Pyruvate dose response studies targeting the vital signs following hemorrhagic shock

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ABSTRACT

Objectives: To determine the optimal effective dose of sodium pyruvate in maintaining the vital signs following hemorrhagic shock (HS) in rats. Materials and Methods: Anesthetized, male Sprague-Dawley rats underwent computer-controlled HS for 30 minute followed by fluid resuscitation with either hypertonic saline, or sodium pyruvate solutions of 0.5 M, 1.0 M, 2.0 M, and 4.0 M at a rate of 5ml/kg/h (60 minute) and subsequent blood infusion (60 minute). The results were compared with sham and non-resuscitated groups. The animals were continuously monitored for mean arterial pressure, systolic and diastolic pressure, heart rate, pulse pressure, temperature, shock index and Kerdo index (KI). Results: The Sham group remained stable throughout the experiment. Non-resuscitated HS animals did not survive for the entire experiment due to non-viable vital signs and poor shock and KI. All fluids were effective in normalizing the vital signs when shed blood was used adjutively. Sodium pyruvate 2.0 M was most effective, and 4.0 M solution was least effective in improving the vital signs after HS. Conclusions: Future studies should be directed to use 2.0 M sodium pyruvate adjuvant for resuscitation on multiorgan failure and survival rate in HS.

Key Words: Blood infusion, kerdo index, sodium pyruvate, hemorrhagic shock, resuscitation

INTRODUCTION

Hemorrhage is a leading cause of death in both civilian and military trauma.[1-3] Despite significant advancement in the pathophysiology of hemorrhagic shock (HS) and its treatment, the mortality rate still remains high.[3] Extensive bleeding due to unintentional injury remains the principle cause of death for US civilians’ ages 1-44.[4] A retrospective study of Trauma Centers from 2006-2010 reported that hemorrhage was the second leading cause of mortality.[5] The military reports 6667 total deaths from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF).[6] Of the potentially survivable deaths from these conflicts, 85% were caused by hemorrhage,[7] and predominantly from extremity vascular injuries.[8] Despite increasing emphasis on early care measures, aggressive fluid resuscitation can exacerbate hemorrhage and decrease survival.[9-11] In light of this, hemorrhagic shock remains a leading cause of morbidity and mortality from battlefield injuries.[12]

The body’s response to infusion is dependent on fluid composition, tonicity, severity of blood loss, and timing of administration.[3] Although the early signs of metabolic failure in HS are reduced oxygen consumption, elevated serum glucose and lactate levels, the restoration of tissue oxygenation with resuscitation does not reliably prevent organ failure and death.[4] The abject failure of improved fluid resuscitation and oxygen enhanced delivery strategies in improving outcome of HS is hallmarkled by the failure of an NIH sponsored trauma trial of HTS and the lack of efficacy of hemoglobin based oxygen carrier resuscitation (cancelled March 2009- http://www.nih.gov/news/health/mar2009/nhlbi-26.htm). When compared to other fluids, HTS, a 5-7.5% saline solution, shows no significant neutrophil excitation in animals.[13] This indicates that the impaired oxygen utilization and glucose metabolism due to dysfunctional mitochondria requires further mechanistic study and new therapeutics to improve outcome.

Pyruvate, a natural product of the reaction in the last step of the glycolytic pathway, plays a role in aerobic and anaerobic
metabolic processes. Pyruvate is a metabolic substrate as well as a potent antioxidant and anti-inflammatory agent.\[^{13}\] These early biochemical events have been implicated in the pathogenesis of organ dysfunction after resuscitation from hemorrhagic shock.

The administration of pyruvate to experimental animals has also been shown to ameliorate organ damage and improve survival in various animal models of hemorrhagic shock\[^{15-18}\]. Its immune modulation coupled with its ability to be transported at smaller volume may provide physiologic and logistical advantages for transport.\[^{13}\] In spite of these promising reports, its optimal dose for maximum benefits in maintaining the vital signs and prevention of organ damage in HS have not been examined. The rat model used in this study compared hypertonic saline solution (HTS) to various concentrations of pyruvate solutions (PYR) with delayed resuscitation after 30 minute of HS. This is a novel approach because it addresses two limitations of previous studies (timing and composition of resuscitative fluid);\[^{16}\] it establishes a concentration of pyruvate solution, which shows maximal benefit with administration after the onset of HS.

