JPET Fast Forward. Published on March 8, 2017 as DOI: 10.1124/jpet.116.239822
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Low Volume Resuscitation for Hemorrhagic Shock: Understanding the Mechanism of PEG-20k*

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Running Title: Actions of Polyethylene Glycol Polymers in Hypovolemic Shock

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Counts:

Number of Text Pages	15
Number of Tables	0
Number of Figures	7
Number of References	29
Abstract Word Count	248
Introduction Word Count	744
Discussion Word Count	1465

Non-Standard Abbreviations:
FITC, Fluorescein Isothiocyanate
JTTS CPG, Joint Theater Trauma Systems Clinical Practice Guidelines
LVR, Low Volume Resuscitation
PEG, Polyethylene Glycol
TICS, Trauma-Induced Capillary Leak Syndrome

Recommended Section Assignment: Cardiovascular

ABSTRACT

Hemorrhagic shock leads to cell and tissue swelling and no reflow from compressed capillaries. Cell impermeants, including polyethylene glycol-20,000 (PEG-20k), reverse ischemia-induced cell swelling, extend low volume resuscitation (LVR) time after shock, and increase tolerance to the low volume state. The purpose of this study was to explore the mechanisms of action of PEG-20k containing LVR solutions. We hypothesized that PEG-20k acts as both an oncotic agent and an impermeant in the microcirculation, which moves water out of the extracellular space and into the capillaries to affect peripheral capillary filling and enhanced perfusion during the low volume state. Rats were hemorrhaged until arterial lactate reached 9-10 mM/L. Then, saline-based LVR solutions containing various impermeant materials were administered (10% blood volume). The LVR times for these solutions was determined by measuring the amount of time required for plasma lactate to climb back to 9-10 mM after LVR administration (low volume tolerance). Capillary blood flow was measured by colored microspheres and blood volume was measured by FITC-labeled albumin dilution. Gluconate (impermeant), albumin (colloid), and PEG-20k (hybrid) increased LVR time over saline by 4-, 3-, and 8-fold, respectively. The combination of impermeant + albumin produced a biological effect that was similar to PEG-20k alone. Capillary blood flow and plasma volume was decreased after shock with saline LVR but increased with PEG-20k, relative to saline. These data are consistent with the hypothesis that PEG-20k may act by establishing multiple osmotic gradients in the microcirculation to drive cell-to-capillary water transfer during hypovolemic shock.

Introduction

Minimizing the use of crystalloids and utilizing blood products after trauma are now becoming mainstream in civilian trauma centers. Damage control resuscitation is also emerging as standard of care for the US Department of Defense, according to the Joint Theater Trauma Systems Clinical Practice Guidelines (JTTS CPG). When blood products are not available for resuscitation, crystalloid solutions are administered. However, only a fraction of infused crystalloid volume stays in the intravascular space and the use of low volume crystalloids has minimal effects on pressure and perfusion (van Lambalgen et al., 1990; Parrish et al., 2015a). The movement of crystalloid fluid from capillary to interstitium is compounded by the increase in capillary permeability from trauma-related inflammation and trauma-induced capillary leak syndrome (TICS) (Stein and Scalea, 2012). Furthermore, crystalloid resuscitation exacerbates TICS, acidosis, hypothermia, and coagulopathy (Duchesne et al., 2010; Stein and Scalea, 2012). Other resuscitation solutions such as hypertonic saline or starch have had disappointing results (Riha et al., 2011; Riha et al., 2013) including concerns and risks associated with their use (Cotton et al., 2006; Duchesne et al., 2010). There remains a need for a better crystalloid fluid that can be given at a low volume to resuscitate patients in hemorrhagic shock awaiting definitive treatment, especially for the prehospital setting. This study tests possible mechanisms of such a solution.

The dominant mechanism of injury in hemorrhagic shock is energy failure secondary to lack of end-organ perfusion and loss of adequate microvascular oxygen transport with subsequent loss of aerobically produced adenosine triphosphate (ATP) (Chaudry et al., 1974). As cells lose ATP due to ischemia, the sodium pump shuts off and sodium ions enter the cell and

accumulate as they run down their electrochemical gradient. Chloride follows electrogenically and water enters the cell osmotically. As water enters ischemic cells, they swell and compress nearby vascular structures, which further aggravates ischemia by reducing local microcirculatory flow (Reffelmann and Kloner, 2002; Rezkalla and Kloner, 2002; Kloner, 2011). Swollen vascular endothelial cells and parenchymal cells compress capillaries to cause no-reflow and promote resuscitation injury and limit oxygen delivery during the low flow state and after full resuscitation (Reffelmann and Kloner, 2002). Reversal of cell swelling with cell impermeants has been used successfully in organ preservation for transplantation (Southard and Belzer, 1980) and recently in shock (Mees et al., 1982; Parrish et al., 2015a; Parrish et al., 2015b). These molecules are permeable to the capillary but impermeable to the parenchymal cell due to size and charge. Thus, this creates an extracellular osmotic gradient that inhibits water entry into the cell.

