

Blood soluble drag-reducing polymers prevent lethality from hemorrhagic shock in acute animal experiments

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Abstract. Over the past several decades, blood-soluble drag reducing polymers (DRPs) have been shown to significantly enhance hemodynamics in various animal models when added to blood at nanomolar concentrations. In the present study, the effects of the DRPs on blood circulation were tested in anesthetized rats exposed to acute hemorrhagic shock. The animals were acutely resuscitated either with a 2.5% dextran solution (Control) or using the same solution containing 0.0005% or 5 parts per million (ppm) concentration of one of two blood soluble DRPs: high molecular weight (MW = 3500 kDa) polyethylene glycol (PEG-3500) or a DRP extracted from *Aloe vera* (AVP). An additional group of animals was resuscitated with 0.0075% (75 ppm) polyethylene glycol of molecular weight of 200 kDa (PEG-200), which possesses no drag-reducing ability. All of the animals were observed for two hours following the initiation of fluid resuscitation or until they expired. We found that infusion of the DRP solutions significantly improved tissue perfusion, tissue oxygenation, and two-hour survival rate, the latter from 19% (Control) and 14% (PEG-200) to 100% (AVP) and 100% (PEG-3500). Furthermore, the Control and PEG-200 animals that survived required three times more fluid to maintain their blood pressure than the AVP and PEG-3500 animals. Several hypotheses regarding the mechanisms underlying these observed beneficial hemodynamic effects of DRPs are discussed. Our findings suggest that the drag-reducing polymers warrant further investigation as a potential clinical treatment for hemorrhagic shock and possibly other microcirculatory disorders.

Keywords: Drag-reducing polymers, hemorrhage, rats, survival, microcirculation, microchannel flow, viscosity

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1. Introduction

Trauma, resulting in hemorrhage and hemorrhagic shock, is an important public health problem. According to National Vital Statistics Reports, approximately 150,000 fatalities and many prolonged illnesses are associated with trauma despite continued advances in the care of trauma patients [25]. Hemorrhage is the major cause of these deaths and complications. In addition, hemorrhage and hemorrhagic shock are major causes of morbidity and mortality in combat casualties [29].

Current treatments for hemorrhagic shock are based on hemorrhage control and volume expansion, including fluid resuscitation with crystalloid and colloid solutions, transfusion of donor blood (if available), or using blood substitutes (not yet available in the US). Transfusion of stored red blood cells may not restore microcirculatory oxygenation [44]. Thus, fluid resuscitation is the only supportive therapy, and currently there are no adequate resuscitation strategies for patients with severe bleeding that may be predisposed to develop irreversible circulatory/hemodynamic collapse. In addition to infusing patients intravenously with large volumes of crystalloid- and/or colloid-based fluids, standard therapies for treatment of hemorrhagic shock include administering sympathomimetic vasopressive agents (to enhance coronary perfusion pressure), inotropic agents (to enhance myocardial contractility), and chronotropic agents (to alter cardiac periodicity), and the use of vasoactive drugs (α -, β -adrenergic agonists, ACE-inhibitors, Ca^{++} -channel blockers), diuretics, anticoagulants, and “blood thinners”. While adequately addressing the problem of insufficient circulating volume and oxygen-carrying capacity, the current therapy for hemorrhagic shock fails to adequately address the issue of impaired microvascular perfusion.

We have tested a new approach for both preventing acute lethality due to hemorrhagic shock and concurrently improving microcirculation wherein blood soluble drag-reducing polymers (DRPs) are added in minute quantities to the resuscitation fluid. This approach is built upon a remarkable 20th century discovery, the Toms effect [41], which described the ability of soluble, high molecular weight ($\text{MW} > 10^6$ Da) polymers to reduce resistance to turbulent flow in a pipe. It was demonstrated that the addition to flowing fluid of a minute quantity of soluble polymers characterized by long chains, relatively linear structure, and unique elastic properties, significantly increased the flow rate under a constant driving pressure drop or decreased the pressure drop under constant flow conditions. However, the polymers have no effect on flow resistance under laminar flow conditions, nor do they change the viscosity of fluid at the effective (extremely low) concentrations. It was also found that DRP additives reduced hydrodynamic resistance in systems with nonturbulent (disturbed laminar) flow, such as pulsating flow in straight and spiral pipes or Couette flow with Taylor vortices at low Reynolds numbers [5,21]. The Toms effect has been intensively investigated for decades and has been utilized for various industrial applications [16,23,24,36,38,39]. However, the exact mechanisms underlying the Toms phenomenon remain incompletely understood.