Reliable indicators of a patient’s hemodynamic status are necessary when evaluating HS and administering fluid resuscitation. Although vital signs are classically monitored, in critical care settings, they may be strikingly normal despite the patient’s unfavorable clinical presentation\[^{19}\]. Additionally, in animal studies, blood pressure variability is often detectable only after an animal has lost over 36% of estimated blood volume\[^{20}\]. In the early HS, sympathetic firing maintains arterial pressure at the expense of decreased tissue perfusion\[^{21}\], which explains why decompensation of vital signs is often a late finding\[^{22}\]. Continuous monitoring of hemodynamic, autonomic, and/or metabolic responses may provide earlier recognition of hemorrhage than standard vital signs and allow interventions before the onset of hypovolemic shock.\[^{23}\] The Shock index (SI) is the ratio of heart rate (HR) to systolic blood pressure (SBP) and may be more useful in early hemorrhage than either vital sign alone.\[^{24}\] The SI and vegetative balance (KI) may be useful in quantification of cardiovascular stress in HS.\[^{25}\]

The purpose of this study is to evaluate the effectiveness of HS to pyruvate solutions (PYR) of various concentrations followed by blood infusions in normalizing vital signs including the SI and KI as adequate fluid resuscitation measure in rats.

**MATERIALS AND METHODS**

The protocol and experimental procedures were reviewed and approved by the Animal Care and Use Committee (IACUC) of the Uniformed Services University of the Health Sciences at Bethesda, MD, with adherence to the Guide for Care and Use of Laboratory Animals. All animals were maintained in accordance with the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

**Animal preparation and surgical procedures**

These procedures were performed according to our established protocol.\[^{26}\] Male Sprague Dawley rats weighing 300-350 grams were purchased from Taconic Farms, Germantown, NY. Spontaneously ventilating rats were anesthetized using 5% isoflurane and 21% oxygen through a fitted nose cone. The femoral artery and femoral vein were cannulated with polyethylene catheters (PE 50- Clay Adams, Piscataway, NJ). The incision was closed with interrupted sutures. Body temperature was monitored and maintained at 37º C with a heating pad-rectal thermometer closed-loop system (Harvard Apparatus, Edenbridge, KT). The isoflurane concentration was reduced to 2.0%. The mean arterial pressure (MAP) was monitored by connecting the femoral arterial catheter to a pressure transducer and a computerized physiograph (Labview 5; National Instruments, Austin, TX). The resuscitative fluid or shed blood was infused through the femoral vein. Animals were allowed to acclimate to the induction of sedation and stress of surgery for about 10 min before the induction of HS.

**Induction of hemorrhagic shock and monitoring the vital signs**

A Wiggers type of isobaric hemorrhage was used\[^{27}\] according to our modified procedure.\[^{28}\] The computer controlled rat model of HS was created by the withdrawal of blood at a rate of 0.5 ml/min for 10-15 min from femoral arterial catheter using an Instech P720 peristaltic pump (Instech Laboratories Inc., Plymouth Meeting, PA) to induce shock (MAP 40 mmHg). Blood pressure was measured with a disposable transducer (Maxim Medical, Athens, TX) and blood pressure analyzer (BPA, Micro-Med, Louisville, KY); its serial port was connected to the PC in which the LabView-based (National Instruments, Austin, TX) hemorrhagic system program, developed at Walter Reed Army Institute of Research (F. Pearce; WRAIR, Silver Spring, MD) and stored the following variables: Mean, systolic, diastolic blood pressures, and HR with 12/minute rate. Additionally, a second PC was used to collect analog signals such as blood pressure, EKG, rectal temperature, exhaled O\(_2\), CO\(_2\) and isoflurane concentration (CapnomacUltima, Datec — Ohmeda, Louisville, CO) with a 16 bit resolution analog-digital converter card (PCI-6052E, National Instruments, Austin,TX) and DataLyser software (Baranyi, WRAIR). The AD sampling rate was 200 Hz. The recording was visualized by DataLyser, and analysis was made with the same software.

