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The Apelin-APJ Axis Is an Endogenous Counterinjury Mechanism in Experimental Acute Lung Injury

Xiao-Fang Fan, MD; Feng Xue, MD; Yue-Qi Zhang, MD; Xue-Ping Xing, MD; Hui Liu, MD; Sun-Zhong Mao, MD, PhD; Xiao-Xia Kong, MD; Yu-Qi Gao, MD, PhD; Shu F. Liu, MD, PhD; and Yong-Sheng Gong, MD, PhD

BACKGROUND: Although the mechanisms and pathways mediating ARDS have been studied extensively, less attention has been given to the mechanisms and pathways that counteract injury responses. This study found that the apelin-APJ pathway is an endogenous counterinjury mechanism that protects against ARDS.

METHODS: Using a rat model of oleic acid (OA)-induced ARDS, the effects of ARDS on apelin and APJ receptor expressions and on APJ receptor binding capacity were examined. The protective effect of activating the apelin-APJ pathway against OA- or lipopolysaccharide (LPS)-induced ARDS was evaluated.

RESULTS: ARDS was coupled to upregulations of the apelin and APJ receptor. Rats with OA-induced ARDS had higher lung tissue levels of apelin proprotein and APJ receptor expressions; elevated plasma, BAL fluid (BALF), and lung tissue levels of apelin-36 and apelin-12/13; and an increased apelin-APJ receptor binding capacity. Upregulation of the apelin-APJ system has important pathophysiologic function. Stimulation of the apelin-APJ signaling using receptor agonist apelin-13 alleviated, whereas inhibition of the apelin-APJ signaling using receptor antagonist [Ala]-apelin-13 exacerbated, OA-induced lung pathologies, extravascular lung water accumulation, capillary-alveolar leakage, and hypoxemia. The APJ receptor agonist inhibited, and the APJ receptor antagonist augmented, OA-induced lung tissue and BALF levels of tumor necrosis factor-α and monocyte chemoattractant protein-1, and plasma and lung tissue levels of malondialdehyde. Postinjury treatment with apelin-13 alleviated lung inflammation and injury and improved oxygenation in OA- and LPS-induced lung injury.

CONCLUSIONS: The apelin-APJ signaling pathway is an endogenous anti-injury and organ-protective mechanism that is activated during ARDS to counteract the injury response and to prevent uncontrolled lung injury.

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ABBREVIATIONS: BALF = BAL fluid; GSH = glutathione; IP = intraperitoneally; LPS = lipopolysaccharide; MDA = malondialdehyde; MPO = myeloperoxidase; OA = oleic acid; PAH = pulmonary arterial hypertension; Sao_2 = arterial blood oxygen saturation

AFFILIATIONS: From the Institute of Hypoxia Medicine (Drs Fan, Xue, Zhang, Xing, H. Liu, Mao, Kong, S. F. Liu, and Gong), Wenzhou Medical University, Zhejiang, China; the Department of Pathophysiology (Dr Gao), Third Military Medical University, Chongqing, China; and The Feinstein Institute for Medical Research (Dr S. F. Liu), Manhasset, NY.

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CORRESPONDENCE TO: Yong-Sheng Gong, MD, PhD, Institute of Hypoxia Medicine, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China; e-mail: fxbwzmc@126.com

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Acute lung injury and ARDS are devastating consequences of many inflammatory and traumatic conditions, including pneumonia, sepsis, trauma, aspiration, pancreatitis, and severe burn.[1,2] Injurious insults trigger cascades of molecular and cellular events, leading to the activation of inflammatory and injury pathways, the activation of leukocytes and platelets, the generation of reactive oxidant species, the release of proteases, and the activation of coagulation pathways.[3-5] These mechanisms act in concert to cause inflammation, increased lung endothelial and epithelial permeability, pulmonary extravascular accumulation of protein-rich fluid, and hypoxia, which are cardinal characteristics of ARDS.[1-5]

In addition to inflammatory and injury pathways, the lungs are endowed with tissue-protective mechanisms.[6,7] These mechanisms counteract the actions of inflammatory and injurious mediators and prevent uncontrolled lung inflammation and injury. Activation of inflammatory and injury pathways may be the driving force for developing ARDS. However, it is the delicate balance between pro- and antiinflammatory factors, and between injury and counterinjury mechanisms, that may determine the extent of lung inflammation and injury and clinical outcomes. Thus, uncovering new mechanisms that protect against ARDS is significant in that it will not only improve our understanding of the pathogenic mechanisms of ARDS, but will also identify a new target for developing therapy to combat ARDS.

