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ORIGINAL ARTICLE

Degradation Products of Polydopamine Restrained Inflammatory Response of LPS-Stimulated Macrophages Through Mediation TLR-4-MYD88 Dependent Signaling Pathways by Antioxidant

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Abstract— Polydopamine (PDA) has a promising application as coating of biomaterials due to its favorable degradability and bioadaptability. However, its bioactivity, such as anti-inflammatory capacity, was still little known. Herein, we investigated whether degradable products of PDA could affect inflammatory response in lipopolysaccharide (LPS)-stimulated human THP-1-derived macrophages. The supernatants containing degradation products of PDA, annotated as PDA extracts, were collected after PDA being immersed in cell culture medium for 3 days. Wherein, the composition of the degradation products was analyzed by HPLC assay. Collected PDA extracts were diluted into 100%, 50%, and 25% of original

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concentration, respectively, to evaluate their anti-inflammatory ability on LPS-induced macrophages from the expression levels of pro-inflammatory cytokines to associated molecular mechanism. Our results showed that the PDA extracts were mainly composed of dopamine, quinine, and PDA segments. Furthermore, macrophages showed no cytotoxicity after PDA extract treatment with or without LPS, while the release levels of TNF-α and IL-6 by LPS-induced macrophages were decreased in dose-dependent by PDA extract treatment. Additionally, TLR-4 and MYD88 expression in protein and RNA level were downregulated by PDA extracts in LPS-induced macrophages. Similarly, PDA extracts effectively inhibited LPS-induced NF-κB trans-locating into nuclear by inactivation of the phosphorylation of IKK-α/β and IKβ-α. Of note, the production of LPS-induced ROS was reduced by PDA extracts in macrophages, while HO-1 expression, a critical protein of antioxidant signaling pathway, was increased. Based on these results, we proposed a potential mechanism by which degradation products of PDA suppressed inflammation of macrophages *via* downregulation TLR-4-MYD88-NFκB pathway and simultaneous activation HO-1 pathway, which might be a possible therapeutic target.

KEY WORDS: polydopamine; macrophages; inflammation; antioxidant; NF-κB.

INTRODUCTION

Biomaterial had been widely used in medical fields for decades, whereas foreign body reaction (FBR), as a unique immune response, was still a potential barrier to biomaterials application [1]. FBR was reported to have adverse clinical effects such as in aseptic loosening of hip or knee arthroplasty [2], gradual functionality loss post-implantation of implantable biosensors [3], or in-stent restenosis of percutaneous coronary intervention [4], *etc.* The general opinions over the FBR against biomaterials implantation were divided into five stages: proteins adsorption, acute inflammation, chronic inflammation, foreign body giant cells (FBGCs) formation, and fibrous capsule formation, from which biomaterials eventually lost function [5].

It was reported that macrophages played an important role throughout the FBR process. Through interacting with biomaterials, macrophages affect post-implantation consequence by polarization of itself (M1 or M2), formation of foreign body giant cells, secretion of cytokines, or regulation of other cells, *etc.* [6]. Especially in the acute inflammation stage, macrophages could be triggered with damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), caused by trauma or infection after implantation, to secret pro-inflammatory chemokines or cytokines such as tumor necrotic factor α (TNF-α) or interleukin-6 (IL-6), *etc.*, which resulted in a local inflammation around biomaterials to impart their function [6, 7]. Furthermore, it was shown that biomaterial encapsulation was enhanced in macrophage depletion of transgenic MaFIA mice in the early period of FBR, indicating the necessary modulation of macrophages to materials at the acute inflammatory stage [8]. Thus, it was necessary to evaluate the changes of macrophages in the acute inflammation phase of FBR in order to predict the function of biomaterials in the entire process of implantation [9].

Recently, there emerged reports on the interactions between Toll-like-4 receptor (TLR-4) and FBR. For instance, graphene oxide could induce TLR-4 expression of macrophages resulting in inflammatory response [10]. TLR-4, basically expressing on the surface of immune cells (macrophages, dendritic cell, B cell, *etc.*), was responsible for sensor DAMPs like high mobility group box 1 (HMGB1), or PAMPs such as LPS, then subsequently activated innate immune response through MYD88 dependent or independent signaling pathway [11]. After that, the nuclear factor kappa-light-chain-enhance of activated B cell (NF-κB) was activated by TLR-4 signal to translate into nuclear to promote the expression of related inflammatory cytokines. Moreover, LPS-induced reactive oxygen species (ROS) contributed to the enhancement of TLR-4 pathway by interacting with NF-κB [12, 13]. On the contrary, the redox systems could effectively mitigate inflammation by counteracting ROS [14]. Among the systems, heme oxygenase-1 (HO-1) played a central role in anti-inflammatory and antioxidant response [15, 16].

PDA constructed by dopamine self-polymerization has attracted attention as coating or nanoparticle in the fields of biomaterial, because of its unique chemical properties [17–19]. For instance, it has been reported PDA

coating could enhance the surface bioactivity of hydroxyapatite formation on Mg-based alloy to promote osteoblast adhesion and proliferation during bone formation [20]. Besides, PDA nanoparticles were an excellent free radical scavenging agent [21]. Most recently, it has been found that PDA nanoparticles could alleviate inflammatory-induced injury *in vitro* and *in vivo* [22]. However, the potential impact of degradable products of PDA to the function of macrophages at the acute inflammatory phase, combining with the infectious environment because of surgery, of FBR was still little known.

Given all that, the present study was designed to study the possible role of the degradation products of PDA in the inflammatory response of LPS-induced THP-1-derived macrophages. The reason why we selected THP-1 cell here was the cause of its high similarity with human primary macrophages [23]. Furthermore, experiments were carry-out to disclose potential mechanisms through investigating TLR-4-MYD88, NF-κB, and HO-1 signaling pathways.

