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Anti-Inflammatory Resuscitation Improves Survival in Hemorrhage With Trauma

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Abstract

Background—Hemorrhage is a common cause of death despite the recent advances in resuscitation and critical care. Conventional resuscitation fluids are designed to reestablish tissue perfusion, but they fail to prevent systemic inflammation. Indeed, resuscitation can promote inflammatory responses, which can be more dangerous than the original hemorrhage. This consideration is relevant in critical care where hemorrhage is normally associated with collateral trauma that can exacerbate the inflammatory responses during resuscitation. Here, we analyzed whether ethyl pyruvate could provide a therapeutic antiinflammatory potential during resuscitation in experimental hemorrhage with trauma.

Methods—Adult male Sprague-Dawley rats were subjected to trauma induced by closed femur fracture. Then, the animals were immediately subjected to lethal hemorrhage during 15 minutes to reach a mean arterial blood pressure of 35 mm Hg to 40 mm Hg and subsequent maintenance of this mean arterial blood pressure for another 15 minutes. Resuscitation was limited to 15 mL/kg Hextend with or without ethyl pyruvate.

Results—Resuscitation with conventional fluids reestablished normal tissue perfusion, but still more than 60% of the animals died. Resuscitation with ethyl pyruvate protected all the animals from lethal hemorrhage with trauma. Trauma exacerbated tumor necrosis factor (TNF) levels in the serum, the spleen, and the heart. Ethyl pyruvate blunted TNF levels in the serum and all the organs but particularly in the lung and the liver during resuscitation. TNF levels in the lung, spleen, and the liver of those animals resuscitated with ethyl pyruvate were statistically similar to those in control animals.

Conclusion—Ethyl pyruvate may attenuate systemic inflammatory responses during resuscitation and improve survival in experimental models of hemorrhage with trauma.

Keywords

Trauma and hemorrhagic shock; Cytokines; Inflammation; Ethyl pyruvate

Hemorrhagic shock is one of the leading causes of death in trauma despite the recent advances in resuscitation and critical care.^{1,2} Conventional resuscitation fluids are designed to reestablish tissue perfusion, but they fail to prevent inflammatory responses. Resuscitation can produce inflammatory responses, which might be more dangerous than the original

hemorrhage. Tumor necrosis factor (TNF) is one of the most characteristic inflammatory and cardiode-pressant factors contributing to cardiovascular shock in hemorrhage and trauma.^{3–5} Moderate TNF production can be beneficial to induce inflammatory responses to confine tissue damage or infection. However, excessive TNF production can contribute to lethal cardiovascular shock and multiple organ failure. TNF seems to play a significant role in “hemorrhagic shock”^{3–5}: (a) TNF is found in patients and experimental models of “hemorrhagic shock”; (b) recombinant TNF pectrum of hemodynamic, metabolic, and pathologic sequelae of “hemorrhagic shock”; and (c) TNF neutralization prevents cardiovascular shock. For these reasons, there is an interest in developing advanced resuscitation fluids capable of restraining TNF production during resuscitation.

Ringer lactated is the most characteristic resuscitation fluid used to restore circulating volume in hemorrhage.⁶ Advanced resuscitation fluids include Hextend, the novel plasma volume expander containing 6% hydroxyethyl starch in Ringer’s lactate.⁷ Hetastarch creates oncotic pressure, which is normally provided by blood proteins and permits retention of intravascular fluid. Hextend is being established as standard care for the Army’s Special Forces because, it seems to prevent multiple organ injury⁸ and improve short-term survival when compared with saline.⁹ Hextend required less intravenous fluid and provided a significant survival benefit in hypotensive resuscitation.^{10,11} Still, advanced and common resuscitation fluids are based on lactated solutions. Lactic acid (2-hydroxypropanoic acid) is used as an inert compound that does not provide any therapeutic value. Moreover, recent studies suggest that lactate in the Ringer’s solution may exacerbate lactic acidosis during hypovolemia.¹² Pyruvate (2-ketopropanoic acid), a molecule structurally similar to lactate, is a potential antioxidant and anti-inflammatory that protects mammalian cells from cytotoxicity.¹³ Pyruvate seems to be beneficial in experimental models of stroke and hemorrhage.¹⁴ However, the clinical use of pyruvate is hampered by its instability and toxicity.¹⁵ Aqueous solutions of pyruvate spontaneously degrade via condensation to form 2,4-dihydroxy-2-methylglutarate, a mitochondrial poison.¹⁵ To circumvent this problem, we used ethyl pyruvate, a stable lipophilic pyruvate derivative. Ethyl pyruvate is also compatible with blood products, but unlike lactate, it can provide a therapeutic benefit to the Ringer’s solution.^{16,17} Ethyl pyruvate is a stable, soluble, nontoxic compound that lacks the potential toxicity of pyruvate and it is classified as general recognized as safe by the FDA. These studies suggest that ethyl pyruvate might provide a beneficial anti-inflammatory potential to resuscitation fluids.

