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Chronic inhibition of farnesyl pyrophosphate synthase improves endothelial function in spontaneously hypertensive rats

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ABSTRACT

Farnesyl pyrophosphate synthase (FPPS, EC 2.5.1.10), an essential enzyme in the mevalonate pathway, catalyzes the synthesis of isoprenoid intermediates. The isoprenoid intermediates are needed for protein isoprenylation of RhoA for its function on regulation of endothelial nitric oxide synthase (eNOS). We previously reported that FPPS were upregulated in spontaneously hypertensive rats (SHR) when compared with Wistar–Kyoto rats (WKY), and this was accompanied by development of endothelial dysfunction. Five-week-old male rats were daily gavaged with vehicle or an FPPS inhibitor (alendronate, 1 or 10 mg/kg). After 12-week administration of alendronate, endothelium-dependent and -independent vasorelaxation were measured in isolated aortic rings. Twelve-week of alendronate (10 mg/kg/day) treatment restored the impaired endothelium-dependent vasodilation in SHR. Furthermore, long-term treatment with an FPPS inhibitor significantly suppressed RhoA activation and increased phospho-eNOS/eNOS ratio. In conclusion, chronic treatment with an FPPS inhibitor improves the endothelial function in SHR, and the upregulation of phospho-eNOS/eNOS ratio with inhibition of RhoA activation may be an important mechanism.

1. Introduction

The vascular endothelium serves as an important autocrine and paracrine organ that regulates homeostasis of the vascular wall, and impaired endothelial function is associated with a series of vascular diseases such as atherosclerosis and hypertension [1–4]. Up to date endothelial dysfunction characterized by an impairment of the production and release of the endothelial-derived nitric oxide (NO) is a strong predictor of cardiovascular disease [5–7].

RhoA, a member of the Rho family of small GTPases, has been shown to negatively regulate the expression and phosphorylation of endothelial nitric oxide synthase (eNOS), thereby negatively regulating the production of NO [7–11]. Two important isoprenoid intermediates of the mevalonate pathway are farnesol pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which are needed for protein isoprenylation of GTPases for their activation [12,13]. Therefore, we reasoned that the mevalonate pathway is an important cellular metabolic pathway in modulating endothelial function (Fig. 1), but direct evidence in support of this hypothesis is insufficient.

In our previous studies, we found that the expressions of key enzymes in mevalonate pathway, including the farnesyl pyrophosphate synthase (FPPS, EC 2.5.1.10), were significantly upregulated in spontaneously hypertensive rats (SHR) [14–16]. FPPS is a key enzyme in mevalonate pathway and directly catalyzes the synthesis of FPP and GGPP [17], which are required for isoprenylation of small GTPases. On the other hand, the SHR has served for decades as one of the preferred animal models of endothelial dysfunction [18]. For instance, SHR aorta exhibited a decrease of eNOS activity and protein expression when compared with those from age-matched Wistar–Kyoto (WKY) rat [19]. However, it remains to be tested whether the decreased synthesis of FPP and GGPP by an FPPS inhibitor leads to an improvement of endothelial function in SHR.

Therefore, the present study was designed to determine whether chronic inhibition of FPPS improves the endothelial function in SHR. For this purpose, we used alendronate, an inhibitor of FPPS in the mevalonate pathway [20–22].

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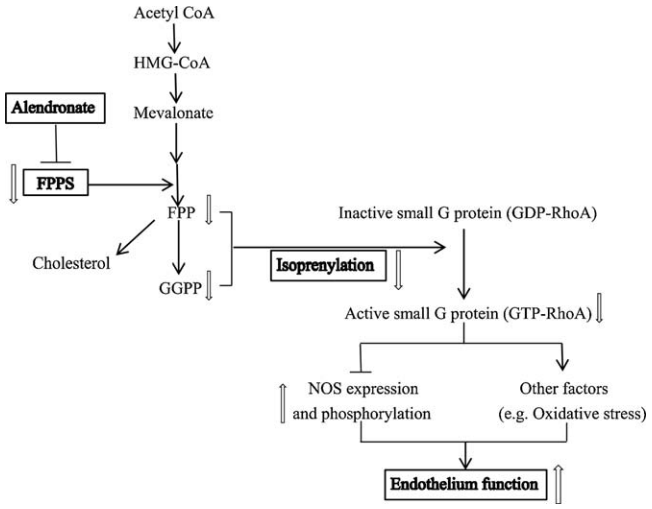

