ambient gas is pumped at a constant flow rate. The product of the gas concentration differences times the flow rate yields Vo<sub>2</sub> and Vco<sub>2</sub>. Measurements of Vo<sub>2</sub> and Vco<sub>2</sub> were obtained every 30 s.

### Microdialysis samples and analysis

Intraperitoneal lactate concentration was measured with a microdialysis device. The principle of microdialysis is to mimic the passive function of a capillary blood vessel by perfusion of a tubular, semipermeable membrane introduced into a tissue. The microdialysis catheter (CMA 20 probe, CMA Microdialysis, Acton, MA) is a double-lumen plastic tube with a 10-mm semipermeable membrane (cutoff 20 kDa) at its distal end. The microdialysis probe was perfused with a neutral solution (Perfusate Fluid T1, CMA Microdialysis; Na<sup>+</sup> 146 mM, K<sup>+</sup> 4 mM, Ca<sup>2+</sup> 3 mM, Cl<sup>-</sup> 156 mM, pH 6) at a flow rate of 1  $\mu$ L/min using a precision multisyringe pump (CMA 102, CMA Microdialysis). Samples were collected continuously over 5-min periods (i.e., 50- $\mu$ L aliquots) by an automated collector (CMA 142, CMA Microdialysis) and immediately frozen at –80°C. Later, the samples were analyzed for lactate and pyruvate with a CMA 600 MD autoanalyzer.

### Data presentation and statistics

All continuously variable data are presented as means  $\pm$  SE and analyzed using Mann-Whitney *U* test. Survival data were analyzed using the log-rank test. *P* values <0.05 were considered statistically significant.

## RESULTS

### Protocol A

Twenty-three animals were used. Three died during hemorrhage and were excluded from the analyses of data. Five of 10 animals in the NS group were alive at the end of the experiment, whereas 8 of 10 animals survived in the DRP-treated group ( $P = 0.14$ ).

Average baseline values for MAP were similar in the control and DRP-treated groups, as were values during shock phase before resuscitation. After injection of the test solutions, MAP in the DRP-treated group was slightly greater than that in the NS-treated group. This difference was statistically significant at T79 (Fig. 1).

Sixty minutes after the end of resuscitation (T70) [Hb] was somewhat lower in the DRP-treated animals as compared with the NS-treated animals ( $P = 0.06$ ) (Table 1). Intraperitoneal [lactate]/[pyruvate] ratio increased steadily in both groups after the onset of hemorrhage (Fig. 2). The peak value was recorded at the end of the shock phase (T5). Subsequently, intraperitoneal [lactate]/[pyruvate] ratio decreased after resuscitation fluid was infused (T10) but never returned to baseline. There were no statistically significant differences in this parameter between the groups.

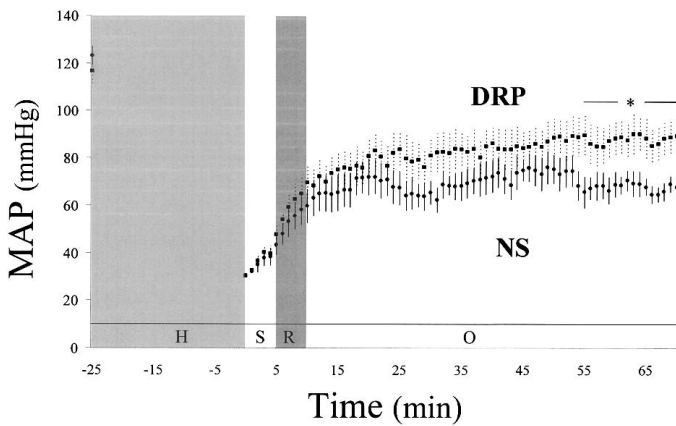


FIG. 1. Time course of MAP in NS and DRP groups in Protocol A. Data points are means  $\pm$  SEM. Circles denote NS group (n = 10); squares denote DRP group (n = 10). H (hemorrhage), light gray; S (shock), white; R (resuscitation), dark gray; O (observation), white. \* $P < 0.05$  versus NS group.

