Biochemical and Biophysical Research Communications 458 (2015) 796–801

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Berberine improves endothelial function by inhibiting endoplasmic reticulum stress in the carotid arteries of spontaneously hypertensive rats

Limei Liu [a, **], Jian Liu [b], Zhengxiang Huang [a], Xiaoxing Yu [a], Xinyu Zhang [a], Dou Dou [a], Yu Huang [b, *]

[a] Department of Physiology and Pathophysiology, Peking University Health Science Center, Peking, China
[b] Institute of Vascular Medicine and Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Hong Kong, China

ARTICLE INFO

Article history:
Received 30 January 2015
Available online 14 February 2015

Keywords:
Carotid artery
Cyclooxygenase
Endoplasmic reticulum stress
Endothelial function
Spontaneously hypertensive rats

ABSTRACT

Activation of endoplasmic reticulum (ER) stress in endothelial cells leads to increased oxidative stress and often results in cell death, which has been implicated in hypertension. The present study investigated the effects of berberine, a botanical alkaloid purified from Coptidis rhizoma, on ER stress in spontaneously hypertensive rats (SHRs) and the underling mechanism. Isolated carotid arteries from normotensive WKYs and SHRs were suspended in myograph for isometric force measurement. Protein phosphorylations and expressions were determined by Western blotting. Reactive oxygen species (ROS) level was measured by DHE staining. SHR carotid arteries exhibited exaggerated acetylcholine-triggered endothelium-dependent contractions (EDCs) and elevated ROS accumulation compared with WKY arteries. Moreover, Western blot analysis revealed the reduced AMPK phosphorylation, increased eIF2α phosphorylation, and elevated levels of ATF3, ATF6, XBP1 and COX-2 in SHR carotid arteries while these pathological alterations were reversed by 12 h-incubation with berberine. Furthermore, AMPK inhibitor compound C or dominant negative AMPK adenovirus inhibited the effects of berberine on above-mentioned marker proteins and EDCs. More importantly, ROS scavengers, tempol and tiron plus DETCA, or ER stress inhibitors, 4-PBA and TUCDA normalized the elevated levels of ROS and COX-2 expression, and attenuated EDCs in SHR arteries. Taken together, the present results suggest that berberine reduces EDCs likely through activating AMPK, thus inhibiting ER stress and subsequently scavenging ROS leading to COX-2 down-regulation in SHR carotid arteries. The present study thus provides additional insights into the vascular beneficial effects of berberine in hypertension.

1. Introduction

Reactive oxygen species (ROS) over-production or increased oxidative stress impairs endothelial function and is one of the primary mediators of the development of hypertension [1–3]. Endothelium-dependent contractions (EDCs) are associated with endothelial dysfunction and correlated with the severity of hypertension [4]. EDCs are induced by endothelium-derived contracting factors including endothelial cyclooxygenase (COX)-derived prostanoids or ROS [5–7]. Reducing oxidative stress can diminish EDCs in renal arteries of spontaneously hypertensive rats (SHR) [6]. Endoplasmic reticulum (ER) stress is among the key players in endothelial dysfunction during hypertension [8] and is caused by the activation of complex cytoplasmic and nuclear signaling pathways, collectively termed the unfolded protein response [9]. Recent studies have shown a close link between ER stress and oxidative stress in cardiovascular pathogenesis [10,11].

Berberine ($[C20H18NO4]^+$), an isoquinoline alkaloid isolated from many medicinal herbs, has long been used in the treatment of gastrointestinal infections and diarrheas, but only until the last few decades its cardiovascular benefits have been reported [12]. Berberine inhibits human immunodeficiency virus protease inhibitor-induced inflammatory response by modulating ER stress

* Corresponding author. School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China. Fax: +852 26035123.
** Corresponding author. Department of Physiology and Pathophysiology, Peking University Health Science Center, No. 38 Xue Yuan Road, Peking 100191, China.
E-mail addresses: liu_limei@126.com (L. Liu), yu-huang@cuhk.edu.hk (Y. Huang).

http://dx.doi.org/10.1016/j.bbrc.2015.02.028
0006-291X/

signaling pathways in murine macrophages [13], ameliorates pro-inflammatory cytokine-induced ER stress in human intestinal epithelial cells [14], and reduces hypoxia/reoxygenation-induced injury by suppressing mitochondria stress and ER stress in human renal proximal tubular cells [15]. Although clinical and experimental studies suggest the anti-hypertensive properties of berberine and its derivative [16–18], the modulatory effects of berberine on ER stress in hypertension remain largely unknown. The present study therefore investigated whether berberine inhibits ER stress to attenuate EDCs in SHR carotid arteries.

