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Luteolin Regulates VCAM-1 Expression in Endothelial Cells *via* Inhibiting p65 NF-κB or Promoting p85 PI3K*

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Abstract Luteolin is able to inhibit the adhesion of neutrophils to microvascular endothelial cells (MEC) and plays an anti-inflammatory role through regulating vascular cell adhesion molecule (VCAM)-1 in MEC. The inhibitory mechanism is related to three signaling pathways: mitogen activated protein kinase (MAPK), nuclear factor-kappa B (NF-κB)/IκB, and phosphatidylinositol-3-kinase (PI3K) /Akt pathways. The pathways (MAPK and NF-κB/IκB) are involved in the positive regulation of VCAM-1, and the PI3K/Akt pathway participates in the negative regulation. We determined the effects of luteolin on phosphorylation of p38 MAPK, p65 NF-κB, and p85 PI3K, which are key proteins in the mentioned pathways, respectively. Luteolin promotes the phosphorylation of p38 MAPK at different time intervals (30 s, and 1 min) and p85 PI3K (30 s, 1 min, and 5 min); but significantly inhibits the phosphorylation of p65 NF-κB (30 s, 1 min, 5 min, and 30 min) in MEC. As our results show, inhibition of p38 MAPK pathway induces downregulation of VCAM-1. Furthermore, inhibition of the phosphorylation of p38 MAPK *via* SB203580, a p38 MAPK inhibitor, also downregulates VCAM-1, suggesting that the regulation of VCAM-1 in the presence of luteolin in MEC is independent upon the p38 MAPK phosphorylation. This is to say luteolin regulates VCAM-1 expression in MEC through either inhibiting p65 NF-κB phosphorylation or promoting p85 PI3K phosphorylation. This work may provide an insight for the molecular mechanism in the anti-inflammatory effect of luteolin.

Key words luteolin, microvascular endothelial cells, NF-κB, MAPK, PI3K, VCAM-1
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Inflammation is a common pathology that occurs in the course of many bacterial and viral infections, as well as other diseases observed in the clinic[1-3]. An excessive inflammatory reaction can seriously endanger the health of humans and animals under certain conditions. Adhesion of leukocytes to endothelial cells is a prerequisite for inflammation and is an important factor in the occurrence and development of inflammation. Luteolin is a phosphodiesterase 4 (PDE_4 is an enzyme that can specifically hydrolyze cAMP) inhibitor that can be found in traditional Chinese medicines, such as *Folium perillae* and *Flos lonicerae*. Studies have shown that luteolin has pharmacological effects that result in anti-inflammatory, anti-anaphylactic, antitumor, antioxidant, and neuroprotective effects[4-6]. Luteolin has been used in the clinic, mainly for the treatment of respiratory inflammatory diseases, cancer, and cardiovascular disease[7]. Our previous studies confirmed that luteolin could help control acute pneumonia in rats, and its mechanism of action involved the inhibition of neutrophils adhesion to

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microvascular endothelial cells (MEC) by blocking lymphocyte function-associated antigen-1 expression in neutrophils[8] and vascular cell adhesion molecule (VCAM) -1 expression in MEC[9].

Recent studies have shown that MEC play a critical role during inflammation[10]. Notably, the excessive expression of adhesion molecules can promote the adhesion and migration of leukocytes, amplify inflammatory responses, and cause damage to the body. VCAM-1 is not expressed in resting endothelial cells. When stimulated by external factors (such as inflammatory factors and oxidative stress), endothelial cells are activated, and several signaling pathways are triggered to produce large amounts of VCAM-1. VCAM-1 is involved in the activation and migration of lymphocytes and monocytes and plays an essential role in pathological processes, such as inflammation, tumor metastasis, and autoimmune diseases[11]. The expression of adhesion molecules in endothelial cells is regulated by the nuclear factor-kappa B (NF-κB) / IκB, mitogen activated protein kinases (MAPK) and phosphatidylinositol-3-kinase (PI3K) / Akt pathways[12], but the pathway used depends on the cellular specificity and stimulating factors received by endothelial cells. Cyclic-AMP is a signal transduction pathway second messenger, and studies show that cAMP is involved in the regulation of the signaling pathways mentioned above[13-19]. Therefore, further research is needed to determine whether luteolin raises cAMP concentrations to ultimately inhibit VCAM-1 expression by altering the phosphorylation of p38 MAPK, p65 NF-κB, or p85 PI3K, key proteins in the above mentioned pathways; this will further clarify the mechanism of the anti-inflammatory effect of luteolin and provide a strategy for the treatment of inflammatory diseases.