**Protocol B**

Fifty rats were used for this protocol. Fourteen rats died during the hemorrhage or resuscitation periods and were excluded from the analysis. All seven of the rats in the CON group that were hemorrhaged but not resuscitated died within 35 min (Fig. 3). Only one of 14 rats in the NS group survived for the entire 2-h period of observation. In contrast, five of 15 rats in the DRP-treated group survived. The difference in the survival times for the NS- and DRP-treated rats was statistically significant ( $P < 0.05$ ).

In all groups,  $VO_2$  decreased at T-15, i.e., when ~10% of the blood volume was withdrawn. During the entire hemorrhage phase, there was a steady decrease in  $VO_2$  that continued during the shock phase. No differences were observed between the NS and DRP groups at baseline and during the shock phase. All of the animals showed an increase in  $VO_2$  (Fig. 4) and  $VCO_2$  (data not shown) after resuscitation fluids were administered, although the increases in these parameters were more durable in the DRP group.

The maximum  $VO_2$  after resuscitation in the NS group was  $6.3 \pm 1.0$  mL/kg/min, whereas the maximum  $VO_2$  after resuscitation in the DRP group was  $8.7 \pm 1.0$  mL/kg/min ( $P < 0.05$ ;

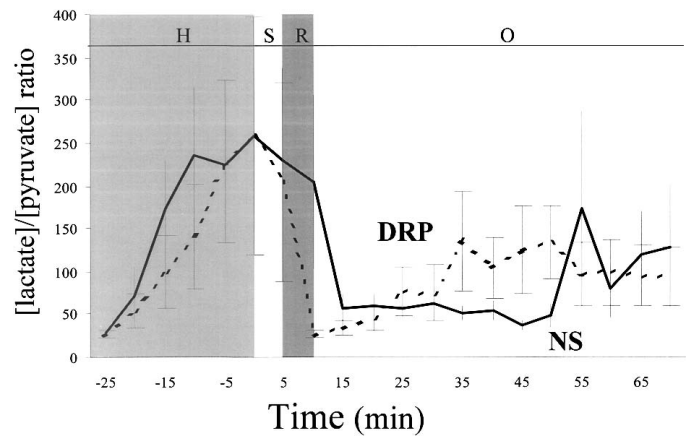


FIG. 2. Intraoperative [lactate]/[pyruvate] ratios in the NS and DRP groups in Protocol A. Data points are means  $\pm$  SEM. Full line denotes NS group (n = 10); dashed line denotes DRP group (n = 10); H (hemorrhage), light gray; S (shock), white; R (resuscitation), dark gray; O (observation), white.

Fig. 5). The results for  $VCO_2$  measurements were similar. After resuscitation, the peak  $VCO_2$  was  $6.0 \pm 1.0$  mL/min/kg in the NS group as compared with  $9.0 \pm 1.1$  mL/kg/min in the DRP group ( $P < 0.05$ ; Fig. 6).

As in Protocol A, the intraoperative [lactate]/[pyruvate] ratio increased rapidly during the hemorrhage and shock phases and decreased following resuscitation (data not shown). There were no significant differences between the NS and DRP groups.

**DISCUSSION**

The principal finding from this study is that survival after acute massive blood loss was significantly prolonged when rats were resuscitated with a small volume of a DRP solution in the absence of resuscitation with blood or any other fluids. In addition, we showed that resuscitation with an aloe vera-derived DRP significantly increased systemic  $VO_2$  relative to the values recorded following resuscitation with a similar volume of normal saline (vehicle for DRP). In the less severe model of hemorrhagic shock, resuscitation with the DRP solu-

TABLE 1. Effect of hemorrhage and resuscitation with vehicle (NS) or DRP on arterial blood gases and other biochemical parameters