Parrish et al.(Parrish et al., 2015a) have demonstrated reduced ischemia-induced cell swelling, increased tolerance to the low volume state, and higher survival rates with administration of cell impermeant-based low volume resuscitation (LVR) solutions in a rodent model of severe hemorrhagic shock. It was reasoned that if this occurs because of the creation of an osmotic gradient for fluid movement during ischemia, then a second gradient created with the addition of an oncotic agent in the resuscitation solution would augment the response. Indeed, when gluconate (a cell impermeant) was combined with polyethylene glycol 20,000 (PEG-20k, a colloid) in a low volume resuscitation crystalloid solution, a marked potentiation in low volume tolerance and blood pressure was observed (Parrish et al., 2015a). Surprisingly, when PEG-20k was used alone, it was equally effective as PEG-20k with the impermeant (gluconate). Additional studies demonstrated that PEG-20k, originally believed to be an oncotic agent, has

both oncotic and impermeant effects because some of the material escapes the capillary space (impermeant effects) (Parrish et al., 2015b). This rather rare molecular behavior may explain how PEG-20k alone increased the low volume resuscitation time (tolerance to the low volume state) 8 fold compared to either saline, mixtures of cell impermeants alone, or pure oncotic agents alone (albumin). Specifically, this one agent may be doing double duty as both an oncotic and an impermeant molecule to generate a double gradient for fluid movement in the microcirculation. Therefore, we hypothesized that PEG-20k in shock acts via biophysical effects on water movement in the microcirculation through both cell impermeant and oncotic properties. These properties prevent cell swelling during ischemia, reload the capillaries with isotonic fluid from the interstitial space, and decompress the microcirculation, which all leads to increased capillary perfusion and oxygen transfer in the low volume state. Figure 1 shows the hypothesized biophysical mechanisms of PEG-20k based LVR solutions in low flow and shock states. This study presents the results of experiments that support this hypothesis.

Methods

All animal work was conducted under a protocol approved by the VCU Institutional Animal Care and Use Committee, which is governed by the rules and regulations set forth in the NIH guide and the USDA.

Rodent Shock Model

A low volume resuscitation (LVR) model was used in adult rats to test the impermeant-based LVR solutions used for pre-hospital resuscitation during severe hemorrhagic shock (Parrish et al., 2015a; Parrish et al., 2015b). Adult male Sprague Dawley rats were anesthetized and maintained in a light surgical plane of anesthesia with isoflurane during the study. Isoflurane was delivered through a nose cone with a fraction of inspired oxygen of 100%. The animals were allowed spontaneous respirations to control their own ventilation and carbon dioxide levels. Polyethylene catheters were placed in both femoral arteries for blood pressure monitoring and blood sampling, and a third catheter was placed in a femoral vein for fluid administration. Heparin (500 U/kg) was given intravenously (IV) to maintain catheter patency. A one centimeter midline incision was created to induce some soft tissue injury and for placement of an intra-abdominal temperature probe. Animals were kept at 38°C using a heating pad and an incandescent light source. Arterial blood pressure, heart rate, and temperature were continuously recorded using PowerLab (ADInstruments, Boston, MA).

After a 15 minute stabilization period, arterial blood was removed at 1 ml/min into a syringe to maintain a mean arterial pressure (MAP) of 30-35 mmHg. More blood was withdrawn as the animal compensated, but a maximum hemorrhage limit of 60% of blood volume was set. Blood volume (ml) was estimated as: (weight (g) x 0.06) + 0.77 as previously

described (Arora et al., 2012). A MAP of 30-35 mmHg was maintained until the plasma lactate reached a value between 9-10 mM, as measured every 15 minutes with a hand held lactate analyzer (Lactate Plus, Nova Biomedical, Waltham, MA) and every hour with the ABL-800 blood gas analyzer (Radiometer, Copenhagen, Denmark). Once the target lactate was reached, a low volume resuscitation equal to 10% of the estimated blood volume was given IV over five minutes using a syringe infusion pump. Thirty minutes after LVR, serial lactate measurements were taken until the lactate again climbed back to the 9-10 mM target because the low volume infusions temporarily lower or stall the accumulation of plasma lactate. The main outcome measured was LVR time, which was defined as the length of time from the start of LVR administration to the time when lactate climbed back to 9-10 mM. The LVR time is a surrogate for tolerance to the low volume or shock state. It is the length of time that a patient can safely remain in the low volume state until definitive care and resuscitation are needed, clinically, the "Golden Hour". At the end of the experiment, the animals were euthanized by Euthasol injection or by exsanguination under anesthesia. The maximum obtainable LVR time in this study was fixed to 240 minutes due to an isofluorane exposure rule that limited exposure of the animals to that exposure duration. Figure 2 depicts the experimental protocol.

Fluids used for low volume resuscitation were: 1) Normal saline, 2): 15% gluconate in saline, a cell impermeant, 3) 10% albumin in saline, 4) 10% PEG-20k in saline, and 5) 15% gluconate + 10% albumin in saline. Other outcomes recorded included the lactate at the end of the LVR time, which in most cases was 9-10 mM by definition. Mean arterial pressure (MAP) was also recorded throughout the experiment. Test agents used for IV resuscitation (sodium gluconate, bovine albumin, and PEG-20k were obtained from Sigma-Aldrich, St. Louis, MO).

