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DRAG-REDUCING HYALURONIC ACID INCREASES SURVIVAL IN PROFOUNDLY HEMORRHAGED RATS

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ABSTRACT—We tested the hypothesis that the infusion of a small volume of a drag-reducing polymer (DRP) solution can prolong survival in rats subjected to lethal hemorrhagic shock (HS; shed 51% of estimated blood volume) in the absence of complete resuscitation with fluids or blood. In this set of experiments, we used a newly designed mixture of hyaluronic acid (molecular weight, $\sim 2.0 \times 10^6$ d; 0.4 mg/mL) and polyethylene oxide (molecular weight, $\sim 4 \times 10^6$ d; 0.05 mg/mL) dissolved in sterile phosphate-buffered saline. Anesthetized rats were subjected to a volume-controlled HS. During the first 20 min, blood (21.7 mL/kg) was withdrawn. During the next 40 min, additional blood (14 mL/kg) was withdrawn, and during the final 20 min, saline vehicle or saline + DRP (2.8 mL/kg) was simultaneously infused. The survival rate of the rats treated with the hyaluronic acid/polyethylene oxide was significantly higher ($P < 0.01$). The mean survival times for control and DRP-treated animals were 100.4 ± 9.5 vs. 154.8 ± 7.0 min ($P < 0.001$). MAP was higher ($P < 0.005$) and skin perfusion was significantly improved in the DRP-treated group after the end of the DRP infusion. These results support the use of nanomolar concentrations of DRP to prolong survival in rats after lethal HS in the absence of fluid resuscitation. The DRP formulation studied here warrants further evaluation for the amelioration of critical illness associated with profound shock when access to resuscitation fluids may not be possible or delayed.

KEYWORDS—Hemorrhagic shock, high molecular weight hyaluronic acid, hemodynamics, survival analysis

INTRODUCTION

Hemorrhagic shock (HS) remains an important cause of death among trauma victims (1, 2). Traumatic injuries cause approximately 100,000 deaths per year in the United States alone (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 (4, 5). Current treatments for HS largely rely on controlling bleeding and volume expansion using fluid resuscitation with crystalloid or colloid solutions. Studies suggest that an adequate volume of fluid should be given to avoid death from profound hypotension after massive blood loss but without blood pressure normalization because this may result in increased bleeding with consequential hemodynamic decompensation and increased mortality (6–8). Additional problems with conventional resuscitation using large (i.e. bulky) volumes of fluid leads to logistical issues under certain extreme circumstances such as those encountered on the battlefield or after a natural or man-made civil disaster (9). Based on the these considerations, it is desirable to be able to administer a therapeutic agent for the initial management of profound HS in the prehospital setting so that survival can be prolonged (i.e. extend the "golden hour") with a modest increase in blood pressure that minimizes disruption of endogenous hemostatic mechanisms (10) until more aggressive resuscitation can be undertaken in a clinical setting.

Flow drag reduction caused by soluble long-chain, high molecular weight polymers (so called drag-reducing polymers or DRP) is a well-described hydrodynamic phenomenon observed in turbulent flow in a pipe and is known as the Toms effect (11). In the past few decades, this effect was tested in the vascular system of experimental animals by injecting them with very low concentrations of blood-soluble DRP. These tests revealed that the intravenous administration of nanomolar concentrations of DRP to experimental animals yielded beneficial hemodynamic effects, including increased cardiac output and improved tissue and organ perfusion (12–19). The administration of DRP increased the number of functioning capillaries in diabetic rats (20) and improved tissue oxygenation in rats subjected to severe hemorrhage (15, 16, 19). Additionally, treatment with DRP solutions was shown to improve oxygen consumption after resuscitation from lethal HS (19), enhance coronary perfusion (17), and increase animal survival (18) in acute animal models of myocardial ischemia.

The exact mechanisms responsible for the salutary effects of DRP in the vascular system remain to be incompletely understood. Some hypotheses and their experimental validations were previously presented (17).

Here, we show that the treatment of rats subjected to massive blood loss with a very small volume of a novel DRP solution containing hyaluronic acid (HA) and polyethylene oxide (PEO) significantly enhanced the animal survival. Despite the fact that the volume of fluid infused to deliver the DRP was insignificant in relation to the volume of blood lost, no additional

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resuscitation was provided, shed blood was not replaced, and the animals remained profoundly hypotensive throughout the course of the experiment.