Over the years, blood-soluble drag-reducing polymers have been shown to produce positive hemodynamic effects in various acute and chronic animal models [4,7,9–14,17,18,27,28,32–35,37,40,43]. Specifically, nanomolar concentrations of these polymers injected intravenously caused an increase in aortic and arterial blood flow and a decrease in both blood pressure and peripheral vascular resistance, with no effect on blood viscosity or blood vessel tone [4,10,13,14,18,32–35]. DRPs were found to significantly increase collateral blood flow in rabbits [7] and the number of functional capillaries in normal and diabetic rats [9]; and reduce hydrodynamic resistance in both normal and adenosine dilated rat mesentery arterioles [40]. In canine models of a subcritical stenosis in the aorta [11,27] and carotid artery [17], injection of DRPs reduced poststenotic flow separation and flow disturbances. Increased blood flow through iliac artery stenoses [43] and redistributed wall shear stresses in the area of aortic stenoses [37] have also been measured in dogs. Finally, chronic intravenous injections of DRPs diminished the development of

atherosclerosis in several atherogenic animal models [6,8,11,12,28]. These experiments, which involved regular intravenous injections of DRPs in rabbits and pigeons at concentrations 50–100 times greater than those that produce hemodynamic effects, demonstrated no toxic effects of the polymers. In addition, the absence of acute toxicity was demonstrated in studies of DRP-based perfusion of isolated hearts [30].

DRPs utilized in these aforementioned studies include: polyacrylamides [4,6,10–12,17,27,28,30,33,35,43], polyethylene oxides (or polyethylene glycols) [7–9,13,14,18,34,40,43], a polysaccharide extracted from okra and characterized as a rhamnogalactogalacturonan (RGGu) [32], and calf thymus DNA [13,14].

On the basis of the intravascular effects of DRPs described above, we hypothesized that the use of DRPs for resuscitation might significantly improve microcirculation and increase survival rate or even prevent lethality of the animals exposed to severe hemorrhagic shock.

2. Materials and methods

2.1. Reagents

In this study we tested two drag-reducing polymers, polyethylene glycol (PEG, which is another name for polyethylene oxide) with average molecular weight of 3.5×10^6 Da (PEG-3500) (Aldrich Chemical Co) and a new aloe vera-based DRP discovered in our laboratory. This is the first report on the drag-reducing ability of the polymers extracted from aloe vera leaves.

PEG with much lower average molecular weight of 2×10^5 Da was also used (PEG-200). This molecule possesses the same chemistry and structure of the higher molecular weight PEG, but with no drag-reducing ability. Both PEGs were carefully dissolved in saline and dialyzed using a membrane with 50,000 Da MW cutoff (Regenerated Cellulose Dialysis Membrane, Spectra/Por, Spectrum Laboratories Inc.). The drag-reducing polymer dissolving procedures were previously described in detail in Polimeni et al. [34].

Mucilage from the aloe vera plant was obtained in our laboratory by extraction from fresh aloe leaves. The extract was purified and characterized as a complex of polysaccharides with average molecular weight of about 4×10^6 Da. Purification of the aloe vera drag-reducing polymer (AVP) was accomplished by standard methods of precipitation with 100% ethanol, dialysis (50 kDa MW cutoff), ultracentrifugation, filtration, and size exclusion chromatography (GPC-Triple Detector, Viscotek, Houston, TX). Prior to each animal experiment, the polymers were dissolved in the resuscitation fluid at the desired concentration.

2.2. *In vitro* DRP tests

The ability of the polymers to reproduce the Toms effect was tested *in vitro* in a circulating system consisting of a centrifugal pump (BioMedicus Inc.), a clamp-on flow probe (Transonic Systems, Inc.), flow meter (Transonic Systems, Inc.), pressure transducers (PCB Piezotronics, Inc.), 3/8 inch Tygon interconnecting tubing (Cole-Parmer), a small diameter glass tube (0.49 cm ID, 92 cm length), and a one liter fluid reservoir. Studied flow rates of 1 to 4 l/min provided Reynolds numbers of 4,330 to 17,330; mean shear rates of 2,000 to 20,000 s^{-1} ; and wall shear stresses between 4.0 to 40.0 N/m^2 in the glass tube. In these experiments, PEG-3500 or AVP was added to the circulating fluid (saline) to yield a final concentration of 5 parts per million (ppm). PEG-200 was added to yield final concentrations of either 5 ppm or 75 ppm. All *in vitro* flow experiments were performed at room temperature of $22 \pm 1^\circ C$.