1 Materials and methods

1.1 Materials

Anti-NF-κB p65 (ab194932), anti-p38 (ab38238), goat anti-rabbit IgG H&L (horseradish peroxidase (HRP), ab205718), goat anti-mouse IgG H&L (fluorescein-5-isothiocyanate (FITC), ab6785), platelet endothelial cell adhesion molecule (PECAM) -1 (ab204527) antibodies, and ammonium pyrrolidinedithiocarbamate (PDTC) were purchased from Abcam (Cambridge, United Kingdom). Anti-PI3K p85 (orb106105) antibody was purchased from Biorbyt (Cambridge, United Kingdom). FITC -VCAM-1 (sc-18854) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). SB203580, radio immunoprecipitation assay (RIPA) lysis buffer, and phenylmethylsulfonyl fluoride (PMSF) were purchased from Solarbio (Beijing, China). Rolipram, N-formylmethionyl-leucyl-phenyl-alanine (fMLP), and LY294002 were sourced from Sigma-Aldrich (St. Louis, MO, USA). Fetal calf serum (FBS), Pen Strep, phosphate buffer saline (PBS), D-Hank's, Dulbecco's modified eagle's medium (DMEM) high glucose medium, and 0.25% Trypsin-ethylenediaminetetraacetic acid (EDTA) were from Gibco (Grand Island, NY, USA). Vascular endothelial growth factor (VEGF) was sourced from Peprotech (Rocky Hill, NJ, USA). Luteolin was obtained from The National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Rabbit anti-beta-actin was obtained from Bioss (Beijing, China). Pre-stained colored protein marker and sodium dodecyl sulfate (SDS) -polyacrylamide gel electrophoresis (PAGE) sample loading buffer were purchased from Beyotime Biotechnology (Shanghai, China). All other reagents were of analytical grade.

1.2 Experimental animals

The animal experiments were conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985) and approved by the Animal Welfare and Ethics Committee of Beijing University of Agriculture, Beijing, China (Approved Number: BUAJDX2016001). Six-day-old Sprague-Dawley (SD) rats (males and females) were used as donors for *in vitro* culturing pulmonary MEC. All rats were purchased from the Animal Center of the Academy of Military Medical Sciences (License Number: SCXK-2012 -2004).

1.3 Culture of MEC

Pulmonary MEC from rats were cultured using a direct adherent culture method. Six-day-old SD rats were euthanized by cervical dislocation; then, their bilateral carotid arteries were severed for bloodletting. Lung tissue was removed aseptically and rinsed three times with ice-cold D-Hank's solution containing penicillin and streptomycin. Lung edges were cut after peeling the serous membrane from the lung surface; then, they were transferred to fresh D-Hank's solution and purged repeatedly. The edges were placed in fetal bovine serum, cut into 0.5 mm^3 fragments, spread

evenly on a cell culture plate, and placed inversely in a 5% CO_2 incubator for 2 h at 37℃. Finally, 20% complete medium was added. Cell migration was observed under an inverted microscope after 24 h, and the culture medium was replaced. Tissues were removed after 60 h, and cells could be observed as having a typical morphology resembling a cobblestone or paving stone and being regionally distributed throughout the tissues. The growth state of cells required occasional observation, and fibroblasts and other miscellaneous adherent cells were scraped under the microscope.

Confluent primary cells were digested with 0.05% trypsin for subculturing. The medium was discarded, and an equal volume of D-Hank's solution was added to wash the cells three times, 1 min per wash. Preheated 0.05% trypsin was added to cover the cell layer, and cells were digested for 1−3 min in the culture incubator. The 0.05% trypsin solution was discarded immediately after most of the cells appeared contracted and round, and complete medium containing fetal bovine serum was added to wash the cells off the plate. The cell density was adjusted to 1×10^8 cells/L for subculture. Cells were identified through immunofluorescence staining of the surface antibody PECAM -1, and the purity was determined by observing the ratio of cell number under fluorescence or by light field. Three-to-five passage cells were used in experiments.

1.4　Detection of fMLP−induced phosphorylation of p38 MAPK，p65 NF−κB，and p85 PI3K

Groups of control and fMLP were established. MEC (1×10^9 cells/L in reaction liquid) were added to each group, and preincubated for 30 min at 37℃ in a 5% CO_2 incubator. Three final concentrations of fMLP at 1 nmol/L, 10 nmol/L, and 100 nmol/L were added into three separate wells, except the control wells. A total of 500 μl reaction liquid was added into every group and cells were then incubated at 37℃ in 5% CO_2 for a stimulation culture of 1 min. The contents of each well were then quickly cooled in an ice water bath, and 150 μl cell suspension was centrifuged at 4℃, 2 000 r/min for 8 min. Then, 100 μl of cell lysate (containing a sufficient amount of protease inhibitor and phosphatase inhibitor) and 25 μl loading buffer were added to the cell pellet for cell lysis and protein protection. The phosphorylation states of p38 MAPK, p65 NF-κB, and p85 PI3K in MEC were detected by conventional western blotting, using β-actin as a positive control. The strips were analyzed using GraphPad Prism 5 analysis software, and the relative phosphorylation changes of proteins were presented as the ratio of the target band-to-the β-actin band.

