European Review for Medical and Pharmacological Sciences 2018; 22: 7533-7542

Research on mechanism of PCS in damaging vascular endothelial cells and promoting formation of atherosclerosis via TLR4/TREM-1

Z.-Y. ZHANG[1], C.-F. HU[2], M.-X. WANG[1], J. LIN[1], J.-M. LI[1], R.-Z. WANG[1]

[1]Department of Cardiovascular Medicine, Taizhou People's Hospital, Taizhou, China
[2]ICU, Taizhou People's Hospital, Taizhou, China

Abstract. – OBJECTIVE: The study aimed to explore the effects of p-cresyl sulfate (PCS) of damaging vascular endothelial cells and promoting the formation of atherosclerosis in mice.

MATERIALS AND METHODS: The apolipoprotein E (ApoE)$^{-/-}$ mice were fed normally and with a high-fat diet; the ApoE$^{-/-}$ mice fed with high-fat diet were divided into two groups and treated with blank control and PCS, respectively. The aortic arch in each group was taken and underwent the oil red O staining, and the serum PCS content in each group was detected. The basic components of plaque were observed, including foam cells, lipid deposition, and cholesterol crystal. Moreover, human umbilical vein endothelial cells were cultured and divided into control group, PCS treatment group (PCS), PCS treatment with TLR4 overexpression group (PCS+TLR4+), and PCS treatment with TLR4 knock-out group (PCS+TLR4-). The degree of endothelial cell damage was detected using a cluster of differentiation CD42b-/CD31+ endothelial microparticles (EMPs), and expressions of Toll-like receptor 4 (TLR4), triggering receptor expressed on myeloid cells-1 (TREM-1), phosphorylated-endothelial nitric oxide synthase (p-eNOS), and tumor necrosis factor-α (TNF-α) in cells were detected via Polymerase Chain Reaction (PCR) and Western blotting.

RESULTS: The serum PCS concentration in high-fat ApoE$^{-/-}$ mice was increased, and the aortic arch sections of ApoE$^{-/-}$ mice treated with PCS displayed the evident atherosclerotic plaques. Experimental results of human umbilical vein endothelial cells showed that the activity of human umbilical vein endothelial cells treated with PCS declined, the expression levels of TLR4, TREM-1, and TNF-α were increased, while that of p-eNOS was decreased. After the TLR4 knockout, the above effects of PCS were reversed.

CONCLUSIONS: PCS damages vascular endothelial cells through TRL4/TREM-1, thereby accelerating the formation of atherosclerosis.

Key Words:
P-cresyl sulfate, TLR4, Atherosclerosis.

Introduction

Atherosclerosis is a major cause of human death currently, and the coronary atherosclerotic heart disease becomes increasingly detrimental, which has been the second killer of human only following the tumor, as well as a severe challenge in the cardiovascular field. The pathogenesis of atherosclerosis is complex, and various theories related to it have been proposed, including thrombosis theory, lipid infiltration theory, damage response theory, oxidative stress theory, shear stress theory, smooth muscle cell monoclonal theory, and chronic inflammation theory, but none of them can fully explain the pathogenesis of all atherosclerotic diseases. Therefore, studying the possible breakthrough points from its pathogenesis is of great significance in exploring new intervention measures and improving the overall prevention and treatment levels of atherosclerotic diseases. In recent years, increasingly more basic and clinical research results have supported that endothelial dysfunction, especially the inflammatory response of endothelial cells, is a key initiating and promoting factor of atherosclerosis[1,2].

P-cresyl sulfate (PCS) is one of the nephrotoxins confirmed currently, whose precursor is p-cresol[3]. As a kind of protein-binding toxin hard to be dissociated, PCS plays an important role in the renal damage and the occurrence and development of uremia. PCS has been proved to be a pro-oxidant with oxidative stress, which can promote the respiratory burst of leukocytes and production of reactive oxygen, and damage vascular endothelial cells, thus leading to vascular dys-

Corresponding Author: Chunfang Hu, MM; e-mail: hcf2527@163.com

function[4-6]. PCS can also induce the expression of a variety of cytokines, such as tumor necrosis factor-α (TNF-α), various interleukins (ILs), and colony-stimulating factors, further damaging cells[7,8]. Endothelial microparticles (EMPs) produced by damaged vascular endothelial cells are shed into the blood. EMPs are a kind of tiny vesicular structure with a diameter fewer than 1 μm, which is positively correlated with the degree of endothelial cell damage, so it often serves as a marker for endothelial cell damage[9]. Studies have demonstrated that PCS can increase the number of EMPs in human umbilical vein endothelial cells, confirming the damage of PCS to vascular endothelial cells. PCS cannot only damage vascular endothelial cells through promoting an inflammatory response, but also directly damage vascular endothelial cells and inhibit the proliferation of vascular endothelial cells. Therefore, PCS is an important factor of cardiovascular disease[10].

The Toll-like receptor (TLR) is a kind of pattern recognition receptor, and there are 11 members in the TLR family playing important roles in the identification of pathogenic molecules[11]. TLR can promote the synthesis and release of cytokines, thereby triggering the inflammatory response. TLR4 is a recognition receptor for lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria. Activated TLR4 can activate multiple signal transduction pathways, including adenosine monophosphate-activated protein kinase (AMPK), phosphatidylinositol 3-hydroxy kinase (PI3K), and nuclear factor-κB (NF-κB), and induce a series of inflammatory mediators, thus producing strong inflammatory response[12,13].

