Beneficial Effects of Ethyl Pyruvate in Septic Shock From Peritonitis

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Hypothesis: Infusion of ethyl pyruvate (EP) solution can improve outcome in a clinically relevant, large-animal model of septic shock resulting from fecal peritonitis.

Design: Prospective randomized animal study.

Setting: University hospital animal research laboratory.

Subjects: Fourteen female sheep.

Interventions: Fourteen fasted, anesthetized, invasively monitored, mechanically ventilated female sheep weighing (mean±SD) 30.4±3.8 kg received 0.5 g/kg of feces intraperitoneally to induce peritonitis, without administration of antibiotic agents or vasoactive drugs. After surgical preparation, the ewes were randomized to receive a continuous intravenous infusion at 15 mg/kg per hour of either EP combined with Ringer lactate solution or Ringer lactate solution only. Fluid resuscitation was titrated to maintain the pulmonary artery occlusion pressure at baseline levels throughout the experiment. All animals were monitored until they died spontaneously or for a maximum of 30 hours.

Results: Compared with Ringer lactate solution alone, EP administration resulted in less tachycardia, longer time to development of arterial hypotension and oliguria (median, 27 vs 15 hours and 24 vs 16 hours, respectively; both P<.01), and prolonged survival time (median, 29.5 vs 17.0 hours; P<.001). Animals who received EP also had a smaller decrease in colloid osmotic pressure (P= .05) and a tendency for lower serum interleukin 6 concentrations (P= .08).

Conclusions: In this clinically relevant model of septic shock in ewes, continuous EP infusion prolonged time to development of organ dysfunction and markedly prolonged survival. These findings suggest a potential use for EP in the treatment of severe sepsis and septic shock.

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EPISOS AND SEPTIC SHOCK STILL represent major health issues. A recent European multicenter study including more than 3000 patients showed that sepsis and septic shock are associated with substantially greater morbidity and mortality rates.¹ There is a definitive need for better therapies.

Ethyl pyruvate (EP), a simple aliphatic ester derived from pyruvate, has been proposed to have beneficial effects in various acute diseases including sepsis.² Ethyl pyruvate administration has been shown to prolong survival time in a lipopolysaccharide-challenged mouse model.³ In a cecal ligation and puncture model of aged mice, a single dose of EP inhibited sepsis-induced renal failure and multiple organ failure.⁴ In a mouse cecal ligation and puncture septic shock model, EP was shown to increase survival⁵ even when administered after the insult, a situation closer to the typical clinical scenario.

These studies have stimulated research on a larger, more clinically relevant model in which the hemodynamic effects of EP administration can also be studied. The goal of our study was to investigate the effects of EP administration in a fecal peritonitis septic shock model in sheep. This model has been used to study other therapeutic interventions.⁶,⁷

METHODS

EXPERIMENTAL ANIMALS

The study was conducted in accord with the guidelines established by the Institutional Review Board for Animal Care of the Free University of Brussels, Brussels, Belgium. Care and handling of the animals were in accord with...
National Institutes of Health guidelines (Institute of Laboratory Animal Resources). Fourteen ewes weighing (mean±SD) 30.4±3.8 kg were fasted for 24 hours with free access to water before the experiment.

