International Immunopharmacology 77 (2019) 105969

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Glucagon-like peptide-1 receptor agonist exendin-4 mitigates lipopolysaccharide-induced inflammatory responses in RAW264.7 macrophages

Canrong Lu[1], Tianyu Xie[1], Xin Guo, Di Wu, Shuo Li, Xiongguang Li, Yixun Lu, Xinxin Wang*

Department of General Surgery, Chinese People's Liberation Army General Hospital, Beijing, China

ARTICLE INFO

Keywords:
Inflammatory response
Macrophage
Exendin-4
JNK
NF-κB
AP-1
iNOS
PGE2

ABSTRACT

Macrophages play a critical role in the immune response against pathogen invasion and injury. However, under pathological stress, macrophages could have aberrant roles and contribute to the pathogenesis of inflammatory associated diseases. Exenatide is a glucagon-like peptide 1(GLP-1) agonist, which belongs to the family of synthetic exendin-based incretin mimetic. Exendin related compounds reduce glucose levels in type 2 diabetes patients. The purpose of this study was to examine the anti-inflammatory effects of exendin-4 in LPS-induced activation of macrophages. We show that exendin-4 inhibits LPS-induced expression of inflammatory mediators (iNOS, COX-2, PGE2 and NO) and pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in RAW264.7 macrophages. Exendin-4 pretreatment mitigates LPS induced cellular ROS production. Mechanistically, Exendin-4 suppresses the LPS-induced activation of the JNK and AP-1 pathway. Furthermore, exendin-4 suppresses both nuclear p65 accumulation and transfected NF-κB promoter activity, indicating it inhibits the activation of the NF-κB pathway. Our study demonstrates that the GLP-1 agonist exendin-4 has a potent anti-inflammatory effect independent on its glucose reducing ability, and exendin-4 has the potential implication to treat inflammatory associated diseases.

1. Introduction

An inflammatory response is a physiological response of the immune system against pathogen infection or injury to our body. In the immune system, different immune cells are constantly active to defend our body from the invasions or harmful stimuli. Macrophages engulf pathogen and scavenge the dead cells, which is termed as "phagocytosis". In healthy conditions, macrophages either directly clear up invasion or indirectly activate other immune cells against the alien antigen, and play a significant role in tissue repair and wound healing. The activated macrophages are involved in different defensive processes by generating many responsive mediators and inflammatory cytokines to influence the immune process. However, under pathological stress, abnormally activated macrophages could produce excessive inflammatory mediators, which could contribute to the development of related diseases, such as atherosclerosis, rheumatoid arthritis and sepsis [1].

Lipopolysaccharide (LPS) is a main component of the membrane from Gram-negative bacteria, and it is a strong stimulus to induce inflammatory responses in the case of bacterial infection. LPS causes the excessive release of pro-inflammatory mediators such as prostaglandin E (PGEs) and interleukins (ILs), which contributes to the inflammatory diseases [2]. PGEs are lipid autacoids derived from arachidonic acid, and the stable production of PGEs plays a vital role in whole-body homeostasis and the generation of inflammatory response. COX-2 is a key enzyme to catalyze the biosynthesis of prostaglandin, and it is highly inducible inflammatory stimuli [3]. Studies have shown that the induction of COX-2 is associated with inflammation and related diseases [4]. Many efforts have been focusing on an understanding of the inflammatory modulation in the anticipation to control the inflammatory response and disease progression. The most known regulator of the inflammation is NF-κB, and its activation contributes to many inflammatory diseases and cancer [5]. The activation of NF-κB in macrophage could generate many pro-inflammatory mediators, such as iNOS and COX-2 [6]. NF-κB directly binds to the promoter of these genes, is the key regulator of their induction [7]. Therefore, the

* Corresponding author at: Department of General surgery, Chinese People's Liberation Army General Hospital, 28, Fuxing Road, Haidian district, Beijing 100853, China.
E-mail address: summer774262@163.com (X. Wang).
[1] Co-first authors.

https://doi.org/10.1016/j.intimp.2019.105969
Received 5 July 2019; Received in revised form 2 October 2019; Accepted 7 October 2019
Available online 01 November 2019
1567-5769/

modulations of macrophage activation and inflammatory mediators have important therapeutic implications in the treatment of inflammatory diseases.

Glucagon-like peptide-1 (GLP-1) is an incretin that plays important physiological roles in glucose homeostasis. Produced from intestine upon food intake, GLP-1 stimulates the secretion of insulin and promotes pancreatic β-cells to be proliferative and functional. Exenatide is a glucagon-like peptide 1 (GLP-1) agonist belonging to the family of synthetic exendin-based incretin mimetics and was approved by the FDA for the treatment of type II diabetes mellitus since 2005 [8,9]. From its pathological progression, type II diabetes can be viewed as a chronic inflammatory disease [10]. One type of macrophage residing in adipose tissue is adipose tissue macrophages (ATMs). The abnormal adipose expansion and recruitment of ATMs are closely associated with the inflammation in obesity-induced related type 2 diabetes [11,12]. From past studies, we recognize that macrophages change their roles between the healthy condition and pathological stress. The investigation of modulating macrophage is significant for our understanding and treatment of the related diseases. Herein, we investigated the role of the GLP-1 agonist exendin-4 on LPS induced inflammatory responses in cultured macrophages.

2. Materials and methods

2.1. Cell culture and treatment

We purchased a murine macrophage RAW 264.7 cell line from ATCC (TIB-71™). The cells were cultured with ATCC-formulated Dulbecco's Modified Eagle's Medium (DMEM) supplied with 10% fetal bovine serum and 1X antibiotics containing 100 U/ml penicillin and 100 g/ml streptomycin. All the cells were housed at the atmosphere with 95% air and 5% carbon dioxide (CO_2) at a 37 °C incubator. The cell treatment reagent LPS was from Sigma-Aldrich (USA) and dissolved in PBS with 1000X concentration at 1 mg/ml. For the cell treatment experiment, RAW 264.7 cells were seeded on 6-well plate, 35-mm or 60-cm dishes to allow for growing into full confluence. The cells were then pretreated with 20 nM Exendin-4 for 6 h and followed by the treatment of 1 μg/mL LPS for 24 h, all the control groups were appropriately included.

