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Ginkgolide A inhibits lipopolysaccharide-induced inflammatory response in human coronary artery endothelial cells via downregulation of TLR4-NF-κB signaling through PI3K/Akt pathway

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Ginkgolide A (GA) is a one of the active components of Ginkgo biloba. We aimed to detect the effects GA on the lipopolysaccharide (LPS)-induced inflammatory response in human coronary artery endothelial cells (HCAECs) and whether the effects are associated with the inhibition of toll-like receptor 4 (TLR4)-NF-κB signaling through PI3K/Akt pathway. HCAECs were stimulated with LPS and treated with GA or TLR4 inhibitor CLI-095. A PI3K/Akt inhibitor LY294002 was used to block the PI3K/Akt pathway. The toxic effects of GA, LPS and LY294002 on HCAEC were evaluated using MTT assay. Levels inflammatory mediators, TLR4 mRNA, NF-κB signaling activity were valuated. We found LPS stimulation significantly increased the release of IL-6, IL-8, MCP-1 and TNF-α from HCAECs, elevated the TLR4 mRNA expression and activated the NF-κB signaling. GA and CLI-095 abolished the LPS-induced inflammatory mediator release and NF-κB signaling activation, and GA reduced the TLR4 mRNA expression without affecting cell viability. However, PI3K/Akt blocking abolished the effects of GA on HCAECs. We conclude that GA could attenuate the LPS-induced inflammatory response in HCAECs and the anti-inflammatory activity might be associated with the inhibition of TLR4-NF-κB signaling through PI3K/AKT pathway. These findings suggest a therapeutic potential of GA in endothelial inflammation.

1. Introduction

Cardiovascular disease is the leading cause of death and disability worldwide, and is a huge drain on public health expenditure (Zhang et al. 2014; Liu et al. 2015). Endovascular interventional therapy is an effective treatment option for some of the patients with cardiovascular diseases (Biondi-Zoccai et al. 2014; Silver and Behrouz 2015). However, the interventional operation may injure the vascular endothelial cells and induce inflammatory response, which might subsequently lead to re-blocking of the artery. So, agents that can inhibit endothelial inflammatory response seem necessary for patients with cardiovascular diseases.

Vascular endothelial cells, lining the entire circulatory system, thus separating blood and extra-vascular tissues, play a crucial role in providing the proper haemostatic balance. Under physiological conditions, endothelial cells prevent thrombosis by means of different anticoagulant and antiplatelet mechanisms (Rajendran et al. 2013; Wang and Ding 2015). Yet, endothelial cells are involved in the initiation and progression of atherosclerosis when injured, ultimately leading to blocking of arteries. Accumulating evidence supports the role of local and systemic inflammation as a common pathophysiological mechanism in cardiovascular diseases including atherosclerosis (Mangge et al. 2014; Roth Flach et al. 2015). Elevated levels of inflammatory mediators, such as IL-6, IL-8, MCP-1 and TNF-α are found in atherosclerosis (Wang et al. 2012; Xu et al. 2013; Yin et al. 2013). The cytokines act on endothelial cells, leading to a cascade of inflammatory reactions and endothelial cell dysfunction. However, there is evidence showing that endothelial cells are not only the targets but, following proper stimulation, contribute to cytokine secretion themselves (Zeuke et al. 2002). Based on the effects of inflammatory response in endothelial cell dysfunction and atherosclerosis, anti-inflammatory management is regarded as a potential therapeutic strategy for cardiovascular diseases (Li et al. 2011; Siti et al. 2015).

Lipopolysaccharide (LPS) can activate inflammatory processes. In preclinical studies, it is widely used in to induce inflammatory response in animals and cellular systems, and to evaluate anti-inflammatory effects of medications (Zeuke et al. 2002; Zhao et al. 2015). Toll-like receptor (TLR) 4, a pattern-recognition receptor, is identified as the most important transmembrane signaling receptor for LPS. After binding to its ligand such as LPS, TLR4 initiates a series of signaling cascades, which may lead to NF-κB activation and inflammatory cytokine release (Song et al. 2016). There is evidence that LPS can influence vascular endothelial cell through TLR4 mediated signaling pathways (Zeuke et al. 2002). However, the expression of TLR4 and the production of inflammatory mediators can be regulated by the PI3K/Akt signaling transduction pathway (Ke et al. 2012; Dong et al. 2014). Activating the PI3K/Akt pathway by medication has been proved to inhibit the LPS-induced inflammatory cytokine release and TLR4 expression in microglia (Ke et al. 2012). Thus, regulating the PI3K/Akt pathway might be also beneficial in inhibiting inflammatory response in other cellular systems.

