Microvascular Research 119 (2018) 98–104

Contents lists available at ScienceDirect

Microvascular Research

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

Ticagrelor protects against AngII-induced endothelial dysfunction by alleviating endoplasmic reticulum stress

Xiaoyu Wang[a], Xuejie Han[a], Minghui Li[a], Yu Han[a], Yun Zhang[a], Shiqi Zhao[a], Yue Li[a,b,*]

[a] Department of Cardiology, The First Affiliated Hospital, Harbin Medical University, Harbin, 150001, Heilongjiang Province, China
[b] Key Laboratory of Cardiac Diseases and Heart Failure, Harbin Medical University, Harbin, 150001, Heilongjiang Province, China

ARTICLE INFO

Keywords:
Ticagrelor
Endothelial dysfunction
AngII
Endoplasmic reticulum stress

ABSTRACT

Ticagrelor has been reported to decrease cardiovascular mortality compared with clopidogrel. This benefit cannot be fully explained by the more efficient platelet inhibition. Many studies demonstrated that ticagrelor improved endothelial function, leaving the mechanism elusive though. The present study aims to investigate whether ticagrelor protects against endothelial dysfunction induced by angiotensinII (AngII) through alleviating endoplasmic reticulum (ER) stress. Male Sprague Dawley rats were infused with AngII or vehicle and administrated with ticagrelor or vehicle for 14 days. Reactive oxygen species (ROS) was detected. Aortas from normal mice were incubated with endoplasmic reticulum stress inducer tunicamycin with or without ticagrelor. Vasorecactivity was measured on wire myography. Rat aortic endothelial cells (RAECs) were pretreated with ticagrelor followed by AngII or tunicamycin. Endothelial nitric oxide synthase (eNOS) phosphorylation and ER stress markers were determined by western blotting. Impaired endothelial function, induction of ER stress, reduced eNOS phosphorylation and elevated ROS generation was restored by ticagrelor treatment in vivo. In addition, tunicamycin induced endothelial dysfunction was improved by ticagrelor. In vitro, the induction of ER stress and inhibited eNOS phosphorylation in REACs exposed to AngII as well as tunicamycin was reversed by co-culturing with ticagrelor. In conclusion, ticagrelor protects against AngII-induced endothelial dysfunction via alleviating ER stress.

1. Introduction

Ticagrelor, a P_2Y_{12} receptor antagonist, is a vital therapy for coronary artery disease. Although controversial, the platelet inhibition and patient outcomes (PLATO) trial demonstrated that ticagrelor was related to lower incidence of major adverse cardiovascular events compared with clopidogrel in patients with acute coronary syndrome (ACS) and that the promising outcome cannot be accounted for by its more effective platelet inhibiting effect (Ait Mokhtar et al. 2016; Wallentin et al. 2009). Therefore, several studies have investigated the pleiotropic effects of ticagrelor, such as the improvement of endothelial function. However, the mechanism underlying the protective effects of ticagrelor on endothelial function remains obscure. Atherogenesis begins with endothelial dysfunction, which precedes severe cardiovascular diseases (Grassi et al. 2011). Thus, it is necessary to confirm that ticagrelor has a protective effect on endothelial function and to determine its underlying mechanism. The renin-angiotensin-aldosterone system is activated in coronary artery disease, and AngII, the major active product is a risk factor for atherosclerotic pathology and can induce endothelial dysfunction, leading to a proinflammatory, prothrombotic and proatherogenic state (Farmer and Torre-Amione 2001). One of the mechanisms is attributed to endoplasmic reticulum (ER) stress. Huang, et al. (Murugan et al. 2015; San Cheang et al. 2015) demonstrated that AngII impaired endothelial function through activating eIF2α and ATF6 pathways. In addition, abundant evidence shows that ER stress is involved in atherosclerotic plaque formation and rupture (Myoishi et al. 2007; Thorp et al. 2009). Therefore, AngII-induced endothelial dysfunction models were generated. The present study aims to investigate whether ticagrelor could improve AngII-induced endothelial dysfunction and to determine its mechanism which was hypothesized to be alleviating ER stress.