MATERIALS AND METHODS

Surgical procedures

All study procedures using rats followed the guidelines for the use of experimental animals of the US National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) used in these experiments were allowed food and water *ad libitum* until the day of the experiment. All rats were anesthetized with ketamine HCl (Hospira, Inc., Lake Forest, Ill; 30 mg/kg, i.m.) and sodium pentobarbital (Ovation Pharmaceuticals, Inc., Deerfield, Ill; 65 mg/kg, i.p.) and allowed to breathe spontaneously. Animals were positioned in dorsal recumbency on a thermal blanket to maintain body temperature at 37°C. Lidocaine (1%; Abbot Laboratories, Parsippany, NJ) was given locally before gaining vascular access. All catheters were flushed with a saline solution containing heparin (50 U/mL). A cervical incision was made, and the right jugular vein was exposed, ligated distally, and cannulated with polyethylene tubing to withdraw blood and to infuse the test solutions. A cutdown was performed in the right groin area, and the femoral artery was isolated and ligated distally. Polyethylene tubing was inserted and attached to a pressure transducer that allowed instantaneous measurement of MAP that was monitored continuously throughout the experiment. A silicon Cronic Cath catheter (Norfolk Medical, Skokie, Ill) was introduced into the femoral vein and used to withdraw blood during the last phase of hemorrhage. When necessary, high-temperature cautery (Aaron Medical, St. Petersburg, Fla) was used at surgical incisions to control bleeding. The instrumentation of the animals was performed within 30 min. Heparin (500 U/kg) was administered immediately after instrumentation through the femoral vein. The position of the inserted catheters was checked postmortem.

Preparation of DRP solution

The DRP additive in the resuscitation fluid (saline) was composed of HA (Hyvisc; Anika Therapeutic, Inc., Bedford, Mass; 0.4 mg/mL) and PEO (Dow Chemical, Russellville, Ark; 0.05 mg/mL). Fraser et al. reported that the half-life of high molecular weight HA in circulating plasma in rabbits and humans averaged between 2.5 and 5 min (21, 22). Therefore, HA was formulated together with PEO to confer a prolonged DRP intravascular rheological effect assuming that HA might be cleared relatively quickly from the blood. We assumed that the clearance rate for HA would be 5 to 15 μg/h. Therefore, considering that the HA concentration in blood after the end of the infusion/blood withdrawal was approximately 30 μg/mL, we anticipated that after 2 to 3 h, the HA concentration would be less than the level needed for optimal therapeutic effects. Accordingly, a small amount of PEO was included to prolong the action of this DRP fluid.

Survival of rats subjected to severe volume-controlled HS

After surgical preparation and a 5-min stabilization period to obtain baseline readings, rats were subjected to HS. Bleeding was carried out in two phases. Initially, 21.7 mL/kg of blood was withdrawn over 20 min (T_{20}) from the jugular vein. Immediately thereafter, an additional 7 mL/kg of blood was withdrawn over 20 min (T_{40}) from the jugular vein, and this was followed by removal of an additional 7 mL/kg of blood over 20 min (T_{60}) from the femoral vein. Thus, hemorrhage occurred over a period of 60 min, and the blood loss was 35.7 mL/kg or 51% of the blood volume.

Rats were randomized into either group (control or DRP) before they were enrolled in the experiment. Those in the control group (n = 14) received 2.8 mL/kg body weight of the control vehicle (saline); those in the HA/PEO group (n = 21) received 2.8 mL/kg body weight of saline containing the DRP. HA/PEO or saline was administered as a continuous infusion during the last 20 min of the hemorrhage period. The solutions were infused via the jugular vein cannula using a syringe pump (KD100; KD Scientific, New Hope, Pa). Rats were observed for 3 h or until expiration (defined by apnea for >1 min). At the end of the 3-h observation period, surviving animals were euthanized with an overdose of KCl.

Blood pressure was continuously monitored using a commercial strain-gauge transducer, amplifier, and monitor (S90603a; SpaceLabs, Redmond, Wash) and recorded with a PowerLab data acquisition system (ADInstruments, Colorado Springs, Colo) connected to a laptop PC. Blood samples (0.3 mL) were collected through the arterial catheter at the beginning of (T_0) and 30 min after (T_{90}) the hemorrhage period to determine blood pH, hemoglobin concentration, base excess, P_{O_2}, P_{CO_2}, lactate concentration, and glucose concentration using a commercial blood gas analyzer (model ABL 725; Radiometer Copenhagen, Westlake, Ohio).