Ginkgo biloba, a herb, has a long history of medical use for various diseases including cardiovascular diseases. Several extracts from *Ginkgo biloba* were obtained and used to treat inflammatory disease, cardiovascular diseases, neurologic diseases, liver diseases and tumor. Some of them exert their effects though regulating the NF-κB signaling pathway (Li et al. 2009). Ginkgolide A (GA), one of the major constituents of *Ginkgo biloba*, is reported to have multiple pharmacological activities (Chen et al. 2012). It has been tested in several cardiovascular cellular systems. Zhou et al. (2006) found GA attenuated homocysteine-induced endothelial dysfunction in porcine coronary arteries. Zhao et al. (2015) reported GA reduced inflammatory response in high-glucose-stimulated human umbilical vein endothelial cells through the STAT3-mediated pathway. HCAECs were shown to respond to LPS (Raveendran et al. 2011).

However, no study investigated the effects of GA on the LPS induced cell damage and inflammatory response in HCAECs. Thus, this study aimed to detect whether GA would exhibit anti-inflammatory effects in LPS-treated HCAEC, and whether this effect might be associated with regulating the PI3K/Akt mediated TLR4 -NF-κB signaling pathway.

2. Investigations and results

2.1. Toxic effects of GA, LPS and LY294002 on the viability of HCAECs

The toxic effects of GA, LPS and LY294002 on HCAECs were evaluated using MTT assay. HCAECs were pretreated with or without the presence of GA (10 μM or 20 μM). Then cells were stimulated by LPS (10 ng/mL) with or without the presence of the PI3K/Akt inhibitor LY294002 (10 μM) and the TLR4 inhibitor CLI-095 (5 μM) for 24 h. The results of the viability evaluation showed that GA 10 μM, GA 20 μM, LPS, LPS+CLI-095, LPS+GA 10 μg, LPS+GA 20 μg, LPS+GA 20 μg+LY294002 had no obvious toxic effects on HCAECs (Table 1).

Table 1: Toxic effects of GA, LPS and LY294002 on the viability of HCAECs

	Cell viability
Control	1±0.09
GA 10 μM	1.03±0.08
GA 20 μM	1.09±0.11
LPS	0.96±0.12
LPS+ CLI-095	1.06±0.09
LPS+GA 10 μM	1.05±0.13
LPS+GA 20 μM	1.08±0.10
LPS+GA 20μM +LY294002	0.98±0.08

Data are presented as mean±SD. [a]$P<0.05$, vs. the control group.

2.2. GA inhibited the LPS-induced TLR4 mRNA expression in HCAECs

As shown in Table 2, treatment with LPS for 24 h led to a marked increase in TLR4 mRNA expression in HCAECs. GA (10 μM and 20 μM) partially abolished the LPS-induced TLR4 mRNA expression, and GA 20 μM was more potent than GA 10 μM. However, the inhibitory effect of GA (20 μM) on the LPS-induced TLR4 mRNA expression was abolished by the PI3K/Akt inhibitor LY294002, which confirmed the role of the PI3K/Akt pathway in the inhibition of TLR4 mRNA expression by GA.

2.3. GA and CLI-095 inhibited the LPS-induced activation of NF-κB signaling in HCAEC

As shown in Tables 2 and 3, treatment with LPS for 24 h significantly increased levels of NF-κB p65 (both protein and mRNA), p-NF-κB p65 and p-IκBα in HCAECs. Additionally, LPS treatment significantly increased the NF-κB p65 DNA binding activity in HCAECs. The data suggested that LPS activated NF-κB signaling in HCAECs. The specific TLR4 inhibitor CLI-095 completely reversed the increases of NF-κB p65 (both protein and mRNA), p-NF-κB p65, p-IκBα and NF-κB p65 DNA binding activity in HCAECs, suggesting that TLR4 played a crucial role in the LPS-induced NF-κB signaling activation (Tables 2, 3).
Similarly to the TLR4 inhibitor, GA (10 μM and 20 μM) partially abolished the LPS-induced NF-κB signaling activation in a dose-dependent manner. However, the inhibitory effects of GA (20 μM) on the LPS-induced NF-κB signaling activation were abolished by PI3K/Akt inhibitor LY294002 (Tables 2, 3), which confirmed the role of the PI3K/Akt pathway in the inhibition of NF-κB signaling activation by GA in HCAECs.