2.2. Real-time PCR

We purified total RNA from treated RAW 264.7 cells by a high pure plasmid kit (Roche). Total 1 μg of RNA was used to synthesize cDNA by one-step RT-PCR kit (Superscript II, Applied Biosystems Inc.) The real-time PCR was performed on an ABI prism 7900HT sequence detection system. The PCR reaction was set up by using a universal SYBR green PCR master mix (Applied Biosystems Inc). We used β-actin as the internal control and calculated the relative gene expression level by ΔΔCt method.

2.3. Western blot analysis

We obtained whole cell lysate by lysing RAW 264.7 cells with RIPA buffer plus protease and phosphatase inhibitors. To isolate the nuclear portion of protein, we used a commercial NE-PER nuclear and cytoplasmic extraction kit (Thermo Fisher Scientific, USA) by following the product's instruction. We then measured the concentration of total cell lysate or nuclear extract using a BCA method (Biorad). A total of 20 μg of cell lysate or nuclear extract were loaded to precasted NU-PAGE gel to reach optimized separation. The gel was then transferred to 0.2 μM PVDF membrane by a gel transfer apparatus and followed by 1-hour blocking, 2-hour primary antibody incubation and another 1-hour incubation with corresponding fluorescent dye-labeled secondary antibody. Finally, we scanned the blots by Odyssey CLx Imager (Li-Cor). The data ware quantitated and presented as fold change.

2.4. ELISAa

We measured the secreted protein level of TNF-α, IL-1β, and IL-6 in our study. Briefly, we collected RAW 264.7 culture media and spun to remove cell debris, and measured the concentration of supernatants by BCA protein assay kit (Bio-Rad, USA). We purchased the following ELISA kits from R&D Systems: TNF-α, IL-1β, and IL-6. We added diluted standards and our samples to the 96-well plate and exactly followed other steps for ELISA assay according to the manufactory' instructions. The reaction data were read by a micro-plate spectrometry. The recorded values of standards were used to plot a linearized curve. The relative level of each experimental sample was extrapolated from the standard sample plot. The final sample value was presented by normalized to total protein amounts.

2.5. Intracellular NO production

We measured the intracellular NO production based on the DAF-FM staining method. In brief, RAW 264.7 growing in 96-well plates were washed and stained with DAF-FM DA dye for 10 min. We used a microplate fluorescence reader to capture the NO reacted green fluorescence at 488 nm wavelength.

2.6. Dihydroethidium (DHE) staining

We measured the cellular ROS level by dihydroethidium dye (DHE) staining method. In brief, RAW 264.7 cells were washed and reacted with 2 μM DHE dye for 10 min. The stained cells were then collected in black plates and visualized using a fluorescent microscope at an excitation wavelength at 480 nM.

2.7. Luciferase activity of AP-1 and NF-κB

We purchased luciferase incorporated NF-κB promoter and AP-1 promoter vectors from Clontech, USA. For plasmid transfection, RAW 264.7 cells were grown on 24-well plates for 40–50% confluence, and the vectors were transfected into cells by Lipofectamine LTX Reagent (Life technology). 24-hour post the transfection, the transfected cells were pretreated with 10 and 20 nM exendin-4 for 6 h and followed by the treatment of 1 μg/ml LPS for another 24 h. The cells were then quickly lysed and transferred to a 96 well plate to measure the luciferase activity by a Luminometer (Promega). The relative luciferase activity was obtained by normalizing the measured luciferase value to the sample protein concentration. We presented the data as fold change.

2.8. Statistical analysis

We repeated all the experiments at least three times or more in this study. The results were presented as the mean ± standard deviation. The comparison for more than two groups was tested by one-way analysis of variance (ANOVA) with Bonferroni post hoc test for multiple comparisons. $P < 0.05$ denotes statistically significant differences for the specific test.

3. Results

3.1. Exendin-4 suppresses LPS- induced expression of PGE_2 and COX-2

To explore the role of exendin-4 in RAW264.7 macrophages, we pretreated RAW 264.7 cells with 10 and 20 nM exendin-4 for 6 h and then treated the cells with 1 μg/mL LPS for 24 h. We measured the expression of PGE_2 mRNA (Fig. 1A), COX-2 mRNA (Fig. 1B) and COX-2 protein levels (Fig. 1C) to evaluate the effect of exendin-4 on these inflammatory mediators. Our results indicated that exendin-4 had a dose-dependently inhibitive effect on LPS-induced PGE_2 and COX-2 expression.

