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Research Article

Inhibition of NLRP3 Inflammasome Contributes to Protective Effect of 5,14-HEDGE Against Lipopolysaccharide-induced Septic Shock

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Abstract

Background and Objective: Nucleotide binding domain and leucine-rich repeat protein 3 (NLRP3) is reported to be involved in the pathogenesis of numerous inflammatory diseases including Alzheimer disease, Parkinson disease, Prion disease and type 2 diabetes mellitus. Previous studies have demonstrated that a stable synthetic analog of 20-hydroxyeicosatetraenoic acid (20-HETE), N-(20-hydroxyeicosa-5[Z],14[Z]-dienoyl)glycine (5,14-HEDGE), prevents vascular hyporeactivity, hypotension, tachycardia, inflammation and mortality in a rodent model of septic shock. This study was aimed to assess effect of 5,14-HEDGE on the changes in NLRP3/apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC)/pro-caspase-1 inflammasome in lipopolysaccharide (LPS)-induced septic shock in rats. **Methodology:** Rats were injected with saline (4 mL kg^{-1}) or LPS (10 mg kg^{-1}) at time 0. Blood pressure and heart rate were measured using a tail-cuff device. 5,14-HEDGE (30 mg kg^{-1}) was administered to rats 1 h after injection of saline or LPS. The rats were sacrificed 4 h after saline or LPS injection and kidney, heart, thoracic aorta and superior mesenteric artery were isolated for measurement of caspase-1/11 p20, NLRP3, ASC and β-actin proteins as well as interleukin-1β (IL-1β) levels. Data were analysed by one-way ANOVA followed by Student-Newman-Keuls test for multiple comparisons, Kruskal-Wallis test followed by Dunns test for multiple comparisons and Student's test or Mann-Whitney U tests when appropriate. **Results:** Blood pressure decreased by 33 mmHg and heart rate increased by 63 bpm in the LPS-treated rats. In the LPS-treated rats, tissue protein expression of caspase-1/11 p20, NLRP3 and ASC in addition to IL-1β levels were increased. The 5,14-HEDGE prevented the LPS-induced changes. **Conclusion:** These findings suggest that inhibition of renal, cardiac and vascular formation/activity of NLRP3/ASC/pro-caspase-1 inflammasome involves in the protective effect of 5,14-HEDGE on LPS-induced septic shock in rats.

Key words: 20-HETE, 5,14-HEDGE, blood pressure, heart rate, inflammation, IL-1β, lipopolysaccharide, NLRP3 inflammasome

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammasomes localized in the cytoplasm of the cells are responsible for the maturation of proinflammatory cytokines and activation of an extremely inflammatory form of cell death "pyroptosis[1,2]". Essential components of inflammasomes are reported to be nucleotide binding and oligomerization domain-like receptor proteins, the adaptor molecule apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) and caspase-1. By far the most investigated inflammasome, nucleotide binding domain and leucine-rich repeat (NLR) protein 3 (NLRP3) inflammasome, consists of NLRP3, ASC and pro-caspase-1. NLRP3 responds to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Activation of the NLRP3 inflammasome requires two separate signals[1,2]: (1) Basal expression of the precursor of IL-1β in addition to the NLRP3 protein is scarcely detectable, thus a priming step (signal 1) is required to drive their transcription. (2) Once primed, NLRP3 inflammasome activation (signal 2), results in its oligomerization and inflammasome assembly, ultimately leading to caspase-1-dependent cleavage and secretion of pro-IL-1β and pro-IL-18. In the canonical pathway, NLRP3 inflammasome activation in macrophages requires two signals[3]. The first signal (priming), is initiated by toll-like receptors (TLRs), nucleotide-binding and oligomerization domain 2, tumor necrosis factor (TNF) receptor (TNFR) 1 or TNFR2, which engages nuclear factor-κB (NF-κB)-mediated expression of NLRP3. The second signal is ensured by PAMPs or DAMP which activate NLRP3 to innitiate inflammasome assembly, release of IL-1β and IL-18 and pyroptosis. An alternative or "noncanonical NLRP3 inflammasome pathway", described by its requirement for caspase-11 is activated by Gram-negative bacteria, including *Escherichia coli* (*E. coli*). The upstream mechanism ruling activation of this pathway involves T LR4-Toll-IL-1 receptor domain-containing adapter-inducing interferon-β-mediated recognition of extracellular lipopolysaccharide (LPS), at least in part, to engage expression of caspase-11.

A w-hydroxylation product of arachidonic acid (AA), 20-hydroxyeicosatetraenoic acid (20-HETE), is produced by cytochrome P450 (CYP), mainly by the CYP4A and CYP4F isoforms and involved in LPS-induced acute systemic inflammation[4,5]. Previous studies have demonstrated that increased CYP4A1 expression and activity participates in the beneficial effects of a 20-HETE mimetic, N-(20-hydroxyeicosa-5[Z],14[Z]-dienoyl)glycine(5,14-HEDGE) in a rodent model of septic shock[6-11]. The results also showed that down regulation of the myeloid differentiation factor 88 (MyD88)/transforming growth factor-activated kinase 1 (TAK1)-dependent signalling pathway participates in the protective effect of 5,14-HEDGE[7]. However, the effect of 20-HETE and/or its analogs on the NLRP3 inflammasome pathway *in vitro* and *in vivo* conditions especially during endotoxemia has not been investigated. Therefore, the aim of this study was to determine whether inhibition of renal, cardiac and vascular formation/activity of NLRP3/ASC/pro-caspase-1 inflammasome participates in the protective effect of 5,14-HEDGE in a rat model of endotoxemia.

MATERIALS AND METHODS

Animals: Experiments were carried out on Wistar rats (male; 220-260 g; n = 24) (Research Center of Experimental Animals, Mersin University, Mersin, Turkey) fed a standard chow. They were synchronized by maintenance of controlled environmental conditions during the experiments. The circadian rhythmicity of the animals was entrained by a standardized 12 h light and 12 h dark cycle. All experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Ethics Committee of Mersin University School of Medicine. The study was carried out from May to October, 2016.