2. Materials and methods

2.1. Chemicals

Anti-phospho-AMPKα (Thr172), anti-eIF2α, and anti-AMPKα antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-phospho-eIF2α (Ser51) and anti-GAPDH antibodies were obtained from Bioword Technology (Louis Park, MN, USA). Anti-COX-1 and anti-COX-2 antibodies were purchased from Cayman Chemical (Ann Arbor, MI, USA). Anti-ATF3 and anti-XBP1 antibodies were obtained from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA). Anti-ATF6 antibody was from Abcam (Cambridge, MA). HRP-conjugated swine anti-rabbit and anti-mouse IgG were from DakoCytomation (Carpinteria, CA, USA). Immobilon-P polyvinylidenedifluoride (PVDF) membrane was from Millipore (Billerica, MA, USA) and chemiluminescence (ECL reagents) was obtained from Amersham Pharmacia (GE Healthcare Life Sciences, Buckinghamshire, UK). Berberine, compound C, N^G-nitro-L-arginine methyl ester (L-NAME), acetylcholine (ACh), tempol, tiron, diethyldithiocarbamate (DETCA), and 4-phenyl butyric acid (4-PBA) were purchased from Sigma–Aldrich Chemical (St Louis, MO, USA). s18886 was a gift from the Institute RecherchesServier (Suresnes, France). SC560 and NS398 were from Tocris Bioscience (Bristol, UK). Dihydroethidium (DHE) was from Invitrogen (Carlsbad, CA, USA). Tauroursodeoxycholic acid (TUDCA) was from Calbiochem-Novabiochem Corp. (San Diego, CA). L-NAME and ACh were dissolved in distilled water. Other drugs were dissolved in DMSO. DMSO (0.1% v/v) did not modify ACh-induced contraction.

2.2. Animal protocols

Male SHR (32–40 weeks old) and WKY rats (32–40 weeks old) were used in compliance with the Institutional Authority for Laboratory Animal Care, Peking University Health Science Center, China. This study 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).

2.3. Artery preparation and functional assay

WKY and SHRs were sacrificed by CO_2 suffocation. Carotid arteries from rats were removed and placed in ice-cold Krebs solution (mmol/L): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11 D-glucose. Arteries were cleaned of adhering tissue and cut into ring segments of 2 mm in length and suspended in myograph (Danish Myo Technology, Aarhus, Denmark) for recording of changes in isometric tension. Briefly, to visualize the endothelium-dependent contractions, rings with endothelium were exposed for 30 min to 100 μmol/L L-NAME to eliminate the relaxant effect of endothelium-derived nitric oxide before the application of ACh (0.01–100 μmol/L). The effects of COX-1 inhibitor (SC560, 10 nmol/L), COX-2 inhibitor (NS398, 1 μmol/L) or TP receptor antagonist (s18886, 0.1 μmol/L) were tested on ACh-induced contractions following 30-min incubation with L-NAME. In some experiments, SHR carotid arteries were incubated with ROS scavengers [tempol (100 μmol/L) or tiron (1 mmol/L) plus DETCA (100 μmol/L)], ER stress inhibitors [4-PBA (10 μmol/L) or TUDCA (20 μmol/L)] and compound C (10 μmol/L, AMPKα inhibitor) along with berbeine (1 μmol/L) for 12 h in Dulbecco's Modified Eagle's Media (DMEM) with 10% fetal bovine serum, 100 IU penicillin, and 100 μg/mL streptomycin.

2.4. Western blot analysis

Carotid arteries from WKY and SHRs were cultured and then homogenized in RIPA lysis buffer containing 1 μg/mL leupeptin, 5 μg/mL aprotinin, 100 μg/mL PMSF, 1 mmol/L sodium orthovanadate, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L sodium fluoride, and 2 μg/mL β-glycerolphosphate. The homogenated were centrifuged at 20,000 × g for 20 min at 4 °C. Protein lysates (10 μg) were separated by electrophoresis and transferred onto PVDF membrane. Blots were blocked with 1% bovine serum albumin or 5% non-fat milk for 1 h and incubated overnight at 4 °C with primary antibodies. After washing, blots were incubated with HRP-conjugated swine anti-rabbit or anti-mouse IgG. Immunoreactive bands were visualized by chemiluminescence and exposed to Kodak Image Station 440 for densitometric analysis.

2.5. Dihydroethidium (DHE) staining

Frozen sections of rat carotid arteries on glass coverslips were loaded with 5 μmol/L DHE at 37 °C for 10 min. ROS fluorescence was measured by a confocal scanning unit (Olympus) at excitation 515 nm and emission 585 nm. Data were analyzed by the Fluoview software (Olympus).

