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ORIGINAL ARTICLE

Basic Study

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

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Author contributions: Jiang MP, Xu C and Guo YW contributed equally to this work and performed most of the experiments; Luo QJ, Li L, Liu HL and Jiang J bred the animals and collected the animal material; Chen HX analyzed the data; and Wei XQ designed the study and wrote the paper.

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Abstract

AIM

To study the role and the possible mechanism of β-arrestin 2 in lipopolysaccharide (LPS)-induced liver injury *in vivo* and *in vitro*.

METHODS

Male β-arrestin $2^{+/+}$ and β-arrestin $2^{-/-}$ C57BL/6J mice were used for *in vivo* experiments, and the mouse macrophage cell line RAW264.7 was used for *in vitro* experiments. The animal model was established *via* intraperitoneal injection of LPS or physiological sodium chloride solution. Blood samples and liver tissues were collected to analyze liver injury and levels of pro-

inflammatory cytokines. Cultured cell extracts were collected to analyze the production of pro-inflammatory cytokines and expression of key molecules involved in the TLR4/NF-κB signaling pathway.

RESULTS

Compared with wild-type mice, the β-arrestin 2 knockout mice displayed more severe LPS-induced liver injury and significantly higher levels of pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and IL-10. Compared with the control group, pro-inflammatory cytokines (including IL-1β, IL-6, TNF-α, and IL-10) produced by RAW264.7 cells in the β-arrestin 2 siRNA group were significantly increased at 6 h after treatment with LPS. Further, key molecules involved in the TLR4/NF-κB signaling pathway, including phospho-IκBα and phosho-p65, were upregulated.

CONCLUSION

β-arrestin 2 can protect liver tissue from LPS-induced injury *via* inhibition of TLR4/NF-κB signaling pathway-mediated inflammation.

Key words: Lipopolysaccharide; Liver injury; β-arrestin 2; TLR4/NF-κB signaling pathway; Pro-inflammatory cytokines

Core tip: The role and mechanism of β-arrestin 2 in lipopolysaccharide (LPS)-induced liver injury remain unclear. In this study, β-arrestin 2 knockout mice displayed more severe LPS-induced liver injury and significantly higher levels of pro-inflammatory cytokines than wild-type mice. Further, RAW264.7 cells treated with β-arrestin 2 siRNA expressed significantly higher pro-inflammatory cytokines and molecules involved in the TLR4/NF-κB signaling pathway (including phospho-IκBα and phosho-p65) than the control group at 6 h after treatment with LPS. Therefore, β-arrestin 2 could protect liver tissue from LPS-induced injury *via* inhibition of TLR4/NF-κB-mediated inflammation and may serve as a therapeutic target.

Jiang MP, Xu C, Guo YW, Luo QJ, Li L, Liu HL, Jiang J, Chen HX, Wei XQ. β-arrestin 2 attenuates lipopolysaccharide-induced liver injury *via* inhibition of TLR4/NF-κB signaling pathway-mediated inflammation in mice. *World J Gastroenterol* 2018; 24(2): 216-225 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i2/216.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i2.216

INTRODUCTION

Lipopolysaccharide (LPS, also called endotoxin)-induced hepatic injury is the pathological basis of varied hepatic diseases, and Kupffer cells are the key components in LPS-induced injury[1]. Researchers found that endogenous LPS derived from the intestine could promote the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β by activating Kupffer cells and accelerate the damage to the liver[2]. Meanwhile, LPS could also aggravate liver damage in nonalcoholic steatohepatitis by increasing the production of TNF-α by Kupffer cells[3]. Additionally, studies of a genetic mouse model of obesity suggested that the mice were more prone to steatohepatitis if they were constantly exposed to LPS[4,5]. When Kupffer cells were eliminated from this model, the mice showed decreased hepatic injury and lower mortality following LPS treatment[6]. LPS and Kupffer cells are the two essential key points in the development of varied hepatic diseases.

β-arrestin 2 is an important protein that plays a well-established role in regulating signaling downstream of the G-protein-coupled receptor (GPCR) pathway. Its recruitment and binding to the ligand-stimulated receptor are essential for signal transduction, sequestration, desensitization, and cell proliferation and differentiation. Recently, accumulating evidence has shown that β-arrestin 2 is a key regulator of not only GPCR-related signaling pathways but also of pathways downstream of major cell surface receptors and receptor tyrosine kinases, including insulin receptor, insulin-like growth factor type 1 receptor, epidermal growth factor receptor, and Toll-like receptor 4 (TLR4). Of these, the TLR4-related signaling pathway has gained much attention for its role in LPS-induced inflammation and host defense. Current views postulate that stimulation with LPS enhances production of pro-inflammatory cytokines from macrophages *via* the TLR4/NF-κB signaling pathway, while silencing of TLR4 or β-arrestin 2 can both inhibit this increase in pro-inflammatory cytokines and negatively regulate TLR4-mediated inflammatory reactions[7]. Based on these findings, we hypothesized that β-arrestin 2 should have great effects on LPS-induced inflammation and hepatic injury *via* a TLR4-related signaling pathway. To explore this hypothesis, we investigated the role and the possible mechanisms of β-arrestin 2 in LPS-induced hepatic injury. We found that deletion of β-arrestin 2 in mice aggravated LPS-induced liver injury by increasing macrophage production of pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, and IL-10. Further, this mechanism might be involved in TLR4/NF-κB-mediated inflammation.

