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**Title:** β-arrestin 2 attenuates lipopolysaccharide-induced liver injury via inhibition of TLR4/NF-κB signaling pathway mediated inflammation in mice

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Lipopolysaccaride induced hepatic injury is the pathological basis of varied hepatic diseases, and kupffer cells are the key components in LPS-induced injury. β-arrestin 2 is an important protein that plays an important role in regulating TLR4/NF-κB signaling pathway. We propose that β-arrestin 2 should have great effects on lipopolysaccharide-induced inflammation and hepatic injury via TLR4-related signal pathway. To clarify that, we investigated the role and the possible mechanisms of β-arrestin 2 in LPS-induced hepatic injury in this study as the following.

Firstly, it was proved that deletion of β-arrestin 2 aggravated LPS-induced liver injury and that deletion of β-arrestin 2 facilitated the expression of inflammatory factors induced by LPS in vivo. We established animal model of LPS-induced liver injury by intraperitoneal injection of LPS (5mg/kg) using β-arrestin 2 WT and β-arrestin 2 KO mice. At 4 hours after administration of LPS, the mice were sacrificed, liver tissues and blood was collected for histopathology score of liver injury and detection of ALT, AST in serum via HE stain, IHC, Tunnel, RT-PCR and ELISA. The results suggested that decreasing of β-arrestin 2 aggravated LPS-induced liver injury and increased pro-inflammatory cytokines in both live tissue and serum.

Secondly, it was proved that decreasing level of β-arrestin 2 promoted production of pro-inflammatory factors from RAW264.7 in vitro. We investigated whether a genetic reduction of β-arrestin 2 in RAW264.7 via RNA interference could increase production of pro-inflammatory factors. Six hours after transfection of β-arrestin 2 siRNA, expression of β-arrestin 2 was significantly downregulated. Meanwhile, another 6 hours after treatment with LPS, RAW264.7 cells treated with β-arrestin 2 siRNA increased production of IL-1β，IL-6，TNF-α and IL-10 which were determined by RT-PCR significantly.

Lastly, we identified the mechanism that decreasing of β-arrestin 2 promoted production of pro-inflammatory factors. We detected expression of key molecules in TLR4/NF-κB signal pathway by Western Blot in this study. The results showed that the key molecules including TRAF6、IKKβ、phospho-IкBα and phospho-p65 produced by RAW264.7 cells increased obviously after treated with LPS for 6 hours, which suggested LPS-induced liver injury was involved with activation of TLR4/NF-κB signaling pathway. Moreover, there were phospho-IкBα and phospho-p65 but not TRAF6、IKKβ presenting significantly increasing in the cells treated with β-arrestin 2 siRNA and LPS, which indicated decreasing of β-arrestin 2 also be involved in activation TLR4/NF-κB signaling pathway.

In conclusion, β-arrestin 2 attenuates lipopolysaccharide-induced liver injury via inhibition of TLR4/NF-κB signaling pathway mediated inflammation in mice. β-arrestin 2 may serve as a therapeutic target for prevention and treatment of lipopolysaccharide-induced liver injury.