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Lycopene protects against LPS-induced proinflammatory cytokine cascade in HUVECs

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Abstract: The aim of the study was to explore whether lycopene protects against the activation of human umbilical vein endothelial cells (HUVECs) induced by a proinflammatory stimulus. HUVECs were pretreated with different concentrations of lycopene (1 μm or 10 μm), then incubated with 1 μg/ml LPS for 24 h. After an incubation, the mRNA and protein levels of proinflammatory cytokines (MCP-1, IL-6, VCAM-1), the expression KLF2, TLR4, ERK1/2 and NF-κB were assayed. The result showed that lycopene treatment significantly suppressed the response of HUVECs to LPS and reduced the levels of proinflammatory cytokines. Also, lycopene increased KLF2 expression, while it inhibited the activation TLR4 and its downstream ERK and the NF-κB signaling pathway in HUVECs.

1. Introduction

The pathogenesis of atherosclerosis is multifactorial (Meng X et al. 2012). Much attention has been given to the role of inflammation in the initiation and progress of atherosclerosis. Vascular endothelial cells act as a central biological barrier, maintaining vascular tonus, protecting monocyte and platelet adhesion, and synthesizing and secreting vasoactive substances, such as endothelin and NO, which respond to various pathophysiological stimuli to protect the integrity of vascular vessels. In endothelial cells, inflammatory cytokines are considered to be an important factor contributing to cell damage, as they induce cell dysfunction and promote cells to change their quiescence into activated phenotypes (Pober et al. 2007). Injured endothelial cells are involved in all the phases of the inflammation process, and are considered to be the critical event in the initiation and development of atherosclerosis. So, protection of endothelial cell function has been shown to be a beneficial strategy for preventing and treating atherosclerosis.

In recent years, the association between dietary habits and the risk factors of atherosclerosis has gained increasing attention. Supplementation of diets with specific protective components may be an attractive alternative to prevent atherosclerosis (Verschuren et al. 2011). Lycopene is a red carotenoid pigment and has unique structural and chemical features. As a potent antioxidant in vitro, lycopene has recently attracted substantial interest because of its potential beneficial role in preventing cardiovascular disease (Mordente et al. 2011). A high intake of lycopene from tomato products is inversely proportional to the risk of atherosclerosis (Palozza et al. 2010). In animal models, lycopene markedly attenuates the formation of atherosclerotic plaques (Hu et al. 2008). In human study, a low concentration of lycopene levels in the serum was shown to be associated with an increase of intima-media thickness (IMT) of the carotid artery (Riccioni et al. 2009).

However, the inherent mechanism of lycopene against atherosclerosis is still elusive. In addition, little is known about the detailed effects of lycopene on amelioration of inflammatory damage in endothelial cells. In the present study, we explored whether lycopene protects against the activation of human umbilical vein endothelial cells (HUVECs) induced by a proinflammatory stimulus. We hypothesize that lycopene treatment may inhibit the expression of inflammatory cytokines through suppressing the activation of TLR4 and its downstream ERK and NF-κB signaling pathway in HUVECs.

2. Investigations and results

2.1. Lycopene down-regulated the inflammatory response of HUVECs to LPS

HUVECs were incubated with or without lycopene in the presence of lipopolysaccharide (LPS). After incubation, it was found that pretreatment with 10 μm lycopene significantly reduced the mRNA level of MCP-1, IL-6, and VCAM-1 in HUVECs in comparison with the control group ($P < 0.05$). However, though 1 μm lycopene slightly reduced proinflammatory cytokines mRNA expression, the difference was not markedly significant compared to the control group. At the same time, consistent with the mRNA levels detected by RT-PCR, similar results were acquired in the detection by ELISA. So, the result showed that lycopene down-regulated the inflammatory response of HUVECs to LPS in a dose-dependent manner (Fig. 1).

