

## New low-volume resuscitation solutions containing PEG-20k

Dan Parrish, MD, Valerie Plant, MD, Susanne L. Lindell, Ashley Limkemann, MD, Heather Reichstetter, LVT, Michel Aboutanos, MD, and Martin J. Mangino, PhD, Richmond, Virginia

- BACKGROUND:** Hypovolemic shock reduces oxygen delivery and compromises energy-dependent cell volume control. Consequent cell swelling compromises microcirculatory flow, which reduces oxygen exchange further. The importance of this mechanism is highlighted by the effectiveness of cell impermeants in low-volume resuscitation (LVR) solutions in acute studies. The objectives of this study were to assess impermeants in survival models and to compare them with commonly used crystalloid solutions.
- METHODS:** Adult rats were hemorrhaged to a pressure of 30 mm Hg to 35 mm Hg, held there until the plasma lactate reached 10 mM, and given an LVR solution (5–10% blood volume) with saline alone (control) and saline with various concentrations of polyethylene glycol-20k (PEG-20k), Hextend, or albumin. When lactate again reached 10 mM following LVR, full resuscitation was started with crystalloid and red blood cells. Rats were either euthanized (acute) or allowed to recover (survival). The LVR time, which is the time from the start of the LVR solution until the start of full resuscitation, was measured as was survival and diagnostic laboratory values. In some studies, the capillary oncotic reflection coefficient was determined for PEG-20k to determine its relative impermeant and oncotic effects.
- RESULTS:** PEG-20k (10%) significantly increased LVR times relative to saline (eightfold), Hextend, and albumin. Lower amounts of PEG-20k (5%) were also effective but less so than 10% doses. PEG-20k maintained normal arterial pressure during the low-volume state. Survival of a 180-minute LVR time challenge was 0% in saline controls and 100% in rats given PEG-20k as the LVR solution. Surviving rats had normal laboratory values 24 hours later. PEG-20k had an oncotic reflection coefficient of 0.65, which indicates that the molecule is a hybrid cell impermeant with significant oncotic properties.
- CONCLUSION:** PEG-20k-based LVR solutions are highly effective for inducing tolerance to the low-volume state and for improving survival. (*J Trauma Acute Care Surg.* 2015;79: 22–29. Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.)
- KEY WORDS:** Cell swelling; shock; polyethylene glycol; rats.

Deaths caused by injury in the United States reached more than 190,000 and cost more than \$400 billion a year in health care costs and lost productivity in 2012.<sup>1</sup> Deaths from trauma are the number one cause of death for people younger than 44 years in the United States and the third leading cause of death overall for all age groups. Trauma accounts for approximately 30% of all life-years lost in the United States, compared with cancer (16%), heart disease (12%), and human immunodeficiency virus (2%).<sup>2</sup> For all traumatic injuries, hemorrhagic shock is responsible for more than 35% of prehospital deaths and more than 40% of all deaths within the first 24 hours. This is second only to trauma deaths induced by severe central nervous system injury.<sup>3</sup> Finally, hemorrhagic hypotension exposes the patient to immediate complications of life-threatening infections, coagulopathies, and multiple-organ failure.<sup>4,5</sup>

Early resuscitation strategies include the use of low volumes of intravenous blood products to increase oxygen

delivery and to replace lost coagulation and clotting factors (coagulation proteins and platelets). While this approach is fine for hospital emergency departments, it is not currently practical in prehospital settings where early intervention may be the key to preventing future complications following more definitive resuscitation. Crystalloids are available for prehospital use because they can be safely transported and stored but they are generally limited in their effectiveness. Attempts to modify basic intravenous crystalloids for prehospital resuscitation by adding hypertonic NaCl or starch (Hextend) as a volume expander have had disappointing results.<sup>6,7</sup> The future use of effective spray dried blood products will be a valuable tool in prehospital settings since they replace chemical coagulation precursors and factors. The use of fresh frozen plasma in the field, which is currently being tested at many centers, will also be useful, but it too is limited by the need for refrigeration.<sup>8</sup> There remains a need for a better crystalloid to resuscitate patients with severe hemorrhagic shock, especially in a prehospital setting. The successful design of such a solution is highly dependent on understanding the pathophysiologic mechanisms that lead to injury during hemorrhagic hypotension and subsequent resuscitation. The optimal solution will likely be an effective new stable crystalloid that targets these mechanisms used together with reconstituted dried plasma products for the replacement and reconstitution of coagulation potential.

The predominant root mechanism of injury in hemorrhagic shock is energy failure. While global ischemia and reperfusion injury are causally based at many levels, they all arise

Submitted: December 2, 2014, Revised: February 19, 2015, Accepted: February 23, 2015.  
From the Department of Surgery, Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, Virginia.

\*D.P. and V.P. equally contributed as first authors.

This work was presented, in part, at the 28th Annual Scientific Assembly of the Eastern Association for the Surgery of Trauma, January 13–17, 2015, in Lake Buena Vista, Florida.