Fig. 1. Mevalonate pathway and endothelial dysfunction. Farnesyl pyrophosphate synthase (FPPS) catalyzes the synthesis of isoprenoid intermediates such as farnesol pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), leading to an isoprenylation of RhoA. This negatively regulates the expression and phosphorylation of endothelial nitric oxide synthase (eNOS), thereby negatively regulating the bioavailability of NO. Theoretically, inhibition of FPPS by alendronate could reverse this process and improve endothelial function.

2. Materials and methods

2.1. Animals and reagents

Five-week-old male SHR and normotensive Wistar–Kyoto (WKY) rats were purchased form the Shanghai Laboratory Animal Center, Chinese Academy of Sciences, and housed in a pathogen-free laboratory at the First Affiliated Hospital of Zhejiang University. The procedures and protocols of the study conformed to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the guidelines of the Animal Care and Use Committee of Zhejiang University.

Eighteen SHR were randomly divided into three groups consisting of the distilled water group (SC), SHR treated with low-dose alendronate (1 mg/kg/day) group (SL), and SHR treated with high-dose alendronate (10 mg/kg/day) group (SH). Six age- and weight-matched WKY were used as WKY control (WC). Alendronate (Fosamax, a gift from Merck, NJ, USA) was administrated every day for 12 weeks by the intragastric route.

Reagents and antibodies were purchased as follows: phenylephrine (PE), acetylcholine (ACh), sodium nitroprusside (SNP), Nω-nitro-L-arginine methyl ester (L-NAME), geranylgeraniol (GGOH), and farnesol (FOH) were from Sigma (St Louis, MO, USA); rabbit anti-eNOS polyclonal antibody was from Santa Cruz Biotechnology (USA); rabbit anti-phospho-eNOS (Ser1177) polyclonal antibody was from Cell Signaling Technology (CST, USA); the Rho pull-down kit was from Cytoskeleton Co. (Germany).

2.2. Systolic blood pressure (SBP) and mean arterial pressure (MAP) measurement

Systolic blood pressure (SBP) was measured every other week in all animals using the tail-cuff method [23,24]. After 12 weeks of drug intervention, after each rat was anesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg body weight), a polyethylene tube (PE50, Becton-Dickinson) was introduced via the right carotid artery to measure the mean arterial pressure (MAP).

2.3. Aortic rings preparation and vasomotor studies

After MAP measurement, blood samples were collected. The descending thoracic aorta was dissected and carefully cleaned of connective tissue. A 15-mm segment was immediately frozen in liquid nitrogen and store at −80 °C until used in immunoblot or biochemical analyses. Another segment was cut into 3–4 mm rings, which were mounted in an organ bath system (Med Lab 6.0 polygraph, Nanjing Medease, China) for vasomotor studies. The aortic rings were equilibrated for 90 min under a resting tension of 2 g in oxygenated (95% O_2 and 5% CO_2) Krebs–Henseleit (KH) solution, containing (mmol/l): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 1.25, and glucose 11. All experiments were performed on aortic rings with endothelium. The presence of functional endothelial was confirmed by the occurrence of relaxations in response to ACh (10^{-5} mol/l) in rings contracted with PE (10^{-6} mol/l).

