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Low Volume Resuscitation Using Polyethylene Glycol-20k in a Pre-Clinical Porcine Model of Hemorrhagic Shock

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Abstract

Introduction—Polyethylene glycol-20k (PEG-20k) is highly effective for low volume resuscitation (LVR) by increasing tolerance to the low volume state. In our rodent shock model, PEG-20k increased survival and expanded the “golden hour” 16-fold compared to saline. The molecular mechanism is largely attributed to normalizations in cell and tissue fluid shifts after low flow ischemia resulting in efficient microvascular exchange. The objective of this study was to evaluate PEG-20k as a low volume resuscitation solution for hemorrhagic shock in a pre-clinical model.

Methods—Anesthetized male Yorkshire pigs (30–40 kg) were hemorrhaged to a MAP of 35–40 mmHg. Once lactate reached 7 mM/L, either saline (n = 5) or 10% PEG-20k (n = 5) was rapidly infused at 10% calculated blood volume. The primary outcome was LVR time, defined by the time from LVR administration to the time when lactate again reached 7 mM/L. Other outcomes measured included: MAP, heart rate (HR), cardiac output (CO), mixed venous oxygen saturation (SvO₂), splanchnic blood flow, and hemoglobin.

Results—Relative to saline, PEG-20k given after controlled hemorrhage increased LVR time by 16-fold, a conservative estimate given that the lactate never rose after LVR in the PEG-20k group. Survival was 80% for PEG-20k LVR compared to 0% for the saline controls (P<0.05). PEG-20k also significantly decreased HR after hemorrhage and increased CO, MAP, splanchnic flow, and

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SvO₂. Falling hemoglobin concentrations suggested sizable hemodilution from fluid shifts into the intravascular compartment.

Conclusions—In a pre-clinical model of controlled hemorrhagic shock, PEG-20k-based LVR solution increased tolerance to the shock state 16-fold compared to saline. PEG-20k is a superior crystalloid for low volume resuscitation that may increase safe transport times in the prehospital setting and find use in hospital emergency departments and operating rooms for patients awaiting volume replacement or normalization of cell, tissue, and compartment fluid volumes.

Keywords

Osmotic changes; microcirculation; reperfusion; polymers; crystalloid

Introduction

Hemorrhage causing severe tissue hypoxia is a leading cause of death in military and civilian populations (1–3). Yet care for wounded military combatants is quite different compared to civilian patients and is limited by available resources and delayed evacuation or prolonged transport to definitive care (2;4). Medical supplies carried in the field must be of low weight, chemically stable, and small volume (2). The recommendation for hemorrhagic shock in *Fluid Resuscitation*, a report published by the Committee on Fluid Resuscitation for Combat Casualties and Institute of Medicine in 1999, is a 250 ml bolus of 7.5% saline. However, a subsequent randomized, double-blinded study by the Resuscitation Outcomes Consortium did not show improved survival outcomes after treatment with hypertonic saline (5;6). In fact, hypertonic saline worsened hypo-coagulation and hyper-fibrinolysis (7). There remains a need for an ideal fluid that can be given at a low volume to resuscitate patients in hemorrhagic shock awaiting definitive treatment, especially in the austere prehospital setting.

Parrish et al. have demonstrated reduced ischemia-induced cell swelling, increased tolerance to the low volume state, improved capillary blood flow, and higher survival with administration of a low volume solution containing polyethylene glycol-20k (PEG-20k) compared to saline in a rodent model of severe hemorrhagic shock (8;9). The resuscitation effects were extraordinary in this model. These effects are attributed to the specific polymer radius and osmotic reflection coefficient that accelerate non-energetic fluid movement into capillaries by multiple osmotic gradients. The next step was to test PEG-20k in a pre-clinical porcine model. The objective of this study was to establish PEG-20k as a low volume resuscitation agent in a pre-clinical porcine model of hemorrhagic shock. We hypothesized that pigs in hemorrhagic shock receiving PEG-20k would have increased tolerance to the shock state, as measured by the low volume resuscitation (LVR) time, compared to pigs receiving saline. We also hypothesized that PEG-20k treated pigs would have improved cardiovascular outcomes relative to saline-treated pigs.