Parameter	Group	T -25 (baseline)	T0 (shock)	T70 (resuscitation)
pH	NS	7.29 $\pm$ 0.01	7.23 $\pm$ 0.06	7.17 $\pm$ 0.04
	DRP	7.26 $\pm$ 0.05	7.24 $\pm$ 0.04	7.19 $\pm$ 0.04
Base excess (mmol/L)	NS	-8.4 $\pm$ 0.4	-18.2 $\pm$ 1.2	-16.5 $\pm$ 1.1
	DRP	-10.3 $\pm$ 1.3	-17.1 $\pm$ 0.9	-18.2 $\pm$ 1.3
[Glucose] (mg/dL)	NS	123 $\pm$ 8	228 $\pm$ 17	275 $\pm$ 36
	DRP	116 $\pm$ 6	236 $\pm$ 13	227 $\pm$ 31
[Lactate] (meq/L)	NS	1.0 $\pm$ 0.1	5.7 $\pm$ 0.8	5.2 $\pm$ 0.6
	DRP	1.1 $\pm$ 0.1	5.7 $\pm$ 0.3	4.5 $\pm$ 0.6
Pco <sub>2</sub> (mmHg)	NS	36 $\pm$ 1	27 $\pm$ 3	25 $\pm$ 1
	DRP	34 $\pm$ 2	26 $\pm$ 3	25 $\pm$ 3
Po <sub>2</sub> (mmHg)	NS	85 $\pm$ 4	113 $\pm$ 4	122 $\pm$ 6
	DRP	92 $\pm$ 5	123 $\pm$ 6	132 $\pm$ 9
[Hb] (g/dL)	NS	12.1 $\pm$ 0.5	10.4 $\pm$ 0.4	9.6 $\pm$ 0.2
	DRP	11.9 $\pm$ 0.4	10.3 $\pm$ 0.3	8.8 $\pm$ 0.3*
Saturation (%)	NS	0.94 $\pm$ 0.02	1.00 $\pm$ 0.01	1.01 $\pm$ 0.01
	DRP	0.95 $\pm$ 0.01	1.00 $\pm$ 0.01	1.00 $\pm$ 0.01

Data presented are mean  $\pm$  SEM. NS group (n = 10). DRP group (n = 10). \* $P = 0.06$  compared with NS controls.

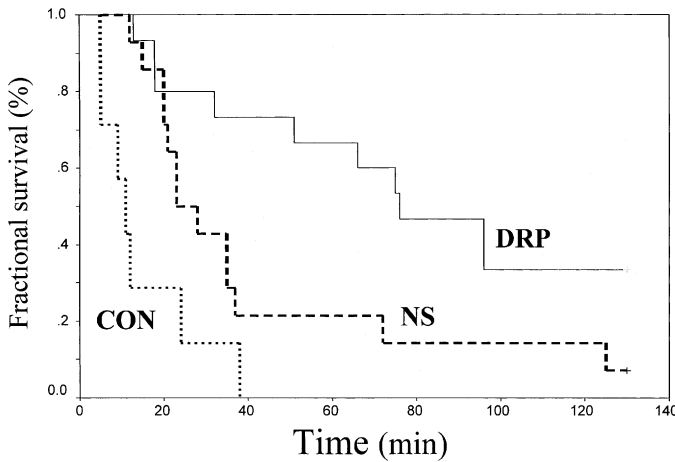


FIG. 3. Fractional survival (%) over time of animals in the CON, NS, and DRP groups in Protocol B. CON (n = 7); NS (n = 14); DRP (n = 15). \*P < 0.05 versus NS group.