Thereafter, aortic rings were preconstricted with PE (10^{-6} mol/l), and once the plateau was attained, cumulative concentration–response curves were obtained by adding the endothelium-dependent vasodilator ACh (10^{-9} to 10^{-6} mol/l) or the endothelium-independent vasodilator SNP (10^{-10} to 10^{-5} mol/l). ACh-induced relaxations were also constructed in the absence or in the presence of the indicated inhibitor. The following inhibitors were used separately: the NOS inhibitor (10^{-4} mol/l L-NAME) and the isoprenoid intermediates of mevalonate pathway (3×10^{-5} mol/l FOH and 6×10^{-5} mol/l GGOH). L-NAME was incubated with the aortic rings for 20 min before contracted with PE. FOH and GGOH were used at a maximally active concentration as previously reported [25], and were incubated for 2 h prior to pre-contraction with PE. All results are expressed as a percentage of the maximal contraction of PE-induced responses.

2.4. Western blotting analysis of eNOS and phospho-eNOS (Ser1177)

The procedure of Western blot analysis was performed as described in our previous reports [14,16], and the expressions of eNOS and phospho-eNOS (Ser1177) were detected using their specific antibodies: eNOS (1:1000), or phospho-eNOS (Ser1177) (1:1000). To ensure equal protein loading, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control.

2.5. RhoA pull-down assay

RhoA activation was determined by pull-down assay using a RhoA activation assay Biochem Kit (Cytoskeleton, USA) as described in our previous report [16]. Briefly, aortas were homogenized in lysis buffer. Equal volumes and amounts of proteins (about 300 μg) were incubated at 4 °C for 1 h with GST-tagged Rhotekin-Rho binding domain (RBD) agarose beads (50 μg), which specifically recognize the GTP-bound form of RhoA. The beads were washed three times with wash buffer. GTP-bound RhoA proteins were detected by Western blotting using the RhoA mouse polyclonal antibody (1:1000). Western blotting of the total amount of RhoA was performed for comparison with the RhoA activity (level of GTP-bound RhoA) in the same samples.

2.6. Measurements of serum NO end products

Blood samples were standing at least 2 h at 4 °C, and then centrifuged at 3000 rpm for 15 min. The supernatants were collected as serum. Serum nitrite and nitrate as a measure of NO were determined using a NO assay kit (Nanjing Jiancheng Bioengineering Institute, China) according to the Griess method. Briefly, after the conversion of nitrate to nitrite with nitrate

reductase, total nitrite concentrations as expressed as μmol/l were determined at an optical density of 550 nm by comparison to standard solutions of sodium nitrite.

2.7. Statistical analysis

Values are reported as mean ± SEM. The concentration–response curves resulting from vasomotor study were analyzed by nonlinear regression (curve fit). One-way analysis of variance (ANOVA) followed by Bonferroni post hoc test was used to determine significant differences among groups. Repeated measures ANOVA followed by the Bonferroni post hoc test was used in the results of blood pressure obtained by the tail-cuff method every other week. Differences were considered statistically significant at a value of $p < 0.05$.

3. Results

3.1. Alendronate reduced SBP and MAP in SHR

SBP values were summarized in Fig. 2A. As expected, before therapy, the SBP of SHR was already higher than that of WKY (124.1 ± 3.0 mm Hg versus 112.8 ± 3.5 mm Hg, $p < 0.01$), and the difference persisted as time progressed. Over the 12-week period, SBP further increased in the SC group compared to the WC group (227.4 ± 5.2 mm Hg versus 118.5 ± 3.4 mm Hg, $p < 0.01$, at the end of the study). Twelve-week administration of high-dose alendronate (10 mg/kg/day) slightly reduced the SBP in SHR, and this effect reached statistically significance from 8th week of treatment. However, the SBP-lowering effect was not found in SL group (1 mg/kg/day).

As to MAP (Fig. 2B), SHR had higher values than WKY (148.0 ± 4.2 mm Hg versus 90.9 ± 4.1 mm Hg, $p < 0.01$). MAP was also reduced in SHR group with high-dose alendronate compared with that in the SC group (126.3 ± 4.1 mm Hg versus 148.0 ± 4.2 mm Hg, $p < 0.01$).