Recently, we described that ethyl pyruvate inhibits TNF production in human and murine macrophages.^{18,19} Previous studies indicated that ethyl pyruvate attenuated systemic inflammation and improved survival in experimental models of sepsis^{18–23} and other classical models of systemic inflammation.^{17,24–26} Unlike previous classical experimental models, hemorrhage in critical care is normally associated with collateral trauma and tissue injury that exacerbate the inflammatory responses during hemorrhage and resuscitation.^{1,2} Here, we intended to determine whether ethyl pyruvate can improve survival in experimental models of hemorrhage with trauma. In addition to survival, blood and organs were subjected to analyses to determine a potential mechanism of action. Given the implications of TNF in hemorrhagic shock, we analyzed the potential of ethyl pyruvate to restrain systemic TNF concentrations during resuscitation.

MATERIALS AND METHODS

Animal Experiments

Adult male Sprague-Dawley rats (350–450 g) were purchased from Harlen Sprague-Dawley (Indianapolis, IN) and acclimated for 7 days at 25°C on a 12-hour light/dark cycle. All animal experiments were performed in accordance with the National Institutes of Health

Guidelines under protocols approved by the Institutional Animal Care and Use Committee of the North Shore University Hospital. Animals were randomized and investigators were blinded to the experimental resuscitation treatment. Animals were analyzed in the following groups: control (no surgical procedure), H (hemorrhage without resuscitation or trauma), TH (trauma + hemorrhage but no resuscitation), TH + resuscitation with Hextend, TH + resuscitation with Hextend supplemented with different concentrations of ethyl pyruvate (5, 25, or 50 mmol/L).

Hemorrhage With Trauma

First, animals underwent trauma, immediately before the surgical catheter placement. Then, after 15 minutes of recording the baseline, animals underwent the hemorrhagic procedure. Trauma was induced by closed femur fracture prior hemorrhagic shock as previously described by Bonnarens and Einhorn.²⁷ Hemorrhagic shock was performed similar to that described by Handrigan et al.¹⁰ with the modifications described in Cai et al.²⁸ Briefly, animals were anesthetized by inhalation of isoflurane (Minrad, Buffalo, NY). With the animal supine, the right femur was placed in abduction and positioned on the center of the support stage. The stage was raised until the blunt guillotine blade was snug with the thigh. The travel of the ramming system was set at 1.5 mm. The 500 g sliding weight was dropped from a height of 25 inches. The complete transverse fracture was confirmed by palpation. The animals were immediately subjected to surgical catheter placement into the femoral artery and vein under sterilized condition with catheter R-FAC (Braintree Scientific, Braintree, MA). After inserting the catheter, the blood pressure and the heart rate were monitored and recorded for 15 minutes as a physiologic baseline prior the hemorrhage procedure. Then, the animals underwent hemorrhage from femoral artery for 15 minutes to reach a mean arterial blood pressure of 35 mm Hg to 40 mm Hg and subsequent maintenance of this blood pressure by continuous blood withdrawal for another 15 minutes. Shed blood was considered lost and it was not reinfused. After the shock phase, the animals of resuscitation received specific resuscitation treatment during 40 minutes with a total volume of 15 mL/kg (equivalent to 1,000 mL of Hextend in a 70 kg man). Heart rate, mean arterial blood pressure, shed blood volume, and intravenous fluid volume were continuously monitored and recorded. After surgical procedure, the animals were allowed to recover from anesthesia and housed individually in regular cages.

Blood Chemistry and Cytokine Measurement

Blood was collected at 2 hours after the hemorrhagic shock and analyzed using i-Stat blood analyzer (Abbot Laboratories, IL). Metabolic changes in hemorrhagic shock were analyzed by the detection of electrolytes, pH, bicarbonate (HCO_3^-), base excess of extracellular fluid (BE_{ecf}), anion gap (AnGap), glucose, hematocrit, and hemoglobin as well as total plasma protein. Lung and kidney functions were assessed by blood gases including total and partial carbon dioxide (TCO_2 , PCO_2) and blood urea nitrogen. TNF was measured by ELISA following the instruction of the manufacturer's kit (R&D Systems, Minneapolis, MN). TNF concentrations in serum were related to volume. Major organs were homogenized in Cell lysis buffer (Cell signaling, Danver, MA) 1:3 weight/volume. Homogenates were centrifuged at 12,000 rpm for 25 minutes at 4°C. The protein concentration in the supernatant was analyzed using Bradford protein assay (Biorad, Hercules, CA). Samples were normalized to the same total protein concentration. Then, TNF concentration was measured by ELISA and correlated with to total organ protein.