Endotoxic shock model: Endotoxic shock was induced in rats as previously described by Tunctan *et al.*[12]. Rats were randomly separated into saline (n = 6), LPS (n = 6), saline plus 5,14-HEDGE (n = 6) and LPS plus 5,14-HEDGE (n = 6) groups. In the saline- and 5,14-HEDGE groups, rats were injected with saline (4 mL kg^{-1}, intraperitoneally [i.p.]) at time 0. Rats in the LPS- and LPS plus 5,14-HEDGE-treated groups were injected with LPS (*E. coli* LPS, O111:B4; Sigma Chemical Co., St. Louis, MO, USA) (10 mg kg^{-1}, i.p., sublethal dose) at time 0. In the 5,14-HEDGE- and LPS plus 5,14-HEDGE-treated groups, rats were injected with 5,14-HEDGE (30 mg kg^{-1}, subcutaneously [s.c.])[6-11] 1 h after injection of saline or LPS, respectively. The 5,14-HEDGE was provided by one of the authors John R. Falck. mean arterial pressure (MAP) and heart rate (HR) of the animals were measured using a tail-cuff device (MAY 9610 Indirect Blood Pressure Recorder System, Commat Ltd., Ankara, Turkey) at time 0 and 1, 2, 3 and 4 h. Rats were sacrificed 4 h after the saline or LPS injection and kidney, heart, thoracic aorta and superior mesenteric artery were isolated from all animals. The tissues were homogenized as previously described by Tunctan *et al.*[12] and the samples were used for measurement of caspase-1 p20, caspase-11 p20,

NLRP3, ASC and β-actin proteins and IL-1β levels as described below. Total protein in these fractions was determined by the coomassie blue method using bovine serum albumin as standard[13].

Immunoblotting: Immunoblotting for caspase-1 p20, caspase-11 p20, NLRP3, ASC and β-actin proteins were carried out according to the method described previously[6-11,14]. Briefly, tissue homogenates (108-160 mg of protein) were exposed to a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) and there after proteins were transferred to a nitrocellulose membrane. The membranes were blocked with non-fat dry milk in Tris-buffered saline (5%) and incubated with primary antibodies in bovine serum albumin (BSA) (1:500 in 5% BSA) overnight at 4°C. The following primary antibodies were used: (1) caspase-1 p20 antibody (D-4) (sc-398715, Santa Cruz); (2) caspase-11 p20 antibody (A-2) (sc-374615, Santa Cruz); (3) NLRP3 antibody (MBS2526381, MyBioSource) and (4) ASC antibody ([N-15]-R) (sc-22514-R, Santa Cruz). After that, the membranes were incubated with sheep anti-mouse IgG-horseradish peroxidase (RPN4201, Amersham, Life Sciences, Cleveland, OH, USA) for caspase-1 p20 and caspase-11 p20. Goat anti-rabbit IgG-horseradish peroxidase (RPN4301, Amersham) was also used for NLRP3 and ASC in BSA (1:1.000 in 0.1%). The blots were exposed to enhanced chemiluminescence (ECL Prime Western Blotting Detection Reagent) (RPN2232, Amersham) according to the instructions of the manufacturer. A gel-imaging system (EC3-CHEMI HR imaging system; Ultra-Violet Products, UVP, Cambridge, UK) was used for visualization of immunoreactive proteins. Densitometric analysis was performed by using NIH image software (Image J 1.46r, Wayne Rasband, National Institute of Health, Bethesda, MD, USA). The same membranes were probed with mouse monoclonal anti-β-actin antibody (A1978, Sigma) (1:500 in 5% BSA) as a control followed by incubation with sheep anti-mouse IgG-horseradish peroxidase (1:1.000 in 0.1% BSA). Relative densities of immunoreactive blots for caspase-1 p20, caspase-11 p20, NLRP3 and ASC proteins were normalized to the corresponding band densities for β-actin.

Measurement of IL-1β levels: Tissue IL-1β levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer in the Rat IL-1 beta ELISA Kit (ELR-IL1b-CL, RayBiotech).

Statistical analysis: Data are expressed as Mean±Standard Error of Means (SEM). Data were analysed by one-way ANOVA followed by Student-Newman-Keuls test for multiple comparisons, Kruskal-Wallis test followed by Dunns test for multiple comparisons and Student's t test or Mann-Whitney U tests when appropriate. A $p<0.05$ was considered to be statistically significant[15].

RESULTS

Effects of 5,14-HEDGE MAP and HR: In the LPS-treated rats, MAP was decreased (Fig. 1a) and HR was increased (Fig. 1b) over the 4 h course of the experiment ($p<0.05$). These changes reached a maximum 4 h after LPS administration. The MAP decreased by 33 mmHg and HR increased by 63 bpm in the LPS-treated rats. 5,14-HEDGE prevented the LPS-induced changes in MAP and HR ($p<0.05$). The MAP and HR were not changed inthe rats treated with saline and 5,14-HEDGE ($p>0.05$).

Effects of 5,14-HEDGE on caspase-1 p20 and caspase-11 p20 protein expression: To investigate effect of 5,14-HEDGE on caspase-1 and caspase-11 (as an index for formation/activity of NLRP3/ASC/procaspase-1 inflammasome), caspase-1 p20 and caspase-11 p20 protein levels were measured in the tissues of endotoxemic rats. The LPS increased expression of caspase-1 p20 and caspase-11 p20 proteins in the kidney (Fig. 2a,b), heart (Fig. 2c, d), thoracic aorta (Fig. 2e, f) and superior mesenteric artery (Fig. 2g, h) ($p<0.05$). 5,14-HEDGE prevented the increase in caspase-1 p20 and caspase-11 p20 protein expression produced by LPS kidney (Fig. 2a, b), heart (Fig. 2c, d), thoracic aorta (Fig. 2e, f) and superior mesenteric artery (Fig. 2g, h) ($p<0.05$). Protein expression of caspase-1 p20 and caspase-11 p20 in the saline- and 5,14-HEDGE-injected rats were not changed by 5,14-HEDGE (Fig. 2) ($p>0.05$).

Effects of 5,14-HEDGE on NLRP3 protein expression: To investigate effect of 5,14-HEDGE on NLRP3 expression, NLRP3 protein levels were measured in the tissues of endotoxemic rats. LPS increased expression of NLRP3 protein in the kidney (Fig. 3a), heart (Fig. 3b), thoracic aorta (Fig. 3c) and superior mesenteric artery (Fig. 3d) ($p<0.05$). 5,14-HEDGE prevented the increase in NLRP3 protein expression produced by LPS in the kidney (Fig. 3a), heart (Fig. 3b), thoracic aorta (Fig. 3c) and superior mesenteric artery (Fig. 3d) ($p<0.05$). Protein expression of NLRP3 in the saline- and 5,14-HEDGE-injected rats was not changed by 5,14-HEDGE (Fig. 3) ($p>0.05$).

