Effect of urokinase on disseminated intravascular coagulation

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Vasquez, Yvonne, Charles H. Williams, and Robert M. Hardaway. Effect of urokinase on disseminated intravascular coagulation. J. Appl. Physiol. 85(4): 1421–1428, 1998.—Our study evaluated the possible therapeutic effect of urokinase in treating the microthrombic effects of disseminated intravascular coagulation by assisting the activation of endogenous plasminogen. Twenty-six pigs were anesthetized, intubated, mechanically ventilated, and surgically catheterized. Septic shock was induced in all 26 pigs by an intravenous infusion of heat-killed Escherichia coli. The pigs were divided into two sets of experiments: in experiment 2 (n = 14), one-half received an intravenous dose of urokinase 1 h after heat-killed E. coli infusion, and in experiment 3 (n = 12) one-half received an intravenous bolus dose and a continuous drip of urokinase 2 h after heat-killed E. coli infusion. The untreated pigs served as controls. Hemodynamic parameters, blood chemistries, and blood gases were analyzed. Urokinase given 1 h after bacterial toxin infusion significantly restored blood flow, resulting in an increase in cardiovascular and pulmonary function and improved survival rate (43% control vs. 100% treated, 24-h experimental period). Treatment given after 2 h showed some significant effect on pulmonary function; however, within 10 h of E. coli infusion, mortality rates in control and treated groups were 100 and 83%, respectively. Early administration of urokinase after onset of disseminated intravascular coagulation restored blood flow and helped resolve organ damage.

septic shock; heat-killed Escherichia coli; swine

DISSEMINATED INTRAVASCULAR COAGULATION (DIC) is commonly reported in septic shock. How DIC is initiated is not clearly understood. It has been postulated that the presence of endotoxin activates the coagulation system, resulting in a marked increase in thrombin and fibrin production (2). Endotoxin may trigger the coagulation system by causing damage to the endothelium (20) and/or by releasing procoagulant materials (2). Fibrinolysis is inhibited during sepsis by elevated levels of plasminogen-activator inhibitors and may contribute to the pathogenesis of DIC (3, 19). Subsequent microvascular thrombosis and occlusion may also lead to the development of multiple-organ failure (6, 10, 11). The treatment of thrombosis can be approached by lysing blood clots via the intravenous infusion of pharmacological plasminogen activators. Studies have shown urokinase and tissue-type plasminogen activator to be useful thrombolytic agents in animal models (12, 13).

Urokinase is a proteolytic enzyme produced by the kidney and found in urine. A low-molecular-weight urokinase, which was isolated from human kidney cell culture, was obtained from Abbott Laboratories, Chicago, IL (Abbokinase). Urokinase acts on the endogenous fibrinolytic system by converting plasminogen to plasmin, which, in turn, degrades fibrin clots (9). Based on the therapeutic principle of urokinase, we investigated whether this thrombolytic agent would be able to treat the microthrombic effects of DIC by lysing microclots and restoring circulation, thus minimizing organ failure and mortality in pigs infused with heat-killed Escherichia coli.