Skin blood flow measurements

Skin blood perfusion measurements were completed using a state-of-the-art noninvasive technique. A laser Doppler tissue perfusion disk probe (probe type DI; Transonic Systems, Inc., Ithaca, NY) was used to measure skin blood flow by placing the probe on the left groin area. The probe touched the skin with minimal contact to minimize applying pressure to avoid occlusion of the underlying microvessels that could cause a reduction of perfusion in the area of interest. The left groin area was chosen to minimize movement artifact, and we also avoided placement of the probe on a site containing large blood vessels. The skin perfusion readout in tissue perfusion units was obtained using a dual-channel BLF21D laser Doppler flow meter (Transonic Systems Inc.) and recorded with the PowerLab data acquisition system.

Data presentation and statistics

All continuously variable data are presented as a mean ± SE and analyzed using a Student *t* test. Survival data were analyzed using the log-rank test. A value of $P < 0.05$ was considered statistically significant.

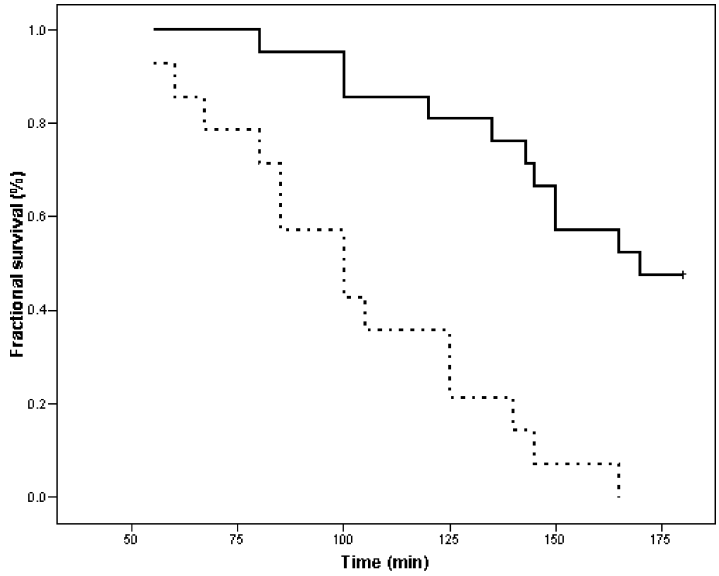

FIG. 1. **Effect of intravenous treatment with DRPs on survival of rats subjected to volume-controlled HS**. Comparison of the survival curves was performed using the log-rank test. Solid line denotes DRP group (n = 21); dashed line denotes control group (n = 14), $P < 0.01$ vs. control group.

RESULTS

A total of 35 animals were used in this study and were randomized to receive either the saline vehicle (2.8 mL/kg) or a similar volume of the DRP in saline. Of 21 animals infused with the DRP solution, 10 (47.6%) survived 3 h after the start of hemorrhage, whereas 0 of 14 animals in the control group survived this period (Fig. 1). Thus, infusion with the HA/PEO DRP cocktail significantly prolonged survival in the absence of resuscitation with a colloid or shed blood ($P < 0.01$). The difference in the mean survival times for control and DRP-treated rats was statistically significant (154.8 ± 7.0 vs. 100.4 ± 9.5 min, respectively; $P < 0.001$).

In both groups, MAP decreased abruptly during the first 20 min of the hemorrhage protocol. The difference in MAP between the two groups at 40 min of hemorrhage was statistically insignificant ($P = 0.215$) before the start of saline or saline + HA/PEO infusion. MAP remained in the range of 40 mmHg until 30 min after the hemorrhage period was completed (T_{90}). At 100 min from the onset of hemorrhage, MAP was significantly greater in the HA/PEO group (45 ± 4 vs. 29 ± 3 mmHg; $P < 0.005$).

Skin perfusion decreased sharply in all animals after the induction of hemorrhage. At approximately 60 min, which coincided with the end of the bleeding and fluid infusion period, animals that received the DRP solution tended to have better skin perfusion compared with those receiving the vehicle (saline) alone. The difference in skin perfusion was statistically significant at the 40-min posthemorrhage time point (3.7 ± 1.2 vs. 0.8 ± 0.3 tissue perfusion units; $P < 0.05$). Except for base excess, blood physiology variables did not differ significantly between the two groups 90 min after initiating shock (Table 1).

