Toll-Like Receptor 4 Signaling Mediates Inflammatory Activation Induced by C-Reactive Protein in Vascular Smooth Muscle Cells

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Key Words

C-reactive protein • Toll-like receptor 4 • Inflammation • Vascular smooth muscle cells • Atherosclerosis

that CRP exerts a proinflammatory action via TLR4-dependent signaling pathway (AT₁R-p38 MAPK-TLR4-PKC α) in VSMCs.

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Abstract

C-reactive protein (CRP) is considered to induce the inflammatory response during atherosclerosis. Toll-like receptor 4 (TLR4)-mediated inflammatory signaling has been shown to facilitate atherogenesis. It remains thoroughly unknown whether TLR4 mediates the proinflammatory effect of CRP. Thus, the study was designed to explore TLR4-related mechanism of proinflammatory effect of CRP in rat vascular smooth muscle cells (VSMCs). CRP increased mRNA levels and protein expressions of vascular endothelial growth factor A (VEGF-A) and inducible nitric oxide synthase (iNOS) in VSMCs, and enhanced NO secretion in the medium. But, CRP hindered nuclear translocation of glucocorticoid receptor (GR) and decreased mRNA level and protein phosphorylation of GR in VSMCs. TLR4 small-interfering RNA (siRNA) significantly reversed the effects of CRP. These suggest that CRP is able to induce inflammatory responses via TLR4 in VSMCs. More importantly, our data provide novel evidence

Introduction

Inflammation is an important event in the development of cardiovascular diseases such as atherosclerosis, hypertension and restenosis [1]. C-reactive protein (CRP), the prototypic marker of inflammation, has been shown in numerous studies to predict cardiovascular events. In addition to being a powerful risk marker [2, 3], much data suggest that CRP exerts direct proinflammatory and proatherogenic effects on endothelial cells [4], vascular smooth muscle cells (VSMCs) [5] and monocytes [6] through increasing expressions of adhesion molecules, cytokines, chemokines and growth factors *in vitro*. Moreover, CRP deposits in the lesions of human and experimental atherosclerosis, and colocalizes with the terminal complement complex and foam cells [7, 8]. On the basis of its pleiotropic roles

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1015-8987/10/0255-0467\$26.00/0

Accessible online at:
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in cardiovascular diseases, CRP provides a pivotal link between inflammation and atherogenesis. Recently, there are increasing evidences that atherosclerosis is an inflammatory disease with immune response [9, 10].

The family of toll-like receptors (TLRs) plays a crucial role in inflammatory diseases like sepsis syndrome, asthma, rheumatoid arthritis, atherosclerosis and inflammatory bowel disease through initiating an innate immune response after recognition of pathogen-associated molecular patterns [11, 12]. Accumulating evidences have indicated that TLRs provoke the inflammatory responses via the nuclear factor- κ B (NF- κ B) pathway to favor the initiation and progression of atherosclerosis. Particularly, the lipopolysaccharide (LPS) receptor TLR4 is highly upregulated in human atheroma, and NF- κ B activation has been noted to co-localize with cells expressing TLR4 within plaques [13, 14]. Therefore, TLR4 serves as a link between inflammation and atherosclerosis.

While roles of CRP in cardiovascular diseases are well established, little is known about proinflammatory effect of CRP in VSMCs. Thus, this study was conducted to evaluate the effect of CRP on productions of inflammatory factors such as vascular endothelial growth factor A (VEGF-A), inducible nitric oxide synthase (iNOS), NO and glucocorticoid receptor (GR) in rat VSMCs. Since TLR4-dependent signaling pathway is known to govern inflammatory responses in atherosclerosis, we also investigated whether TLR4-dependent signaling mediated inflammatory responses induced by CRP in VSMCs.

Materials and Methods

Reagents

Recombinant human CRP and Gö 6976 were purchased from Calbiochem (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were produced by Gibco BRL (Carlsbad, CA, USA). LPS from Escherichia coli 0111:B4, PD123319, SP600125, SB203580 and PD098059 were obtained from Sigma (St Louis, Missouri, USA). Losartan was from Merck (Rahway, NJ, USA). Antibodies against glyceraldehyde-3-phosphate dehydrogenase (GAPDH), VEGF-A, iNOS, PKC α and TLR4 were bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies to GR, phospho-GR, p38 and phospho-p38 were from Cell Signaling Technology (Beverly, MA, USA). The small-interfering RNA (siRNA) to target the gene of interest, the siGENOME SMARTpool TLR4 (M-090819-00), siCONTROL Non-targeting siRNA (a negative control siRNA, NC siRNA) (D-001210-02-20), siGlo RISC-free siRNA (D-001600-01) and DharmaFECT 2 transfection reagent (T-2002-01) were obtained from Dharmacon (Lafayette, CO, USA).