**ANESTHESIA**

On the day of the experiment, the animals were initially weighed and premedicated with a combination of intramuscular xylazine hydrochloride, 75 µg/kg, and ketamine hydrochloride, 6 mg/kg (Imalgine; Merial, Lyon, France) and placed in the supine position. The cephalic vein was cannulated with a peripheral venous 18-gauge catheter (Surflo IV Catheter; Terumo Corp, Leuven, Belgium). After intravenous administration of a combination of 30 µg/kg of fentanyl citrate (Janssen Pharmaceuticals, Beerse, Belgium) and 0.1 mg/kg of pancuronium bromide (Pavulon; Organon, Oss, the Netherlands), an 8-mm tracheal tube (Hi-Contour; Mallinckrodt Medical, Athlone, Ireland) was placed. Mechanical ventilation was started in controlled volume mode (Servo 900 C ventilator; Siemens-Elema, Solna, Sweden) with tidal volume of 9 mL/kg, positive end-expiratory pressure of 10 cm H₂O, fraction of inspired oxygen (FIO₂) of 1, ratio of inspiratory time to expiratory time of 1:2, and a squarewave pattern. Respiratory rate was adjusted to maintain end-tidal carbon dioxide pressure (47210 A Capnometer; Hewlett Packard GmbH, Boeblingen, Germany) between 35 and 45 mm Hg. A 14F Foley catheter (Beiersdorf AG, Hamburg, Germany) was placed to measure urine output. The right femoral artery and vein were exposed. A 4F arterial catheter (Vygon, Cirencester, England) was introduced and connected to a pressure transducer (Edwards LifeSciences Corp, Irvine, Calif) zereed at the midthorax level. An introducer was inserted through the femoral vein, and a 7F Swan-Ganz catheter (Edwards LifeSciences) was advanced into the pulmonary artery with monitoring of pressure waveforms. A midline laparotomy was performed. After cecotomy, 0.5 g/kg body weight of feces was collected and spilled into the abdominal cavity. The cecum was then closed and returned to the abdominal cavity. Mechanical ventilation was started in controlled volume mode (Servo 900 C ventilator; Siemens-Elema, Solna, Sweden) with tidal volume of 9 mL/kg, positive end-expiratory pressure of 10 cm H₂O, fraction of inspired oxygen (FIO₂) of 1, ratio of inspiratory time to expiratory time of 1:2, and a squarewave pattern. Respiratory rate was adjusted to maintain end-tidal carbon dioxide pressure (47210 A Capnometer; Hewlett Packard GmbH, Boeblingen, Germany) between 35 and 45 mm Hg. A 14F Foley catheter (Beiersdorf AG, Hamburg, Germany) was placed to measure urine output. The right femoral artery and vein were exposed. A 4F arterial catheter (Vygon, Cirencester, England) was introduced and connected to a pressure transducer (Edwards LifeSciences Corp, Irvine, Calif) zereed at the midthorax level. An introducer was inserted through the femoral vein, and a 7F Swan-Ganz catheter (Edwards LifeSciences) was advanced into the pulmonary artery with monitoring of pressure waveforms. A midline laparotomy was performed. After cecotomy, 0.5 g/kg body weight of feces was collected and spilled into the abdominal cavity. The cecum was then closed and returned to the abdominal cavity. All sheep were sedated with intravenous administration of a combination of 0.2 mg/kg per hour of midazolam maleate (Dormicum; Roche Pharmaceuticals, Attikis, Greece) and 3 µg/kg per hour of fentanyl citrate (Janssen Pharmaceuticals). Muscular blockade was achieved using 30 µg/kg per hour of pancuronium bromide. Controlled mechanical ventilation was adjusted to ensure normoxia (80 mm Hg ≤ PaO₂ ≤ 120 mm Hg) and normocapnia (35 mm Hg ≤ PaCO₂ ≤ 45 mm Hg) according to repeated blood gas analysis (ABL800 OSM3; Radiometer). Hemoglobin concentration and oxygen saturation were measured with an analyzer calibrated for animals (OSM3; Radiometer).

**PROCEDURES**

After surgical preparation, the animals were placed in the prone position and baseline measurements were recorded. The animals were randomized to receive a constant dose of 15 mg/kg per hour of EP (Critical Therapeutics Inc, Cambridge, Mass) with Ringer lactate solution (RL) or RL only. The dose of EP was based on previous animal observations. Additional RL was titrated as needed in both groups to maintain pulmonary arterial occlusion pressure at baseline levels during the observation period.

All monitored variables were recorded every 60 minutes. Pressure measurements including mean arterial pressure, pulmonary arterial pressure, right atrial pressure, and pulmonary arterial occlusion pressure were referenced to the midchest level and obtained at end expiration (Sirecust 404; Siemens, Erlan- gen, Germany). Heart rate, mean arterial pressure, pulmonary arterial pressure, core temperature, and cardiac output (Vigilance monitor; Baxter Healthcare Corp, Irvine, Calif), and minute volume, plateau pressure, expiratory tidal volume, and end-tidal carbon dioxide pressure were continuously monitored. Body surface area was calculated using the following equation:

Body surface area = 0.084 × (body weight in kilograms)^(0.725) × (0.419)²

Cardiac index (L·min⁻¹·m⁻²), stroke volume index (mL/m²), systemic vascular resistance (dyne·s·cm⁻⁵), pulmonary vascular resistance (dyne·s·cm⁻⁵), left ventricular stroke work (g·m·m⁻²/beat), oxygen delivery (mL·kg⁻¹·min⁻¹), oxygen consumption (mL·kg⁻¹·min⁻¹), and oxygen extraction were calculated using standard formulas.