MATERIALS AND METHODS

Reagents and chemicals

LPS (Cat. L2630), Trizol reagent (Cat. T9424), and rat tail collagen (Cat. L2630) were purchased from Sigma (St Louis, MO, United States). Dulbecco's modified Eagle's medium (DMEM, Cat. C11995500B), Roswell Park Memorial Institute 1640 Medium (RPMI-1640, Cat. C11875500BT), fetal bovine serum (Cat. 10270-106), penicillin and streptomycin (Cat.

15140122), and Trypsin (Cat. 25200-056) were obtained from Gibco (Rockville, MD, United States). Real-time PCR Master Mix kit-SYBR Green (Cat. AQ141-04) was from Transgen (Beijing, China). siRNA-β-arrestin 2 (Cat. sc-29208), anti-glyceraldehyde-3-phosphate dehydrogenase antibody (GAPDH, Cat. sc-25778), horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG secondary antibody (Cat. sc-2004), anti-p65 (Cat. sc-372), anti-phospho-IkBα (Cat. sc-8404), anti-IkBα (Cat. sc-371), and anti-TRAF6 (Cat. sc-7221) antibodies were all purchased from Santa Cruz (Santa Cruz, CA, United States). Anti-myeloperoxidase (MPO) primary antibody (Cat. ab9535), anti-β-arrestin 2 (Cat. ab54790), anti-phospho-p65 (Cat. ab86299), and anti-phospho-Akt (Cat. ab38449) antibodies were from Abcam (Abcam, Cambridge, MA, United States). Lipofectamine 3000 (Cat. L3000-015) was from Invitrogen (Invitrogen, Carlsbad, CA, United States). Heparin was from Wanbang (Xuzhou, Jiangsu, China). First Strand cDNA Synthesis Kit ReverTra Ace-α-TM (Cat. FSK-100) was from Toyobo (New York, NY, United States). Alanine transaminase (ALT, Cat. CSB-E16539m), aspartate transaminase (AST, Cat. CSB-E12649m), and TNF-α (Cat. CSB-E04741m) enzyme-linked immunosorbent assay (ELISA) kits were all from Cusabio (Wuhan, Hubei, China).

Animal model and treatments

All animal experiments were approved by the Institutional Animal Care and Use Committee of The Third Affiliated Hospital of Sun Yat-sen University (certification no.: IACUC-F3-17-0801). The original β-arrestin $2^{+/-}$ heterozygous C57BL/6J mice were a gift from Dr. Robert J Lefkowitz (Duke University Medical Center, Durham, NC). Male β-arrestin $2^{-/-}$ and β-arrestin $2^{+/+}$ mice aged 6 to 8 wk and weighing 20-25 g were randomly divided into four groups with six mice in each group. To establish an LPS-induced liver injury model, the mice were intraperitoneally injected with LPS (5 mg/kg) or physiological solution of sodium chloride. Four hours later, the mice were killed by intraperitoneal injection of 10% chloral hydrate (350 mg/kg). A 0.5 mL blood sample was collected from the inferior vena cava. Serum was stored at -20 ℃ before testing. The liver was carefully isolated from each mouse. Part of the liver tissue was immediately fixed in 10% neutral buffered formalin before embedding to prepare paraffin sections and the other part was stored at -80 ℃ for further analysis.

Cell culture and treatment

The mouse macrophage cell line RAW264.7 was obtained from American Type Culture Collection. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) at 37 ℃ in a 5% CO_2-humidified incubator. Cells at 70% confluence were collected and seeded at 2 × 10^5 cells per well in a six-well plate for further experimentation. Transfections of cells with β-arrestin 2 siRNA RNAoligo and the control RNAoligo were performed with Lipofectamine 3000 according to the manufacturer's instructions. At 24 h after transfection, the medium was replaced with regular culture medium and cells were then treated with LPS (1000 ng/mL). At 6 h after administration of LPS, the cells were collected for further experiments.

Histopathology score of liver injury

Hematoxylin and eosin (HE) staining and TUNEL staining were performed to generate a histopathology score of liver injury. H&E staining was performed as described in our previous study[8] and TUNEL staining was performed using an in-situ cell death detection kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Briefly, sections were scored in a blinded manner for apoptosis and hemorrhage in five 200 × magnified fields according to Hoque's report[9]. Apoptosis was scored from 0-4 according to the rate of hepatocyte apoptosis (0: ⩽ 1%; 1: 1%-5%; 2: 5%-10%; 3: 10%-20%; and 4: ⩾ 20%) per 200 × field. Hemorrhage was also scored as 0-4 based on the hemorrhage rate (0: 0%; 1: 1%-5%; 2: 5%-20%; 3: 20%-50%; and 4: ⩾50%) per 200 × field.

Immunohistochemistry

To evaluate LPS-induced hepatic injury, immunohistochemistry was used to detect the expression of MPO in neutrophils, and the number of MPO positive cells was counted in 20 randomly selected 200 × magnified fields of each section. Immunohistochemistry was performed as previously described[10], and MPO was detected using the anti-MPO primary antibody and horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG secondary antibody. The liver tissues of six mice from each group were studied.

