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Short-term effect of ascorbate on bacterial content, plasminogen activator inhibitor-1, and myeloperoxidase in septic mice

Scott B. Swarbreck, PhD,[a,b] Dan Secor, PhD,[a,b] Christopher G. Ellis, PhD,[a,c] Michael D. Sharpe, MD,[d] John X. Wilson, PhD,[e] and Karel Tyml, PhD[a,b,c,*]

[a] Critical Illness Research Program, Lawson Health Research Institute, London, Ontario, Canada
[b] Department of Physiology and Pharmacology, University of Western Ontario, London, Canada
[c] Department of Medical Biophysics, University of Western Ontario, London, Canada
[d] Department of Anesthesia and Perioperative Medicine, Critical Care Western, University of Western Ontario, London, Canada
[e] Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, New York

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ABSTRACT

Background: Sepsis, a potential risk associated with surgery, leads to a systemic inflammatory response including the plugging of capillary beds. This plugging may precipitate organ failure and subsequent death. We have shown that capillary plugging can be reversed rapidly within 1 h by intravenous injection of ascorbate in mouse skeletal muscle. It is unknown whether, in parallel with this effect, ascorbate negatively affects the protective responses to sepsis involving the fibrinolytic and immune systems. We hypothesized that treatment with ascorbate for 1 h does not alter bacterial content, plasminogen activator inhibitor 1 (PAI-1), and neutrophil infiltration in lung, kidney, spleen, and liver (organs with high immune response) of septic mice.

Materials and methods: Sepsis was induced by feces injection into the peritoneum. Mice were injected intravenously with ascorbate at 6 h (10 mg/kg), and samples of peritoneal fluid, arterial blood, and organs collected at 7 h were subjected to analyses of bacterial content, PAI-1 messenger RNA and enzymatic activity, and myeloperoxidase (MPO) (a measure of neutrophil infiltration).

Results: Sepsis increased bacterial content in all fluids and organs and increased PAI-1 messenger RNA and enzymatic activity in the lung and liver. Sepsis increased the myeloperoxidase level in the lung and liver, and lowered it in the spleen. Except for decreasing the bacterial content in blood, these responses to sepsis were not altered by ascorbate.

Conclusions: The rapid effect of ascorbate against capillary plugging in the septic mouse skeletal muscle is not accompanied by alterations in PAI-1 or myeloperoxidase responses in the organs with high immune response.

* *Corresponding author*. Critical Illness Research Program, Victoria Research Laboratories, 6th Floor, 800 Commissioners Road East, London, Canada, N6C 2V5. Tel.: +1519 685 8500x55076; fax: +1519 685 8341.
E-mail address: ktyml@lhsc.on.ca (K. Tyml).

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1. Introduction

Abdominal and other surgeries can increase the risk of developing sepsis. Sepsis, a systemic inflammatory response to an infection, has many detrimental effects on the cardiovascular system. These include hypotension, reduced vascular reactivity, and reduced capillary bed perfusion [1–4]. The precise mechanism of reduced capillary perfusion is unknown, but an activated coagulation system including microthrombi formation in capillaries has been suggested [2,5]. Sepsis elevates plasminogen activator inhibitor 1 (PAI-1), which may lead to inhibited fibrinolysis and subsequent stabilization of microthrombi and plugging of capillaries [6–8]. However, data from PAI-1 knockout mice suggest elevated PAI-1 during sepsis may be beneficial as survival from sepsis was reduced in these knockout mice [9,10]. These studies indicate the complexity of the effects of sepsis on the coagulation system in various organs.

