Activated protein C attenuates cardiopulmonary bypass–induced acute lung injury through the regulation of neutrophil activation

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Objective: Cardiopulmonary bypass is known to induce systemic inflammatory responses that injure multiple organs, especially the lungs. Activated protein C has been demonstrated to play an important role in the regulation of inflammation in addition to coagulation. We investigated the anti-inflammatory effects of activated protein C in a rat model of cardiopulmonary bypass.

Methods: Rats were randomized to receive an intravenous bolus of vehicle (control), 0.1 mg/kg diisopropyl fluorophosphate-activated protein C, or 0.1 mg/kg activated protein C 10 minutes before the initiation of cardiopulmonary bypass. Rats underwent cardiopulmonary bypass for 60 minutes followed by another 60-minute observation.

Results: The activated protein C group showed significantly higher mean arterial oxygen pressure and lower mean lung wet-to-dry weight ratio after cardiopulmonary bypass than the control and diisopropyl fluorophosphate-activated protein C groups. Furthermore, lung pathology revealed minimal inflammatory change in the activated protein C group. A marked increase in CD11b expression and a decrease in CD62L expression after cardiopulmonary bypass were observed in the control and diisopropyl fluorophosphate-activated protein C groups. However, administration of activated protein C significantly attenuated these changes. Lung content of tumor necrosis factor-α and interleukin-1β in the activated protein C group tended to be lower than in the other groups. Lung content of macrophage inflammatory protein-2 in the activated protein C group was significantly lower than in the diisopropyl fluorophosphate-activated protein C group.

Conclusions: Administration of activated protein C before cardiopulmonary bypass attenuates acute lung injury induced by cardiopulmonary bypass at least in part through the inhibition of neutrophil activation and possibly via the attenuation of proinflammatory cytokine production in this rat model of cardiopulmonary bypass. (J Thorac Cardiovasc Surg 2011;141:1246-52)

ET/BS

Cardiopulmonary bypass (CPB) is a common procedure in heart surgery. Despite advances in surgical procedures and material technology, CPB induces both coagulopathy and inflammation when blood touches the bypass circuit. Because the lung is vulnerable to attack by neutrophils, CPB is often followed by pulmonary dysfunction, which varies widely, ranging from a clinically undetectable condition to the most severe form, acute respiratory distress syndrome.[1] These responses are associated with endotoxin release; accelerated thrombin generation; activation of complement, leukocytes, and platelets; and the production of cytokines and other inflammatory mediators.[2-5] This complex chain of events seems to have similarities to sepsis, which results from a generalized inflammatory and procoagulant response to an infection.[6] These findings raise the possibility that adequate regulation of coagulation and inflammation may prevent CPB-induced lung injury, which is strongly related to an adverse outcome.[1]

Activated protein C (APC) is an important natural anticoagulant that is generated from protein C by the action of the thrombin-thrombomodulin complex on the endothelial cell.[7] APC inactivates factors Va and VIIIa, and neutralizes the activity of plasminogen activator inhibitor-1, thereby increasing fibrinolysis. Apart from the anticoagulant effects of APC, its direct anti-inflammatory properties have recently been identified.[7] In vitro studies demonstrated that APC down-regulates proinflammatory cytokines, such as interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α release, from both endothelial cells and leukocytes through a specific surface molecule, endothelial protein C receptor (EPCR).[13,16] In addition, expression of intercellular adhesion molecule (ICAM)-1 stimulated by IL-1β and TNF-α was suppressed by APC.[12] In animal models, APC reduced ischemia–reperfusion injury in a variety of organs by inhibiting monocyte activation and cytokine-induced neutrophil chemoattractant expression.[8-11] In a clinical study, Lamarche and colleagues[24] reported successful

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Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication March 14, 2010; revisions received April 21, 2010; accepted for publication May 31, 2010; available ahead of print July 5, 2010.

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0022-5223/$36.00

doi:10.1016/j.jtcvs.2010.05.043

Abbreviations and Acronyms

ACT = activated clotting time
APC = activated protein C
CPB = cardiopulmonary bypass
DIP = diisopropyl fluorophosphate
EPCR = endothelial protein C receptor
ICAM = intercellular adhesion molecule
IL = interleukin
MIP = macrophage inflammatory protein
TNF = tumor necrosis factor
W/D = wet-to-dry

treatment of severe acute respiratory distress syndrome and septic shock by the combined use of extracorporeal membrane oxygenation and APC. Furthermore, a past trial showed that APC significantly reduced 28-day mortality rate when compared with placebo control in patients with severe sepsis.[14] These findings suggest that APC may have beneficial effects on acute organ damage. However, the effect of APC on CPB-induced acute lung injury remains unknown.

The purposes of this study were to (1) investigate whether the administration of APC has a protective effect on acute lung injury in a rat model of CPB and, if so, (2) verify its mechanism.