Quantitative real-time RT-PCR

Total RNA was collected using Trizol reagent according to the manufacturer's instructions. Then, 2 μg of RNA was used for reverse transcription to produce first-strand cDNA with the First Strand cDNA Synthesis Kit ReverTra Ace-α-TM according to the manufacturer's instructions. Real-time PCR was performed for genes of interest on an ABI7700 System (Applied Biosystems, Foster City, CA) using DyNAmo SYBR Green Master Mix. β-actin was used as an internal reference to normalize the genes of interest. The melting curve for each gene was analyzed to ensure the specificity of amplification. The genes of interest and the primers are as follows: *TNF-α*: forward, 5′-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3′ and reverse, 5′- GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3′; *IL-1β*: forward, 5′-ATGGCAACTGTTCCTGAACTCAACT-3′ and reverse, 5′-CAGGACAGGTATAGATTCTTTCCTTT-3′; *IL-6*: forward, 5′-AGGATACCACTCCCAACAGACCT-3′ and reverse, 5′-CAAGTGCATCATCGTTGTTCATAC-3′; *IL-10*: forward, 5′-GCTCTTACTGACTGGCATGAG-3′ and

reverse, 5′-CGCAGCTCTAGGAGCATGTG-3′; *β-actin*: forward, 5′-GGCTGTATTCCCCTCCATCG-3′ and reverse, 5′-CCAGTTGGTAACAATGCCATGT-3′.

Western blot analysis

Western blot analysis was used to test for the expression of proteins of interest in cultured cells or liver tissues and was performed as previously described[11]. Briefly, equal amounts of protein were separated by electrophoresis and then transferred to polyvinylidene difluoride membranes. After blocking for 1 h at room temperature, the membrane was incubated with primary antibodies against β-arrestin 2, TRAF6, IKKβ, IκBα, phospho-IκBα, p65, phosho-p65, or GAPDH at 4 ℃ overnight. After washing, the membrane was then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. The bands were visualized using an enhanced chemiluminescence system. Image-Pro Plus 6.0 software (Media Cybernetics, 8484 Georgia Avenue Silver Spring, MD, United States) was used for densitometry analyses.

ELISA

The levels of ALT, AST, LDH, and TNF-α in the culture supernatants and mouse serum were determined with the ELISA kits according to the manufacturer's instruction.

Statistical analysis

SPSS version 13.0 (SPSS Inc., Chicago, IL, United States) was used for statistical analyses. Data are expressed as the mean ± SD and differences between groups were assessed by Student's *t*-test or one-way analysis of variance, followed by Bonferroni's post hoc tests. A two-sided *P*-value < 0.05 was considered significant.

RESULTS

Deletion of β-arrestin 2 aggravates lipopolysaccharide-induced liver injury in vivo

To investigate the role of β-arrestin 2 in LPS-induced liver injury, we first established an animal model of LPS-induced liver injury by intraperitoneal injection of LPS (5 mg/kg) into β-arrestin 2 wild type (WT) and β-arrestin 2 knockout (KO) mice. Four hours after administration of LPS, the mice were sacrificed and liver tissues and blood were collected for histopathological scoring of liver injury and detection of ALT and AST in serum. As shown in Figure 1, we observed a significant difference in AST and ALT levels (Figure 1A and B) between β-arrestin 2 WT and β-arrestin 2 KO mice treated with LPS, whereas no significant difference was observed in the mice treated with physiological solution of sodium chloride. Similarly, histopathological scores of liver injury, including hemorrhage score (Figure 1D) and apoptosis score (Figure 1F), were significantly higher in β-arrestin 2 KO mice than in β-arrestin 2 WT mice after administration of LPS. Moreover, the MPO index (Figure 1G and H) presented similar results to the AST, ALT, and histopathology scores. These results suggested that decreased β-arrestin 2 aggravated LPS-induced liver injury.

Deletion of β-arrestin 2 facilitates the expression of lipopolysaccharide-induced inflammatory factors

As mentioned in the Introduction section, LPS-induced liver injury involves an increase in pro-inflammatory cytokines *via* activation of Kupffer cells. We therefore evaluated mRNA levels of *IL-1β*, *IL-6*, *TNF-α*, and *IL-10* in liver tissue and serum. We discovered that mRNA levels of *IL-1β*, *IL-6*, *TNF-α*, and *IL-10* were noticeably increased after treatment with LPS. The mRNA levels of the four pro-inflammatory cytokines mentioned above were significantly higher in liver tissue from β-arrestin 2 KO mice as compared with liver tissue from the β-arrestin 2 WT mice (Figure 2 A-D). Meanwhile, protein analysis of IL-6 and TNF-α in serum was consistent with the results in liver tissue (Figure 2E and F). These results indicated that increased pro-inflammatory cytokines in both liver tissue and serum might be associated with decreased β-arrestin 2.

Decreasing levels of β-arrestin 2 promote the production of pro-inflammatory factors in RAW264.7 cells in vitro

Based on the above results, we supposed that decreasing β-arrestin 2 might promote the production of pro-inflammatory factors *via* macrophage activation. To confirm this, we investigated whether a genetic reduction of β-arrestin 2 in RAW264.7 cells could increase the production of pro-inflammatory factors. As shown in Figure 3A and B, 6 h after transfection with β-arrestin 2 siRNA, expression of β-arrestin 2 was significantly down-regulated. Meanwhile, at another 6 h after treatment with LPS, RAW264.7 cells treated with β-arrestin 2 siRNA showed significantly increased production of IL-1β, IL-6, TNF-α, and IL-10 (Figure 3C-F). These results revealed that decreasing β-arrestin 2 in RAW264.7 cells promoted the *in vitro* production of pro-inflammatory factors.