Viscosities of all tested polymer solutions and rat blood with polymer additives were measured at room temperature using a rotational viscometer (Contraves Low Shear 30, Contraves, Switzerland) and by capillary viscometers with a capillary radius of 0.0235 cm (Cannon Instrument Co.) at shear rates of approximately $300\text{--}400\text{ s}^{-1}$. Osmolality and oncotic pressures were determined using a freezing point Automatic Osmometer (μ Osmette, Precision Systems, Inc.) and Colloid Osmometer (Wescor, Inc.).

2.3. *In vitro* microchannel experiments

Suspensions of rat RBCs were prepared using washed RBCs at the hematocrit of 20%. RBCs were washed three times with a phosphate buffer solution (PBS, Sigma Chemical Co) and resuspended in PBS containing 1.0% bovine serum albumin with or without a DRP (polyethylene glycol with molecular weight of about 4×10^6 Da) at the concentration of 10 ppm. In addition, RBC suspension was prepared in PBS containing 1.0% bovine serum albumin with PEG-200. The suspensions were driven through a rectangular glass channel ($100 \times 100 \times 25000\ \mu\text{m}$) using a syringe pump (PHD 2000, Harvard Apparatus Co.) at flow rates of 0.01–0.5 ml/min (corresponding Reynolds numbers of 0.5–20). The channel was mounted on a specially designed flow chamber, which was connected via Tygon tubing to a syringe pump (PHD 2000, Harvard Apparatus, MA). The discrete motion of RBCs under shear was visualized *in situ* through an inverted research microscope (IX70, Olympus, NJ) with stroboscopic light. Images of RBCs flowing through the microchannel were recorded by using a digital high-resolution CCD camera (1328×1024 pixels, Kodak Megplus 1.4) and an image acquisition board (PIXCI, EPIX Inc., IL) hosted in a computer. The exposure time for imaging was set at about 5 microseconds with the stroboscope so that the potential image blur of individual RBCs caused by the RBC motion was minimized. The thickness of the cell-free layer was determined from 20 different images using the image analysis software (Scion Image, Scion Corporation, MD).

2.4. *Animals*

Male, pathogen-free Sprague-Dawley rats (350–400 g) were obtained from Charles-River Co. (Boston, MA). All animals received humane care in compliance with the National Society of Medical Research Principles of Laboratory Animal Care and with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The experiments were approved by the University's Institutional Animal Care and Use Committee.

2.5. *Experimental protocol*

The animals were anesthetized with an IM injection of ketamine hydrochloride (90 mg/kg)/xylazine (10 mg/kg) and then anticoagulated with sodium heparin (SQ, 20 IU). The animals were placed on a temperature-controlled surgical pad (38°C). A tracheotomy was performed and a catheter segment was inserted through the trachea to maintain the airway. The rats were allowed to breathe spontaneously.

Arterial blood pressure was measured by cannulating the right carotid artery and connecting the catheter to a pressure transducer amplifier system (Abbott Laboratories, North Chicago, IL). The catheters and cannulae were flushed with heparinized saline solution (20 IU) before use. A laser-Doppler flowmeter (Transonic Systems Inc., Ithaca, NY) was used to continuously monitor tissue perfusion (TP) in the lip mucosa. Tissue oxygenation (TcpO_2) was measured at the beginning and end of eleven Control and fifteen DRP (AVP, $n = 9$, and PEG-3500, $n = 6$) experiments using a transcutaneous oxygen tension

measurement system (TCM3, Radiometer, Denmark). This instrument was not available for the other experiments in this series. As suggested by the manufacturer, after thoroughly cleansing the shaved skin with alcohol, the measurement probe was placed on the surface of the thigh. A calibration against air was performed before each experiment. The probe was heated to the preset temperature of 44°C providing maximal vasodilation. Subsequently, the T_{cpO_2} measurements were automatically temperature-corrected to 37°C. These methods are described in detail elsewhere [1,3,31].