2.6. Adenoviral infection

Recombinant virus was produced in human embryonic kidney (HEK) 293A cells. SHR carotid arteries were infected with a dominant negative AMPK (DN-AMPK) adenovirus, using a protocol of 4-h exposure to 1.5 μl of adenovirus to 24-well plate (1×10^8 plaque forming units/ml). 4 h after infection, arteries were cultured for 20 h in full DMEM medium and then treated with or without berberine (1 μmol/L) for additional 12 h. Thereafter, arteries were collected for functional study, Western blotting, and DHE staining.

2.7. Statistical analysis

The contraction was expressed as percentage of 60 mmol/L KCl-induced tension. Results are means ± SEM and n represents carotid arteries from different rats. Statistical significance was determined by two-tailed Student's t-test and nonparametric test. $P < 0.05$ was considered significantly different.

3. Results

3.1. Berberine attenuates endothelium-dependent contractions in SHR carotid arteries

Endothelium-dependent contractions (EDCs) are associated with endothelial dysfunction in hypertension [4,6]. ACh-triggered EDCs in carotid arteries from SHR were exaggerated compared with WKY arteries, and 12-h incubation with berbeine (1 μmol/L) markedly attenuated EDCs from SHR arteries without affecting those from WKY (Fig. 1A). The EDCs were likely mediated through COX-2-dependent mechanism since they were inhibited or abolished by selective COX-2 inhibitor NS398 (1 μmol/L) or selective TP

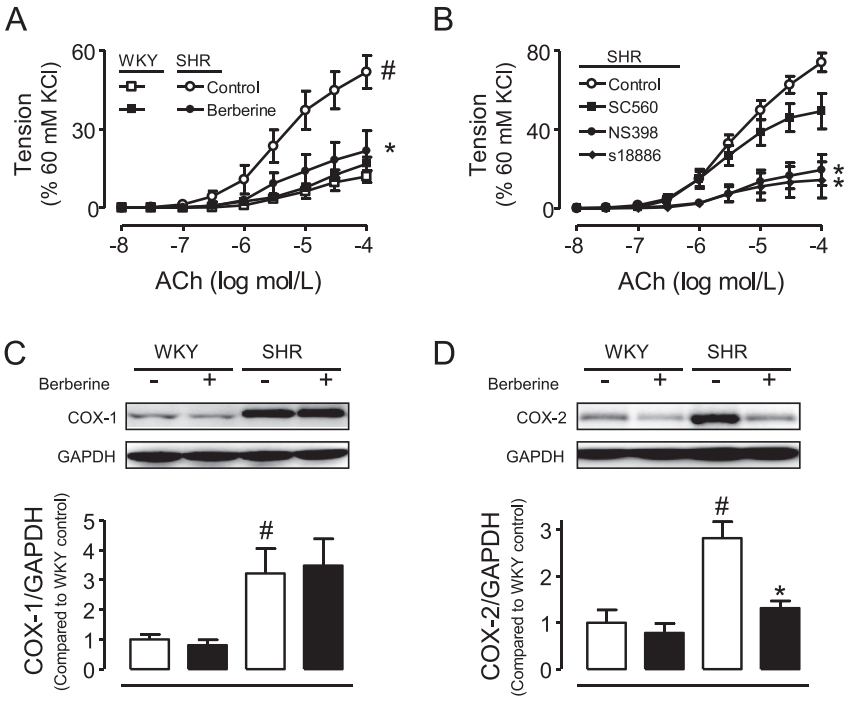

Fig. 1. (A) ACh elicited endothelium-dependent contractions (EDCs) of L-NAME-treated SHR carotid arteries, which were inhibited by 12 h-incubation with berberine (1 μmol/L). (B) EDCs in SHR arteries were inhibited substantially by treatment with selective COX-2 inhibitor NS398 (1 μmol/L) or selective TP receptor antagonist s18886 (0.1 μmol/L) but slightly reduced by COX-1 inhibitor SC560 (10 nmol/L). COX-1 (C) and COX-2 (D) expressions in carotid arteries. $^{*}P < 0.05$ vs SHR control; $^{\#}P < 0.05$ vs WKY control. Results are means ± SEM (n = 6 for functional study, n = 4 for Western blot).

receptor antagonist s18886 (0.1 μmol/L) but slightly reduced by COX-1 inhibitor SC560 (10 nmol/L) (Fig. 1B). Western blot analysis revealed the elevated levels of COX-1 (Fig. 1C) and COX-2 (Fig. 1D) in SHR carotid arteries compared with WKY. However, berberine reduced the expression of COX-2 (Fig. 1D) but not COX-1 (Fig. 1C) in SHR arteries, suggesting that berberine attenuates EDCs in SHR arteries probably through down-regulating COX-2 expression.