Table 2: Levels of TLR-4 mRNA, NF-κB p65 mRNA and NF-κB p65 DNA binding activity

	TLR-4 mRNA	NF-κB p65 mRNA	NF-κB p65 DNA binding activity
Control	1.00±0.08	1.00±0.12	0.20 ±0.02
LPS	5.72±0.85 [a]	7.51±1.30 [a]	0.86±0.10a [a]
LPS+ CLI-095	6.85±1.20 [a]	1.29±0.23	0.23±0.03
LPS+GA 10 μM	4.06±0.51 [a,b]	4.85±0.69 [a,b]	0.60±0.08b [a,b]
LPS+GA 20 μM	2.75±0.36 [a,b,c]	2.08±0.33 [a,b,c]	0.39±0.05bc [a,b,c]
LPS+GA 20 μM +LY294002	7.09±1.40 [a,d]	8.49±1.50 [a,d]	0.90±0.11 [a,d]

Data are presented as mean±SD. [a]$P<0.05$, vs. the control group; [b]$P<0.05$, vs. the LPS group; [c]$P<0.05$, vs. the LPS+GA 10 μM group; [d]$P<0.05$, vs. the LPS+GA 20 μM group.

Table 3: Levels of NF-κB p65, p-NF-κB p65 and p-IκBα

	NF-κB p65	p-NF-κB p65	p-IκBα
Control	1.00±0.08	1.00±0.06	1.00±0.08
LPS	6.39±1.14 [a]	9.16±1.55 [a]	6.21±0.90 [a]
LPS+ CLI-095	1.15±0.21	1.09±0.13	1.11±0.16
LPS+GA 10 μM	4.16±0.63 [a,b]	7.05±1.18 [a,b]	4.70±0.64 [a,b]
LPS+GA 20 μM	2.30±0.41 [a,b,c]	3.07±0.50 [a,b,c]	2.38±0.43 [a,b,c]
LPS+GA 20 μM +LY294002	8.08±1.37 [a,d]	11.90±2.11 [a,d]	7.40±1.31 d [a,d]

Data are presented as mean±SD. [a]$P<0.05$, vs. the control group; [b]$P<0.05$, vs. the LPS group; [c]$P<0.05$, vs. the LPS+GA 10 μM group; [d]$P<0.05$, vs. the LPS+GA 20 μM group.

2.4. GA and CLI-095 inhibited the LPS-induced production of cytokines in HCAEC

Levels of some inflammatory mediators were determined. As shown in Tables 4 and 5, LPS significantly increased the production of IL-6, IL-8, MCP-1 and TNF-α from HCAECs. However, the TLR4 inhibitor CLI-095 completely inhibited the increases of IL-6, IL-8, MCP-1 and TNF-α in the LPS-treated HCAECs, suggesting that TLR4 played a crucial role in the LPS-induced production of these inflammatory mediators in HCAECs.
Similarly to the TLR4 inhibitor, GA (10 μM and 20 μM) down-regulated the LPS-induced production of IL-6, IL-8, MCP-1 and TNF-α from HCAEC in a dose-dependent manner. However, the down-regulatory effects of GA (20 μM) on the LPS-induced production of these inflammatory mediators were abolished by PI3K/Akt inhibitor LY294002 (Tables 4, 5).

3. Discussion

The anti-inflammatory effects of GA have been reported in some animal models and cellular systems. Yet, little is known about its effects on the endothelial cell associated inflammatory response.

