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Prevention of LPS-induced lung inflammation by PLAG administration via blocking of IL-6-STAT3-MIP-2 pathway

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Abstract

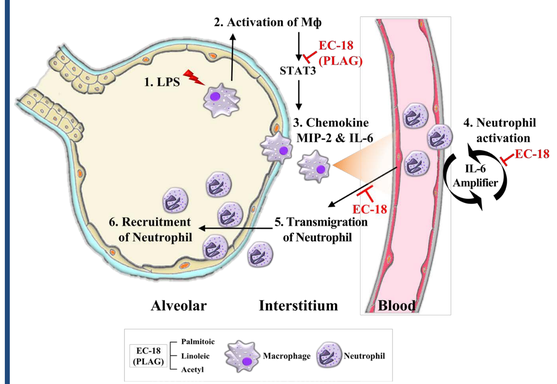
Neutrophil plays a key role in the innate immune system through as the first leukocyte to be migrated to the acute inflammatory region. Lung inflammation is an acute respiratory failure and links closely to excessive neutrophil recruitment and severe mortality. The intranasal LPS introduced mice were shown the over-flowed neutrophils into bronchoalveolar. EC-18 (PLAG) is a synthetic monoacetyldiacylglycerol (acetyl-DAG). DAG is a lipid second messenger for PKC activator which is associated with an important for cellular functions. The transmigrated neutrophils in alveolar by LPS stimulation were completely blocked by EC-18 administration. LPS-induced cell migration associated factors such as IL-6, MIP-2 and S100A8/9 were decreased by addition of EC-18. STAT3 phosphorylation which related to neutrophil migration was significantly down regulated in EC-18 dieted mice. Interestingly, the selectivity of EC-18 in the ameliorating of excessive neutrophil migration was verified when compared with EC-18 analogues and metabolites. Post treatment of EC-18 has also therapeutic effect on LPS-induced lung inflammation. These data could be utilized as a most potential therapeutic agent for lung inflammation through regulation of neutrophil transmigration via blocking of IL-6-STAT3-MIP-2 pathway.

Introduction

Acute lung injury (ALI) is a severe respiratory inflammation with an increased permeability of the alveolar-capillary barrier. Neutrophil migration into the lung is the critical step in the early progression of ALI. Neutrophil recruitment into the lung is occurred by the massive pro-inflammatory cytokines and chemokines, and leads to lung edema, endothelial and epithelial injury. ALI is still associated with a high mortality, and a specific therapy is not available. There are several factors involved in neutrophil migration into the lung of ALI model mouse. **MIP-2 (CXCL2)** is secreted by resident macrophages in the lung, and it has the chemotactic effect for neutrophil recruitment toward inflammation sites. **S100A8** and **S100A9**, which are known as inflammatory protein complex, exist in high amounts in the cytoplasm of neutrophils. They are able to regulate neutrophil activation and migration into the target tissue. **IL-6** is a critical pro-inflammatory cytokine that contributes to the initiation and extension of the inflammatory response. Furthermore, IL-6 autocrine signaling loop shows the enhanced self-amplification through STAT3 phosphorylation. They play an important role in host defense, however, the excessive production of these factors can cause the severe inflammation such as acute lung injury and are controlled by several signal factors. **STAT3** activation in the lung is associated with acute phage of lung injury. STAT3 is commonly phosphorylated by IL-6, but also by TLR4->MyD88->p38 MAPK->STAT3 pathway. In this study, we investigated whether EC-18 has the therapeutic potential in LPS injected mouse model and examined what molecule and the signal pathway was regulated by EC-18.