3.2. Berberine attenuates endothelium-dependent contractions through inhibiting endoplasmic reticulum stress in SHR carotid arteries

Berberine was reported to activate AMPK in endothelial cells [19], smooth muscle cells [20], and pancreatic β cells [21]. The present study shows a reduced phosphorylation of AMPK in SHR carotid arteries. Treatment with berberine increased AMPK phosphorylation in arteries from both WKY and SHR (Fig. 2A). Since the activation of AMPK inhibits endoplasmic reticulum (ER) stress in high-fat-induced obesity [22], we next explored the effect of berberine on ER stress-associated proteins. We found that phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) (Fig. 2B) and levels of activating transcription factor-3 (ATF3) (Fig. 2C), activating transcription factor-6 (ATF6) (Fig. 2D), and X-box binding protein-1 (XBP1) (Fig. 2E) were all elevated in SHR arteries, which were reversed by 12 h-treatment with berberine (Fig. 2B–E). AMPK inhibitor compound C and dominant negative AMPK (DN-AMPK) adenovirus reversed the beneficial effect of berberine by increasing AMPK phosphorylation (Fig. 3A), decreasing eIF2α phosphorylation (Fig. 3B), and reducing levels of ATF3 (Fig. 3C), ATF6 (Fig. 3D), XBP1 (Fig. 3E) and COX-2 (Fig. 3F). In addition, the inhibitory effect of berberine on EDCs was also reversed by compound C or DN-AMPK adenovirus (Fig. 3G). Taken together, the present results indicate that berberine attenuates EDCs probably through AMPK activation and subsequent inhibition of ER stress and reduction of COX-2 expression in SHR carotid arteries.

3.3. Berberine reduces COX-2 expression via scavenging ROS production in SHR carotid arteries

Alleviation of ER stress inhibits ROS production [22]. Next, we determined ROS levels in rat carotid arteries. ROS production was increased in carotid arteries from SHR compared with those from WKY, while treatment with berberine (1 μmol/L) or ER stress inhibitors 4-PBA (10 μmol/L) or TUCDA (20 μmol/L) reduced the ROS levels in SHR arteries (Fig. 4A and B). ROS suppression reduces the COX-2 up-regulation in SHR renal arteries [6]. This study also demonstrates that ROS scavengers tempol (100 μmol/L) or tiron (1 mmol/L) plus DETCA (100 μmol/L) normalized the elevated ROS production in SHR arteries (Fig. 4A and B). Moreover, these inhibitors of ROS or ER stress reduced COX-2 up-regulation (Fig. 4C) and inhibited EDCs in SHR carotid arteries (Fig. 4D). Taken together, the present results suggest that berberine-induced reduction of COX-2 expression and/or activity is most likely mediated through inhibiting ER stress and associated ROS in SHR carotid arteries.

4. Discussion

Although several studies suggest the therapeutic potential of berberine and its derivatives for hypertension [17,18,23], the effects of berberine on endoplasmic reticulum (ER) stress and endothelium-dependent contractions (EDCs) in hypertension remain elusive. The present study provides *in vitro* evidence that berberine confers vascular protection probably by its activation of AMPK in SHR carotid arteries. Berberine counteracts several pathological features of hypertension, including the suppression of ER stress, inhibition of ROS accumulation, and attenuation of EDCs. This alkaloid compound inhibits ER stress-related proteins eIF2α