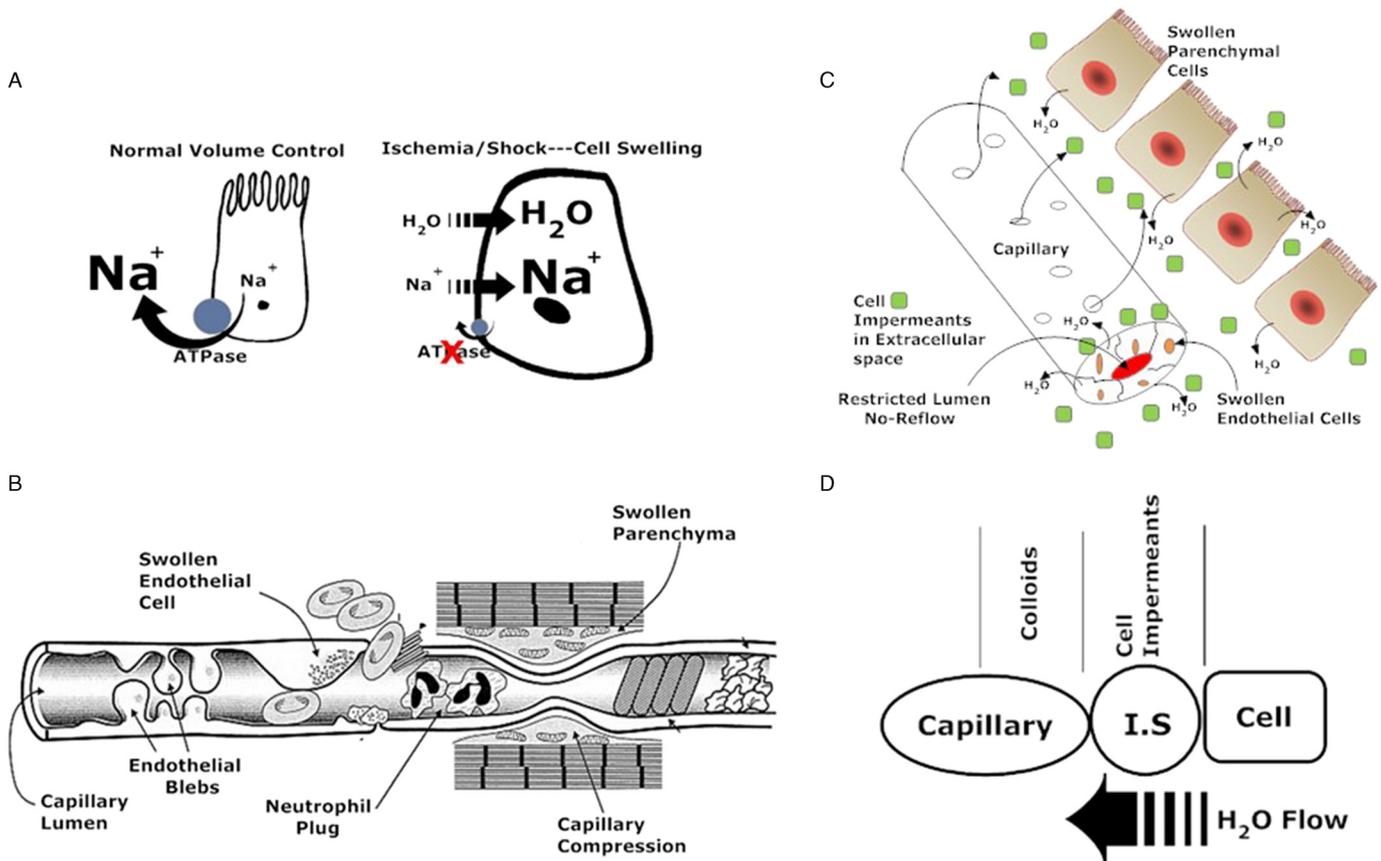
Address for reprints: Martin J. Mangino, PhD, Virginia Commonwealth University; Medical College of Virginia Campus; Department of Surgery; 1101 E Marshall St, Richmond, VA 23298; email: mjmangino@vcu.edu.

DOI: 10.1097/TA.0000000000000682

from changes that occur when the cell energetics drops because of a loss of adequate microvascular oxygen transport and subsequent loss of aerobically produced high-energy adenine nucleotides.<sup>9-11</sup> One mechanism of cell, tissue, and organ injury is cell swelling that occurs from the loss of adenosine triphosphate (ATP)-dependent cell volume regulatory control mechanisms. In most cells, the single highest energy consuming process is the running of the Na/K ATPase pumps in the cell membrane. These pumps actively transport sodium ions out of the cell to maintain membrane potentials and to run numerous Na<sup>+</sup>-dependent facilitated membrane transport processes such as calcium, glucose, amino acids, and organic cation transporters. In the absence of ATP to run those pumps, as occurs in ischemia following hemorrhagic shock, the Na/K ATPase turns off, and sodium enters the cell as it runs back down its electrochemical gradient. The elevated intracellular sodium futilely stimulates the sodium pump that cannot run

because of the loss of ATP.<sup>12</sup> Chloride then enters the cell down an electrical gradient, and water follows the sodium chloride down a developing osmotic gradient, which causes the cell to swell. Hydropic degeneration from energy failure damages membrane and mitochondrial structures,<sup>13</sup> which may lead to cell death. Swelling of parenchymal cells can also compress local capillaries, leading to further reduced capillary flow and oxygen delivery causing a self-amplifying cycle. Figure 1 shows how this mechanism occurs and how novel cell impermeant molecules can passively reverse this dangerous water flow.