Conclusion

- **EC-18 (PLAG) effectively blocked the neutrophil transmigration into the lung alveolar.**
- **EC-18 could be utilized as a potential therapeutic agent for prevention of acute and chronic inflammation related disease like ALI.**



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Results

Figure 1. EC-18 attenuated the LPS-induced ALI

In mice that had been administered LPS, lung tissues showed strong inflammation through a large amount of Evans Blue accumulation (Fig 1A). In mice treated with LPS and EC-18 or dexamethasone, Evans Blue accumulation was decreased in the lungs. These findings were confirmed by quantitative analysis of Evans Blue-labeled albumin extraction from the lungs (Fig 1B, ***p<0.001). Approximately 16 h after LPS induction, leukocytes were counted in whole blood and BALF samples. LPS challenge significantly increased neutrophil infiltration into BALF compared to the control. LPS/EC-18 and LPS/dexamethasone co-treated animals exhibited significantly decreased neutrophil migration into BALF (Fig 1C, ***p<0.001). Neutrophils in the LPS/EC-18- or LPS/dexamethasone-treated groups were more prevalent in the blood compared to those in the LPS-treated group (Fig 1D). The composition of BALF cells was analyzed (Fig 1E). LPS treatment increased neutrophil population to 86.27% of leukocytes, while EC-18 treatment significantly reduced this population to 32.17%. EC-18 (55.87%) relatively induced the lymphocyte population compared to that induced by LPS (3.13%) in mice (Fig 1E). **These findings suggest that EC-18 significantly suppresses LPS-induced ALI progression.**

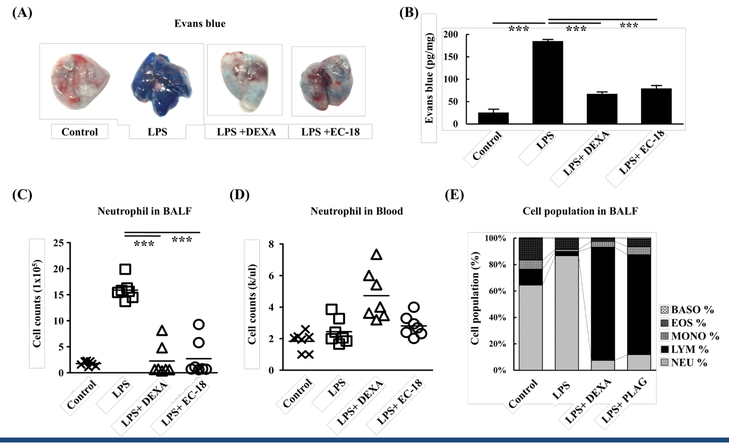

Figure 2. EC-18 inhibits LPS-induced inflammatory cell infiltration in the lungs

LPS treatment induced vast inflammatory cell infiltration into the lung tissue compared to control treatment (Fig 2A). LPS/EC-18 co-treated mice exhibited considerably attenuated inflammatory cell infiltration into the alveolar and had normal alveolar morphology. More precisely, the massive amounts of neutrophil transmigration from blood into the lungs were blocked by EC-18 administration (Fig 2B). In mice treated with LPS and EC-18 or dexamethasone, the histological score was also shown the treatment effect (Fig 2C, ***p<0.001). MPO activity was substantially increased in LPS-treated mice and significantly decreased in the LPS/EC-18 co-treated mice (Fig 2D, ***p<0.001**). These data demonstrate that EC-18 plays a protective role in ALI through the blockade of neutrophil migration into the lung.**

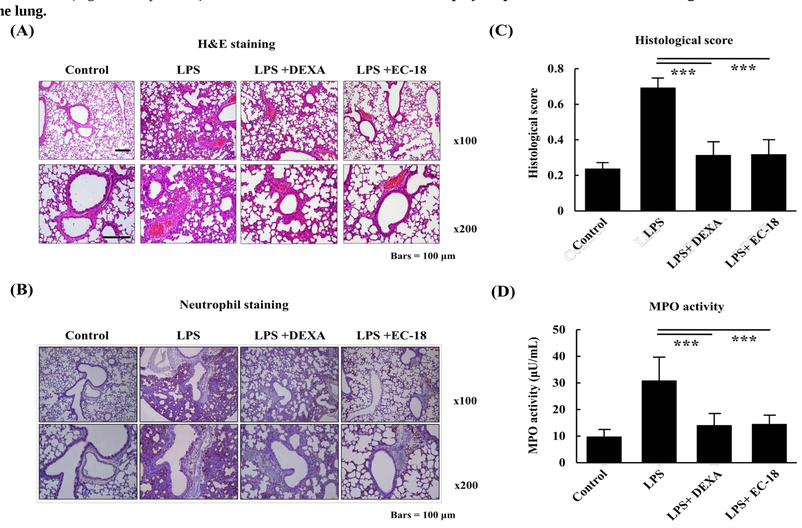

Figure 3. IL-18 downregulates the production of pro-inflammatory cytokines in BALF and serum