2. Methods and materials

2.1. Animal treatment

All experimental procedures involving animals in the present study were performed in accordance with the ethical standards of the Guide

* Corresponding author at: The First Affiliated Hospital, Harbin Medical University, Youzheng Street No. 23, Nangang District, Harbin, 150001, Heilongjiang Province, China.
E-mail address: ly99ly@vip.163.com (Y. Li).

https://doi.org/10.1016/j.mvr.2018.05.006
Received 28 January 2018; Received in revised form 23 April 2018; Accepted 13 May 2018
Available online 17 May 2018

for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH Publications No. 8023, revised 1978) and approved by the Animal Care and Use Committee of Harbin Medical University. Male Sprague–Dawley rats (230–250 g) were purchased from Vital River Company (Beijing, China) and housed in a temperature-controlled condition with a 12-h light/dark cycle. Rats were randomly divided into the control group ($n = 15$), the AngII group (n = 15) and the ticagrelor group (n = 15). Rats were anaesthetized with sodium pentobarbital (60 mg/kg ip), and osmotic mini-pumps (0.5 μL/h, 14 days, model2002; Alzet) were subcutaneously implanted on the back. The control group was infused with saline and gavaged with vehicle. The AngII and ticagrelor groups were continuously infused with AngII (biovision, US, 60 ng/min) for 14 days (Giachini et al. 2010). The ticagrelor group received ticagrelor (150 mg/kg/day, gavage) and AngII group received vehicle for 14 days (Nanhwan et al. 2014). Systolic blood pressure was measured with tail cuff plethysmography (IITC Life Science MRBP Blood Pressure System). After 14 days, rats were sacrificed and thoracic aortas were dissected for the following experimental procedures.

2.2. Ex vivo culture of rat aortic rings

Thoracic aortas was isolated and cut into ring segments in sterile physiological salt solution (PSS) containing (mM)) NaCl, 130; KCl, 4.7; MgSO47H2O, 1.17; KH2PO4, 1.18; NaHCO3, 14.9; CaCl2, 1.6; and D-glucose, 5.5 (pH 7.35–7.45). Aortic rings were incubated in Dulbecco's Modified Eagle's Media (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA), 100 μg/mL streptomycin and 100 U/mL penicillin and co-incubated with tunicamycin (ER stress inducer, 2 μg/mL), with or without ticagrelor (1 μM) at 37 °C for 16 h. Ticagrelor was pretreated 30 min before tunicamycin and DMSO was administrated as vehicle.

2.3. Vasoreactivity

This part of experiment was performed according to our previous study (Wang et al. 2016). Briefly, thoracic aortas were dissected into 3–5-mm-long segments in ice-cold PSS and suspended between 2 triangular steel hooks and incubated in PSS aerated with 95% O_2 and 5% CO_2 at 37 °C. The resting tension of all rings was adjusted to 2.0 g for a period of 60-min equilibration. Then aortas were contracted with high-KCl PSS (60 mM). After rinsing with PSS, 1 μmol/L phenylephrine (PE) was added to cause a sustained contraction. Endothelial dependent relaxation to acetylcholine (Ach; 10^{-9}–10^{-5} μmol/L; Sigma, St. Louis, MO, USA) or endothelial independent relaxation to sodium nitroprusside (SNP; 10^{-10}–10^{-7} μmol/L) were performed cumulatively. Vasoconstriction was induced by PE (10^{-9}–10^{-6} μmol/L) with or without pre-incubation with N^G-Nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 μM; Beoytime, Shanghai, China).