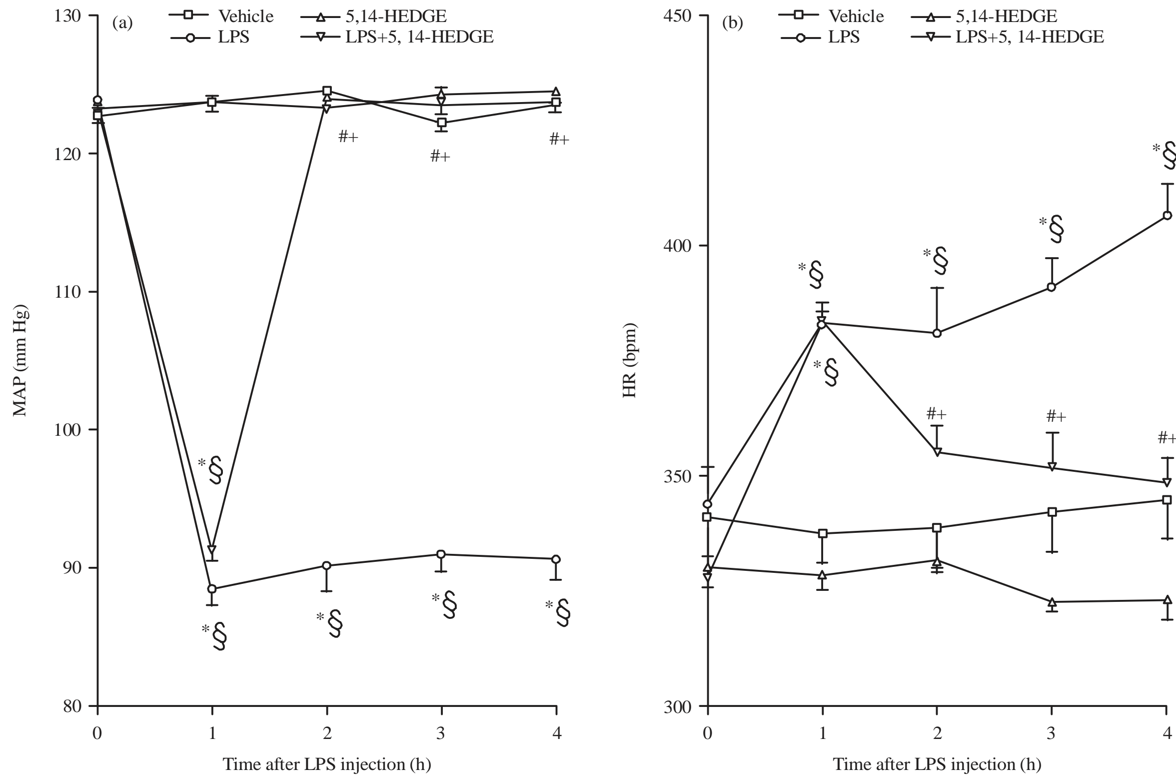
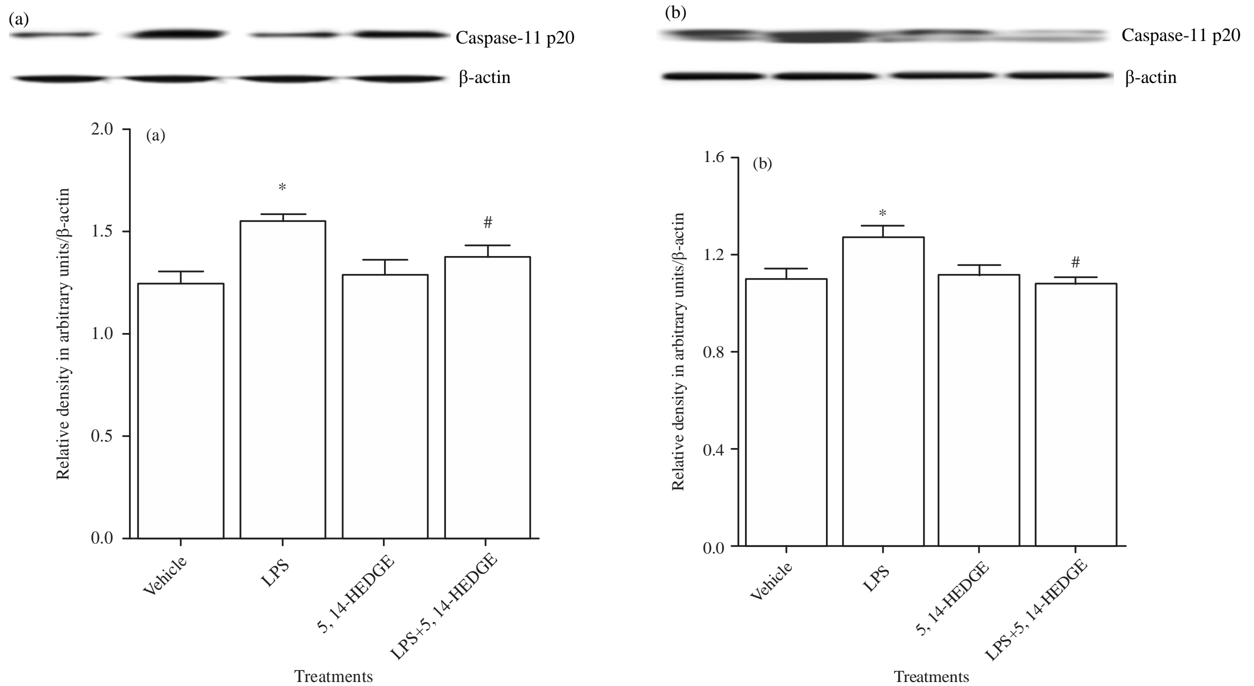


Fig. 1(a-b): Time course of the effects of 5,14-HEDGE on (a) MAP and (b) HR following injection of saline (vehicle) or LPS to rats
Data represent the Mean±SEM of 6 animals, *Statistically different from the corresponding value seen in rats treated with saline ($p<0.05$), #Statistically different from the corresponding value seen in the rats treated with LPS ($p<0.05$), §Statistically different from the time 0 h value within a group ($p<0.05$), +Statistically different from the time 1 h value within a group ($p<0.05$)