MATERIALS AND METHODS

Animals and Reagents

Adult male Sprague-Dawley rats (aged 14–17 weeks, weighing 400–580 g) from a licensed vendor (Japan SLC Inc, Shizuoka, Japan) were used in the present study. APC and diisopropyl fluorophosphate (DIP)-APC, an inactive derivative of APC, were provided by the Chemo-sero-therapeutic Research Institute, Kumamoto, Japan. Rats were randomized to receive intravenous bolus of vehicle, 0.1 mg/kg DIP-APC, or 0.1 mg/kg APC 10 minutes before the initiation of CPB. This protocol resulted in the creation of 3 groups: rats given vehicle before CPB (control group, n = 6), rats given DIP-APC (DIP group, n = 6) before CPB, and rats given APC (APC group, n = 6) before CPB. The Animal Care Committee of the National Cardiovascular Center approved this experimental protocol.

Surgical Procedure

Animal procedure was a modification of the technique described previously.[15] In brief, with the animals under anesthesia via intraperitoneally administered pentobarbital sodium (50 mg/kg) and artificial ventilation at a tidal volume of 8 mL/kg, the right jugular vein and left carotid artery were exposed for CPB. Then, an 18-gauge cannula with side holes and a 22-gauge cannula were placed for venous drainage and arterial blood return, respectively. A 22-gauge cannula was inserted into the right femoral vein to infuse saline with 5% bovine serum albumin, and a 24-gauge cannula was inserted into the femoral artery to monitor arterial blood pressure. The bypass circuit was primed with 9 mL of saline with 5% bovine serum albumin. After administration of heparin (200 U/kg), CPB was established with the perfusion rate maintained at 60 mL/kg/min for 60 minutes. Just before the establishment of CPB, clamping of the left pulmonary hilum was performed via anterolateral thoracotomy to simulate the lung condition when total extracorporeal circulation was performed, and the tidal volume was decreased to 6 mL/kg during CPB. When CPB was terminated, the pulmonary clamp was released and the tidal volume was returned to the initial level. The rat was observed for another 60 minutes after CPB. Arterial blood samples were obtained 3 times (before CPB, at the end of CPB, and at the end of the experiment) to measure arterial oxygen pressure and blood hemoglobin level.

Wet-to-Dry Weight Ratio of Left Lung

The left lung was excised immediately after animal sacrifice and divided into 3 parts. The superior third was weighed before and after desiccation in a dry oven at 70°C, and wet-to-dry (W/D) weight ratio was calculated as a marker for lung edema.

Measurement of Lung Cytokine Content

The middle third of the left lung sample was used to measure the lung contents of cytokines. The sample was homogenized in 0.5 mL phosphate-buffered saline containing a protease inhibitor, sonicated, and then centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatants were stored at –80°C until the cytokines were measured. Lung levels of rat TNF-α, IL-1β, and macrophage inflammatory protein (MIP)-2 were measured using corresponding enzyme-linked immunosorbent assay kits (BioSource International, Inc, Camarillo, Calif). The results were corrected by the protein content determined by the Bradford method.[18]

Expression of Adhesion Molecules on Circulating Neutrophils

The expression of CD11b and CD62L on the neutrophil surface was analyzed by fluorescence-activated cell sorting (FACS SCAN flow cytometer; Becton Dickinson, San Jose, Calif) before CPB, at the end of CPB, and at the end of the experiment. Whole blood was incubated with phycoerythrin-conjugated anti-rat CD11b (Serotec Ltd, Oxford, England) and fluorescein isothiocyanate-conjugated anti-rat CD62L (BD Bioscience Pharmingen, San Diego, CA). Erythrocytes were then destroyed by incubation with lysing solution (BD Bioscience Pharmingen). After washing twice with phosphate-buffered saline and centrifuge, neutrophils were fixed with CELLFIX (Becton Dickinson). Isotype-identical antibodies served as controls.

Activated Clotting Time

The measurement of activated clotting time (ACT) (Hemocron Jr Signature; International Technidyne Corporation, Edison, NJ) was performed before CPB and at the end of the experiment. In the APC and DIP groups, the first blood samples were collected 10 minutes after the administration of drugs.

Serum Fibrinogen Level

Serum level of fibrinogen was measured by the thrombin method at the end of the experiment by SRL, Inc, Tokyo, Japan.

Pathologic Study

The inferior third of the lung was fixed with 10% formalin, embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin–eosin for pathologic study.

Statistical Analysis

Numeric values are expressed as mean ± standard error of the mean unless otherwise indicated. Comparisons of parameters among the 3 groups were made using 1-way analysis of variance, followed by the Newman–Keuls test. Comparisons of changes in parameters among the 3 groups were made by 2-way analysis of variance for repeated measures, followed by the Newman–Keuls test.