Statistical Analyses

All data were expressed as mean $\pm$ SD. Analyses of normality and variance were performed to verify the assumptions of analysis of variance (ANOVA). Data were logtransformed where applicable. Statistical analyses were performed using the one-way ANOVA with the

Bonferroni correction. ANOVA was used to compare all treatments and specific pair-wise comparisons as stated in the experiments particularly to compare Control versus Hextend, Hextend versus HEP (Hextend containing 50 mmol/L EP), and Control versus HEP. The Student's *t* test was used to compare mean values between the two experimental groups. Statistical analyses of survival were determined using the Logrank test. Kaplan-Meier product-limit method was used for survival graphs. The values of $p < 0.05$ were considered statistically significant.

RESULTS

Ethyl Pyruvate Provided Therapeutic Potential to Resuscitation Fluids

Hemorrhagic shock required withdrawing 18.2 ± 3.6 mL blood/kg body weight and the maintenance of that blood pressure for another 15 minutes required another 6 ± 2.5 mL blood/kg body weight. Animals without resuscitation (NR) did not reestablish normal blood pressure (Fig. 1, A) and died within the first 5 hours after hemorrhage (Fig. 1, B). Resuscitation with Hextend (HXT) allowed the animals to recover normal blood pressure and reestablish tissue perfusion, but still more than 60% of the animals died within the first 5 hours after hemorrhagic shock. Resuscitation with Hextend protected 40% of the animals in a statistically significant manner ($n = 9$; $p < 0.001$, Logrank Test; HXT vs. control). Animals resuscitated with Hextend supplemented with 50 mmol/L ethyl pyruvate reestablished blood pressure significantly earlier than the animals resuscitated with Hextend alone. The addition of 50 mmol/L ethyl pyruvate to Hextend solution provided a therapeutic potential to rescue all the animals from lethal hemorrhage ($p < 0.05$, Logrank Test; HEP vs. HXT). All the animals resuscitated with Hextend supplemented with 50 mmol/L ethyl pyruvate survived ($n = 7$ per group). Ethyl pyruvate improved survival in a concentration dependent manner (Fig. 1, C). The addition of 25 mmol/L but not 5 mmol/L ethyl pyruvate to Hextend induced a statistically significant protection ($p = 0.01$ and $p = 0.6$, respectively, Logrank test) when compared with Hextend solution without ethyl pyruvate. Addition of 25 mmol/L ethyl pyruvate to Hextend protected 80% of the animals with hemorrhagic shock, whereas 5 mmol/L ethyl pyruvate protected 50% of the animals ($n = 7$ per group). Our analyses excluded the animals that died during the surgical procedure and counted only those animals that completed the resuscitation treatment. All nonsurvival animals died within the first 5 hours after hemorrhagic shock. All the animals that passed that critical period, survived the hemorrhagic shock, and no late deaths were found, although the animals were followed for up to 7 days. These results also suggest that ethyl pyruvate may induce a lasting protection and it did not merely delay the pathologic onset.

Blood Chemistry Analyses During Resuscitation

Characteristic pathologic markers of hemorrhage were assessed by blood chemistry analyses. Blood was collected at 2 hours after the hemorrhagic load, which is ~ 30 minutes before the average time of death for the control animals without resuscitation treatment (161 ± 26 minutes). Animals without resuscitation are characterized by uremia, metabolic acidosis, and hyperglycemia. Animals without resuscitation showed characteristic high levels of blood urea nitrogen representing a moderate renal failure. Although resuscitation with Hextend or Hextend supplemented with 50 mmol/L ethyl pyruvate improved survival, they did not prevent uremia (Fig. 2, A). Hemorrhagic animals without resuscitation showed metabolic acidosis (Fig. 2, B) associated with elevated anion gap (AnGap) (Fig. 2, C), hypochloremia (Fig. 2, D), and low levels of bicarbonate (Fig. 2, E). Likewise, hemorrhage caused hyperglycemia that was not prevented by either resuscitation with Hextend or Hextend supplemented with 50 mmol/L ethyl pyruvate (Fig. 2, F). Resuscitation with Hextend or Hextend supplemented with ethyl pyruvate induced similar effects in these markers.

