学位論文抄録

Enhanced Cellular Polysulfides Negatively Regulate TLR4 Signaling
and Mitigate Lethal Endotoxin Shock
（細胞内ポリスルフィドの亢進はTLR4シグナルを阻害し致死性エンドトキシンショックを軽減させる）

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Background and Purpose: During bacterial infection, macrophage-derived inflammatory mediators such as tumor necrosis factor-α (TNF-α), play critical roles in immune responses. Cysteine persulfide (CysSSH) and cysteine polysulfides ($Cys[S]_nH$, n>2) are cysteine derivatives having sulfane sulfur atoms bound to cysteine thiol. Accumulating evidence has suggested that cysteine persulfides/polysulfides are abundant in prokaryotes and eukaryotes and play important roles in diverse biological processes such as antioxidant host defense and redox-dependent signal transduction. In this study, I examined the effects of chemically synthesized polysulfide donors on lipopolysaccharide (LPS)-induced innate immune responses in macrophages and on pathogenicity of endotoxin shock in mice.

Methods: *N*-Acetyl-L-cysteine (NAC) connecting with polysulfur chains were synthesized as novel polysulfide donors (NAC polysulfides). The murine macrophage cell line RAW264.7 cells were stimulated with Toll-like receptors (TLRs) ligands in the absence or presence of NAC polysulfides. Formation of the intracellular persulfides/polysulfides were determined by means of liquid chromatography-tandem mass spectrometry (LC-MS/MS). TNF-α and interferon-β (IFN-β) were measured by ELISA. Quantitative RT-PCR was used for analysis of IFN-β mRNA transcripts. Cellular signal transduction was studied by Western blotting. To establish endotoxin shock model mice, male 9-week-old C57BL/6 mice were received intraperitoneal injections of phosphate buffered saline (PBS) or LPS (20 mg/kg). Thirty minutes later, mice were further received intraperitoneal injection of physiological saline and NAC polysulfides (120 μmol/kg). The serum and tissues were collected from mice post 30 h LPS injection.

Results: LC-MS/MS analyses revealed that NAC polysulfides rapidly reacted with reduced glutathione (GSH) to form a variety of sulfur containing metabolites such as GSH persulfide (GSSH), GSH tetrasulfide (GSSSH), hydrogen sulfide, and thiosulfate, through sulfur transfer from NAC polysulfides to GSH. Importantly, NAC polysulfides treatment markedly increased intracellular persulfide/polysulfide levels of cysteine and GSH in RAW264.7 cells, suggesting that NAC polysulfides can be delivered to cytosol and act as very potent sulfur donors inside the cells. Furthermore, NAC polysulfides treatment strongly inhibited the production of pro-inflammatory cytokines including TNF-α and IFN-β from macrophages stimulated with TLR ligands such as LPS, polyinosinic-polycytidylic acid sodium salt (poly I:C), and zymosan A, for TLR4, TLR3, and TLR2, respectively. Western blotting analyses demonstrated that NAC polysulfides significantly suppressed NF-κB and STAT1 signaling. The current *in vivo* animal study also proved that NAC polysulfides ameliorated LPS-induced endotoxin shock in mice.

Conclusions: The present study clearly showed that NAC polysulfides can act as potent persulfide/polysulfide donors in biological system. Cellular polysulfides negatively regulate TLRs-mediated proinflammatory signaling and hence constitute a potential target for inflammatory disorders including lethal endotoxin shock.