MATERIALS AND METHODS

Materials and Sample Preparation

PDA was synthesized with a slightly modified protocol based on the method reported by Lee H, et al. [24]. In brief, dopamine solution was prepared by dissolving dopamine hydrochloride (2 mg/mL, alfa-aesar, UK) in ethanol with an optimized amount (3%, *v*/*v*) of ammonia water (25-28 wt.%). Afterward, 5 mL of dopamine solution was added into six-well culture plate for 24 h at room temperate for self-polymerization, and the plate was displaced with solution, rinsed with distilled water for three times, blown dry and stored in desiccator until for further use. PDA extracts were harvested according to ISO-10993 guideline. Briefly, the above-mentioned plate was added with cell culture medium, RPMI 1640 (Gibco, USA), with 4 mL for 3 days at 37 °C in a 5% CO_2 humidified environment. By the end of time, the collected supernatant was respectively diluted into 100%PDA, 50%PDA, and 25%PDA samples with PRMI 1640 medium and kept at 4 °C for further experiments. Additionally, the pH value and osmotic pressure of the PDA extracts were measured by pH detector (PB-10, Sartorius, Germany) and freezing point osmometer (Osmomat 3000, USA) and meanwhile the chemical composition of PDA extracts was analyzed by high-performance liquid chromatography assay (HPLC, Agilent 1260, USA). Briefly, the column temperature was controlled at 37 °C and the flow rated was maintained at 1.2 mL/min with an injection volume of 10 μL and UV detection absorbance. Finally, data was processed with Empower pro software.

Cells Culture and Treatment

THP-1 cell line was obtained from cell culture collection of the Chinese Academy of Sciences, Shanghai, China. The THP-1 cells were cultured with RPMI 1640 medium supplementary 10% inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). Then, cells were treated with 50 ng/mL Phorbol 12-myristate 13-acetate (PMA, sigma, USA) for 24 h in order to differentiate into THP-1-derived macrophages and were replaced with fresh cell culture medium to incubate for another 24 h.

For assessing the possible cytotoxicity of PDA extracts, THP-1 cells were treated by various PDA extracts with or without LPS (1 μg/mL, sigma, USA) for 24 h. The supernatants were collected and tested using a lactate dehydrogenase (LDH) cytotoxicity assay kit (Nanjin Jiancheng, China) according to the protocol. Meanwhile, cells were measured with CCK-8 assay kit (Dojindo, Japan) in line with the manufacturer's instruction.

Flow Cytometry Analysis (FACS)

THP-1 cells were harvested after with LPS for 24 h prior to treatment with various PDA extracts. Then, cells were stained with the apoptotic kit (BD, USA) containing annexin V-FITC and propidium iodide (PI) or TLR-4-PE (BioLegend, USA). After incubation for 30 min, cells were subjected for flow cytometry (Canto II, BD, USA).

For the intracellular ROS detection, macrophages were pretreated with various PDA extracts for 3 h prior to LPS stimulation for another 90 min. Afterward, cells were stained by dichlorodihydrofluorescein diacetate (DCFH, Beyotime, China) for 30 min according to the protocol. Finally, cells were harvested and analyzed by FACS or directly visualized using a fluorescence microscope (DFC310, LECI, Germany). Data of FACS were processed using Flowjo 7.6.

Real-Time Quantitative PCR Analysis (RT-qPCR)

Macrophages were seeded in 6-well plate treated with or without LPS for various hours after pretreatment with PDA extracts for 3 h. The total RNA extraction and cDNA synthesis were performed by related kits (TOYOBO, Japan). Bio-Rad C100 was employed for RT-qPCR analysis using SYBR green (TOYOBO, Japan). The levels of target genes were normalized to GAPDH, a housekeeping gene,

for calculation. The primer sequences of genes were list in Table S1.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was carried out to determine the expression of pro-inflammatory cytokines (IL-6 and TNF-α). Macrophages were treated with same method as mentioned above. The level of cytokine in supernatant was determined using an ELISA kit (DAKWE, China) according to the manufacturer's instructions. The minimal concentrations of TNF-α and IL-6 were 30 pg/mL and 6.25 pg/mL, respectively.

Western Blotting Analysis

Macrophages were treated as mentioned above. Protein lysates extracting from cells were loaded into 10% or 12% sodium dodecyl sulfate-[olyacrylamide gel electrophoresis (SDS-PAGE). After that, proteins were transferred to PVDF transfer membranes (Millipore, Billerica, USA). Primary antibody including MYD88, P65, Histone H3, p-IKK-α/β, IKK-α/β, p-IκBα, IκB and HO-1 (CST, USA) were separately incubated overnight after blocking with 7% skimmed milk. Secondary antibody, IRDye® 800CW Goat anti-Mouse IgG and IRDye® 800CW Goat anti-Rabbit IgG (Li-COR, USA) were incubated with membranes for 1 h. Finally, results were acquired by an infrared imaging system (Li-Cor odyssey, Li-COR, USA).

Immunofluorescence Staining

The effects of PDA extracts on NF-κB activity were evaluated with NF-κB activation-nuclear translocation assay kit (Beyotime, China). Macrophages were seeded in 6-well plate with LPS before the addition of PDA extracts for 3 h. After rinsing, fixation and blocking, cells were incubated with p-65 primary antibody at 4 °C overnight. Macrophages were subsequently incubated with cy3-conjugated secondary antibody for 1 h and then stained with DAPI for 5 min at room temperature. Finally, cells were visualized by fluorescence microscope.

Statistical Analysis

The statistical analyses were performed by using one-way ANOVA with Turkey HSD of SPSS software. Results were interpreted as means ± standard deviation (SD) and two-tailed $P < 0.05$ was considered as statistical significance.

RESULTS

Characterization of PDA Extracts

HPLC analysis of compounds of PDA extracts/degradation products showed an additional peak at retention time of 3.244 min as compared with the control group(cell culture medium) (Fig. 1a). The spectrum of the peak from 200 to 600 nm revealed obviously an intense peak at 283 nm and a weak peak at 340 nm as shown in Fig. 1b. Qin Y, *et al* reported that during the production of PDA, dopamine, quinine and PDA could be detected at 283, 320 and 425 nm, respectively [25]. Thereby, it could be reasonably speculated the most dominate compounds in PDA extracts were dopamine and some intermediate products such as quinine and PDA. Additionally, the pH value and osmolality of PDA extracts showed no obvious difference with that of the control group (Fig. 1c).

Cytotoxicity of PDA Extracts on THP-1 Cells

As shown in Fig. 2a, b, the cellular growth and cell viability of THP-1 cells were not inhibited by PDA extracts at various concentrations with or without LPS stimulation. Furthermore, no apoptotic or necrotic cells were observed in the all PDA extract groups and control group (Fig. 2c). Our results demonstrated that PDA extracts had no cytotoxicity on macrophages.