Trauma Increased Inflammatory Responses During Hemorrhage

The effects of trauma in hemorrhage were determined by analyzing both control hemorrhagic animals (without trauma) and hemorrhagic animals with trauma. TNF, a characteristic inflammatory and cardiodepressant factor contributing to cardiovascular shock, is a characteristic common response to both hemorrhage and trauma. Animals with hemorrhage and trauma had almost double TNF levels in the serum when compared with the control hemorrhagic animals without trauma (Fig. 3). TNF was also analyzed in the major organs, and TNF concentration was normalized according to total protein concentration. Among the major organs, TNF concentration was higher in the heart and the spleen in both hemorrhage and hemorrhage with trauma. The highest systemic TNF levels found in hemorrhage with trauma associated with significantly higher TNF levels in the heart, the spleen, and the liver but not in the lung. Although the increase of hepatic TNF levels were slightly lower, the animals with trauma have almost double concentration of cardiac and splenic TNF, a proportion similar to that found in the serum.

Ethyl Pyruvate Attenuated Systemic Inflammation During Resuscitation

The most significant effect correlating with the therapeutic potential of ethyl pyruvate was its anti-inflammatory potential to restrain serum TNF levels. Hemorrhage induced a characteristic lethal systemic TNF response that was not prevented by resuscitation with Hextend. Resuscitation with Hextend slightly attenuated serum TNF levels by 10%. This effect could be explained by the dilution of serum TNF induced by the volume of resuscitation. These results also suggest that systemic TNF levels may not reflect the levels of that cytokine in major organs because of the TNF production by circulating immune cells. However, resuscitation with Hextend containing ethyl pyruvate prevented this lethal response, and these animals had circulating TNF levels statistically similar to those of control normal rodents (Fig. 4). To characterize its anti-inflammatory mechanism, we analyzed the effects of ethyl pyruvate in specific organs (Fig. 5). Although resuscitation with Hextend did not inhibit systemic TNF levels, resuscitation itself attenuated TNF levels in many organs and particularly in the lung and the liver. Resuscitation with ethyl pyruvate induced a major effect inhibiting TNF levels by 50% and 60% in the heart and the spleen, respectively. TNF levels in the lung, spleen, and the liver of those animals resuscitated with ethyl pyruvate were statistically similar to those in control animals (without hemorrhage).

DISCUSSION

Conventional resuscitation fluids are designed to reestablish blood pressure and tissue perfusion, but they fail to prevent deleterious inflammatory responses. Resuscitation with conventional fluids such as Hextend reestablished tissue perfusion, but still more than 60% of the animals died within the first 5 hours after hemorrhage. Indeed, resuscitation can cause systemic inflammatory responses that can be more dangerous than the original hemorrhage. This consideration is particularly relevant in critical care where hemorrhage is normally associated with collateral trauma that can exacerbate inflammatory responses. Here, we report that ethyl pyruvate may provide a therapeutic potential to resuscitation fluids. All the animals resuscitated with Hextend supplemented with 50 mmol/L ethyl pyruvate survived hemorrhage with trauma. There are two considerations that may define the clinical implications of these studies. First, our experimental model intends to mimic significant aspects of critical care including trauma and tissue injury. Similar to hemorrhaging humans who either die quickly or survive long term, all the control animals either died quickly within the first 5 hours or survived long term. The animals were followed for up to 7 days and no late deaths were observed. Second, our experimental models included about 50% of total blood volume loss. Shed blood was considered lost and it was not reinfused. Resuscitation included a small volume of 15 mL/kg (equivalent to 1,000 mL of Hextend in a

70 kg patient) that represents ~50% of the shed blood volume. High-volume, normotensive resuscitation may lead to increased hemorrhage volume and markedly higher mortality when shock is associated with major vascular injury.²⁹ These considerations may have implications in several clinical settings of rural areas, military combats, or mass casualty characterized by low supplies of resuscitation fluid, long time of evacuation to a medical facility, and hemorrhage associated with severe trauma.^{11,30}