The formula of SI calculation is: ([HR in beats per minute] / (SBP in mmHg]). In interpreting results, the normal is 0.5 to 0.7; elevated SI: (>0.9) was found helpful by Rady et al. (1994) to identify patients in the emergency care units requiring admission and/or intensive care despite apparently stable vital signs.\[^{28}\] The KI can be calculated by using the equation (1 – diastolic blood pressure/heart rate) + 100.
Experimental groups
At the end of the shock phase (30 minute), the animals were resuscitated with fluid over 60 min (T90) followed by subsequent reinfusion of shed blood (T150).

Group 1. Sham (9 animals): Instrumented time control rats were anesthetized and instrumented in the same manner as rats in the other shock groups, but did not undergo arterial hemorrhage or resuscitation except the withdrawal of blood for laboratory investigation.

Group 2. HS (9 animals): HS without resuscitation. Animal was monitored till death.

Group 3. HTS (10 animals): Resuscitated with 7.5% hypertonic saline (HTS) at the rate of 5 ml/kg as a control for hypertonic sodium pyruvate group (HSP).

Group 4-7. HSP (8 animals in each gp): Resuscitated with various doses of sodium pyruvate (pyruvate 0.5 M, pyruvate 1 M, pyruvate 2 M, and pyruvate 4 M) infused at a rate of 5 ml/kg/hr.

Data analyses
Data from hemorrhagic system program was transferred to Excel spreadsheet, and were grouped by the type of infusion fluids, and compared to Sham and each other. Statistical manipulations involved calculations of group mean and standard errors as well as a one-way ANOVA with GraphPad Prism (La Jolla, CA) followed by Tukey Kramer post hoc test. A P value <0.05 was considered significant.

RESULTS
Mean arterial pressure (MAP) response to hemorrhage, HTS, pyruvate 0.5 M, pyruvate 1.0 M, pyruvate 2.0 M, pyruvate 4.0 M
Data depicted in Figure 1 shows that MAP was similar in all the animals at the baseline (0-5 minute), and decreased to approximately 40 mmHg in all groups during the 10-15-minute period that put them into HS. The MAP of the Sham group remained stable between 95 and 103 mmHg throughout the experiment. The animals with HS who received no fluids did not survive to the end of the experiment, but remained at a MAP of 40 mmHg before they died. Because of similar weights of the rats 323.39 ± 18.08 g, the total shed blood volume (2.43 ± 0.66% of total weight of the rat) did not differ significantly among the different rats. After resuscitation with the various intravenous fluids, all solutions increased the MAP, but still remained below the baseline. Mean arterial pressure was measured at the 105-110-minute mark and differences were noted among the different fluids. Overall, HTS increased the MAP the most by 44 mmHg. For pyruvate solutions, the 1.0 M solution increased the MAP the most by 37 mmHg, 2.0 M was the second highest with 33 mmHg, 0.5 M increased the MAP the next highest by 31 mmHg, and finally, 4.0 M increased the MAP the least with a 24 mmHg increase. However, the blood infusion that followed all solutions was effective in restoring the MAP to near baseline only in the HTS, 0.5 M, and the 1.0 M. The 2.0 M overshot the baseline, and the 4 M did not reach baseline.

