Acute Amelioration of Inflammatory Activity Caused by Endothelin-2 Deficiency during Acute Lung Injury

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学 位 論 文 の 内 容 要 旨

Acute Amelioration of Inflammatory Activity Caused by Endothelin-2 Deficiency during Acute Lung Injury

急性肺損傷におけるエンドセリン-2 欠損による炎症反応改善

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Background and purpose:

Acute lung injury (ALI) is an acute inflammation of the lung characterized by increased vascular permeability and leukocyte infiltration that can progress to acute respiratory distress syndrome and ultimately respiratory failure. Among the main strategy to treat it is the control of inflammation. Endothelin-2 (Edn2) is an isoform of endothelin-1 (Edn1) that is known to be important in lung development and maintenance through mechanism that remains elusive. Various factors could disturb lung homeostasis and development and lead to alveolar destruction. One such example is lung inflammation. Inflammation in the mice lung just after birth hampers alveolar development. Similarly, induction of acute inflammation using ALI mice model decrease alveolar complexity. As such, I hypothesized that Edn2 physiology involves inflammatory pathway. In this study, I investigated whether knock out of Edn2 could alter the inflammatory activity and which pathway was involved.

Methods

For in vivo study, 8 weeks old Edn2-floxed or Edn2 non-specific inducible knock out (Edn2-iKO) C57BL6J mice received either intratracheal 75ul of PBS or Lipopolysaccharide (LPS) 1mg/ml to induce ALI. After 24 hours, mice underwent pulmonary function test and were then sacrificed. The bronchoalveolar lavage (BAL) fluid was collected for enzyme-linked immunosorbent assay (ELISA), and white blood cell total and differential count. The lung was harvested for western blotting, quantitative real-time PCR, and histological analysis.

Results

After 24 hours, total leukocytes count in Edn2-iKO mice tend to decrease compared to the Edn2-floxed counterpart. After calculation of the differential count, I found that neutrophil number was significantly decreased in Edn2-iKO mice. Additionally, protein concentration of BAL of Edn2-iKO lung was also lower. As these findings indicate lower inflammation in Edn2-iKO mice, I investigated inflammatory markers further. The mRNA expression of the major inflammatory markers such as IL-6,

TNFα, IL-1β in Edn2-iKO lung was downregulated compared to Edn2-floxed mice, although it was not statistically significant. However, the mRNA expression of IFNγ was significantly blunted in Edn2-iKO mice. I further investigated genes that regulated by IFNγ, commonly known as Interferon Stimulated Genes but did not find significantly different expression.

However, I found that a transcription factor downstream of IFNγ, STAT1, was significantly decreased in Edn2-iKO mice. Furthermore, using ELISA of the BAL fluid, I found that IL-1β levels in Edn2-iKO was similarly reduced, potentially as a result of STAT1 downregulation. Next, I investigated a transcription factor that can be activated by IL-1β and play a role in neutrophil recruitment, nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). Phosphorylation of NFκB at the p65 site was significantly suppressed in Edn2-iKO mice. Taken together, these finding suggest that deficiency of Edn2 could affect IFNγ/STAT1/IL-1β pathway and lowers the severity of inflammation in Edn2-iKO lungs.

Interestingly, the pulmonary function of Edn2-iKO lung was unaltered compared to the Edn2-floxed, indicating that the reduced inflammation severity did not improve lung function. Further, Edn1 was widely known to have pro-inflammatory effect. As there is a possibility that knock out of Edn2 could result in compensatory effect from Edn1 or other component of the endothelin system, the expression of Edn1 and two of its receptors were also checked. Edn1 expression was surprisingly unaffected either by LPS or Edn2 knock out. Additionally, compared to mice given PBS, in mice given LPS, both Endothelin Receptor Type A (ETAR) and Endothelin Receptor Type B (ETBR) were downregulated.

Discussion

ALI is a condition that can quickly progress to respiratory failure and death without proper treatment. Its pathophysiology involves inflammatory responses in the alveolar bed, leading to leukocyte infiltration and edema. Endothelin, mainly Edn1, is involved in pathological conditions related to dysregulated inflammation, such as pulmonary hypertension, myocardial infarction, chronic obstructive pulmonary disease, and ALI. Additionally, several proinflammatory cytokines, such as interleukins,

interferons, and TNFα, are induced by the overabundance of Edn1. As Edn1 and Edn2 bind the same ETAR and ETBR receptors, Edn2 potentially modulate inflammation as well.

In the present study, I investigated the relation between Edn2 and inflammation using an in vivo ALI model. Loss of Edn2 adversely affect lung development and maintenance. Therefore, I initially assumed that deleting Edn2 will worsen inflammation. Surprisingly, I found that with Edn2 deficiency, LPS-induced inflammation generally improved. There was lower neutrophil recruitment in lung of Edn2-iKO mice. The IFNγ/STAT1/IL-1β axis was downregulated, accompanied with reduction of NFκB activity further suggesting that Edn2 deficient lung exhibited lesser inflammatory response. These findings may provide an important and novel insight into the mechanism of ALI.

Evidently, Edn1 is known as a proinflammatory molecule, and it shares the same receptor with Edn2. Thus, blockade of Edn2 receptor, which will concurrently block Edn1 using Endothelin Receptor Antagonist (ERA) may prove to be a promising treatment option during ALI. Previous studies indicated that ERA administration could lead to anti-inflammatory effect in various organ, including the lung. Additionally, several ERAs have been clinically approved for the treatment of pulmonary arterial hypertension and more recently subarachnoid hemorrhage. In other conditions, such as diabetic nephropathy and essential hypertension, ERA have also showed some efficacy. Therefore, ERA could potentially prove beneficial for ALI.

