Introduction: Pifithrin-α (PFT-α), a pharmacological inhibitor of p53, reversely inhibits a p53 transcriptional activity. As far as is known, there is no report on the in vitro effect of PFT-α on lipopolysaccharide (LPS)-induced inflammatory response including production of cytokines and nitric oxide (NO). Recently, PFT-α is reported to prevent LPS-induced hepatic injury. LPS leads to the production of large amounts of nitric oxide (NO) via the expression of an inducible isoform of NO synthase (iNOS) and the excessive NO release is involved in LPS-induced cell death and tissue injury. LPS triggers the NO production through activating both MyD88-dependent and independent pathways after the binding to toll like receptor (TLR) 4. In particular, LPS-induced interferon (IFN)-β production in the MyD88-independent pathway, including TRAF3, TRIF, IKK-ε and IRF3, is a key event and correlated with the production of NO. In the present study, we studied the effect of PFT-α on LPS-induced NO production. Incidentally, we found that PFT-α inhibited LPS-induced NO production in vitro, independent of p53.

Materials and methods: The murine macrophage cell line, RAW 264.7, was obtained from Riken Cell Bank (Tsukuba, Japan) and maintained in α-MEM medium containing 5% heat inactivated fetal calf serum, antibiotics, antifungocides and non essential amino acid at 37°C under 5% CO2. Culture medium supernatant was collected to analyze NO and cytokines with Grease reagent and enzyme linked immune absorbent assay, respectively. The expression and activation of signal molecules were determined by immunoblotting. The expression of mRNA was quantified by real time polymerase chain reaction.

Results: 1) PFT-α at 5 and 10 μM inhibited LPS-induced NO production whereas it did not affect the TNF-α production.

2) PFT-α significantly reduced the expression of iNOS protein and mRNA in LPS-stimulated cells (approximately 50% inhibition).

3) To confirm that PFT-α downregulates LPS-induced NO production via inhibition of p53, the effect of p53-specific siRNA on LPS-induced iNOS expression was examined. Silencing of p53 with siRNA does not inhibit LPS-induced iNOS expression. Other hand, PFT-α reduced LPS-induced iNOS protein expression in cells transfected with p53-specific or control siRNA.

4) PFT-α at the concentrations ranging from 1 to 10 μM inhibited LPS-induced IFN-β production. The expression of IFN-β mRNA was also inhibited by PFT-α, suggesting that PFT-α might inhibited LPS-induced NO production via downregulation of the IFN-β production in the MyD88-independent pathway.

5) PFT-α reduced the level of IKK-ε and IRF3 phosphorylation. PFT-α reduced the expression level of TRAF3 but not TRIF. PFT-α gradually reduced the TRAF3 expression in the presence or absence of LPS.

6) PFT-α inhibited the NO production in response to poly I:C as well as LPS. In other hand, PFT-α did not inhibit IFN-β-induced NO production.

Discussion: In the present study we have demonstrated that PFT-α inhibits LPS-induced NO production via impairment of the MyD88-independent pathway. PFT-α does not affect the production of TNF-α mainly dependent on the MyD88-dependent pathway of LPS signaling. On the other hand, it reduces the production of IFN-β that is characteristic of the MyD88-independent one. Moreover, PFT-α inhibits the NO production in response to poly I:C, which utilizes TRIF, TRAF3 and IRF3 in the NO production pathway, like the MyD88-independent pathway. Collectively, PFT-α is strongly suggested to impair the MyD88-independent pathway but not MyD88-dependent pathway and attenuate LPS-mediated inflammatory response.