MATERIALS AND METHODS

Animal preparation. This study was approved by the Animal Care Committee of Texas Tech University Health Science Center. Twenty-six commercial pigs weighing 29–45 kg were anesthetized with ketamine hydrochloride (10 mg/kg im), acepromazine (0.2 mg/kg), xylazine (1 mg/kg), and pentothal (3 mg/kg iv), and they continued to receive a dilute dose of pentothal (0.5 mg) as needed. The animals were intubated and mechanically ventilated (North America Drager; Telford, PA) with 30% oxygen-70% room air. The left carotid artery was catheterized for blood pressure monitoring, blood sampling, and fluid administration. A pressure-sensor catheter (Millar; Houston, TX) was also inserted into the left carotid artery and guided into the left ventricular cavity. The left jugular vein was cannulated for a balloon-tipped pulmonary artery catheter (Opticath; Abbott, Mountain View, CA), which was floated into the pulmonary artery, and the following hemodynamic and physiological parameters were recorded: central venous pressure (CVP), mean pulmonary arterial pressure (MPAP), mean pulmonary capillary wedge pressure (MPCWP), and cardiac output (CO) by thermodilution (9520A; Edwards Laboratories; Santa Ana, CA); core temperature via the thermocouple; and venous oxyhemoglobin (HbO2) saturation via an oximeter (OS/880, Oximetrix; Mountain View, CA). A second catheter was also slipped into the left jugular vein for blood sampling and fluid administration. All catheters were connected to a pressure transducer (1290A; Hewlett-Packard; Wallingford, MA) and monitored via a multichannel recorder (7758A; Hewlett-Packard). Thermoluidation CO was determined by injecting 10 cm3 of iced dextrose via a central venous line. This procedure was performed in duplicates at 0.5-h intervals. End-expiratory CO2 was measured with a capnograph (7050, Marquette Electronics, Milwaukee, WI). A cutaneous three-lead electrocardiograph was used, and lead II was monitored (78432A monitor and 78172A recorder; Hewlett-Packard). The urinary bladder was catheterized for urine output. Body temperature was monitored via a rectal thermistor (43TA Tele-Thermometer; Yellow Spring Instruments; Yellow Springs, OH). The femoral vein was probed for blood flow rate and was monitored on a meter (T101 Ultrasonic Bloodflow Meter; Transonic Systems, Ithaca,
NY). Blood volume and CVP were maintained at normal levels by an intravenous drip of isotonic saline (0.9% sodium chloride). Blood was analyzed for arterial and mixed venous blood gases, fibrin degradation products, fibrinogen, activated partial thromboplastin time, prothrombin time, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, hemoglobin, red blood cell count, white blood cell count, glutamic-pyruvic transaminase (GPT), lactic acid, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, and Lee-White clot time. Blood gases were measured by CO-oximeter analysis (Radiometer America, Westlake, OH), and blood chemistries were analyzed at Thomason General Hospital Laboratory (El Paso, TX).

Heat-killed E. coli preparation. E. coli (EC, serotype 078:H11; American Type Culture Collection, Rockville, MD) were grown in Lennox L broth at 37°C in a shaking water bath for 24 h. Organisms were washed three times and resuspended in isotonic saline. Inocula were adjusted to 2 × 10^{10} cells/ml by a hemacytometer and then killed by boiling for 30 min. After sterility (complete killing) verification, they were stored at −70°C until needed. The dose of E. coli was selected to cause a lethal effect on the lung and death at ~8 h after the animal was removed from the ventilator.

Experimental protocol. After catheter insertions, animals were stabilized for 30 min, and then baseline measurements were recorded. Control measurements were recorded 1 h after stabilization. All pigs were then injected with 2 ml/37.5 kg of heat-killed E. coli in 20 ml isotonic saline over a 20-min period, with the use of a mechanical injector (341A; Sage Instruments; Cambridge, MA). The pigs were divided into two sets of experiments: experiment 2 (n = 14) and experiment 3 (n = 12). Seven of the fourteen pigs in experiment 2 were given an intravenous bolus dose of 250,000 U/37.5 kg of urokinase (Abbokinase, Abbott Laboratories) diluted in 20 ml of pentobarbital sodium. Blood chemistries and blood gases were counted as survivors and killed by lethal injection of 1 ml/37.5 kg of E. coli. The dose of E. coli was selected to cause a lethal effect on the lung and death at ~8 h after the animal was removed from the ventilator.

Results

Mean arterial pressure (MAP)

\[ \text{MAP} = \frac{(systolic AP - diastolic AP)}{3} + \text{diastolic AP} \]

where MAP is arterial pressure

Cardiac index (CI) = CO/kg body wt

Stroke volume product (RPP) = HR × systolic AP

Pulmonary vascular resistance (PVR) = \[(\text{MPAP} - \text{PCWP}) \times 80)/\text{CO}\]

Systemic vascular resistance index (SVRI) = \[(\text{MAP} - \text{CVP})/\text{CI}\]

Total peripheral resistance (TPR) = \[(\text{MAP} - \text{CVP}) \times 80)/\text{CO}\]

Right ventricular stroke work index (RVSWI) = \[(\text{MAP} - \text{CVP}) \times 0.0136\]

Left ventricular stroke work index (LVSWI) = \[(\text{MAP} - \text{PCWP}) \times 0.0136\]

where PCWP is pulmonary capillary wedge pressure.