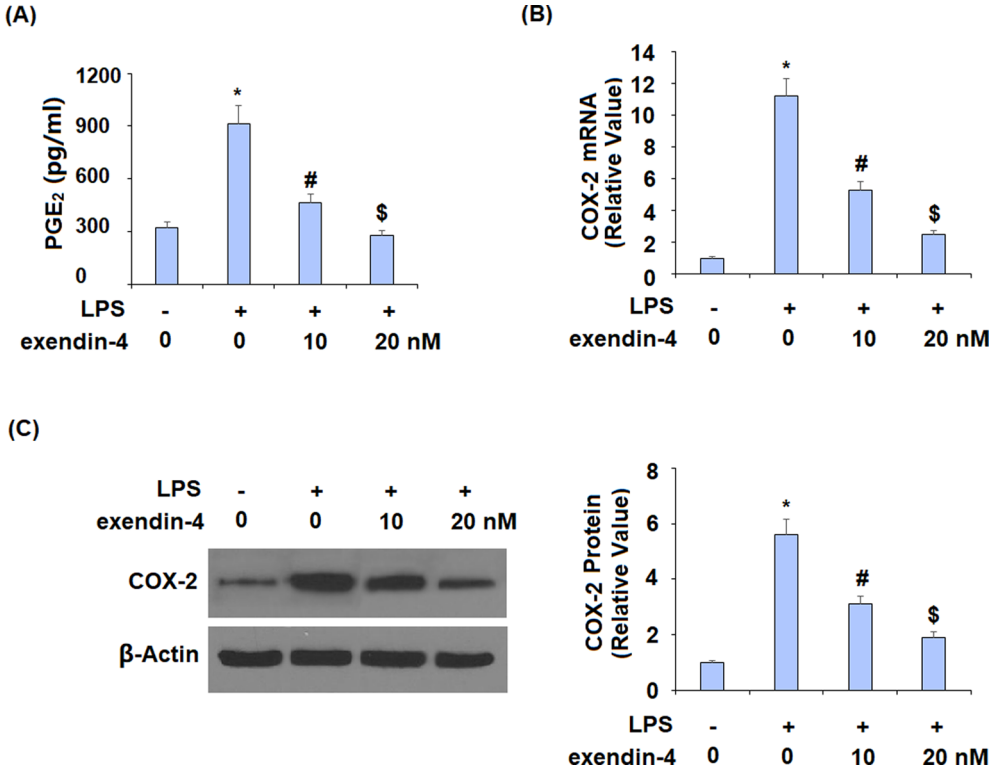

Fig. 1. Exenatide reduced Lipopolysaccharide (LPS)- induced production of prostaglandin E2 (PGE_2) and the expression of cyclooxygenase 2 (COX-2) in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 (a type of Exenatide) for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A). The secretion of PGE_2 was measured by ELISA analysis; (B). The expression of COX-2 at the mRNA levels; (C). The expression of COX-2 at the protein levels was determined by western blot analysis (*, #, $, $P < 0.01$).

3.2. Exendin-4 suppresses LPS induced NO and iNOS expression

In macrophages, the major synthesis source of nitric oxide (NO) is from inducible nitric oxide synthase (iNOS). Inflammation-induced NO can damage the normal cellular function and is involved in the development of varied diseases [13]. We measured the influence of exendin-4 on intracellular NO level and iNOS expression. The result showed that exendin-4 pretreatment significantly reduced the production of NO (Fig. 2A) and the expression of iNOS at the mRNA (Fig. 2B) and protein (Fig. 2C) levels.

3.3. Exendin-4 inhibits LPS-induced pro-inflammatory cytokines

It has been known that inflammatory cytokines play important roles in the progression of inflammatory diseases. Upon exposure to LPS (1 μg/mL) for 24 h, the secretion of pro-inflammatory increased conspicuously. But when RAW264.7 macrophages were treated with 10 and 20 nM exendin-4 for 6 h, it showed significant suppression on LPS-induced expression on TNF-α mRNA (Fig. 3A), IL-1β mRNA (Fig. 3B) and IL-6 mRNA (Fig. 3C). By ELISA assay, we got similar results that exendin-4 suppressed LPS induced secretion of TNF-α (Fig. 3D), IL-1β (Fig. 3E) and IL-6 (Fig. 3F).

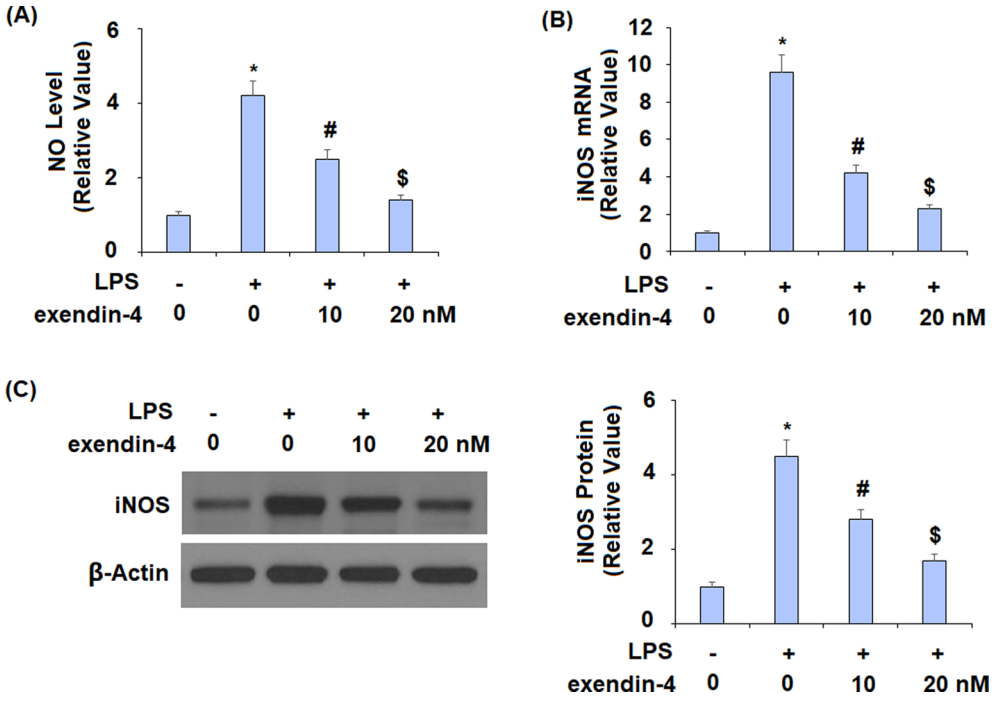

Fig. 2. Exenatide reduced Lipopolysaccharide (LPS)- induced production of nitric oxide (NO) and the expression of inducible nitric oxide synthase (iNOS) in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A). Production of NO was measured by DAF-FM staining; (B). mRNA levels of iNOS were determined by real-time PCR; (C). Protein levels of iNOS were determined by western blot analysis (*, #, $, $P < 0.01$).