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a member of the TREM family, which is mainly expressed in neutrophils, mature monocytes, and macrophages. TREM-1 can act on the occurrence and development of inflammation, thus amplifying the inflammation[14]. Studies[15-17] have found that the overexpression of TREM-1 can reduce the release of the anti-inflammatory factor IL-10, and increase the secretion and release of inflammatory factors and chemokines, thus positively feeding back the inflammatory response.

It has been suggested that PCS can produce inflammatory response through TLR4 and the downstream TREM-1 pathway, thereby causing the cardiovascular endothelial cell dysfunction and ultimately leading to atherosclerosis.

Materials and Methods

Animals and Reagents

A total 80 apolipoprotein E (ApoE)$^{-/-}$ mice aged 8 weeks old weighing (22.3 ± 0.5) g in normal nutritional status and mental status were provided by the Laboratory Animal Center of Nanjing University (Nanjing, China). This study was approved by the Animal Ethics Committee of Taizhou People's Hospital Animal Center. TLR4, TREM-1, TNF-α, and phosphorylated-endothelial nitric oxide synthase (p-eNOS) antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA); TLR4, TREM-1, TNF-α, and p-eNOS primers were purchased from Sangon (Shanghai, China); oil red O staining kit was purchased from Beyotime (Shanghai, China); TLR4 lentivirus was bought from Shanghai HanBio Co., Ltd. (Shanghai, China); fluorescein isothiocyanate (FITC)-labeled mouse anti-human leukocyte differentiation antigen platelet-endothelial cell adhesion molecule (CD31) monoclonal antibody, FITC-labeled isotype control antibody immunoglobulin G1 (IgG1), phycoerythrin (PE)-labeled mouse anti-human selectin (CD42b) monoclonal antibody, PE-labeled isotype control antibody IgG1, FITC-labeled Annexin V, and Propidium Iodide (PI) dye were bought from BD (Franklin Lakes, NJ, USA); ribonucleic acid (RNA) reverse transcription kit and quantitative Polymerase Chain Reaction (qPCR) kit were purchased from TaKaRa (Otsu, Shiga, Japan).

Modeling and Grouping

A total of 20 ApoE$^{-/-}$ mice were fed with a normal diet, while 20 ApoE$^{-/-}$ mice were fed with a high-fat diet supplemented with 0.15% cholesterol and 21% lard oil for 6 weeks to prepare the mouse model of hyperlipidemia. Another 20 ApoE$^{-/-}$ mice were randomly selected as control group and underwent gavage with distilled water, and 20 ApoE$^{-/-}$ mice were taken as PCS treatment group and underwent gavage with distilled water containing 17 μL/g PCS (100 mg/kg). Mice were fed at (21±2)°C under the relative humidity of (50±15)% and 12/12 h light/dark cycle, and executed after 8 weeks for further data analysis.

Oil Red Staining

After fasting for solids not liquids overnight, mice were anesthetized, and the chest was opened to expose the heart. Under aseptic conditions, the heart and aorta were taken, fixed with 10% formaldehyde, dehydrated, routinely

embedded into paraffin, and sliced into about 5 μm-thick sections at 60°C overnight, followed by dewaxing with xylene and dehydration with gradient alcohol. The same four sections were selected from the aortic root of each mouse, and two sections were serially taken at an interval of 100 μm, followed by oil red O staining and observation under a light microscope. The plaque area (PA) and cross-sectional vascular area (CVA) were detected using the Image-Pro Plus IPP (Media Cybernetics, Inc., Bethesda, MD, USA), and the average of 4 sections was taken for each sample.

Detection of PCS

After 15 μL 6M hydrochloric acid and 100 mg NaCl were added into 100 μL serum, the mixture was shaken violently for 5 min, added with 1 mL isopropyl ether, fully mixed and shaken for 1 min, followed by centrifugation at 3000 g and room temperature for 10 min. Then, 800 μL supernatant was taken into an Eppendorf (EP) tube, added with 100 μL NaOH dissolved in methanol, fully mixed and blown dry with nitrogen. 80 μL methanol was added into the residual dry powder, dissolved and mixed evenly. Finally, 5 μL samples were taken for the high-performance liquid chromatography-mass spectrometry (HPLC-MS).

Determination of CD42b-/CD31+EMPs

The cell culture supernatant was collected and centrifuged at 5000 g and 4°C for 10 min to remove the cells and cell debris. After centrifugation, 50 μg supernatant was added into an absolute counter tube, and then, 5 μL specific antibodies (anti-CD31-PE, anti-CD42b-FITC) or an equal amount of isotype control antibodies were also added, followed by incubation at room temperature in a dark place for 20 min and detection *via* flow cytometry. 0.3-0.8 μm microparticles were collected, FITC and PE fluorescence intensity was further analyzed at 525 nm and 575 nm, and 20000 fluorescence-labeled absolute counter microspheres were collected. The concentration of EMPs was calculated as follows: number of fluorescence-labeled EMPs × 20000 × 20/number of absolute counter microspheres collected.

Western Blotting

Tissues extracted were ground with liquid nitrogen, diluted with normal saline and placed on ice. Then, the supernatant was taken and centrifuged at 4°C for 5 min, and the supernatant was discarded. The precipitate was resuspended using the radioimmunoprecipitation assay (RIPA) lysis solution containing phenylmethanesulfonyl fluoride (PMSF; Beyotime, Shanghai, China), lysed and centrifuged at 16000 g and 4°C for 15 min. The supernatant was taken for protein quantification. The protein was added with loading buffer and heated for denaturation, followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, the protein was transferred onto a membrane, sealed with 5% skim milk for 2 h, added with the primary antibody for incubation at 4°C overnight, and washed with Tris-buffered saline with Tween-20 (TBST) 3 times (10 min/time). The corresponding secondary antibody was added for incubation at room temperature for 1 h, and the protein was washed again with TBST 3 times (10 min/time). Finally, the protein expression in different samples was detected using the ECL method.