Effect of PDA Extracts on Pro-inflammatory Response of LPS-Induced Macrophages

Inflammatory cytokines such as TNF-α and IL-6 were able to be released by macrophages after LPS stimulation [26]. The effect of PDA extracts on the secretion of inflammatory cytokines was investigated by ELISA assay. As shown in Fig. 3a, b, PDA extracts suppressed the expression of TNF-α and IL-6 in a dose-dependent manner. To further examine whether PDA extracts inhibited LPS-induced inflammatory response *via* TLR-4, an anti-TLR-4 monoclonal antibody (MTS510) was used to treat macrophages prior to stimulation with LPS. The results revealed that MTS510 with or without PDA extracts depressed TNF-α and IL-6 release after LPS stimulation. However, there was no difference on the expression of TNF-α of LPS-induced macrophages between MTS510 alone group and the combination of various PDA extracts and MTS510 groups. Of note, treatment of macrophages with a combination of MST510 and 100%PDA extract,

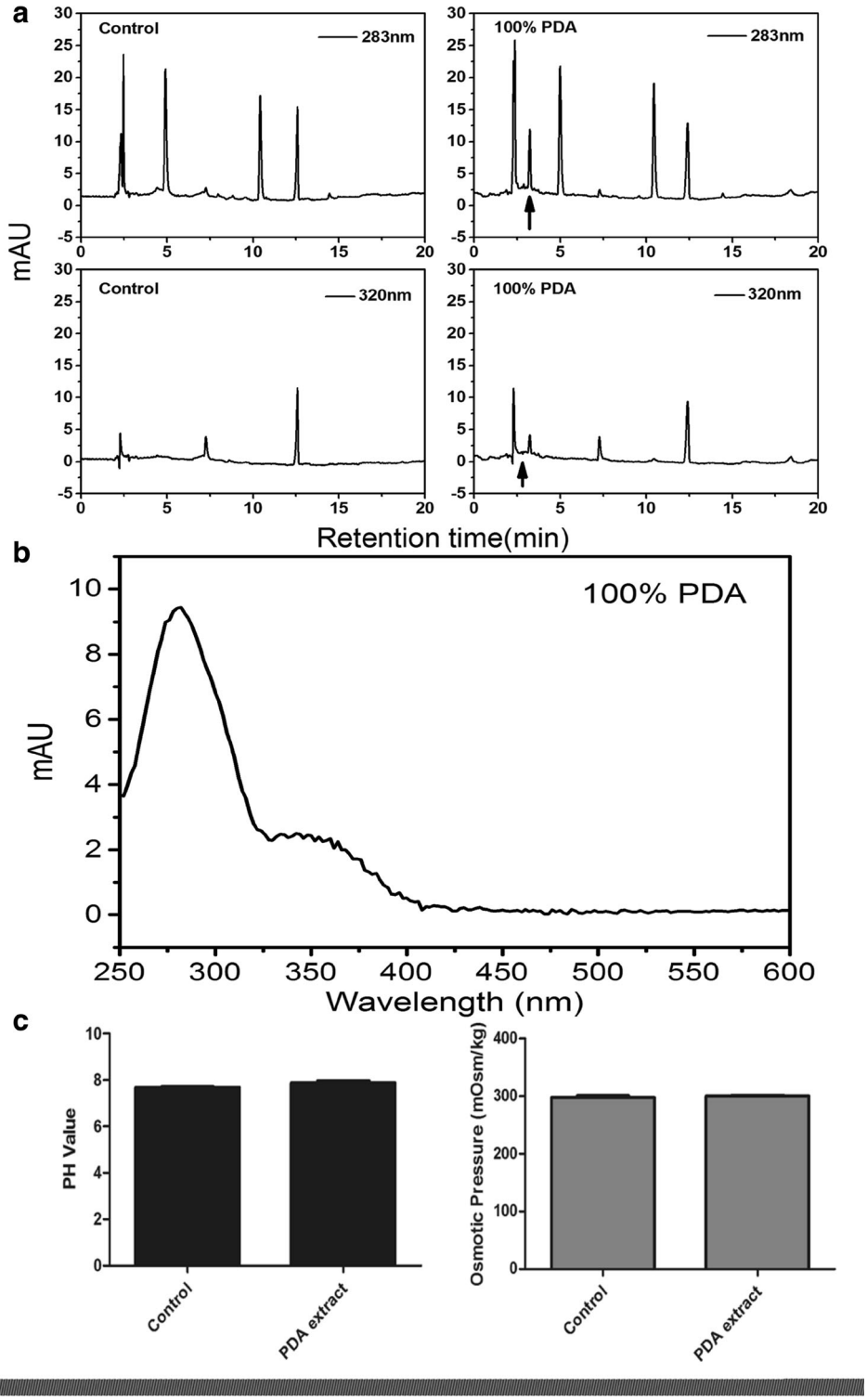

Fig. 1. The characterization of degradation products in PDA extracts. **a** 100% PDA extracts were detected at 283 and 320 nm of UV absorbance by HPLC; the results (black arrow) were shown as through compared with control medium (RPMI 1640). **b** The spectrums of PDA extract contents were visualized by the spectra-dependent UV absorbance of HPLC. **c** The pH value and osmotic pressure were analyzed of PDA extracts and control.