1.5　Detection of luteolin on the phosphorylation of p38 MAPK，p65 NF−κB，and p85 PI3K

Blank control, control, luteolin, and rolipram groups were used. MEC (1×10^9 cells/L in reaction liquid) and test compound or PBS was added to each group of cells, and the cells were then preincubated for 30 min at 37℃ in a 5% CO_2 incubator. Formyl-MLP at a final concentration of 10 nmol/L was added into each well except the blank control. A total of 500 μl of the reaction liquid from each group was again incubated at 37℃ under 5% CO_2 for a stimulation culture of 30 s, 1 min, 5 min, or 30 min. The content of each well was quickly cooled in an ice water bath, and the 150 μl cell suspension was centrifuged at 4℃, 2 000 r/min for 8 min. Then, 100 μl of the cell lysate (containing a sufficient amount of protease inhibitor and phosphatase inhibitor) and 25 μl of the loading buffer were added to the cell pellet for cell lysis and protein protection. The phosphorylation states of p38 MAPK, p65 NF-κB, and p85 PI3K in MEC were detected by conventional western blotting using β-actin as a positive control. Bands were analyzed using the GraphPad Prism 5 analysis software, and the relative phosphorylation changes of proteins were presented as the ratio of the target band-to-the β-actin band.

1.6　Measurement of VCAM−1 expression in MEC

Digested cells were rinsed with PBS, and the cell concentration was adjusted to 1×10^6 cells/400 μl. MEC suspension (400 μl) and 50 μl of luteolin, SB203580, PDTC, or LY294002, at a final concentration of 10 μmol/L, were respectively added to each well of a 24-well cell culture plate. The plate was preincubated for 30 min at 37℃ in a 5% CO_2 incubator, and 50 μl of fMLP (100 nmol/L) was added to each well except for the blank control; 100 μl PBS was added to the blank control wells. A total of 500 μl of reaction liquid from each treatment group was incubated again at 37℃ under 5% CO_2 for 30 min or 1 h. The content of each well was centrifuged for 5 min at 1 200 r/min, and cells were suspended in

PBS. Next, 40 μl of FITC-VCAM-1 was added to the wells, and the mixture was incubated at 37℃ for 30 min in the dark and gently shaken every 10 min. The contents were centrifuged at 1 200 r/min for 8 min, and rinsed twice with PBS; then, they were suspended in 500 μl PBS. The expression of VCAM-1 was detected using flow cytometry for 20 000 cells.

1.7 Statistical analysis

Statistical analysis was performed using the GraphPad Prism 5 software. Statistical significance of differences among different treatments was assessed using Student's *t*-test. A *P*-value of less than 0.05 was considered statistically significant. Data were expressed as the $\bar{x} + s$ of three independent experiments.

2 Results

2.1 Identification of MEC

The proliferation of cultured MEC was assessed, and these cells could reach confluence within 3−5 days of passage and exhibited typical morphology resembling a paving stone (Figure 1). Greater than 95% purity could be achieved for cultured MEC. However, the growth and proliferation of MEC decreased as the passage number increased. After the fifth passage, the cellular adhesion rate and purity decreased, and the cells were no longer suitable for experiments.

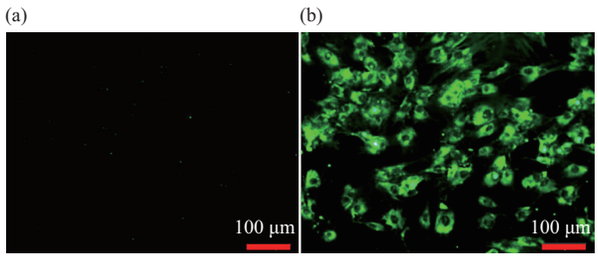

Fig. 1　Micrograph of pulmonary MEC in rats

The cell membrane and cytoplasm were stained green using specific immunofluorescence staining of the surface antibody PECAM -1; the nuclei were not stained (b). The cell membrane and cytoplasm stained nothing without using PECAM -1 in the control (a).

2.2 The effect of fMLP on the phosphorylation of p38 MAPK, p65 NF−κB, and p85 PI3K

Formyl-MLP dose-dependently promoted the phosphorylation of both p38 MAPK and p65 NF-κB in MEC, and 10 nmol/L and 100 nmol/L fMLP could significantly promote the phosphorylation of p38 MAPK and p65 NF-κB ($P < 0.05$). By contrast, fMLP had no discemible effect on p85 PI3K phosphorylation in MEC ($P > 0.05$) (Figure 2).