Arterial samples were obtained at baseline and at 1, 4, 5, 9, 13, 17, and 21 hours after feces spillage, and were sampled in heparinized syringes and centrifuged at 3000 rpm for 15 minutes at 4°C. Plasma was extracted and saved at −78°C for later interleukin 6 (IL-6) measurements. Colloid osmotic pressure was measured (Onkometer BMT 923; BMT, Berlin, Germany).

Mouse antiovine IL-6 monoclonal antibody (MCA1659; Serotec Ltd, Kidlington, England), rabbit antiovine IL-6 polyclonal antibody (AH6p+4; Serotec), and sheep antirabbit horseradish peroxidase conjugated antibody (STARp; Serotec) were used to measure IL-6 levels. In short, the monoclonal antibody was used as a coating antibody with a concentration of 1:200, diluted in phosphate-buffered saline solution, and incubated overnight on 96-well plates for enzyme-linked immunosorbent assay (ELISA plate; Greiner Bio-One, Frickenhausen, Germany) at 4°C. After discarding the coating solution, 250 µL of blocking buffer (phosphate-buffered saline solution per 1% bovine serum albumin) was added for 2 hours at room temperature, then rinsed 3 times with phosphate-buffered saline solution—0.05% polysorbate 20 (Tween 20; ICI Americas Inc, Wilmington, Del). A 50-µL serum sample was diluted with 50 µL of phosphate-buffered saline solution per 1% body surface area, placed in plate wells, and incubated for 1 hour at room temperature. The plates were then washed 3 times before adding detection polyclonal antibody (STARp) and incubated for 1 hour at room temperature. After being rinsed 3 times, the substrate for the conjugated horseradish peroxidase was added to the plate and allowed to react for 10 minutes. The optical density of the plate wells was then read on an ELISA plate reader at 450 nm.

**POSTMORTEM STUDIES**

Immediately after the sheep died, randomized lung autopsy was performed, taking 2 samples from the top, middle, and bottom of the right lung and 1 sample from the small intestine. One sample was saved in 4% buffered formalin solution, routinely processed into paraffin blocks, sectioned at 4-µm intervals, and stained with hematoxylin-eosin for light microscopy. A pathologist (M.R.) blinded to the conditions of the study examined all fields of the slides. Lesion severity was graded according to the degree of interstitial congestion, neutrophil accumulation, and septal thickening, and alveolar hemorrhage was graded on a scale of 0 (normal) to 3 (most severe). The second lung sample was weighed and dried at 200°C for 24 hours and the lung wet-dry ratio was calculated.

**STATISTICAL ANALYSIS**

Data are expressed as mean±SD. Baseline variables and grade of lesion severity were compared with the t test. Kaplan-Meier curves were constructed for overall survival and time to devel-
Partial improvement of hypotension and oliguria, and the curves were compared using the log rank test. A 2-way (time and group) multivariate analysis of variance with repeated measures taking into account missing values was used to make comparisons for other continuous variables. Statistical analysis was performed using the JMP software package (SAS Institute Inc, Cary, NC). P < .05 was considered statistically significant.

RESULTS

There was no difference between groups in baseline measurements (Table). The amount of RL infused was larger in the EP and RL group (hereafter referred to as the EP group) than in the RL only group (hereafter referred to as the RL group) (13.4 ± 2.1 vs 10.6 ± 2.0 L; P = .006), but the difference was due to the longer survival times in the EP group because there was no difference in fluid accumulation between groups in the first 16 hours.

