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# Stearoyl lysophosphatidylcholine enhances the phagocytic ability of macrophages through the AMP-activated protein kinase/p38 mitogen activated protein kinase pathway

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ABSTRACT

A previous study showed that stearoyl lysophosphatidylcholine (sLPC) suppressed extracellular high mobility group box 1 translocation in macrophages stimulated with lipopolysaccharide through AMP-activated protein kinase (AMPK) activation. In the present study, we investigated whether sLPC-induced AMPK activation could enhance macrophages phagocytosis of bacteria. We found that sLPC increased phosphorylation of AMPK and acetyl-CoA carboxylase, a downstream target of AMPK, in a time- and dose-dependent manner in macrophages. Furthermore, sLPC increased the uptake of FITC-conjugated *Escherichia coli* by macrophages in a dose-dependent manner, and treatment with an AMPK inhibitor (compound C) or siRNA to AMPKα1 reversed this uptake. sLPC increased the phosphorylation of p38 mitogen-activated protein kinase (MAPK), but inhibition of AMPK activity with compound C or siRNA to AMPKα1 prevented the sLPC-induced increase in p38 MAPK phosphorylation. SB203580, a p38 MAPK inhibitor, decreased sLPC-induced phagocytosis. In vivo, systemic administration of sLPC to mice led to increased AMPK and p38 MAPK activity in the lung and to increased phagocytosis of fluorescent *E. coli* in bronchoalveolar lavage cells. These results suggest that sLPC increases macrophages phagocytosis through activation of the AMPK/p38 MAPK pathway. Therefore, sLPC is a candidate pharmacological agent for the treatment of bacterial infections in clinically relevant conditions.



## 1. Introduction

Phagocytes, such as macrophages, play important roles in the removal of invading pathogens, tissue repair, and immune modulation. The engagement of specific receptors on the surface of macrophage, such as the Fcγ receptor or complement receptor, by pathogens lead to the activation of diverse signaling molecules, which induce actin cytoskeleton remodeling [1,2]. During the internalization of bacteria, the Rho family of GTPases, Cdc42, Rac1, and RhoA regulate cytoskeletal rearrangement. Previous studies showed that several kinases, including AMP-activated protein kinase (AMPK), p38 mitogen activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3-K), are implicated in cytoskeletal reorganization [3–5].

In addition to the role of AMPK in the regulation of lipid and glucose metabolism, cell migration, cell growth, and cell polarity [6–8], there is growing evidence that AMPK is also involved in modulating inflammatory conditions. For example, AMPK activation reduced LPS-induced production of inflammatory cytokines in vitro and in vivo [9–11] and suppressed a carbon tetrachloride-induced increase in the expression of TNF-α, interleukin (IL)-6, and inducible nitric oxide synthase [12]. LPS- or free fatty acid-induced activation of nuclear factor (NF)-κB signaling and expression of inflammatory cytokines, such as TNF-α and IL-6, were suppressed by a constitutively active form of AMPK and stimulated by a dominant-negative form [13,14].

In the circulation, lysophosphatidylcholine (LPC) is a highly abundant lysophospholipid that is found in a mixture of fatty acids with different lengths and saturations [15,16]. LPC is a bioactive lipid with diverse functions in various cell types [17]. For example, LPC stimulated the production of inflammatory mediators, such as cyclooxygenase-2 and IL-8, in vascular endothelial cells [18,19] and of macrophage inflammatory protein-2 in macrophages [20]. LPC also led to the release of IL-1β in microglial cells [21]. However, stearoyl lysophosphatidylcholine (sLPC) (18 carbons at the sn-1 position) has a potent anti-inflammatory

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effect in acute inflammatory settings, such as sepsis. sLPC reduced organ dysfunction and mortality in various animal models of sepsis, even when it was administered after the establishment of sepsis [22,23].

Previous studies showed that AMPK could modulate cytoskeletal rearrangement, including actin polymerization and microtubule formation, in various cell types, such as macrophages, during phagocytosis [3,8]. In macrophages, sLPC increased AMPK activation and inhibited LPS-induced HMGB1 translocation from the nucleus to the extracellular space through AMPK activation [24,25]. These results suggest sLPC may have a potential therapeutic effect in acute inflammatory conditions associated with bacterial infection. Therefore, we investigated whether sLPC could enhance the phagocytosis of bacteria by macrophages through AMPK activation.

## 2. Materials and methods

### 2.1. Mice

Male BALB/c mice (20–25 g, 8–10 weeks old) were obtained from Samtako Science (Daejeon, Korea). The mice were kept on a 12-h light/dark cycle with free access to food and water. All experiments were conducted in accordance with protocols approved by the Animal Care and Ethics Committee of Chonnam National University Medical School.