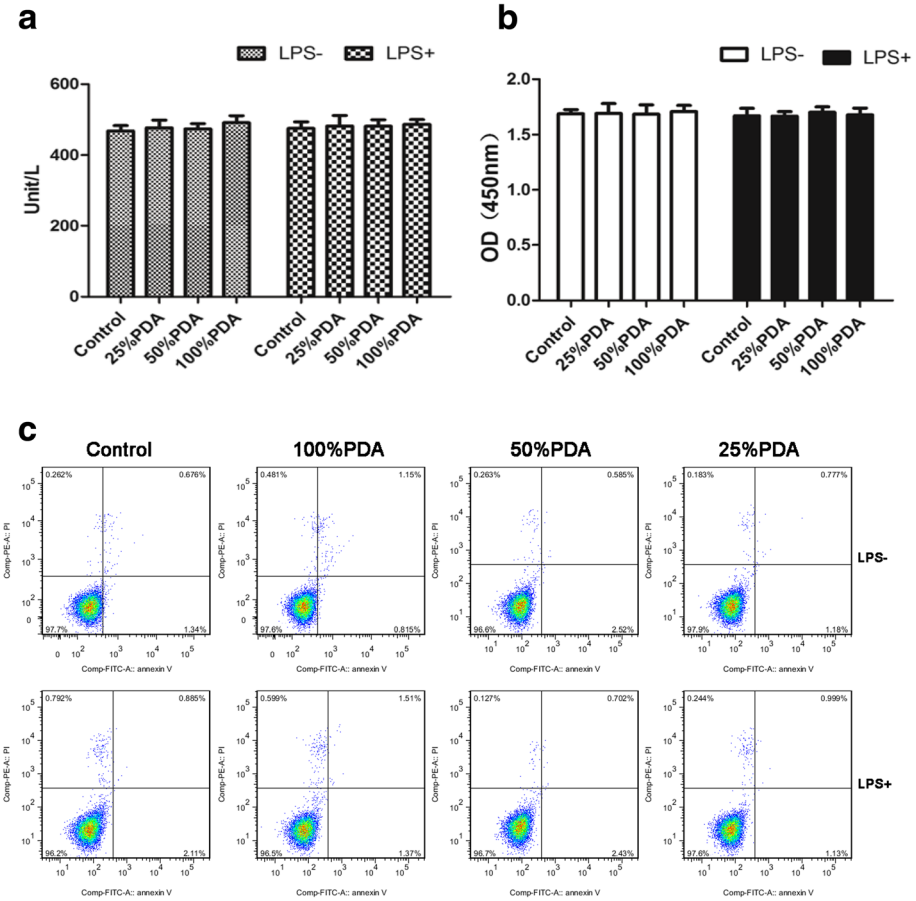

Fig. 2. Effect of PDA extracts on cytotoxicity of THP-1 cells. THP-1 cells were pretreated with PDA extracts (100%PDA, 50%PDA, and 25%PDA, respectively) for 3 h and stimulated with or without LPS for 24 h. **a** The concentration of LDH in supernatant determined by LDH test. **b** The cell viability measured with CCK-8 assay. **c** The probable apoptotic and necrotic cells analyzed by FACS.

neither 25%PDA extract nor 50%PDA extract, synergistically inhibited IL-6 expression elicited by LPS as compared with that of treatment MTS510 alone (Fig. 3c, d). Therefore, PDA extracts inhibited LPS-induced inflammatory response that was dependent on TLR-4.

Effect of PDA Extracts on TLR-4-MYD88 Dependent Signaling Pathway in LPS-Induced Macrophages

To further determine how PDA degradation products affected TLR-4 signaling pathway, we examined the expression of TLR-4 and MYD88 in LPS-induced macrophages for 24 h after PDA extract pretreatment for 3 h. The results demonstrated a decrease of TLR-4 and MYD88 expression at both the mRNA and protein levels in 50% and 100% PDA extract groups compared to LPS-induced control group. Remarkably, the 100% PDA extract even resulted in TLR-4 and MYD88 expression, in both mRNA and protein level, lower than that of the control group. However, 25%PDA extract showed no inhibition except MYD88 expression at protein level (Fig. 4). Altogether, these data showed that PDA extracts could downregulate TL-4/MYD88 signaling pathway in a dose-dependent manner.

Inhibition of PDA Extracts to the NF-κB Signaling Pathway in LPS-Induced Macrophages

The NF-κB played a central role in the production of inflammatory mediators. After IKK-α/β, a crucial protein as the upstream of NF-κB, was activated by MYD88, the IκBα, a repressor of NF-κB, was phosphorylated and

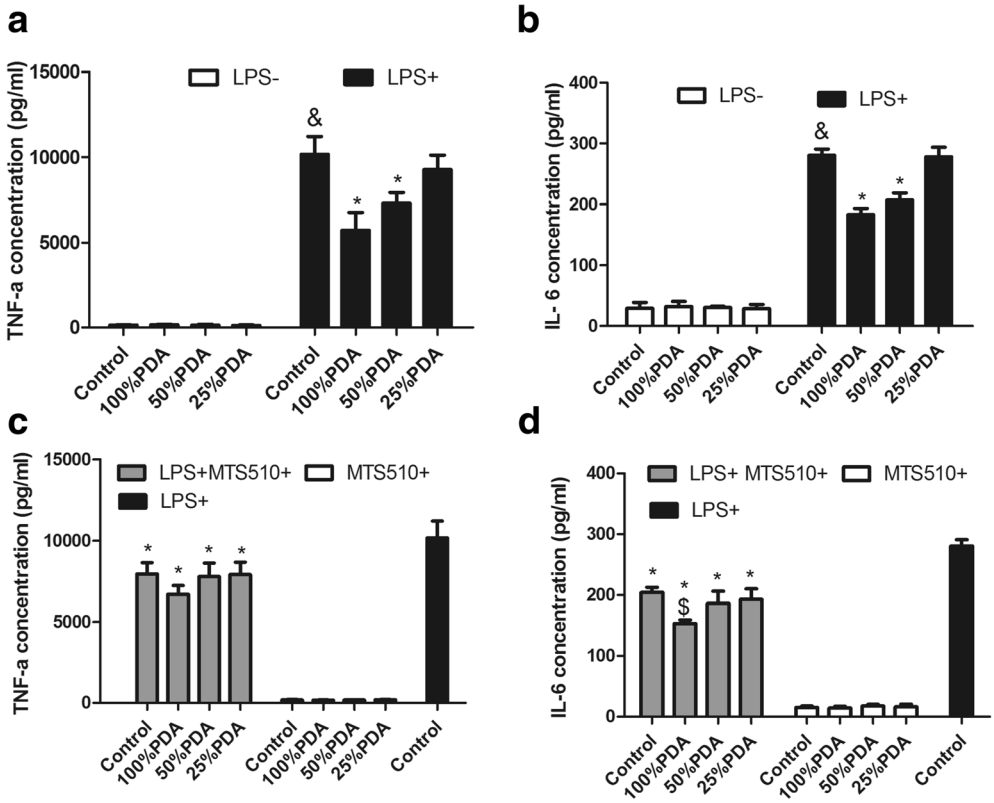

Fig. 3. Effect of PDA extracts on the expression of pro-inflammatory cytokines of macrophages through LPS binding TLR-4 receptor. Macrophages were pretreated with various PDA extracts for 3 h and stimulated with LPS for 24 h. The production of cytokines TNF-α (a) and IL-6 (**b**) in supernatant was determined with ELISA. Macrophages were pretreated with anti-TLR-4 antibody (MTS510, 3 μg/mL) for 1 h prior to the addition of PDA extracts for another 3 h, and then stimulated with LPS for 24 h. The concentration of cytokines TNF-α (**c**) and IL-6 (**d**) in medium was measured by ELISA. $^{\&}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* LPS-induced control group; $^{\$}P < 0.05$ *vs.* LPS+ MTS510 control group.