Sepsis is associated with marked reduction in plasma ascorbate (reduced form of vitamin C), leading to poor outcome in sepsis [11]. Importantly, studies in mouse models of sepsis demonstrated that injection of an ascorbate bolus reduces organ injury and mortality in sepsis [1,12,13]. We have shown that ascorbate injection at the onset of sepsis prevents capillary plugging seen at 7–48 h of sepsis, and also that ascorbate rapidly reverses (within 1 h) capillary plugging in the septic mouse and rat skeletal muscle; both the prevention and reversal involved reduced platelet activation and platelet-endothelial cell adhesion [1,2,5,14,15]. Antioxidant therapy in septic rats with N-acetylcysteine reduced organ dysfunction from oxidative damage caused by neutrophils [16]; this reduced organ dysfunction is consistent with the beneficial effect of ascorbate. However, because ascorbate could alter the coagulation system [5,17], the question arises as to whether it could negatively affect the beneficial role of PAI-1 in sepsis. For example, Jaulmes *et al.* [17] showed that PAI-1 expression depends on the activity of nicotinamide adenine dinucleotide phosphate oxidase, which can be inhibited by ascorbate [18]. Given the apparent requirement for PAI-1 in survival during sepsis [9], ascorbate could thus indirectly reduce survival by inhibiting PAI-1. Also, because PAI-1 is required for normal neutrophil efferocytosis [19] and infiltration [20], inhibition of PAI-1 by ascorbate could compromise the neutrophil-mediated immune response in infected organs. Ascorbate has bactericidal properties at very high levels, raising the possibility that the reduced mortality observed with ascorbate treatment could be because of reduced bacterial loads [21,22]. However, no apparent bactericidal effect of ascorbate in the peritoneum of septic mice has been reported [13].

In the present study, we focused on the rapid beneficial effect of ascorbate seen during the course of sepsis [1,2], because this effect maybe clinically relevant to patients already presenting with sepsis. Our objective was to determine if, in parallel to the rapid effect of ascorbate on the microcirculation, there were also rapid effects of ascorbate on the bacterial content, PAI-1 levels, and neutrophil infiltration in septic mice. Based on the fact that ascorbate reduces mortality in sepsis, and that PAI-1 is needed for survival, we hypothesized that ascorbate does not alter the PAI-1 expression and neutrophil infiltration in organs with presumed high immune response in sepsis (lung, kidney, spleen, and liver), preserving the beneficial effect from PAI-1.

2. Materials and methods

2.1. Animal model of sepsis

All experiments were performed with approval of the University of Western Ontario Council on Animal Care. Male C57BL/6 mice were bred in our animal facility from mice purchased from Jackson Laboratory (Bar Harbor, ME) or Charles River (Sherbrooke, Quebec, Canada), and were housed in a controlled environment with a 12 h light–dark cycle and access to food and water *ad libitum*. The mice used were between 2–3 mo old and between 20–25 g in weight. Feces was collected from the cecum of donor mice and mixed in sterile saline at a concentration of 75 mg/mL and kept at 4°C. Mice were anesthetized using a ketamine (80 mg/kg) and xylazine (4 mg/kg) mixture given via intraperitoneal injection. Sepsis was induced in our mice by injecting the feces mixture into peritoneum (FIP) as described previously [1]. Alternatively, control sham mice were injected with 1 mL sterile saline intraperitoneally. All mice were fluid resuscitated subcutaneously with 1 mL saline containing 2 μg/mL buprenorphine as an analgesic.

2.2. Experimental design

We have reported that intravenous injection of ascorbate delayed to 6 h of sepsis (i.e., when capillary plugging is prevalent) rapidly restores capillary blood flow in skeletal muscle by 7 h [2]. This restoration of flow (or reversal of plugging) could be due to dissolution of microthrombi through enhanced fibrinolysis due to inhibition of PAI-1 by ascorbate. In the present study, we chose to study the effect of this delayed ascorbate injection. At 6 h after FIP, mice were anesthetized and then injected intravenously with 0.1 mL of sterile saline or ascorbate (10 mg/kg); they remained under anesthesia for 1 h. On completion of the 1 h treatment time (7 h after FIP), the blood, peritoneal fluid, lung, kidney, spleen, liver, heart, and the extensor digitorum longus skeletal muscle were harvested. We chose lung, kidney, spleen, and liver because these organs were suspected to be involved in neutrophil recruitment and immune response during sepsis [23,24]. We chose the skeletal muscle because in our previous studies we focused on the microcirculatory response of this organ during sepsis [1,2]; the heart presented another type of muscle to compare with skeletal muscle.

2.3. Tissue and blood collection

All chemicals were from Sigma–Aldrich (Oakville, Ontario, Canada) unless otherwise noted. The carotid artery blood was collected 9:1 in acid citrate dextrose solution whereas peritoneal fluid was diluted with 2 mL sterile saline and collected to determine the bacterial load. The organs located in the peritoneum were washed twice with sterile saline to remove