An appropriate amount of tissue samples was taken, added with TRIzol (Invitrogen, Carlsbad, CA, USA) and lysed at room temperature for 5 min. The tissue lysate was added with 1/5 volume of chloroform, fully shaken until full emulsification without layers, and placed at room temperature for 5 min, followed by centrifugation at 12000 g and 4°C for 15 min. The supernatant was taken into the EP tube, and an equal volume of isopropanol was also added, mixed evenly and placed at room temperature for 10 min, followed by centrifugation at 12000 g and 4°C for 10 min. The supernatant was discarded, the precipitate was washed with 75% ethanol and centrifuged for another 5 min, the ethanol was discarded, and the precipitate was dried for 2-3 min and dissolved with 20 μL diethyl pyrocarbonate (DEPC)-treated water. After the RNA concentration was measured, reverse transcription was performed according to instructions of the reverse transcription kit. PCR system (a total of 20 μL) is as follows: 10 μL TB Green Premix Ex TaqII (2×), 0.4 μL ROX Reference Dye or Dye II (50×), 0.8 μL forward primers and 0.8 μL reverse primers (10 μmol/L), 2 μL cDNA solution and 6 μL sterilized ddH_2O. PCR cycle parameters are as follows: pre-denaturation at 95°C for 30 s, 95°C for 5 s, 60°C for 34 s, collection of fluorescence at 60°C, a total of 40 cycles. Data were collected using the Applied Biosystems 7500 Fast Real Time-PCR System. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene, and the relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Data were expressed as mean ± standard deviation and analyzed *via* paired or unpaired *t*-test. One-way analysis of variance was used for the comparison among groups, and SNK post-hoc test was adopted for the pairwise comparison. $p < 0.05$ suggested that the difference was statistically significant. Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for data analysis, and GraphPad software (La Jolla, CA, USA) was used for plotting.

Results

The serum PCS level was increased in mice fed with a high-fat diet, and atherosclerotic plaques were more severe in mice treated with PCS.

The gross specimen of the aorta of mice in each group underwent oil red O staining, and the atherosclerotic lesion sizes were compared. The oil red O staining of aortic sections in the four groups of mice is shown in Figure 1A, and the atherosclerotic lesion size is shown in Figure 1B. According to the microscopic observation, there were aortic plaques formed in $ApoE^{-/-}$ mice fed normally, and its average size was about 348×10^3 um^2, while evident aortic plaques were formed in $ApoE^{-/-}$ mice fed with a high-fat diet, and its average size was about 420×10^3 um^2.

Moreover, the serum PCS content in the wild-type mice and $ApoE^{-/-}$ mice fed with a high-fat diet was significantly increased ($p < 0.01$) (Table I).

Besides, in the two group of $ApoE^{-/-}$ mice fed with a high-fat diet, the group treated with PCS showed increased serum PCS level and elevated atherosclerotic lesion size compared to the mice treated with distilled water ($p < 0.05$) (Figure 1A and 1B; Table I).

Damage of PCS to Human Umbilical Vein Endothelial Cells

Human umbilical vein endothelial cells were divided into control group and PCS treatment group. After PCS at a concentration of 0 μg/mL, 20 μg/mL, 40 μg/mL, and 80 μg/mL acted on human umbilical vein endothelial cells for 24 h, the cell culture supernatant was collected to determine the release amount of CD42b-/CD31+EMPs. Results revealed that PCS could significantly increase the release of CD42b-/CD31+EMPs in human umbilical vein endothelial cells compared with that in control group, and the increased level of CD42b-/CD31+EMPs was positively proportional to PCS concentration (Figure 2).

Then, human umbilical vein endothelial cells were divided into control group and PCS treatment group. After PCS at a concentration of 80 μg/mL acted on human umbilical vein endothelial cells for 24 h, RNA and protein were collected to detect expression levels of p-eNOS and TNF-α. Results revealed that the expression level of p-eNOS was decreased in human umbilical vein endothelial cells in PCS treatment group, while that of TNF-α was increased ($p < 0.01$) (Figure 3A and 3B). These results prove the damage of PCS to human umbilical vein endothelial cells.

PCS Damaged Human Umbilical Vein Endothelial Cells Via TLR4/TREM-1

Human umbilical vein endothelial cells were divided into control group, PCS treatment group (PCS), PCS treatment with TLR4 overexpression group (PCS+TLR4+), and PCS treatment with TLR4 knock-out group (PCS+TLR4-). After PCS at a concentration of 80 μg/mL acted on human umbilical vein endothelial cells for 24 h, the cell culture supernatant was collected to determine the release amount of CD42b-/CD31+EMPs. RNA and protein were collected to detect expression levels of TLR4, TREM-1, p-eNOS, and TNF-α. The release amount of CD42b-/CD31+EMPs is shown in Figure 4, and results manifested that TLR4 knockout could effectively reduce the release amount of CD42b-/CD31+EMPs under PCS treatment, while TLR4 overexpression could increase the release amount of CD42b-/CD31+EMPs. PCS treatment could increase the expression levels of TLR4 and TREM-1, and the expression level of TREM-1 declined after the TLR4 knockout, while that was increased after TLR4 overexpression (Figure 5A and 5B). TLR4 knockout led to the increased expression of p-eNOS and decreased expression of TNF-α, while TLR4 overexpression could further promote the increased expression of TNF-α and decreased expression of p-eNOS caused by PCS (Figure 3A and 3B). To sum up, PCS can damage human umbilical vein endothelial cells *via* TLR4/TREM-1.