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. The procedures were conducted as previously described, except lactate controlled endpoint were used (10).

Male Yorkshire pigs (30–40 kg, Archer Farms) were fasted the night before the experiment, but access to water was allowed. Anesthesia was induced with an intramuscular injection of ketamine and Xylazine and intravenous injection of propofol. After endotracheal intubation, isoflurane at 1–2% was used to maintain anesthesia with a fraction of inspired oxygen of 30% (Narkomed 2 Ventilator, Dräger, Lubeck, Germany). If needed, a circulating water warming pad was used to maintain temperature of 38°C.

Once appropriate anesthesia was achieved, vessel cannulations were performed using cut-down method. The right femoral artery was cannulated with a (PE 280) cannula and was connected to a hemodynamic monitoring system (PowerLab, ADInstruments, Boston, MA). The right carotid artery was cannulated for blood withdrawal. The right external jugular vein was cannulated with a 7 Fr. pulmonary artery catheter (Swan-Ganz Continuous Cardiac Output Thermodilution Catheter, Edwards Lifesciences, Irvine, CA) with the tip in a wedge position when the balloon was inflated. The PowerLab data acquisition system was used to monitor pressure waveforms during pulmonary artery catheter placement. The left external jugular vein was cannulated for fluid administration. A midline abdominal incision from xiphoid to pubis was then made to cause a measured surgical trauma injury and to gain access to a ~2 mm branch of the superior mesenteric artery. After careful dissection, a flow probe was placed around the artery and connected to transit-time blood flowmeter (Transonics model T403, Ithaca, NY) that was monitored by the PowerLab. For three animals, pulmonary artery catheterization and/or mesenteric artery dissection were not performed due to technical complications. Arterial blood pressure, heart rate, mesenteric artery flow, and temperature were continuously monitored by PowerLab, and pulmonary artery catheter variables, including mixed venous oxygen saturation (SvO₂) and cardiac output, were continuously measured by a cardiac output computer (Edwards Lifesciences Vigilance Irvine, CA).

In most animals, pre-hemorrhage saline was administered intravenously until the capillary wedge pressure reached 5–8 mmHg. This was done to normalize baseline volumes before hemorrhage. An arterial blood gas (ABG) sample was obtained, and adjustments were made to the ventilator to maintain pCO₂ between 38–42 mmHg. Additional ABG samples were obtained if needed to assess interventions.

After a 15 minute stabilization period, arterial blood was removed at an initial rate of 4 ml/kg using a Masterflex[®] peristaltic roller pump (Cole-Parmer, Chicago, IL) until the mean artery pressure (MAP) reached 35–40 mmHg. More blood was withdrawn at a subsequent rate of 2 ml/kg as the animal compensated. A MAP of 35–40 mmHg was maintained until plasma lactate reached a value between 7–8 mM/L, as measured every 15 minutes with a hand held lactate analyzer (Lactate Plus, Nova Biomedical, Waltham, MA) and with the ABL-800 blood gas analyzer (Radiometer USA, Cleveland, OH). Once the target lactate was reached, a low volume resuscitation (LVR) solution equal to 10% of calculated blood volume of either saline (n = 5) or 10% w/v PEG-20k (n = 5) was given IV over 5 minutes

using a Masterflex[®] roller pump. Circulating blood volume was estimated as 70 ml/kg body weight.

Fifteen minutes after LVR, serial lactate measurements were taken until the lactate again climbed to the 7–8 mM/L target. The main outcome was LVR time, which was defined as the length of time from start of LVR administration to the time at which lactate again reached 7–8 mM/L. If lactate did not decrease after LVR and only increased in subsequent measurements, LVR time was arbitrarily set at 15 minutes, which was the minimum time sampling interval. If lactate decreased and did not reach the target of 7–8 mM/L by 240 minutes, the maximum LVR time was arbitrarily set at 240 minutes. The LVR time is a surrogate for tolerance to the low volume or shock state (8;9). Clinically, it would be similar to the length of time that a patient can safely remain in the low volume state until definitive care and full resuscitation are needed, i.e. the “Golden Hour.” At the end of the experiment, animals were euthanized by administration of I.V. Euthasol.