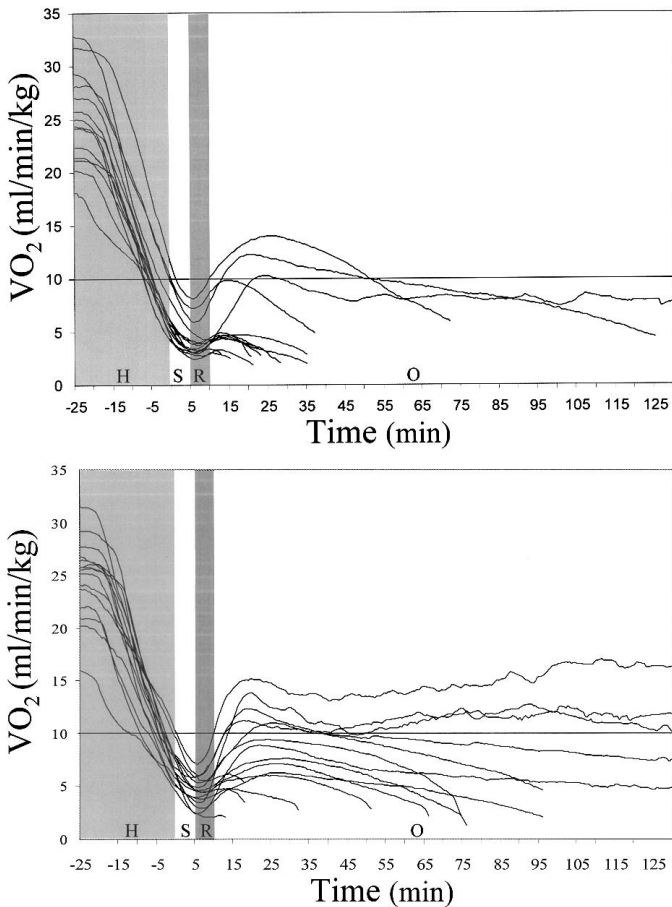


FIG. 4. Changes in  $V_{O_2}$  over time for animals in the NS (panel A) and DRP (panel B) groups in Protocol B. NS (n = 14); DRP (n = 15); H (hemorrhage), light gray; S (shock), white; R (resuscitation), dark gray; O (observation), white.  $V_{O_2}$  decreases during hemorrhage as perfusion of the tissues and lungs is impaired, and it increases with resuscitation.

tion 5 min after the completion of the bleeding phase increased MAP to a statistically significantly greater extent than did resuscitation with the saline vehicle.

The primary motivation for these animal studies was the need to develop a resuscitation fluid that can be carried by military fighters or medics during combat. Packed erythrocytes

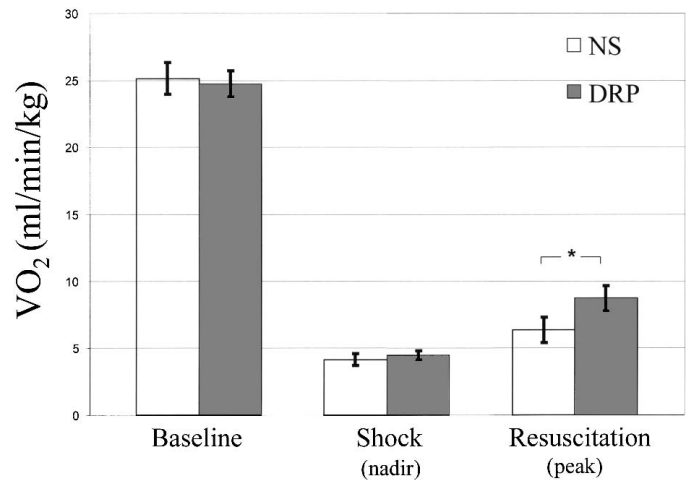


FIG. 5. Mean  $V_{O_2}$  of NS and DRP groups during the baseline (T–25), shock (T0–T5), and resuscitation–observation periods (T5–T130). Bars are means  $\pm$  SEM. NS (n = 14); DRP (n = 15). \*P < 0.05 versus NS group.