Discussion

The incidence rate of cardiovascular disease is increased significantly in patients with chronic renal disease, abnormality of cardiac structure

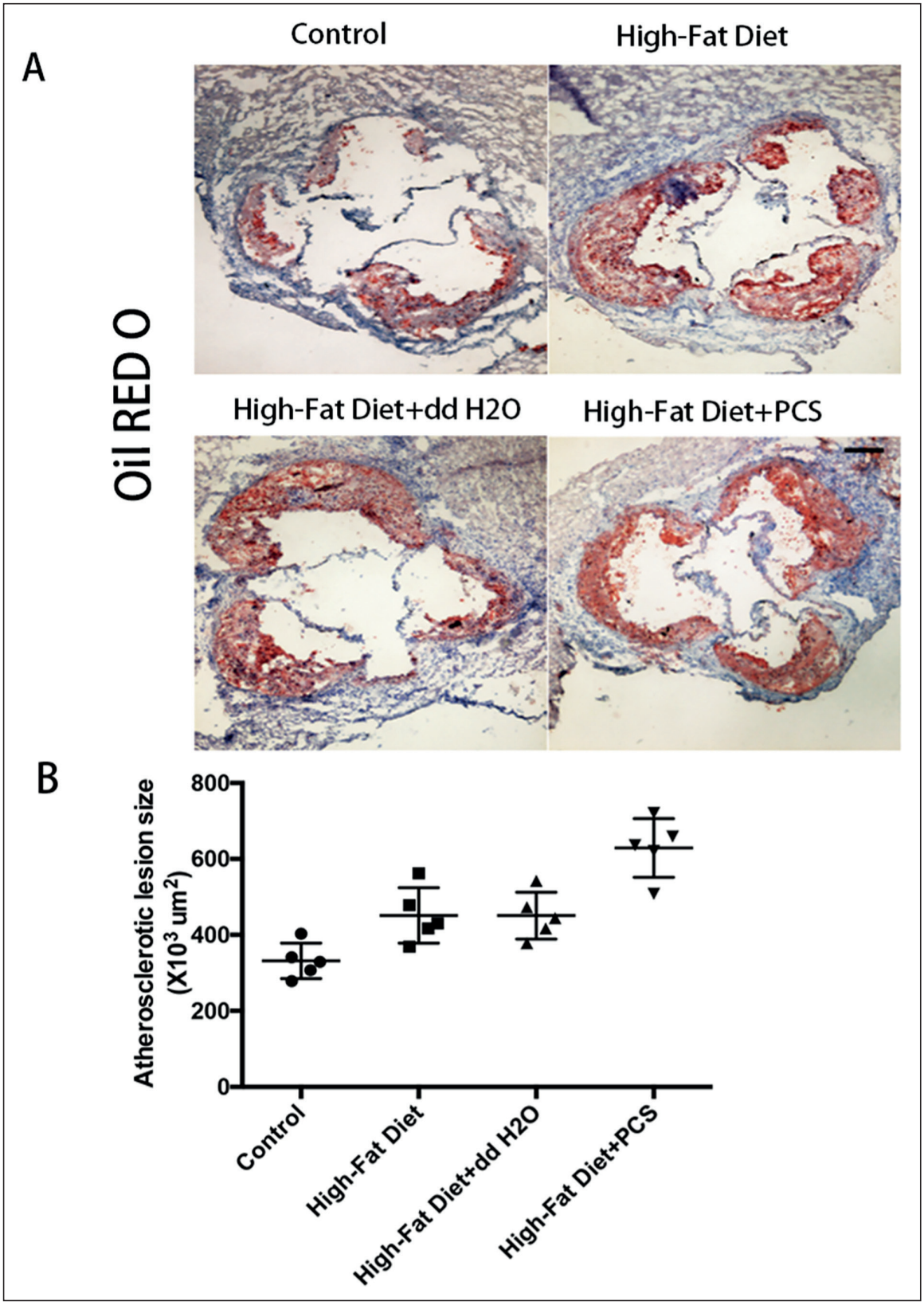

Figure 1. ***A,*** Representative sections of aortic sinus stained with Oil Red O in ApoE$^{-/-}$ mice in different groups (Control, High-Fat Diet, High-Fat Diet+dd H_2O, High-Fat Diet+PCS) at 16 weeks. ***B,*** Quantitative analysis of lesion size staining in different groups. Values are presented as the mean SD, n=5.

Table I. PCS level in ApoE$^{-/-}$ mice serum of different groups.

Group	Weight (g)	PCS (μg/mL)
Normal diet	26.59 ± 1.45	0.75 ± 0.88
High-Fat diet	29.04 ± 2.57	2.91 ± 3.04
Control	30.73 ± 1.79	3.28 ± 4.17
PCS	32.60 ± 2.08	6.83 ± 3.36

and coronary artery-related lesions, including atherosclerosis and myocardial infarction, occur in many patients with end-stage renal disease[18,19], which may be related to more common traditional risk factors for cardiovascular disease in patients with chronic renal disease. Hypertension, diabetes mellitus, lipid metabolism disorder, hyperhomocysteinemia, and abnormal activation of the renin-angiotensin system are more likely to occur in patients with chronic renal disease[20-23].