Arterial and venous blood samples were withdrawn at the beginning and end of experiment for determination of hematocrit, blood gases, pH, and lactate concentration (ABL 555, Radiometer, Copenhagen, Denmark).

Rats were subjected to hemorrhagic shock and then resuscitated. During resuscitation the animals were randomized to four groups. The Control group ($n = 26$) received resuscitation fluid consisting of a mixture of Plasma-Lyte and 2.5% dextran-40 (Baxter Healthcare Corp., Deerfield, IL). The colloid (dextran) was added to slow the crystalloid flux into the interstitial space. No DRPs were added to the resuscitation fluid. The AVP group ($n = 26$) received the same resuscitation fluid containing 0.0005% (5 ppm) AVP. The PEG-3500 group ($n = 20$) received the same resuscitation fluid containing 0.0005% (5 ppm) PEG-3500, while the PEG-200 group ($n = 21$) was infused with the same resuscitation fluid containing 0.0075% (75 ppm) PEG-200. Viscosities of all resuscitation fluids were measured by a rotational viscometer as described above.

To induce hemorrhagic shock, blood was withdrawn from the right carotid artery until mean arterial pressure (MAP) decreased to 25 mm Hg with no compensation (average withdrawal time was 28.6 ± 2.6 min). At this point, tissue perfusion had typically decreased to 20–25% of baseline. After 5 min of uninterrupted hemorrhagic shock, animals were resuscitated with the fluids described above over a 15 min period to replace lost blood volume. Then, the animals were infused with the same fluid for two hours or until they expired. Neither assisted ventilation nor additional medications were utilized during the hemorrhagic shock and resuscitation periods in any group. Animals, which expired prior to the start of resuscitation (total of 17 animals in all four groups) or at less than 15 min after beginning resuscitation (9 in Control group and 3 in PEG-200 group), were excluded from the data and statistical analyses presented below. None of the animals of the AVP and PEG-3500 groups expired at less than 15 min from the onset of resuscitation. Thus all the animals resuscitated with two DRP solutions are included in the data analyses below.

After the two-hour post hemorrhagic shock period, the experiments were discontinued by a bolus injection of supersaturated potassium chloride. This procedure is consistent with recommended practices of the American Veterinary Medical Association Council on Euthanasia.

Data are presented as the mean \pm standard deviation. ANOVA with repeated measures was performed for all parameters. A two-tailed Student t test for unpaired observations was used to compare each polymer group with Control at each time interval. A two-tailed paired t test was applied to analyze differences between baseline and final endpoint values within each group (Statview; Abacus Concepts, Berkeley, CA). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. *In vitro* circulating system experiments

The addition of 5 ppm of PEG-3500 and AVP to circulating saline produced a marked reduction (up to 60%) in the pressure drop required to achieve a particular flow rate (Fig. 1) signifying a reduction in

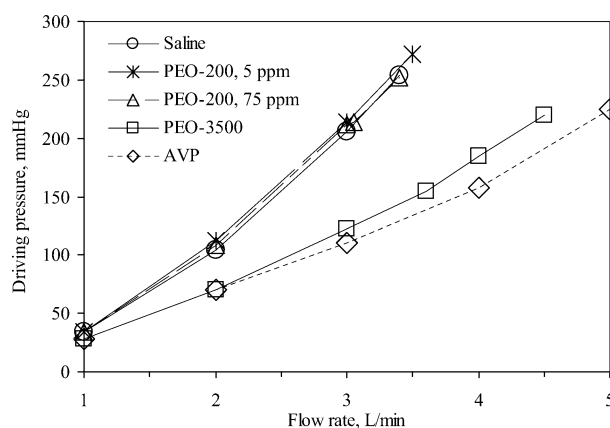


Fig. 1. Driving pressure (pressure drop) vs. flow rate in the mock circulatory system. Nomenclature: saline (\circ), PEG-200 at a concentration of 5 ppm ($*$), PEG-200 at a concentration of 75 ppm (Δ), PEG-3500 (\square), and AVP (\diamond) both at a concentration of 5 ppm.

flow resistance. Polyethylene glycol with lower molecular weight, PEG-200, failed to reduce resistance to flow at both applied concentrations of 5 ppm and 75 ppm, demonstrating the same flow characteristics as for saline (Fig. 1).