I was curious to see whether alteration in endothelin signaling could affect the expression of the other component of endothelin family during inflammation. Interestingly, the mRNA level of the two receptors of Edn2, the Ednra and Ednrb, was suppressed in inflammation. Apparently, Edn1 was unchanged regardless of inflammation or Edn2 expression. This suggested that any differences seen between Edn2-iKO mice and Edn2-floxed mice is exclusively due to the loss of Edn2, and not due to any compensatory changes of Edn1. To conclude, Edn2 deficiency caused amelioration of inflammatory reactions in ALI, and blocking this pathway could prove beneficial as a novel treatment target in ALI condition.

神戸大学大学院医学研究科（博士課程）

論文審査の結果の要旨

受付番号	甲第 3326 号	氏名	AHMAD MUSTHAFA
論文題目 Title of Dissertation	Acute Amelioration of Inflammatory Activity Caused by Endothelin-2 Deficiency during Acute Lung Injury 急性肺損傷におけるエンドセリン-2 欠損による炎症反応改善		
審査委員 Examiner	主査 Chief Examiner　坂口一彰 副査 Vice-examiner　古屋敷智之 副査 Vice-examiner　眞庭謙昌		

（要旨は１，０００字～２，０００字程度）

ALI is a severe inflammation of the lung that can lead to respiratory failure. Edn2 is important for lung development and maintenance, but its mechanism is unclear. Inflammation can disrupt lung homeostasis and development, leading to alveolar destruction. This study investigates the role of Edn2 in inflammation and whether its knock out can alter inflammatory activity. For the in vivo study, 8-week-old C57BL6J mice were used, either with the Edn2 gene floxed or with non-specific inducible knock out (Edn2-iKO). These mice were given intratracheal injections of either PBS or LPS to induce ALI. After 24 hours, the mice underwent pulmonary function tests and were then sacrificed. The bronchoalveolar lavage (BAL) fluid was collected for ELISA and white blood cell count, while the lung was harvested for western blotting, quantitative real-time PCR, and histological analysis. After 24 hours, leukocyte and neutrophil counts were significantly decreased in Edn2-iKO mice compared to Edn2-floxed mice, indicating lower inflammation. The protein concentration of BAL in Edn2-iKO lungs was also lower. The mRNA expression of major inflammatory markers such as IL-6, TNFα, and IL-1β was downregulated in Edn2-iKO lungs, although not statistically significant.

The mRNA expression of IFNγ was significantly decreased in Edn2-iKO mice, but there was no significant difference in the expression of genes regulated by IFNγ, known as Interferon Stimulated Genes. However, Edn2-iKO mice showed significantly decreased levels of STAT1 and IL-1β, potentially leading to lower inflammation. Phosphorylation of NFκB at the p65 site was also significantly suppressed in Edn2-iKO mice. These findings suggest that deficiency of Edn2 could affect IFNγ/STAT1/IL-1β pathway and lower the severity of inflammation in Edn2-iKO lungs.The study found that the pulmonary function of Edn2-iKO lung was not improved despite reduced inflammation severity. Edn1 was known to have a pro-inflammatory effect, but Edn1 expression was unaffected by LPS or Edn2 knock out. Both Endothelin Receptor Type A (ETAR) and Endothelin Receptor Type B (ETBR) were downregulated in mice given LPS compared to those given PBS. Additionally, the pathophysiology of ALI involves inflammatory responses in the alveolar bed, leading to leukocyte infiltration and edema.

Endothelin, mainly Edn1, is involved in pathological conditions related to dysregulated inflammation, such as pulmonary hypertension, myocardial infarction, chronic obstructive pulmonary disease, and ALI. Edn1 and Edn2 bind the same ETAR and ETBR receptors, and Edn2 potentially modulates inflammation as well. In the study, Musthafa found that Edn2 deficiency improved LPS-induced inflammation, with lower neutrophil recruitment in the lungs of Edn2-iKO mice. The IFNγ/STAT1/IL-1β axis was downregulated, accompanied by a reduction of NFκB activity, suggesting that Edn2-deficient lungs exhibited a lesser inflammatory response.

These findings provide important and novel insights into the mechanism of ALI. Evidently, Edn1 is a proinflammatory molecule that shares the same receptor with Edn2. Blocking the Edn2 receptor, which will concurrently block Edn1 using Endothelin Receptor Antagonist (ERA), may prove to be a promising treatment option during ALI. Previous studies have shown that ERA administration could lead to anti-inflammatory effects in various organs, including the lung. Several ERAs have been clinically approved for the treatment of pulmonary arterial hypertension and more recently subarachnoid hemorrhage. Therefore, ERA could potentially prove beneficial for ALI.

To further explore the effects of endothelin signaling on the expression of the endothelin family during inflammation, Musthafa found that the mRNA level of the two receptors of Edn2, the Ednra and Ednrb, was suppressed in inflammation. Interestingly, Edn1 was unchanged regardless of inflammation or Edn2 expression. This suggests that any differences seen between Edn2-iKO mice and Edn2-floxed mice are exclusively due to the loss of Edn2, and not due to any compensatory changes of Edn1. Overall, Edn2 deficiency caused amelioration of inflammatory reactions in ALI, and blocking this pathway could prove beneficial as a novel treatment target in ALI condition.

本研究は全く新しい方法で、動物モデルを用いて Acute lung Injury のメカニズム解明を目指したもので、従来あまり知られていなかったエンドセリン 2 の機能を明らかにし、難病の治療につながりうる重要な知見を得たものとして価値ある集積と認める。

よって、本研究者は、博士（医学）の学位を得る資格があると認める。