According to recent studies, however, after traditional risk factors for cardiovascular diseases, such as hypertension and diabetes mellitus, are controlled, and dyslipidemia in patients with the chronic renal disease is improved, the cardiovascular complications in patients fail to be improved[24]. Therefore, uremia toxin, as a unique cardiovascular risk factor in chronic renal disease, has been a research focus at present. Protein-binding small-molecule toxins cannot be removed from the body due to dialysis, so it has been paid more attention to[25]. Scholars[26-28] have demonstrated that PCS, as a kind of protein-binding small-molecule toxin, is related to the cardiovascular mortality and all-cause mortality rates of patients with chronic renal disease in different degrees. However, the specific mechanism of PCS in inducing cardiovascular system diseases has not been fully studied yet. The influence of PCS on the occurrence and development of atherosclerosis is a major focus, so this research mainly aims to study the specific mechanism of PCS in inducing atherosclerosis.

The formation of atherosclerotic plaque is a multi-factor complex process, in which an inflammatory response is an important promoting factor. The inflammatory response leads to the release of a large number of cells and inflammatory factors, activates multiple inflammatory response pathways, causes damage to the structure and organelle of vascular endothelial cells in different degrees, and results in dysfunction of vascular endothelial cells, thereby accelerating the plaque formation[2]. TLR4, a member of the TLR family, is also a pattern recognition receptor for LPS, which plays an important role in the initiation and progression of inflammation[12]. Some authors[29,30] have proved that TLR4 can promote the formation of atherosclerotic plaque, such an effect may be related to the activation of NF-κB signaling pathway by TLR4, and TLR4 also regulates the role of oxidized low-density lipoprotein during the formation of atherosclerosis. However, there is a lack of study on the role of TLR4 in the process of promoting atherosclerosis by PCS. In this investigation, the possible correlation between PCS and TLR4 in the formation of atherosclerosis was studied.

TREM-1 is a kind of pro-inflammatory factor, which amplifies the inflammation. It has been demonstrated in existing investigations

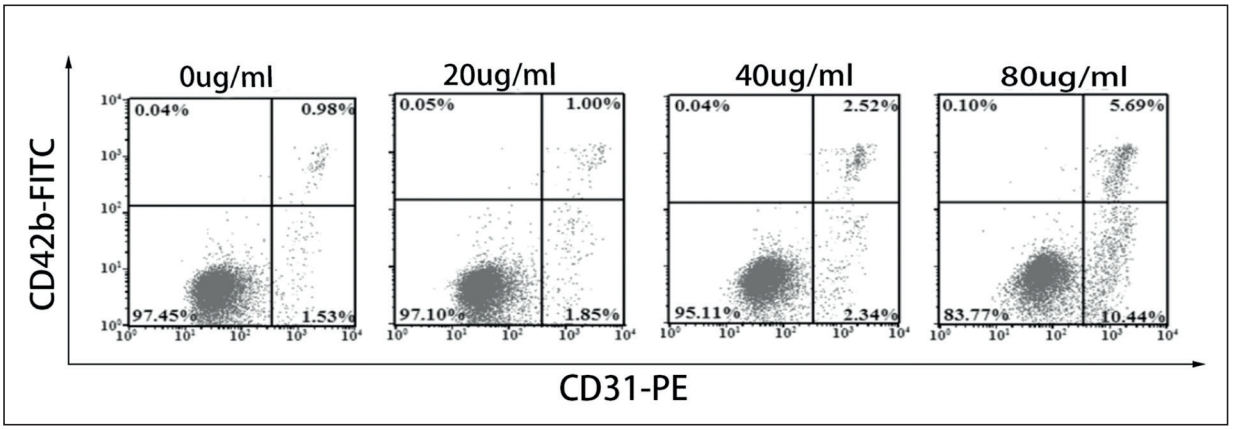

Figure 2. Flow cytometry analysis of CD42b-/CD31+EMPs was determined using FITC/PI staining in human cells umbilical vein endothelial cells treated with PCS with different concentration. Data are typical of three similar experiments. The percentage of FITC and/or PI positive cells was depicted with cytofluorometer quadrant graphs.

