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Activated protein C inhalation: A novel therapeutic strategy for acute lung injury

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Acute lung injury (ALI) is a critical illness syndrome with a mortality rate of 25–40%. Despite recent advances of our understanding of the pathophysiology of ALI, no pharmacologic therapies have been proven effective. The key pathogenesis of ALI is the activation of the coagulation cascade and impaired fibrinolysis, resulting in extensive fibrin and hyaline membrane deposition. Activated protein C (APC), an endogenous protein that promotes fibrinolysis and inhibits thrombosis, can modulate the coagulation and inflammation associated with ALI. It is therefore reasonable to suggest that preventing the progression of pulmonary coagulopathy, by restoring normal intraalveolar levels of protein C, will be of therapeutic benefit to patients with ALI. However, a recent clinical trial demonstrated that APC did not improve outcomes from ALI, raising the possibility that the method of APC administration, intravenous infusion or inhalation, may influence the outcomes. In this article we propose the hypothesis that APC inhalation might be a promising and novel choice in the treatment of ALI.

key words:

acute lung injury • activated protein C • acute respiratory distress syndrome

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BACKGROUND

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are major causes of morbidity and mortality in critically ill patients, with an incidence rate of approximately 200,000 patients per year in the United States [1] and a mortality rate of 25–40%. Despite recent advances in our understanding of the pathophysiology of ALI, there are currently no effective pharmacologic therapies for ALI or ARDS [2].

One of the main pathogeneses in ALI includes the activation of the coagulation cascade and impaired fibrinolysis, resulting in extensive fibrin and hyaline membrane deposition [3]. Activation of the coagulation cascade, specifically thrombin formation, can induce inflammatory events, including expression of IL-1, IL-6, and IL-8, and transmigration of inflammatory cells across the lung endothelium [4].

The protein C pathway is of vital importance for regulation of the coagulation system. Activated protein C (APC), an endogenous protein that promotes fibrinolysis and inhibits thrombosis, is an important modulator of the coagulation and inflammation associated with ALI. APC is converted from its inactive precursor protein C, by thrombin coupled to thrombomodulin (TM) [5].

APC has many potential beneficial properties in ALI, including inhibition of leukocyte adhesion and chemotaxis, and suppression of inflammatory cytokines. Various studies have also demonstrated that APC can inhibit bronchoalveolar coagulation, neutrophil accumulation in alveoli, anti-apoptotic, and endothelial barrier-enhancing properties [6].

One recent study demonstrated that normal alveolar epithelium contains TM and can activate protein C [7]; however, this conversion can be impaired by down-regulation of TM. Current evidence implies that APC is at a low level in the plasma of patients with ALI/ARDS and that lower levels are associated with worse outcomes. This indicates that the protein C pathway is suppressed due to APC defective production or increased breakdown in patients with ALI/ARDS [8].

It is therefore reasonable to suggest that preventing the progression of pulmonary coagulopathy by restoring normal intraalveolar levels of protein C will be therapeutically beneficial for patients with ALI. Preliminary reports showed that systemic administration of recombinant activated protein C leads to a high intraalveolar level. APC, with a sustained half-life in the lung, may be of vital value if exogenously administered [9].

Currently, one randomized controlled trial (PROWESS) has shown that intravenous infusions of recombinant human (rh)-APC significantly reduced the mortality of patients with severe sepsis [10], a major cause of ALI and sharing many pathophysiological similarities. However, the use of rh-APC in less sick patients or those with single-organ failure has been questioned as bringing higher risk than benefit [11]. Several concerns have been raised regarding the results of the original PROWESS trial. First, the overall significant beneficial effect of rh-APC occurred primarily in patients with a high risk of death. Second, the risk of hemorrhage with

rh-APC therapy *vs.* placebo during the trial did not reach significance; serious hemorrhaging occurred during drug infusion, and was significantly increased with rh-APC [12].

Several reports have already addressed a beneficial effect of rh-APC in experimental ALI animal models by intratracheal or systematic administration. The first randomized controlled trial reporting the effect of rh-APC in patients with ALI was published in 2008 [13]; however, the results showed that rh-APC did not improve outcomes from ALI. No statistically significant difference was observed in terms of the number of ventilator-free days or 60-day mortality. The results of the trial did not support a large clinical trial of APC for ALI in the absence of severe sepsis and high disease severity.