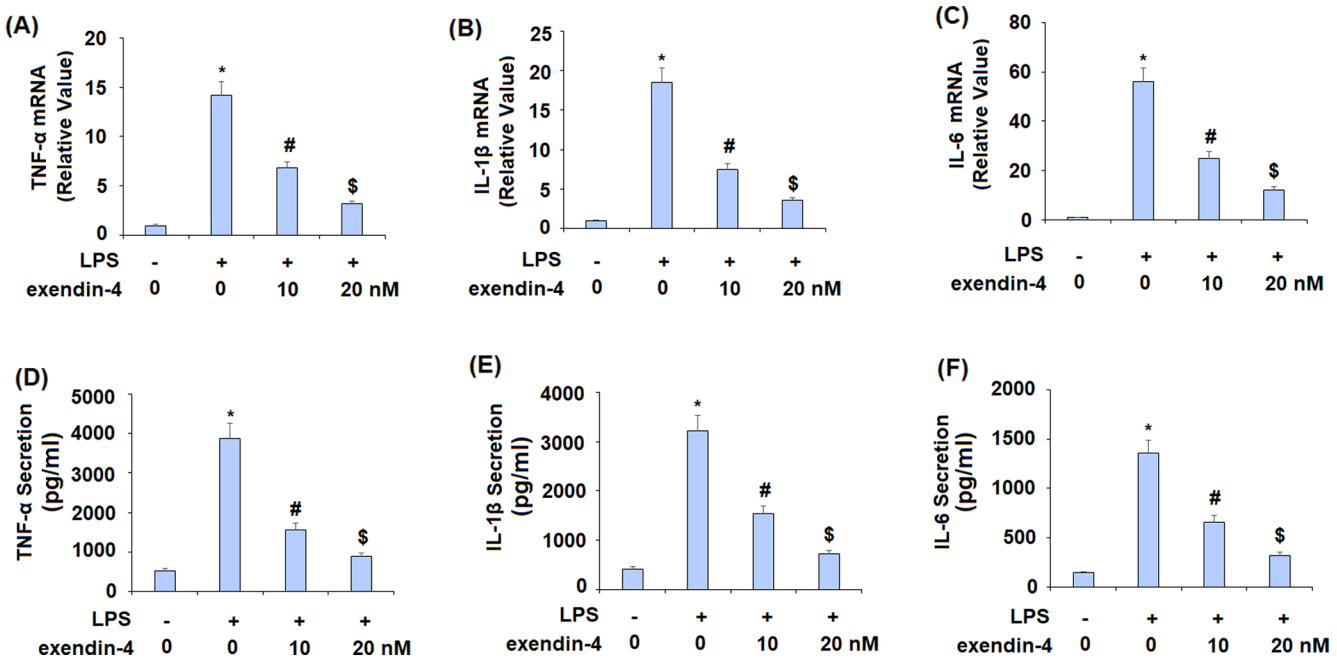

Fig. 3. Exenatide reduced Lipopolysaccharide (LPS)- induced expression and secretion of pro-inflammatory cytokines in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A) mRNA level of TNF-α; (B) mRNA levels of IL-1β; (C) IL-6; (D) Secretion of TNF-α as measured by ELISA; (E) Secretion of IL-1β was measured by ELISA; (F) Secretion of IL-6 was measured by ELISA (*, #, $, $P < 0.01$).

3.4. Exendin-4 inhibits LPS- induced expression of MMP-2 and MMP-9

Both MMP-2 and MMP-9 are recognized as the important collagenases in the degradation of the extracellular matrix in inflammatory diseases. Next, we assessed the expression profile of MMP-2 and MMP-9. The results showed that exendin-4 inhibited LPS-induced expression of MMP-2 (Fig. 4A) and MMP-9 mRNA (Fig. 4B). By examining their protein expression, we confirmed this inhibitory role of exendin-4 on MMP-2 (Fig. 4C) and MMP-9 (Fig. 4D).

3.5. Exendin-4 mitigates LPS induced oxidative stress

We then assessed the effect of exendin-4 on LPS- induced oxidative stress by measuring the cellular ROS level. When compared to non-treated cells, exendin-4 showed a dose-dependent suppression on LPS-induced ROS production (Fig. 5).