Increased pro-inflammatory factors are involved in activation of the TLR4/NF-κB signaling pathway

The TLR4/NF-κB signaling pathway is considered to be involved in LPS-induced liver injury, and silencing of β-arrestin 2 can negatively regulate TLR4-mediated inflammatory reactions. To identify the mechanism whereby decreased β-arrestin 2 promotes production of pro-inflammatory factors, we then detected the expression of key molecules in the TLR4/NF-κB signaling pathway. The results showed that key molecules, including TRAF6, IKKβ, phospho-IκBα, and phospho-p65, produced by RAW264.7 cells increased noticeably after treatment with LPS for 6 h (Figure

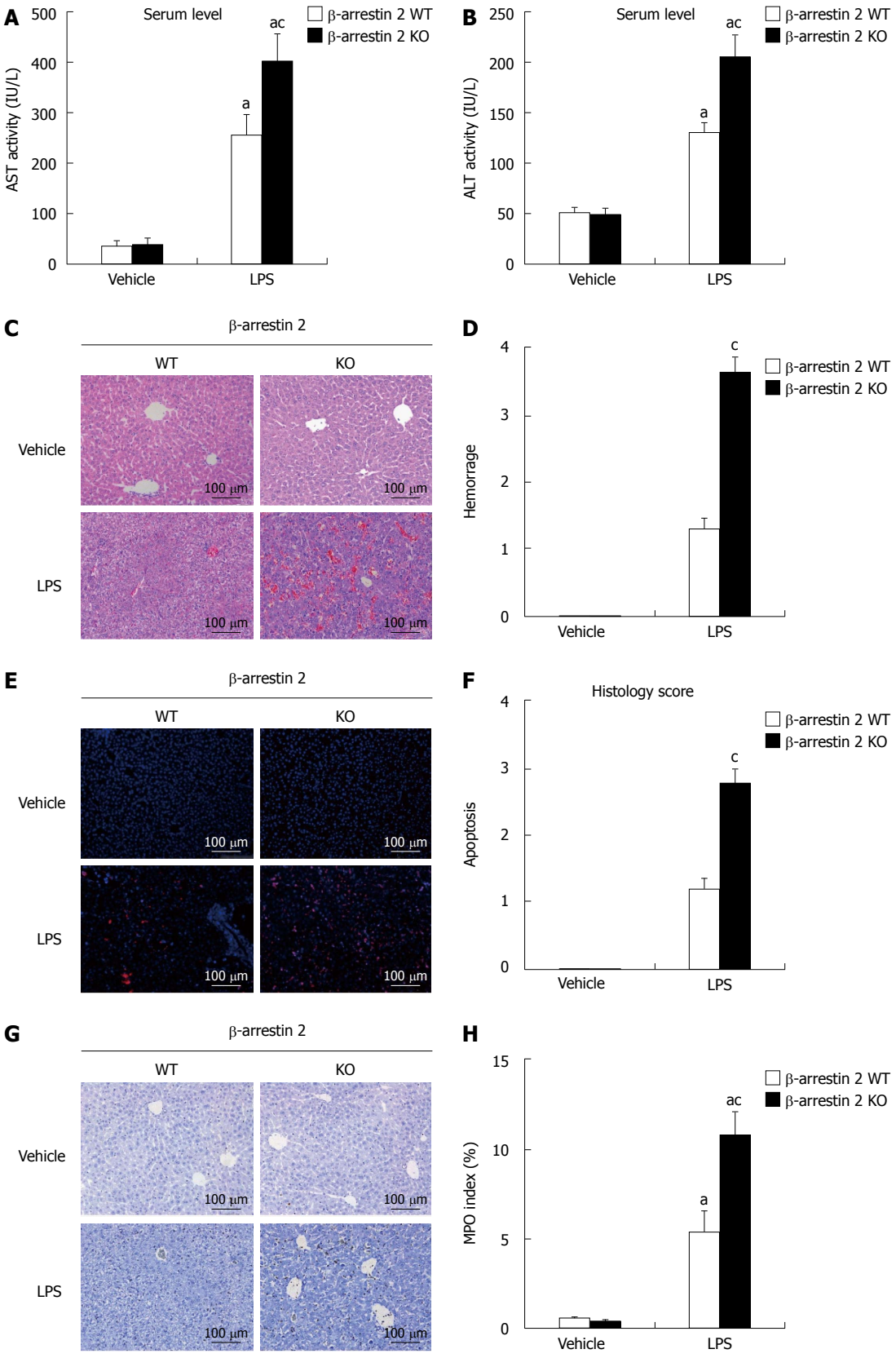

Figure 1 Evaluation of lipopolysaccharide-induced liver injury *in vivo* . A and B: Serum levels of ALT and AST were detected using ELISA kits in β-arrestin 2 WT and β-arrestin 2 KO mice treated with LPS and vehicle; C: HE staining of liver tissue (magnification, × 200); D: Histology score of hemorrhage in β-arrestin 2 WT and KO mice treated with LPS and vehicle; E: TUNEL staining of liver tissue (magnification, × 200); F: Histology score of apoptosis in β-arrestin 2 WT and KO mice treated with LPS and vehicle; G: Immunohistochemical staining for MPO expression in liver tissues from β-arrestin 2 WT and β-arrestin 2 KO mice treated with LPS and vehicle, (magnification, × 200); H: MPO index in β-arrestin 2 WT and β-arrestin 2 KO mice treated with LPS and vehicle. $^{a}P < 0.05$ *vs* vehicle control group; $^{c}P < 0.05$ *vs* β-arrestin 2 WT mice. LPS: Lipopolysaccharide.