Resuscitation with Hextend with or without ethyl pyruvate induced a very similar effect in the most characteristic metabolic markers. These markers may represent a moderate organ dysfunction because ethyl pyruvate induced a lasting survival although it did not prevent these effects. These results are in agreement with previous studies indicating that ethyl pyruvate does not affect early hemodynamics in swine or rodents with classical hemorrhage without trauma.²⁶ Resuscitation with ethyl pyruvate reestablished mean arterial blood pressure significantly earlier than resuscitation with Hextend. Since TNF is a characteristic cardiodepressant factor contributing to cardiovascular shock,^{3,4} this effect could be explained, at least in part, by lower serum TNF levels. TNF is a common response to both trauma and hemorrhage. This is a significant consideration because unlike classical experimental models, clinical hemorrhage is normally associated with collateral trauma that exacerbates the inflammatory responses contributing to cardiovascular shock during resuscitation. Trauma induced by broken femur increased serum TNF levels by twofold in experimental hemorrhage. This response correlated with higher TNF levels in most of the organs but particularly in the spleen and the heart. These results concur with previous studies suggesting that the spleen can be a major source of systemic TNF and inhibition of TNF production in the spleen can prevent cardiovascular shock.³¹ For instance, nicotinic agonists attenuates systemic inflammation in control but not in splenectomized endotoxemic rodents.³¹ However, ethyl pyruvate also attenuates systemic inflammation in splenectomized animals²⁸ suggesting that it triggers a different anti-inflammatory mechanism independent of the spleen. In agreement with this hypothesis, our study indicates that ethyl pyruvate attenuates TNF levels in all the major organs. Indeed, the most significant results correlating with its therapeutic potential were that, unlike Hextend, ethyl pyruvate blunted systemic TNF levels. Ethyl pyruvate was particularly efficient in the spleen, lung, and liver, where TNF levels after resuscitation were statistically similar to those in control animals. These results concur with other studies suggesting that ethyl pyruvate can ameliorate organ injury in different clinical settings.³²

Little is known about the therapeutic mechanism of ethyl pyruvate. Our previous studies indicate that ethyl pyruvate prevents endotoxin-induced NF- κ B activation in RAW264.7 macrophage cells.^{18,19} Similar studies in kidney 293 cells indicated that 40 mmol/L of ethyl pyruvate inhibits p65RelA DNA-binding.³³ In a cell-free system, ethyl pyruvate failed to inhibit the DNA-binding activity of both p50 homodimers and the mutant form of p65RelA with substitution of serine for cysteine 38.³³ In addition to different cell lines, we also reported that ethyl pyruvate modulates splenic NF- κ B.²⁸ A potential mechanism is that ethyl pyruvate can attenuate TNF production by inhibiting NF- κ B. However, these results represent a conundrum because NF- κ B modulates cytokine production, but it also protects parenchyma cells from cytotoxicity and cell death.^{34–36} The most characteristic example is that p65RelA^{37–39} and IKK^{40,41} knockout mice exhibit embryonic death resulting from extensive TNF-mediated fetal hepatocyte apoptosis. Consistently, disruption of the TNF signaling, either by removing TNF⁴² or TNF-R1,⁴¹ prevents this hepatocyte apoptosis in *rela*^{-/-} mice, allowing embryonic development to birth. In agreement with these studies, NF- κ B inhibition after partial hepatectomy results in massive hepatocyte apoptosis and impaired liver function,³⁹ and NF- κ B activation in the liver, by neutralizing HMGB1, prevents hepatic injury during ischemia and reperfusion.⁴³ However, HMGB1 inhibition produces different effects in enterocytes. Anti-HMGB1 antibodies inhibit NF- κ B in

HMGB1-challenged enterocytes and prevent intestinal derangements during experimental sepsis.⁴⁴ These studies suggest that ubiquitous NF- κ B inhibition may not generate an overall beneficial effect especially in the liver, unless the therapy targets specific organs, immune cells, or NF- κ B isoforms.⁴⁵ We recently reported that ethyl pyruvate can also inhibit poly(ADP-ribose) polymerase (PARP) activation during resuscitation.²⁸ These results could have significant implications as PARP inhibition prevents organ injury and improves survival in hemorrhagic shock.^{46–49}

Since PARP regulates NF- κ B, and PARP inhibitors suppress TNF production in vitro and in vivo,⁴⁸ it is possible that the therapeutic potential of ethyl pyruvate is, at least in part, mediated by the modulation of PARP. However, it is unknown how ethyl pyruvate can modulate PARP. One hypothesis is that PARP can be modulated by the antioxidant potential of ethyl pyruvate.⁵⁰ During hemorrhage oxidant stress causes DNA strand breakage, which it is the main activator of PARP. The broad-spectrum antioxidant potential of ethyl pyruvate can reduce oxidative stress and less DNA breakage can prevent PARP activation.⁴⁸ Future studies are needed to determine the anti-inflammatory mechanism of ethyl pyruvate and also to define its clinical potential in different settings of critical care. These studies are of particular interest since a phase-II trial using ethyl pyruvate in cardiopulmonary bypass was recently terminated after the enrollment of 102 patients, yet the final results have not been reported.⁵¹ One recent study indicated that ethyl pyruvate decreased NF- κ B but worsened survival in LPS-challenged mice.⁵² Mice were treated with a single dose of different concentrations of ethyl pyruvate (0.01–100 mg/kg, n = 204) at the same time that LPS was giving. The individual experiments did not provide a statistically significant effect, but it was statistically significant if all the experiments were combined together.⁵² One potential explanation for this effect is that ethyl pyruvate was given in a single dose at the same time as LPS. Other studies reported therapeutic benefits with repeated administration of ethyl pyruvate started at different time points even before the endotoxemic challenge.^{18,53} Together, these results warrant caution and reveal the need to study the factors affecting the mechanism and therapeutic potential of ethyl pyruvate in different experimental models before its effective use in clinical applications.^{52,53}