This basic mechanism of cell ischemic injury has been well described in organ preservation associated with transplantation.<sup>14-16</sup> Effective modern organ preservation solutions were developed around this concept and contain high concentrations of cell impermeants.<sup>17</sup> These are classes of nontoxic molecules, usually saccharides and small organic cations and anions, which are small enough to freely egress the capillary space in the



**Figure 1.** Proposed mechanism of action of cell impermeants in the nonenergetic rebalancing of water movements during low-volume shock states. *A*, The original defect is caused by the energy-dependent collapse of the Na/K ATPase activity during shock because of low oxygen delivery and loss of ATP. As the pump fails, Na<sup>+</sup> enters the cell followed by water. *B*, Swollen parenchymal cells compress local capillary networks in the tissue that increase the resistance to capillary blood flow and further impede microcirculatory oxygen delivery. This allows local lactates to rise. *C*, Loading the interstitial space with cell impermeants such as gluconate or raffinose prevents ischemia-induced water movement (swelling) by osmotically holding water in the interstitial space. This prevents capillary compression and preserves local exchange capacity, even under low-volume conditions. *D*, The inclusion of an oncotic molecule with an impermeant establishes an osmotic-oncotic gradient between the intracellular-interstitial-capillary compartments, which promotes further the energy independent flow of water from the cell (where it should not be) into the capillary (where it should be). The movement of capillary water with oncotic agents then 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.

microcirculation but are too large or too charged to cross the cell membrane. As such, they preferentially load into the interstitial space where they create an osmotic force that prevents the movement of water into the cell as the sodium concentrations rise during ischemia. They prevent lethal cell swelling. Cell impermeant, as a class of agents, are one of the most effective components of organ preservation solutions used today.<sup>18</sup> The University of Wisconsin solution contains high amounts of raffinose, lactobionic acid, sulfate, and phosphate, which all act as cell impermeants to prevent water movement. The Belzer-UW MPS solution uses gluconate, and HTK solution uses both high concentrations of histidine and mannitol as impermeants. Water movement in organ preservation is slower than ischemia at normal mammalian temperatures because hypothermia is used to preserve organs, which slows down the process. Since cell swelling during ischemia induced by hemorrhagic hypotension also occurs<sup>19</sup> and at a much faster rate than in organ preservation because of the warmer temperatures, it was hypothesized that loading the interstitial space with nontoxic cell impermeants during the low-volume period would prevent lethal cell swelling and increase the tolerance of the patient to the low-volume state and improve outcomes at resuscitation. In fact, acute studies in rodents with severe hemorrhagic shock indicate that small cell impermeants double the tolerance of animals to the low-volume state.<sup>20</sup> This study further found that one particular molecule, polyethylene glycol-20k (PEG-20k) increased the tolerance to the low-volume state 6 fold, compared with saline controls. It was hypothesized that PEG-20k superiority was due to the molecule behaving as hybrid where some escapes the capillary space to act as an impermeant to prevent water movement into the cell and a large portion of the molecule stays behind in the capillary to exert oncotic force that draws the interstitial water into the capillary. This was supported by observations that PEG-20k in LVR solutions also normalizes the arterial blood pressure in the low-volume state immediately after administration.<sup>20</sup> This previous study neither assessed the effects of PEG-20k based LVR solutions in a survival model nor compared them with standard crystalloid solutions used today. This was the objective of the current study. We hypothesize that PEG-20k added to LVR solutions possesses both impermeant and colloidal properties that greatly improve outcomes in a low-volume resuscitation (LVR) model of severe hemorrhagic shock.

## MATERIALS AND 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 National Institutes of Health guide and the US Department of Agriculture.

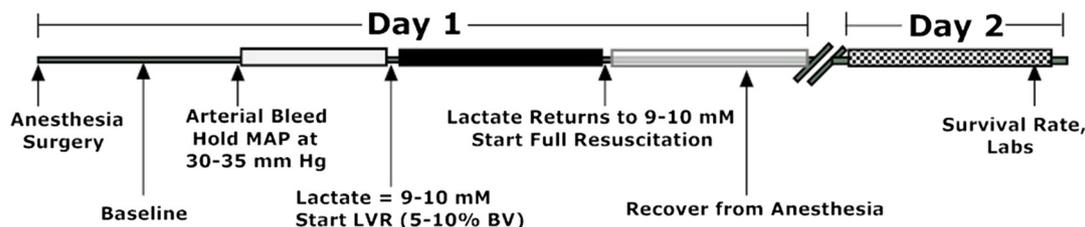
### Rodent Shock Model

An LVR model was used in adult rats to test the impermeant-based LVR solution used for prehospital resuscitation of rats with severe hemorrhagic shock. Adult Sprague-Dawley rats were anesthetized with isoflurane and maintained in a light surgical plane of anesthesia during the study. Polyethylene catheters were placed in both femoral arteries for blood pressure monitoring and blood sampling, and a catheter was placed in one femoral vein for