2.4. Vascular reactive oxygen species and superoxide determination

Reactive oxygen species (ROS) production was estimated from the ratio of dihydroethidium (DHE) to 4′,6-diamidino-2-phenylindole (DAPI) fluorescence. Fresh-frozen aorta sections of 10-μm thickness were prepared on a cryostat and were incubated with 5 μmol/L DHE (Sigma, St. Louis, MO, USA)-containing PSS for 15 min. Sections were counterstained with the nuclear stain DAPI (Beoytime, Shanghai, China), followed by three washes with PSS. Fluorescence were observed and recorded with a Zeiss fluorescence microscope. Vascular superoxide anion production was evaluated by lucigenin-enhanced chemiluminescence method. Briefly, a 5-mm segment of aorta was incubated in Krebs-HEPES buffer (in mM: NaCl 99.0, NaHCO3 25, KCl 4.7, KH2PO4 1.0, MgSO4 1.2, glucose 11.0, CaCl2 2.5 and Na-HEPES 20.0) with 10 μM lucigenin (Sigma, St. Louis, MO, USA) and 100 μM NADPH (Beoytime, Shanghai, China). Luminescence was measured using multi-technology microplate reader (Thermo Scientic, USA). The data were expressed as RLU per mg of tissue dry weight.

2.5. Cell culture

Primary rat aortic endothelial cells (RAECs) were isolated from rat thoracic artery by an explant technique and cultured with rat endothelial growth medium (ECM-r, Sciencell 1021, US) containing 5% fetal bovine serum,1% endothelial cell growth factor and 1% penicillin/streptomycin. Cells from the third to fifth passages were pretreated with ticagrelor (Aladdin, Shanghai, China, 1 μmol/L) or PBA (100 μmol/L Sigma, St. Louis, MO, USA) for 30 min before AngII (0.5 μmol/L, 48 h) or tunicamycin (Aladdin, Shanghai, China, 2 μg/mL, 16 h) exposure. DMSO was administrated as vehicle.

2.6. Western blot

Aortas and RAECs were homogenized in RIPA (Beyotime, Shanghai, China) lysis buffer containing PhosSTOP (Roche, Switzerland) and PMSF (Beyotime, Shanghai, China) on ice. Protein supernatant was obtained by centrifuging. Protein was denatured by boiling after qualified by BCA kit. Protein samples of equal weight were separated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis and blotted to a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). After a block with 5% non-fat milk in 0.05% Tween-20 Tris-buffered saline for 1 h, membranes were incubated with primary antibodies against phosphorylated eNOS at Ser1177 (p-eNOS, Cell Signaling Technology, Danvers, MA, USA), total eNOS (Cell Signaling Technology, Danvers, MA, USA), phosphorylated eIF2α (p-eIF2α, Cell Signaling Technology, Danvers, MA, USA), total eIF2α (Cell Signaling Technology, Danvers, MA, USA), ATF6 (Abcam, Cambridge, UK), ATF4 (Proteintech, Wuhan, China), CHOP (Proteintech, Wuhan, China), GRP78 (Proteintech, Wuhan, China) and β-actin (ZSBG-BIO, Beijing, China) overnight at 4 °C, followed by appropriate secondary antibodies for 1 h on the next day. After wash, the membranes were exposed to the ChemiDoc XRS gel documentation system (Bio-Rad, Hercules, CA, USA) with an ECL kit (Beyotime, Shanghai, China). The protein bands were analysed with Image Lab software.

2.7. Statistical analysis

Results are presented as mean ± SEM. Relaxation was expressed as percentage reduction in contraction induced by PE. Data was analysed via one-way ANOVA followed by Newman-Keuls tests (GraphPad Software, San Diego, USA). Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Ticagrelor improved endothelial function in AngII-infused rats

AngII infusion induced a remarkable elevation of blood pressure, and ticagrelor administration did not have an effect on blood pressure changes (Fig. 1A). As is shown in Fig. 1B, the endothelial dependent relaxation induced by Ach was significantly reduced in aortas from AngII infused rats and was restored by ticagrelor administration. Endothelial independent relaxation to SNP was not significantly different among groups (Fig. 1C). Compared with the control, vasoconstriction induced by PE was increased in aortas from AngII infused rats, which was normalized by ticagrelor (Fig. 1E). NO synthase inhibitor L-NAME abolished the difference in constriction among groups (Fig. 1F), suggesting that the protective effect of ticagrelor might be associated with NO bioavailability.