Coronary Perfusion pressure (CPP) = diastolic AP – PCWP

Table 1. Disseminated intravascular coagulation changes in experiments 2 and 3

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Treated Group</th>
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<tbody>
<tr>
<td></td>
<td>0 h IAC 1 h</td>
<td>3 h 5 h 8 h</td>
</tr>
<tr>
<td>Fibrinogen, mg/dl</td>
<td>154 ± 15</td>
<td>126 ± 18</td>
</tr>
<tr>
<td>Fibrinogen, 10^7/jul</td>
<td>± 15 ± 18</td>
<td>± 19 ± 22</td>
</tr>
<tr>
<td>FDP, µg/dl</td>
<td>± 5.6 ± 6.5</td>
<td>± 5.8 ± 4.9</td>
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Data are means ± SE. All pigs were intravenously injected with 2 ml of killed Escherichia coli (2 × 10^{10} cells/ml) at a rate of 20 ml/min over a 20-min period. Urokinase treatment was delayed in experiment 2 and 3 for 1 and 2 h, respectively. FDP, fibrin degradation products; IAC, immediately after killed E. coli; IAK, immediately after urokinase infusion.
RESULTS

Mortality rate. In experiment 2, four out of seven control pigs (57%) died soon after the mechanical ventilator was removed, whereas all treated pigs (100%) survived the full 24-h experimental period. In experiment 3, all control pigs (100%) died within 10 h after infusion of killed E. coli, and five out of six treated pigs (83%) died within 10 h.

Necropsy and histological findings. At necropsy, the lungs of all control pigs were hemorrhagic, congested, cyanotic, and edematous with involvement of most of both lungs. In some cases, the stomach and gut showed congested and hemorrhagic area. Liver and spleen often showed congestion. Histopathology reports showed lung atelectasis, focal accumulation of neutrophils in the alveolar spaces, proteinaceous alveolar edema, scattered bronchi filled with neutrophils, and swelling and proliferation of alveolar septal cells. The spleen had congestion of red pulp and lymphocytic necrosis of the germinal centers with karyorrhexis and pyknosis. The liver had lymphocytes and eosinophils in the portal tracts, hepatocellular swelling and cytoplasmic vacuolation, paraportal edema, and early bile duct hyperplasia. The pancreas had homogeneous eosinophilic fluid in the interlobular spaces, necrosis of acinar cells (pyknosis), few scattered neutrophils, loss of zymogen granules, and scattered acinar cells with clear cytoplasmic vacuolation. The stomach had hyperemia, necrosis, and hemorrhage. The kidney had interstitial accumulation of lymphocytes, proteinuria, and congestion of glomeruli. Heart, duodenum, brain, and skeletal muscle showed no significant lesions. Treated pigs in both

![Fig. 1. Coronary perfusion pressure (CPP; A and B), left ventricular stroke work index (LVSWI; C and D), and systemic vascular resistance index (SVRI; E and F) during killed Escherichia coli infusion. All pigs were intravenously injected with 2 ml of killed E. coli (2 × 10^{10} cells/ml) at a rate of 20 ml/min over a 20-min period. Urokinase treatment was delayed in experiment 2 (A, C, and E) and experiment 3 (B, D, and F) for 1 and 2 h, respectively. Data are means ± SE. IAC, immediately after killed E. coli infusion. *P < 0.05 from baseline (Base); +P < 0.001 from baseline; and #P < 0.05 between groups.](http://jap.physiology.org/)}
experiment showed mild lesions; however, lesions in the animals from experiment 3 were greater compared with those from experiment 2.

DIC parameter changes. The onset of DIC was indicated by a fall in fibrinogen levels, fall in platelet count, and appearance of fibrin-degradation products (Table 1). In experiments 2 and 3, fibrinogen and fibrin-degradation product levels showed no significant difference between control and treated pig groups. Platelet levels were lower in the treated group 8 h after killed E. coli infusion in experiment 2. No significant difference between groups was noted in experiment 3. Urokinase treatment did not correct DIC changes in either experiment.