HYPOTHESIS

What is the reason behind the failure of rh-APC in patients with ALI? Several issues of the trial should be considered. Firstly, the trial specifically excluded patients with sepsis-induced ALI because it was considered standard of care to treat these types of patients with rh-APC. The mortality rate of patients in this trial was therefore very low. Secondly, rh-APC therapy was initiated within 72 hours after onset of ALI/ARDS. Furthermore, the number of patients enrolled into the trial may have been too small ($n=75$) and the study may have been relatively underpowered to detect modest potential benefits on the primary endpoints. In addition, we propose that the route of rh-APC administration, whether intravenous infusion or inhalation, may influence the outcomes.

APC is 461 amino acids long with a molecular weight of 55 kDa. APC possesses several properties, including inhibition of coagulation, inflammation and apoptosis. However, APC has a short biological half-life time of only 13 minutes when administered intravenously [14]. In order to maintain a relatively high alveolar concentration of APC, its intravenous dose should be sufficiently high. This raised the issue that intravenous high dose may cause more adverse effects. More importantly, intravenous rh-APC infusion in the original PROWESS trial reduced the mortality rate in severely septic patients, but this is limited in clinical utility due to increased risk of bleeding.

Consequently, an alternative routine of APC administration may be helpful to elucidate the issue. We proposed that APC applied directly to the airways and alveoli through inhalation, while minimizing systematic anticoagulant effects, might be of value in clinical settings. Recently, the effect of APC inhalation has been explored in several animal studies. In a murine model of asthma, APC inhalation showed an anti-inflammatory effect and improved bronchial hyperresponsiveness [15]. In a mouse model of acute lung injury, APC inhalation appeared to attenuate lung inflammation, without reversing the observed increases in lung permeability and bronchoalveolar lavage fluid (BALF) cytokines. It was suggested that this effect may be associated with leukocyte trafficking modifications [16]. In an anesthetized sheep model of endotoxin-induced lung injury, it was shown that inhalation of APC attenuates lung injury by preventing a decline in the volume of aerated lung tissue and improving oxygenation [17]. As the first rh-APC inhalation in a larger animal model, the attenuation of ALI was thought

to be caused by anticoagulant, anti-inflammatory and pro-fibrinolytic effects of APC. It is noticeable that up to 24 h after APC inhalation, APC could be still detected in BALF, indicating that it may provide a long-acting direct means of treating ALI. In a mouse model of ventilator-induced lung injury (VILI), topical application of APC by inhalation was demonstrated to prevent endothelial barrier disruption, attenuated hypoxia and inflammatory response [18].

Heslet et al. [19] reported the first case using APC inhalation for severe ARDS. A 48-year-old woman diagnosed with T-cell lymphoma was treated with chemotherapy. After the last course, the patient developed respiratory insufficiency and then met the diagnostic criteria for severe ARDS. Because of the acute life-threatening state, APC inhalation therapy, with a dosage schedule of 190 µg/Kg 3 times per day for 7 days, was experimentally explored. Although the patient died suddenly from a cardiac arrest, APC inhalation, as a "first in man" therapy, seemed to demonstrate a positive response. A reduction of infiltrates on chest X-ray and a 138% increase in oxygenation capacity by the PaO₂/FiO₂ ratio on day 7 were observed. Meanwhile, no adverse effects of APC inhalation were observed. No signs of systemic bleeding or alveolar hemorrhage were present.

Taken together, we propose the hypothesis that APC inhalation might be a promising and novel choice in the treatment of ALI. Local delivery of APC to the lungs will allow high concentrations of APC in the alveolar space, which will minimize the significant systemic effects. More laboratory and clinical studies are critically needed to determine whether APC inhalation benefits subjects with acute lung injury. The question of whether APC inhalation will show a beneficial effect in other animal models of ALI needs further investigation. The distribution of inhaled APC within the lungs and how to optimize the APC delivery should also be investigated. The appropriate dosage and course of APC for inhalation should be clarified. More research is needed to clarify the exact role of protein C pathway in ALI, providing new insights into APC therapy for ALI.

CONCLUSIONS

In conclusion, since delivery of APC by inhalation is easy to use, APC inhalation should be further investigated as a potential therapeutic strategy for ALI.

Conflict of interest statement

None declared.

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