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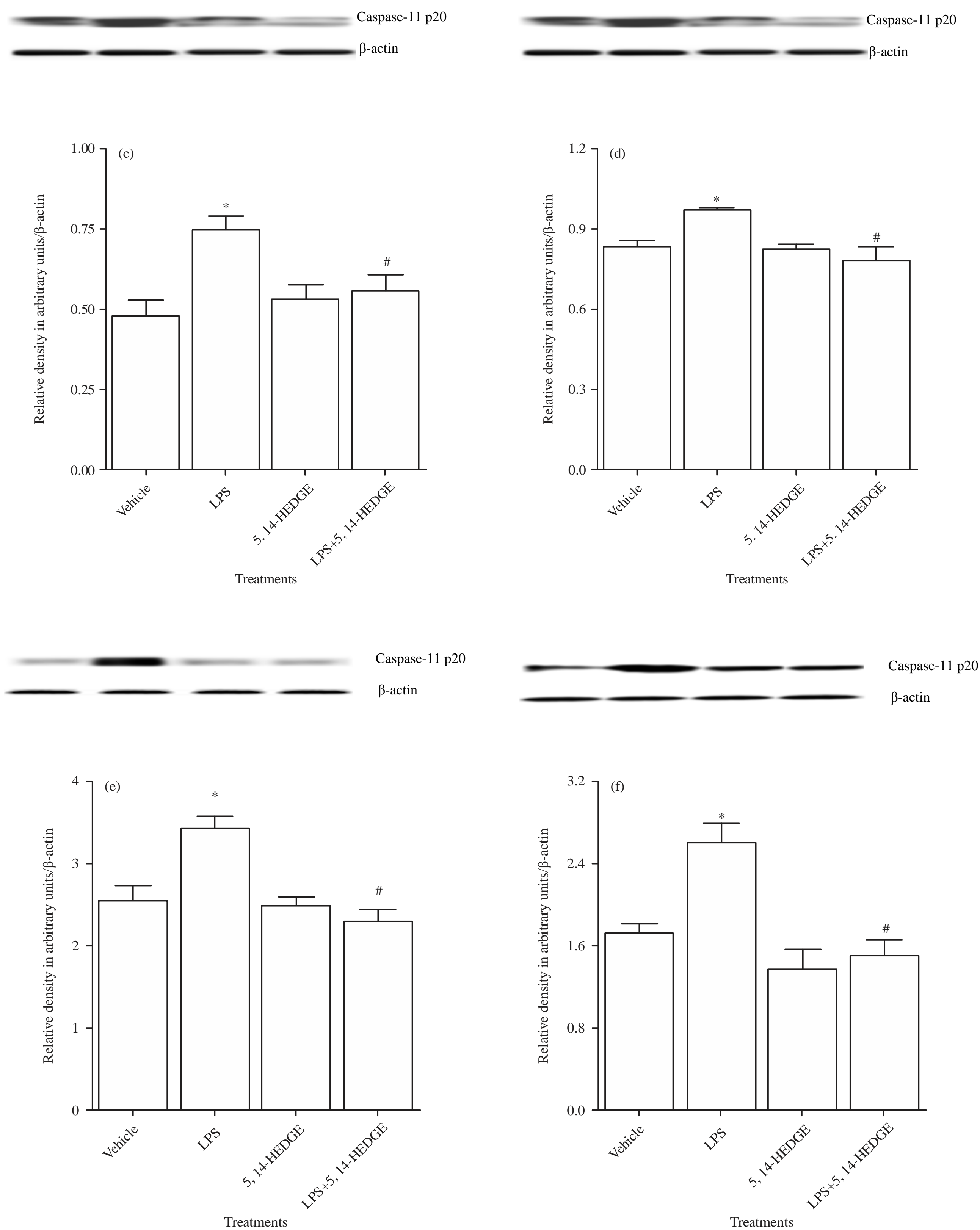

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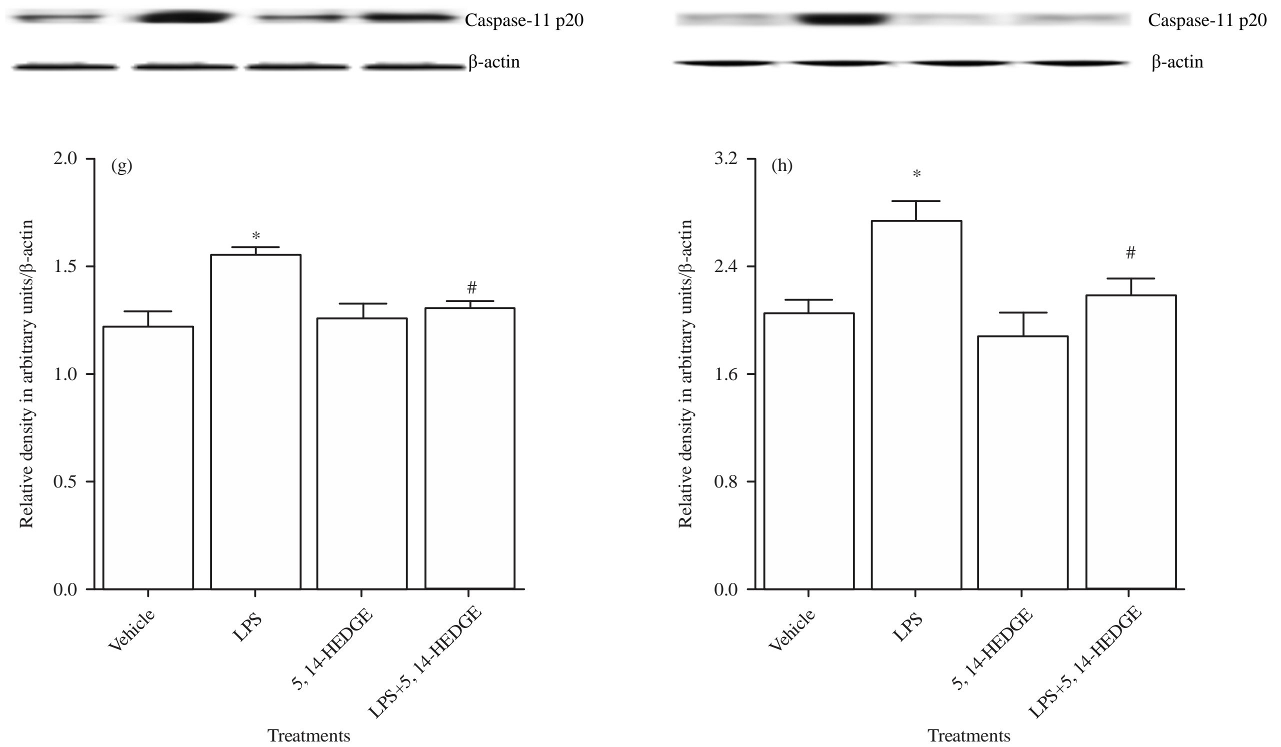
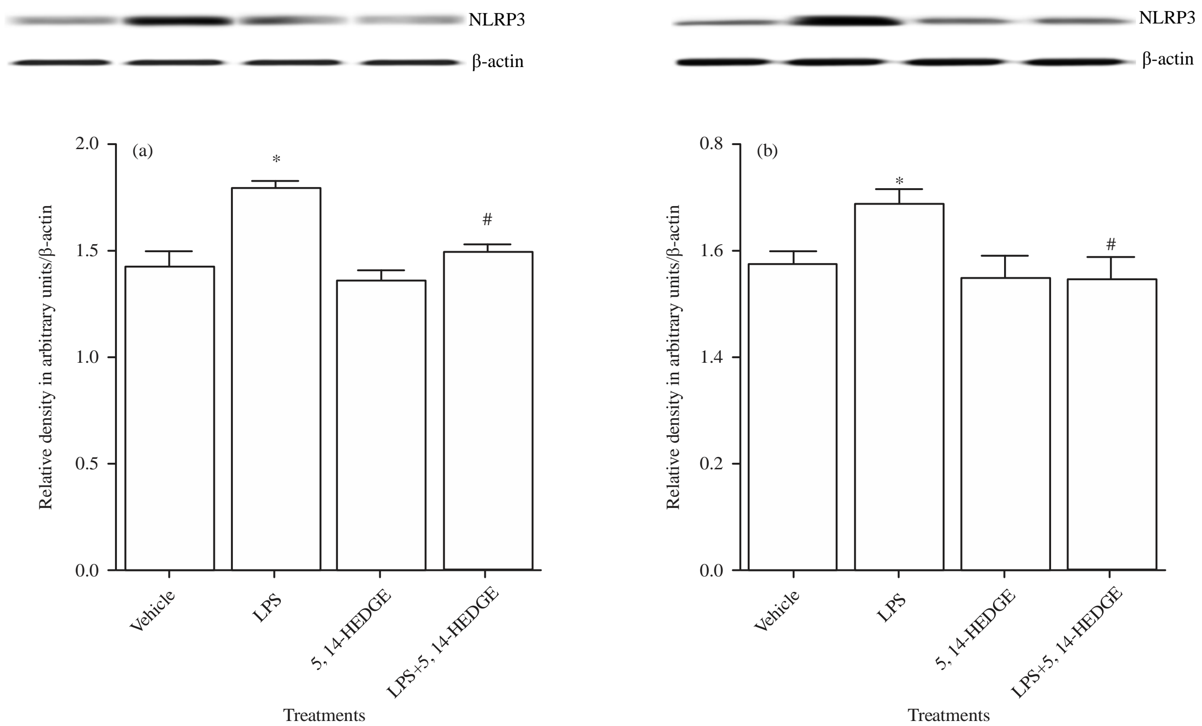