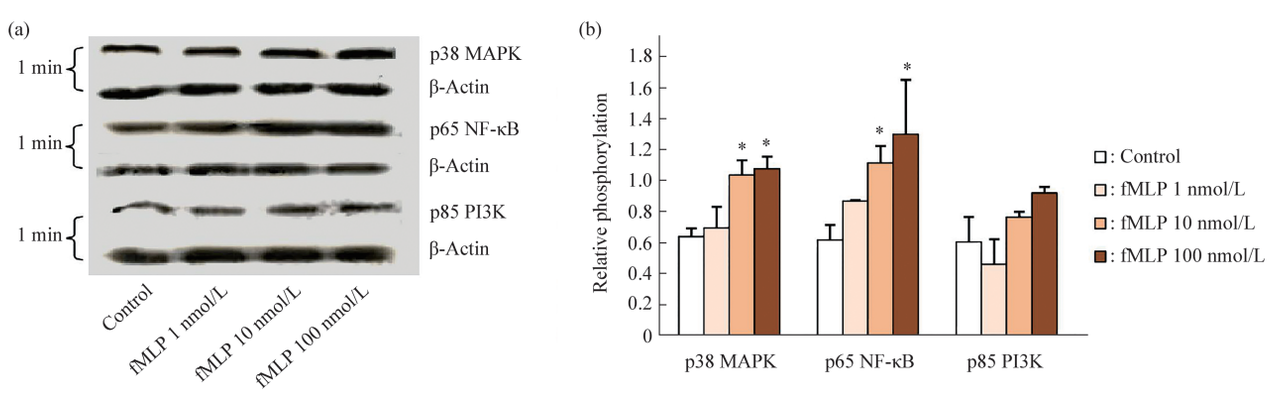

Fig. 2　The effect of fMLP on phosphorylation of p38 MAPK, p65 NF−κB, and p85 PI3K

The relative phosphorylation of proteins was presented as the ratio of the target band-to-the β-actin band. Representative figures (a) and densitometric analysis (b) of the phosphorylation of p38 MAPK, p65 NF-κB, and p85 PI3K were shown. Drug group versus control group, * $P < 0.05$.

2.3 The effect of luteolin on p38 MAPK phosphorylation

10 nmol/L of fMLP promoted p38 MAPK phosphorylation in MEC at specific time points and significantly promoted p38 MAPK phosphorylation at 1 and 5 min ($P < 0.05$ or $P < 0.01$). Luteolin promoted p38 MAPK phosphorylation in MEC at different time points, and both 100 μmol/L luteolin at 30 s and 10 μmol/L and 100 μmol/L luteolin at 1 min significantly promoted p38 MAPK phosphorylation

($P < 0.05$ or $P < 0.01$). The positive control treatment with rolipram inhibited p38 MAPK phosphorylation at different time points and significantly inhibited p38 MAPK phosphorylation at 5 min ($P < 0.05$) (Figure 3).

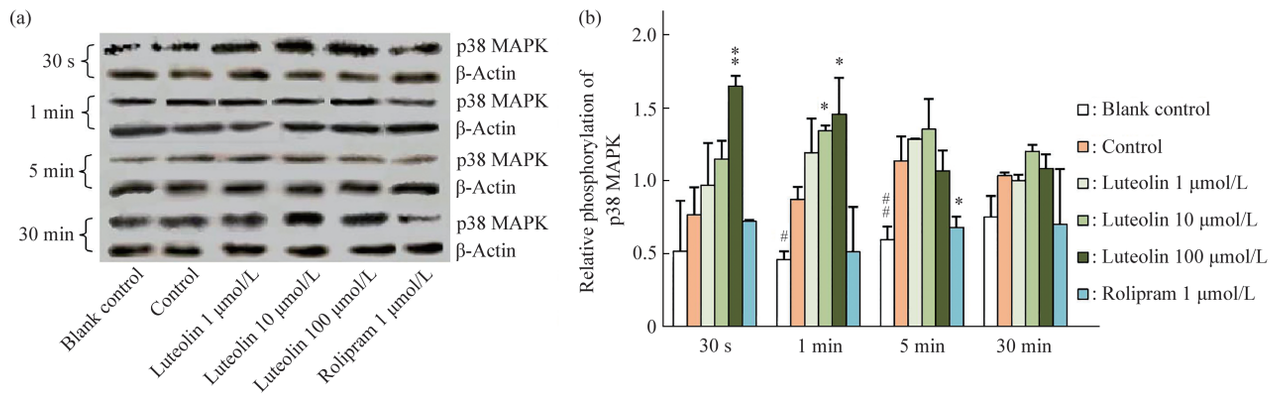

Fig. 3　The effect of luteolin on p38 MAPK phosphorylation

The relative phosphorylation of p38 MAPK was presented as the ratio of the target band-to-the β-actin band. Representative figures (a) and densitometric analysis (b) of p38 MAPK phosphorylation were shown. Blank control group versus control group, $^{\#}P < 0.05$, and $^{\#\#}P < 0.01$; and drug group versus control group,$^{*}P < 0.05$, and $^{**}P < 0.01$.