Table 4: Levels of mRNA expression of IL-6, IL-8, MCP-1 and TNF-α in HCAECs

	IL-6 mRNA	IL-8 mRNA	MCP-1 mRNA	TNF-α mRNA
Control	1.00±0.08	1.00±0.12	1.00±0.08	1.00±0.10
LPS	13.27±1.85 [a]	10.34±1.31 [a]	9.01±1.55 [a]	5.03±0.82 [a]
LPS+ CLI-095	1.10±0.13	1.20±0.22	1.17±0.25	1.06±0.11 a
LPS+GA 10 μM	9.86±1.90 [a,b]	7.05±1.03 [a,b]	6.68±0.91 [a,b]	3.27±0.49 [a,b]
LPS+GA 20 μM	4.09±0.50 [a,b,c]	3.53±0.65 [a,b,c]	3.01±0.51 [a,b,c]	1.85±0.41 [a,b,c]
LPS+GA 20 μM +LY294002	14.08±2.10 [a,d]	10.02±1.86 [a,d]	12.11±2.30 [a,d]	6.83±1.08 [a,d]

Data are presented as mean±SD. [a]$P<0.05$, vs. the control group; [b]$P<0.05$, vs. the LPS group; [c]$P<0.05$, vs. the LPS+GA 10 μM group; [d]$P<0.05$, vs. the LPS+GA 20 μM group.

Table 5: Levels of of IL-6, IL-8, MCP-1 and TNF-α

	IL-6	IL-8	MCP-1	TNF-α
Control	1.00±0.11	1.00±0.15	1.00±0.13	1.00±0.11
LPS	12.04±2.05 [a]	11.28±1.59 [a]	13.30±2.08 [a]	7.59±1.04 [a]
LPS+ CLI-095	1.08±0.15	1.14±0.20	1.17±0.24	1.10±0.16 a
LPS+GA 10 μM	8.21±1.70 [a,b]	7.69±1.13 [a,b]	8.93±1.36 [a,b]	4.05±0.63 [a,b]
LPS+GA 20 μM	3.83±0.48 [a,b,c]	3.01±0.57 [a,b,c]	4.51±0.88 [a,b,c]	2.10±0.33 [a,b,c]
LPS+GA 20 μM +LY294002	14.66±2.31 [a,d]	13.55±2.40 [a,d]	15.63±2.79 [a,d]	8.91±1.77 [a,d]

Data are presented as mean±SD. [a]$P<0.05$, vs. the control group; [b]$P<0.05$, vs. the LPS group; [c]$P<0.05$, vs. the LPS+GA 10 μM group; [d]$P<0.05$, vs. the LPS+GA 20 μM group.