Fig. 2(a-h): Effects of 5,14-HEDGE on the LPS-induced increase in expression of caspase-1 p20 and caspase-11 p20 proteins in (a, b) Kidney, (c, d) Heart, (e, f) Thoracic aorta and (g, h) Superior mesenteric artery of control and endotoxemic rats
Caspase-1 p20 and caspase-11 p20 protein expression in tissue homogenates was measured by immunoblotting. Data represent the Mean±SEM of 4 animals, *p<0.05 vs. control group, #p<0.05 vs. LPS group



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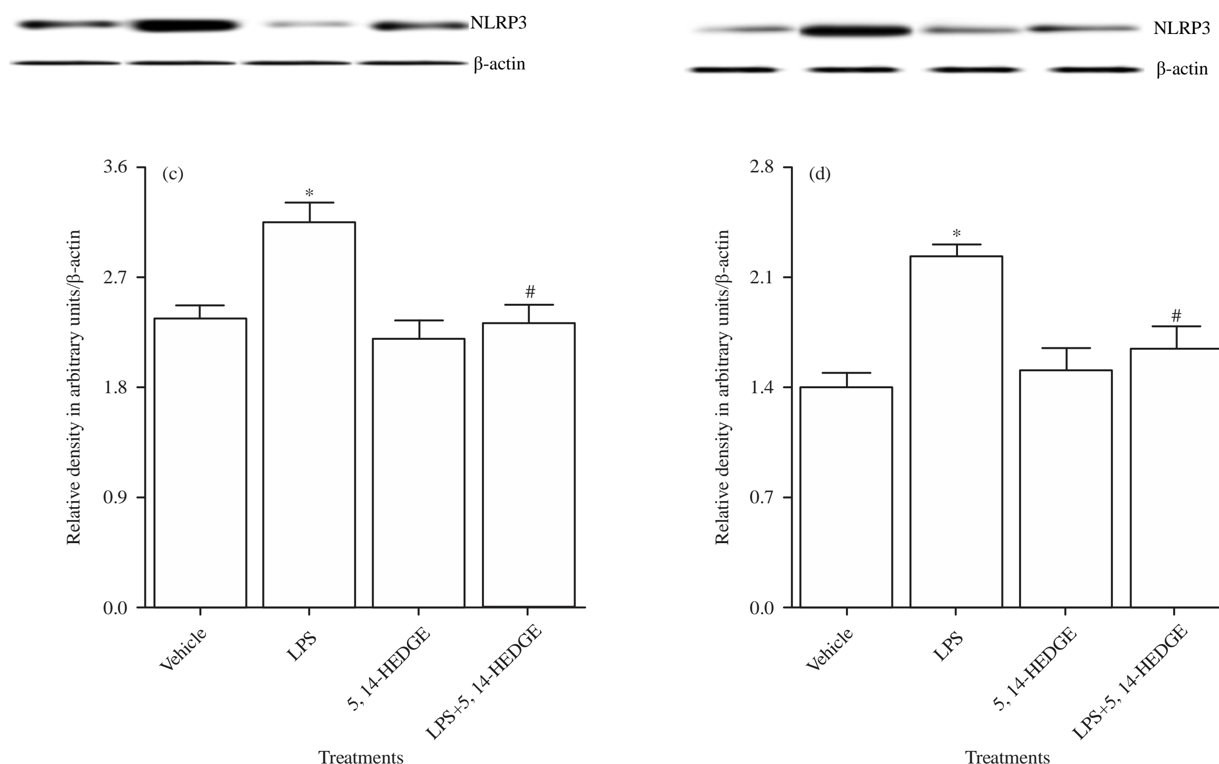

Fig. 3(a-d): Effect of 5,14-HEDGE on the LPS-induced increase in expression of NLRP3 protein in (a) Kidney, (b) Heart, (c) Thoracic aorta and (d) Superior mesenteric artery of control and endotoxemic rats

NLRP3 protein expression in tissue homogenates was measured by immunoblotting. Data represent the Mean±SEM of 4 animals, *p<0.05 vs. control group, #p<0.05 vs. LPS group

Effects of 5,14-HEDGE on ASC protein expression: To investigate effect of 5,14-HEDGE on ASC expression, ASC protein levels were measured in the tissues of endotoxemic rats. LPS increased expression of ASC protein in the kidney (Fig. 4a), heart (Fig. 4b), thoracic aorta (Fig. 4c) and superior mesenteric artery (Fig. 4d) (p<0.05). 5,14-HEDGE prevented the increase in ASC protein expression produced by LPS in the kidney (Fig. 4a), heart (Fig. 4b), thoracic aorta (Fig. 4c) and superior mesenteric artery (Fig. 4d) (p<0.05). The ASC protein expression was not changed in the saline- and 5,14-HEDGE-injected rats (Fig. 4) (p>0.05).

Effects of 5,14-HEDGE on IL-1β formation: To investigate the effect of 5,14-HEDGE on IL-1β formation (as an index for NLRP3/ASC/procaspase-1 inflammasome activity), IL-1β levels were measured in the tissues endotoxemic rats. LPS increased IL-1β levels in the kidney (Fig. 5a), heart (Fig. 5b), thoracic aorta (Fig. 5c) and superior mesenteric artery (Fig. 5d) (p<0.05). The increase in IL-1β levels produced by LPS was prevented by 5,14-HEDGE in the kidney (Fig. 5a), heart (Fig. 5b), thoracic aorta (Fig. 5c) and superior mesenteric artery (Fig. 5d) (p<0.05). Tissue levels of IL-1β were not changed in the rats treated with saline and 5,14-HEDGE (Fig. 5) (p>0.05).