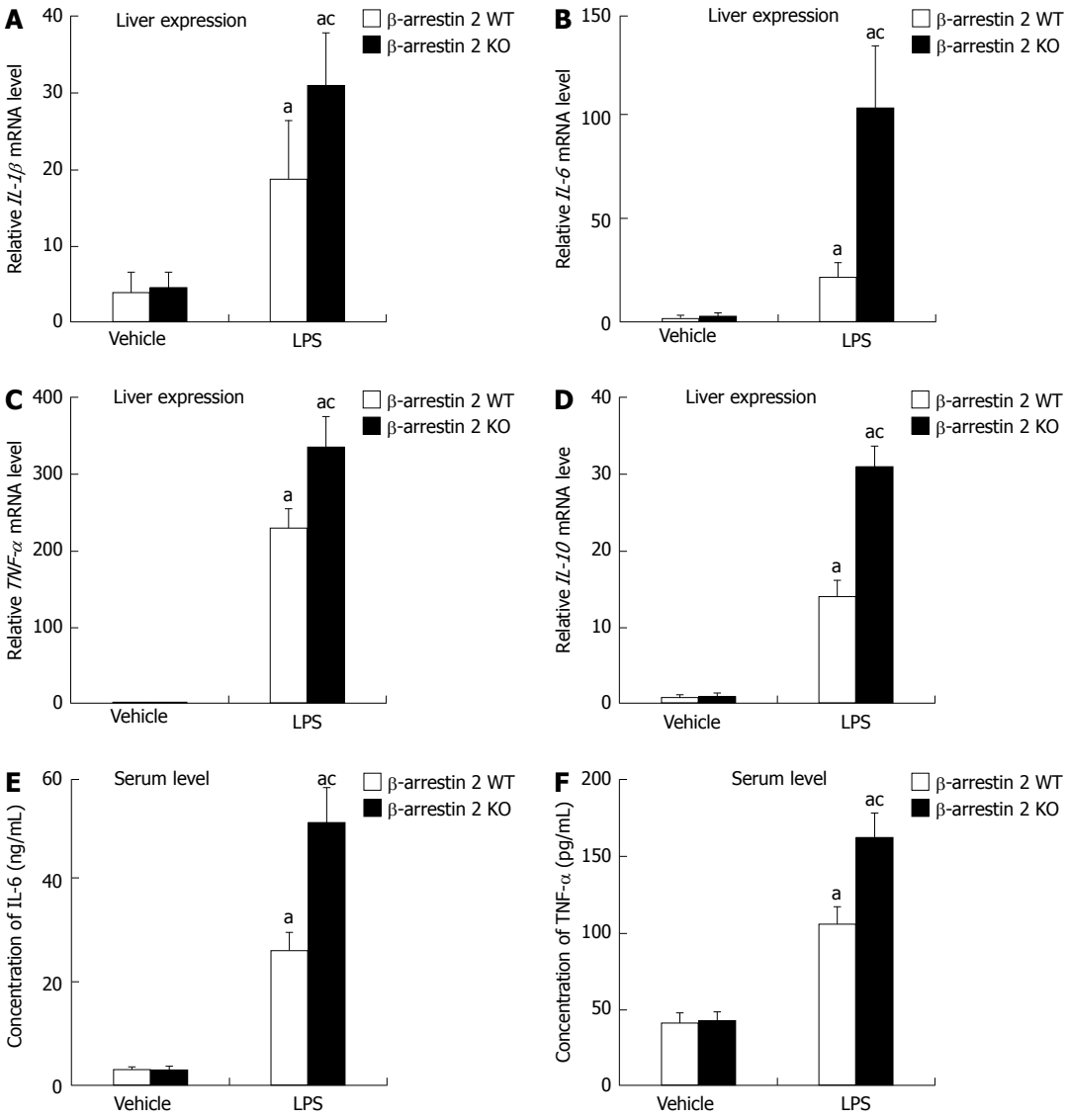

Figure 2 Expression of inflammatory factors induced by lipopolysaccharide *in vivo*. qRT-PCR was used to determine relative mRNA levels of IL-1β (A), IL-6 (B), TNF-α (C), and IL-10 (D) in liver tissues; E and F: ELISA was used to determine levels of IL-6 and TNF-α in serum. $^{a}P < 0.05$ *vs* control group; $^{c}P < 0.05$ *vs* β-arrestin 2 WT mice.

4A), suggesting that LPS-induced liver injury is related to activation of the TLR4/NF-κB signaling pathway. Moreover, phospho-IκBα and phospho-p65 (but not TRAF6 or IKKβ) were significantly increased in the cells treated with β-arrestin 2 siRNA and LPS (Figure 4B), indicating that decreased β-arrestin 2 might also be involved in activation of the TLR4/NF-κB signaling pathway, therefore promoting the production of pro-inflammatory factors in RAW264.7 cells.