All animals developed arterial hypotension associated with increased cardiac index, reflecting a hyperkinetic state with low systemic vascular resistance (Figure 1). Compared with animals in the RL group, those in the EP group developed less severe tachycardia (P < .03) (Figure 1) and a tendency toward a higher left ventricular stroke work index (P = .08). Median time to development of arterial hypotension (27.0 vs 13.0 hours, respectively) and oliguria (24.0 vs 16.0 hours, respectively) (Figure 2) was significantly longer in the EP group than in the RL group (both P < .01). Blood lactate levels increased less in the EP-treated animals at the end of the experiment, although the differences did not reach statistical significance (Figure 1). Colloid osmotic pressure decreased in both groups. Despite somewhat lower values at baseline, colloid osmotic pressure was higher in the EP group after 9 hours (Figure 3). Interleukin 6 tended to be lower in the EP group (P = .08, Figure 3). Survival time was longer in the EP than in the RL group (median time, 29.5 vs 17.0 hours, respectively; P < .001; Figure 4).

Respiratory system compliance, resistance, PaO2/FIO2 ratio, and lung wet-dry ratio were similar in both groups. The autopsy examination revealed marked bowel distention with large quantities of free peritoneal fluid. Histologic examination of the lungs revealed infiltration by microorganisms, alveolar edema, congestive atelectasis, neutrophil infiltration, dilated microvessels, and alveolar thickening. Histologic examination of the small intestine showed edema and congestion. There was no statistical difference in the grade of lesion severity between groups for the lung or the gut.

Table. Baseline Variables in EP and RL Groups*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>P Value</th>
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<tbody>
<tr>
<td></td>
<td>EP and RL</td>
<td>RL</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>31.6 ± 4.7</td>
<td>29.3 ± 2.4</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>38.0 ± 0.9</td>
<td>38.2 ± 0.9</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>101 ± 15</td>
<td>107 ± 11</td>
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<tr>
<td>Mean pulmonary arterial pressure, mm Hg</td>
<td>18 ± 5</td>
<td>16 ± 4</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>90 ± 7</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>Cardiac index, L min⁻¹ m⁻²</td>
<td>3.88 ± 0.29</td>
<td>3.76 ± 0.31</td>
</tr>
<tr>
<td>PaO2/FIO2</td>
<td>303 ± 57</td>
<td>300 ± 49</td>
</tr>
<tr>
<td>Lactic acid level, mEq/L</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.2</td>
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Abbreviations: EP, ethyl pyruvate; RL, Ringer lactate solution.  
SI conversion factor: To convert lactic acid to millimoles per liter, multiply by 0.1110.  
*Data are given as the mean ± SD.

Figure 1. Heart rate (A), mean arterial pressure (B), cardiac index (C), systemic vascular resistance (D), and lactic acid (E) concentration between groups. Squares indicate those who were treated with a combination of ethylene pyruvate and Ringer lactate solution; circles, those who were treated with Ringer lactate solution only (control group). *P < .05.
The main finding of our study is that EP administration delayed the onset of hypotension and oliguria and prolonged survival in a peritonitis septic shock model. These effects suggest that treatment with EP could be beneficial in severe sepsis and septic shock.

With our model, an attempt was made to reproduce the clinical condition as closely as possible and resulted in a hyperdynamic pattern with fever, arterial hypotension, elevated cardiac index, decreased systemic vascular resistance, and lactic acidosis, all typical clinical features of septic shock. Without antibiotic or vasopressor therapy, death occurs after 16 to 24 hours. The evolution to death is related to multiple organ failure characterized by hypotension, oliguria, and respiratory failure.

As a key glycolytic intermediary, pyruvate is an endogenous antioxidant and free radical scavenger. It can interact with hydrogen peroxide and scavenger hydroxyl radical. The production of reactive oxygen species has been shown to be a key pathogenic mechanism involved in sepsis. The use of pyruvate as a therapeutic agent is limited by aqueous instability from spontaneous condensation and cyclic reaction to form parapyruvate, which is toxic to cells. Pyruvate-containing solutions such as sodium pyruvate have been used successfully in various disease models involving oxidative stress, ischemia-reperfusion–induced injury, stroke, and hemorrhagic shock. With the concentration of pyruvate that needs to be reached, sodium pyruvate promotes the development of hypernatremia. As an ester derived from pyruvic acid and ethanol, EP circumvents the potential problems associated with the aqueous instability of pyruvate or hypernatremia secondary to sodium pyruvate. Administration of EP has been effective in various models of acute systemic inflammatory response such as endotoxemia, cecal ligation and puncture, and acute necrotizing pancreatitis in rodents. Ethyl pyruvate has been shown to be superior to sodium pyruvate in in vitro ex-
perperiments of cellular injury and in providing epithelial protection in in vivo models including endotoxia. This suggests that EP may have additional properties to those of pyruvate alone. Ethyl pyruvate has been demonstrated to have antioxidant and anti-inflammatory capacities. Through modification of p65, EP inhibits signaling via the nuclear factor-kB pathway. In our study, the somewhat lower IL-6 concentrations in the EP-treated animals support possible anti-inflammatory effects.