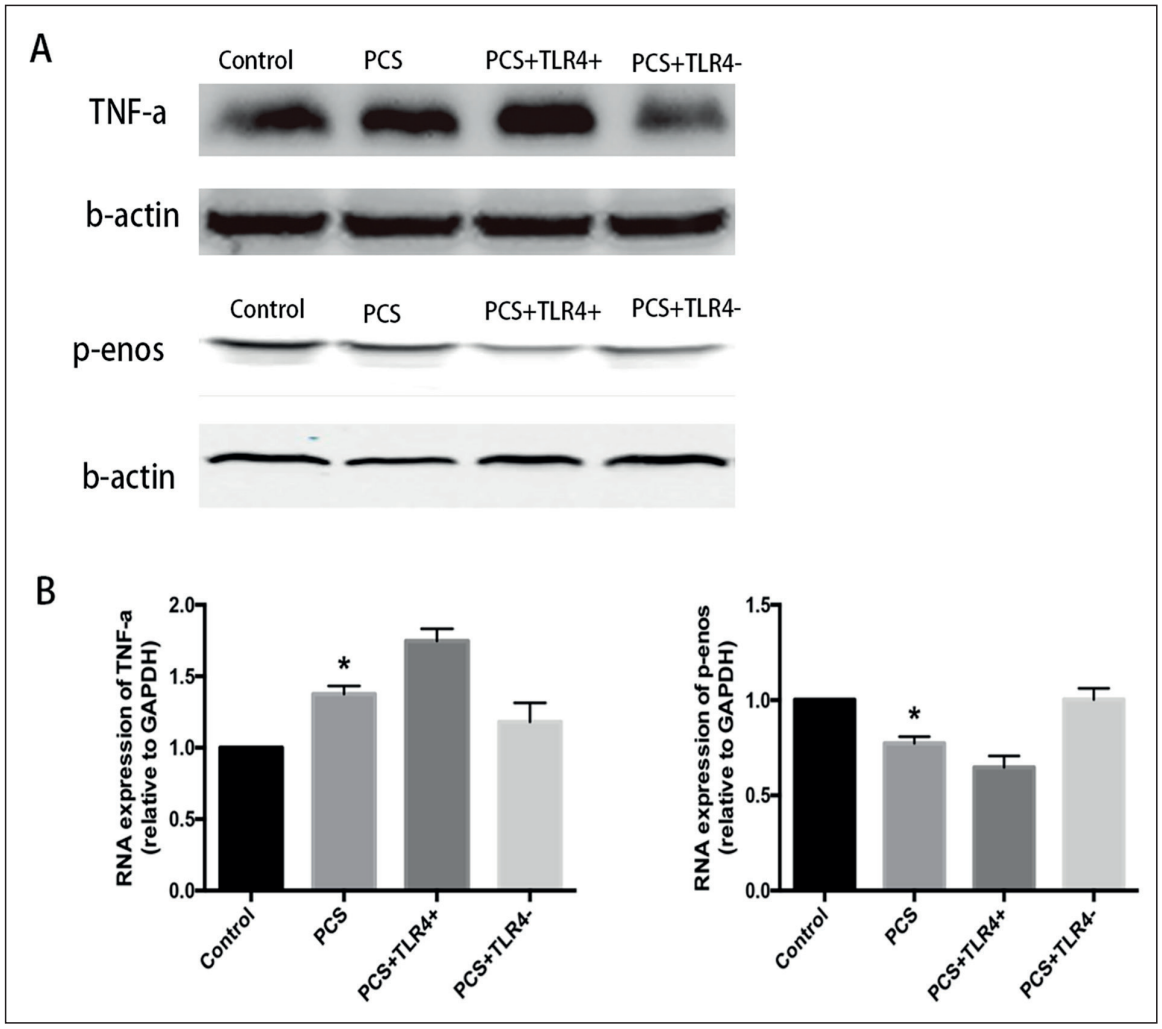

Figure 3. ***A,*** Images of TLR4 protein and mRNA content in Control, PCS, PCS+TLR4+, -PCS+TLR4- *via* Western blotting detection and real-time PCR detection. **p* < 0.05 *vs.* Control (n = 5). ***B,*** Images of TREM-1 protein and mRNA content in Control, PCS, PCS+TLR4+, -PCS+TLR4- *via* Western blotting detection and real-time PCR detection. **p* < 0.05 *vs.* Control (n = 5).

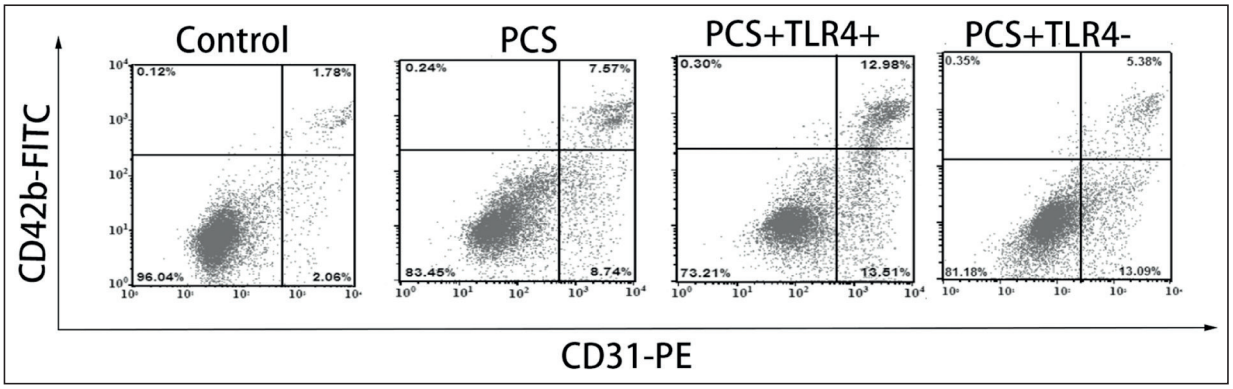

Figure 4. Flow cytometry analysis of CD42b-/CD31+EMPs was determined using FITC/PI staining in human cells umbilical vein endothelial cells. Data are typical of three similar experiments. The percentage of FITC and/or PI positive cells was depicted with cytofluorometer quadrant graphs.