### 2.2. Reagents and antibodies

sLPC (LPC 18:0) and lauroyl LPC (LPC 12:0) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Bovine serum albumin (BSA), RPMI 1640 was supplemented with L-glutamine, penicillin-streptomycin and fetal bovine serum (FBS) (GIBCO, Gaithersburg, MD, USA). Fluorescein-conjugated *Escherichia coli* (K-12 strain) was purchased from Invitrogen (Eugene, OR, USA). Bicinchoninic acid (BCA) protein assay reagent was purchased from Pierce (Rockford, IL, USA). Antibodies specific for p-AMPK (No. 2531), p-ACC (No. 3662), p-p38 (No. 9211), p-Akt (No. 9271), AMPK (No. 2532), p38 (No. 9212), and compound C were purchased from Cell Signaling Technology (Beverly, MA, USA). SB203580 was purchased from Calbiochem (La Jolla, CA, USA). Control siRNA and siRNA to AMPKα1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

### 2.3. Western blotting

Western blotting was performed as described previously [26,27]. Briefly, the protein concentration of each sample was assayed using a BCA protein assay kit standardized to BSA according to the manufacturer's protocol. Equal amounts of protein were separated by 10% SDS-PAGE and electrotransferred onto polyvinylidene difluoride (PVDF). To determine the levels of total and phosphorylated proteins, membranes were probed with specific primary antibodies followed by detection with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibodies. Bands were detected using enhanced chemiluminescence (ECL) Western blotting detection reagents (Millipore, Billerica, MA, USA) and imaged on a LAS-3000 (Life Science Systems, Fujifilm Global) imager. To determine the ratio of phosphorylated to total protein, densitometry was performed using a multi-gauge V3.0 chemiluminescence system with analysis software (Life Science Systems, Fujifilm Global).

### 2.4. Cell isolation and culture

Murine peritoneal macrophages were isolated as described previously [27]. Briefly, cells were isolated 4 days after intraperitoneal injection with 4% Brewer thioglycollate and then cultured in 6- or 12-well plates. After 2 h, cells were washed three times with culture medium to remove non-adherent cells and then cultured at 37 °C in RPMI 1640 medium containing 5% FBS. Murine macrophage RAW 264.7 cells

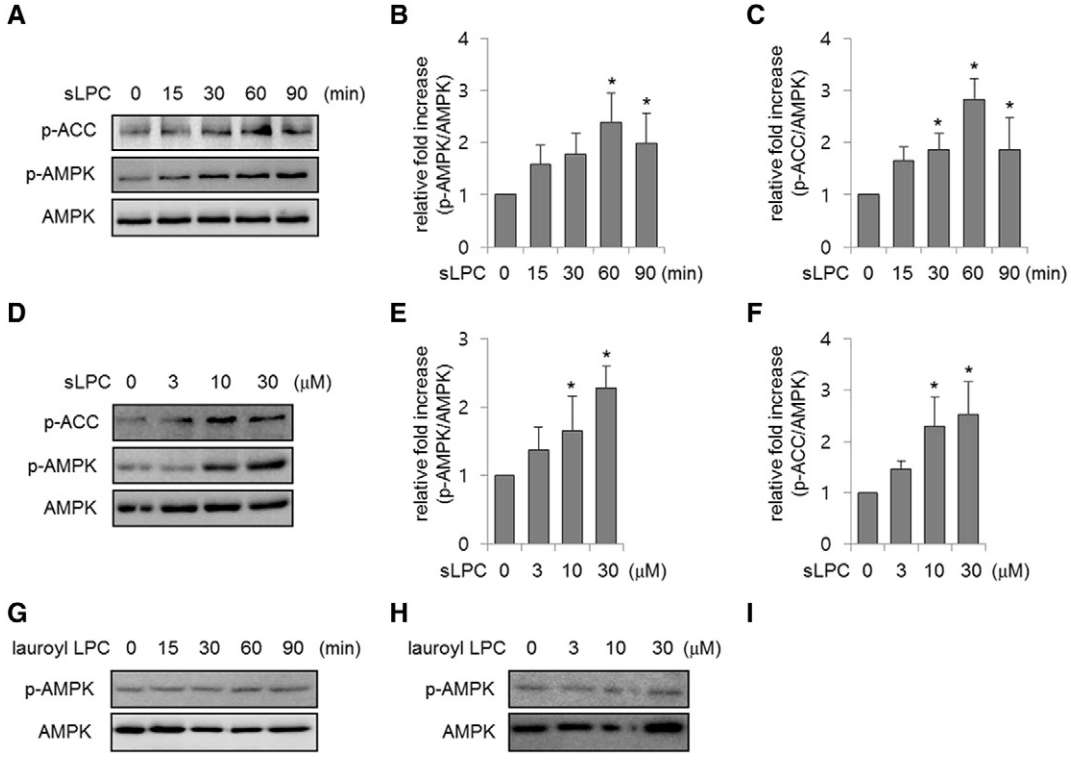


**Fig. 1.** Effects of stearoyl lysophosphatidylcholine (sLPC) on AMP-activated protein kinase (AMPK) activation. Mouse peritoneal macrophages were cultured with sLPC (30 μM) for the indicated time periods (A–C) or with sLPC (0, 3, 10, or 30 μM) for 1 h (D–F). A and D: Representative Western blots show levels of phosphorylated AMPK and acetyl-CoA carboxylase (ACC) and total AMPK. B, C, E, and F: The ratio of phosphorylated ACC or AMPK to total AMPK calculated with data from four experiments. Each bar represents the mean ± SD. * $P < 0.05$ compared with the control. G and H: Cells were cultured with lauroyl LPC (30 μM) for the indicated time periods or with lauroyl LPC (0, 3, 10, or 30 μM) for 1 h. Representative gels are shown, and a second independent experiment provided consistent results.