2.4　The effect of luteolin on p65 NF−κB phosphorylation

Formyl-MLP at 10 nmol/L promoted p65 NF-κB phosphorylation in MEC at different time points and significantly promoted p65 NF-κB phosphorylation at 1 min, 5 min, or 30 min ($P < 0.05$ or $P < 0.01$). Luteolin inhibited p65 NF-κB phosphorylation in MEC at different time points ($P < 0.05$ or $P < 0.01$) except for 1 μmol/L luteolin at 30 s and 30 min. Rolipram significantly inhibited p65 NF-κB phosphorylation at 30 s, 1 min, and 5 min ($P < 0.01$), and promoted p65 NF-κB phosphorylation at 30 min ($P < 0.01$) (Figure 4).

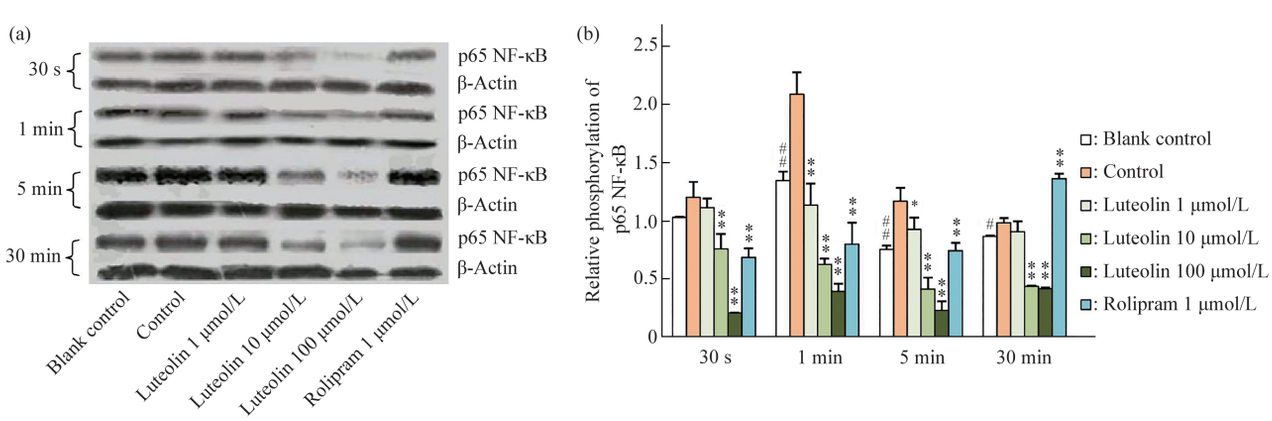

Fig. 4　The effect of luteolin on p65 NF−κB phosphorylation

The relative phosphorylation of p65 NF-κB was presented as the ratio of the target band-to-the β-actin band. Representative figures (a) and densitometric analysis (b) of p65 NF-κB phosphorylation were shown. Blank control group versus control group,$^{\#}P < 0.05$, and $^{\#\#}P < 0.01$; and drug group versus control group,$^{*}P < 0.05$, and $^{**}P < 0.01$.

2.5　The effect of luteolin on p85 PI3K phosphorylation

10 nmol/L fMLP treatment showed a trend toward the promotion of p85 PI3K phosphorylation in MEC at different time points, but none of the changes were significant ($P > 0.05$). Luteolin promoted p85

PI3K phosphorylation in MEC at different time points and 10 μmol/L of luteolin at 30 s, 1 min, or 5 min treatment or 1 μmol/L luteolin at 1 min treatment significantly promoted p85 PI3K phosphorylation ($P < 0.05$ or $P < 0.01$). Rolipram did not affect p85 PI3K phosphorylation ($P > 0.05$) (Figure 5).