Expression of MIP-2 (CXCL2), IL-6, and S100A8/9 was increased in BALF cells from mice treated with LPS compared to that in control animals (Fig 3A). This increased gene expression was significantly downregulated in mice treated with LPS and EC-18 together. Other chemokines exhibited either a moderate decrease or no change following treatment with these agents. EC-18 treatment did not significantly change MIP-2 mRNA expression in the lungs (Fig 3B). Furthermore, MIP-2 and IL-6 secretion was significantly increased in BALF and serum following LPS administration, and EC-18 treatment markedly decreased secretion of MIP-2 and IL-6 (Fig 3C and D, ***p<0.001, **p<0.005, *p<0.05). **These data indicate that EC-18 effectively suppresses production of the anti-inflammatory cytokines in the inflammatory region via blockade of neutrophil transmigration.**

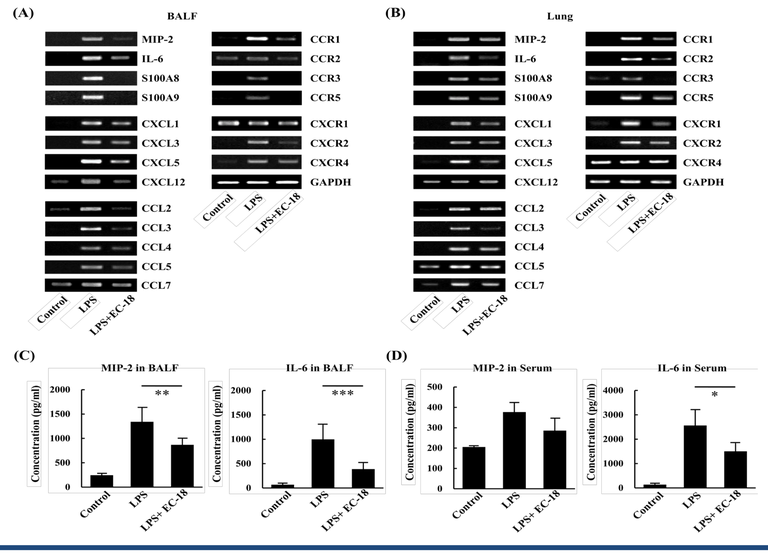

Figure 4. EC-18 inhibits LPS-induced activation of STAT3 and PKCδ

STAT3 was phosphorylated by LPS challenge in the lung, and EC-18 co-treatment downregulated this STAT3 phosphorylation (Fig 4A). The phosphorylation of STAT4 and 5 was also slightly downregulated in the LPS/EC-18-treated group. Thus, EC-18 exerts an anti-inflammatory effect mainly through the suppression of LPS-induced STAT3 activation. PKCδ is also well known as a critical regulator in the inflammatory response and mediator in LPS-induced response [21]. LPS-induced PKCδ phosphorylation was also inhibited in the LPS/EC-18 co-treated group (Fig 4B). To confirm, Raw 264.7 mouse macrophage cell line were incubated with EC-18 or DMSO as the solvent control, and stimulated with LPS for 4 h. LPS-induced STAT3 activation was significantly decreased by EC-18 pretreatment in dose-dependent manner (Fig 4C). The phosphorylation of PKCδ was also inhibited by EC-18 treated Raw 264.7 and A549 (Fig 4D and E). LPS-induced productions of MIP-2 and IL-6 were significantly downregulated in the LPS/EC-18-treated group in dose dependent manner (Fig 4F and G, ***p<0.001, **p<0.005). **Taken together, these data indicate that EC-18 may play a critical role in the resolution of the inflammatory response.**