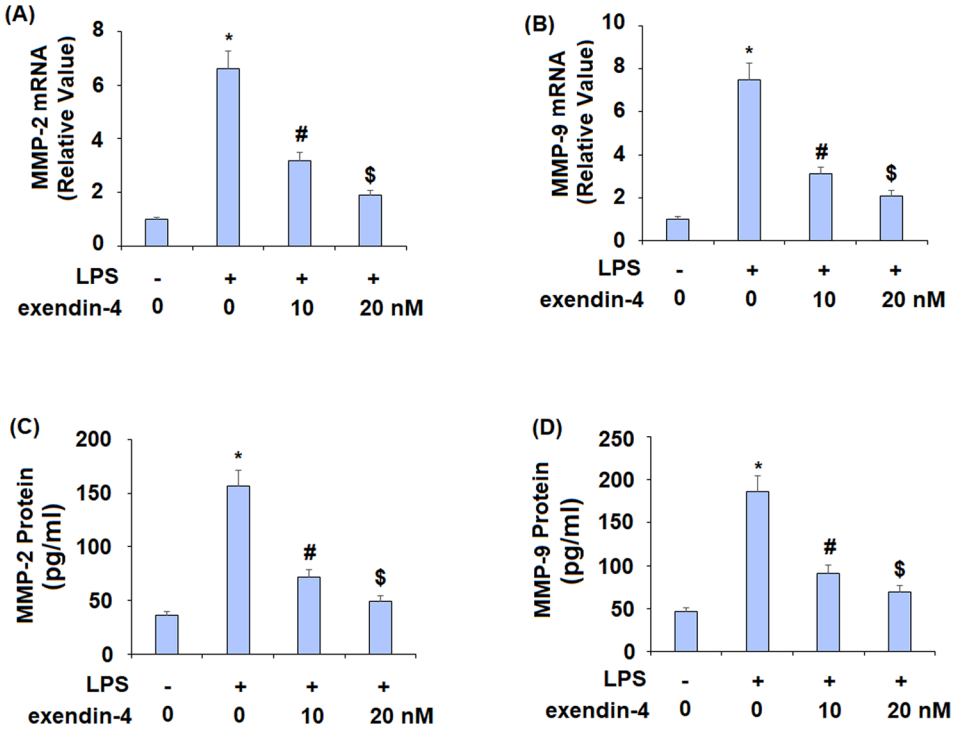

Fig. 4. Exenatide inhibited Lipopolysaccharide (LPS)- induced expression of MMP-2 and MMP-9 in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A). mRNA of MMP-2; (B). mRNA of MMP-9; (C). The protein of MMP-2 was measured by ELISA; (D). The protein of MMP-9 was measured by ELISA (*, #, $, $P < 0.01$).

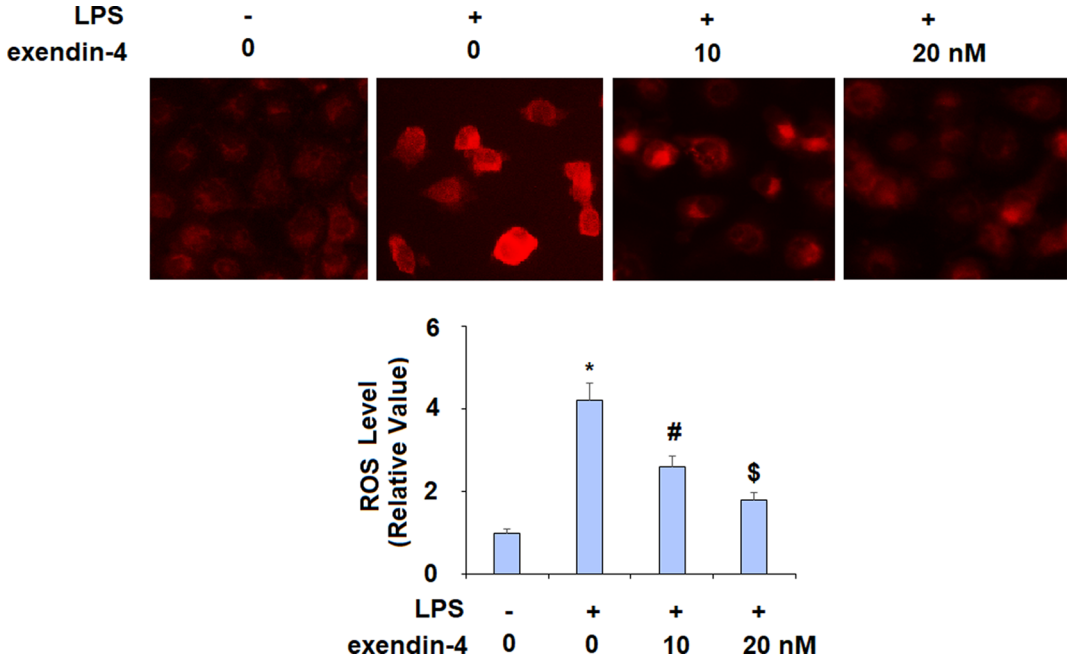

Fig. 5. Exenatide reduced Lipopolysaccharide (LPS)- induced oxidative stress in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. Intracellular levels of reactive oxygen species (ROS) were measured by dihydroethidium (DHE) staining (*, #, $, $P < 0.01$).

3.6. Exendin-4 suppresses LPS- induced activation of JNK

We then examined the molecular pathways by which exendin-4 is involved. Stress-induced JNK phosphorylation is known to be responsive to LPS treatment. By examining the phosphorylated and total JNK level, we found that exendin-4 had a dose-dependent inhibitive effect on LPS- induced JNK phosphorylation (Fig. 6), indicating that exendin-4 suppressed the activation of the JNK pathway.

3.7. Exendin-4 suppresses LPS-induced activation of the transcription factor AP-1

Next, we assessed the effect of exendin-4 on transcriptional factor AP-1. By measuring it's two subunits c-fos and c-jun. We showed exendin-4 reduced LPS induced both c-fos and c-jun expression (Fig. 7A), suggesting its inhibitive effect on AP-1 activation. Consistently, the luciferase assay demonstrated that exendin-4 suppressed the transcriptional activity of AP-1 (Fig. 7B).

3.8. Exendin-4 suppresses LPS- induced activation of NF-kB

NF-κB is the key regulator of the inflammation, and its activation is closely associated with the development of inflammatory diseases. We examined the influence of exendin-4 on NF-κB activation. Firstly, we found that exendin-4 treatment attenuated the nuclear translocation of NF-κB p65 (Fig. 8A). Secondly, we found that exendin-4 suppressed activation of the NF-κB luciferase promoter by LPS (Fig. 8B). This data indicates that exendin-4 suppresses LPS- induced activation of NF-κB in macrophages.

3.9. Exendin-4 reduces LPS- induced expression of TLR4

Since the pro-inflammatory effects of LPS are mediated by toll-like receptors (TLRs), we examined whether exendin-4 antagonizes the effects of LPS by down-regulating TLR4 expression. We used quantitative PCR to measure levels of TLR4 mRNA and Western blotting to measure levels of TLR4 protein in RAW264.7 macrophages. LPS up-regulated TLR4 expression at both mRNA and protein levels, while exendin-4 significantly repressed this up-regulation (Fig. 9).