Systemic hemodynamics. The intravenous injection of killed E. coli resulted in a fall in MAP, CI, CPP, LVSWI, SVRI, SV, and CO in both experiments. No significant difference in MAP and CI was noted between control and treated pig groups in either experiment 2 or 3. CPP, LVSWI, and SVRI, levels were higher in the treated group 2 h after killed E. coli infusion in experiment 2 ($P < 0.05$), and in experiment 3 no significant difference was noted between groups (Fig. 1). SV tried to recover to baseline levels after urokinase treatment, whereas control group levels continued to drop ($P < 0.05$) in experiment 2 (Fig. 2A). SV in experiment 3 showed no attempt for recovery in the treated group (Fig. 2B). CO also tried to recover in treated and control groups, but treated levels in experiment 2 and control levels in experiment 3 decreased ($P < 0.05$) 2 h after killed E. coli infusion for the remainder of both experiments (Fig. 2, C and D). HR increased 2 h after killed E. coli infusion, which persisted until the end of both experiments (Fig. 2, E and F).

![Graphs showing hemodynamic changes](http://jap.physiology.org/)
MCVP changes were inconsistent in control and treated groups for both experiments.

Pulmonary hemodynamics. MPAP, PVR, and RVSWI peaked immediately after the onset of killed E. coli infusion and then dropped but remained above baseline levels for the remainder of both experiments. After the administration of urokinase, MPAP levels were lower (P < 0.05) in the treated group at 3 h compared with the control group in experiment 2 (Fig. 3, A and B). In experiment 3, a difference (P < 0.05) in MPAP levels was noted until 6 h after E. coli infusion. PVR showed no significant difference between groups for experiment 2; however, a significant difference was noted between groups after the administration of urokinase in experiment 3 (Fig. 3, C and D). RVSWI levels were significantly lower 2.5 h after killed E. coli infusion in the treated group for experiment 2. Experiment 3 showed no difference between groups for RVSWI levels (Fig. 3, E and F). MPCWP changes were inconsistent in control and treated groups for both experiments.

Blood gases. A progressive fall in arterial oxygen tension (Pao2) was observed after killed E. coli infusion in both pig groups. In experiment 2, Pao2 dropped (P < 0.01) from 366 Torr at baseline to 39.8 Torr at 8 h in the control group and from 316.5 Torr at baseline to 104.1 Torr at 8 h in the treated group. Both groups maintained minimal levels for the remainder of the experiment (Fig. 4A). In experiment 3, Pao2 dropped (P < 0.01) from 327 Torr at baseline to 28.1 Torr at 6–8 h in the control group and from 315.3 Torr at baseline to 37.1 Torr at 6–8 h in the treated group (Fig. 4B). The marked fall, observed in both pig groups and experiments, in Pao2 at 8 h was due to the discontinuation of oxygen supplementation at 7 h.

Fig. 3. Mean pulmonary artery pressure (MPAP; A and B), pulmonary vascular resistance (PVR; C and D), and right ventricular stroke work index (RVSWI; E and F) during killed E. coli infusion. All pigs were intravenously injected with 2 ml of killed E. coli (2 × 10^10 cells/ml) at a rate of 20 ml/min over a 20-min period. Urokinase treatment was delayed in experiment 2 (A, C, and E) and experiment 3 (B, D, and F) for 1 and 2 h, respectively. Data are means ± SE. *P < 0.05 from baseline; †P < 0.001 from baseline; and #P < 0.05 between groups.
Arterial HbO₂ levels were maintained at baseline levels for the 7-h experimental duration on the mechanical ventilator but rapidly dropped after discontinuation of oxygen. In experiment 2, arterial HbO₂ dropped (\(P < 0.01\)) from 97.9% at baseline to 37.6% at 8 h in the control group and from 97.9% at baseline to 67.7% at 8 h in the treated group. Both groups remained below baseline levels for the duration of the experiment (Fig. 4C). In experiment 3, arterial HbO₂ dropped (\(P < 0.01\)) from 97.9% at baseline to 28.0% at 6–8 h in the control group and from 98.4% at baseline to 43.9% at 6–8 h in the treated group (Fig. 4D).

In comparing control and treated groups for experiment 2, a progressive decrease in arterial pH (from 7.48 to 7.32 and from 7.48 to 7.34, respectively, \(P < 0.05\)) was observed during the 5-h period subsequent to killed E. coli infusion (Fig. 4E). Arterial pH levels in both groups then returned to baseline levels by the conclusion of the experiment. In experiment 3, arterial pH also dropped (\(P < 0.01\)) from 7.4 at baseline to 7.2 at 6–8 h in the control group and from 7.5 at baseline to 7.2 at 6–8 h in the treated group (Fig. 4F). Supplemental oxygen was discontinued at 7 h.