Systolic blood pressure (SBP) response to hemorrhage, HTS, pyruvate 0.5 M, pyruvate 1.0 M, pyruvate 2.0 M, pyruvate 4.0 M
Data depicted in Figure 2 shows that SBP was similar in all of the rats at the baseline measurement (0-5 minute) and decreased to approximately 62.33 ± 5.24 mmHg in all groups during the 10-15-minute period that put the rats into HS. The SBP of the Sham group remained stable between 124 ± 0.46 and 130 ± 0.34 mmHg throughout the experiment. The rats with HS who received no fluids did not survive to the end of the experiment, but remained at a SBP between 66 ± 0.39 and 70 ± 0.28 mmHg before they died. Because of similar weights of the rats 323.39 ± 18.08 g, the total shed blood volume (2.43 ± 0.66% of total weight of the rat) did not differ significantly among the different rats. After resuscitation with the various intravenous fluids, all solutions increased the SBP, but still remained below baseline. SBP was measured at the 105-110-minute mark and differences were noted among the different fluids. Overall, the HTS had the greatest increase from hemorrhage to resuscitation with an increase of 58 mmHg. For pyruvate, the 1.0 M solution increased the SBP the most by 56 mmHg, 0.5 M was the second highest with 53 mmHg, 2.0 M increased the SBP the next highest by 49 mmHg, and finally, 4.0 M increased the SBP the least with a 39 mmHg increase. However, the blood infusion that followed all solutions was effective in restoring the SBP to near baseline only in the 0.5 M and the 1.0 M. The 2.0 M overshot the baseline, and
the 4 M did not reach baseline.

**Diastolic Blood Pressure (DBP) response to hemorrhage, HTS, pyruvate 0.5 M, pyruvate 1.0 M, pyruvate 2.0 M, pyruvate 4.0 M**

Data depicted in Figure 3 as well as the third section of that DBP was similar in all of the rats at the baseline measurement (0-5 minute) and decreased to approximately 32.33 ± 1.03 mmHg in all groups during the 10-15 minute period that put the rats into HS. The DBP of the Sham group remained stable between 74 ± 0.35 and 87 ± 0.29 mmHg throughout the experiment. The rats with HS who received no fluids did not survive to the end of the experiment, but remained with an HR between 340 ± 0.27 and 439 ± 2.54 bpm before they died. Because of similar weights of the rats 323.39 ± 18.08 g, the total shed blood volume (2.43 ± 0.66% of total weight of the rat) did not differ significantly among the different rats. After resuscitation with the various intravenous fluids, all solutions increased the DBP near or above baseline. Heart rate was measured at the 105-110 minute mark and differences were noted among the different fluids. The 4.0 M solution increased the HR the most by 178 bpm and overshot the baseline by 125 bpm, 1.0 M was the second highest with 87 bpm, 2.0 M increased the HR the next highest by 85 bpm, and finally, 0.5 M increased the HR the least with a 56 bpm increase. Overall, HTS had the third greatest increase with 68 bpm. However, the blood infusion that followed all solutions did not significantly change the HR from the IVF HR.

**Pulse pressure (PP) response to hemorrhage, HTS, pyruvate 0.5 M, pyruvate 1.0 M, pyruvate 2.0 M, pyruvate 4.0 M**

Data depicted in Figure 5 shows that PP was similar in all of the rats at the baseline measurement (0-5 minute) and decreased to approximately 30.00 ± 5.68 mmHg in all groups during the 10-15 minute of hemorrhage period. The PP of the Sham group remained stable between 44 ± 0.21 and 53 ± 0.14 mmHg.
throughout the experiment. The rats with HS who received no fluids did not survive to the end of the experiment, but remained at a PP between 36 ± 0.30 and 38 ± 0.30 mmHg before they died. Because of similar weights of the rats 323.39 ± 18.08 g, the total shed blood volume (2.43 ± 0.66% of total weight of the rat) did not differ significantly among the different rats. After resuscitation with the various intravenous fluids, all solutions increased the PP, and all overshot the baseline HR. Pulse pressure was measured at the 105-110-minute mark and differences were noted among the different fluids. The 0.5 M solution increased the PP the most by 40 mmHg, which also overshot the baseline HR; 0.5 M solution had the least most significant increase (+56 bpm), but it did not overshoot the baseline HR value. Blood infusion made no impact on the post-IVF resuscitation values. *P < 0.05 compared with sham.