administration of fluids. The animals were allowed to ventilate on their own to establish normal arterial blood gas (ABG) values. A 1-cm midline incision was created to induce soft tissue injury and for the placement of a temperature probe in the abdomen. The animals were kept at 38°C using a heating pad and an incandescent light source above them. Arterial blood pressure, heart rate, and temperature were continuously recorded using a PowerLab (ADInstruments, Boston, MA). After a 30-minute stabilization period, heparin was given (500 U/kg), and arterial blood was slowly removed at 1 mL/min into a syringe to maintain blood pressure at 30 mmHg to 35 mm Hg. This hypotension was maintained until the plasma lactate reached a value between 9 mM and 10 mM, as measured every 15 minutes with a handheld lactate analyzer (Lactate Plus, Nova Biomedical, Waltham, MA) and every hour with a blood gas analyzer (Radiometer 800). In preliminary studies, 9 mM to 10 mM was the highest plasma lactate level achievable without mortality during the LVR period. Once the target lactate was reached, an LVR equal to 5% to 10% of the calculated blood volume<sup>21</sup> of saline was administered intravenously over a 10-minute period using a syringe infusion pump. When the blood lactate again reached 9 mM to 10 mM, full resuscitation was started, which consisted of a volume of saline equal to the volume of the blood loss (approximately 55–60% of total blood volume) plus 30% of the removed red blood cells (washed) infused intravenously over 10 minutes. After 1 hour of full resuscitation, the animals were euthanized by an anesthetic overdose, and terminal blood was removed for analysis. The time from the start of the LVR period until the start of full resuscitation is called the LVR time, and it represents the tolerance of the animal to the low-volume state or the maximum amount of time that a shocked subject can safely remain in the low-volume state until more definitive resuscitation is required. This was a major outcome used in the study. In some experiments, survival from severe shock was studied with impermeant-based LVR and compared with saline controls. In these studies, the animals were held in the low-volume state for 180 minutes receiving either 10% saline as a control or 10% saline containing 10% PEG-20k impermeant. After 180 minutes, the animals were given full resuscitation and were awakened from anesthesia after the catheters were removed. These surviving subjects were studied the following day (24 hours) to determine the rate of survival, blood pressure, lactates, base excess, PaO<sub>2</sub> (A-a gradient), and other blood laboratory values. The shock and LVR protocol is illustrated in Figure 2.

### Oncotic Reflection Coefficient

The oncotic reflection coefficient ( $\sigma_d$ ) of PEG-20k in rodent capillaries was determined to characterize the biophysical characteristics of this impermeant in capillary networks. The  $\sigma_d$  describes the relative convective solvent drag transport of a molecule across capillary pores. This characteristic is diffusion independent and is measured by determining the ratio of a compound's lymph concentration to the plasma concentration at high lymph flow rates. In these studies, rats were anesthetized as before, and a PE10 cannula was introduced into the thoracic duct as previously described<sup>22</sup> to direct the lymphatic flow into a collection tube. Heavy cream (5 mL) was injected into the stomach to help visualize the duct after the lipid was absorbed. A



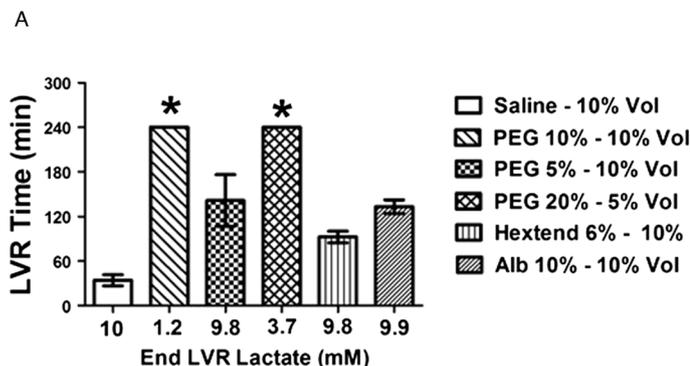
**Figure 2.** Diagram showing the shock, resuscitation, and recovery protocol used for these studies. The LVR time is the time from the start of the LVR (after the lactate during hemorrhagic shock reaches 9–10 mM) until the time after the LVR infusion when the lactate rises back up to 9 mM to 10 mM again. This immediately precedes full resuscitation. The LVR time is a measure of the tolerance to the low-volume state and is a function of the microcirculatory effectiveness since it is dependent on the rate of change of the plasma lactate.

saline infusion (intravenous) was started at 0.25 mL/min to accelerate lymph flow. Then, a single-bolus injection of 5-mg FITC-labeled PEG-20k (Nanocs, New York, NY) in saline was given. Blood plasma and lymphatics were collected every 10 minutes for an hour. FITC-PEG was then quantitated by direct measurement of the FITC fluor using a fluorescence plate reader (Biotek FL-800) with an excitation wavelength at 485 nm and an emission wavelength at 520 nm. The  $\sigma_d$  was estimated to be 1-L/P of FITC-PEG-20k as previously described.<sup>23</sup> The values for  $\sigma_d$  are from 0 to 1 where 0 means no reflection into the capillary or complete freedom of passage through the capillary pores (impermeant characteristics). A  $\sigma_d$  of 1.0 means total reflection back into the capillary or complete oncotic properties.

Statistical evaluations of mean values were performed using parametric analysis of variance with multiple comparisons corrections using the Dunnnett or Bonferroni test for more than two groups or an unpaired *t* test for comparison of data with only two values (control and test groups). Fisher's exact test was used for survival testing, and a *p* value of 0.05 was set as a cutoff for statistical significance.