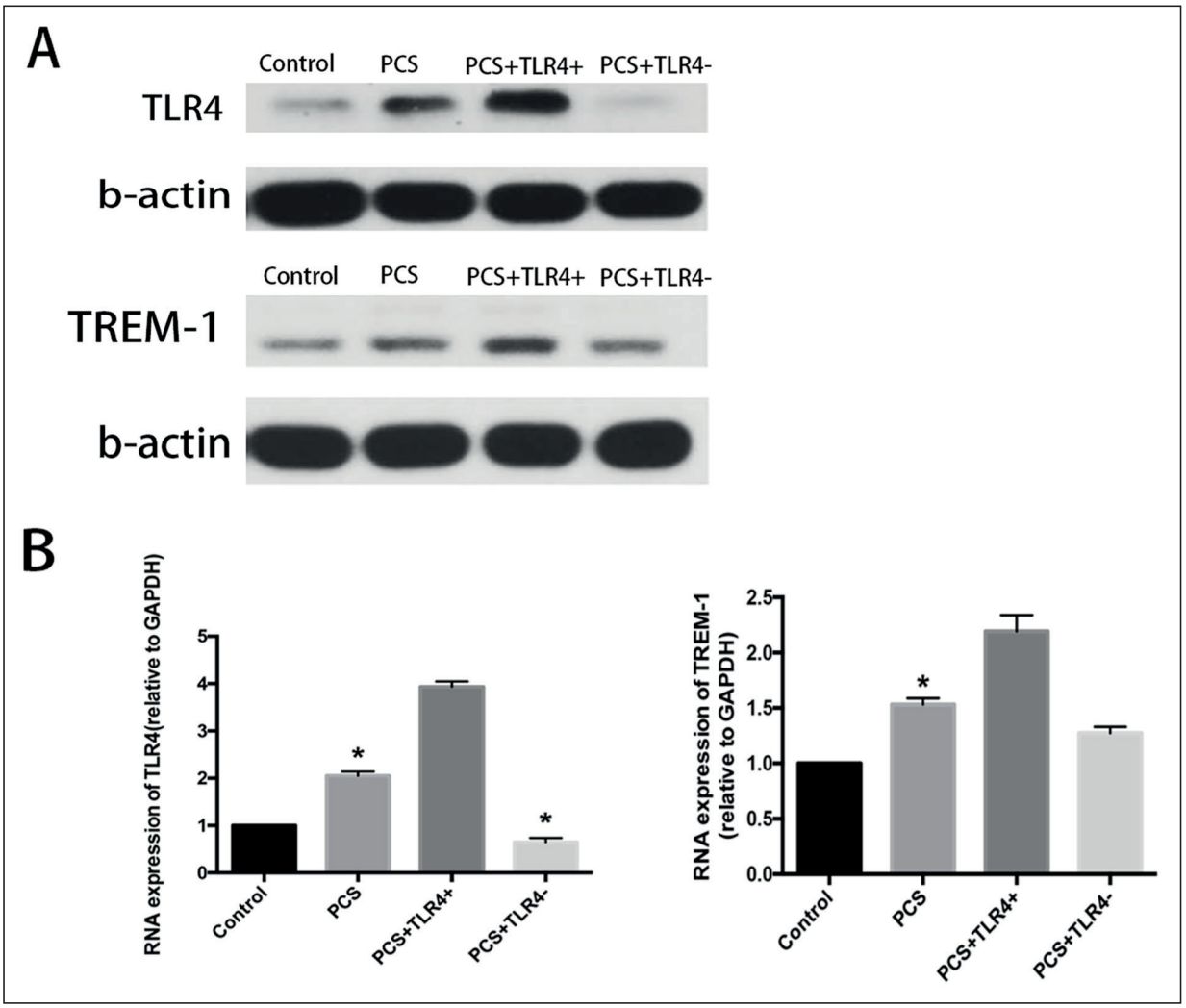

Figure 5. ***A,*** Images of TNF-a protein and mRNA content in Control, PCS, PCS+TLR4+, PCS+TLR4- *via* Western blotting detection and real-time PCR detection. *$p < 0.05$ *vs.* Control (n=5). ***B,*** Images of p-enos protein and mRNA content in Control, PCS, PCS+TLR4+, PCS+TLR4- *via* Western blotting detection and real-time PCR detection. *$p < 0.05$ *vs.* Control (n = 5).

that TREM-1 can bind to TLR4 to promote the inflammatory response under the action of TLR4[31]. TREM-1 plays an important role that cannot be ignored in the action of TLR4, so the related changes in TLR4/TREM-1 under the action of PCS were explored in this study.

Results of this work showed that PCS promotes the formation of atherosclerotic plaque in mice, and the cell experiment provided a theoretical basis for it. In human umbilical vein endothelial cells, PCS can significantly promote the expressions of TLR4 and TREM-1, and lead to the increased expression level of the inflammatory factor TNF-α and decreased expression level of NO-related gene p-eNOS. However, the cell environment *in vitro* is so single that the complex environment *in vivo* cannot be simulated. Therefore, the role of PCS in the formation of atherosclerosis and its specific mechanism are still pending further study.