Gene	Primer sequence	Accession number
iNOS	5'-TGCCCTGCAGACTGGATTG-3' 5'-TCCTGCCAGATGTGGGTCTTC-3'	NM_012611
VEGF-A	5'-GACAGAAGGGGAGCAGAAAGC-3' 5'-CCGCACACCGCATTAGGG-3'	NM_031836
GR	5'-GGGTACTCAAGCCCTGGAATG-3' 5'-CCCGTAATGACATCCTGAAGCT-3'	NM_012576
TLR4	5'-GGCATCATCTTCATTGTCCTG-3' 5'-AGCATGTCTCTCCCACTCG-3'	NM_019178
GAPDH	5'-GCCTTCTCCATGGTGGTGAA-3' 5'-GGTCGGTGTGAACGGATTG-3'	NM_017008

Table 1. Primer sequence used for PCR

Endotoxin detection by Limulus assay

Endotoxin level in CRP was tested using chromogenic Limulus ameocyte lysate assay (WinKQCL, Bio-Whittaker, MD, USA) [15]. The average endotoxin level in CRP was ~0.006 endotoxin unit/ml, which was not sufficient to induce TLR4 expression. The purity of CRP was >99%, as confirmed by 12% SDS-PAGE.

VSMCs culture

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male Sprague-Dawley (SD) rats were obtained from the Laboratory Animal Center of School of Medicine of Xi'an Jiaotong University. VSMCs were prepared from the thoracic aorta of 2–3-month-old male SD rats by the explant method [16]. The cells were grown in DMEM supplemented with 10% FBS in a 5% CO₂ atmosphere at 37 °C. Finally, identity of VSMCs was confirmed by the morphological examination and showed 99 % purity as estimated with immunocytochemical staining for α -actin. The cells at passage 3-8 were used for the experiments. After reaching confluence, medium was replaced with serum-free DMEM for an additional 24 h culture before the experiments.

Immunofluorescence analysis of GR

VSMCs were plated on cover slips and grown to confluence. Then, the treated cells were fixed in 4% formaldehyde-PBS for 15 min. The cell membrane was fenestrated with 0.1% Triton-100-PBS for 20 min, and nonspecific binding sites were blocked with 10% goat serum. After incubation with mouse anti-rat GR antibody, the cells were further incubated with the secondary antibody conjugated to rhodamine. Next, the cellular nuclei were stained with DAPI [17]. Finally, the cells were observed under a fluorescent microscope (BX-51 Olympus).

Nitrite assay

Nitrite accumulation was measured in the culture medium of confluent VSMCs as previously described [18]. Nitrite was colorimetrically quantified after adding 100 μ l of Griess reagent (1% sulfanilamide and 0.1% naphthylendiamine in 5% phosphoric acid) to 100 μ l culture medium. Absorbance at

Fig. 1. CRP induces VEGF-A expression in VSMCs. VSMCs were stimulated with the different concentrations of CRP or LPS for 9 h and then, mRNA (A) and protein (B) expression of VEGF-A was determined by RT-PCR, quantitative real-time PCR and western blot. Values are expressed as the mean $\pm$ SEM (n=3). * P <0.05, ** P <0.01, *** P <0.001 vs control.

550 nm was determined with a microplate reader (Molecular Devices). Nitrite concentrations were calculated by comparison with the absorbance of standard solutions of sodium nitrite prepared in the cell culture medium.