Regional Blood Flow

In another series of studies (n = 6), local capillary blood flow was studied using the colored microsphere technique (Adams et al., 2001; Parrish et al., 2015a). Animals were prepared as previously described but a catheter was also placed into the aortic root, using real time pressure and pressure waveforms as indicators of catheter tip location by identifying the aortic valve. During the stabilization/baseline period, 300 µL colored microspheres (Triton Technologies, San Diego, CA) were rapidly injected into the aortic root as a calibrated arterial reference blood sample was simultaneously removed from the femoral artery catheter with a withdrawal pump at a constant rate of 0.25 ml/min. A different color microsphere was injected 30 minutes after LVR. After the study, tissue samples were removed from major organs, and microspheres were recovered from the tissue samples and reference arterial blood samples by alkaline digestion and repeated centrifugations. Dye coating the purified colored microspheres was extracted with acidified 2-ethoxyethyl acetate and quantitated using a UV-VIS spectrophotometer (Shimadzu). Individual colors were resolved using a matrix inversion algorithm from the composite spectra. Blood flow was calculated by the tissue dye content using the reference blood draw as a blood flow standard. Correction for microsphere loss occurred using the recovery of blue microspheres that were added to the tissues as an internal standard prior to digestion (10,000 spheres added per sample). All flows were normalized to 100 g tissue weight and expressed as the change from baseline values before shock and low volume resuscitation.

Blood Volume Determinations:

Total blood volume of rats after shock and after various times following LVR were calculated using the indicator dilution technique (Iijima et al., 1998; Ertl et al., 2007). An I.V.

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bolus of FITC-Albumin (Sigma-Aldrich, St. Louis, MO) of known volume and activity was administered and a reference I.V. sample was taken 15 minutes later for estimation of the volume dilution effect. Plasma volume was calculated by the degree of FITC dilution using a standard dilution curve with saline. Plasma volume was divided by 1-Hct to determine the circulating blood volume. Blood volume was also assessed by the same indicator dilution principle using hematocrit during the LVR period. The assumptions of this method were: 1.) The red blood cells stay in the vascular compartment during LVR; 2.) The volume of the packed cell component remains constant during LVR (because no further bleeding is allowed), and; 3.) Changes in Hct during LVR are inversely proportional to changes in the plasma volume component of the intravascular space. Baseline blood volumes before shock were estimated using a formula as previously described (Parrish et al., 2015a).

Circulating cytokines:

To determine a possible role for early Th1 cytokines in this model, we measured plasma levels of IL-1β and TNFα because they are representative of early Th1 cytokines that may be formed during hypovolemic shock and early resuscitation injury and play a role in later autolytic inflammation (Sato et al., 2008). Cytokines from plasma samples were determined by standard ELISA using commercially available kits (Boster Bio, Pleasanton, CA).

Statistical Analysis:

Data are expressed as mean ± standard deviation. Each group consisted of 5-9 rats, which was derived from power analysis and the known variance of the data from similar studies. Data were analyzed by one-way or two-way analysis of variance (ANOVA) and Bonferroni's multiple

comparison correction using the InStat program (GraphPad Software, Inc., La Jolla, CA). A *p*-value < 0.05 was considered statistically significant.

Results

The effects of a variety of chosen LVR solutions on LVR time are shown in Figure 3. Normal saline was the control LVR fluid, which produced a LVR time of 34 ± 8 minutes. The LVR time significantly increased to 114 ± 10 minutes and 92 ± 20 minutes in the gluconate and albumin groups, respectively, compared to saline. The LVR time for the PEG-20k group was 240 ± 0 minutes ($p < 0.05$, relative to saline, gluconate, and albumin groups). This LVR time was cut off for technical reasons and would have been higher because the lactate at 240 minutes was only 1.2 mM, which is well below the target cutoff of 9-10 mM. There was no significant difference in LVR times between PEG-20k and the albumin + gluconate treated groups (240 ± 0 min and 225 ± 24 min, respectively). However, the true comparison between these two groups is unknown because the full LVR time in the PEG-20k group was not realized because the lactate target was not reached due to an isofluorane anesthesia duration of exposure rule (240 min). This was technically the result of time dependent anesthesia problems in the animals after 4 hours. Therefore, the reported magnitude of the PEG-20k effect during shock in Figure 3 is underestimated when measured by the LVR time.

Similar to the LVR times, the mean arterial blood pressure in the impermeant, PEG-20k, and albumin groups were much higher throughout the LVR period, compared to the saline LVR control (Figure 4). Generally, the mean arterial blood pressures during LVR correlated with the

LVR times such that the groups with the longest LVR time (PEG-20k) also had the highest MAP and vice-versa (Figure 4).

Reductions in capillary blood flow after shock and LVR, as measured by the colored microsphere technique, were significantly less in all organs and tissues (except the ileum) during the LVR period in PEG-20k resuscitated animals, relative to the saline resuscitated control animals (Figure 5). All flow values (except in the left ventricle) in the saline group after LVR were statistically lower than their paired baseline values and all flow values in the PEG-20k group after LVR were statistically unchanged from their paired baseline values. In other words, shock and LVR with saline caused significant reductions in local tissue blood flow, which was prevented when PEG-20k was used as the LVR solution.

Blood volume measurements made after hemorrhage and at 15, 30, and 60 minutes after LVR administration in saline and PEG-20k groups are shown in figure 6. Blood volume was estimated prior to shock. Shock significantly reduced blood volume in both groups, relative to baseline. Resuscitation with PEG-20k significantly increased blood volume at all times after LVR compared to the saline control LVR solution using either indicator dilution technique. Resuscitation with low volumes of 10% PEG-20k, but not saline, caused blood volume to significantly increase above values observed after hemorrhage.