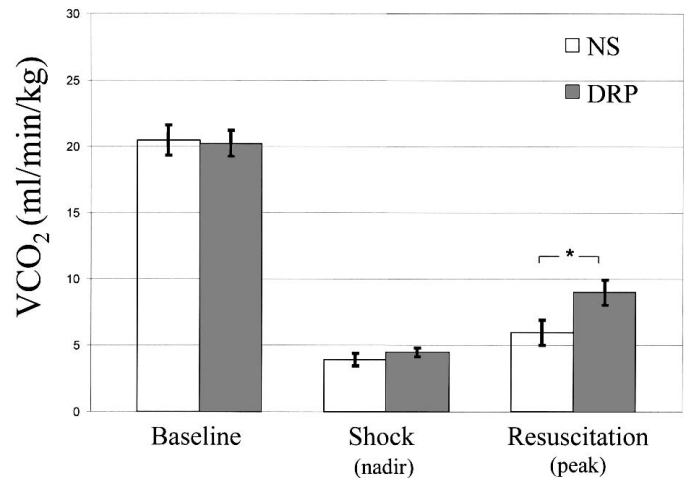


FIG. 6. Mean  $V_{CO_2}$  of NS and DRP groups during the baseline (T–25), shock (T0–T5), and resuscitation–observation periods (T5–T130). Bars are means  $\pm$  SEM. NS (n = 14); DRP (n = 15).

or other blood products cannot be used in the field because these resuscitation fluids need to be refrigerated. Because of the practical limits of how much additional weight can be carried by combatants already heavily burdened by other equipment and materiel, the amount of currently used asanguinous resuscitation fluids (e.g., 6% hetastarch in normal saline or Ringer’s lactate solution) that can be brought onto the field of battle is insufficient to be life-saving for victims of major blood loss (15). An ideal solution would “buy” a few extra hours of time to permit evacuation to a site where more definitive resuscitation and hemostasis could be provided but would provide this benefit even when only a very small volume is infused (15). A solution with these characteristics also would be of benefit under certain circumstances for the initial management of victims of civilian trauma (15).

Although resuscitation with DRP solution increased MAP to a statistically significantly greater extent than did resuscitation with NS, blood pressure remained relatively low. In pilot studies, we observed that MAP actually decreased when normal rats were injected with DRP. Inspection of the data depicted in

Figure 1 reveals that MAP tended to be higher in the DRP-treated as compared with the NS-treated animals toward the end of the observation period (i.e., during the interval when substantial mortality was occurring in the latter group). Higher MAP might simply have reflected better physiological status of the DRP-treated rats.

Several studies have shown that systemic  $\text{VO}_2$  is a more reliable and accurate indicator of severity and a better predictor of mortality after hemorrhagic shock than traditionally monitored parameters such as heart rate or MAP (16–18). During the initial phase of hemorrhage,  $\text{VO}_2$  is defended by increased extraction of oxygen from arterial blood, leading to widening of the arteriovenous oxygen content difference. However, after a critical volume of blood is lost from the intravascular compartment,  $\text{VO}_2$  decreases to such an extent that oxidative metabolism is compromised. Anaerobic metabolism increases, leading to increased production of lactic acid (and other organic acids), and, as a consequence, the [lactate]/[pyruvate] ratio increases in blood and tissues (19). Using a combination of indirect calorimetry and microdialysis, we confirmed herein that profound hemorrhagic shock in rats is associated with both decreased systemic  $\text{VO}_2$  and increased [lactate]/[pyruvate] in blood and peritoneal fluid. Administration of a small volume of DRP solution increased systemic  $\text{VO}_2$  to a significantly greater extent than did resuscitation with an equivalent volume of NS (Figs. 4 and 5). Although the absolute value of the incremental increase in maximum postresuscitation  $\text{VO}_2$  achieved by using DRP instead of NS was small (2.4 mL/min/kg), the relative incremental increase was substantial (37%). Given the importance of  $\text{VO}_2$  as a predictor of mortality in hemorrhagic shock (see above), we suspect that the significant improvement in survival time associated with resuscitation with DRP solution was related to the effect of this fluid on tissue perfusion and oxygen delivery.

Aloe-based DRP is most likely a predominantly linear carbohydrate with high molecular mass and specific viscoelastic properties that allow this compound to reduce resistance to turbulent flow when added to a fluid at minute concentrations. Thus, DRP belongs to a class of DRPs demonstrating the Toms effect. Although DRPs have been tested and used primarily in nonbiological settings (e.g., to reduce energy requirements for pumping petroleum) (10), several studies have shown that microvascular perfusion in the mammalian circulation is improved after intravenous injection of synthetic or natural DRPs (11–14, 20, 21). Although many investigators have attributed their *in vivo* results to decreased turbulence after injection of the DRPs, blood flow in the mammalian circulation is thought to be largely laminar (except for the aorta and great vessels during exercise) (22, 23). Thus, although the mechanisms underlying the beneficial effects of DRPs on blood circulation remain to be identified, they are most likely different from the original Toms drag-reducing effect because there is minimal turbulent flow in the vascular system.