separated from NF-κB. Then, the activated NF-κB entered into nuclear to promote gene expression [27]. Therefore, we evaluated whether the inhibition of inflammation by PDA extracts was mediated through NF-κB pathway. As shown in Fig. 5a, the protein p65 was transferred into nuclear from cytosol in the presence of LPS, whereas 100% PDA extract remarkably alleviated the process, and meanwhile 25%PDA extract did not show change with LPS-induced control. In addition, the western blot results were also in agreement with the results of immunofluorescence (Fig. 5b–d). Subsequently, we assessed *IκBα* gene expression. Since LPS activation led to an increase of *IκBα* expression at mRNA level, while 100%PDA extract could attenuate this tendency from 2 h after LPS stimulation (Fig. 6a). Furthermore, western blot data proved that the activation of IκBα and IKK-α/β stimulated by LPS was significantly reversed in the presence of 100%PDA extract but not 25%PDA extract (Fig. 6b–d). All these results suggested PDA extracts were able to inactivate NF-κB signaling pathway.

Elimination ROS Production in LPS-Induced Macrophage by PDA Extracts

The above findings prompted us to explore how PDA extracts affected the TLR-4-MYD88-NF-κB signaling pathway. We hypothesized the PDA extracts to the regulation of ROS level in LPS-induced macrophages might be a possible clue. Thus, the intracellular ROS level was evaluated by FACS and fluorescence microscope. The results illustrated that 100% PDA extracts could effectively abate ROS production induced by LPS (Fig. 7a–c). Furthermore, the protein level of HO-1 expression in LPS-induced macrophages with western blot was also evaluated and the results, as shown in Fig. 7d, e, revealed that

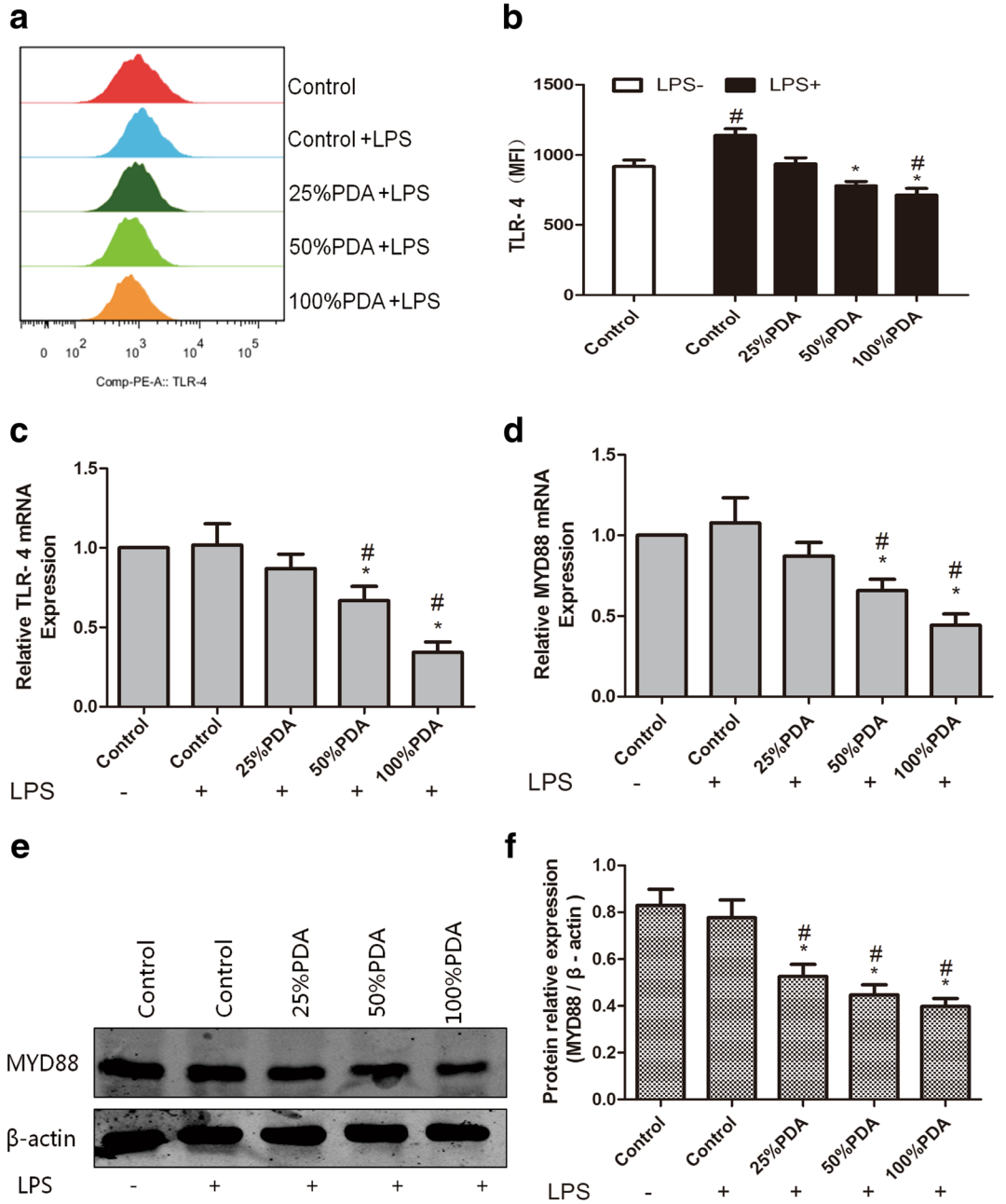

Fig. 4. Regulation by PDA extracts on LPS-induced TL-4-MYD88 dependent signaling pathway in macrophages. Protein expression of TLR-4 analyzed by FACS (**a**, **b**) and MYD88 was detected using western blot (**e**, **f**). The mRNA levels of TLR-4 and MYD88 were measured using real-time PCR (**c**, **d**). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* LPS-induced control group.

100%PDA extract promoted HO-1 expression with or without LPS stimulation. Thereby, we speculated PDA extracts might either act as a scavenger of ROS or activate antioxidant protein to inhibit inflammatory response.