Data are expressed as mean $\pm$ standard deviation. Data were analyzed by two-way ANOVA and Bonferroni’s multiple comparison correction and the Fisher’s Exact test using the InStat program (GraphPad Software, Inc., La Jolla, CA). A p -value < 0.05 was considered statistically significant.

Results

The consistency of the two groups of animals used in the study is shown, in part, by the similarities in the times required to complete milestones during the protocol. Table 1 shows that baseline characteristics as well as surgery and hemorrhage times and volumes were not different between the two groups. The primary outcome, LVR time, was much longer for the PEG-20k treated pigs, compared to the saline-treated pigs (* $P < 0.05$). Survival in the saline group 0% (0/5) was significantly lower than the survival in the PEG-20k treated group 80% (4/5), as determined by Fisher’s Exact test ($P = 0.047$).

The effects of hemorrhage and low volume resuscitation on plasma lactate concentrations in the two pig groups are shown in Figure 1A. Lactate in both groups rose steeply at the same rate during hemorrhagic shock to reach the predetermined target and then continued to climb after low volume resuscitation with saline. However, LVR using PEG-20k caused the plasma lactate concentrations to steadily decline over the next 4 hours to end at values that were the same as the baseline before hemorrhage. The low volume resuscitation (LVR) time, which is derived from the plasma lactate values during the LVR period, is shown in Figure 1B. By definition, the LVR time is the time required for the plasma lactate to reach 8 mM after giving the LVR solution. In saline pigs, the plasma lactate never declined after LVR so we assigned an LVR value of 15 minutes, which is our shortest monitoring interval during this phase of the study. By contrast, LVR time in the PEG-20k group was 240 minutes or 16 fold higher than the control. Since the lactate in that group fell and never rose, the real LVR time was much greater than 240 min, which was arbitrarily cut off for technical reasons. In fact, the end lactate after LVR in the PEG-20k group was only 1.80 ± 0.44 mM, far less than the end point of 8 mM. By contrast, the end lactate value in the control group receiving a saline LVR was 8.02 ± 0.5 mM (Figure 1B).

In addition to extending the tolerance to the low volume state (LVR time), PEG-20k produced profound and rapid effects on cardiovascular performance in pigs after low volume fluid resuscitation. Figure 2 shows that mean arterial blood pressure (MAP) and cardiac output significantly increased after PEG-20k LVR, relative to the saline control group. Heart rate (HR) significantly declined in the PEG-20k group, relative to control. Absolute MAP values settled in at about 60 mmHg in the PEG-20k group and below 30 mmHg in the saline group, which was fatal.

Mixed venous blood oxygen saturation (SvO_2) reported from the optical pulmonary artery catheter (Figure 3A) fell steadily during hemorrhage but significantly increased back to baseline with PEG-20k LVR, compared to the saline LVR pigs where SvO_2 continued to drop until death. Hemoglobin (Figure 3B) fell rapidly after PEG-20k LVR after all controlled bleeding had stopped. In the control pigs, hemoglobin didn't change after saline LVR.

Blood flow through a branch of the superior mesenteric artery (SMA) was monitored continuously throughout these studies and is shown in Figure 4 for both saline and PEG-20k resuscitated pig groups. The mesenteric blood flow, expressed as a percentage of the pre-hemorrhage baseline, fell continuously in both groups during the hemorrhage period to about 50% of the original baseline values. After saline resuscitation, the flow remained unchanged after LVR. In the PEG-20k resuscitated group, however, mesenteric blood flow rose significantly to over 2 times the baseline blood flow after LVR. This hyperemia persisted throughout the 240 minute LVR period in the PEG-20k group.

Arterial blood gas (ABG) values are shown in Figure 5. Bicarbonate (HCO_3^-) in the arterial blood (panel A) fell during hemorrhage in both groups of pigs. Bicarbonate quickly rose to baseline values after LVR with PEG-20k compared to saline resuscitated pigs where the values remained low after LVR. The arterial CO_2 levels remained steady in both groups during hemorrhage (panel B). In the PEG-20k LVR group, the $PaCO_2$ significantly rose after resuscitation above baseline relative to the values in the saline LVR group where the carbon dioxide content continued to fall. Arterial blood pH fell in both groups during hemorrhage and normalized after LVR only in the PEG-20k group (data not shown).