2.2. Lycopene Induced resistance to suppress KLF2 expression in HUVECs

Previous studies showed that KLF2 levels were markedly suppressed in HUVECs after LPS treatment (He et al. 2010). In

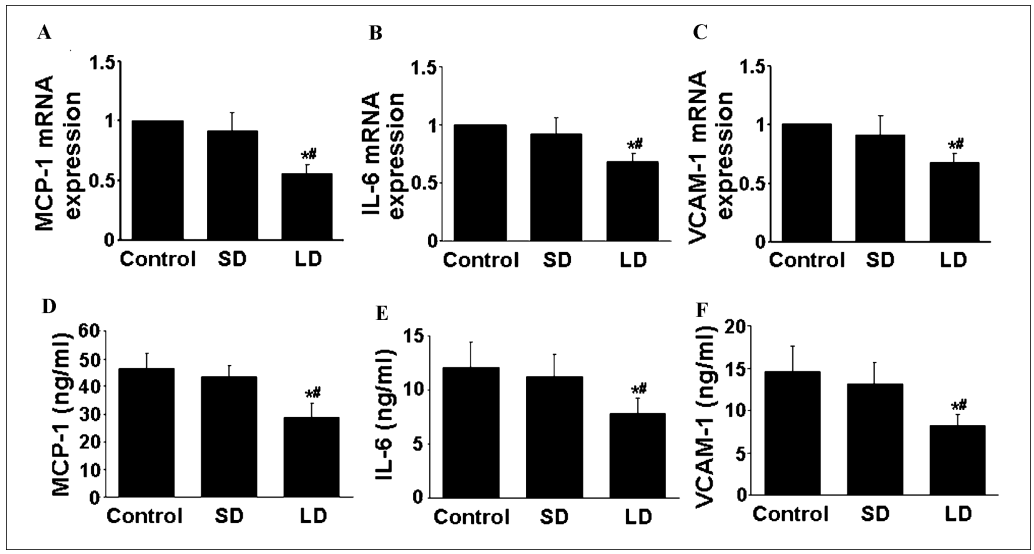

Fig. 1: Effects of lycopene on LPS-induced expression of inflammatory cytokine in HUVECs**.** A, mRNA expression of MCP-1; B, mRNA expression of IL-6; C, mRNA expression of VCAM-1; D, Levels of MCP-1 in cell supernatants; E, Levels of IL-6 in cell supernatants; F, Levels of VCAM-1 in cell supernatants. SD, low-dose Tregs group; LD, large-dose Tregs group; $^{*}P<0.05$ vs. control group, $^{\#}P<0.05$ vs. SD group.

this study, we investigated KLF2 mRNA and protein levels in HUVECs and found that the decrease in KLF2 expression induced by LPS was noticeably reversed when 10 μm lycopene was added ($P<0.05$). However, there was not any difference between the control group and the 1 μm lycopene group (Fig. 2).

2.3. Lycopene suppressed expression of TLR4 in HUVECs

We examined TLR4 mRNA expression in HUVECs by RT-PCR and found that the TLR4 mRNA level was more significantly attenuated in the 10 μm lycopene group than that in the control and 1 μm lycopene groups (Fig. 3, $P<0.05$). The protein