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REFERENCES

1. Mannucci PM, Levi M. Prevention and treatment of major blood loss. *N Engl J Med* 2007;356:2301–2311. [PubMed: 17538089]
2. Rushing GD, Britt LD. Reperfusion injury after hemorrhage: a collective review. *Ann Surg* 2008;247:929–937. [PubMed: 18520219]
3. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol* 1993;9:317–343. [PubMed: 8280464]
4. Tracey KJ, Cerami A. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. *Annu Rev Med* 1994;49:1–503. [PubMed: 8198398]
5. Ulloa L, Tracey KJ. The “cytokine profile”: a code for sepsis. *Trends Mol Med* 2005;11:56–63. [PubMed: 15694867]
6. Cordell AR. Milestones in the development of cardioplegia. *Ann Thorac Surg* 1995;60:793–796. [PubMed: 7677535]
7. Gan TJ, Bennett-Guerrero E, Phillips-Bute B, et al. Hextend, a physiologically balanced plasma expander for large volume use in major surgery: a randomized phase III clinical trial. Hextend Study Group. *Anesth Analg* 1999;88:992–998. [PubMed: 10320157]

8. Nielsen VG, Tan S, Brix AE, Baird MS, Parks DA. Hextend (hetastarch solution) decreases multiple organ injury and xanthine oxidase release after hepatoenteric ischemia-reperfusion in rabbits. *Crit Care Med* 1997;25:1565–1574. [PubMed: 9295833]
9. Kellum JA. Fluid resuscitation and hyperchloremic acidosis in experimental sepsis: improved short-term survival and acid-base balance with Hextend compared with saline. *Crit Care Med* 2002;30:300–305. [PubMed: 11889298]
10. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL. Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. *Shock* 2005;23:337–343. [PubMed: 15803057]
11. Mapstone J, Roberts I, Evans P. Fluid resuscitation strategies: a systematic review of animal trials. *J Trauma* 2003;55:571–589. [PubMed: 14501908]
12. Baskett TF. The resuscitation greates: Sydney Ringer and lactated Ringer's solution. *Resuscitation* 2003;58:5–7. [PubMed: 12867303]
13. Montgomery CM, Fairhurst AS, Webb JL. Metabolic studies on heart mitochondria. III. The action of parapyruvate on alpha-ketoglutaric oxidase. *J Biol Chem* 1956;221:369–376. [PubMed: 13345826]
14. Slovin PN, Huang CJ, Cade JR, et al. Sodium pyruvate is better than sodium chloride as a resuscitation solution in a rodent model of profound hemorrhagic shock. *Resuscitation* 2001;50:109–115. [PubMed: 11719137]
15. Vonkorff RW. Pyruvate-C14, purity and stability. *Anal Biochem* 1964;8:171–178. [PubMed: 14186671]
16. Fink MP. Ethyl pyruvate: a novel anti-inflammatory agent. *Crit Care Med* 2003;31(1 Suppl):S51–S56. [PubMed: 12544977]
17. Fink MP. Ethyl pyruvate: a novel treatment for sepsis and shock. *Minerva Anesthesiol* 2004;70:365–371. [PubMed: 15181417]
18. Ulloa L, Ochani M, Yang H, et al. Ethyl pyruvate prevents lethality in mice with established lethal sepsis and systemic inflammation. *Proc Natl Acad Sci USA* 2002;99:12351–12356. [PubMed: 12209006]
19. Ulloa L, Fink MP, Tracey KJ. Ethyl pyruvate protects against lethal systemic inflammation by preventing HMGB1 release. *Ann NY Acad Sci* 2003;987:319–321.
20. Yang R, Uchiyama T, Watkins SK, Han X, Fink MP. Ethyl pyruvate reduces liver injury in a murine model of extrahepatic cholestasis. *Shock* 2004;22:369–375. [PubMed: 15377894]
21. Sappington PL, Cruz RJ Jr, Harada T, et al. The ethyl pyruvate analogues, diethyl oxaloproprionate, 2-acetamidoacrylate, and methyl-2-acetamidoacrylate, exhibit anti-inflammatory properties in vivo and/or in vitro. *Biochem Pharmacol* 2005;70:1579–1592. [PubMed: 16226725]
22. Su F, Wang Z, Cai Y, Rummelink M, Vincent JL. Beneficial effects of ethyl pyruvate in septic shock from peritonitis. *Arch Surg* 2007;142:166–171. [PubMed: 17309968]
23. Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nat Med* 2003;9:517–524. [PubMed: 12724763]
24. Tawadrous ZS, Delude RL, Fink MP. Resuscitation from hemorrhagic shock with Ringer's ethyl pyruvate solution improves survival and ameliorates intestinal mucosal hyperpermeability in rats. *Shock* 2002;17:473–477. [PubMed: 12069183]
25. Yang R, Gallo DJ, Baust JJ, et al. Ethyl pyruvate modulates inflammatory gene expression in mice subjected to hemorrhagic shock. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G212–G221. [PubMed: 12065309]
26. Mulier KE, Beilman GJ, Conroy MJ, Taylor JH, Skarda DE, Hammer BE. Ringer's ethyl pyruvate in hemorrhagic shock and resuscitation does not improve early hemodynamics or tissue energetics. *Shock* 2005;23:248–252. [PubMed: 15718923]
27. Bonnarens F, Einhorn TA. Production of a standard closed fracture in laboratory animal bone. *J Orthop Res* 1984;2:97–101. [PubMed: 6491805]
28. Cai B, Chen F, Lin X, et al. Anti-inflammatory adjuvant in resuscitation fluids improves survival in hemorrhage. *Crit Care Med* 2009;37:860–868. [PubMed: 19237889]