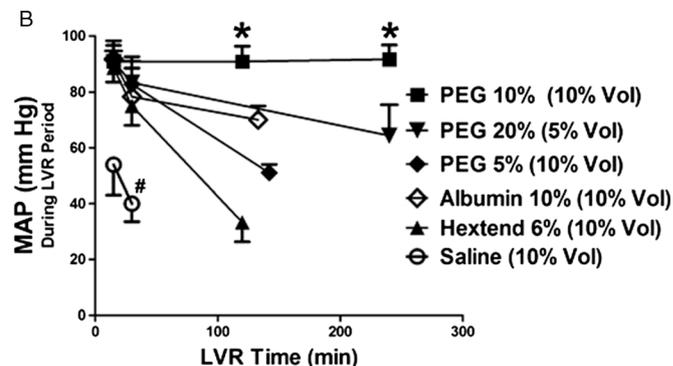
## RESULTS

The effects of LVR solutions on the LVR time are shown in Figure 3A. The LVR time in this model is an index measuring



the tolerance of the individual to the low-volume state. It is the length of time that a patient can safely remain in the low-volume state until definitive medical care and resuscitation is needed (golden hour), as indexed by the accumulation of a critical level of oxygen debt (lactate). With the use of normal saline (10% blood volume) as a control base crystalloid, the LVR time was determined to be approximately 30 minutes. Specifically, the time from the start of the low-volume infusion (triggered when the patient accumulated a lactate of 10 mM) until the time when the patient reaccumulated the same lactate level was determined to be an average of 30 minutes. This was significantly increased to 240 minutes (eightfold increase) when the same volume of saline contained 10% PEG-20k. Compared with PEG-20k, the LVR times for traditional resuscitation solutions such as 10% Hextend or 10% albumin were significantly lower. The plasma lactate at the end of the LVR period was close to 10 mM in all groups because this level of oxygen debt triggered the end of the LVR period by definition. However, the plasma lactate in the PEG-20k group at the end of 240 minutes was only 1.2 mM, which is far lower than the 10 mM trigger. Thus, the LVR time in the PEG-20k group (10%) was arbitrarily cut off and is a significant underestimation of its true value.

During the low-volume state after the LVR solution is administered, mean arterial blood pressure (MAP) was measured for the duration of the LVR period for a variety of LVR



**Figure 3.** A, LVR times for rodents in acute studies comparing the effectiveness of LVR solutions containing PEG-20k, Hextend, albumin, and saline controls. The numbers on the x-axis are the corresponding lactate concentrations in the plasma at the end of the LVR period. Most are close to 10 mM because that was the definition of the end of the LVR period. All agents were at a concentration of 5% to 20% by weight and were delivered at a volume equal to 10% or 5% of the calculated blood volume. \**p* < 0.05 relative to all other values, all treated groups are significantly different from saline (control), all values are mean (SD), *n* = 6 to 10 per group. B, MAP measured at 15 minutes, 30 minutes, and at the end of the LVR period for (6) groups of rats treated with various amounts of PEG-20k, albumin, Hextend, or saline as the LVR solution. \**p* < 0.05 relative to the other corresponding groups, #*p* < 0.05 relative to all other corresponding values, *n* = 6 to 10 per group, all values are mean (SD).

**TABLE 1.** The Effects of PEG-20k LVR Solution on Hemorrhagic Shock Values During the LVR Period and After Full Resuscitation in Survival Animals

During LVR					
	LVR Time, min	MAP, mm Hg	Lactate, mM	HCO <sub>3</sub> <sup>-</sup> , mM	Pao <sub>2</sub> , mm Hg
Saline (10%)	34 (18)	49.3 (11)	9.53 (2.1)	11.9 (2.1)	389 (72)
PEG-20k (10%)	180 (0)*	95.0 (3.5)*	1.42 (0.6)*	25.3 (3.4)*	465 (31)
Next-day survival					
	Survival, %	MAP, mm Hg	Lactate, mM	HCO <sub>3</sub> <sup>-</sup> , mM	Pao <sub>2</sub> , mm Hg
Saline (10%)	0 (0)	NA	NA	NA	NA
PEG-20k (10%)	100 (0)*	85.6 (6.2)*	1.2 (0.1)*	25.6 (2.6)*	475 (80)*

\**p* < 0.05.Values are mean (SD). Relative to corresponding saline values, n = 5, Pao<sub>2</sub> measured with an FIO<sub>2</sub> of 0.9.

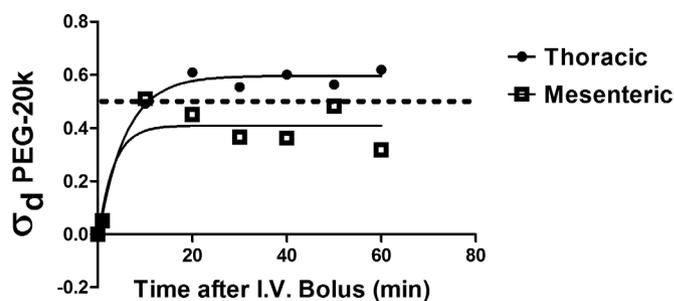
solutions (Fig. 3B). Saline solution (10%) resulted in low MAP values during the 30-minute LVR time with values less than 60 mmHg and 40 mmHg at 15 minutes and 30 minutes, respectively. These pressures were improved with Hextend and albumin and completely normalized with PEG-20k (90–100 mm Hg).

Survival studies were conducted in a series of animals to determine the long-term survival effects of impermeant-based LVR solutions after a severe blood loss (55–60%) and a high accumulation of oxygen debt (lactate 10 mM). These results are shown in the Table 1. All animals were required to undergo a controlled 180-minute LVR time with a 10% LVR solution following the hemorrhagic shock protocol (Fig. 2). When saline was used, 0% survived for 24 hours, and most died within 30 minutes to 45 minutes after the LVR solution was administered. By comparison with the control, survival was 100% with the same volume of saline containing 10% PEG-20k. Furthermore, all of the animals that survived 24 hours after full resuscitation had normal blood pressure and ABG values, both during the 180-minute LVR period and after 24 hours of recovery. Saline-treated animals had very low pressures during the LVR period, they demonstrated aberrant ABG values that were characteristic of severe metabolic acidosis, and they did not report 24-hour values since they all died during the LVR period.