Apelin is a group of small peptides derived from a common precursor, preproapelin. Several active apelin peptides, including apelin-36, apelin-13 and apelin-12, have been reported.[8,9] All apelin peptides exert their biologic effects by binding to a G-protein-coupled receptor, the APJ receptor, leading to biologic responses.[8,9] Apelin and its receptor have a wide range of tissue distribution and have diverse physiologic functions.[8,9] Apelin causes vasodilatation,[10] lowers BP,[11] antagonizes angiotensin- or vasopressin-induced vasoconstriction,[9,12] and improves cardiac contractility.[13] Apelin-APJ signaling regulates the embryonic development of the cardiovascular system[14] and modulates angiogenesis and neovascularization in adult mice.[15,16] The apelin and APJ receptor are upregulated during tissue injury.[17-21] However, the role of this upregulation varies with the organs. Apelin upregulation protects against tissue injury in the heart and pancreas[17-19] but mediates tissue injury in the liver and dorsal root.[20,21] Levels of apelin and APJ receptor expressions are high in the lungs[22] but are downregulated under some pathologic conditions. Patients and animals with pulmonary arterial hypertension (PAH) have lower plasma apelin levels and a reduced apelin expression in lung endothelial cells[23] or lung tissue,[24] which may contribute to the development of PAH.[23-25] In a rat model of 100% oxygen exposure-induced bronchopulmonary dysplasia, apelin is up- or downregulated depending on cell type.[26] Despite all this evidence, the effects of ARDS on apelin and APJ receptor expression, and the modulatory role of the apelin-AJP system in ARDS, have not been studied.

In this study, we tested the hypothesis that the apelin-APJ system serves as an endogenous counterinjury mechanism in a rat model of oleic acid (OA)-induced ARDS. We chose an OA-induced ARDS model for two reasons: (1) OA-induced ARDS exhibits histopathologic and physiologic features that are similar to those of human ARDS[27] and (2) OA-induced ARDS is severe, which allows us to reliably test the protective effects of the apelin-APJ system. We demonstrated, we believe for the first time, that apelin-APJ signaling is activated during ARDS, and that it serves as an endogenous counterinjury mechanism that protects against experimental ARDS.

Materials and Methods

Animal Experiments

All animal studies were approved by the animal care and use committee of Wenzhou Medical University and complied with US National Institutes of Health guidelines. Male rats (of about 250 g) were randomly divided into six groups (five to 10 per group). Rats in the control (Con) or ARDS group were injected with saline (0.2 mL/kg, IV) or OA (0.2 mL/kg, IV). Rats in the Con + apelin or Con + Ala group, or in the ARDS + apelin or ARDS + Ala group, were injected with apelin-13 or [Ala]-apelin-13 (both at 10 nmol/kg, intraperitoneally [IP]) (Phoenix Pharmaceuticals, Inc) 1 h before and 2 h after saline or OA injection. In the postinjury treatment studies, apelin (10 nmol/kg, IP) was administered 1 h after OA injection and 3 h after the initial dose of apelin-13. At 6 h after OA injection, arterial blood gas was analyzed. This was followed by BAL, and blood and lung samples were collected. For the lipopolysaccharide (LPS) model, rats were intratracheally instilled with *Escherichia coli* LPS (5 mg/kg), and were injected with apelin (10 nmol/kg, IP) 4 h after LPS installation, followed by an additional dose of apelin-13 every 6 h. Measurements were made 24 h after LPS. Details of the materials and methods are given in e-Appendix 1.