3.2. Viscosity data

No measurable effect of either polymer additive on solution viscosity (1.0 cP at 20°C) was found. All polymer solutions demonstrated Newtonian flow behavior; solution viscosity was constant at all applied shear rates (1.28–94.5 s⁻¹). The viscosity of the resuscitation fluid (Plasma Lyte with 2.5% of dextran-40) with or without DRPs was 1.5 cP at 22°C. We measured viscosity of rat blood obtained from a donor animal after the addition of PEG-3500 or AVP at a concentration of 5 ppm, and PEG-200 at a concentration of 75 ppm. Figure 2 shows the data of rat blood viscosity plotted against shear rate. As one can see, there is no quantifiable effect of any additive on blood viscosity at all the investigated shear rates.

No effects of any polymer on either the osmotic or oncotic pressure of the resuscitation fluids and rat blood were found.

3.3. In vitro microchannel experiments

Figure 3 shows the changes in the thickness of the near wall cell-free layer with increase in flow rate during microchannel flow of 20% RBC suspensions. One can see that the thickness of the marginal cell-free layer in the RBC suspension containing 10 ppm of the DRP is significantly thinner than the cell-free layer in the control RBC suspension with no DRP added ($p < 0.001$ at all flow rates). The presence of PEG-200 in RBC suspension did not change near-wall cell-free layer (not shown in figure) similar to the control RBC suspension with no DRPs.

3.4. In vivo experiments

All 46 animals from the AVP and PEG-3500 groups survived the two-hour post hemorrhagic shock period. Only five of the 26 Control animals and three of the 21 PEG-200 animals survived the two-hour period. Moreover, the Control and PEG-200 animals that survived required three times more fluid

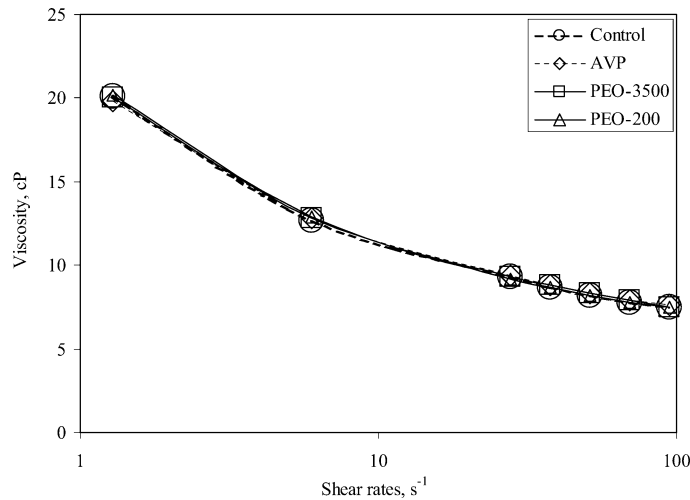


Fig. 2. Viscosity of rat blood ($H_t = 46\%$), vs. shear rate measured by a rotational viscometer (Contraves, Low Shear 30) at room temperature of 22°C . Nomenclature: control (whole blood) (\circ), whole blood with PEG-200 at a concentration of 75 ppm (Δ), PEG-3500 (\square), and AVP (\diamond) at concentrations of 5 ppm.

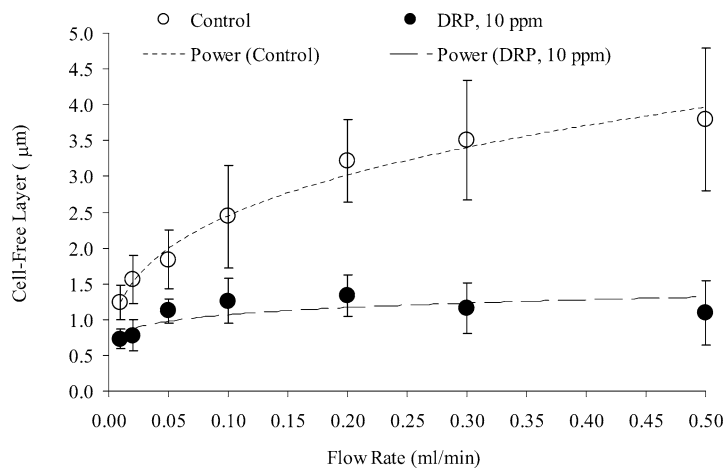


Fig. 3. Thickness of marginal near-wall cell-free layer (in μm) vs. flow rate measured during flow of 20% RBC suspensions with (DRP, 10 ppm) and without (Control) drag-reducing polymers through a microchannel.

to maintain their blood pressure during the post-resuscitation period compared to the AVP and PEG-3500 animals. The mean survival times and volumes of withdrawn blood and infused fluids are given in Table 1.