The capillary oncotic reflection coefficient for PEG-20k was measured in rats (Fig. 4). The reflection coefficient was determined to be approximately 0.65, which indicates that some of the fluorescently labeled PEG-20k marker was pushed across the capillary into the interstitial space and lymphatics while much of the label was also clearly detected in the capillary space (plasma). The oncotic reflection coefficients were measured under high lymphatic flow rates by administering an intravenous infusion of saline during the 1-hour study. These conditions unmask the convective solvent drag transport potential of the tracer rather than the diffusional transport characteristics.<sup>24</sup>

## DISCUSSION

Prehospital resuscitation of patients in the field with severe trauma and hypovolemic shock is challenging since the first responders are often forced to work with low volumes of simple crystalloid solutions that are both transportable and



**Figure 4.** The oncotic reflection coefficient ( $\sigma_d$ ) for PEG-20k was measured in six rats to determine the impermeant and the oncotic effects of this molecule in both the mesenteric vascular bed and the thoracic bed. FITC-labeled PEG-20k was used as a tracer molecule, and the reflection coefficient was determined by measuring the lymph (L) to plasma (P) concentrations of FITC-PEG after an intravenous injection of the tracer under conditions of high lymph flow induced by volume loading with intravenously administered saline infusions (0.25 mL/min). Lymph was sampled from a cannula placed into the thoracic duct to drain either the thoraces or the mesentery. FITC-labeled PEG-20k was measured by excitation-emission spectrofluorometry. The oncotic reflection coefficient was calculated as 1-L/P for PEG-20k at high lymph flow rates to make transport across the capillary totally dependent on convective solvent drag transfer and independent of diffusion. A coefficient of 1.0 indicates complete reflection back into the capillary and describes a pure oncotic agent. A reflection coefficient of 0 indicates no reflection at high lymph flow rates and describes a pure impermeant molecule (provided it is impermeant to cell membranes). The actual measured  $\sigma_d$  for PEG-20k was approximately 0.60 in the thoracic tissues and 0.40 in the mesenteric tissues, which suggest that the molecule is in fact behaving as a hybrid where some escapes into the interstitial space to act as an impermeant and a large amount of the material stays in the capillary where it behaves as an oncotic agent. The lower values in the mesentery is consistent with the known fenestrated “leaky” capillaries in the gut. The hybrid behavior is consistent with its physiologic effects on blood pressure and low-volume tolerance following shock. This property also explains why PEG-20k is effective by itself without classic impermeants (such as gluconate) added with it. The values of  $\sigma_d$  at each time point represent the average of three independent values from three animals for each vascular bed.

stable under field conditions. A recently described advancement in this area uses high concentrations of cell impermeant molecules in saline solutions such as LVR solutions for pre-hospital management of severely hypovolemic shock patients. In acute studies, these solutions prevent ischemia-induced cell swelling, which alleviates both the harmful effects on cell and mitochondrial membranes and greatly improves microcirculatory capillary flow and exchange by preventing the occlusion of the microcirculation by swollen parenchymal and endothelial cells. This study extends those findings by testing the effects in survival shock models and compares these effects to crystalloid solutions that are considered standard of care in the field today. Finally, this study explores further the unique mechanism of action of PEG-20k, which has been determined to be the most superior impermeant molecule yet tested for LVR in severe hypovolemic shock.

Cell impermeants are useful in severe shock because they load the interstitial space with osmotically active molecules that are impermeant to the cell membrane but freely escape the capillary space. The increased osmotic force generated outside of the cell prevents intracellular water accumulation, cell swelling, and secondary capillary compression (Fig. 1). The addition of an oncotic agent to a cell impermeant solution was hypothesized to potentiate the effect of the impermeant alone by establishing a second oncotic gradient between the capillary space and the interstitial compartment, thereby augmenting the translocation of water accumulated in the interstitial space by the impermeants into the capillary space. This nonenergetic movement of water into the capillary raises capillary pressure and increases capillary perfusion by both reducing the resistance to capillary flow (by preventing diameter changes from compression) and by increasing the capillary pressure gradient for flow. To our surprise, the addition of the oncotically active impermeant, PEG-20k, to LVR solutions containing simple impermeants such as gluconate geometrically potentiated the impermeant effect. The total response of the two components was reproduced by the PEG-20k component alone and much less than a pure oncotic agent alone (albumin). This suggested that PEG-20k may have a hybrid effect where the molecule acts both as a capillary permeable cell impermeant and as a traditional oncotic agent. Therefore, this study focused solely on the PEG-20k molecule.