Quantitative real-time PCR and RT-PCR

Total RNA was extracted with TRIzol reagent kit (Invitrogen, CA, USA), and cDNA was synthesized from 1 µg of total RNA using Revert Aid™ First Strand cDNA synthesis kit (Fermentas, St. Leon-Rot, Germany) following the manufacturer's instructions. Real-time PCR was performed with the SYBR® Premix Ex Taq™ II (Perfect Real Time) kit (Takara, Japan) on an iCycler iQ™5 thermocycler (Bio-Rad, USA) following the manufacturer's instructions. Primers were designed with Beacon Designer 4.0 (Premier Biosoft, USA) (see Table 1 for the sequences). GAPDH was used as an internal control. Traditional PCR was performed according to the manufacturer's instructions. RT-PCR products were analyzed by electrophoresis on 2% agarose gel containing ethidium bromide. A melting point dissociation curve generated by the instrument was used to confirm that only a single product was present. Quantization of relative gene expression was calculated by the comparative Ct method ($2^{-\Delta\Delta C_T}$) as described by the manufacturer. Results were expressed as relative to control [19].

Western blot analysis

As described early [20], protein samples (20 µg) were subjected to 12% SDS-PAGE gel and transferred onto a polyvinylidene difluoride membrane in semi-dry system (Bio-Rad, Hercules, CA). After blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20, the membranes were incubated with the specific antibodies against iNOS (1:200), VEGF-A (1:200), total-GR (1:50), phospho-GR (1:50), TLR4 (1:200), total-p38 (1:200), phospho-p38 (1:200), PKCα (1:200) and GAPDH (1:400). Reagents (Pierce) for strengthening chemiluminescence were applied to the blots, and the light signals were detected by X-ray film. Optical density of the bands was scanned and quantified with Gel Doc 2000 (Bio-Rad). Results were expressed as relative to control.

RNA interference

Forty to fifty percent of confluent VSMCs were transiently transfected with TLR4 siRNA (100 nM) and NC siRNA using DharmaFECT 2 transfection reagent following the manufacturer's protocol. The transfection efficiency was estimated by measuring uptake of siGlo RISC-free siRNA 48 h after transfection, and 60%-70% of the cells were positive for siGlo RISC-free siRNA. And the silencing effect of TLR4 was confirmed by quantitative real-time PCR and western blot 48 h after transfection. All assays were performed at least 48 h after RNA transfection.

Statistical analysis

Data were expressed as mean $\pm$ SEM from three individual experiments. Statistical evaluation was performed by Student *t* test and one- or two-way ANOVA followed by Dunnett test. Value of P <0.05 was considered statistically significant.

Results

CRP induces VEGF-A expression in VSMCs

To study the effect of CRP on VEGF-A expression in VSMCs, the cells were incubated with CRP (10, 25, 50 µg/ml) or LPS (100 ng/ml) for 9 h and then, VEGF-A expression was determined by quantitative RT-PCR, quantitative real-time PCR and western blot. As shown in Fig. 1A and B, LPS increased VEGF-A expression at mRNA and protein levels in VSMCs. Similarly, CRP also significantly stimulated VSMCs to express VEGF-A in a concentration-dependent fashion.

CRP increases iNOS expression and NO production in VSMCs

To assess whether CRP induces iNOS expression in VSMCs, the cells were incubated with CRP (10, 25,

Fig. 2. CRP increases iNOS expression and NO production in VSMCs. The cells were stimulated with the different concentrations of CRP or LPS for 9 h and then, mRNA (A) and protein (B) expression of iNOS was determined by RT-PCR, quantitative real-time PCR and western blot. Following pretreatment of VSMCs with the different concentrations of CRP or LPS for 12, 24, 36 or 48 h, nitrite concentration (C) in the medium was measured using Griess reaction. Values are expressed as the mean $\pm$ SEM (n=3). * P <0.05, ** P <0.01 vs control.

Fig. 3. CRP hinders nuclear translocation of glucocorticoid receptor (GR) in VSMCs. After VSMCs were incubated with CRP (25 μg/ml) or LPS (100 ng/ml) for 30 min, GR expression was identified with immunocytofluorescence and observed under fluorescent microscope. The results were representative of three independent experiments.

50 μg/ml) or LPS (100 ng/ml) for 9 h and then, iNOS expression was measured. As seen from Fig. 2A and B, mRNA and protein expression of iNOS was hardly detectable in the unstimulated VSMCs, but the expression of iNOS was markedly upregulated in the cells stimulated with CRP or LPS.

Consistent with iNOS, NO generation was time-dependently increased after stimulating VSMCs with the different concentrations of CRP or LPS for 12, 24, 36 or 48 h (Fig. 2C).