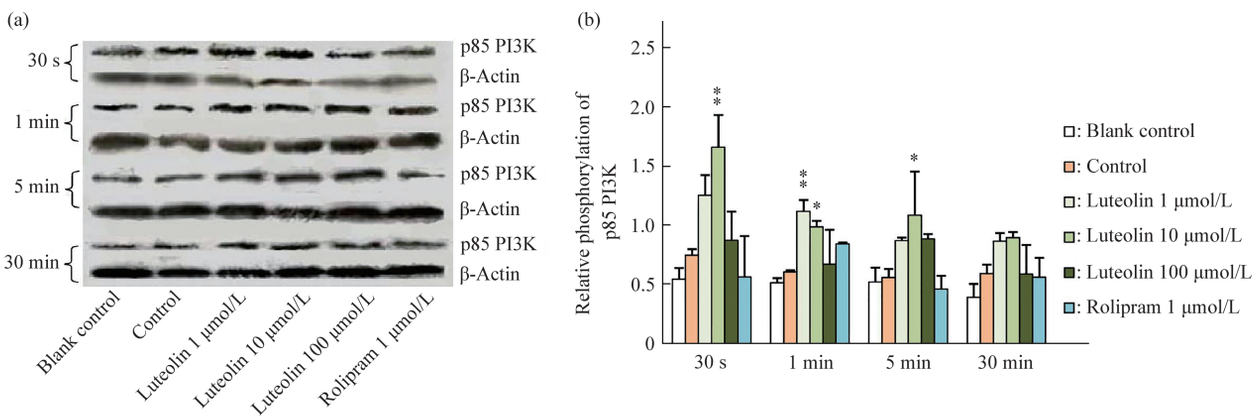

Fig. 5 The effect of luteolin on p85 PI3K phosphorylation

The relative phosphorylation of p85 PI3K was presented as the ratio of the target band-to-the β-actin band. Representative figures (a) and densitometric analysis (b) of p85 PI3K phosphorylation were shown. Drug group versus control group, * $P < 0.05$, and ** $P < 0.01$.

2.6 Effects of the inhibitors on VCAM−1 expression

Formyl-MLP significantly promoted VCAM-1 expression in MEC at 30 min or 1 h ($P < 0.01$). SB203580, a p38 MAPK (phosphorylation) inhibitor, inhibited fMLP-induced VCAM-1 expression in MEC 30 min or 1 h post-treatment ($P < 0.01$). The NF-κB inhibitor, PDTC, significantly inhibited VCAM-1 expression in MEC at 1 h ($P < 0.05$). However, LY294002, a PI3K inhibitor, did not alter VCAM-1 expression in MEC ($P > 0.05$). Luteolin, as a positive control, inhibited VCAM-1 expression in MEC at 30 min or 1 h ($P < 0.01$) (Figure 6).

3 Discussion

MAPK is a highly conserved eukaryotic signaling pathway that plays an important role in the inflammatory responses of various cell types. The MAPK signaling pathway, especially the p38 pathway, plays a crucial role in the regulation of cytokine-induced VCAM-1 expression. Studies show that blocking the MAPK pathway can greatly inhibit VCAM-1 expression[20-23]. Luteolin is involved in the regulation of the p38 MAPK pathway and generally exerts an inhibitory effect on p38 MAPK in healthy tissues or cells[24-27]; however, it can promote p38 MAPK activity in abnormal tissue or cells, such as tumors[28-29]. There are four subtypes of p38 MAPK that can be activated by different mediators and have different biological effects. Moreover, different p38 MAPK subtypes react differently to the same stimulus and can form a complex and diverse network of signal transduction pathways. After activation by phosphorylation, p38 MAPK translocates into the nucleus or other sites, acts on intracellular targets, and can play regulatory roles. Our findings showed that fMLP could promote p38 MAPK phosphorylation. Luteolin generally promoted the p38 MAPK phosphorylation of MEC and significantly promoted p38 MAPK phosphorylation 30 s or 1 min post-treatment ($P<0.05$ or $P<0.01$). However, SB203580, a p38 MAPK inhibitor, strongly inhibited VCAM-1 expression in MEC ($P<0.01$), and its mechanism involves the inhibition of p38 MAPK phosphorylation[30]. Therefore, blocking p38 MAPK phosphorylation could inhibit VCAM-1 expression in MEC. Thus, the role of luteolin in regulating VCAM-1 expression in MEC is either independent of its p38 MAPK phosphorylation-promoting effect or is unrelated to the p38 MAPK signaling pathway.