3.2. Alendronate improved endothelium-dependent relaxation in SHR aortas

ACh (10^{-9} to 10^{-6} mol/l) caused endothelium-dependent relaxation in a dose-dependent manner in both SHR and WKY rats (Fig. 3A). However, ACh-induced relaxation in SC group was seriously impaired compared with that in WC group. The maximal relaxation induced by ACh in SC group was 47.4%, while it was 93.3% in WC group. Twelve-week treatment of high-dose alendronate (10 mg/kg/day) significantly improved this impairment and improved the maximal relaxation to 57.3% in SHR ($p < 0.01$ versus SC group). However, ACh-induced maximal relaxation in SL group was only 49.4% ($p > 0.05$, compared with SC group). These data suggested that high-dose alendronate exerted more pronounced beneficial effect on endothelium-dependent relaxation in SHR. Meanwhile, SNP (10^{-10} to 10^{-5} mol/l) induced endothelium-independent relaxation in a dose-dependent manner in all groups. No significant differences were found among groups ($p > 0.05$, Fig. 3B), indicating that alendronate had no effect on endothelium-independent relaxation of SHR.

Pretreatment with L-NAME (10^{-4} mol/l) abolished ACh-induced relaxations in aortic rings from both WKY and SHR, without significant differences among them (Fig. 4A and B). Treatment with either 1 or 10 mg/kg alendronate did not significantly modify the

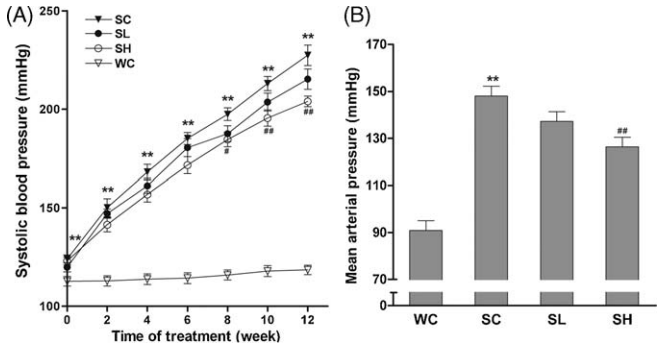

Fig. 2. Effects of alendronate treatment on systolic blood pressure (SBP) and mean arterial pressure (MAP). SBP was measured by the tail-cuff method (A) every other week and MAP was recorded by carotid catheter method (B). SHRs were gavaged daily with distilled water (SC, $n = 6$), 1 mg/kg alendronate (SL, $n = 6$), or 10 mg/kg alendronate (SH, $n = 6$), and WKY rats were gavaged with distilled water as control (WC, $n = 6$). Data are shown as mean ± SEM. $^{**}p < 0.01$ compared with the WC group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with the SC group.

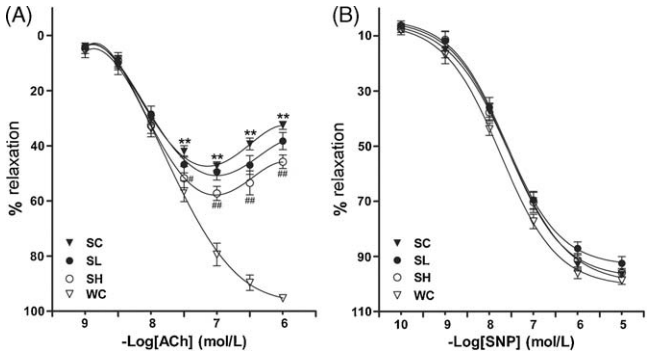

Fig. 3. Influences of alendronate treatment on endothelium-dependent and -independent vasorelaxation. (A) Endothelium-dependent relaxations induced by acetylcholine (ACh) and (B) endothelium-independent relaxations induced by sodium nitroprusside (SNP) in isolated aortic rings. SHRs were gavaged daily with distilled water (SC, $n = 6$), 1 mg/kg alendronate (SL, $n = 6$), or 10 mg/kg alendronate (SH, $n = 6$), and WKY rats were gavaged with distilled water as control (WC, $n = 6$). Values are shown as mean ± SEM. $^{**}p < 0.01$ versus WC group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus SC group.