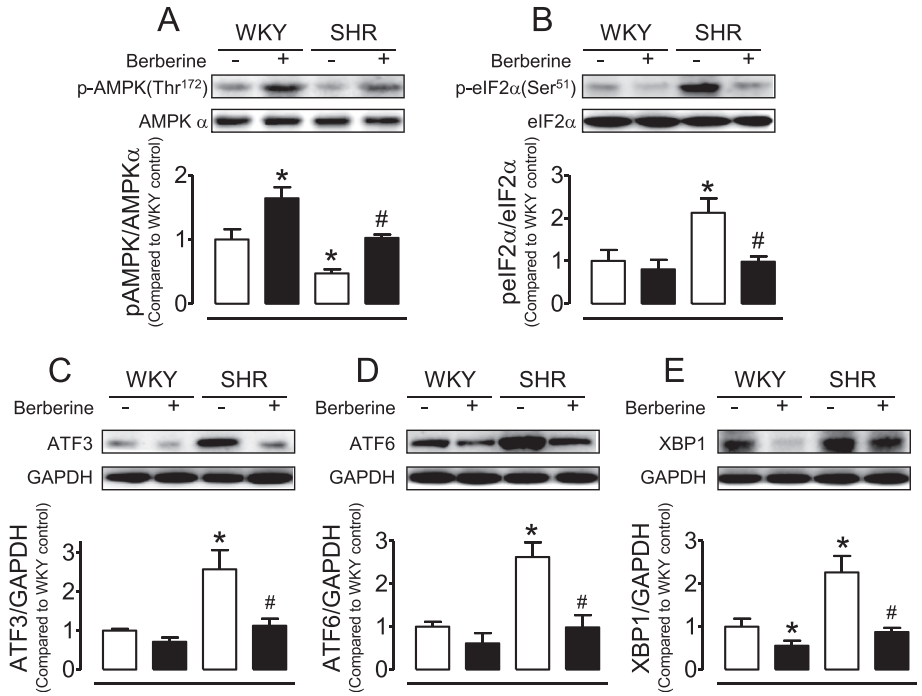

Fig. 2. (A) Phosphorylation and expression of AMPK, (B) phosphorylation and expression of eIF2α, and the levels of ATF3 (C), ATF6 (D), and XBP1 (E) in carotid arteries from WKY and SHR. $^{*}P < 0.05$ vs WKY control; $^{\#}P < 0.05$ vs SHR control. Results are means ± SEM (n = 4).

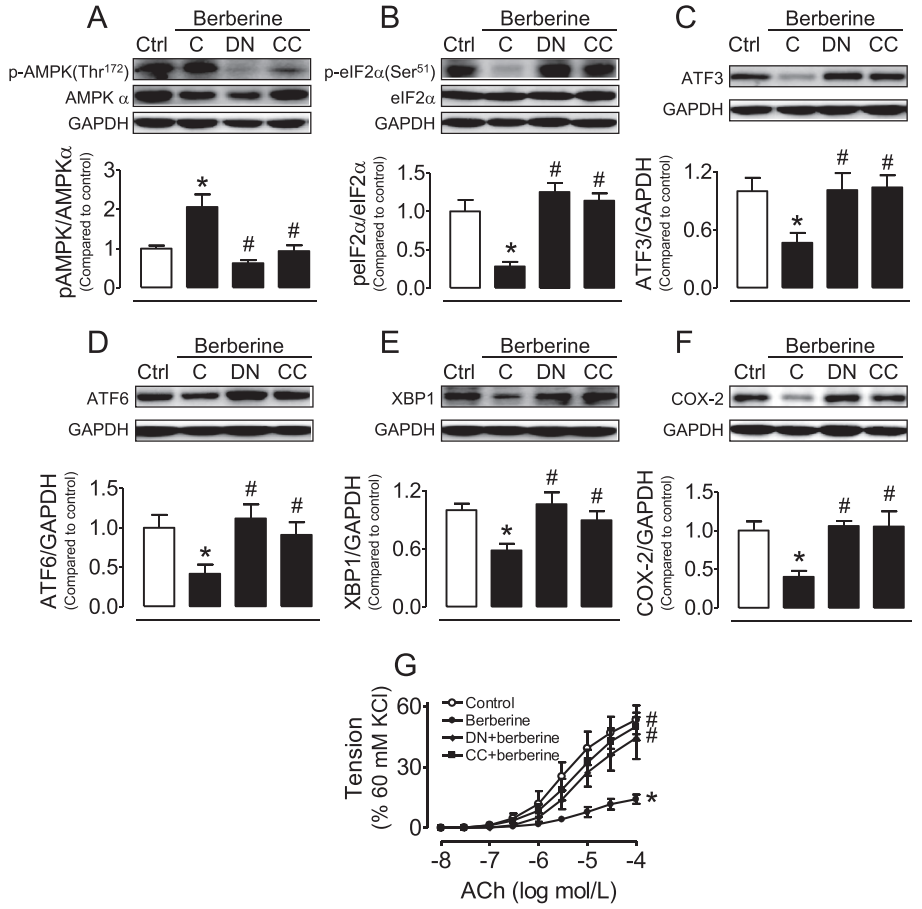

Fig. 3. (A) Phosphorylation and expression of AMPK, (B) phosphorylation and expression of eIF2α, and the levels of ATF3 (C), ATF6 (D), XBP1 (E) and COX-2 (F) in SHR carotid arteries. (G) EDCs in SHR carotid arteries. $^{*}P < 0.05$ vs control; $^{\#}P < 0.05$ vs berberine control. Results are means ± SEM (n = 6 for functional study, n = 4 for Western blot). Ctrl, control; C, berberine control; DN, DN-AMPK; CC, compound C.