NF-κB is an important transcription factor for driving VCAM-1 expression. Normally, NF-κB binding with IκB occurs in the cytoplasm, and the complex

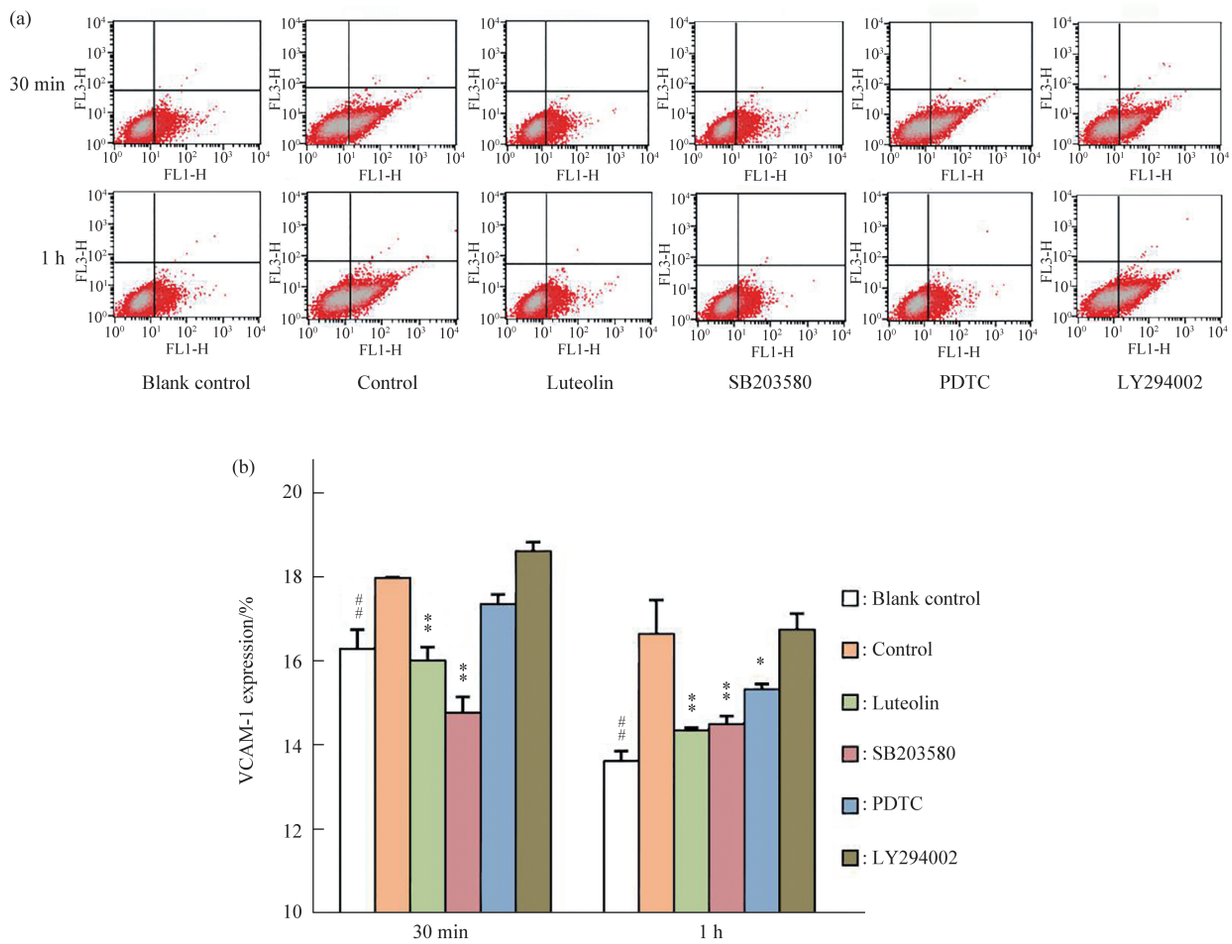

Fig. 6　Results of the inhibitors on VCAM-1 expression

Representative scatter diagrams (a) and fluorescence density analysis (b) of VCAM-1 expression were shown. Luteolin was used as a positive control. Blank control group versus control group, $^{\#\#}P < 0.01$; and drug group versus control group, $^{*}P < 0.05$, and $^{**}P < 0.01$.

formed produces no transcriptional activity. IκB can be phosphorylated by IκB kinase upon stimulation by external factors. NF-κB is then detached from IκB, and the nuclear binding site of the p65 subunit becomes exposed. Then, NF-κB translocates to the nucleus, binds to the VCAM-1 promoter, and VCAM-1 transcription is induced. Studies have shown that many traditional Chinese medicines exhibit the anti-inflammatory effects by inhibiting NF-κB activity. Crocetin is a natural carotenoid compound and produces various pharmacological effects, such as anti-oxidant and anti-atherosclerotic effects. Crocetin can effectively inhibit the expression of VCAM-1 and the adhesion of monocytes to endothelial cells; the mechanism of its action involves enhancing antioxidant ability, reducing intracellular reactive oxygen species levels, and inhibiting the NF-κB activation of endothelial cells[31]. Tanshinone IIA is an effective treatment for inflammation and atherosclerosis, which may involve the down-regulation of VCAM-1 and ICAM-1 through the partial blockade of TNF-α-induced NF-κB activation and IκB-α phosphorylation in endothelial progenitor cells[32]. Buddleoside treatment can decrease NF-κB expression and increase IκBα expression during the inflammatory injury of vascular endothelial cells, down-regulate the expression of ICAM-1 and VCAM-1, and reduce the degree of inflammatory injury of vascular endothelial cells[33]. Our findings showed that fMLP robustly promoted p65 NF-κB phosphorylation. Luteolin significantly inhibited p65 NF-κB phosphorylation in MEC at different time points (P<0.05 or P<0.01). Additionally, PDTC, a NF-κB inhibitor, also inhibited VCAM-1 expression of MEC ($P < 0.05$). Therefore, blocking NF-κB phosphorylation could inhibit the