Baseline hemodynamic parameters (MAP, TP), blood gases, pH, and lactate concentration did not differ significantly among the groups (Tables 2 and 3). All animals showed a significant decrease in MAP and TP after induction of hemorrhage (Table 2). Restoration of the shed blood volume with resuscitation fluid increased blood pressure, but failed to restore tissue perfusion (TP) in the majority of the Control and PEG-200 animals. Control and PEG-200 animals (92% and 90%, respectively) developed severe metabolic acidosis and elevated blood lactate concentrations (Table 3). In contrast, in all AVP and PEG-3500 animals both blood pressure and TP improved significantly after initiating fluid resuscitation

Table 1

Mean values of survival time and withdrawn/infused fluid volumes: ^a*p* < 0.001 vs. PEG-200; ^b*p* < 0.001 vs. CONTROL

Group	Survival time, min	Shed blood volume, ml	Infused fluid volume, ml
AVP	120	9.8 ± 0.6	19.8 ± 1.8 ^{a,b}
PEG-3500	120	9.4 ± 0.9	18.4 ± 3.1 ^{a,b}
PEG-200	71 ± 35.4	9.2 ± 0.6	33.2 ± 18.9
CONTROL	57 ± 35.5	9.3 ± 0.7	27.7 ± 15.2

Table 2

Mean arterial pressure (MAP, mmHg) and tissue perfusion (TP, TPU) at baseline (BASE), at the end of blood withdrawal (Hemorrhagic shock), after replacement of the shed blood volume with resuscitation fluid (Restoration), and at the termination of the experiment (END): ^a*p* < 0.05 vs. PEG-200, ^b*p* < 0.05 vs. CONTROL, ^c*p* < 0.005 vs. BASE, ^d*p* < 0.05 vs. Hemorrhagic shock

DRP	BASE		Hemorrhagic shock		Restoration of the lost blood volume		END	
	MAP	TP	MAP	TP	MAP	TP	MAP	TP
AVP	86.5 ± 5.5	6.4 ± 1.5	24.5 ± 1.1 ^c	1.6 ± 0.6 ^c	61.5 ± 8.1 ^{a,b,c,d}	7.4 ± 1.4 ^{a,b,d}	58.0 ± 4.2 ^{a,b,c,d}	7.6 ± 2.1 ^{a,b,d}
PEG-3500	86.1 ± 5.2	6.2 ± 1.2	24.7 ± 0.8 ^c	1.4 ± 0.6 ^c	62.4 ± 5.0 ^{a,b,c,d}	8.8 ± 3.5 ^{a,b,d}	59.4 ± 4.2 ^{a,b,c,d}	8.7 ± 2.4 ^{a,b,d}
PEG-200	85.8 ± 4.4	5.4 ± 1.0	25.0 ± 0.6 ^c	1.4 ± 0.6 ^c	53.5 ± 9.4 ^{c,d}	2.7 ± 1.2 ^c	33.8 ± 9.6 ^c	1.5 ± 0.8 ^c
CONTROL	87.2 ± 5.9	5.9 ± 1.2	24.9 ± 0.3 ^c	1.4 ± 0.6 ^c	56.0 ± 13.7 ^{c,d}	2.9 ± 1.0 ^c	29.3 ± 9.2 ^c	1.5 ± 0.6 ^c

Table 3

Mean values for blood pH, pO₂ (mmHg) and lactate (mmol/l) measured at baseline and the termination (end) of these experiments: ^a*p* < 0.001 vs. PEG-200, ^b*p* < 0.001 vs. CONTROL, ^c*p* < 0.001 vs. Base