In this study, we for the first time demonstrated that GA inhibited the LPS-induced inflammatory response in HCAECs via downregulation of TLR4-NF-κB signaling through the PI3K/Akt pathway. Inflammation is involved in the pathophysiology of endothelial cell damage and atherosclerosis (Botti et al. 2012; Roth Flach et al. 2015). In the inflammatory process, the artery endothelial cell is not only the target but, following proper stimulation, contributes to cytokine secretion itself (Zeuke et al. 2002), which may further exacerbate the inflammatory cascade reactions. LPS can activate inflammatory processes in animals and cellular systems. HCAECs have been shown to respond to LPS (Raveendran et al. 2011). In this study, we treated the HCAECs with LPS and found that LPS significantly promoted the release of IL-6, IL-8, MCP-1 and TNF-α, which are cytokines that are involved in the pathophysiology of atherosclerosis (Wang et al. 2012; Yin et al. 2013; Xu et al. 2013; Lin et al. 2014). GA has exhibited anti-inflammatory properties in recent *in vivo* and *in vitro* studies (Zhao et al. 2015; Ye et al. 2016). However, the effects of GA on LPS treated HCAECs have not been investigated. In this study, we demonstrated that GA could reduce the production of cytokines IL-6, IL-8, MCP-1 and TNF-α from the LPS-treated HCAECs in a dose-dependent manner. This would be beneficial for the attenuation of the inflammatory cascade reactions. The inhibition of these inflammatory mediators has exhibited protective effects on cardiovascular system (Li et al. 2011; Siti et al. 2015). Our findings suggest a therapeutic potential of GA in inflammation- associated cardiovascular damage. In order to detect the mechanism of the inhibitory effects of GA on the release of the cytokines in the LPS-treated HCAECs, we investigated the changes of TLR4-NF-κB signaling. LPS may induce inflammatory response via activating several types of receptors, such as TLR2 and TLR4 (Lüttgenau et al. 2016). TLR4 is the main receptor of LPS, serving as the primary mediator of innate immune responses to LPS by triggering a cascade of pro-inflammatory events. A key downstream signal of TLR4 is mediated by NF-κB. NF-κB is located in the cytoplasm in its inactive form, combined with its inhibitor IκB. The binding between the LPS-activated TLR4 and MyD88 ultimately results in the phosphorylation of IκB. The phosphorylated IκB loses its inhibitory effect on NF-κB and NF-κB is activated by phosphorylation. The phosphorylated NF-κB, the active form of NF-κB, moves into the nucleus and induces the genes transcription, leading to the production of the pro-inflammatory cytokines. It has been demonstrated that IL-6, IL-8, TNF-α and MCP-1 are all downstream inflammatory mediators of NF-κB signaling (Lei et al. 2016). The expression of TLR4 on HCAECs surface has recently been confirmed (Zeuke et al. 2002; Raveendran et al. 2011). In this study, we found that LPS significantly increased the expression of TLR4 mRNA in HCAECs. And consistently, LPS significantly increased the levels NF-κB p65, p-NF-κB p65 and p-IκBα in HCAECs. The results indicated that LPS might activate the NF-κB signaling via promoting TLR4 expression in the HCAECs. In order to confirm the role of TLR4 signaling in the NF-κB activation and the release of IL-6, IL-8, TNF-α and MCP-1 by LPS in HCAECs, we cultured the LPS-treated HCAECs in the presence of a specific TLR4 inhibitor CLI-095. We found the TLR4 inhibitor to completely reverse the increases in levels of NF-κB p65, p-NF-κB p65, p-IκBα, IL-6, IL-8, TNF-α and MCP-1. The findings confirmed that the LPS-induced NF-κB activation and inflammatory mediator release were mediated through TLR4 signaling. Our results are consistent with the findings in a study that reported TLR4-mediated inflammatory activation of HCAECs by LPS (Zeuke et al. 2002). Additionally, results presented in our study showed that GA inhibited TLR4 mRNA expression, NF-κB activation and cytokine production in a dose-dependent manner in the LPS-treated HCAECs. These findings suggested that the anti-inflammatory activity of GA in the LPS-treated HCAECs might be associated with downregulation of TLR4-NF-κB signaling.

It has been demonstrated that TLR4 expression can be regulated by the PI3K/Akt pathway (Ke et al. 2012; Dong et al. 2014). PI3K/Akt was reported to be involved in inflammatory response regulation. Studies showed that PI3K/Akt activation had a role in the negative regulation of LPS-induced IL-6, IL-8 and TNF-α release from microglial cells (Tarassishin et al. 2011) and bone marrow macrophages (Ke et al. 2012). Other studies demonstrated that PI3K/Akt is a potent suppressor of inflammatory response (Dong et al. 2014; Rong and Xijun 2015; Li et al. 2016). To test the role of PI3K/Akt in the inhibition of TLR4-NF-κB signaling by GA in this study, the PI3K/Akt inhibitor LY294002 (Chang et al. 2016) was used to block the PI3K/AKT signaling. Interestingly, we found that the inhibitory effect of GA on the TLR4 mRNA expression was abolished by the PI3K/Akt inhibitor in the LPS-treated HCAECs. Consistently, the inhibitory effects of GA on the LPS-induced NF-κB activation and cytokine production in the HCAECs were also abolished by the PI3K/Akt inhibitor. All the results indicated that GA might inhibit the TLR4-NF-κB signaling mediated inflammatory response through the PI3K/AKT pathway.

In conclusion, LPS induced inflammatory response by activating TLR4-NF-κB signaling in HCAECs. GA could dose-dependently inhibit the LPS-induced inflammatory response in HCAECs and the anti-inflammatory activity might be associated with the inhibition of TLR4-NF-κB signaling through the PI3K/AKT pathway. These findings suggest that GA may have a therapeutic potential in endothelial inflammation associated cardiovascular disease.