CRP attenuates GR expression/activation in VSMCs

After VSMCs were incubated with CRP (25 μg/ml) or LPS (100 ng/ml) for 30 min, GR expression was identified with immunocytofluorescence. The result in Fig. 3 disclosed that CRP or LPS hindered GR nuclear translocation in VSMCs, thereby potentially repressing activation of GR. On account of the described result above, we further examined the effect of CRP (10, 25, 50 μg/ml) on GR expression by quantitative RT-PCR,

Fig. 4. CRP decreases mRNA expression and protein phosphorylation of glucocorticoid receptor (GR) in VSMCs. The cells were stimulated with the different concentrations of CRP or LPS for 9 h (for mRNA) or 30 min (for protein) and then, mRNA expression (A) and protein phosphorylation (B) of GR was determined by RT-PCR, quantitative real-time PCR and western blot. And t-GR and p-GR represent total and phosphorylated GR, respectively. Values are expressed as the mean $\pm$ SEM (n=3). * P <0.05, ** P <0.01 vs control.

quantitative real-time PCR and western blot in VSMCs. Along with LPS, CRP downregulated mRNA expression and protein phosphorylation of GR in a concentration-dependent manner (Fig. 4A and B).

TLR4 mediates CRP-modulated VEGF-A, iNOS and GR expressions in VSMCs

After VSMCs were transiently transfected with TLR4 siRNA for 48 h, mRNA and protein levels of TLR4 were obviously downregulated, and the knockdown efficiency of TLR4 was 67% as illustrated in Fig. 5A and B. To prove the association between TLR4 and CRP-modulated expressions of VEGF-A, iNOS and GR, the cells were stimulated with CRP (25 μg/ml) for 9 h after transfecting with TLR4 siRNA or NC siRNA for 48 h. The results displayed that CRP-induced increases of VEGF-A and iNOS in mRNA and protein levels were abolished by TLR4 siRNA but not NC siRNA (Fig. 5C-F), and knockdown of TLR4 gene substantially reversed the effect of CRP on GR expression/activation in VSMCs (Fig. 5G and H). These demonstrate that CRP regulates VEGF-A, iNOS and GR expressions through TLR4.

CRP stimulates TLR4 expression via angiotensin II type 1 receptor (AT₁R)-MAPK pathways in VSMCs

We found that TLR4 mRNA and protein levels in VSMCs were increased after exposure of the cells to CRP (25 μg/ml) for 9 h (Fig. 6A and B). To discern the relationship of AT receptor, MAPK and TLR4 activation triggered by CRP, the cells were stimulated with CRP for 9 h after pretreatment for 30 min with AT₁R antagonist losartan, AT₂R blocker PD123319, JNK1/2 inhibitor

SP600125, p38 MAPK inhibitor SB203580 and ERK1/2 inhibitor PD098059 alone or in the different combinations. Then, TLR4 expression was determined. As described in Fig. 6A and B, CRP-induced mRNA and protein expression of TLR4 was significantly inhibited by losartan, SP600125, SB203580 or PD098059 but not PD123319, and the inhibitory effect was more obvious when the cells were pretreated with the combination of losartan and SP600125 or SB203580 or PD098059. These confirm that CRP-induced TLR4 expression in VSMCs involves AT₁R and MAPK.

CRP regulates VEGF-A, iNOS and GR expressions via AT₁R-p38 MAPK-TLR4 pathway in VSMCs

To certify whether AT₁R and MAPK are involved in CRP-caused VEGF-A, iNOS upregulation and GR inactivation in VSMCs, the cells were stimulated with CRP (25 μg/ml) for 9 h (for VEGF-A and iNOS) or 30 min (for GR) after pretreatment for 30 min with losartan, SP600125, SB203580 and PD098059, respectively. As displayed in Fig. 7A and B, losartan and SB203580, but not SP600125 and PD098059, inhibited CRP-induced protein expressions of VEGF-A and iNOS. In addition, AT₁R blocker losartan remarkably inhibited CRP-stimulated p38 MAPK phosphorylation as well (Fig. 7D), suggesting that AT₁R mediates p38 MAPK activation in CRP-stimulated VSMCs. Therefore, we consider that AT₁R-p38 MAPK pathway possibly mediates CRP-induced expressions of VEGF-A and iNOS in VSMCs. Likewise, the blockades of AT₁R and p38 MAPK enhanced protein phosphorylation of GR in response to CRP in VSMCs, indicating that CRP-inhibited GR activation is linked to AT₁R-p38 MAPK signal pathway