DISCUSSION

The results of the study suggest that LPS administration increases expression of caspase-1 p20, caspase-11 p20, NLRP3 and ASC proteins in addition to IL-1β levels in the kidney, heart, thoracic aorta and superior mesenteric artery. These findings also suggest that 5,14-HEDGE, a 20-HETE mimetic, prevents hypotension, tachycardia and inflammation in the LPS-induced septic shock model in rats. These effects of 5,14-HEDGE may be due to decreased expression of caspase-1 p20, caspase-11 p20, NLRP3 and ASC in addition to IL-1β levels. Overall, these findings suggest that inhibition of formation/activity of NLRP3/ASC/pro-caspase-1 inflammasome contributes to the protective effect of 5,14-HEDGE against hypotension, tachycardia and inflammation during endotoxemia (Fig. 6).

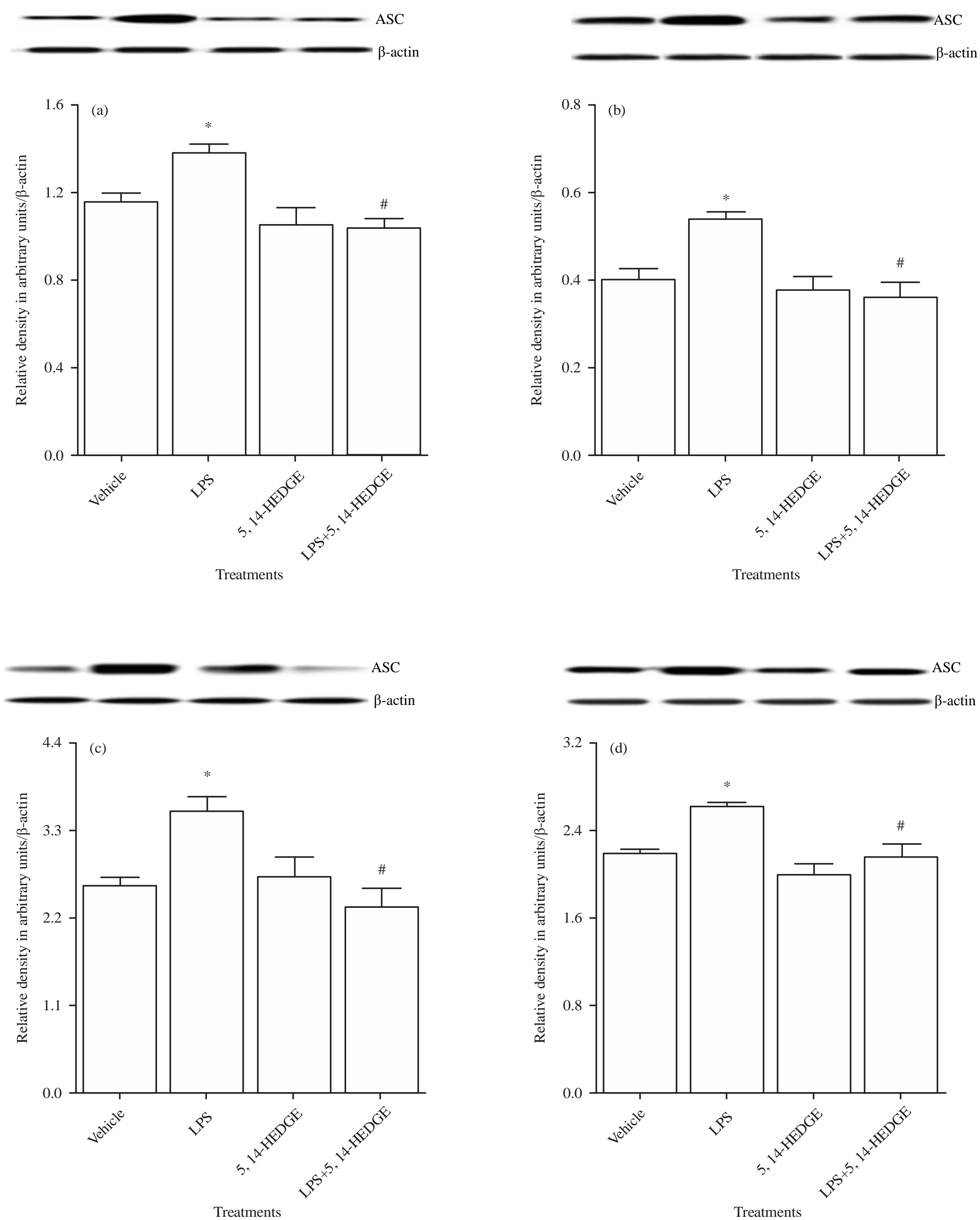

Fig. 4(a-d): Effect of 5,14-HEDGE on the LPS-induced increase in expression of ASC protein in (a) Kidney, (b) Heart, (c) Thoracic aorta and (d) Superior mesenteric artery of control and endotoxemic rats

ASC protein expression in tissue homogenates was measured by immunoblotting. Data represent the Mean±SEM of 4 animals, *p<0.05 vs. control group, #p<0.05 vs. LPS group