DISCUSSION

LPS-induced hepatic injury is the pathological basis of varied hepatic diseases. However, the injury is considered indirect and induced by the production of pro-inflammatory cytokines from activated Kupffer cells[12]. In this current study, we found that serum levels of AST and ALT were visibly high in both β-arrestin 2 KO and WT mice treated with LPS for 4 h (Figure 1A and B). Meanwhile, the histopathology scores and count of MPO positive cells were also higher in the LPS group compared with the control group. These results showed obvious liver damage not only in β-arrestin 2 KO mice but also in WT mice, which suggested that our animal model was successful. In addition, mRNA levels of *IL-1β*, *IL-6*, *TNF-α*, and *IL-10* in liver tissue and serum levels of IL-6 and TNF-α were significantly higher after treatment with LPS, which also indicated that LPS-induced liver injury was mediated by pro-inflammatory cytokines. All these results were consistent with previous reports by Wang *et al*[13] and Seregin *et al*[14].

β-arrestin 2 is an important negative regulator of the TLR4 signaling pathway and could protect mice from TLR4-mediated endotoxic shock and lethality *via* down-regulation of inflammatory cytokines[13]. However, it is unclear whether β-arrestin 2 could attenuate LPS-induced liver injury *via* regulation of

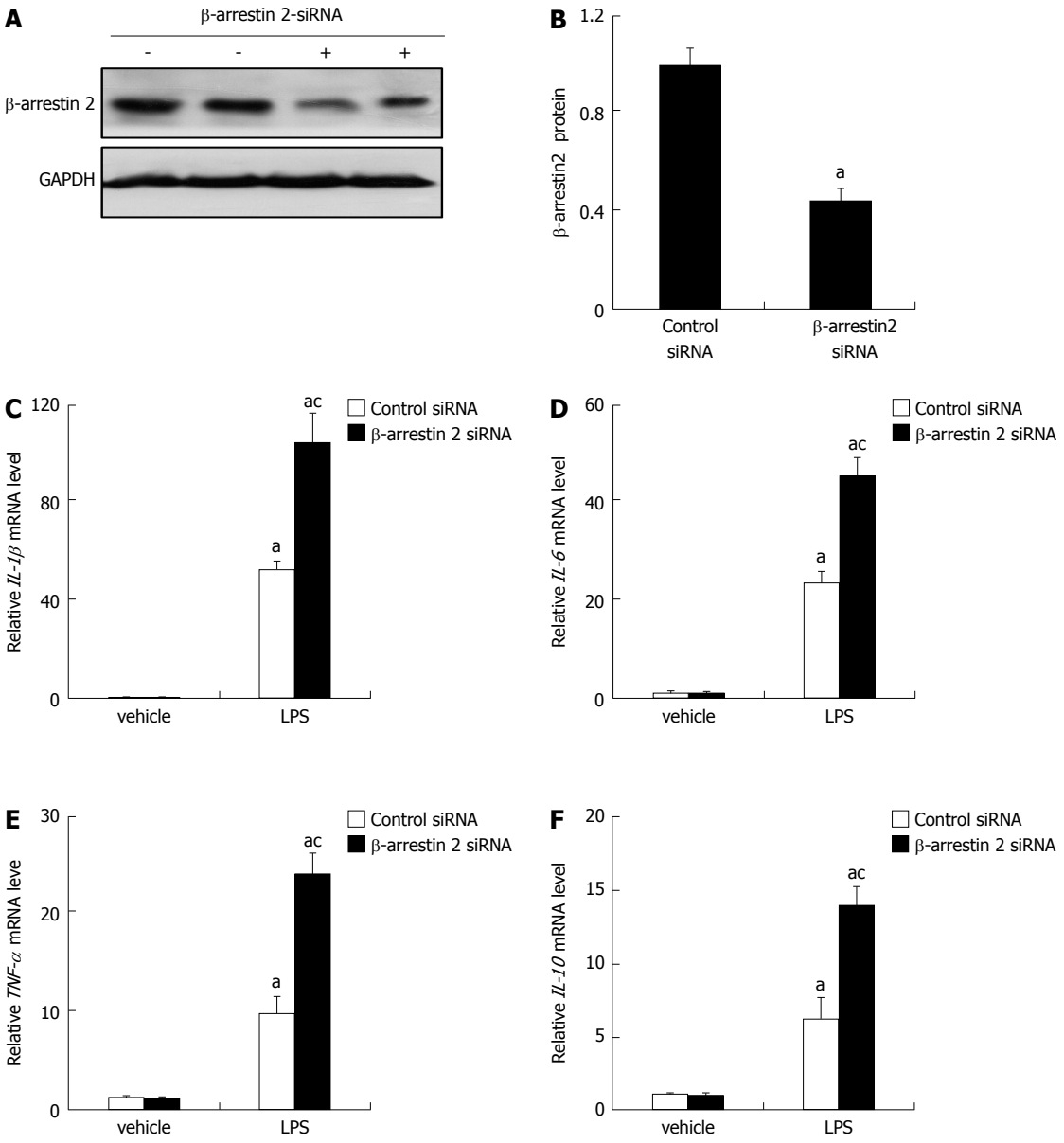

Figure 3 Expression of inflammatory factors by RAW264.7 cells *in vitro* . A: Expression of β-arrestin 2 in RAW264.7 cells was detected by Western blot. Levels of GAPDH are shown as a loading control; B: Relative quantitative evaluation of the Western blot analysis for β-arrestin 2 expression with ImageJ software, C-F: qRT-PCR was used to determine relative mRNA levels of IL-1β (C), IL-6 (D), TNF-α (E), and IL-10 (F) produced by RAW264.7 cells. $^{a}P < 0.05$ *vs* vehicle group; $^{c}P < 0.05$ *vs* control siRNA group.