Group	pH		pO ₂		Lactate	
	Base	End	Base	End	Base	End
AVP	7.39 ± 0.03	7.36 ± 0.05 ^{a,b}	83.0 ± 11.1	88.7 ± 8.1 ^{a,b}	0.7 ± 0.2	1.8 ± 0.4 ^{a,b,c}
PEG-3500	7.38 ± 0.03	7.42 ± 0.03 ^{a,b}	80 ± 8.2	84.2 ± 8.5 ^{a,b}	0.8 ± 0.3	1.8 ± 0.6 ^{a,b,c}
PEG-200	7.38 ± 0.03	7.11 ± 0.13 ^c	82.2 ± 8.4	57.5 ± 18.8 ^c	0.8 ± 0.3	6.1 ± 2.8 ^c
CONTROL	7.40 ± 0.04	7.16 ± 0.15 ^c	80.5 ± 8.6	59.8 ± 20.8 ^c	0.9 ± 0.3	5.6 ± 2.2 ^c

(Table 2). At the end of the AVP and PEG-3500 experiments, although MAP remained relatively depressed below baseline values, TP, pO₂, pCO₂, and pH in the final blood samples did not differ notably from the baseline values (*p* < 0.001 vs. Control and PEG-200 final blood samples). Blood lactate values were increased in the final blood samples but remained within the normal range in the AVP and PEG-3500 animals. Hematocrit decreased from the base level of 47 ± 2% in all groups to 26 ± 5% in the AVP animals; to 26 ± 2% in the PEG-3500 group; to 21 ± 6% in the Control animals; and to 20 ± 7% in the PEG-200 group. As noted above, all resuscitation fluids had the same viscosity of 1.5 cP at 22°C and osmolality of 290 ± 5 mOsm.

After considerable reduction due to hemorrhage and shock, tissue pO₂ returned to baseline in animals resuscitated with the two DRP solutions (*n* = 15; Base: 12.5 ± 4.1 mmHg, End: 13.1 ± 5.2 mmHg) but remained significantly depressed in control animals (*n* = 11; Base 12.8 ± 4.6 mmHg, End 2.7 ± 0.7 mmHg, *p* < 0.001).

4. Discussion

In the present study, we demonstrated that two DRPs, natural AVP and synthetic PEG-3500, produced the same beneficial effects on survival rate, microcirculatory flow and tissue oxygenation in animals subjected to hemorrhagic shock. These two polymers are chemically dissimilar, but both possess drag-reducing properties as demonstrated *in vitro*. The polymers produced no effects on osmotic and oncotic pressure. Thus, the mechanisms underlying the described phenomenon of recovery from hemorrhagic shock are most likely based on the fluid dynamic effects and/or viscoelastic properties of the DRPs versus the actual chemistry of these polymers. This statement is supported by the observation that PEG-200, which has the same chemistry as PEG-3500 but lacks the ability to reduce flow resistance *in vitro* or to decrease cell-free layer in microchannels, failed to improve hemodynamics and prevent animal mortality from hemorrhagic shock.

While the exact mechanisms underlying the intravascular DRP effects remain to be identified, they are likely different from the original Toms drag-reducing effect, since there is no turbulent flow in the rat vascular system. Certain hypotheses regarding the microcirculatory enhancement observed in our study and in previous animal experiments can be drawn from other *in vitro* studies regarding DRP effects on flow separation in models of bifurcating vessels. It has been demonstrated *in vitro* [19,20] that under flow conditions corresponding to realistic vascular hemodynamics ($1 \leq Re \leq 400$), DRPs reduce the size of the flow separation and delay the development of vortices at bifurcations. *In vivo*, this mechanism may reduce pressure loss in arterial vessels and thus increase precapillary pressure thereby promoting the elimination of capillary stasis caused by hemorrhagic shock and increasing the density of functional capillaries (tissue perfusion).