Discussion

The objective of this study was to establish preliminary data for the use of PEG-20k in low volume resuscitation solutions in a pre-clinical porcine model of hemorrhagic shock. The data support our hypothesis that PEG-20k based low volume resuscitation solutions improve tolerance to the shock state as demonstrated by an increased LVR time, the surrogate for tolerance in our studies. The data also support our secondary hypothesis that cardiovascular outcomes are improved in the PEG-20k treated pigs, as compared to saline. The data are similar to the results found in the rodent hemorrhagic shock model that were previously reported by our group.

The rodent model of severe hemorrhagic shock was translated to a parallel porcine model for pre-clinical testing of novel PEG-20k based resuscitation solutions (8;9). There are some

differences between the two models. The target MAP during hemorrhagic shock was 5 mmHg higher for pigs than it was for the rats (35 Vs. 30 mmHg). This was to prevent myocardial injury in the pigs that would otherwise occur during very low MAPs (10). Plasma lactate accumulation was used as a marker for reporting oxygen debt in both models which served as our metabolic endpoint of shock severity (11–14). The target lactates used to assess end-oxygen debt accumulation after hemorrhage was lower in pigs (7–8 mM) compared to the rat model (9–10 mM). Furthermore, the time needed to accumulate the target lactate in the pig was almost twice as long compared to the rat model (60 Vs. 115 min). These differences are attributable to differences to low volume and ischemia tolerance between the species and probably animal sizes. Some rats in the previous studies and more pigs in the current study, failed to reach the target lactate level during hemorrhage and were not included in the study. The disassociation of oxygen debt with plasma lactate during hemorrhage (lactate non-responders) in some animals underscores the shortcomings of using lactate to report oxygen debt (14). Just because lactate was not proportionally reported by some non-responding animals, does not indicate that they are not in fact accumulating oxygen debt during shock. The reasons for this limitation are unclear but it shouldn't affect the utility of the model in demonstrating differences in shock tolerance outcomes between the two treatment groups because the lactate non-responders occur randomly in both animal groups independent of the treatment and they were disqualified from the analysis because of their failure to build lactate timely. Future studies using direct oxygen debt measurements as the primary outcome are appropriate.

Hemorrhage causes oxygen debt to accumulate in patients and in animal models. This model controlled the amount of oxygen debt induced by hemorrhage by setting predetermined plasma lactate targets, after which low volume resuscitation was started. While we acknowledge problems with this technique, it attempts to ensure each resuscitated animal starts off with similar amounts of global ischemia (oxygen debt). This ensures a fair comparison of the two treatment arms if we assume that mismatches in lactate and oxygen debt occur randomly, when they do occur. The potential to re-establish oxygen exchange and transfer in the microcirculation by the type of LVR solution is indexed by the time it takes for the plasma lactate to again rise back to the target level after the start of the low volume infusion. This is defined as the LVR time and represents tolerance to the low volume state. In the saline controls, the saline LVR never reduced the rate of ascent of plasma lactate, unlike what we observed in the rat model when the saline bolus caused a temporary plateau. By contrast, pigs receiving the same volume of saline LVR containing PEG-20k continued to increase plasma lactate for the first 15 minutes after LVR but then began clearing lactate all the way to baseline. The end plasma lactate 240 minutes after LVR in the PEG treated pigs was only 1.80 mM while the lactate in the controls exceeded the target value 0–15 minutes after LVR (8.02 mM). Therefore, the true LVR time in the PEG group would have been much higher if the experiment were allowed to go on until the pigs began to build lactate to reach the 8 mM target. It is likely that they may never have re-accumulated enough lactate after PEG-20k LVR to reach the target value that ends the LVR period (8 mM) because their lactate values after 4 hours of hypovolemia were so low and their hemodynamic status was so stable. Therefore, the 240 min LVR time in the PEG group is arbitrary and grossly underestimates the true value. The PEG-20k crystalloid LVR alone without further

resuscitation using blood products may be sufficient to induce total ischemic tolerance to the low volume state but inadequate to handle traumatic and dilutional coagulopathies. Uncontrolled shock studies in pigs will highlight the importance of combining solutions like PEG-20k that enhance tissue perfusion and oxygen transfer with blood products that control coagulation and hemostasis.