Our study findings suggest that administration of EP may improve cardiac function in sepsis. Animals treated with EP had less severe tachycardia and a trend to higher left ventricular work stroke index. Previously, in a rat model of cardiac ischemia-reperfusion, Woo et al showed that EP enhanced myocardial adenosine triphosphate levels, attenuated myocardial oxidative injury, and preserved cardiac function. These beneficial effects can be traced back to EP. In animal models, pyruvate preserved cardiac function in ischemia-reperfusion lesions, attenuated stunning, and reduced infarct size. In patients with congestive heart failure and after cardiopulmonary bypass surgery, pyruvate has been shown to preserve both systolic and diastolic function. Antioxidant effects likely have a key role in the cardiovascular effects of EP via direct neutralization of peroxides or enhancement of the intracellular glutathione-reduced nicotinamide adenine dinucleotide phosphate antioxidant system.

Another important observation was the lesser decrease in plasma colloid osmotic pressure in the EP group than in the RL group. Our model of septic shock due to peritonitis is associated with a significant decrease in colloid osmotic pressure, as can be observed in human beings. The decrease in colloid osmotic pressure largely reflects the alterations in capillary permeability in addition to the administration of large amounts of crystalloids. Inasmuch as all animals received only crystalloids and EP has a low molecular weight and is not expected to influence albumin synthesis, especially in a short-term study, decreased capillary egress of large molecules is the most likely explanation for this observation. Hence, this finding suggests that EP may preserve endothelial integrity and decrease capillary leakage syndrome in severe sepsis. This suggestion is largely compatible with the antioxidant effects of the molecule because reactive oxygen species have an important role in the development of these permeability alterations. Likewise, Sims et al reported that EP ameliorated structural and functional damage to the intestinal mucosa in a model of mesenteric ischemia-reperfusion in the rat. In our study, there was no significant difference in histologic findings, likely because tissue samples were obtained after different times of evolution. Further experiments should involve sacrifice and sampling of animals at fixed time points to show potential protective effects of EP on tissue histology.

The prolonged time to develop oliguria also suggests some renal protective effects in the EP group. Miyaji et al showed that EP administered 12 hours after cecal ligation and puncture in mice decreased plasma tumor necrosis factor α and kidney messenger RNA for tumor necrosis factor α, and increased messenger RNA for urokinase-like plasminogen activator. Ethyl pyruvate decreased serum creatinine concentration, tubular damage, and multiple organ injury at 24 hours. Ethyl pyruvate may protect against or accelerate recovery from acute renal failure. Ethyl pyruvate has no known toxicity and is inexpensive. We did not observe any adverse effects in our study.

Our study has limitations. First, although the animal model reproduces well the hemodynamic alterations of septic shock in human beings, the animals were initially healthy and their response may be different from that in acutely ill patients with compromised cardiorespiratory reserve. Second, we opted not to give antibiotic or vasoactive agents to avert any influence of these additional variables and to obtain a lethal model. Third, we did not investigate the dose effects of EP infusion in our model. Different doses of EP, from 24 mg/kg to 360 mg/kg, have been used in mice and rats. Hauser et al demonstrated that a 30-mg/kg loading dose of EP followed by a 12-hour infusion at 30 mg/kg per hour improved hemodynamic stability and ameliorated acid-base derangement induced by chronic endotoxia in pigs. Because the average survival time in our model was approximately 18 to 20 hours in the control animals, we thought a dose of 15 mg/kg per hour would be reasonable.

We conclude that, in this animal model of septic shock from peritonitis, administration of EP may delay the development of hypotension and oliguria, and prolong survival. These observations suggest a potential use for EP in the treatment of severe sepsis and septic shock.

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REFERENCES