4. Experimental

4.1. Cell culture

HCAECs were obtained from Cell Applications Inc (San Diego, CA, USA) and grown in ECM endothelial growth medium (ScienCell, San Diego, CA, USA) supplemented with 5% fetal bovine serum and 100 U/mL penicillin/streptomycin with 5% CO_2 at 37 °C. Cells from passages 3-5 were used for further experiments.

4.2. Toxic effects of GA, LPS and LY294002 on the viability of HCAEC

The effect of on cell viability was determined using 3-(4,5-dimethylthazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) assay. After grown in 96-well plates for 24 h, cells were pretreated with or without GA (10 μM or 20 μM) for 1 h. Then they were stimulated by LPS (10 ng/mL) with or without the presence of PI3K/Akt inhibitor LY294002 (10 μM) and TLR4 inhibitor CLI-095 (5 μM) for 24 h. After that, MTT (Amresco, Solon, OH, USA) at a final concentration of 0.5 μg/mL was added to the wells. The cells were incubated with MTT for 4 h at 37 °C in the dark. The medium was removed and the crystals were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). The absorbance was measured at 570 nm using a microplate reader (Thermo Scientific, Waltham, MA, USA). Cell viability = (experimental group absorbance value/control group absorbance value) × 100%.

4.3. ELISA assay

The cell-free culture medium was collected for measurements of inflammatory cytokines IL-6, IL-8, TNF-α-1 and MCP. The supernatants of HCAECs lysates were collected for the investigations of NF-κB p65, phosphorylated NF-κB p65 (p-NF-κB p65) and phosphorylated IκB-α (p-IκBα). Levels of these parameters were determined using commercially available enzyme-linked immunosorbence assay (ELISA) kits according to the instructions provided by the manufacturers. ELISA kits: NF-κB p65 (Cusabio Company, Wuhan, China); p-NF-κB p65 and p-IκBα (Cell Signaling, Beverly, Massachusetts, USA); IL-6, IL-8, MCP-1 and TNF-α (R&D Systems, Inc., Minneapolis, MN, USA).

4.4. NF-κB p65 DNA binding activity assay

Nuclear protein was extracted from HCAEC using a nuclear extraction kit (Active Motif, Carlsbad, CA, USA). NF-κB p65 DNA-binding activity was measured using TranAM™ NF-κB p65 Chemi transcription factor assay kit was used (Active Motif, Carlsbad, CA, USA). The NF-κB p65 DNA-binding activity was determined by measuring the absorbance at 450.

4.5. PCR assay

Total RNA was isolated from HCAECs using Trizol reagents (Invitrogen Corp, Eugene, OR). Total RNA was converted to cDNA using the iScript cDNA synthesis kit (Thermo Scientific, Shanghai, China). cDNA was amplified by real-time polymerase chain reaction (RT-PCR). Reactions were run on a 7500 Real Time PCR system (Thermo Scientific, Beijing, China) using the following conditions: 95 °C for 3 min followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s. The primers were as follows: GAPDH forward, 5′ ACAACTTTGGTATCGTGGAAGG 3′ and reverse, 5′ GCCATCACGCCACAGTTTC 3′; TLR4 forward, 5′ GCCTTTCAGGGAATTAAGC TCC 3′ and reverse, 5′ GATCAACCGATGGACGTGTAAA 3′; NF-κB p65 forward, 5′- TCAATGGCTACACAGGACCA-3′ and reverse, 5′- CACTGTCACCTGGAAGC AGA -3′; TNF-α forward, 5′ GTAGCCCATGTTGTAGCAAACC 3′ and reverse, 5′ C TGATGGTGTGGGTGAGGAG 3′; IL-6 forward, 5′ AGAGGCACTGGCAGAA AACAAC 3′ and reverse, 5′ AGGCAAGTCTCCTCATTGAATCC 3′; IL-8 forward, 5′ TTTCAGAGACAGCAGAGCACACAA3′ and reverse, 5′CACACAGAGCTGCA GAAATCAGG3′; MCP-1 forward, 5′ GCTCATAGCAGCAGCCACCTT 3′ and MCP-1 reverse, 5′ GGAATCCTGAACCCACTT 3′.

4.6. Statistical analysis

All data were expressed as mean±SD. Statistical comparisons were conducted by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Differences were considered statistically significant if $P < 0.05$.