Fig. 5. CRP modulates VEGF-A, iNOS and GR expressions via TLR4 in VSMCs. After VSMCs were transiently transfected with TLR4 siRNA (100 nM) or NC siRNA for 48 h, mRNA (A) and protein (B) level of TLR4 was determined by RT-PCR, quantitative real-time PCR and western blot. Following exposure of the transfected cells to CRP for 9 h (for mRNA) or 30 min (for GR protein), mRNA and protein expressions of VEGF-A (C and D), iNOS (E and F) and GR (G and H) were evaluated. Values are expressed as the mean $\pm$ SEM (n=3). ^{ss} $P<0.01$, ^{sss} $P<0.001$ vs NC siRNA.

(Fig. 7C). Considering the results from Fig. 6 and 7, we conclude that CRP regulates VEGF-A, iNOS and GR

expressions in VSMCs principally through AT₁R-p38 MAPK-TLR4 pathway.

Fig. 6. CRP increases TLR4 expression in VSMCs through angiotensin II type 1 receptor (AT₁R)-MAPK signal pathways. The cells were stimulated with CRP for 9 h after pretreated for 30 min with losartan, PD123319, SP600125, SB203580 and PD098059 alone or in different combinations and then, mRNA (A) and protein (B) expression of TLR4 was determined by RT-PCR, quantitative real-time PCR and western blot. Values are expressed as the mean $\pm$ SEM (n=3). *** P <0.001 vs control; # P <0.05, ## P <0.01, ### P <0.001 vs CRP.

Fig. 7. CRP regulates VEGF-A, iNOS and GR expressions through angiotensin II type 1 receptor (AT₁R)-p38 MAPK signal pathway in VSMCs. The cells were stimulated with CRP for 9 h (for VEGF-A and iNOS) or 30 min (for GR) after pretreated for 30 min with losartan, SP600125, SB203580, PD098059 and then, protein expressions of VEGF-A (A), iNOS (B) and GR phosphorylation (C) were determined using western blot. In another experiment, VSMCs were stimulated with CRP for 30 min after pretreated for 30 min with losartan and then, p38 MAPK phosphorylation (D) was measured by western blot. Values are expressed as the mean $\pm$ SEM (n=3). ## P <0.01, ### P <0.001 vs CRP.

Involvement of PKC α in CRP-adjusted VEGF-A, iNOS and GR expressions in VSMCs

As depicted in Fig. 8A, TLR4 siRNA potentially reversed the effect of CRP on PKC α activation in

VSMCs, suggesting that TLR4 mediates PKC α activation in CRP-stimulated VSMCs. Next, we investigated whether PKC α regulates the effect of CRP on expressions of VEGF-A, iNOS and GR in VSMCs.

Fig. 8. Involvement of PKC α in CRP-adjusted VEGF-A, iNOS and GR expressions in VSMCs. After transiently transfected with TLR4 siRNA (100 nM) or NC siRNA for 48 h, the cells were exposed to CRP for another 9 h and then, protein expression of PKC α (A) was measured by western blot. In another experiment, the cells were stimulated with CRP for 9 h (for VEGF-A and iNOS) or 30 min (for GR) after pretreated with PKC α antagonist Gö 6976 for 1 h and then, protein expression of VEGF-A (B), iNOS (C) and GR phosphorylation (D) were determined using western blot. And m-PKC α represents membrane protein of PKC α . Values are expressed as the mean $\pm$ SEM (n=3). ^{ss} P <0.01 vs NC siRNA; [#] P <0.05, ^{##} P <0.01, ^{###} P <0.001 vs CRP.

The cells were stimulated with CRP (25 μ g/ml) for 9 h (for VEGF-A and iNOS) or 30 min (for GR) after pretreatment for 1 h with PKC α inhibitor Gö 6976, protein expressions of VEGF-A, iNOS and GR were determined. The results showed that Gö 6976 attenuated VEGF-A and iNOS expressions (Fig. 8B and C) and enhanced GR activation in CRP-stimulated VSMCs (Fig. 8D), confirming that CRP induces the inflammatory response through TLR4-PKC α signal pathway in VSMCs. Additionally, the activated PKC α is regarded as an upstream event of NF- κ B activation, thus regulating expressions of NF- κ B-dependent genes [21, 22]. Altogether, we get into the conclusion that CRP induces inflammatory responses through TLR4-dependent signal pathway (AT₁R-p38 MAPK-TLR4-PKC α) in VSMCs.