Other hypotheses may reflect DRP effects on the flow behavior of RBCs in microvessels. Our recent experiments on the flow of RBCs in 100-micron diameter channels showed a significant decrease in the near-wall cell-free layer thickness in the presence of minute concentrations (5–10 ppm) of DRPs in suspensions [46]. The same polymers with lower molecular weight and with no ability to produce the Toms effect did not change flow behavior of RBCs in our microchannels. In the vascular system, this rheological effect can attenuate the plasma “skimming” and both Fåhræus and Fåhræus–Lindqvist effects in small vessels and, thus, increase local hematocrit, apparent viscosity and wall shear stress in microvessels and capillaries. As demonstrated in the work of Tsai and Intaglietta [42], the increase in viscosity helps to maintain functional capillary density (FCD), which was proven to be the primary factor of survival in hemorrhagic shock. Intaglietta studies “have shown that maintenance of FCD in shock is a critical parameter in determining outcome in terms of survival vs. non-survival, independently of tissue pO_2 , suggesting that extraction of products of metabolism may be a more critical function of capillaries than oxygenation” [22]. As was mentioned in the Introduction, DRPs were previously shown to significantly increase the number of functional capillaries in normal and, especially, in diabetic rats [9]. In diabetic animals, the injection of 1 ppm of polyethylene oxide with MW of 4,000 kDa caused an increase in the total number of open capillaries by nearly 50%, including a considerable increase in the number of functional capillaries of diameter of 3–4 microns, and an increase in the velocity of capillary flow by over 50% [9]. The decrease in the near wall cell-free layer associated with the DRPs is a new phenomenon which has yet to be investigated in detail and explained (this investigation is obviously beyond the scope of this report). We can speculate that alignment and stretch of the polymers along the flow paths may diminish RBC rotation. RBC rotation is important contributor to the formation of a near-wall plasma layer in microvessels. In addition, an increase in blood viscoelasticity due to the presence of DRPs may strengthen non-Newtonian patterns of the axial velocity profile in vessels

(increasing the profile bluntness, decreasing the velocity near the central axis and increasing the velocity near channel/vessel wall). This would change the RBC distribution in the axial core and shift their mean velocity closer to the mean velocity of blood.

Another possible explanation for our present results may be that due to their elasticity, DRPs can enhance the mixing efficiency in capillaries, as was shown in *in vitro* experiments by Groisman and Steinberg [15] at flow conditions similar to those observed at the capillary levels (very low Reynolds numbers and relatively high shear rates). With reference to post-hemorrhagic shock, this effect (increased plasma mixing) might be responsible for the increased transport of oxygen in capillaries, especially, in the so-called “unstirred boundary layer of plasma [26]” surrounding RBCs through which oxygen must diffuse. Using a mathematical model of oxygen transport in a capillary, Wang and Popel [45] demonstrated that the fractional oxygen transport resistance in the plasma region accounts for 65–80% of the total intracapillary resistance. Using a computational analysis, Bos et al. [2] also indicated that mixing in plasma may enhance oxygen transport in capillaries.

We also believe that the mechanisms underlying our observed intravascular DRP effects may be different in various locations within the circulatory system. However, these potential mechanisms will be directly related to the drag-reducing ability of these polymers or particular physico-chemical properties of these polymers which enable the drag-reducing phenomenon. The major support for this statement is that two blood soluble polymers (PEG-3500 and AVP) with completely unrelated chemical structure and occurrence (synthetic versus natural) produced exactly the same effects on blood circulation in our study. Their only shared physical property is that both reduce resistance to turbulent flow (the Toms effect). Moreover, a polymer of the same chemical structure as one of our DRPs (PEG-200), but with lower molecular weight (200 kDa) such that the Toms effect is not produced, is not associated with any salutary hemodynamic effects. As a consequence, PEG-200 does not improve animal hemodynamics and survival in hemorrhagic shock compared to Control animals.

Tissue hypoperfusion is associated with a number of clinical maladies. Most of the standard therapies for treatment of tissue hypoperfusion during various low-flow states are based largely on efforts to improve global hemodynamics (i.e., cardiac output) via infusion of volume-expanding solutions and/or inotropic drugs. This largely overlooks the serious damage that diminished microcirculation may cause in these patients. Often, hypoperfusion effects associated with hemorrhagic shock persist for many hours and sometime days, especially depressed intestinal and renal blood flow. In the present acute experiments, we found that the blood soluble DRPs significantly improved animal hemodynamic parameters and two-hour post-resuscitation survival rate. Further studies are planned to examine the long-term effects of the DRPs on animal post-hemorrhagic shock survival and homeostasis.

In summary, the present work demonstrates that blood soluble DRPs, when injected at nanomolar concentrations (as low as 5 ppm) in animals in hemorrhagic shock, rapidly increased blood pressure and restored microcirculatory flow, resulting in increased oxygen delivery to tissues. These results suggest further investigations of possible applications for DRPs as a new clinical treatment for tissue hypoperfusion caused by hemorrhagic shock.

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