Plasma levels of IL-1β and TNFα at baseline, after hemorrhage, and 60 min after low volume resuscitation are shown in Figure 7. IL-1β concentrations after saline LVR were statistically higher compared to the corresponding levels after PEG-20k LVR. All other values were not significantly different either during the shock protocol for either cytokine or between treatment groups for any corresponding time points during the protocol for either cytokine.

Discussion

Our previous studies have demonstrated efficacy of a novel platform of low volume resuscitation crystalloid solutions in extending the tolerance to the low volume state or the amount of time that a severely shocked patient can safely remain in the low volume state until definitive resuscitation and medical care are delivered. These solutions contain cell impermeants and are designed specifically to reduce the amount of ischemia-induced cell swelling during shock. Low volume resuscitation solutions that used the specific polymer PEG-20k produced striking biological effects that increased the tolerance to the low volume state (LVR time) 4-8 fold over previous classes of smaller cell impermeants or conventional saline based resuscitation fluids. The objective of this study was to explore the mechanisms of action of PEG-20k that account for these strong biological and pre-clinical effects.

Simple cell impermeant molecules like gluconate, trehalose, and raffinose have been used in organ preservation solutions to prevent tissues from swelling in cold ischemic environments. Ideal cell impermeants are molecules that have a unique size enabling them to freely escape the capillary space but not cross the cell membrane because they are too big or charged. Thus, they accumulate outside of the cell and osmotically hold water from entering the cell, which is its normal propensity during shock and ischemia when energy dependent volume control mechanisms fail (Na^+/K^+ ATPase). These simple impermeants prevented cell and tissue swelling in rodent models of hemorrhagic shock when introduced into low volume resuscitation

solutions. Their low toxicity and chemical inertness allows them to be successfully used in high concentrations capable of exerting these necessary biophysical effects on water shifts during shock. Gluconate quadrupled the low volume resuscitation time compared to saline. In an attempt to optimize this effect, we added colloidal molecules to create a second osmotic gradient in the microcirculation, which was designed to pull water into the capillary space. The first studies used PEG-20k as a colloid together with the simple impermeant gluconate. This increased the LVR time 7-fold in the rodent model, which suggested that the double gradient approach may have worked. However, subtraction experiments using only PEG-20k without the gluconate produced the same effect as the two together. It was then hypothesized that the larger PEG-20k molecule may be acting as both a cell impermeant and a colloidal molecule.

Further studies indeed determined that this was true since the osmotic reflection coefficient for PEG-20k was determined to be 0.5 in the rat microcirculation (thoracic and mesenteric beds) under non-shock conditions (Parrish et al., 2015b). This means that roughly 1/3 of the PEG-20k escapes the capillary space to load into the interstitium where it acts like a simple cell impermeant (gluconate) and 2/3 of the molecules in the circulation remains behind in the capillary space where it acts as a colloidal agent to produce the second osmotic gradient. While this double gradient effect of PEG-20k is completely and unambiguously supported by the biophysical osmotic reflection coefficient data, the translation of these properties into the strong biological effects seen with PEG-20k remain less certain. To make this biological link and support the hypothesis that the second osmotic gradient indeed contributes to the very long LVR time of PEG-20k during shock, we attempted to recapitulate the biological effect of PEG-20k with two distinct molecules used together: a small ideal cell impermeant (gluconate) that produces an osmotic gradient between the intracellular and the extracellular space and a classic

colloidal agent (albumin) that produces a second osmotic gradient between the interstitial space and the capillary (intravascular) space.

Indeed, the use of these two distinct molecules (albumin and gluconate) produced a biological effect during low volume resuscitation that was similar as PEG-20k when used alone. This supports the hypothesis that the biological effect seen with PEG-20k may be due, in part, to its unique biophysical attributes that allow it to behave as both an ideal impermeant and a colloid in the microcirculation during shock states. However, since we do not know the exact LVR time with PEG-20k alone because the period was terminated early, biological differences between this group and the albumin + gluconate group probably exist, which suggests that not all of the PEG-20k effect may be attributable to osmotic gradients. While other biological effects of PEG-20k are likely in these settings, they currently remain unknown. Protection and hydration of the shock-eroded glycocalyx and induction of cell surface immuno-camouflage by PEG-20k polymers remain strong possibilities.

Rats receiving saline had a transient increase in MAP during LVR infusion, but the pressure started to drop as soon as the infusion stopped. This is because saline physiologically distributes unequally between the vascular and interstitial compartments. About 20% of the administered saline volume will remain in the intravascular space and 80% goes elsewhere (Haupt, 1986; Haupt, 1989). Since only the 20% remaining in the capillaries supports arterial pressure, it is not surprising that saline given in low volumes during shock produce poor or absent effects on the arterial pressure. When PEG-20k based LVR solutions are administered, it may prevent saline from loading into the interstitium while simultaneously returning the fluid that leaked into the interstitium back into the capillary spaces during the shock period (Gosling, 2003; Keel and Trentz, 2005; Kumar et al., 2010). These passive volume shifts reduce local

tissue swelling, decompress the microcirculation, reduce resistance to flow, and reload the capillaries. All of these changes drive local tissue perfusion and provide cardiac preload. This was demonstrated directly by the increase in MAP and indirectly by the rapid clearance of lactate in LVR and by direct measurements of increased capillary blood flow with PEG-20k compared to saline. This is further supported by the significant effects of PEG-20k solutions on expansion of the plasma and blood volumes after their administration during the LVR period. PEG-20k maintenance of perfusion pressure and lactate clearance in the low volume state can extend up to eight hours (unpublished data), which was the longest period so far examined.