VCAM-1 expression of MEC. Thus, luteolin can inhibit VCAM-1 expression in MEC through the suppression of p65 NF-κB phosphorylation or by blocking the NF-κB signaling pathway.

PI3K mainly refers to a class of PI3K composed of p85/p110, which can interact with all inositol phosphates and phosphatidylinositol. PI3K is activated when it binds to its receptor, resulting in downstream signaling cascade reactions. A study has shown that the PI3K/Akt pathway plays an important negative feedback role in excessive immune responses and Toll-like receptor-mediated proinflammatory responses. For example, vascular endothelial growth factor (VEGF)-inducing VCAM-1 expression mainly occurs *via* NF-κB activation with PI3K-mediated suppression in human umbilical vein endothelial cells[34]. These findings indicated that differences in the concentrations of fMLP did not affect p85 PI3K phosphorylation. Luteolin generally promoted the p85 PI3K phosphorylation of MEC, and 1 μmol/L or 10 μmol/L luteolin could strongly promote p85 PI3K phosphorylation after 30 s, 1 min and 5 min treatments ($P < 0.05$ or $P < 0.01$). LY294002, a PI3K inhibitor, did not inhibit VCAM-1 expression in MEC. Therefore, blocking p85 PI3K phosphorylation does not affect VCAM-1 expression in MEC. Because of the negative feedback effect exerted by PI3K on excessive immune responses and proinflammatory responses, luteolin's inhibition of VCAM-1 expression in MEC may come from the negative regulation of PI3K phosphorylation. Our findings also show that both SB203580 and PDTC have a synergetic effect on the luteolin-mediated inhibition of VCAM-1 expression, while LY294002 does not affect luteolin-mediated inhibition of VCAM-1 expression.

In conclusion, luteolin regulates VCAM-1 expression in MEC *via* inhibiting p65 NF-κB phosphorylation or promoting p85 PI3K phosphorylation, which may provide an insight for the molecular mechanism in the anti-inflammatory effect of luteolin. Currently, one therapeutic strategy to guard against inflammation involves inhibiting or blocking the abnormal expression of key signaling proteins in inflammation related signaling pathways. Therefore, further studies on the changes in p65 NF-κB phosphorylation in MEC and the mechanism of luteolin action may provide new targets and effective drugs for the treatment of inflammatory diseases.

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木犀草素通过抑制p65 NF-κB、促进p85 PI3K调节微血管内皮细胞VCAM-1表达*

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摘要　通过抑制微血管内皮细胞血管细胞黏附分子（VCAM）-1的表达，木犀草素可阻遏中性粒细胞与微血管内皮细胞的黏附，起到抗炎作用. 木犀草素调节VCAM-1表达与三条信号通路有关：丝裂原活化蛋白激酶（MAPK）、核因子kappa B（NF-κB）/IκB和磷脂酰肌醇3激酶（PI3K）/Akt通路. 其中，MAPK和NF-κB/IκB通路参与VCAM-1正向调节，PI3K/Akt通路参与VCAM-1负向调节. 本文研究了木犀草素对微血管内皮细胞该三条通路中的关键蛋白p38 MAPK、p65 NF-κB、p85 PI3K磷酸化. 结果表明：木犀草素在反应的30 s和1 min促进p38 MAPK磷酸化，在30 s、1 min和5 min促进p85 PI3K磷酸化，而在30 s、1 min、5 min和30 min抑制p65 NF-κB磷酸化. 阻抑p38 MAPK通路导致VCAM-1表达下调，而p38 MAPK抑制剂SB203580可通过抑制p38 MAPK磷酸化也下调VCAM-1，提示木犀草素对微血管内皮细胞VCAM-1的调节作用独立于p38 MAPK磷酸化. 由此可知，木犀草素通过抑制p65 NF-κB磷酸化或促进p85 PI3K磷酸化调节微血管内皮细胞VCAM-1表达. 本文为木犀草素抗炎作用的分子机制研究提供了新的线索.

关键词　木犀草素，微血管内皮细胞，NF-κB，MAPK，PI3K，VCAM-1

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