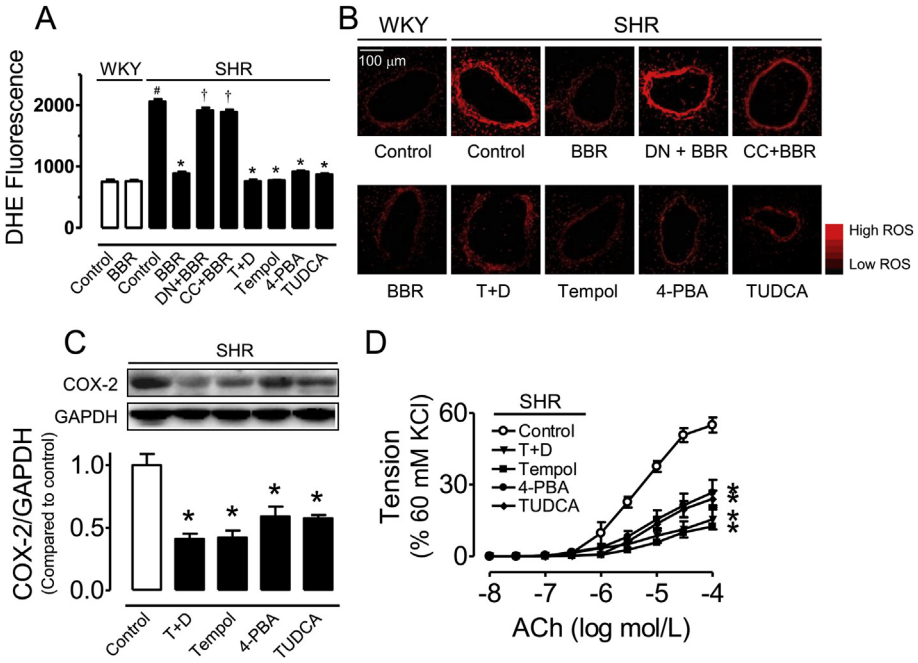

Fig. 4. (A & B) Dihydroethidium (DHE) fluorescence intensity in rat carotid arteries. (C) Effects of ROS scavengers and ER stress inhibitors on COX-2 expression in SHR carotid arteries. (D) Effects of ROS scavengers and ER stress inhibitors on EDCs in SHR carotid arteries. $^{*}P < 0.05$ vs SHR control; $^{\#}P < 0.05$ vs WKY control; $^{\dagger}P < 0.05$ vs SHR + berberine. Results are means ± SEM (n = 6 for functional study, n = 4 for Western blot and DHE staining). BBR, berberine; DN, DN-AMPK; CC, compound C; T + D, tiron plus DETCA.

phosphorylation and ATF3, ATF6, and XBP1 expressions. Futhermore, the EDCs in SHR carotid arteries were mainly mediated by COX-2 and berberine attenuated EDCs via reducing COX-2 expression. These new findings suggest that berberine might be useful for the treatment of vascular dysfunctions associated with hypertension.

The formation of endothelium-derived contracting factors depends on the activity of endothelial COX isoforms, COX-1 and COX-2, which contributes to the induction of EDCs [24]. The present study reveals the elevated vascular expression of COX-1 and COX-2 in SHR compared with WKY. The exaggerated EDCs in SHR arteries was inhibited by COX-2 inhibitor NS398 but only slightly attenuated by COX-1 inhibitor SC560, suggesting that the EDCs in SHR arteries is more likely to be mediated by COX-2 up-regulation. Although several previous studies evaluated the direct endothelial effects of berberine [12,19,25,26], the present study demonstrates that berberine suppresses EDCs accompanied by reduction of COX-2 expression but not COX-1 expression in SHR arteries.

In addition to its beneficial metabolic effects [27], more evidence suggests that AMPK is an important regulator of vascular homeostasis [6,28,29]. We observed that AMPK phosphorylation in carotid arteries was reduced in SHR compared with WKY. While, berberine increased AMPK phosphorylation in arteries from both WKY and SHR. Metformin protects endothelial function by inhibition of ER stress through AMPK activation in diet-induced obese mice [22] and prevents ER stress-induced apoptosis through AMPK activation in a mouse pancreatic β cell line NIT-1 cells [30]. ER stress is mediated by three ER membrane-associated proteins that engage complex downstream signaling pathways, including cleavage of ATF6, activation of eIF2α/ATF3 pathway, and splicing of XBP1 [31]. Next, we explored whether AMPK was involved in the suppression of ER stress in SHR. Western blot analysis shows the enhanced eIF2α phosphorylation and increased expressions of ATF3, ATF6 and XBP1 in carotid arteries from SHR compared to those from WKY, which were reversed by berberine. Furthermore, AMPK inhibitor compound C or dominant negative AMPK adenovirus suppressed the inhibitory effects of berberine on both ER membrane-associated proteins and EDCs in SHR arteries. Finally, we examined the relationship between ER stress and COX-2. ROS mediate COX-2-dependent contraction in renovascular hypertension [32]. Bone morphogenic protein-4 up-regulates COX-2 through ROS elevation [33] and glucagon-like peptide-1 down-regulates COX-2 expression via scavenging mitochondrial ROS [6]. Here, we show that ROS scavengers and ER stress inhibitors reduced ROS production, inhibited COX-2 expression, and suppressed EDCs in SHR arteries. In addition, the ROS reduction, COX-2 down-regulation, and EDCs inhibition by berberine were reversed by either AMPK inhibitor compound C or dominant negative AMPK adenovirus.