Hemorrhagic shock decreases oxygen delivery, which results in the accumulation of oxygen debt during the low volume state. Using lactate clearance directly and the LVR time indirectly, which relies on lactate levels, we were able to clearly demonstrate that PEG-20k based LVR solutions both stop accumulation of oxygen debt and rapidly repay the debt even during the low volume state. This is supported by the rapid drop in lactate levels after PEG-20k based LVR and the extremely long LVR times, relative to the values seen with conventional saline LVR. While about 50% of this drop in lactate can be attributable to dilution from an expanding intravascular volume, much of the remaining lactate clearance may be due to increased efficiency of microvascular capillary oxygen transfer and/or an overall increase in oxygen delivery, which drives the conversion of lactate back to pyruvate for subsequent aerobic ATP synthesis. In fact, preliminary studies in a large animal porcine model of shock and low volume resuscitation indicate that PEG-20k based LVR under similar low volume conditions leads to a hyperdynamic cardiovascular response characterized by cardiac output increasing 50% higher than pre-shock baseline values over much of the low volume state (Plant et al., 2016). These combined factors likely account for the apparent rapid oxygen debt repayment and the

100% overnight survival (Parrish et al., 2015a) seen in PEG-20k treated rodents. In patients with long pre-hospital transport times, this can limit further ischemic and reperfusion injury and possibly begin debt repayment during the transport period.

A likely mechanism of action of PEG polymers in LVR not addressed by this study include reconstruction of the endothelial glycocalyx by PEG-20k. Shock and crystalloid resuscitation are known to erode the glycocalyx, thus promoting resuscitation injury by promoting cellular inflammation (Torres et al., 2013a; Torres et al., 2013b). Polyethylene glycol polymers are known to bind to the cell membrane with their accompanying water layers (Neu et al., 2003) that could effectively rebuild the glycocalyx during the low volume resuscitation and reperfusion period (Hauet and Eugene, 2008). While this likely happens, it seems that such effects would be expressed after longer periods of resuscitation since cellular inflammation may require hours rather than minutes, which is the time period where rapid capillary blood flow and lactate clearance were observed with PEG-20k in this study. It is reasonable to suggest that PEG-20k LVR may reload peripheral capillaries by early osmotic water transfer while having later effects on glycocalyx-mediated cellular inflammation. Our cytokine data also support this.

In conclusion, 10% PEG-20k is a novel low volume resuscitation fluid with encouraging potential for pre-hospital use in hemorrhagic shock. By improving local oxygen delivery and capillary perfusion, these solutions increases tolerance to the shock state during prolonged pre-hospital and transport periods. This study tests a likely biophysical mechanism for its efficacy, namely, the osmotic cell-to-capillary transfer of accumulated water that drives efficient local perfusion under low volume conditions.

Authorship Contributions:

Participated in the research design: Plant, Parrish, Limkemann, and Mangino

Conducted Experiments: Plant, Parrish, Limkemann, and Mangino

Performed Data Analysis: Plant and Mangino

Wrote or contributed to the writing of the manuscript: Plant, Parrish, Limkemann, Ferrada, Aboutanos, and Mangino

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Footnotes

* This work was supported by grants from the Department of Defense (W81XWH-12-1-0599) and (W81XWH-16-2-0040) to Dr. Mangino. This study was presented, in part, at the 74th Annual Meeting of the American Association for the Surgery of Trauma, September 9-12, 2015, in Las Vegas, Nevada

Figure Legends

Figure 1. Hypothetical mechanism of action for PEG-20k in low volume resuscitation for shock. Cell impermeants can escape the capillary but are too large or charged to enter cells, and thus create an osmotic gradient to prevent cell swelling during shock. We hypothesized that PEG-20k acts via biophysical effects on water movement through both cell impermeant and oncotic properties. To test our hypothesis, we tried to recapitulate the PEG-20k effect by combining a cell impermeant (gluconate) with a colloid (albumin). The movement of capillary water with oncotic agents increases capillary pressures that promote capillary flow even under low-volume states. The sum effect is to promote effective and efficient capillary transport and oxygen delivery in the low-volume state. I.S., Interstitial space.

Figure 2. Timeline diagram of the hemorrhagic shock and resuscitation protocol. Rats were hemorrhaged to a mean arterial pressure of 30-35 mmHg. Once lactate reached 9-10 mM, low volume resuscitation (LVR) was administered. The primary outcome was LVR time, which is the length of time from start of LVR to the time at which lactate climbed back to 9-10 mM.

Figure 3. The effect of different LVR solutions on LVR time. Data are presented as mean (SD). Numbers below bars indicate mean lactate at the end of LVR time, which by definition should be 9-10 mM. Numbers above bars indicate sample size. All the treatment groups had significantly

higher LVR times than the saline (control) group. There was no significant difference between PEG-20k and the albumin + impermeant group.

Figure 4. Mean arterial pressures after LVR administration, measured at 15 minutes, 30 minutes, throughout the LVR period, and at end of LVR time. * $p < 0.05$ relative to the other values at the same corresponding times in the other groups. # $p < 0.05$ relative to all other values.

Figure 5. Capillary blood flow measured 30 minutes after LVR. Data are presented as mean (SD) as % change from baseline. Each rat served as its own baseline. * $p < 0.05$ compared to saline group. For the saline group, all flows except left ventricle were statistically less than paired baseline flows. For PEG-20k, all flows were not different from baseline. n = 5.

