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4-Phenylbutyric acid suppresses inflammation through regulation of endoplasmic reticulum stress of endothelial cells stimulated by uremic serum

Wei Zeng, Yan-Hong Guo, Wei Qi, Ji-Gang Chen, Li-Ling Yang, Zhi-Feng Luo, Jiao Mu, Bing Feng *

Institute of Nephrology of Chongqing and Department of Nephrology, Xinqiao Hospital, The Third Military Medical University, Chongqing 400037, PR China

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ABSTRACT

Aims: Endoplasmic reticulum (ER) stress is involved in the pathogenesis of atherosclerosis (AS). Endothelial cell (EC) dysfunction and monocyte migration to the subendothelium are considered to be essential manifestations of AS. We conducted this study to determine whether ER stress was involved in uremic serum-induced EC dysfunction and whether the regulation of ER stress using a chemical chaperone 4-phenylbutyric acid (4-PBA) had a preventative effect.

Main methods: Human umbilical vein endothelial cells (HUVECs) were divided into 4 groups: a control serum group (C.S), a uremic serum group (U.S), a uremic serum plus 4-PBA (5 mM) treatment group (4-PBA), and a uremic serum plus pyrrolidine dithiocarbamate (PDTC:50 μM) treatment group (PDTC).

Key findings: Lower concentrations of uremic serum (<10%) facilitated the proliferation of HUVECs. In contrast, the proliferative capability of HUVECs was gradually decreased when we continuously increased the concentration of uremic serum. Compared with C.S, HUVEC incubation with uremic serum had high expression levels of GRP78, p-PERK, NF-κB, MCP-1, and VEGF. THP-1 migration was markedly higher than C.S over the indicated time. These alterations were inhibited by the administration of 4-PBA.

Significance: These findings suggest that regulation of ER stress coupled with inflammatory activation by 4-PBA would be a promising therapy to reverse the process and development of uremic serum-induced EC dysfunction.

Introduction

Cardiovascular disease (CVD) is one of the most serious complications associated with a high mortality in end-stage renal disease (ESRD) (Tonelli et al., 2006). It is well established that endothelial dysfunction is involved in renal function-associated cardiovascular mortality (Amabile et al., 2005). Endothelial cells (ECs) that line the blood vessels contact uremic toxins in ESRD; theoretically, ECs are the primary victims of uremic toxin. Endothelial dysfunction develops at the earliest stages of kidney disease when the glomerular filtration rate (GFR) begins to decline and blood pressure increases (Sima et al., 2009; Morris et al., 2000), which includes impaired endothelium-dependent vasodilation, increased adhesion of platelets and leukocytes, inflammatory activation, and decreased bioavailability of nitric oxide (Stam et al., 2006; Amann and Ritz, 2000; Blacher et al., 2002; Dickhout et al., 2005). Importantly, activated monocytes migrate to the subendothelium and differentiate into macrophages, and eventually become foam cells, constituting the fundamental pathological change of atherosclerosis (AS). In our previous research, we observed continuous inflammatory activation of endothelial cells induced by uremic serum. Moreover, the proteasome inhibitor MG132 alleviated the inflammation, which suggested that the ubiquitin proteasome pathway (UPP) plays an important role in the vascular complications associated with uremia (Feng et al., 2011). However, the UPP makes up only a small part of the inflammatory signaling pathways. Most studies suggest that there is crosstalk between endoplasmic reticulum (ER) stress and UPP, and ER stress can cause a series of pathological cellular changes, including the activation of a local inflammatory response, cell proliferation, and apoptosis (Austin, 2009; Kitamura, 2008; Rasheva and Domingos, 2009). ER stress couples oxidative stress and mitogen-activated protein kinases (MAPK), along with inflammation (Fornoni et al., 2008; Zhang and Kaufman, 2008; Sekine et al., 2006). Therefore, ER stress would be involved in the pathogenesis of EC dysfunction induced by uremic serum.