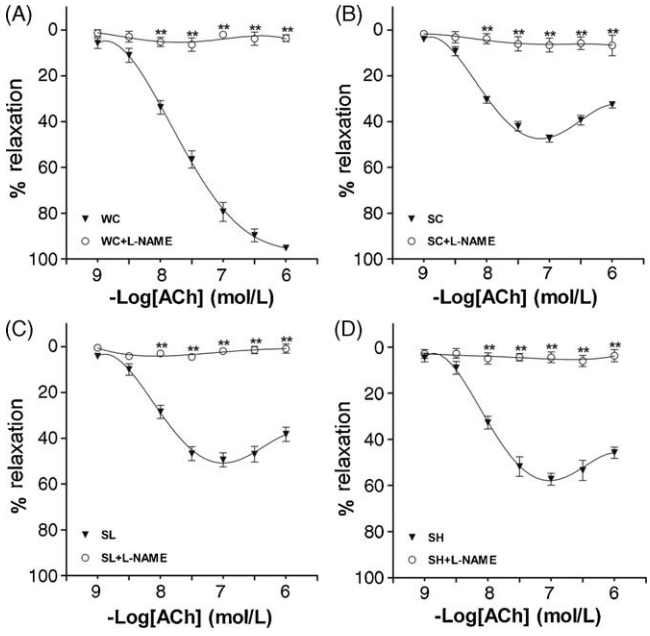

Fig. 4. Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME) in alendronate-induced endothelial protection. Effects of L-NAME (10^{-4} mol/l) on endothelium-dependent relaxation induced by acetylcholine (ACh) were tested in isolated aortic rings. (A) WKY rats were gavaged with distilled water as control (WC, $n = 6$); (B) SHRs were gavaged daily with distilled water (SC, $n = 6$); (C) SHRs were gavaged daily with 1 mg/kg alendronate (SL, $n = 6$); (D) SHRs were gavaged daily with 10 mg/kg alendronate (SH, $n = 6$), Values are shown as mean ± SEM, in the absence or presence of L-NAME. $^{**}p < 0.01$ versus ACh dose–response curve in the absence of the inhibitor.

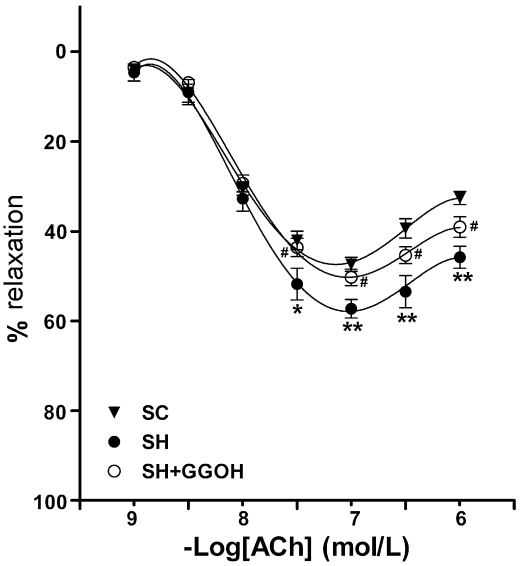

Fig. 5. Role of geranylgeraniol (GGOH) in alendronate-induced endothelial protection. Effects of GGOH (6×10^{-5} mol/l) on endothelium-dependent relaxation induced by acetylcholine (ACh) were tested in isolated aortic rings from 10 mg/kg/day alendronate-treated SHR (SH group). Data represent mean ± SEM from 6 rats. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus values obtained in SHR-control (SC) group, and $^{\#}p < 0.05$ versus SH group.