Figure 6. Circulating blood volume measured in rats by the indicator dilution technique using a FITC-labelled albumin probe (A) or hematocrit (B) to estimate the size of the intravascular fluid compartment during low volume resuscitation with saline or saline containing 10% PEG-20k. Blood volume values were estimated in the rats before shock (Baseline, BL), and measured with indicator dilution after the hemorrhagic shock (HS) period, and after the low volume resuscitation (LVR) period. Values are mean ± SD for 4 rats in each group. * $P<0.05$ relative to the corresponding value in the saline group. Both LVR solutions were given at a volume of 10% of the estimated baseline blood volume. Saline was 0.9% NaCl solution and PEG-20k was a 10% weight to volume solution of polyethylene glycol (mean mw = 20,000 Da) dissolved in saline.

Figure 7. Plasma IL-1β (A) and TNFα (B) concentrations in rats at baseline, after hemorrhagic shock, and after 60 minutes of low volume resuscitation (LVR-60). Rats received either a saline LVR or a PEG-20k LVR after shock equal to 10% of the calculated blood volume administered

over 10 minutes. All values are mean ±SD, n = 6, *P<0.05 relative to the corresponding saline value.

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Figures

Figure 1

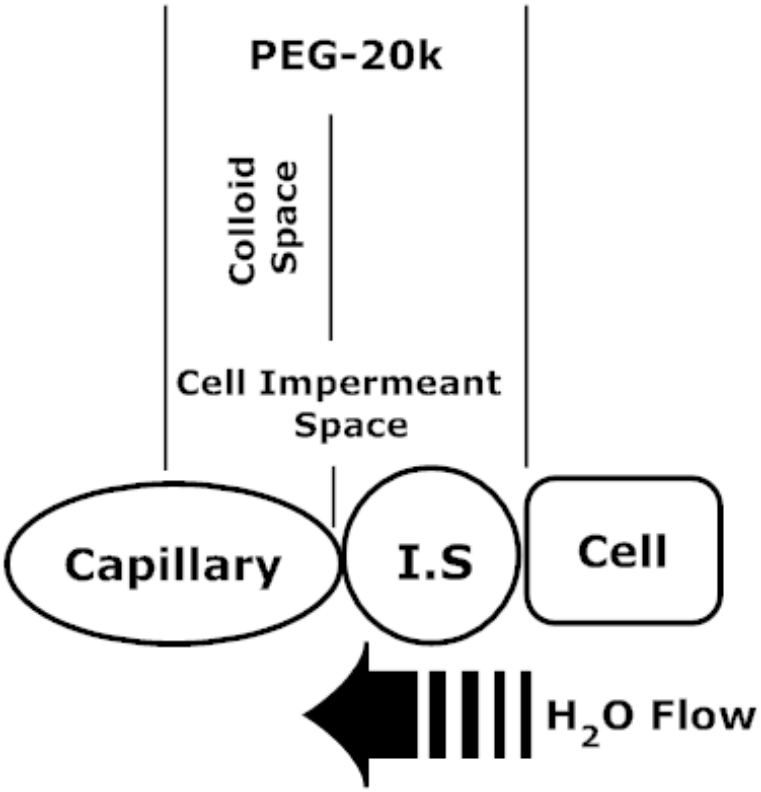

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Figure 2

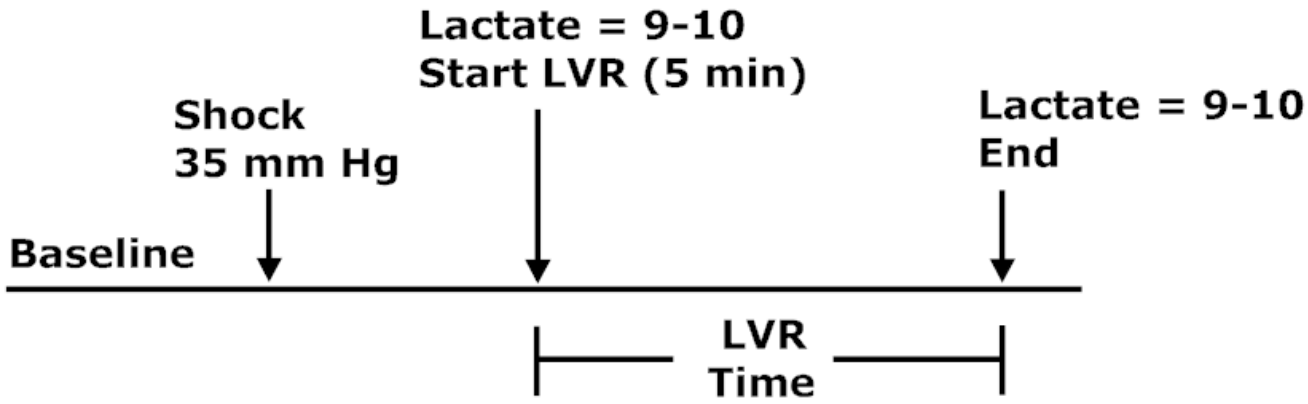

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Figure 3

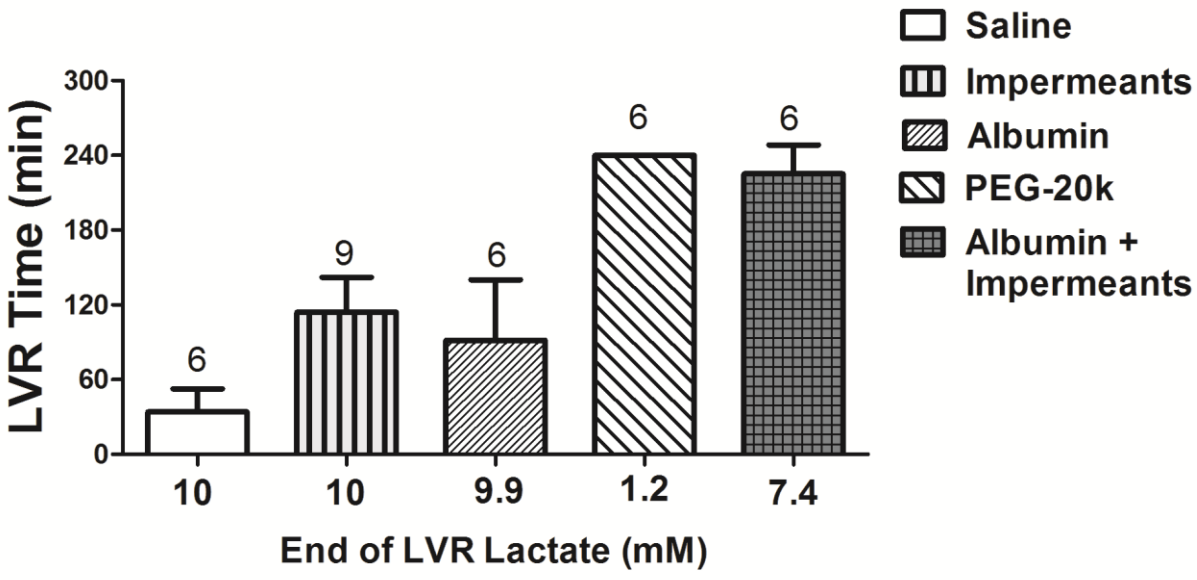

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Figure 4

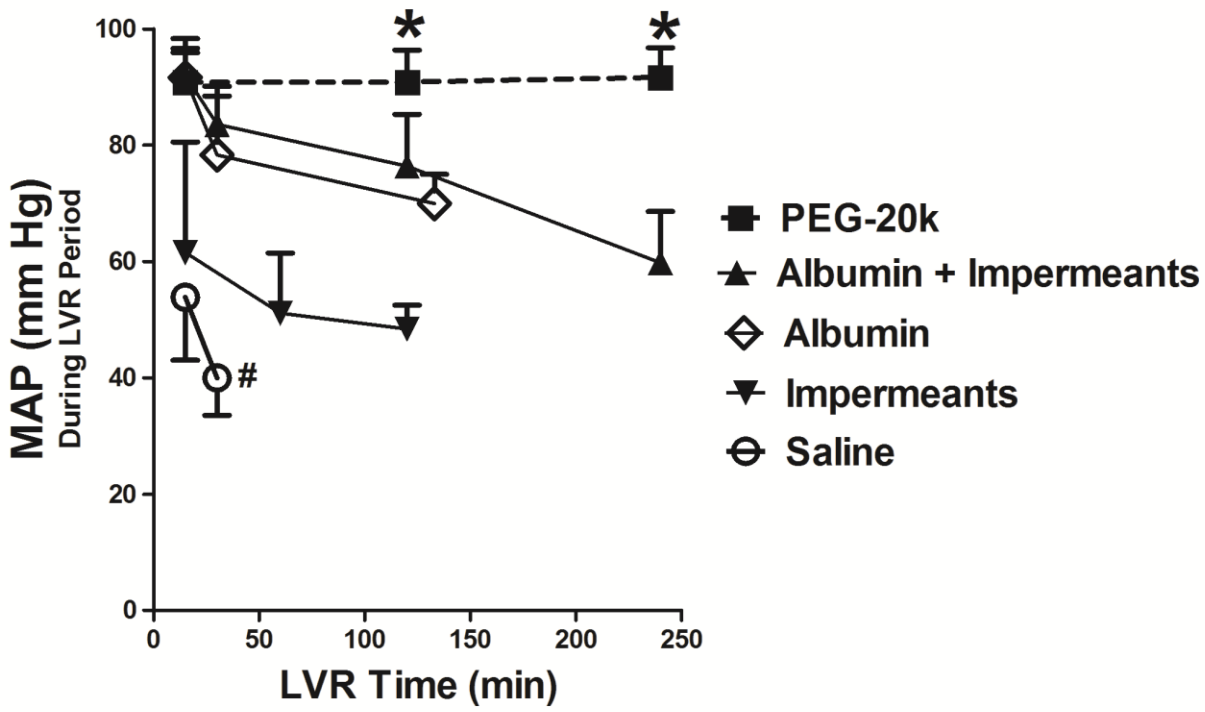

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Figure 5

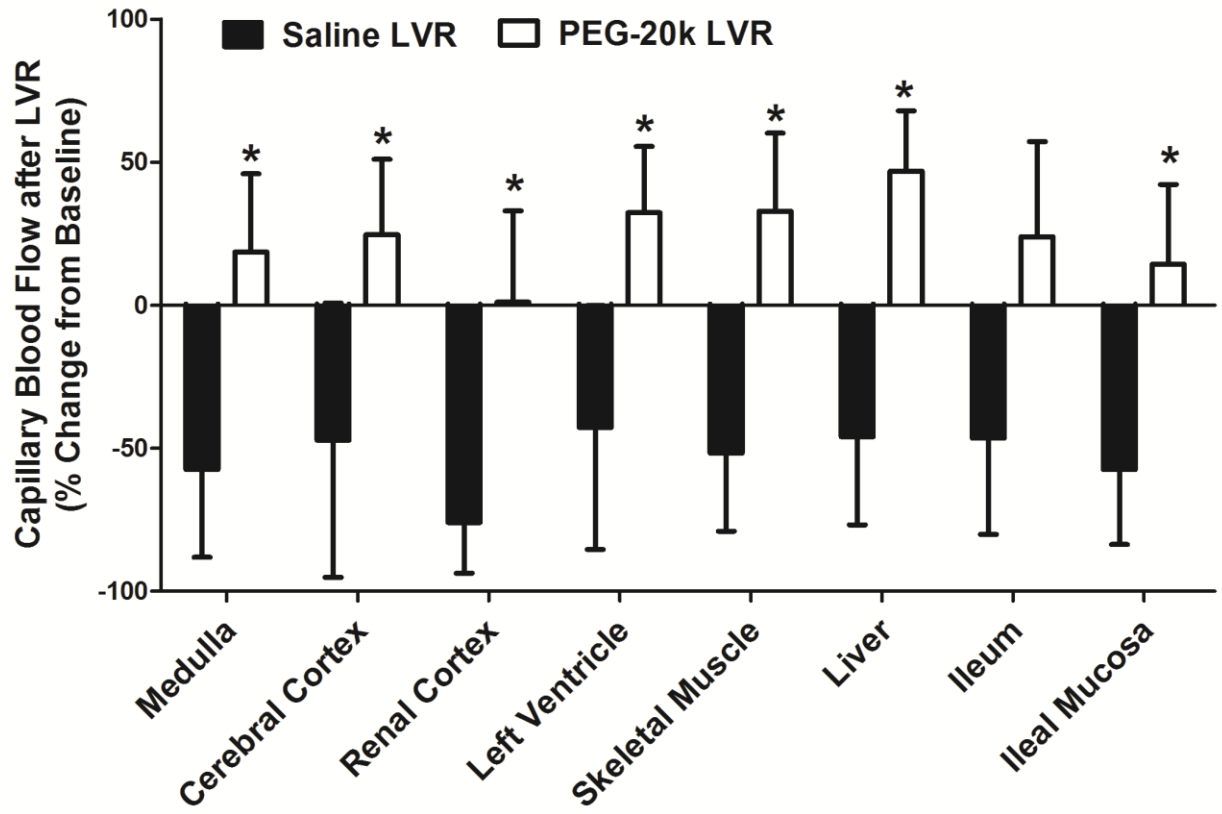

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Figure 6

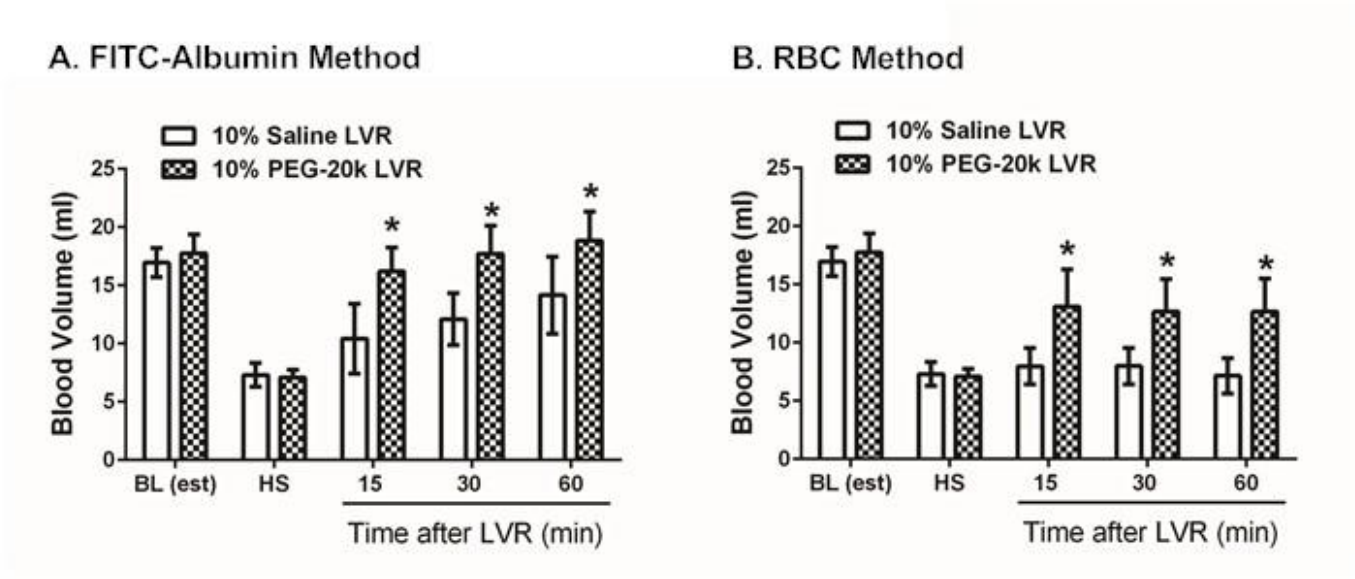

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Figure 7