In this study, we incubated human umbilical vein endothelial cells (HUVECs) with uremic serum for the purpose of investigating the effect of uremic serum on activation of the ER stress in HUVECs and the role of the ER stress in inflammation of HUVECs.

* Corresponding author. Tel.: +86 23 68774021; fax: +86 23 68774321.
E-mail address: fxb12@aliyun.com (B. Feng).

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Materials and methods

Serum preparation

The blood samples from chronic renal failure patients were drawn from 10 patients who received maintenance hemodialysis before the dialysis session and heparin administration. Five patients were men and 5 were women; their mean age was 45.75 ± 18.19 years, and their dialysis period lasted for 4 h 3 times weekly. In all of these patients, renal failure was attributed to chronic glomerulonephritis. Blood samples were collected and centrifuged at 3000 rpm for 5 min at 4 °C. Serum samples were pooled and inactivated at 56 °C for 30 min. They were filtered through 0.22 μm filters (Millipore, USA) and stored at −20 °C. The mean values for the biochemical parameters measured were as follows: BUN, 18.018 ± 4.916 mmol/L; Cr, 832.425 ± 176.055 μmol/L; CO_2-CP, 15.000 ± 1.633 mmol/L; Na^+, 139.650 ± 2.541 mmol/L; K^+, 4.148 ± 0.780 mmol/L; ALB, 34.475 ± 8.528 mmol/L; TG, 1.433 ± 0.388 mmol/L; and CHO 5.280 ± 1.216 mmol/L. We recruited 4 age- and sex-matched healthy subjects who served as healthy controls. The mean values for the biochemical parameters measured were as follow: BUN, 7.105 ± 0.415 mmol/L; Cr, 91.4253 ± 6.210 μmol/L; CO_2-CP, 23.250 ± 3.304 mmol/L; Na^+, 142.075 ± 7.317 mmol/L; K^+, 4.525 ± 0.392 mmol/L; Alb, 42.078 ± 2.859 mmol/L; TG, 2.063 ± 0.762 mmol/L; and CHO. 4.435 ± 0.219 mmol/L. All participants provided their informed consent.

Cells and culture

HUVECs and human monocytic THP-1 cells from the Institute of Burn Research (The Third Military Medical University, China) were grown in RPMI 1640 medium (Salt Lake City, UT, USA) supplemented with 10% fetal bovine serum (Sijiqing, Hangzhou, China), at 37 °C in a humidified incubator (Heraeus, Germany) with 95% air and 5% CO_2. Cells (passages 2–3) grown to sub-confluence were randomly divided into the uremic serum group (U.S) and the control serum group (C.S). They were treated with uremic serum or control serum as appropriate at the engineered concentrations for predetermined durations of time. After induction of uremic cells, we divided the cells into 3 groups: HUVEC incubation with 10% uremic serum (10% U.S), HUVECs in 10% uremic serum with 4-PBA treatment (4-PBA), and HUVECs in 10% uremic serum with pyrrolidine dithiocarbamate treatment (PDTC). The cells were treated with uremic serum plus 5 mM 4-PBA (St. Louis, MO) or 50 μM PDTC (Sigma, USA), as appropriate.

Cell viability assays

The cell viability was measured as described previously (Trevisi et al., 2006). Briefly, HUVECs were plated on M96-well plates at 1×10^4cells/mL. After the corresponding treatments, we incubated the cells for 4 h with 0.5 mg/mL of MTT (Amersham, LON, UK) and then lysed the cells with dimethylsulfoxide (DMSO). Absorbance was measured at 490 nm in a microplate reader (Sunrise, Austria).

Flow cytometry

Cell cycle was analyzed by flow cytometry as previously described (Lin et al., 2004). We collected adherent cells from each group and fixed them on ice in 95% ethanol for 30 min. We washed the cells with PBS and resuspended them in 1 mL of cell cycle buffer (50 μg/mL propidium iodide (PI) in PBS containing 1 mg/mL RNase) for 30 min at 37 °C in the dark. After staining, we evaluated the cells for their positions in the cell cycle using flow cytometry (BD FACSCalibur™, USA).