29. Stern SA, Dronen SC, Birrer P, Wang X. Effect of blood pressure on hemorrhage volume and survival in a near-fatal hemorrhage model incorporating a vascular injury. *Ann Emerg Med* 1993;22:155–163. [PubMed: 8427424]
30. Institute of Medicine, Committee on Fluid Resuscitation for Combat Casualties. *Protocols of Care at the Site of Injury*. National Academy Press; Washington, DC: 1999. p. 97-108.
31. Huston JM, Ochani M, Rosas-Ballina M, et al. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 2006;203:1623–1628. [PubMed: 16785311]
32. Yang R, Uchiyama T, Alber SM, et al. Ethyl pyruvate ameliorates distant organ injury in a murine model of acute necrotizing pancreatitis. *Crit Care Med* 2004;32:1453–1459. [PubMed: 15241088]
33. Han Y, Englert JA, Yang R, Delude RL, Fink MP. Ethyl pyruvate inhibits nuclear factor-kappaB-dependent signaling by directly targeting p65. *J Pharmacol Exp Ther* 2005;312:1097–1105. [PubMed: 15525791]
34. Baueurle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol* 1994;12:141–179. [PubMed: 8011280]
35. Li Q, Verma IM. NF-kappaB regulation in the immune system. *Nat Rev Immunol* 2002;2:725–734. [PubMed: 12360211]
36. Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. *Oncogene* 2006;25:6758–6780. [PubMed: 17072327]
37. Li ZW, Chu W, Hu Y, et al. The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. *J Exp Med* 1999;189:1839–1845. [PubMed: 10359587]
38. Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM. Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. *Science* 1999;284:321–325. [PubMed: 10195897]
39. Tanaka M, Fuentes ME, Yamaguchi K, et al. Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKKbeta-deficient mice. *Immunity* 1999;10:421–429. [PubMed: 10229185]
40. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 1995;376:167–170. [PubMed: 7603567]
41. Alcamo E, Hacohen N, Schulte LC, Rennert PD, Hynes RO, Baltimore D. Requirement for the NF-kappaB family member RelA in the development of secondary lymphoid organs. *J Exp Med* 2002;195:233–244. [PubMed: 11805150]
42. Doi TS, Marino MW, Takahashi T, et al. Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. *Proc Natl Acad Sci USA* 1999;96:2994–2999. [PubMed: 10077625]
43. Tsung A, Kaizu T, Nakao A, et al. Ethyl pyruvate ameliorates liver ischemia-reperfusion injury by decreasing hepatic necrosis and apoptosis. *Transplantation* 2005;79:196–204. [PubMed: 15665768]
44. Sappington PL, Yang R, Yang H, Tracey KJ, Delude RL, Fink MP. HMGB1 B box increases the permeability of Caco-2 enterocytic monolayers and impairs intestinal barrier function in mice. *Gastroenterology* 2002;123:790–802. [PubMed: 12198705]
45. Mantell LL, Parrish WR, Ulloa L. HMGB1 as a therapeutic target for infectious and inflammatory disorders. *Shock* 2006;25:4–11. [PubMed: 16369179]
46. Szabó C. Potential role of the peroxynitrate-poly(ADP-ribose) synthetase pathway in a rat model of severe hemorrhagic shock. *Shock* 1998;9:341–344. [PubMed: 9617883]
47. Virág L, Szabó C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev* 2002;54:375–429. [PubMed: 12223530]
48. Jagtap P, Szabó C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat Rev Drug Discov* 2005;4:421–440. [PubMed: 15864271]
49. Liaudet L, Soriano FG, Szabó E, et al. Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose)polymerase. *Proc Natl Acad Sci USA* 2000;97:10203–10208. [PubMed: 10954738]