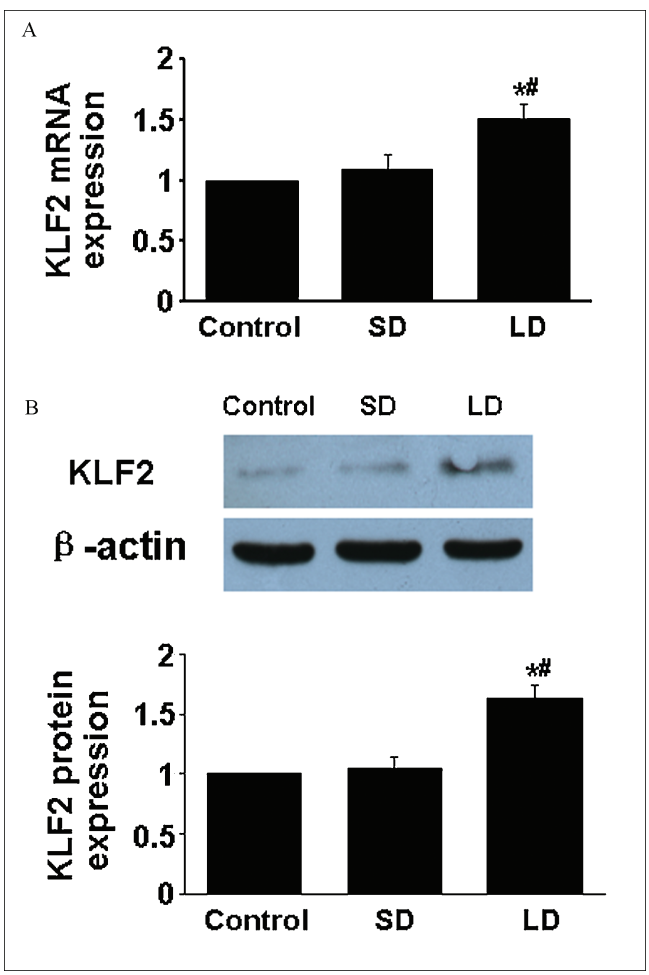

Fig. 2: Effects of lycopene on the mRNA and protein expression of KLF2 in HUVECs**.** A, mRNA expression of KLF2; B, Protein expression of KLF2 and quantitative analysis. $^{*}P<0.05$ vs. control group, $^{\#}P<0.05$ vs. SD group.

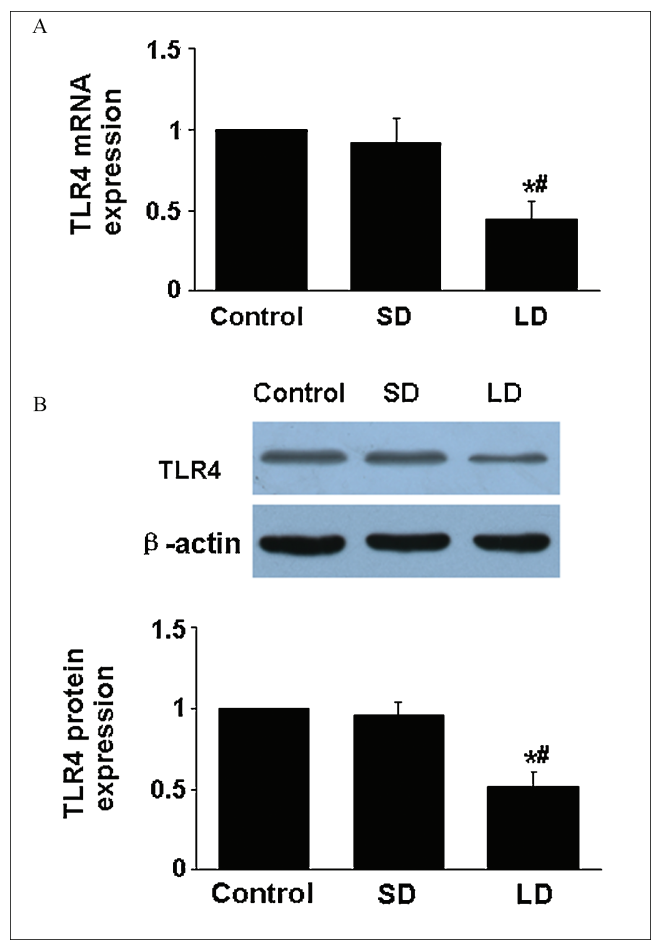

Fig. 3: Effects of lycopene on the mRNA and protein expression of TLR4 in HUVECs. A, mRNA expression of TLR4; B, Protein expression of TLR4 and quantitative analysis. $^{*}P<0.05$ vs. control group, $^{\#}P<0.05$ vs. SD group.

Table 1: Primers for RT-PCR

	Sence	Anti-sence
MCP-1	GCTGACCCCAGCAGAAGTG	TCTTCGGAGTTGGGTTTGC
IL-6	ATGAACTCCTTCTCCACAAGCGC	GAAGAGCCCTCAGGCTGGACTG
VCAM-1	CATCCACAAAGCTGCAAGAA	CCTGGATTCCCTTTTCCAGT
KLF2	GCACGCACACAGGTGAGAAG	ACCAGTCACAGTTTGGGAGGG
TLR4	AGGATGAGGACTGGGTAAGGA	CTGGATGAAGTGCTGGGACA
β-actin	CCTGTACGCCAACACAGTGC	ATACTCCTGCTTGCTGATCC

level of TLR4 was also detected by Western blot. As shown in Fig. 3, TLR4 protein expression was more suppressed in the 10 μm lycopene group than that in the control and 1 μm lycopene groups ($P < 0.05$). So, the result showed that lycopene suppressed mRNA and protein expression of TLR4 in HUVECs exposed to LPS in a dose-dependent manner.