The near normalization of plasma lactate with PEG-20k based LVR solutions was attributable to both dilution and clearance. About a 50% dilution effect from the PEG-induced osmotic movement of isotonic water into the vascular space was observed. The remaining drop in plasma lactate was attributable to non-dilutional mechanisms. The hemoglobin data suggest intravascular dilution because the hemoglobin concentration after LVR with PEG-20k was about 50% of the baseline. Since no further bleeding or loss of RBCs was allowed to occur after the end of hemorrhage (because it was a controlled bleed model), the hemoglobin concentration reduction likely represents a proportional dilution effect with non-hemoglobin containing fluid from outside of the vascular compartment. The remaining 50% non-dilutional drop in plasma lactate that was observed after PEG-20k LVR likely represents a combination of clearance by renal filtration into the urine and by the metabolic oxidation of lactate to pyruvate by LDH as the peripheral microcirculation opens (8;9) and aerobic metabolism resumes.

Oxygen debt induced by severe hemorrhagic shock was not directly measured in this study but was indexed by plasma lactate concentrations. Plasma lactate was metabolically cleared by PEG-20k LVR solutions and prevented from rising again, even in the low volume state, presumably because the oxygen debt was being repaid. Furthermore, bioenergetic (lactate conversion) and cardiovascular homeostasis was maintained by these solutions alone, which indicate the animals were induced to tolerate the new low volume state. This is in stark contrast to the saline volume controls where the pigs continued to rapidly accumulate oxygen debt (lactate) and displayed very low cardiovascular function after LVR until they died shortly thereafter. The oxygen repayment after PEG-20k LVR is further evidenced by the return of cardiovascular function and even hyper-dynamic responses seen 1–2 hours after LVR. The increased cardiac output above baseline values and the rapid return of SvO_2 represents an increase in oxygen delivery to the periphery, which is consistent with debt repayment. Other data supporting debt repayment include hyperemia in the splanchnic bed and normalization of bicarbonate, lactate, and blood pH. Rapid oxygen debt repayment, as occurs with PEG-20k LVR, is indicative of survival after resuscitation (13).

This study was a preliminary scientific experiment that tested PEG-20k based low volume resuscitation in a large animal model. Our scientific objective was to compare the PEG-20k containing low volume treatment to its saline vehicle at the same volume to dissect out only its effects on low volume tolerance. This is why PEG-20k LVR was compared to saline LVR. The authors acknowledge that normal saline is not an effective low volume resuscitation fluid at the low volumes used. As such, our comparative data amplifies the significance and effectiveness of the PEG-20k component. In the future, clinically relevant studies will be performed where PEG-20k will be compared to traditional low volume resuscitation fluids in the pig model as was done recently in rodent studies where PEG-20k LVR solutions were compared to both Hextend and albumin (8).

Unlike traditional resuscitation solutions like starch, albumin, or even hypertonic saline, PEG-20k has hybrid partitioning properties because it distributes to both the vascular space and the interstitial space in a ratio of about 2:1 due to its unique osmotic reflection coefficient (8). At equilibrium, its concentrations are higher in the vascular space than in the interstitial space, which are both higher than in the intracellular space. Therefore, it creates two osmotic gradients. The first gradient is from cell to interstitium, which draws water out of ischemic cells to reverse ischemic cell swelling. The second gradient is from interstitium to the capillary space, which drives capillary filling with interstitial water that was pulled from the cells. Thus, PEG-20k solutions pull isotonic fluid out of the extravascular spaces more effectively than other solutions, thereby decompressing and filling capillaries, which improves oxygen delivery and debt repayment even during the low volume state. We propose that these effects are due to the creation of these multiple osmotic gradients by the unique osmotic reflection coefficient of this size of PEG polymer, an attribute not shared by other clinical LVR solutions. This biophysical difference is seemingly very important because the biological effects observed with HES and albumin are dramatically different than to PEG-20k (8). Hypertonic saline is completely different from PEG-20k because it is a solution of small ions permeable to the cell. The concern with hypertonic saline is that it will cause only a *transient* increase in intravascular volume because it eventually distributes to total body water, especially during periods of cell ischemia common in shock when the Na/K pump fails. Unless cellular bioenergetics are fully restored, there isn't enough oxygen and ATP production for the Na/K pump to remove all of the sodium load from the hypertonic saline. This causes intracellular sodium entry followed by intracellular chloride and water movements, which exacerbate the initial cell swelling problem and the secondary problems with capillary compression. A further concern with hypertonic saline is that rapid sodium entry into ischemic cells during shock drives the sodium pump activity further, which rapidly hydrolyzes an already low and depleted intracellular ATP pool. This exacerbates metabolic demand in the face of a bioenergetic crisis, worsens ischemia, and should be avoided.