An LVR solution containing 10% by weight of PEG-20k (given at 10% blood volume) has been shown to be optimal in our shock models. This was the criterion standard to compare other solutions to size up their clinical potential. A 10% PEG solution given at 10% of the calculated blood volume (approximately 500 mL for an adult patient) produced the longest tolerance to the low-volume state as compared with 6% Hextend and 10% albumin solutions. Since clinical formulations of albumin are generally approximately half strength (6% by weight), the values observed in this study probably are overestimations of the effects observed clinically. Furthermore, the effect of the PEG-20k group has been significantly underestimated in this study since the LVR times were cut off at only 240 minutes, which is lower than the true LVR time because the trigger of 10 mM was never achieved in this group. Had the LVR time been increased until the lactate in the PEG-20k group reached 10 mM, the final LVR time would have been much greater than 240 minutes. Therefore, the true limits of PEG-20k-based

LVR solutions, regarding tolerance to the low-volume state, are not yet known.

In a dose deescalation trial, we compared 10% PEG-20k given at either a lower volume (5% blood volume) of the same concentration (10%) or a lower volume (5%) at twice the concentration (20%). The lower total PEG-20k dose was less effective, while the same dose but given at the lower volume was still very effective. This suggests that even lower volumes of LVR solutions can be achieved down to 5% of calculated blood volume. This is approximately 250 mL for an adult patient and may find use in combat casualty care on the battlefield where carry volumes of intravenous fluids for resuscitation are more of a concern.

The previous trials of impermeant-based LVR solutions in shock were acute studies. The effects on survival are an important consideration for possible clinical use. When the LVR time was controlled to 180 minutes, all of the animals resuscitated with 10% PEG-20k as the LVR solution survived 24 hours compared with 0% survival in the saline control group. Furthermore, the surviving rodents were perfectly normal both in terms of physiologic laboratory values and behaviorally. There were no apparent adverse effects of the PEG-20k LVR solutions except a temporary diuresis immediately after administration of the solution and a temporary metabolic alkalosis. Since it is believed that PEG-20k acts as a hybrid molecule, it is likely that some of the material passes across Bowman's space in the glomerulus where it acts as an impermeant in the tubules to increase osmotic water clearance and cause a diuresis, similar to a mannitol effect. Furthermore, the increased excretion of water and likely electrolytes too could prevent hydrogen ion reabsorption and increase renal acid excretion, thereby causing a metabolic alkalosis. This is a favorable effect in shocked patients that are experiencing severe metabolic lactacidosis and obviates the requirement of bicarbonate administration to correct acidosis during resuscitation.

The impermeant effect in LVR solutions is greatly augmented when a colloid is also present. Since the putative colloidal agent, PEG-20k, works as well by itself as it does with typical small-molecule impermeants such as gluconate, it was hypothesized that this size PEG polymer may act as a hybrid and possess both impermeant and colloidal properties. To support this hypothesis, the capillary oncotic reflection coefficient for PEG-20k was measured in rats (Fig. 4). The reflection coefficient was determined to be approximately 0.65, which clearly suggests that some of the material escapes into the interstitial space (impermeant characteristics) while a large portion of the material stays behind in the capillary to act oncologically. This strongly indicates a hybrid nature of PEG-20k, which supports the direct observations of its superior utility alone as an LVR solution and its apparent ability to cross the glomerulus to cause a diuresis. Further studies are needed to characterize the renal handling of PEG-20k, but its combined impermeant and colloid effects are now well supported.

There are three major limitations of the study. First, the model was for a controlled hemorrhage event and needs to be evaluated also in an uncontrolled model since many clinical conditions have an uncontrolled component. The current rodent model needs to be translated to a large animal preclinical study, and finally, the toxicologic effects of PEG-20k need more

evaluation, especially any possible platelet and coagulation responses (nothing has been identified to date in survival studies). Human trials are pending an Food and Drug Administration regulatory pathway decision.

In conclusion, PEG-20k used at 10% weight and administered at 10% calculated blood volume during severe hypovolemic shock produces striking salutary benefits. These effects dramatically prolong survival in a controlled hemorrhage model and are likely due to the molecules hybrid impermeant and oncotic properties.

#### AUTHORSHIP

Each author contributed significantly to and is willing to take public responsibility for one or more aspects of the study: its design, data acquisition, and analysis and interpretation of data.

#### DISCLOSURE

This work was supported by grants from the National Institutes of Health R01 DK087737 and the Department of Defense W81XWH-12-1-0599 to M.J.M.