In summary, the present results demonstrate that berberine attenuates EDCs most likely through AMPK activation; the latter alleviates ER stress and subsequently normalizes ROS overproduction and down-regulates COX-2 expression in SHR carotid arteries. The present study thus provides additional mechanisms underlying beneficial effects of berberine to preserve vascular function in hypertension.

Author disclosure statement

No competing financial interests exist.

Conflict of interest

None.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 81200183), National Basic Research Program of China (2012CB517805) and National Fundamental Fund Project Subsidy Funds of Personnel Training of China (J1030831/J0108).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.028.

References

[1] A. Montezano, R.M. Touyz, Reactive oxygen species, vascular Noxs and hypertension: focus on translational and clinical research, Antioxid. Redox Signal. 20 (2014) 164–182.
[2] M. Araujo, C.S. Wilcox, Oxidative stress in hypertension: role of the kidney, Antioxid. Redox Signal. 20 (2014) 74–101.
[3] W.T. Wong, S.L. Wong, X.Y. Tian, Y. Huang, Endothelial dysfunction: the common consequence in diabetes and hypertension, J. Cardiovasc. Pharmacol. 55 (2010) 300–307.
[4] M. Feletou, Y. Huang, P.M. Vanhoutte, Vasoconstrictor prostanoids, Pflugers Arch. 459 (2010) 941–950.
[5] M. Feletou, Y. Huang, P.M. Vanhoutte, Endothelium-mediated control of vascular tone: COX-1 and COX-2 products, Br. J. Pharmacol. 164 (2011) 894–912.
[6] L. Liu, J. Liu, X.Y. Tian, W.T. Wong, C.W. Lau, A. Xu, et al., Uncoupling protein-2 mediates DPP-4 inhibitor-induced restoration of endothelial function in hypertension through reducing oxidative stress, Antioxid. Redox Signal. 21 (2014) 1571–1581.
[7] S.L. Wong, F.P. Leung, C.W. Lau, C.L. Au, L.M. Yung, X. Yao, et al., Cyclooxygenase-2-derived prostaglandin F2alpha mediates endothelium-dependent contractions in the aortae of hamsters with increased impact during aging, Circ. Res. 104 (2009) 228–235.
[8] M. Galan, M. Kassan, P.J. Kadowitz, M. Trebak, S. Belmadani, K. Matrougui, Mechanism of endoplasmic reticulum stress-induced vascular endothelial dysfunction, Biochim. Biophys. Acta 1843 (2014) 1063–1075.
[9] J. Groenendyk, P.K. Sreenivasaiah, H. Kim do, L.B. Agellon, M. Michalak, Biology of endoplasmic reticulum stress in the heart, Circ. Res. 107 (2010) 1185–1197.
[10] C.C. Glembotski, Endoplasmic reticulum stress in the heart, Circ. Res. 101 (2007) 975–984.
[11] T. Minamino, I. Komuro, M. Kitakaze, Endoplasmic reticulum stress as a therapeutic target in cardiovascular disease, Circ. Res. 107 (2010) 1071–1082.
[12] F. Cheng, Y. Wang, J. Li, C. Su, F. Wu, W.H. Xia, et al., Berberine improves endothelial function by reducing endothelial microparticles-mediated oxidative stress in humans, Int. J. Cardiol. 167 (2013) 936–942.
[13] W. Zha, G. Liang, J. Xiao, E.J. Studer, P.B. Hylemon, W.M. Pandak Jr., et al., Berberine inhibits HIV protease inhibitor-induced inflammatory response by modulating ER stress signaling pathways in murine macrophages, PLoS One 5 (2010) e9069.
[14] X. Hao, A. Yao, J. Gong, W. Zhu, N. Li, J. Li, Berberine ameliorates pro-inflammatory cytokine-induced endoplasmic reticulum stress in human intestinal epithelial cells in vitro, Inflammation 35 (2012) 841–849.
[15] W. Yu, M. Sheng, R. Xu, J. Yu, K. Cui, J. Tong, et al., Berberine protects human renal proximal tubular cells from hypoxia/reoxygenation injury via inhibiting endoplasmic reticulum and mitochondrial stress pathways, J. Transl. Med. 11 (2013) 24.
[16] N. Li, D.Z. Dai, Y. Dai, CPU86017 and its isomers improve hypoxic pulmonary hypertension by attenuating increased ETA receptor expression and extracellular matrix accumulation, Naunyn Schmiedebergs Arch. Pharmacol. 378 (2008) 541–552.