The observed effects were not related to major volume expansion from an increment in blood osmotic pressure after the addition of DRP because the osmolarity of a 50  $\mu\text{g/mL}$  DRP solution in distilled water is  $1.25 \times 10^{-5}$  mOsm (unpub-

lished observations). This concentration has been previously shown to have no effects on the osmotic pressure of blood (9).

It has been demonstrated that DRP reduces the size and delays the development of flow separations and vortices at vessel bifurcations under flow conditions corresponding to realistic vascular hemodynamics with Reynolds numbers  $1 \leq \text{Re} \leq 400$  (23). *In vivo*, this effect may reduce pressure loss in arterial vessels and thus increase precapillary pressure, promoting increased perfusion of blood through the capillary network.

*In vitro* studies of the flow of erythrocyte suspensions in microchannels showed that there is a significant decrease in the near-wall cell-free layer in the presence of minute concentrations of DRP in the suspension medium (9). This phenomenon could alter the distribution of red blood cells within the circulation. Specifically, the local hematocrit might increase because the effect of so-called “plasma skimming” at bifurcations of microvessels would be attenuated. Conversely, the central hematocrit would be expected to decrease. As a result, the density of functioning capillaries would be increased, as would oxygen delivery. In the present series of experiments, we did not directly investigate the effects of DRP on the microcirculation. Nevertheless, we think this phenomenon is a plausible explanation of the relatively lower postresuscitation hemoglobin concentration in DRP-treated as compared with NS-treated animals ( $8.8 \pm 0.3$  vs.  $9.6 \pm 0.2$  g/dL, respectively;  $P = 0.06$ ).

As suggested by Kameneva et al. (9), DRP may provide better diffusion of oxygen molecules from erythrocytes to tissues because of a better mixing in plasma surrounding red blood cells. This hypothesis is based on the results of *in vitro* experiments, which demonstrated that DRPs were capable of increasing mixing efficiency of fluids at hydrodynamic conditions ( $\text{Re} < 1$ ) corresponding to those seen in microvessels (24). With the addition of DRP to circulation, delivery of  $\text{O}_2$  could be enhanced because of an efficient mixing at the plasma layer surrounding red blood cells and also because of the previously mentioned redistribution of erythrocytes from the macrocirculation to capillaries.

One potential weakness in the experimental design for the present series of experiments is that heparin was administered to all of the animals before they were subjected to hemorrhagic shock. Heparin, of course, can influence the response to hemorrhagic shock (25, 26). Heparin was used to minimize problems related to clotting of the arterial and venous catheters because our goal was to study a model of hemorrhagic shock treated with a very small volume of resuscitation fluid. Clotting would have necessitated infusing additional fluid to flush the catheters to maintain patency, defeating a key objective of the experiment.

Despite this concern, we doubt that heparin influenced our findings. First, both the control and experimental groups received the same dose of heparin. Second, even in the absence of heparin administration, DRPs have been shown to increase blood flow at constant blood pressure in previous animal studies (12, 13). Third, in chronic studies, administration of a DRP in the absence of heparin was shown to significantly reduce atherogenesis, presumably as a result of beneficial hemodynamic effects following intravenous injection (27, 28). Fourth,

we have carried out numerous *in vitro* studies showing that DRPs increase the flow of suspensions of washed red blood cells in the absence of heparin or any other anticoagulant (M. V. Kameneva, unpublished observations).

In summary, we demonstrated that resuscitation with a small volume of solution containing DRP delays death and increases  $VO_2$  and MAP in rats subjected to lethal volume-controlled hemorrhagic shock. Future studies should evaluate the underlying mechanisms and the effects of DRP in larger animals that might more closely replicate the physiology of human hemorrhagic shock.

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