2.4. Lycopene suppressed the activation of NF-κB and ERK1/2 pathway in HUVECs

To further elucidate the downstream signaling pathways of lycopene-induced inhibition of inflammatory response, the role of NF-κB and ERK1/2 activation was assayed. In HUVECs, the protein levels of NF-κB p 65 and P-ERK1/2 were more noticeably decreased in the 10 μm lycopene group than those in control and 1 μm lycopene groups (Fig. 4, $P < 0.05$). The results showed that lycopene had a dose-dependent suppressing effect in the activation of NF-κB and ERK1/2 pathway of HUVECs.

3. Discussion

Lycopene is an acyclic carotenoid with 11 linear conjugated double bonds, which make it a chemically potent antioxidant. It has been shown that high intake of lycopene can influence the progress of atherosclerosis both in human and animal studies (Palozza et al. 2010; Riccioni et al. 2009). However, to date, the inherent mechanism of lycopene against atherosclerosis is still elusive. In addition, little is known about the detailed effects of lycopene on amelioration of inflammatory damage in endothelial cells. In the present study, we investigated the interactions of lycopene with LPS-impaired HUVECs with regard to the intracellular signaling pathway. The result showed that lycopene protected against LPS-induced inflammation, induced resistance to suppressed KLF2 levels through suppressed LPS-induced TLR4 expression and the activation of NF-κB and the ERK1/2 pathway in HUVECs.

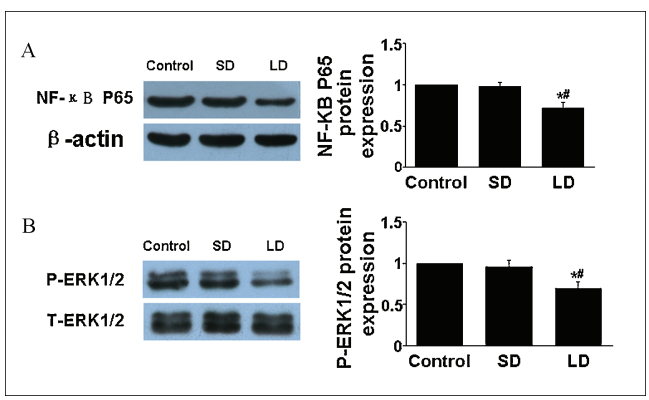

Fig. 4: Effects of lycopene on activation of NF-κB and ERK1/2 pathway in HUVECs. A, protein expression of NF-κB p65 and quantitative analysis; B, protein expression of ERK1/2 and quantitative analysis. $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. SD group.

Inflammation characterizes the pathological process of atherosclerosis. Atherosclerosis has been considered a chronic inflammatory disease of the arterial wall, with the production of inflammatory mediators contributing to the development of atherosclerotic lesions. Endothelial cells have an ability to activate and regulate inflammatory processes and the secretion of proinflammatory cytokines by endothelial cells is an important character of the initiation of atherosclerosis (Sprague et al. 2009). An increased exposure of endothelial cells to circulating proinflammatory mediators may trigger an inflammatory response cascade in the arterial wall (Hung et al. 2008). Inhibition of proinflammatory cytokines production by endothelial cells may be a beneficial tactic in preventing atherosclerosis development. LPS may be linked to vascular disease and LPS plays an active role in vascular inflammation and is thought to be a strong stimulator of the pathogenesis of atherosclerosis (Kiechl et al. 2001). A previous study suggested that HUVECs exposed to LPS have a markedly increased expression of inflammatory cytokines (He et al. 2010). In this study, we found that lycopene effectively down-regulated the levels of proinflammatory cytokines in HUVECs impaired by LPS. The result showed that lycopene has an anti-inflammatory role and provides a protective function to endothelail cells, and this may explain, in part, why lycopene can prevent the development of atherosclerosis.