pro-inflammatory cytokines. Porter *et al*[7] found that deletion of β-arrestin 2 in mice could decrease serum levels of IL-1β, IL-12p40, interferon-γ, IL-2, IL-3, IL-4, and IL-5. Thus, they hypothesized that β-arrestin 2 could reduce LPS-induced inflammation. Conversely, Li *et al*[15] found in another study that overexpression (but not deletion) of β-arrestin 2 could reduce the production of pro-inflammatory cytokines such as TNF-α and reduce experimental arthritis severity. Our results showed that ALT and AST levels and histopathology scores were higher in β-arrestin 2 KO mice than in β-arrestin 2 WT mice, which suggested that decreased β-arrestin 2 can aggravate LPS-induced liver damage. Moreover, the pro-inflammatory cytokines in both liver tissues and serum were higher in β-arrestin 2 KO mice, which revealed that decreased β-arrestin 2 might promote production of pro-inflammatory cytokines. We then investigated the correlation between decreased β-arrestin 2 and production of pro-inflammatory cytokines *in vitro* using siRNA interference technology. As we show in Figure 3, pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, and IL-10 were higher in the cells treated with β-arrestin 2 siRNA and LPS, which revealed that β-arrestin 2 could attenuate LPS-induced liver injury *via* negative regulation of pro-inflammatory cytokines. Other than negative regulation of pro-inflammatory cytokines, Fong *et al*[16] found that β-arrestin 2 might modulate the CXCR4-induced chemotactic migration of lymphocytes. Moreover, Basher *et al*[17] and Fan *et al*[18] found that β-arrestin 2 inhibited chemotactic migration of neutrophils. In our study, the number of MPO

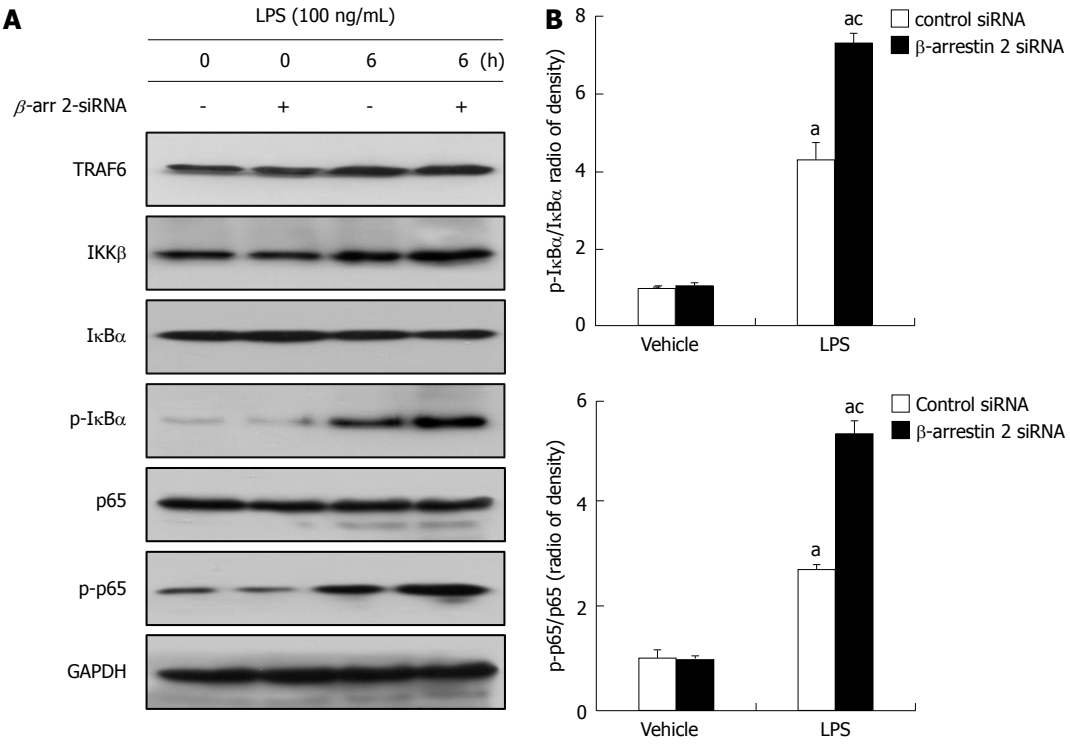

Figure 4 Expression of key molecules involved in the TLR4/NF-κB signaling pathway *in vitro*. A: Western blot analysis of the expression of key molecules involved in the TLR4/NF-κB signaling pathway. The levels of GAPDH are shown as a loading control; B: Relative gray value quantitative evaluation for p-IκBα/IκBα and p-p65/p65. $^{a}P < 0.05$ *vs* vehicle control; $^{c}P < 0.05$ *vs* control siRNA group.

positive cells was larger in liver tissues from β-arrestin 2 KO mice (Figure 1G and H), which indicated that decreased β-arrestin 2 reduce neutrophil infiltration in liver tissue. The results further revealed that inhibition of the chemotactic migration of neutrophils might be another mechanism by which β-arrestin 2 attenuates LPS-induced liver injury.