4. Discussion

The microbial invasion caused inflammation is a complex process between host and pathogen. The pathogen invasion- induced aberrant inflammatory response in the organ often leads to extensive tissue damage, acute respiratory failure, systemic dysfunction, and even life-

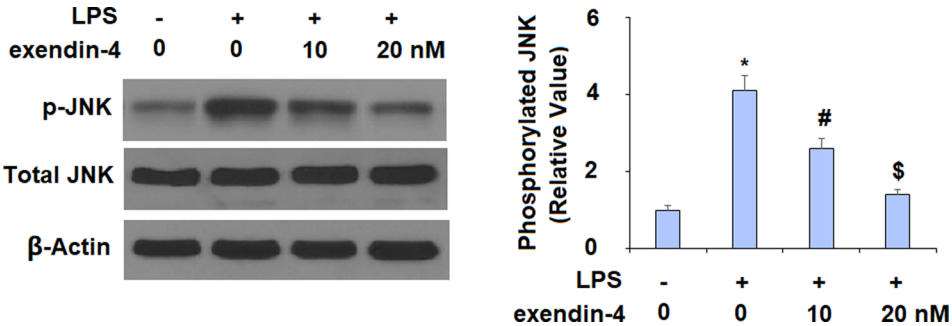

Fig. 6. Exenatide suppressed Lipopolysaccharide (LPS)- induced activation of JNK in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 2 h. Phosphorylated and total JNK has been measured (*, #, $, $P < 0.01$).

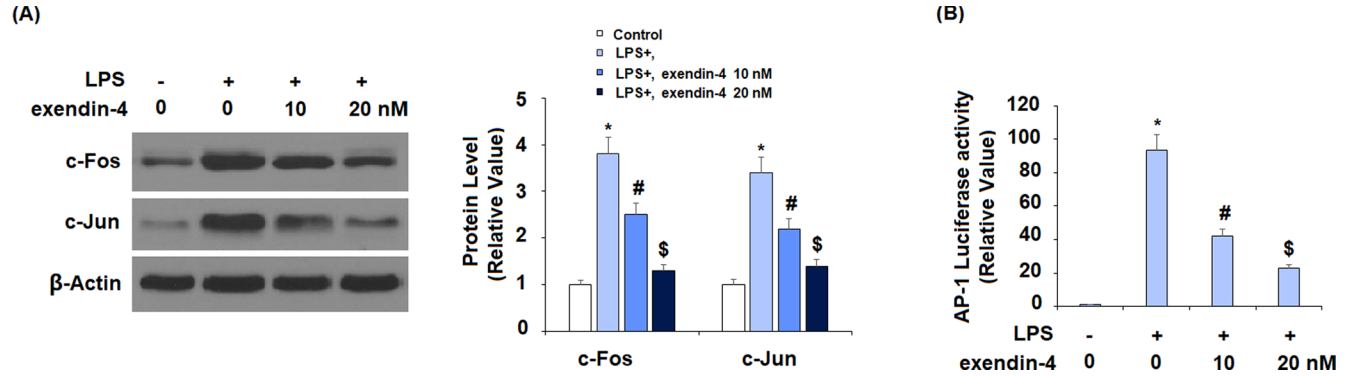

Fig. 7. Exenatide suppressed Lipopolysaccharide (LPS)-induced activation of the transcriptional factor AP-1 in RAW264.7 macrophages. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A). Protein levels of c-Fos (1, 3.8, 2.5, 1.3) and c-Jun were measured by western blot analysis; (B). Luciferase activity of AP-1 (*, #, $, $P < 0.01$).

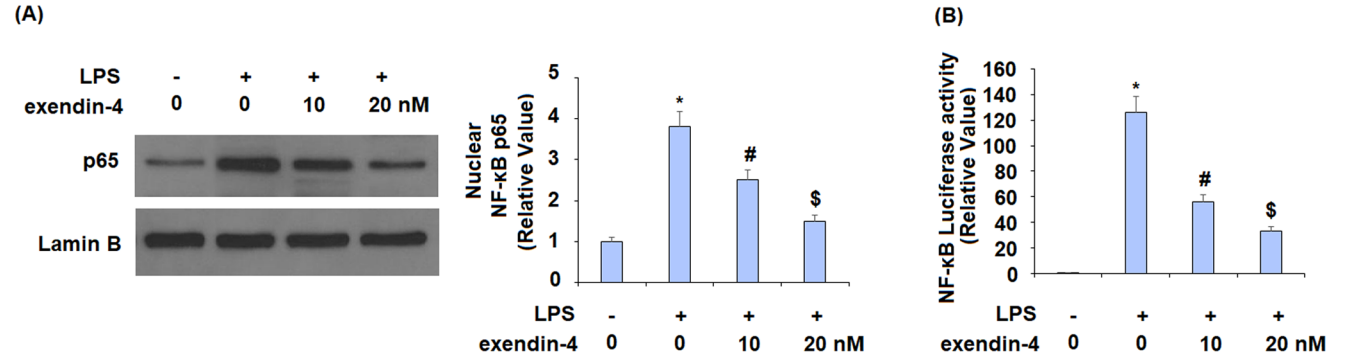

Fig. 8. Exenatide suppressed Lipopolysaccharide (LPS)- induced activation of NF-κB. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A). Nuclear translocation of NF-κB p65; (B). Luciferase activity of NF-κB (*, #, $, $P < 0.01$).

threaten events [14]. For example, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are two frequently encountered diseases with high morbidity and mortality in patients admitted in ICU. A high inflammatory response caused leukocyte infiltration and elevated pro-inflammatory cytokines in the lung are the key features in their pathogenesis [15]. Many chronic diseases are featured as long-lasting inflammation and infection during the pathological progression, such as osteoarthritis and type II diabetes.