DISCUSSION

In this work, we studied the potential capability of the degradation products of PDA to depress inflammatory response and the possible underlying mechanism. Our

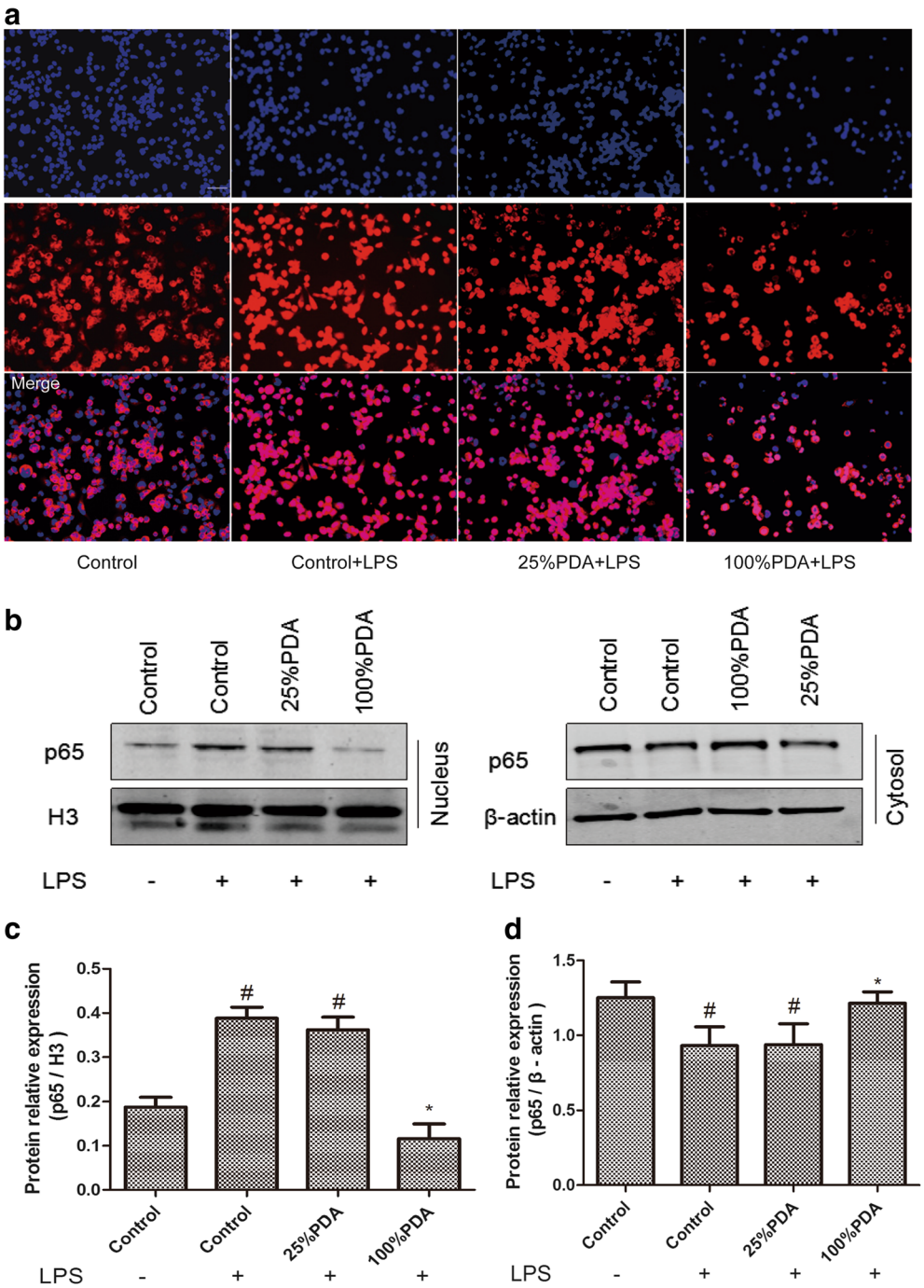

Fig. 5. Effect of PDA extracts on the inhibition of LPS-induced NF-κB translocation in macrophages. Cells were pretreated with PDA extracts (100%PDA and 25%PDA) for 3 h and then stimulated by LPS for 30 min. **a** Immunofluorescence images of control, LPS control, and PDA extracted with LPS groups. Scale bar = 50 μm. The p65 expression in cytosol and nuclear of macrophages were determined by western blot (**b**–**d**). $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* LPS-induced control group.

results demonstrated that pro-inflammatory cytokines secreted by LPS-induced macrophages were reduced by PDA extract treatment. We further disclosed related anti-inflammatory mechanism of PDA in which the LPS-