Conflict of interest: None declared.

References

Biondi-Zoccai G, Abbate A, Peruzzi M, Frati G (2014) Commentary: observations, trials, and meta-analyses: the life cycle of evidence-based endovascular therapy. J Endovasc Ther 21: 693-696.

Botti C, Maione C, Dogliotti G, Russo P, Signoriello G, Molinari AM, Corsi MM, Sica V, Cobellis G (2012) Circulating cytokines present in the serum of peripheral arterial disease patients induce endothelial dysfunction. J Biol Regul Homeost Agents 26: 67-79.

Chang MC, Tsai YL, Chang HH, Lee SY, Lee MS, Chang CW, Chan CP, Yeh CY, Cheng RH, Jeng JH (2016) IL-1β-induced MCP-1 expression and secretion of human dental pulp cells is related to TAK1, MEK/ERK, and PI3K/Akt signaling pathways. Arch Oral Biol 61: 16-22.

Chen Y, Wang C, Hu M, Pan J, Chen J, Duan P, Zhai T, Ding J, Xu C (2012) Effects of ginkgolide A on okadaic acid-induced tau hyperphosphorylation and the PI3K-Akt signaling pathway in N2a cells. Planta Med 78: 1337-1341.

Dong H, Zhang X, Dai X, Lu S, Gui B, Jin W, Zhang S, Zhang S, Qian Y (2014) Lithium ameliorates lipopolysaccharide-induced microglial activation via inhibition of toll-like receptor 4 expression by activating thePI3K/Akt/FoxO1 pathway. J Neuroinflammation 11: 140.

Ke B, Shen XD, Ji H, Kamo N, Gao F, Freitas MC, Busuttil RW, Kupiec-Weglinski JW (2012) HO-1-STAT3 axis in mouse liver ischemia/reperfusion injury: regulation of TLR4 innate responses through PI3K/PTEN signaling. J Hepatol 56: 359-366.

Lei Q, Li L, Cai J, Huang W, Qin B, Zhang S (2016) ORF3 of hepatitis E virus inhibits the expression of proinflammatory cytokines and chemotactic factors in LPS-stimulated human PMA-THP1 cells by inhibiting NF-κB pathway. Viral Immunol Jan 15. [Epub ahead of print]

Li CS, Qu ZQ, Wang SS, Hao XW, Zhang XQ, Guan J, Han F (2011) Effects of suxiao jiuxin pill (see test) on oxidative stress and inflammatory response in rats with experimental atherosclerosis. J Tradit Chin Med 31:107-111.

Lin J, Kakkar V, Lu X (2014) Impact of MCP-1 in atherosclerosis. Curr Pharm Des 20: 4580-4588.

Liu A et al, 2015 1. Liu A, Yu Z, Wang N, Wang W (2015) Carotid atherosclerosis is associated with hypertension in a hospital-based retrospective cohort. Int J Clin Exp Med 8: 21932-21938.

Li R, Chen B, Wu W, Bao L, Li J, Qi R (2009) Ginkgolide B suppresses intercellular adhesion molecule-1 expression via blocking nuclear factor-kappaB activation in human vascular endothelial cells stimulated by oxidized low-density lipoprotein. J Pharmacol Sci 110: 362-369.

Li YH, Fu HL, Tian ML, et al. Neuron-derived FGF10 ameliorates cerebral ischemia injury via inhibiting NF-κB-dependent neuroinflammation and activatingPI3K/Akt survival signaling pathway in mice. Sci Rep. 2016; 6: 19869.

Lüttgenau J, Herzog K, Strüve K, Latter S, Boos A, Bruckmaier RM, Bollwein H, Kowalewski MP (2016) LPS-mediated effects and spatio-temporal expression of TLR2 and TLR4 in the bovine corpus luteum. Reproduction 151: 391-399.

Mangge H, Becker K, Fuchs D, Gostner JM (2014) Antioxidants, inflammation and cardiovascular disease. World J Cardiol 2014; 6: 462-477.

Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, Nishigaki I (2013) The vascular endothelium and human diseases. Int J Biol Sci 9: 1057-1069.