Figure 4: Heart rate mean of the means of all pyruvate solutions. Assuming that Sham represents the normal HR values for an anesthetized rodent. All IVF increased HR toward the baseline with the most significant increase seen in the 4.0 M solution (+178 bpm); however, it overshot the baseline HR by 125 bpm. The second most significant increase was seen in 1.0 M solution (+87 bpm), which also overshot the baseline HR; 0.5 M solution had the least significant increase (+56 bpm), but it did not overshoot the baseline HR value. Blood infusion made no impact on the post-IVF resuscitation values. *P < 0.05 compared with sham.

Figure 5: Pulse pressure mean of means of all pyruvate solution. Assuming that Sham represents the normal PP values for an anesthetized rodent. All IVF increased the PP toward baseline, in fact, all overshot the baseline value. The most significant increase was seen with the 0.5 M solution (+40 mmHg) followed by the 1.0 M solution (+31 mmHg). The least significant increase was seen with 4.0 M solution (+21 mmHg). All IVFs overshot the baseline value in the same order in which they increased the PP: the 0.5 M solution overshot the PP the most (+26 mmHg) with 4.0 M solution overshooting the least (+8 mmHg). Blood infusion brought the overshot PP values closer to baseline. *P < 0.05 compared with sham.

Kerdo index (KI) response to hemorrhage, HTS, pyruvate 0.5 M, pyruvate 1.0 M, pyruvate 2.0 M, pyruvate 4.0 M

Data presented in Figure 7 shows that KI was similar in all animals at the baseline (0-5 minute), and increased to approximately 100.88 ± 0.013 in all groups during the 10-15-minute period that put the rats into HS. The KI of the Sham group remained stable between 100.74 ± 0.001 and 100.78 ± 0.001 mmHg throughout the experiment. The rats with HS who received no fluids did not survive to the end of the experiment, but remained at a KI between 100.90 ± 0.001 and 100.93 ± 0.001 mmHg before they died. Because of similar weights of the rats 323.39 ± 18.08 g, the total shed blood volume (2.43 ± 0.66% of total weight of the rat) did not differ significantly among the different rats. After resuscitation with the various intravenous fluids, all solutions decreased the KI except for the 4.0 M solution. Shock index was measured at the 105-110-minute mark, and differences were noted among the different fluids. Overall, HTS decreased the SI the most with a −1.86 decrease. For the pyruvate solutions, the 0.5 M solution decreased the SI the most by −1.73; 1.0 M decreased the SI the next most with −1.57; 2.0 M solution had the least decrease in SI with a value of −0.88; and 4.0 M increased the SI by 0.27. With the blood infusion that followed, only the HTS, 0.5 M, and 2.0 M solutions brought the SI close to the baseline value; 1.0 M and 4.0 M remained elevated.
was measured at the 105-110-minute mark, and differences were noted among the different fluids. Hypertonic saline solution and the 2.0 M solution decreased the KI the most by −0.07; 1.0 M decreased the SI the next most with −0.05; 0.5 M had the least decrease in KI with a value of −0.04, but 4.0 M increased the KI by 0.01. With the blood infusion that followed, all values further decreased toward the baseline level except for the 4.0 M solution.

**DISCUSSION**

In both civilian and military traumatic injury, severe and prolonged hemorrhage is a leading cause of death.\[1,2\] Hemorrhage leads to HS, which causes the vast majority of preventable deaths.\[7\] The primary interventions in hemorrhage are hemostatic control and intravenous fluid resuscitation.\[12\] Current studies such as this one are aiming to identify intravenous fluids with pyruvate as metabolic adjuvant in normalizing the physiologic measures while reducing cellular injury. While fresh whole blood is known to be the best resuscitation fluid; but it is only available when auto-transfused making its availability very limited.\[29\] Even transfusions of stored red blood cells show an altered immune response and are an independent risk factor for post injury multiple organ failure.\[29\] Previous studies have shown that HTS and PYR solutions may be beneficial in fluid resuscitation.\[16\] The purpose of this observational and descriptive study is to evaluate the effectiveness of HTS to pyruvate solutions (PYR) of various concentrations followed by blood infusions in normalizing vital signs, the SI and KI as adequate fluid resuscitation measure in rats.