Kupffer cells are macrophages located in liver tissue, which have been identified as the major cells that produce pro-inflammatory cytokines and play a critical role in LPS-induced inflammation[19-22]. RAW264.7 macrophages are derived from leukemic mice and have similar characteristics to Kupffer cells in liver tissue. Therefore, we used RAW264.7 cells for our *in vitro* studies. Our results showed that pro-inflammatory cytokines from RAW264.7 cells obviously increased at 6 h after treatment with LPS, which revealed that RAW264.7 could act as Kupffer cells when stimulated by LPS. It is well known that β-arrestin 2 is involved in various cell signaling pathways and plays a key role in the regulation of cell signaling[23]. For example, β-arrestin 2 acts as a scaffold protein and participates in the activation of JNK and ERK in the MAPK pathway[24,25]. Further, β-arrestin 2 can directly bind to TRAF6 and reduce the phosphorylation of NF-κB through inhibition of TRAF6 oligomerization and ubiquitination, further inhibiting activation of the NF-κB pathway[13,26]. A recent study showed that β-arrestin 2 could down-regulate TLR4-mediated production of NLRP3 and IL1-1β, which are involved in inflammation induced by various factors[9]. In our study, we investigated the key molecules involved in the TLR4/NF-κB signaling pathway and found that key molecules, including TRAF6, IKKβ, phospho-IκBα, and phospho-p65, produced by RAW264.7 cells increased noticeably after treatment with LPS (Figure 4). Moreover, siRNA-mediated knockdown of β-arrestin 2 further increased levels of the key molecules mentioned above, including phospho-IκBα and phospho-p65 (Figure 4). This suggested that reductions in β-arrestin 2-induced pro-inflammatory cytokines might be associated with inhibition of the TLR4/NF-κB signaling pathway.

Altogether, our results showed that deletion of β-arrestin 2 in mice aggravated LPS-induced liver injury *via* increasing macrophage production of pro-inflammatory cytokines including IL-1β, IL-6, TNF-α, and IL-10. This mechanism might be involved in TLR4/NF-κB-mediated inflammation. We therefore concluded that β-arrestin 2 could protect liver tissue from LPS-induced injury *via* inhibition of TLR4/NF-κB-mediated inflammation. However, further study of the exact role and possible mechanism of β-arrestin 2 is needed.

ARTICLE HIGHLIGHTS

Research background

Lipopolysaccharide (LPS)-induced liver injury serves as the pathological basis of varied hepatic diseases. LPS does not directly harm hepatocytes, while Kupffer cells serve as the key components of LPS-induced injury through secretion of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. β-arrestin 2 is a protein that plays an important role in regulating the TLR4/NF-κB signaling pathway, which plays a critical role in inflammation. However, the

role of β-arrestin 2 in LPS-induced liver injury remains unclear.

Research motivation

The inhibition of LPS-induced inflammation *via* regulation of the TLR4/NF-κB signaling pathway may be a therapeutic method for modulating LPS-induced injury. β-arrestin 2 is a protein that plays an important role in regulating the TLR4/NF-κB signaling pathway. Therefore, we hypothesized that β-arrestin 2 can play a role in the prevention of LPS-induced liver injury.

Research objectives

The objective of this study was to investigate the role and the possible mechanism of β-arrestin 2 in LPS-induced liver injury *in vivo* and *in vitro*. This is the first study to show that β-arrestin 2 attenuated LPS-induced liver injury in a mouse model induced by injection of pure LPS. β-arrestin 2 may serve as a therapeutic target for the prevention and treatment of LPS-induced liver injury.

Research methods

The animal model was established *via* intraperitoneal injection of LPS or physiological sodium chloride solution in male β-arrestin $2^{+/+}$ and β-arrestin $2^{-/-}$ C57BL/6J mice. Blood samples and liver tissues were collected for analysis of liver injury and levels of pro-inflammatory cytokines. Extracts from the cultured mouse macrophage cell line RAW264.7 treated with various conditions were collected to analyze the production of pro-inflammatory cytokines and expression of key molecules involved in the TLR4/NF-κB signaling pathway.

Research results

The β-arrestin 2 knockout mice displayed more severe LPS-induced liver injury and significantly higher levels of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and IL-10, than the wild-type mice. Compared with the control group, pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and IL-10, produced by the β-arrestin 2 siRNA-treated RAW264.7 cells were significantly higher at 6 h after treatment with LPS. The key molecules involved in the TLR4/NF-κB signaling pathway were also increased, including phospho-IκBα and phosho-p65.

Research conclusions

We hypothesized that β-arrestin 2 could protect liver tissue from LPS-induced injury *via* inhibition of TLR4/NF-κB-mediated inflammation. This hypothesis was proven using an animal model of LPS-induced liver injury in male β-arrestin 2+/+ and β-arrestin 2-/- C57BL/6J mice and a cell model using the mouse macrophage cell line RAW264.7. These findings may be helpful for the prevention and treatment of LPS-induced liver injury in future clinical practice *via* strengthening the function of β-arrestin 2. However, further study on the exact role and possible mechanism is still needed.

Research perspectives

Studies of the role of β-arrestin 2 agonists and methods of up-regulation of β-arrestin 2 in the prevention and treatment of LPS-induced liver injury should be performed.

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