Transcription factor KLF2, as a key transcriptional switch point between the quiescent and activated states of endothelial cells, is thought to exert a leading role in regulating expression of the endothelial cell gene (Dekker et al. 2006). It has been documented that KLF2 suppressed the inflammatory response, and inhibited atherogenic transcription and anti-thrombogenesis (Dekker et al. 2006). KLF2 can be inhibited by inflammatory stimuli, such as LPS. In the study, we found the inhibition of KLF2 by LPS was, in part, reversed by lycopene.

TLR4 is the receptor of LPS and has an important function in regulating the inflammatory response in many cells. TLR4 is active in inflammatory disease and the up-regulation of TLR4 expression is associated with the release of inflammatory cytokines (Yang et al. 2011). On the contrary, deficiency of TLR4 leads to the reduction of macrophage infiltration and local inflammation response in atherosclerotic plaque (Wang et al. 2011). It has been documented that TLR4 levels increase in HUVECs after the stimulus by LPS (Yang et al. 2011). Whether lycopene can suppress LPS-induced up-regulation of TLR4 and its signaling pathway remains unclear. In the present study, it was shown that enhanced expression of TLR4 was largely ablated in high dose lycopene-treated cells.

LPS is able to activate the NF-κB pathway in HUVECs (Smith et al. 2007). A activation of the transcription factor NF-κB has been suggested to enhance the secretion and expression of proinflammatory cytokines and contribute to driving the inflammatory response (Smith et al. 2007). On the contrary, blocking NF-κB activation may lead to a decreased proinflammatory response (Groesdonk et al. 2006). KLF2 and NF-κB may be mutually

antagonistic, and the balance of these factors could predict the degree of cellular activation (He et al. 2010). In line with this, the results showed that lycopene induced the resistance to inhibition of KLF2 and suppressed the activation of transcription factor NF-κB in HUVECs exposed to LPS. So, these findings may help to understand the mechanism of lycopene against the inflammatory response primarily through affection of the NF-κB signaling pathway.

The ERK signaling pathway belongs to the MAPK family, which is associated with vascular inflammation (Lin et al. 2006). The ERK pathway plays a key role in regulating the levels of inflammatory cytokines. LPS induces the activation of the ERK pathway via signal transduction in HUVECs upon LPS stimilation (Smith et al. 2007). In this study, we found that lycopene suppressed the LPS-induced proinflammatory response in HUVECs, accompanied by down-regulating ERK expression.

The result showed that lycopene plays a key role in protecting the function of HUVECs. On the basis of our results, these findings may help to understand the mechanism of lycopene in antiatherogenic properties.

4. Experimental

HUVECs were isolated from human umbilical veins, grown and passaged as previously described (Wagner et al. 2002). HUVECs were cultured in 6-well plates (1×10^6 cells/well) through 3-4 passages. HUVECs were randomly divided into three groups: the control group, small dose lycopene group and large dose lycopene group. Cells in the control group were treated with 1 μg/ml lipopolysaccharide (LPS, Sigma, UK) for 24 h. In the two lycopene groups, the cells were preincubated with lycopene at different concentrations (1 μm or 10 μm) for 1 h, followed by a 24 h treatment with 1 μg/ml lipopolysaccharide. After incubation, the supernatants were collected and HUVECs were harvested.

Real-Time PCR (RT-PCR) for MCP-1, IL-6, VCAM-1, Kruppellike factor 2 (KLF2), TLR4 and western blot for KLF2, TLR4, nuclear factor κB (NF-κB p65), total-extracellular signal-regulated kinase 1/2 antibody (T-ERK1/2), phospho-ERK1/2 antibody (P-ERK1/2) were assessed routinely. Primers for RT-PCR were shown in Table 1. In a addition, The concentrations of MCP-1, IL-6 and VCAM-1 in supernatants were determined by enzyme-linked immunosorbent assay (ELISA) assays.

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