50. Woo YJ, Taylor MD, Cohen JE, et al. Ethyl pyruvate preserves cardiac function and attenuates oxidative injury after prolonged myocardial ischemia. *J Thorac Cardiovasc Surg* 2004;127:1262–1269. [PubMed: 15115981]
51. Critical Therapeutics announces decision to discontinue trial of CTI-01, daily updates. Available at: <http://www.thefreelibrary.com/Critical+Therapeutics+Announces+Results+of+CTI-01+Phase+II+Trial.-a0151425989>
52. Su J, Li X, Cui X, et al. Ethyl pyruvate decreased early nuclear factor-kappaB levels but worsened survival in lipopolysaccharide-challenged mice. *Crit Care Med* 2008;36:1059–1067. [PubMed: 18176313]
53. Tenhunen JJ. Bull's eye missed by the magic bullet: preclinical investigations, publication bias, and promising new interventions. *Crit Care Med* 2008;36:1361–1363. [PubMed: 18379268]

Fig. 1.

Resuscitation with Hextend supplemented with ethyl pyruvate improved survival in hemorrhage with trauma. Adult male Sprague-Dawley hemorrhagic rats received no resuscitation treatment (NR) or were resuscitated with 15 mL/kg (intravenously, [i.v.]) Hextend (HXT) or Hextend supplemented with 50 mmol/L ethyl pyruvate (HEP). (A) Arterial blood pressure was recorded during the hemorrhage and the resuscitation treatment. (B) Resuscitation with Hextend supplemented with 50 mmol/L of ethyl pyruvate (HEP50) statistically protected the animals from experimental hemorrhage with trauma ($n = 7$ per group). (C) Ethyl pyruvate improved survival in hemorrhage with trauma in a concentration dependent manner in the animals treated with 50 mmol/L (HEP50), 25 mmol/L (HEP25), or 5 mmol/L (HEP5) ethyl pyruvate. $p < 0.05$ Survival Logrank Test versus NR (*) or HXT (#).

Fig. 2.

Blood chemistry analyses. Blood from control adult male Sprague-Dawley rats, or hemorrhagic animals without resuscitation treatment (NR) or resuscitated with 15 mL/kg (i.v.) Hextend (HEXT) or Hextend supplemented with 50 mmol/L ethyl pyruvate (HEP) was collected at 2 hours after the hemorrhagic load to analyze (A) *blood urea nitrogen (BUN)*, (B) *pH*, (C) *the anion gap (AnGap)*, (D) *chloride (Cl)*, (E) *bicarbonate (HCO₃)*, and (F) *Glucose*. # represents $p < 0.05$ for HEP versus HXT. * represents $p < 0.05$ versus control (n = 5 per group; One-way ANOVA with Bonferroni's corrections).

Fig. 3.

Trauma increased TNF responses in hemorrhage. Blood and organs from adult male Sprague-Dawley rats subjected to hemorrhagic without (H) or with trauma (TH) were collected at 2 hours after the hemorrhagic load to analyze TNF protein concentration in the serum and specific organs. * represents $p < 0.05$ versus control ($n = 5$ per group; One-way ANOVA with Bonferroni's corrections).

Fig. 4. Resuscitation with Hextend supplemented with ethyl pyruvate attenuated serum TNF levels. Blood from control adult male Sprague-Dawley rats, or hemorrhagic animals without resuscitation treatment (NR) or resuscitated with 15 mL/kg (i.v.) Hextend (HXT) or Hextend supplemented with 50 mmol/L ethyl pyruvate (HEP) was collected at 2 hours after the hemorrhagic load to analyze serum TNF levels. # represents $p < 0.05$ for HEP versus HXT. * represents $p < 0.05$ when compared with control ($n = 5$ per group; One-way ANOVA with Bonferroni's corrections).

Fig. 5.

Resuscitation with Hextend supplemented with ethyl pyruvate attenuated TNF levels in major organs. Organs from control or animals subjected to hemorrhage with trauma were analyzed at 2 hours to determine TNF concentration in the (A) *lung*, (B) *heart*, (C) *spleen*, and (D) *liver*. # represents $p < 0.05$ for HEP versus HXT. * represents $p < 0.05$ when compared with control (n = 5 per group; One-way ANOVA with Bonferroni's corrections).