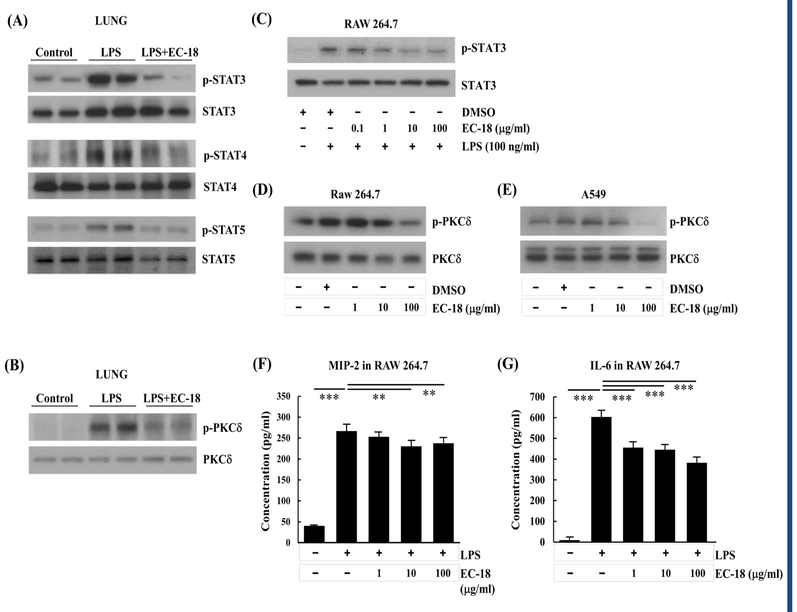

Figure 5. Acetylation of DAG is responsible for the suppressive effect on ALI

To determine whether the acetylation on DAG is the underlying reason for the suppressive effect on ALI, whole blood and BALF cells were counted. EC-18 is composed of palmitic acid, linoleic acid, and acetylated glycerol (Fig 5A), and the EC-18-derivative PLG is a DAG that consists of two fatty acid chains, palmitic acid, and linoleic acid. EC-18 is the acetylated form of PLG. HLH is composed of linoleic acid. Linoleic fatty acid (LA) or palmitoic fatty acid (PA) was used as EC-18 derivative. EC-18 markedly decreased neutrophil infiltration into BALF (Fig 5B, ***p<0.001), whereas the EC-18 derivatives PLG, HLH, LA, and PA had no inhibitory effect on LPS-induced ALI progression. **These findings suggest that the acetylation of DAG is critical in ALI attenuation. In this regard, further studies are needed to examine the mechanism involved in the acetylated DAG-mediated anti-inflammatory response.**

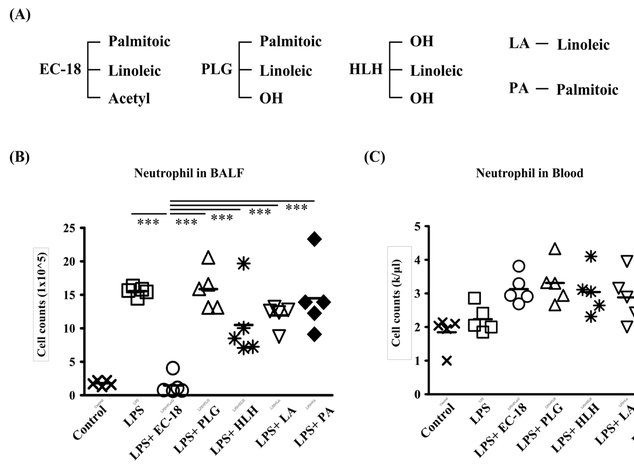

Figure 6. Post treatment of EC-18 have a potential therapeutic agent for lung inflammation

Because we showed that EC-18 exerts a suppressive effect in the ALI mouse model when co-administered with LPS, we next investigated whether EC-18 has the same effect when administered as medication. After 2 h treatment with LPS, EC-18 was then administrated orally. Impressively, neutrophils in BALF were diminished by half after post-treatment with EC-18 (Fig 6A). Thus, **EC-18 may be a therapeutic agent for inflammatory disease, including ALI.**