Discussion

Atherosclerosis is clearly an inflammatory disease. CRP may contribute to development of atherosclerosis and the subsequent cardiovascular events by inducing

inflammatory responses. The proinflammatory cytokines play important roles in all stages of atherosclerosis. VEGF acts not only as a potent angiogenic factor [23], but also as a proinflammatory cytokine by increasing endothelial permeability and inducing expressions of adhesion molecules [24, 25]. Many cytokines are able to enhance the expression of VEGF isoforms, especially VEGF-A, in the vessel wall to promote atherosclerosis [26, 27]. Previous studies showed that iNOS expression is upregulated with subsequent synthesis of large quantity of NO following injury to the vasculature [28]. NO is an important proinflammatory and proatherogenic mediator. The enhancement of NO level likely plays a significant role in the development of atherosclerosis [29]. It is well known that CRP stimulates iNOS expression and NO production in murine macrophages [30, 31]. The results in the study showed that CRP at the concentrations used remarkably stimulated VSMCs to express VEGF-A and iNOS in the mRNA and protein level, and increased NO production in VSMCs. These demonstrate that CRP triggers the inflammatory response through VEGF-A, iNOS and NO in rat VSMCs.

Besides proinflammatory factors, antiinflammatory factors also regulate progression of atherosclerosis. GR is a member of the nuclear hormone receptor superfamily, and plays critical roles in regulating several physiological and pathological processes such as inflammation, metabolism, cell proliferation and immune responses. Previous reports suggested that GR exerts an antiinflammatory effect through transrepressing expressions of NF- κ B and AP-1 regulated genes [32, 33]. Interestingly, aside from glucocorticoids, cytokines can also influence the expression and function of GR [34]. In this study, CRP inhibited nuclear translocation, mRNA expression and protein phosphorylation of GR in rat VSMCs, implying that CRP also causes inflammatory response through inactivating GR in VSMCs.

Although proinflammatory effect of CRP has firmly been established, its proinflammatory signaling pathway, especially in cardiovascular system, remains to be completely defined. The MAPK family members (p38 MAPK, JNK and ERK) are important in regulating the production of cytokines and inflammatory mediators. AT₁R may be an upstream receptor of MAPK and regulates the expressions of NF- κ B-dependent genes [35]. TLR4-mediated inflammatory responses are associated with AT₁R and subsequent MAPK in the different cells [36, 37]. Moreover, CRP has been shown to augment AT₁R expression and activity in VSMCs [38]. In the present experiment, we confirmed that CRP-induced TLR4 expression involved an AT₁R-MAPK-dependent pathway in VSMCs, and the increases of VEGF-A and iNOS and inhibition of GR initiated by CRP were related to

AT₁R-p38 MAPK-TLR4 signal pathway in VSMCs dominantly.

CRP triggers inflammatory response in part via TLR4-dependent signaling pathway and but, the downstream signaling event of TLR4 is still obscure. Previous studies have demonstrated that TLR4 recruits the downstream kinases to lead to the activation of NF- κ B, which controls LPS- and CRP-induced expressions of an array of inflammatory cytokine genes [39, 40]. PKC family members have been regarded as important regulators in NF- κ B activation [21, 22, 36]. Our result manifested that knockdown of TLR4 noticeably inhibited PKC α activation in CRP-stimulated VSMCs. PKC α inhibitor Gö 6976 abolished protein expressions of iNOS and VEGF-A, and enhanced GR activation in CRP-stimulated VSMCs. These confirm that PKC α , a downstream molecular of TLR4, regulates inflammatory response induced by CRP in VSMCs.

In conclusion, our data provide the direct evidence that CRP exerts a proinflammatory action via TLR4-dependent signaling pathway (AT₁R-p38 MAPK-TLR4-PKC α) in VSMCs. These findings may be pivotal for understanding the potential role of TLR4-dependent signaling pathway in modulating inflammatory events in atherosclerosis.

Acknowledgements

This study was supported by National Natural Science Foundation of China (30772567).

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