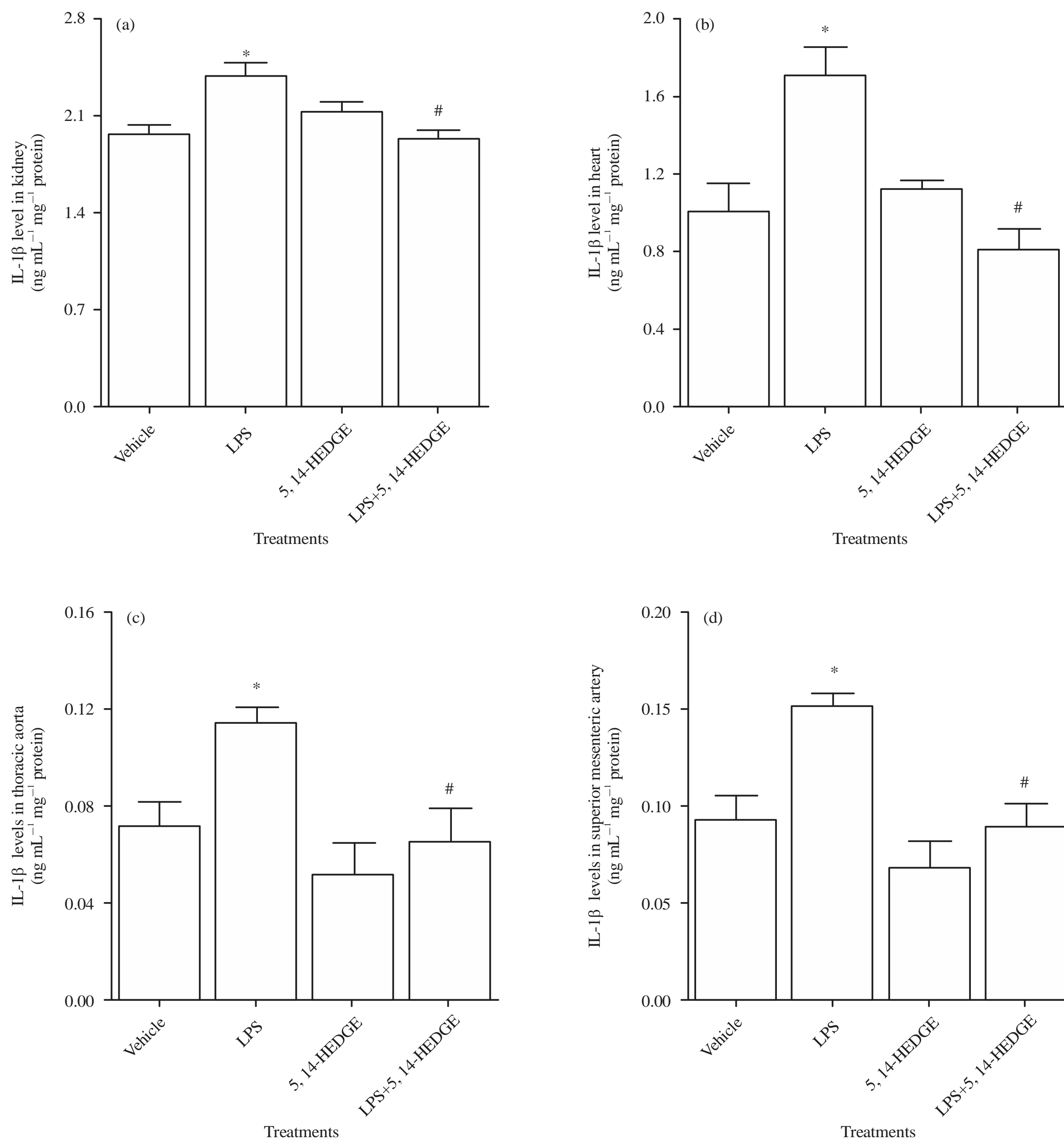

Fig. 5(a-d): Effect of 5,14-HEDGE on the LPS-induced increase in IL-1β levels in (a) Kidney, (b) Heart, (c) Thoracic aorta and (d) Superior mesenteric artery of control and endotoxemic rats

IL-1β levels in tissue homogenates were measured by the Rat IL-1β ELISA Kit following the instructions of the manufacturer. Data represent the Mean±SEM of 5 animals, *p<0.05 vs. control group, [#]p<0.05 vs. LPS group

The LPS-induced systemic inflammation is reported to be involved in the release of proinflammatory mediators to the circulation. These mediatorsaugment the molecular and cellular responses resulting in injury of several organs, primarily kidney, heart, lung and brain, associated with inflammation[5,16,17]. Stimulation of NF-κB in response to LPS leads increased production of proinflammatory mediators produced in certain cells, such as blood-borne cells (BBCs), vascular smooth muscle cells (VSMCs) and endothelial cells resulting in inflammation[4,16,17]. These include: Eicosanoids, cytokines and reactive nitrogen and oxygen species (RNS and ROS)[4,16,17]. Inflammasomes are multi protein complexes which trigger the activation of inflammatory caspases, maturation of proinflammatory cytokines and cell death[1,3,18-20]. Among the NLR family, NLRP3 is reported to be involved in the pathogenesis of several diseases including Alzheimer disease,

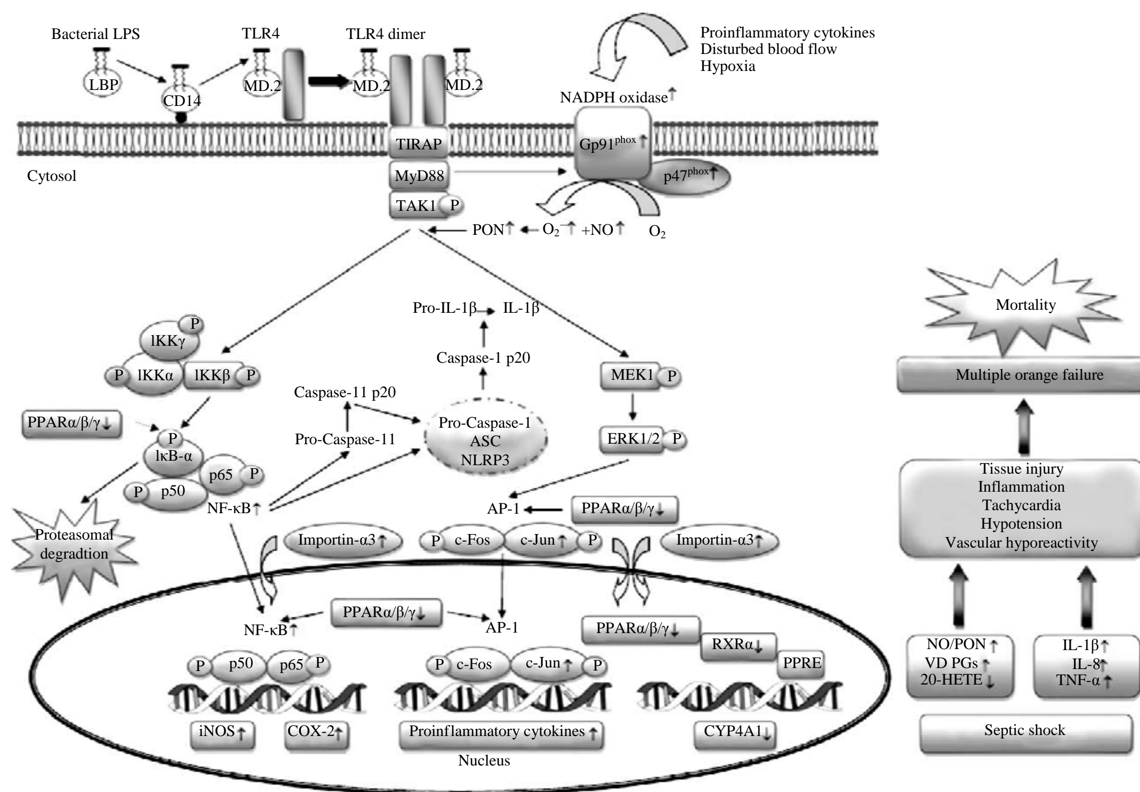

Fig. 6: A schematic model demonstrating involvement of LPS-induced changes in NLRP3/ASC/pro-caspase-1 inflammasome formation/activity based on the findings of the present study and our previous results