#### REFERENCES

- National Center for Injury Prevention and Control. *Web-based Injury Statistics Query and Reporting System (WISQARS)*. 2013. Available at <http://www.cdc.gov/injury/wisqars/>.
- Finkelstein EA, Corso PS, Miller TR. *The Incidence and Economic Burden of Injuries in the United States*. New York, NY: Oxford University Press; 2006.
- Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma*. 2006;60:S3–S11.
- Heckbert SR, Vedder NB, Hoffman W, Winn RK, Hudson LD, Jurkovich GJ, Copass MK, Harlan JM, Rice CL, Maier RV. Outcome after hemorrhagic shock in trauma patients. *J Trauma*. 1998;45:545–549.
- Franklin GA, Boaz PW, Spain DA, Lukan JK, Carrillo EH, Richardson JD. Prehospital hypotension as a valid indicator of trauma team activation. *J Trauma*. 2000;48:1034–1037.
- Riha GM, Kunio NR, Van PY, Hamilton GJ, Anderson R, Differding JA, Schreiber MA. Hextend and 7.5% hypertonic saline with dextran are equivalent to lactated Ringer's in a swine model of initial resuscitation of uncontrolled hemorrhagic shock. *J Trauma*. 2011;71:1755–1760.
- Riha GM, Kunio NR, Van PY, Kremenevskiy I, Anderson R, Hamilton GJ, Differding JA, Schreiber MA. Uncontrolled hemorrhagic shock results in a hypercoagulable state modulated by initial fluid resuscitation regimens. *J Trauma Acute Care Surg*. 2013;75:129–134.
- Holcomb JB, Pati S. Optimal trauma resuscitation with plasma as the primary resuscitative fluid: the surgeon's perspective. *Hematology Am Soc Hematol Educ Program*. 2013;2013:656–659.
- Chaudry IH, Sayeed MM, Baue AE. Depletion and restoration of tissue ATP in hemorrhagic shock. *Arch Surg*. 1974;108:208–211.
- Gomez H, Mesquida J, Hermus L, Polanco P, Kim HK, Zenker S, Torres A, Namas R, Vodovotz Y, Clermont G, et al. Physiologic responses to severe hemorrhagic shock and the genesis of cardiovascular collapse: can irreversibility be anticipated? *J Surg Res*. 2012;178:358–369.
- Chaudry IH. Use of ATP following shock and ischemia. *Ann N Y Acad Sci*. 1990;603:130–140.
- Barlet-Bas C, Khadouri C, Marsy S, Doucet A. Enhanced intracellular sodium concentration in kidney cells recruits a latent pool of Na-K-ATPase whose size is modulated by corticosteroids. *J Biol Chem*. 1990;265:7799–7803.
- Petit PX, Goubern M, Dirolez P, Susin SA, Zamzami N, Kroemer G. Disruption of the outer mitochondrial membrane as a result of large amplitude swelling: the impact of irreversible permeability transition. *FEBS Lett*. 1998;426:111–116.
- Southard JH, Belzer FO. Organ preservation. *Annu Rev Med*. 1995;46:235–247.
- Southard JH, Belzer FO. Principles of organ preservation part I. *Surgical Rounds*. 1993;353–360.
- Southard JH, Belzer FO. Principles of organ preservation Part II. *Surgical Rounds*. 1993;443–448.
- Southard JH, Belzer FO. Control of canine kidney cortex slice volume and ion distribution at hypothermia by impermeable anions. *Cryobiology*. 1980;17:540–548.
- Southard JH, van Gulik TM, Ametani MS, Vreugdenhil PK, Lindell SL, Pienaar BL, Belzer FO. Important components of the UW solution. *Transplantation*. 1990;49:251–257.
- Mees N, Southard JH, Belzer FO. Inhibition of ischemic induced cellular swelling in kidney cortex tissue by lactobionate anions. *J Trauma*. 1982;22:118–120.
- Parrish D, Lindell S, Reichstetter H, Aboutanos M, Mangino MJ. Cell impermeant based low volume resuscitation in hemorrhagic shock: a biological basis for injury involving cell swelling. *Ann Surg*. 2014. In press.
- Arora TK, Malhotra AK, Ivatury R, Mangino MJ. L-arginine infusion during resuscitation for hemorrhagic shock: impact and mechanism. *J Trauma Acute Care Surg*. 2012;72:397–402.
- Ionac M. One technique, two approaches, and results: thoracic duct cannulation in small laboratory animals. *Microsurgery*. 2003;23:239–245.
- Reed RK, Townsley MI, Taylor AE. Estimation of capillary reflection coefficients and unique PS products in dog paw. *Am J Physiol*. 1989;257:H1037–H1041.
- Mortillaro NA, Granger DN, Kviety PR, Rutili G, Taylor AE. Effects of histamine and histamine antagonists on intestinal capillary permeability. *Am J Physiol*. 1981;240:G381–G386.

#### EDITORIAL CRITIQUE

This manuscript produced by Drs. Parrish, Mangino and colleagues continue their investigations using cellular impermeants (in particular PEG-20k) in hemorrhagic shock resuscitation. Cellular impermeant such UW solution and PEG-20 K solutions attempt to reduce cellular edema and organ injury following shock by stabilizing water movement during resuscitation. By using both oncotic pressure and the cellular impermeant function of PEG solution the authors minimize organ edema and endothelial edema and reduce cellular ischemia and organ injury following hemorrhagic shock. Additionally, the authors' prior ex-vivo cellular work has validated a reduction in swelling in both ischemic hepatic and ischemic pulmonary cells treated with impermeants. In the current model, tissue water composition would be an important marker for the mechanism of action of this experimental intervention and the authors continued evaluation of in-vivo organ edema is encouraged.

The question regarding the impact of impermeant resuscitation on hemostasis is an important one. Prior cell impermeants such as UW solution have had significant problems with red blood cell aggregation in living models and as such have not been effective resuscitation fluids. Peg-20K does not appear to have these effects. Investigation into an uncontrolled hemorrhage model and further research into the effect on both platelet aggregation and the impact on fibrinolysis should be conducted to validate the author's impressive findings.

This novel resuscitation pathway could have significant impact on pre-hospital and acute resuscitation following injury by reducing the amount of fluid needed to be carried in the field by medical personnel and by allowing for low crystalloid volume resuscitation to adequately restore organ blood flow. The overall application of this resuscitation strategy is truly limitless. While the author's initial work is in hemorrhagic shock, patients suffer septic shock that require large volumes of crystalloid resuscitation could also benefit from

this line of research. The authors should be commended on their continued research into this novel resuscitation method which has the potential for broad reaching impact on how we resuscitate patients from shock.

**Jason W. Smith, MD, PhD**  
*University of Louisville Hospital*  
*Louisville, Kentucky*