Macrophages are the important immune cells against microbial pathogens. When activated, macrophages secrete a variety of chemokines, cytokines, and chemical mediators [16]. The signaling transduction initiated by TLRs can produce a large number of pro-inflammatory factors such as TNF-α, IL-1β, and IL-6, which promote local or systemic inflammation. However, the aberrant expression of pro-inflammatory factors is the driving force of the deterioration of many diseases [1,17]. Therefore, modulating the activation of macrophages is a wise therapeutic strategy of treating various inflammatory diseases.

TLR4 is one of the most studied TLRs. Activation of TLR4 has been associated with the initiation and progression of T2DM [18,19]. Importantly, TLR4 has been identified as the primary receptor of LPS, mediating the inflammatory responses of LPS via activating the NF-kB signaling pathway in mammalian cells. LPS derived from bacteria can increase GLP-1 secretion and plasma GLP-1 in the gastrointestinal tract [20]. Interestingly, a recent study reported that TLR4 deficiency mice blunted the secretion of GLP-1, suggesting that TLR4 is necessary for glucose-induced GLP-1 secretion *in vivo* [21]. In the current study, we found that the GLP-1 analog exendin-4 inhibited LPS- induced inflammation in RAW264.7 macrophages. In the current study, we found that exendin-4 down-regulated TLR4 mRNA and protein. Based on these findings, we speculated that there is a negative loop between GLP-1 and LPS/TLR4 signaling. However, the underlying mechanism is still unknown. Future studies will provide us with a complete picture.

In this study, we found that exendin-4 inhibited LPS-induced NO and PGE_2 production is due to its inhibition on the transcription and

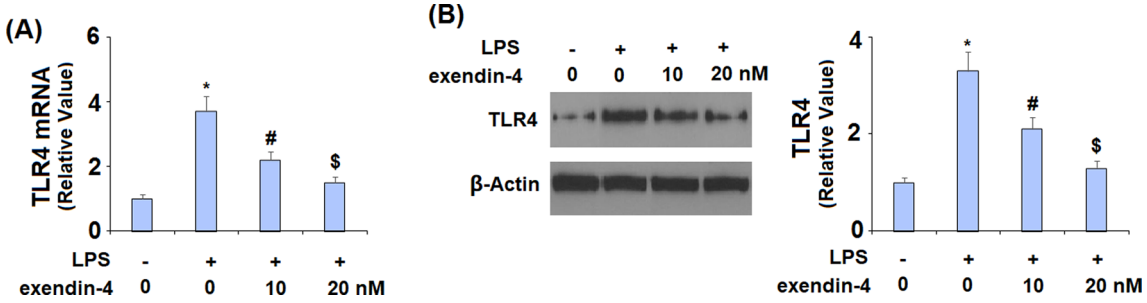

Fig. 9. Exenatide reduced lipopolysaccharide (LPS)- induced expression of TLR4. RAW264.7 macrophages were pretreated with 10 and 20 nM exendin-4 for 6 h, followed by stimulation with 1 μg/mL LPS for 24 h. (A). mRNA of TLR4; (B). The protein of TLR4 (*, #, $, $P < 0.01$).

production of inflammation-related enzymes (iNOS and COX-2). Exendin-4 also exhibited strong inhibition on the expression of pro-inflammatory factors, including TNF-α, IL-1β, and IL-6. Consequently, exendin-4 showed strong suppression of cellular ROS production. These facts indicate that exendin-4 is a potent anti-inflammatory and antoxidant agent, which influences multiple key inflammatory signals.

NF-κB is the master regulatory transcription factor, which plays a key role in regulating the expression of iNOS, COX-2, and pro-inflammatory cytokines such as TNF-α [22–24]. Upon activation, released nuclear factor-NF-κB (heterodimer of P50 and p65) translocate into the nucleus, where it binds to the promoter regions of these inflammatory genes, and induces the expression of a wide range of cytokines, enzymes and adhesion molecules [25–27]. Our study demonstrates that exendin-4 inhibits nuclear translocation of p65. We also show that exendin-4 has an inhibitory effect on LPS-induced activation of NF-κB promoter. These shreds of evidence suggest that the exendin-4 act to mitigate LPS elicited the NF-κB activation. Our data also shows exendin-4 suppresses the activation of the JNK and AP-1 pathway. The inter-connection of JNK, AP-1, and NF-κB has been reported, and the activation of JNK could result in increased nuclear AP-1 and NF-κB response [28]. Exendin-4 likely mediated JNK suppression leading to its inhibition on nuclear AP-1 and NF-κB in macrophage.

An inflammatory response is a major host defense mechanism against invading pathogens, and host cells activate a variety of immune responses to prevent damage to the body. The main limitation of the current study is that we only examined the protective effects of exendin-4 against LPS- induced inflammation in an *in vitro* RAW264.7 macrophages culture model. It should be noticed that the host defense *in vivo* is a complicated process and needs to be elucidated. Future *in vivo* studies with animal models or clinical trials are necessary to verify the anti-inflammatory effect of exendin-4.

In conclusion, our study aims to modulate the inflammatory response in macrophage, and we demonstrate the potent anti-inflammatory effects of exendin-4 and its mechanism in cultured RAW264.7 cells. Our data suggest that exendin-4 is a promising anti-inflammatory compound in the treatment of inflammatory-related diseases.

Declaration of Competing Interest

All authors declared they had none conflict interest need to be disclosed.

References

[1] M.J. Sweet, D.A. Hume, Endotoxin signal transduction in macrophages, J. Leukoc. Biol. 60 (1996) 8–26.

[2] T.L. Gioannini, J.P. Weiss, Regulation of interactions of Gram-negative bacterial endotoxins with mammalian cells, Immunol. Res. 39 (2007) 249–260.

[3] E. Ricciotti, G.A. FitzGerald, Prostaglandins and inflammation, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 986–1000.