Raveendran VV, Tan X, Sweeney ME, Levant B, Slusser J, Stechschulte DJ, Dileepan KN (2011) Lipopolysaccharide induces H1 receptor expression and enhances histamine responsiveness in human coronary artery endothelial cells. Immunology 132: 578-588.

Rong R, Xijun X (2015) Erythropoietin pretreatment suppresses inflammation by activating the PI3K/Akt signaling pathway in myocardial ischemia-reperfusion injury. Exp Ther Med 10: 413-418.

Roth Flach RJ, Skoura A, Matevossian A, Danai LV, Zheng W, Cortes C, Bhattacharya SK, Aouadi M, Hagan N, Yawe JC, Vangala P, Menendez LG, Cooper MP, Fitzgibbons TP, Buckbinder L, Czech MP (2015) Endothelial protein kinase MAP4K4 promotes vascular inflammation and atherosclerosis. Nat Commun 6: 8995.

Silver B, Behrouz R (2015) Endovascular therapy for ischemic stroke. N Engl J Med 372: 2364-2365.

Siti HN, Kamisah Y, Kamsiah J (2015) The role of oxidative stress, antioxidants and vascular inflammation incardiovascular disease (a review). Vascul Pharmacol 71: 40-56.

Song J, Fan HJ, Li H, Ding H, Lv Q, Hou SK (2016) Zingerone ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting Toll-like receptor 4 signaling pathway. Eur J Pharmacol 772: 108-114.

Tarassishin L, Suh HS, Lee SC (2011) Interferon regulatory factor 3 plays an anti-inflammatory role in microglia by activating the PI3K/Akt pathway. J Neuroinflammation 8: 187.

Wang F, Xia W, Liu F, Li J, Wang G, Gu J (2012) Interferon regulator factor 1/retinoic inducible gene I (IRF1/RIG-I) axis mediates 25-hydroxycholesterol- induced interleukin-8 production inatherosclerosis. Cardiovasc Res 93: 190- 199.

Wang Y, Ding Y (2015) Berberine protects vascular endothelial cells in hypertensive rats. Int J Clin Exp Med 8: 14896-14905.

Xu XR, Zou ZY, Xiao X, Huang YM, Wang X, Lin XM (2013) Effects of lutein supplement on serum inflammatory cytokines, ApoE and lipid profiles in early atherosclerosis population. J Atheroscler Thromb 20: 170-177.

Ye N, Wang H, Hong J, Zhang T, Lin C, Meng C (2016) PXR mediated protection against liver inflammation by ginkgolide A in tetrachloromethane treated mice. Biomol Ther (Seoul) 24: 40-48.

Yin K, Tang SL, Yu XH, Tu GH, He RF, Li JF, Xie D, Gui QJ, Fu YC, Jiang ZS, Tu J, Tang CK (2013) Apolipoprotein A-I inhibits LPS-induced atherosclerosis in ApoE(-/-) mice possibly via activated STAT3-mediated upregulation of tristetraprolin. Acta Pharmacol Sin 34: 837-846.

Zhang H, Xu H, Xie H, Li F, Yu X, Sui D (2014) Cardiovascular protective effects of IL-1 ra-Fc-IL-18BP on experimental myocardial infarction by inhibiting oxidative stress and inflammation in a rat model. Pharmazie 69: 769-774.

Zhao P, Wang Y, Zeng S, Lu J, Jiang TM, Li YM (2015) Protective effect of astragaloside IV on lipopolysaccharide-induced cardiac dysfunction via downregulation of inflammatory signaling in mice. Immunopharmacol Immunotoxicol 37: 428-433.

Zhao Q, Gao C, Cui Z (2015) Ginkgolide A reduces inflammatory response in high-glucose-stimulated human umbilical vein endothelial cells through STAT3-mediated pathway. Int Immunopharmacol 25: 242-248.

Zhou W, Chai H, Courson A, Lin PH, Lumsden AB, Yao Q, Chen C (2006) Ginkgolide A attenuates homocysteine-induced endothelial dysfunction in porcine coronary arteries. J Vasc Surg 44: 853-862.

Zeuke S, Ulmer AJ, Kusumoto S, Katus HA, Heine H (2002) TLR4-mediated inflammatory activation of human coronary artery endothelial cells by LPS. Cardiovasc Res 56: 126-134.