Conclusions

We observed that P-cresyl sulfate confers damages vascular endothelial cells through TRL4/TREM-1, thereby promoting the formation of atherosclerosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004; 109: I27-I32.
2) Bai B, Liang Y, Xu C, Lee MY, Xu A, Wu D, Vanhoutte PM, Wang Y. Cyclin-dependent kinase 5-mediated hyperphosphorylation of sirtuin-1 contributes to the development of endothelial senescence and atherosclerosis. Circulation 2012; 126: 729-740.
3) Niwa T. Update of uremic toxin research by mass spectrometry. Mass Spectrom Rev 2011; 30: 510-521.
4) Holdsworth SR, Tipping PG. Leukocytes in glomerular injury. Semin Immunopathol 2007; 29: 355-374.
5) Schepers E, Meert N, Glorieux G, Goeman J, Van der Eycken J, Vanholder R. P-cresylsulphate, the main in vivo metabolite of p-cresol, activates leucocyte free radical production. Nephrol Dial Transplant 2007; 22: 592-596.
6) Palm F, Nangaku M, Fasching A, Tanaka T, Nordquist L, Hansell P, Kawakami T, Nishijima F, Fujita T. Uremia induces abnormal oxygen consumption in tubules and aggravates chronic hypoxia of the kidney via oxidative stress. Am J Physiol Renal Physiol 2010; 299: F380-F386.
7) Lee SB, Kalluri R. Mechanistic connection between inflammation and fibrosis. Kidney Int Suppl 2010: S22-S26.
8) Sun CY, Hsu HH, Wu MS. p-Cresol sulfate and indoxyl sulfate induce similar cellular inflammatory gene expressions in cultured proximal renal tubular cells. Nephrol Dial Transplant 2013; 28: 70-78.
9) Meijers BK, Van Kerckhoven S, Verbeke K, Dehaen W, Vanrenterghem Y, Hoylaerts MF, Evenepoel P. The uremic retention solute p-cresyl sulfate and markers of endothelial damage. Am J Kidney Dis 2009; 54: 891-901.
10) Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, Temmar M, Choukroun G, Vanholder R, Massy ZA; European Uraemic Toxin Work Group (EUTox). Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. Nephrol Dial Transplant 2010; 25: 1183-1191.
11) Akira S, Hoshino K. Myeloid differentiation factor 88-dependent and -independent pathways in toll-like receptor signaling. J Infect Dis 2003; 187 Suppl 2: S356-S363.
12) Calippe B, Douin-Echinard V, Laffargue M, Laurell H, Rana-Poussine V, Pipy B, Guery JC, Bayard F, Arnal JF, Gourdy P. Chronic estradiol administration in vivo promotes the proinflammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. J Immunol 2008; 180: 7980-7988.
13) Chen LJ, Ding YB, Ma PL, Jiang SH, Li KZ, Li AZ, Li MC, Shi CX, Du J, Zhou HD. The protective effect of lidocaine on lipopolysaccharide-induced acute lung injury in rats through NF-kappaB and p38 MAPK signaling pathway and excessive inflammatory responses. Eur Rev Med Pharmacol Sci 2018; 22: 2099-2108.
14) Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 2001; 410: 1103-1107.
15) Schenk M, Bouchon A, Seibold F, Mueller C. TREM-1--expressing intestinal macrophages crucially amplify chronic inflammation in experimental colitis and inflammatory bowel diseases. J Clin Invest 2007; 117: 3097-3106.
16) Gibot S, Massin F, Marcou M, Taylor V, Stidwill R, Wilson P, Singer M, Bellingan G. TREM-1 promotes survival during septic shock in mice. Eur J Immunol 2007; 37: 456-466.
17) Bleharski JR, Kiessler V, Buonsanti C, Sieling PA, Stenger S, Colonna M, Modlin RL. A role for triggering receptor expressed on myeloid cells-1 in host defense during the early-induced and adaptive phases of the immune response. J Immunol 2003; 170: 3812-3818.
18) Nohria A, Hasselblad V, Stebbins A, Pauly DF, Fonarow GC, Shah M, Yancy CW, Califf RM, Stevenson LW, Hill JA. Cardiorenal interactions: insights from the ESCAPE trial. J Am Coll Cardiol 2008; 51: 1268-1274.
19) Keeley EC, Kadakia R, Soman S, Borzak S, McCullough PA. Analysis of long-term survival after revascularization in patients with chronic kidney disease presenting with acute coronary syndromes. Am J Cardiol 2003; 92: 509-514.
20) Locatelli F, Del VL, Pozzoni P. Anemia and cardiovascular risk: the lesson of the CREATE Trial. J Am Soc Nephrol 2006; 17(12 Suppl 3): S262-S266.
21) Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, Reddan D; CHOIR Investigators. Correction of anemia with epoetin alfa in chronic kidney disease. N Engl J Med 2006; 355: 2085-2098.
22) Molitch ME. Management of dyslipidemias in patients with diabetes and chronic kidney disease. Clin J Am Soc Nephrol 2006; 1: 1090-1099.
23) Mix TC, Brenner RM, Cooper ME, de Zeeuw D, Ivanovich P, Levey AS, McGill JB, McMurray JJ, Parfrey PS, Parving HH, Pereira BJ, Remuzzi G, Singh AK, Solomon SD, Stehman-Breen C, Toto RD, Pfeffer MA. Rationale--Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT): evolving the management of cardiovascular risk in patients with chronic kidney disease. Am Heart J 2005; 149: 408-413.
24) Sarnak MJ, Coronado BE, Greene T, Wang SR, Kusek JW, Beck GJ, Levey AS. Cardiovascular disease risk factors in chronic renal insufficiency. Clin Nephrol 2002; 57: 327-335.
25) Martinez AW, Recht NS, Hostetter TH, Meyer TW. Removal of P-cresol sulfate by hemodialysis. J Am Soc Nephrol 2005; 16: 3430-3436.

26) De Smet R, Van Kaer J, Van Vlem B, De Cubber A, Brunet P, Lameire N, Vanholder R. Toxicity of free p-cresol: a prospective and cross-sectional analysis. Clin Chem 2003; 49: 470-478.

27) Meijers BK, Bammens B, De Moor B, Verbeke K, Vanrenterghem Y, Evenepoel P. Free p-cresol is associated with cardiovascular disease in hemodialysis patients. Kidney Int 2008; 73: 1174-1180.

28) Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y. Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. Kidney Int 2006; 69: 1081-1087.

29) Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Arditi M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation 2001; 104: 3103-3108.

30) Walton KA, Hsieh X, Gharavi N, Wang S, Wang G, Yeh M, Cole AL, Berliner JA. Receptors involved in the oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine-mediated synthesis of interleukin-8. A role for Toll-like receptor 4 and a glycosylphosphatidylinositol-anchored protein. J Biol Chem 2003; 278: 29661-29666.