[4] C.S. Williams, M. Mann, R.N. DuBois, The role of cyclooxygenases in inflammation, cancer, and development, Oncogene 18 (1999) 7908–7916.

[5] S.F. Liu, A.B. Malik, NF-kappa B activation as a pathological mechanism of septic shock and inflammation, Am. J. Physiol. Lung Cell. Mol. Physiol. 290 (2006) L622–L645.

[6] S. Shishodia, D. Koul, B.B. Aggarwal, Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF-kappa B activation through inhibition of activation of I kappa B alpha kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis, J. Immunol. 173 (2004) 2011–2022.

[7] R. Glauben, A. Batra, I. Fedke, M. Zeitz, H.A. Lehr, F. Leoni, et al., Histone hyperacetylation is associated with amelioration of experimental colitis in mice, J. Immunol. 176 (2006) 5015–5022.

[8] J.J. Neumiller, Differential chemistry (structure), mechanism of action, and pharmacology of GLP-1 receptor agonists and DPP-4 inhibitors, J. Am. Pharm. Assoc. 49 (5) (2009) S16–S29.

[9] D.D. Mafong, R.R. Henry, Exenatide as a treatment for diabetes and obesity: implications for cardiovascular risk reduction, Curr. Atherosclerosis Rep. 10 (1) (2008) 55–60.

[10] M.J. Kraakman, A.J. Murphy, K. Jandeleit-Dahm, H.L. Kammoun, Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? Front Immunol. 5 (2014) 470.

[11] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante, Jr Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest. 112 (2003) 1796–1808.

[12] S. Virtue, A. Vidal-Puig, Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome - an allostatic perspective, Biochim. Biophys. Acta 1801 (2010) 338–349.

[13] A.M. Stern, J. Zhu, An introduction to nitric oxide sensing and response in bacteria, Adv. Appl. Microbiol. 87 (2014) 187–220.

[14] S. Lin, H. Wu, C. Wang, Z. Xiao, F. Xu, Regulatory T cells and acute lung injury: cytokines, uncontrolled inflammation, and therapeutic implications, Front. Immunol. 9 (2018) 1545.

[15] L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, L. Zhao, Inflammatory responses and inflammation-associated diseases in organs, Oncotarget. 9 (6) (2017) 7204–7218.

[16] N. Fujiwara, K. Kobayashi, Macrophages in inflammation, Curr. Drug Targets Inflamm. Allergy 4 (3) (2005) 281–286.

[17] A.E. Medvedev, K.M. Kopydlowski, S.N. Vogel, Inhibition of lipopolysaccharide-induced signal transduction in endotoxin- tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression, J. Immunol. 164 (2000) 5564–5574.

[18] I. Jialal, H. Kaur, S. Devaraj, Toll-like receptor status in obesity and metabolic syndrome: a translational perspective, J. Clin. Endocrinol. Metab. 99 (2014) 39–48.

[19] N.Q. Huang, H. Jin, S.Y. Zhou, J.S. Shi, F. Jin, TLR4 is a link between diabetes and Alzheimer's disease, Behav. Brain Res. 316 (2017) 234–244.

[20] A.T. Nguyen, S. Mandard, C. Dray, V. Deckert, P. Valet, P. Besnard, D.J. Drucker, L. Lagrost, J. Grober, Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: involvement of the GLP-1 pathway, Diabetes 63 (2) (2014) 471–482.

[21] L. Wang, X. Zhan, Z. Wang, J. Ma, X. Chang, X. Zhu, Toll-like receptor 4 is necessary for glucose-dependent glucagon-like peptide-1 secretion in male mice, Biochem. Biophys. Res. Commun. 510 (1) (2019) 104–109.

[22] V.T. Ha, H.S. Beak, E. Kim, K.S. Baek, M.J. Hossen, W.S. Yang, et al., NF-kappaB/AP-1-targeted inhibition of macrophage-mediated inflammatory responses by depigmenting compound AP736 derived from natural 1,3-diphenylpropane skeleton, Mediators Inflamm. (2014).

[23] Q. Shi, J. Cao, L. Fang, H. Zhao, Z. Liu, J. Ran, et al., Geniposide suppresses LPS-induced nitric oxide, PGE2 and inflammatory cytokine by downregulating NF-kappaB, MAPK and AP-1 signaling pathways in macrophages, Int. Immunopharmacol. 20 (2) (2014) 298–306.

[24] D.J. Kwon, S.M. Ju, G.S. Youn, S.Y. Choi, J. Park, Suppression of iNOS and COX-2 expression by flavokawain A via blockade of NF-kappaB and AP-1 activation in RAW 264.7 macrophages, Food Chem. Toxicol.: An Int. J. Published British Ind. Biol. Res. Assoc. 58 (2013) 479–486.

[25] J. Inflamm. 5 (1) (2008) 7, https://doi.org/10.1186/1476-9255-5-7.

[26] D.J. Kwon, Y.S. Bae, S.M. Ju, G.S. Youn, S.Y. Choi, J. Park, Salicortin suppresses lipopolysaccharide-stimu-lated inflammatory responses via blockade of NF-kB and JNK activation in RAW 264.7 macro-phages, BMB Rep. 47 (6) (2014) 318–323.

[27] A. Lahti, U. Jalonen, H. Kankaanranta, E. Moilanen, c-Jun NH2-terminal kinase inhibitor anthra(1,9-cd) pyrazol-6(2H)-one reduces inducible nitric-oxide synthase expression by destabilizing mRNA in acti-vated macrophages, Mol. Pharmacol. 64 (2) (2003) 308–315.

[28] S. Papa, F. Zazzeroni, C.G. Pham, C. Bubici, G. Franzoso, Linking JNK signaling to NF-kappaB: a key to survival, J. Cell Sci. 117 (Pt 22) (2004) 5197–5208.