Our findings show that both HTS and PYR solutions were most effective at normalizing the vital signs and indices when followed by infusion of shed whole blood. The 2.0 M PYR solution showed the most significant improvement in MAP, SBP, DBP, and the KI when followed by blood resuscitation. Whole blood contains reactive oxygen species scavengers and buffering capacity, minimizing its activation of the immune system. In previous studies, it has been shown that animals resuscitated with fresh whole blood did not have immune-mediated cellular injury caused by neutrophil activation.\[29\] In fact, the infusion of whole blood actually reversed neutrophil activation, which suggests that restoration of blood volume is not the only critical variable in resuscitation.\[29\] Pyruvate’s protective effect on intracellular antioxidants\[16\] and mitochondrial metabolism facilitated by the immune modulation of fresh whole blood may have played an important role in the compensatory changes.\[29\]

The SI is the ratio of the HR to SBP. The index is a sensitive indicator of left ventricular dysfunction and can become elevated following a reduction in left ventricular stroke work. The SI can be used in the emergency care and intensive care units to identify patients needing a higher level of care despite vital signs that may not appear strikingly abnormal.\[30\] Persistent elevation of the SI has been associated with a poor outcome in critically ill patients.\[31\] The Shock index is a sensitive indicator of left ventricular dysfunction\[32\] and displays variability in critical patients displaying normal vital signs\[33\]. Persistently elevated SI is also associated with poor outcomes in critically ill patients.\[32\] In our current study, the greatest decrease in SI was seen with HTS followed by the 0.5 M solution, and the least decrease in SI was seen with 4.0 M solution which remain elevated even after blood infusion. These results indicate the toxicity of higher pyruvate concentrations i.e. 4M which actually increased the SI (+0.27) in comparison to its benefits at lower doses.
The KI values above 100 indicate sympathicotonia, and values below 100 indicate parasympathicotonia. Both SI and modified KI were successfully used to differentiate among hemostatic bandages.[23] Furthermore, the KI values are indicative of autonomic dysfunction.[24] In addition, HTS was the most effective fluid when used alone. It had the greatest improvements at restoring all vital signs and SI and KIs toward the baseline. These findings are significant because HTS showed compensatory change alone in all measures. HTS fluid resuscitation has also shown no significant neutrophil activation suggesting it is also protective against cellular injury.[25]

This study has many strengths to contribute to today’s controversy on fluid resuscitation. First, the study aims to mimic real-life traumatic scenarios; for example, 21% oxygen was used to mimic the inspiration of room air during emergency hemorrhage. Previous studies infused solutions before and during hemorrhage, whereas this study aimed to represent the delay in fluid resuscitation often experienced on the battlefield; therefore, intravenous solutions were administered after the onset of HS. Second, the use of the Shock and Kerdo indices are able to quantify cardiovascular and autonomic stress responses before vital signs are abnormal.[26] Third, this study addresses the limitations of previous studies by establishing the concentration of pyruvate solutions which shows maximal benefit.[16]

Although this study exhibits many strengths, we recognize that certain limitations also exist. First, the use of fresh whole blood infusions after intravenous solutions is unrealistic for battlefield trauma. There is no ability to capture the blood lost on the battlefield for reinfusion. Current TCCG guidelines for Tactical Evacuation Care state that if blood products are available, resuscitate with plasma followed by packed red blood cells.[24] Second, the use of pressure-controlled HS models in not as realistic as uncontrolled HS models.[27] The amount of blood lost in real-life situations is often unknown, and visually, estimated blood loss is often imprecise.[28] An uncontrolled model may be beneficial in the future. Third, the use of anesthetic was uniform in all subjects; however, the effect on cardiovascular responses is unknown. Fourth, at a sustained MAP of 40 mmHg, the body loses its autonomic self-regulatory ability. Future challenges exist for advances in fluid resuscitation models after severe HS. Evidence exists that hypothermia is the most effective method to maintain cellular function in the presence of ischemia, but clinical studies have not been conducted on the benefits of hypothermia in trauma patients.

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