↑: Decrease, ↓: Increase

Parkinson disease, Prion disease and type 2 diabetes mellitus characterized by inflammatory reaction[2,20,21]. The NLRP3 is expressed in some cell types, such as VSMCs, endothelial cells and BBCs. Expression of NLRP3 can be induced by proinflammatory cytokines (e.g., TNF-a) and TLR agonists (e.g., LPS) in a NF-kB-dependent manner[1,3,18-20]. Recently, the NLRP3 inflammasome has been reported to be activated following injection of LPS[22-31]. In the LPS-induced septic shock, compensatory mechanisms have been stated to be responsible for the changes in the production of vasoregulatory molecules which could contribute to decrease in blood pressure[5]. These include: (1) increased sensitivity of baroreceptor reflex mechanisms, (2) activation of the renin-angiotensin-aldosterone system and (3) overproduction of catecholamines, PGs, RNS (e.g., nitric oxide [NO] and peroxynitrite (PON)], ROS [e.g., superoxide (O_{2-})]) and endothelin-1[5]. The NO has also been reported to prevent NLRP3 activation and so has a beneficial effect in the septic shock induced by LPS[31-33]. In addition, it has been reported that ROS derived from NOX enzymes is essential for LPS-induced activation of NLRP3 inflammasome[34-36]. Furthermore, it has been demonstrated that vascular hyporeactivity, hypotension, tachycardia, inflammation and tissue injury were prevented by 5,14-HEDGE in endotoxemic rats and LPS-induced mortality in mice[6-11]. In these experiments, 5,14-HEDGE increased expression of peroxisome proliferator-activated receptor (PPAR) α/β/γ and retinoid X receptor (RXR) α, CYP2C23 and CYP4A1 in addition to formation of antiinflammatory and vasoconstrictor eicosanoids (i.e., epoxyeicosatrienoic acids and 20-HETE, respectively). It also suppressed MyD88/TAK1/IkB kinase (IKK) β/inhibitor of kB (IkB)-α/NF-κB and MyD88/TAK1/mitogen-activated protein kinase kinase (MEK) 1/extracellular signal-regulated kinase (ERK) 1/2/activator protein (AP)-1 pathways. Additionally, it inhibited soluble epoxide hydrolase activity associated with importin-α3 protein expression. Moreover, inducible NO synthase (iNOS), soluble guanylyl cyclase, protein kinase G, cyclooxygenase (COX)-2 and nicotinamide adenine dinucleotide phosphate oxidase (NOX) subunits (NOX2 [gp91^{phox}; a O_{2-}generating NOX enzyme] and NOXO2 [p47^{phox}; organizer subunit of gp91^{phox}]) expression/activity were inhibited by 5,14-HEDGE. Hence, it prevented formation of O_{2-}, NO, PON and vasodilator (VD) prostaglandins (PGs) (i.e., PGI_2 and PGE_2). Furthermore,

decreased formation of proinflammatory cytokines (i.e., TNF-α and IL-8) and and serum levels of microribonucleic acids (miRNAs) (i.e., miR-150, miR-223 and miR-297) by 5,14-HEDGE was observed. These findings also showed that inhibition of formation/activity of NLRP3/ASC/pro-caspase-1 inflammasome reverses inflammatory hyperalgesia in LPS-treated mice in addition to the changes in NF-kB expression/activity, caspase-11 and isoforms of NOX and NOS[12]. In the present study, administration of LPS decreased MAP and augmented HR in addition to increased caspase-1 p20, caspase-11 p20, NLRP3 and ASC expression associated with IL-1β levels in the renal, cardiac and vascular tissues of these animals. Furthermore, administration of 5,14-HEDGE reversed the hypotension and tachycardia induced by LPS in addition to increased caspase-1 p20, caspase-11 p20, NLRP3 and ASC expression and IL-1β levels. In only one previous study, MCC950 (a selective inhibitor of NLRP3 inflammasome) has been shown to prevent rise in serum levels of IL-1β and IL-6 caused by LPS (*E. coli* LPS, 055:B5) whereas it did not noticeably decrease TNF-α levels in C57BL/6 mice[37]. In another study, IL-1β secretion and processing of caspase-1 in response to NLRP3 stimulation and absent in melanoma (AIM)-2 by preventing oligomerization of ASC in LPS-treated murine macrophages was inhibited by MCC950[38]. In the study, MCC950 also prevented AIM2-dependent pyroptosis in the LPS-treated cells[38]. Overall, the results of the present study suggest that inhibition of NLRP3/ASC/pro-caspase-1 inflammasome formation/activity also participates in the beneficial effect of 5,14-HEDGE on LPS-induced septic shock in rats.

Even though, we have not completely characterized the mechanism of inhibitory effect of 5,14-HEDGE on NLRP3 inflammasome, it can be speculated that 5,14-HEDGE interact with NLRP3 and influence a critical step in its activation resulting in IL-1β production and inflammation. It is also possible that 5,14-HEDGE might decrease NLRP3 inflamasome formation and/or activity indirectly by decreasing the effects of RNS, ROS, eicosanoids, cytokines, and/or transcription factors on the NLRP3 inflammasome. However, further experiments should be performed to verify the validity of the hypothesis. Considering that inflammasomes are mainly multiprotein complexes of BBCs, this leads to the interpretation that the primary effect of LPS and 5,14-HEDGE in this study could be on the BBCs existing in these tissues. It is also likely that the changes in the NLRP3 inflammasome and MyD88/TAK1 signalling pathway in the cardiovascular and renal tissues as well as the BBCs remained in the tissues could be inhibited by 5,14-HEDGE directly or indirectly. However, additional studies are required to identify the site of action of 5,14-HEDGE in LPS-stimulated BBCs, VSMCs and endothelial cells in relation with cardiovascular and renal tissues which are extremely vascularised.

CONCLUSION

The data obtained from this study provide an evidence that inhibition of NLRP3/ASC/pro-caspase-1 inflammasome formation/activity in renal, cardiac and vascular tissues of endotoxemic rats involves in the protective effect of 5,14-HEDGE. Considering the critical role of 20-HETE in the control of blood pressure and inflammatory processes, additional experiments with 20-HETE mimetics in animal models of septic shock could provide novel therapeutic strategies to prevent hypotension, tachycardia and inflammation leading to multiple organ failure and death due to endotoxemia.

SIGNIFICANCE STATEMENTS

This study discovers the inhibitory effect of 5,14-HEDGE, a 20-HETE mimetic, on the formation/activity of NLRP3/ASC/pro-caspase-1 inflammasome that can be beneficial for treatment of septic shock. This study will help the researcher to uncover the critical area of controlling of blood pressure and inflammatory processes during endotoxemia that many researchers were not able to explore. Thus a new theory on the decreased production of 20-HETE associated with increased activity of NLRP3 inflammasome leading to hypotension, tachycardia and inflammation which results in multiple organ failure and death, may be arrived at the conclusion regarding the importance of possible use of 20-HETE mimetics in treatment of septic shock.

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