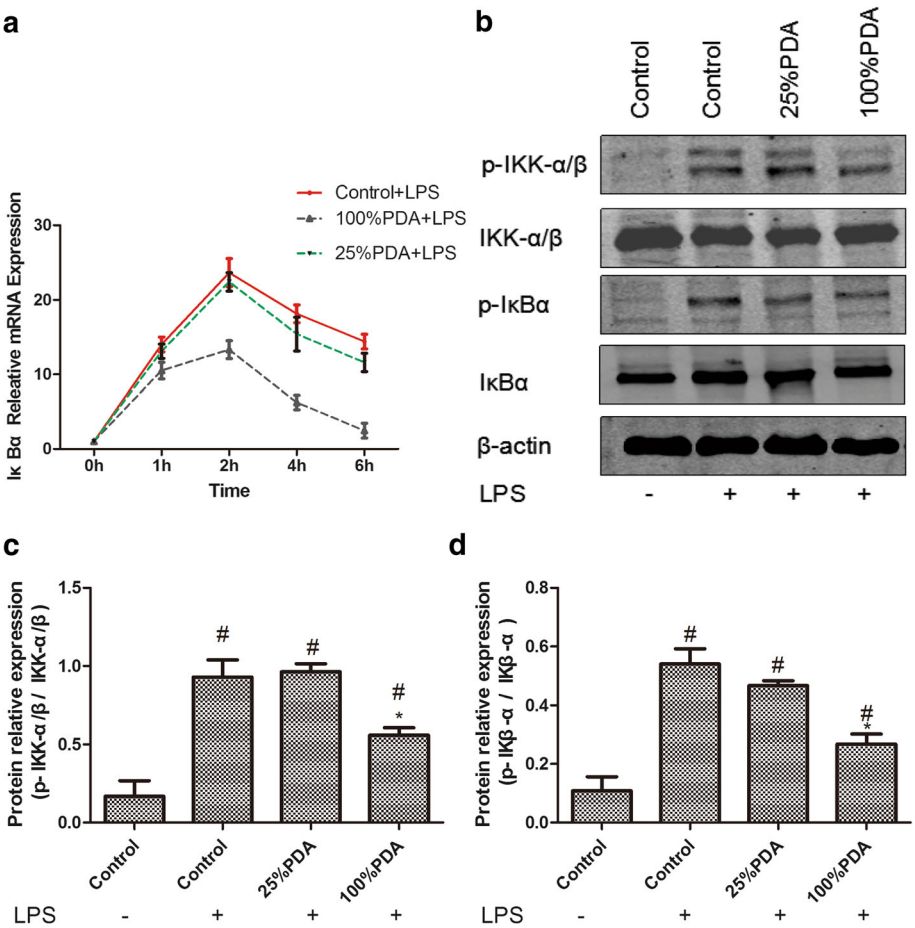

Fig. 6. Effect of PDA extracts on NF-κB inflammatory signaling pathway in LPS-induced macrophages. **a** Cells were pretreated with PDA extracts and then stimulated with LPS at various time points. The increase of LPS-induced *IκB-α* gene expression was alleviated in the presence of 100%PDA extracts analyzed by real-time PCR after normalization GAPDH. **b**–**d** Cells were pretreated with PDA extracts and then stimulated with LPS for 30 min. The relative expression of p-IKβ-α/IKβ-α and p-IKKα/β/IKKα/β was visualized and analyzed by western blot, respectively. $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* LPS-induced control group.

induced TLR-4-MYD88-NF-κB inflammatory signaling pathway could be inactivated by PDA extracts while it was able to scavenge ROS and upregulate HO-1 activity.

PDA extracts consisted of degradation products mainly composed of dopamine, PDA segments and quinine according to the HPLC results. Dopamine had been reported to control inflammation by dopamine receptors (DRs) signaling pathway such as DR2 or DR3 [28, 29]. In addition to dopamine, most recently, Zhao, H et al. found that PDA nanoparticles were able to therapy acute inflammation-induced injury [22]. Therefore, we reasonably speculated that the dopamine and PDA segments in the PDA extracts might play dominant roles in anti-inflammatory response.

It was reported that a variety of inflammatory cytokines played important roles in FBR after implantation [30]. For instance, TNF-α was a necessary mediator in the formation of foreign body giant cells during FBR [31]. Besides, the high expression of IL-6 would prolong FBR by altering macrophages polarization, promoting monocyte differentiation and enhancing Janus Kinase/Signal transducer and activator of transcription (JAK/STAT3) activation, a critical inflammatory signaling pathway [32–34]. Thus, the expression of TNF-α and IL-6 was evaluated and results indicated release of them were decreased in a dose-dependent manner by PDA extract treatment in LPS-induced macrophages, which suggested PDA might be an excellent agent to limit FBR.