TABLE 1. **Effect of hemorrhage and resuscitation on blood parameters measured at baseline and at T = 90 min**

Parameter	Group	T_0	T_{90}
pH	Control	7.33 ± 0.05	7.33 ± 0.06
	DRP	7.34 ± 0.03	7.37 ± 0.07
P_{O_2}, mmHg	Control	82.2 ± 9.5	88.3 ± 7.4
	DRP	90.4 ± 7.9	91.9 ± 13.0
P_{CO_2}, mmHg	Control	50.5 ± 6.2	24.8 ± 9.7
	DRP	45.1 ± 4.8	27.2 ± 4.7
Hemoglobin concentration, g/dL	Control	13.4 ± 1.1	8.4 ± 2.3
	DRP	13 ± 0.9	9.2 ± 0.9
Glucose, mg/dL	Control	133.3 ± 22.7	169.3 ± 88.9
	DRP	119.1 ± 18.8	163.6 ± 12.9
Lactate, meq/L	Control	1.0 ± 0.2	3.3 ± 0.7
	DRP	1.1 ± 0.4	2.3 ± 0.5
Base excess, mM	Control	0.9 ± 1.6	−16.6 ± 4.9*
	DRP	−0.2 ± 2.5	−8.2 ± 5.05
Bicarbonate, mM	Control	24.2 ± 1.29	15.2 ± 5.5
	DRP	23.6 ± 1.85	18 ± 3.9

Data are presented as mean ± SE. DRP group (n = 21); control group (n = 14).
*$P < 0.05$ compared with DRP-treated animals.

DISCUSSION

This is the first study to demonstrate that a significant survival improvement can be achieved using a simple polymer solution of HA and PEO delivered in a volume of saline that was too small to be considered as a fluid resuscitation method. Remarkably, we found that resuscitation with a volume of the DRP solution representing 4% of the estimated blood volume of a normal rat resulted in approximately 50% 3-h survival after experiencing more than 50% blood loss. Our shock model was 100% lethal in animals receiving the saline vehicle alone. This finding suggests that treatment with a polymer possessing drag-reducing properties might be useful for prolonging the period of time patients survive after losing large quantities of blood due to traumatic injuries or other catastrophes (e.g. rupture of an abdominal aortic aneurysm).

We previously reported that rats resuscitated after a pressure-controlled hemorrhage (31.5 mL/kg) with 7 mL/kg of isotonic sodium chloride solution containing 0.05 mg/mL of an *Aloe vera*–derived DRP had a significantly higher 2-h survival rate (33% vs. 7%) compared with controls resuscitated with isotonic sodium chloride solution (19). Polymers isolated from *A. vera* are a poorly characterized mixture of compounds and, thus, would be hard to develop for clinical use. Here, we report for the first time that HA can be used for resuscitation. High molecular weight HA is a naturally occurring polymer that was first reported to have drag-reducing properties by Hoyt in 1965 (23). Hyaluronic acid is a linear high molecular weight (10^5 – 10^7 d) mucopolysaccharide composed of repeating disaccharide units, each unit consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine. Hyaluronic acid formulations are currently approved by the US Food and Drug Administration for orthopedic, ophthalmic, and topical drug-delivery applications. In this work, we successfully applied HA as a polymeric component of a resuscitation fluid supplemented with nanomolar concentrations of another DRP, PEO.

Our study has a number of limitations. We examined the effects of DRPs only in rats that were subjected to shock but never fully resuscitated with blood or asanguinous fluids. Although ischemia during the shock phase can injure tissues, it is well established that reperfusion also contributes to cellular damage. It remains to be determined whether early treatment with DRPs that prolongs survival during the shock phase followed by standard resuscitation with blood and crystalloid solutions would lead to improved long-term survival. Finally, we performed all of our *in vivo* studies using rats that were anesthetized with ketamine and sodium pentobarbital. We recognize that these anesthetic agents have numerous pharmacologic effects, and that treatment with DRPs might be more or less beneficial in unanesthetized animals or humans.

By extending the time window before irreversible shock develops, treatment in the field with a DRP solution might "buy" enough time to allow transport of severely injured patients to locations where definitive care, including the

control of bleeding and resuscitation with blood products and asanguinous fluids, can be provided. The findings presented here also support the general concept that the enhancement of microvascular perfusion is a reasonable therapeutic strategy for the management of HS.

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