[17] J.C. Liu, P. Chan, Y.J. Chen, B. Tomlinson, S.H. Hong, J.T. Cheng, The antihypertensive effect of the berberine derivative 6-protoberberine in spontaneously hypertensive rats, Pharmacology 59 (1999) 283–289.
[18] J. Lan, F. Dong, Z. Yan, W. Zheng, J. Fan, G. Sun, Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension, J. Ethnopharmacol. 23 (2015) 69–81.
[19] Y. Wang, Y. Huang, K.S. Lam, Y. Li, W.T. Wong, H. Ye, et al., Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilatation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase, Cardiovasc. Res. 82 (2009) 484–492.
[20] K.W. Liang, S.C. Yin, C.T. Ting, S.J. Lin, C.M. Hsueh, C.Y. Chen, et al., Berberine inhibits platelet-derived growth factor-induced growth and migration partly through an AMPK-dependent pathway in vascular smooth muscle cells, Eur. J. Pharmacol. 590 (2008) 343–354.
[21] L. Liu, J. Liu, Y. Gao, X. Yu, G. Xu, Y. Huang, Uncoupling protein-2 mediates the protective action of berberine against oxidative stress in rat insulinoma INS-1E cells and in diabetic mouse islets, Br. J. Pharmacol. 171 (2014) 3246–3254.
[22] W.S. Cheang, X.Y. Tian, W.T. Wong, C.W. Lau, S.S. Lee, Z.Y. Chen, et al., Metformin protects endothelial function in diet-induced obese mice by inhibition of endoplasmic reticulum stress through 5' adenosine monophosphate-activated protein kinase-peroxisome proliferator-activated receptor delta pathway, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 830–836.
[23] T.T. Zhang, B. Cui, D.Z. Dai, W. Su, CPU 86017, p-chlorobenzyltetrahydroberberine chloride, attenuates monocrotaline-induced pulmonary hypertension by suppressing endothelin pathway, Acta Pharmacol. Sin. 26 (2005) 1309–1316.
[24] S.L. Wong, W.T. Wong, X.Y. Tian, C.W. Lau, Y. Huang, Prostaglandins in action indispensable roles of cyclooxygenase-1 and -2 in endothelium-dependent contractions, Adv. Pharmacol. 60 (2010) 61–83.
[25] M. Zhang, C.M. Wang, J. Li, Z.J. Meng, S.N. Wei, R. Bucala, et al., Berberine protects against palmitate-induced endothelial dysfunction: involvements of upregulation of AMPK and eNOS and downregulation of NOX4, Mediat. Inflamm. 2013 (2013) 260464.
[26] F. Affuso, A. Ruvolo, F. Micillo, L. Sacca, S. Fazio, Effects of a nutraceutical combination (berberine, red yeast rice and policosanols) on lipid levels and endothelial function randomized, double-blind, placebo-controlled study, Nutr. Metab. Cardiovasc. Dis. 20 (2010) 656–661.
[27] C. Chen, Y. Zhang, C. Huang, Berberine inhibits PTP1B activity and mimics insulin action, Biochem. Biophys. Res. Commun. 397 (2010) 543–547.
[28] M.H. Zou, Y. Wu, AMP-activated protein kinase activation as a strategy for protecting vascular endothelial function, Clin. Exp. Pharmacol. Physiol. 35 (2008) 535–545.
[29] L. Liu, J. Liu, W.T. Wong, X.Y. Tian, C.W. Lau, Y.X. Wang, et al., Dipeptidyl peptidase 4 inhibitor sitagliptin protects endothelial function in hypertension through a glucagon-like peptide 1-dependent mechanism, Hypertension 60 (2012) 833–841.
[30] T.W. Jung, M.W. Lee, Y.J. Lee, S.M. Kim, Metformin prevents endoplasmic reticulum stress-induced apoptosis through AMPK-PI3K-c-Jun NH2 pathway, Biochem. Biophys. Res. Commun. 417 (2012) 147–152.
[31] L. Yang, P. Li, S. Fu, E.S. Calay, G.S. Hotamisligil, Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance, Cell. Metab. 11 (2010) 467–478.
[32] X.Y. Tian, W.T. Wong, F.P. Leung, Y. Zhang, Y.X. Wang, H.K. Lee, et al., Oxidative stress-dependent cyclooxygenase-2-derived prostaglandin f(2alpha) impairs endothelial function in renovascular hypertensive rats, Antioxid. Redox Signal. 16 (2012) 363–373.
[33] W.T. Wong, X.Y. Tian, Y. Chen, F.P. Leung, L. Liu, H.K. Lee, et al., Bone morphogenic protein-4 impairs endothelial function through oxidative stress-dependent cyclooxygenase-2 upregulation: implications on hypertension, Circ. Res. 107 (2010) 984–991.