Conclusion

An induction of complete metabolic and cardiovascular tolerance to the low volume state in the absence of full resuscitation may be a huge advantage to shock patients in the pre-hospital military and civilian setting where the golden hour needs to be expanded in the absence of whole blood or blood products. Therefore, PEG-20k based crystalloid low volume resuscitation solutions may be very useful under those emergent conditions.

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Figure 1.

Plasma lactate concentrations in pigs during the time course of the shock and resuscitation protocol (Panel A). PEG, 10% Polyethylene Glycol-20k LVR solution. * $P < 0.05$ between corresponding values or compared to the last value recorded in the saline group before death. Panel B shows the LVR times for both groups of pigs. * $P < 0.05$ relative to saline for both LVR time and the end lactate values. Values are mean $\pm$ SD, $n=5$ for each group.

Figure 2.

Cardiovascular endpoints in pigs during the shock and resuscitation protocol in pigs given an LVR solution consisting of saline or 10% PEG-20k. A. Mean Arterial Pressure (MAP), B. Cardiac Output (CO), C. Heart Rate (HR). All values are mean $\pm$ SD, $n=5$ for each group, * $P<0.05$ between corresponding values or to the last value recorded in the saline group before death.

Figure 3.

Systemic venous oxygen saturation (SvO₂) measured in the mixed systemic venous blood of the pulmonary artery by the optical catheter (panel A) and hemoglobin from ABGs (panel B) during the shock and resuscitation protocol in pigs given a saline or a PEG-20k based LVR solution. Values are mean $\pm$ SD, n=5 for each group, * P<0.05 between corresponding values or to the last value recorded in the saline group before death.

Figure 4.

Blood flow measured in a branch of the superior mesenteric artery (SMA) during the shock and resuscitation protocol in pigs given an LVR solution consisting of saline or 10% PEG-20k. Values are mean $\pm$ SD, $n=5$ for each group, * $P<0.05$ between corresponding values or to the last value recorded in the saline group before death.

Figure 5.

Arterial blood gas HCO₃⁻ (panel A) and pCO₂ (panel B) measured during the shock and resuscitation protocol in pigs given a saline or a 10% PEG-20k based LVR solution. Values are mean $\pm$ SD, n=5 for each group, * P<0.05 between corresponding values or to the last value recorded in the saline group before death.

Characteristics of the two treatment groups of pigs in the study

	Control: 0.9% NaCl	Treatment: 10% PEG-20k
Weight (kg)	35.5 $\pm$ 2.6	35.3 $\pm$ 3.8
Blood Volume (ml)	2375 $\pm$ 172	2363 $\pm$ 257
Intubation/Surgery Time (min)	88.0 $\pm$ 20	82.2 $\pm$ 15
Loading Volume (ml)	306 $\pm$ 95	383 $\pm$ 211
Hemorrhage Time (min)	103 $\pm$ 8.9	122
Hemorrhage Volume (ml)	1298 $\pm$ 145	1220 $\pm$ 262
Hemorrhage Volume (% Total)	54.6 $\pm$ 5.5	51.5 $\pm$ 7.7

All values are mean $\pm$ SD. No significant differences exist between the groups for each corresponding attribute, n=5 each.