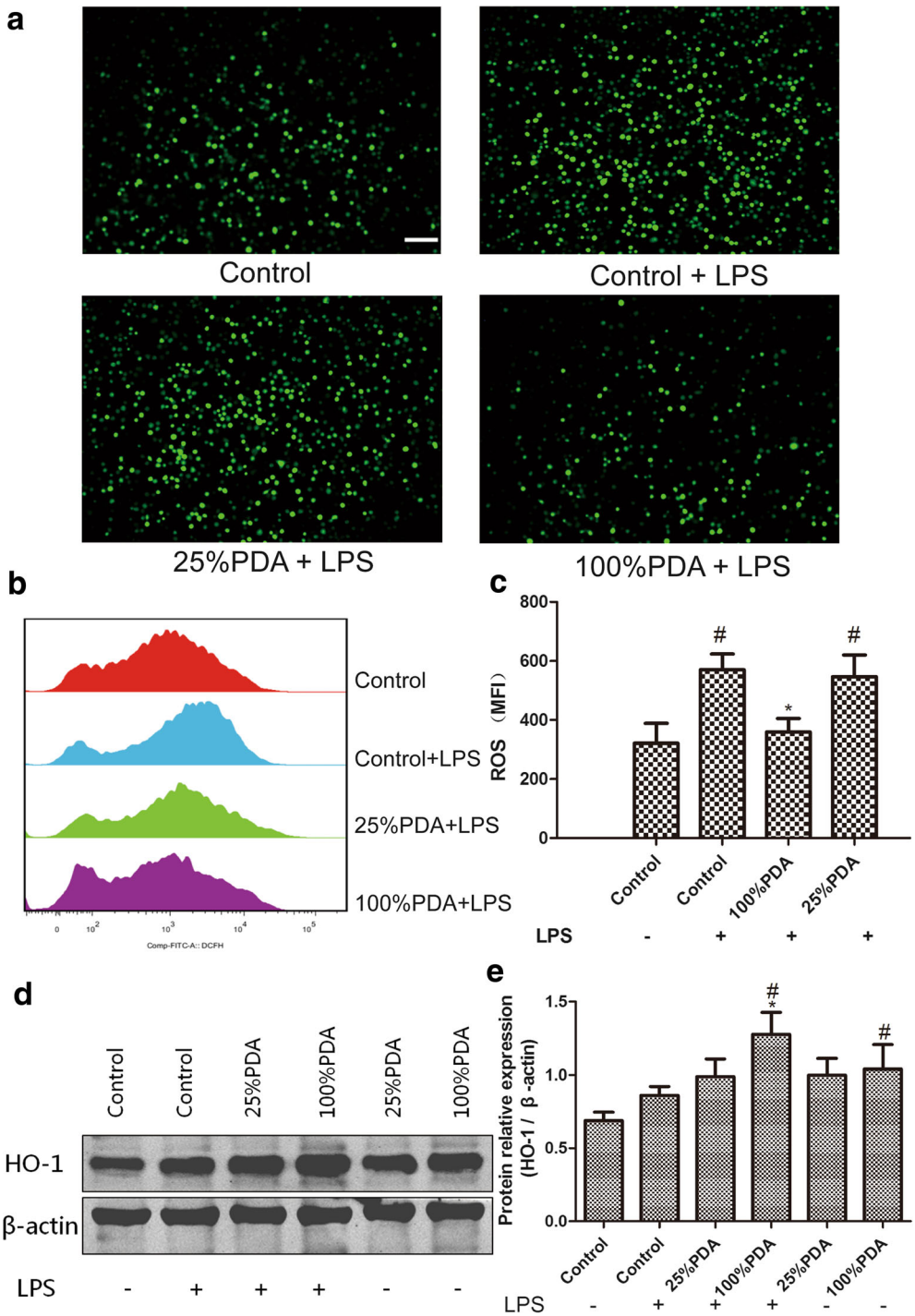

Fig. 7. Effect of PDA extracts on antioxidant in LPS-induced macrophages. Cells were pretreated with various PDA extracts for 3 h and then stimulated with LPS for 1 h. **a** The ROS level of macrophages was assayed by fluorescence microscope using DCFH probe. Scale bar = 100 μm. **b**, **c** Intracellular ROS expression was also detected by FACS using DCFH probe. **d**, **e** Macrophages were subjected to the same treatment as above-mentioned for 48 h. The protein expression of HO-1 in macrophages was determined by western blot. $^{\#}P < 0.05$ *vs.* control group; $^{*}P < 0.05$ *vs.* LPS-induced control group.

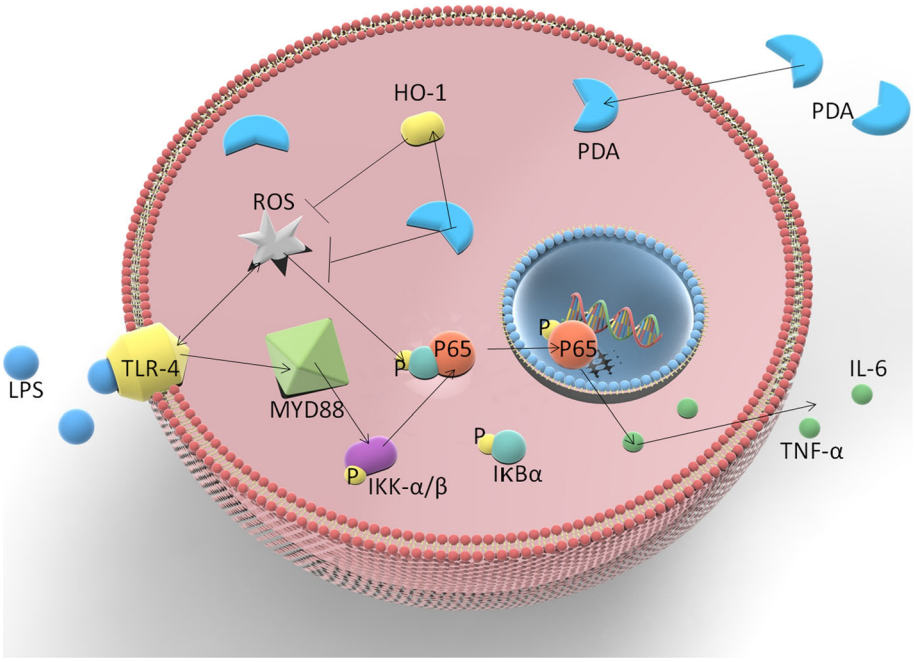

Fig. 8. Schematic illustration about the potential regulatory mechanism of the degradation products of PDA to LPS-induced inflammatory responses in macrophages. The PDA extract could scavenge intracellular ROS and upregulate the HO-1-related antioxidant signaling pathway, which lead to the inactivation of LPS-induced TLR-4-MYD88-NF-κB signaling pathway in macrophages.

Toll-like receptors (TLRs) were a group of pattern recognition receptors which represented critical elements in the initiation and progression of inflammatory cytokine production in response to PAMPs or DAMPs [35]. TLR-4 was one of most important TLRs which induced downstream signals, MYD88 dependent and independent, that resulted in cytokine production and initiated an immune response after implantation [36]. So far, there was little report about the relationship between PDA and TLR-4 signaling pathway. Our study demonstrated the TLR-4 and MYD88 expression in LPS-induced macrophages were reduced by the PDA extracts, indicating that PDA might have a protective effect against inflammation due to TLR-4 activation. Furthermore, previous reports had shown NF-κB signaling pathway was a crucial downstream of TLR-4 dependent pathway during innate immune [37]. Thereby, we evaluated the levels of NF-κB pathway components, and the results indicated that the phospho-IKK-α/β and phospho–IκBα levels were partially decreased, which proved the NF-κB signaling pathway with LPS stimulation was inactivated after PDA extract treatment. Based on these results, the PDA extract-induced decrease of TNF-α and IL-6 production after LPS stimulation might be related to the inactivation of TLR-4/MYD88/NF-κB pathway in macrophages.

The increase of ROS could enhance NF-κB activity by inducing p65 translocation into nuclear to bind target genes [38, 39], while LPS treated macrophages exhibited slightly elevated ROS expression as shown in Fig. 7a–c. Thus, it was reasonable that decreased ROS level would result in an anti-inflammatory effect. Accordingly, Zhao, H et al. reported that the production of ROS in LPS-induced Raw 264.7 macrophages was diminished by PDA nanoparticles [22] and Quan, W *et al*. also found PDA coating owned antioxidant properties [40]. In addition to the antioxidant of PDA, Liebscher, J *et al*. reported that dopamine showed obvious reducibility in terms of its containing phenol hydroxyl group [41]. Thus, our results indicated PDA extracts were able to decrease ROS production, which were in line with these previous reports. Interestingly, we first found PDA extracts could increase HO-1 activity which, in turn, inhibited inflammatory response through redox systems [42]. Although, in the present study, the degradation products of PDA exhibited an anti-inflammatory capacity through mediating with TL-4/NF-κB signaling pathway in the LPS-induced macrophages, the rest of inflammatory signaling pathways have not been studied yet. In addition, further experiments on its *in vivo* capacity of anti-inflammation should be implemented.

Altogether, these findings might provide some degree of evidence to promote the wide use of PDA in medical fields in terms of anti-inflammation application.

CONCLUSION

The present study proved that PDA negatively mediated inflammatory response of macrophage triggered by LPS. Our results first demonstrated PDA limited the release of pro-inflammatory cytokines by down-regulating TLR-4/MYD88 expression and NF-κB signaling pathway, which might be through controlling ROS production and activating HO-1-related antioxidant signaling pathway (Fig. 8). Altogether, these findings provided supports to evaluate the optimal effects of PDA application in clinic.

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COMPLIANCE WITH ETHICAL STANDARDS

Competing Interests. The authors declare that they have no competing interests.

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