Real-time fluorescence PCR

Total RNA was extracted with TRIzol (TIANGEN, Beijing, China) according to the manufacturer's instructions. A standard reverse transcriptase reaction kit (Toyobo, Japan) was used to synthesize cDNA. The primer sequences for GRP78 (200 bp) were CAAGCAGAAGTGGG TTCA (sense) and GGGAAAGCTAGGGGAAAATAAG (antisense); for P^{21} (531 bp), the primer sequences were AGGAGGCGCCATGTCAGAAC (sense) and AGGACTGCAGGCTTCCTGTG (antisense); for NF-κB (121 bp), the primer sequences were GGCTATAACTCGCCTAGTGA (sense) and CGAAGGAGCTGATCTGACTCA (antisense); for MCP-1 (147 bp), the primer sequences were CAGCCAGATGCAATCAATGC (sense) and GTGGTCCATGGAATCCTGAA (antisense); for VEGF (489 bp), the primer sequences were CCTGGTGGACATCTTCCAGGAGTACC (sense) and GAAGCTCATCTCTCCTATGTGCTGGC (antisense); GAPDH (159 bp) was used as a reference gene: the primer sequences were CCACCCATGG

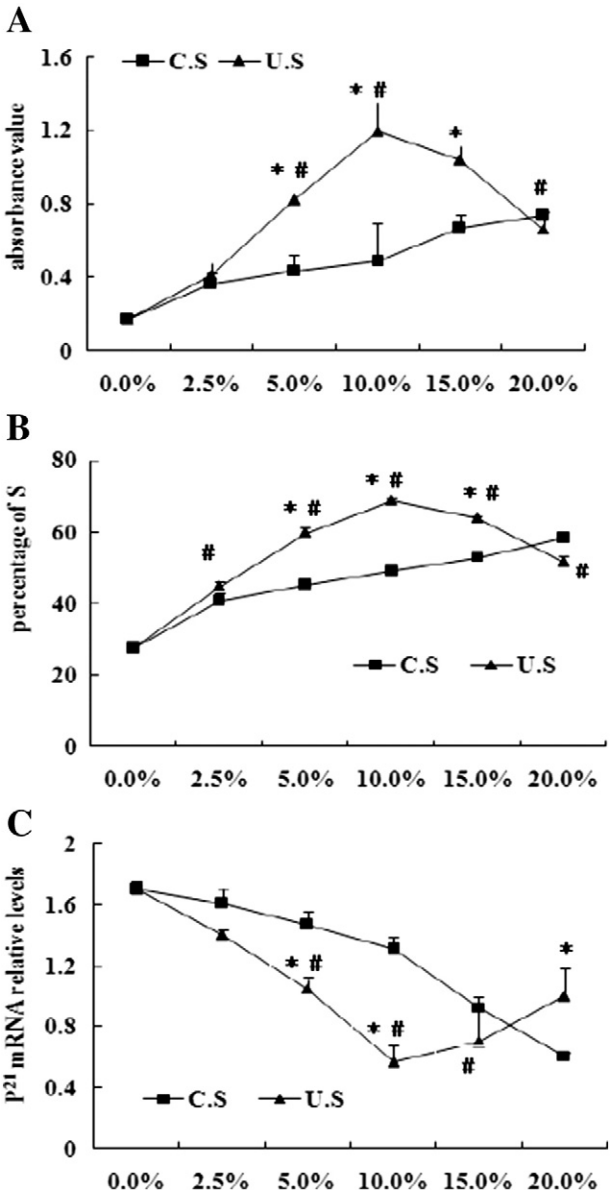

Fig. 1. Proliferation of HUVECs exposed to the different types of serum. HUVECs in C.S or U.S groups exposed to different serum concentrations (0%–20%) for 24 h; subsequently, cell vitality was determined by the MTT assay (A), cell cycle phase was determined by flow cytometry (B), expression of P^{21} mRNA was determined by RT-PCR (C). (n = 5/group). $^*P < 0.05$ versus the C.S group; $^\#P < 0.05$ versus the previous concentration.