Blood chemistries. Creatinine, BUN, and GPT levels were monitored as indicator of renal failure, impaired kidney function, and liver malfunction, respectively. Creatinine, BUN, and GPT levels had a minimal change, and no significant difference was noted between control and treated pig groups in experiment 2 and 3.
DISCUSSION

The administration of heat-killed E. coli resulted in changes typical of severe septic shock, with DIC changes characterized by a fall in fibrinogen levels, fall in platelet count, and appearance of fibrin-degradation products. Septic shock changes included a fall in MAP, a rise in mean pulmonary pressure, a fall in CO, and an increase in HR. These DIC (2, 11, 20) and septic-shock (1, 16) changes are in agreement with previous experimental reports in patients; however, the DIC value changes in our swine model are moderate compared with human value changes.

Using this swine model of septic shock, we found that a bolus dose of urokinase administered 1 h after killed E. coli infusion improved cardiovascular and pulmonary function. Pulmonary hypertension and vasoconstriction are some of the initial responses to killed E. coli. During the second phase, which occurs at ~3 h, urokinase reduced pulmonary hypertension but not PVR. Low CPP, LVSWI, and SVRI levels were improved 1 h after urokinase treatment (P < 0.05), and several attempts of recovery were noted for SV and CO levels. RVSWI levels were lowered 1.5 h (P < 0.05) after urokinase administration and remained below control pig levels for the remainder of the experiment. Systemic hypotension was not affected by urokinase.

Urokinase administered in a bolus dose, followed by a continuous drip, 2 h after killed E. coli infusion improved pulmonary performance but did not have much of an effect on cardiovascular dysfunction. The second phase of pulmonary hypertension and vasoconstriction was reduced by urokinase treatment. CO remained at normal levels after treatment and stayed above control pigs levels until the end of the experiment. Urokinase had no effect on systemic hypotension.

In a previous experiment (7), we also found that in swine challenged with killed E. coli and treated 20 min later with a bolus dose of urokinase cardiovascular and pulmonary function was favorably affected. High MPAP levels were returned to normal after urokinase treatment.

After the mechanical ventilator was discontinued (7 h after killed E. coli infusion), arterial blood gases were affected. Significant changes were observed: a fall in oxygen tension and HbO2 and a rise in hydrogen ion concentration. Pigs treated 20 min and 1 h after killed E. coli infusion maintained higher levels of oxygen tension and HbO2, and hydrogen ion concentrations returned to normal values. All pigs treated 2 h after killed E. coli infusion died shortly after ventilator removal.

We also found that urokinase administration during swine septic shock prolonged survival. In our present study, all treated pigs in experiment 2 (1-h lapse before urokinase treatment) survived the 24-h experimental period, whereas only 43% of the control pigs lived for 24 h. In experiment 3 (2-h lapse before urokinase treatment), all control pigs died within 10 h of killed E. coli infusion, and 83% of treated pigs died within 10 h. Our previous study (20-min lapse before urokinase treatment) (8) showed a 100% survival rate in treated pigs for the 24-h experimental duration, and 88% of the control pigs died before 24 h.

Other thrombolytic agents, such as streptokinase (17) and tissue-type plasminogen activator (14), have also improved survival in endotoxemic animals. Heparin (18) has been shown to prevent organ dysfunctions associated with sepsis in rabbits, and saruplase (15), a recombinant single-chain urokinase-type plasminogen activator, is able to dissolve microthrombosis associated with DIC in endotoxemic rats. The aforementioned thrombolytic therapies have either been administered as a pretreatment or simultaneously with endotoxin infusion. Patient reports have shown that the administration of urokinase is successful in the treatment of pulmonary thromboembolism (4, 5).

Our previous study (8) in combination with the present study suggest that the beneficial effects of urokinase on survival may depend on how early the treatment is administered after the onset of DIC. Subsequent microvascular thrombosis and occlusion lead to impeded blood flow, tissue hypoxia, and microinfarction in such vital organs as the liver, kidneys, and lung (8). Microinfarction would progressively impair function and ultimately lead to organ failure. If microclots are lysed shortly after their formation, damage is kept to a minimum. The critical point in which microinfarction leading to organ failure is irreversible seems to be within 2 h after killed E. coli infusion in pigs. Early administration of urokinase may be of benefit in restoring blood flow and helping resolve organ damage.

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