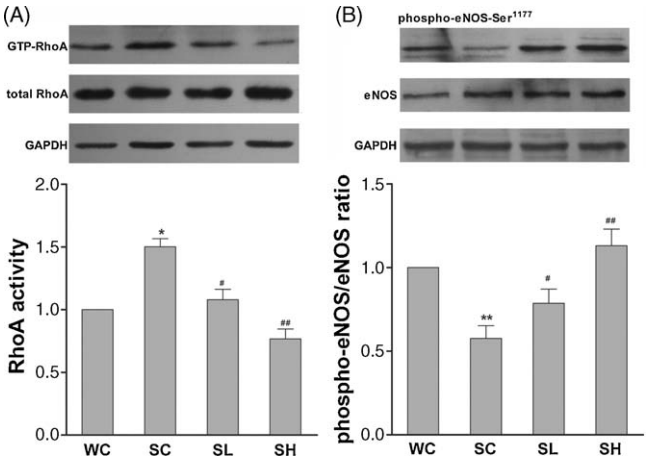

Fig. 6. Effects of alendronate treatment on RhoA activation and eNOS phosphorylation in aortas. SHRs were gavaged daily with distilled water (SC, $n = 6$), 1 mg/kg alendronate (SL, $n = 6$), or 10 mg/kg alendronate (SH, $n = 6$), and WKY rats were gavaged with distilled water as control (WC, $n = 6$). (A) RhoA expression and activity were examined by pull-down assay. RhoA activation is expressed as the ratio between GTP-binding and total RhoA protein. (B) Immunoblot analysis of eNOS and phospho-eNOS (Ser1177) were tested by Western blot. Relative activated eNOS is represented as the ratio between phosphorylated eNOS and total eNOS. Data represent as mean ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus WC group, and $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus SC group. GAPDH was used as a loading control.

inhibition caused by L-NAME (Fig. 4C and D). In all groups, ACh (10^{-9} to 10^{-6} mol/l) concentration–response curves in the presence of L-NAME were significantly different from those obtained in the absence of the inhibitor ($p < 0.01$, Fig. 4).

One of the FPPS downstream products, GGOH (6×10^{-5} mol/l), abolished the improvement of endothelium-dependent relaxation in SH group (Fig. 5). After pre-incubation with GGOH (6×10^{-5} mol/l) for 2 h, ACh-induced maximal relaxation in SH group was reduced to 49.7% ($p < 0.05$ versus 57.3% in absence of GGOH). GGOH pretreatment did not affect the ACh-induced relaxations in WC, SC and SL groups (data not shown). These data suggested that FPPS and its isoprenoid products were important in the impairment of endothelium-dependent relaxation in SHR. In our studies, the response to ACh was determined after contraction of the aortic rings with PE. To allow accurate and reliable determination of the response, contraction must be stable and in a sufficient amplitude. With FOH (3×10^{-5} mol/l), another important downstream product of FPPS, these optimal conditions could not be reliably archived in all groups (data not shown). These results are in agreement with recent studies by Roullet et al. [25], which showed dysfunction of norepinephrine (NE)-induced contraction in rat aortic rings in presence of FOH. Thus, the effect of FOH on aortic ring relaxation could not be evaluated in our experiment.

3.3. Alendronate suppressed Rho activation

Rho function depends on its conversion from the GDP- to the GTP-bound state. The levels of the GTP-bound active form of RhoA in aortas were determined by an affinity pull-down assay using Rhotekin GST-RBD. The levels of activated RhoA were higher in SC group compared to those in WC (Fig. 6A, $p < 0.05$). Treatment with alendronate decreased GTP-RhoA levels in a dose-dependent manner in aortas from SL ($p < 0.05$ versus SC group) and SH groups ($p < 0.01$ versus SC group). However, there were no significant changes in the expression levels of total RhoA between SHR and WKY, and between alendronate-treated and -untreated SHR.

3.4. Alendronate increased phospho-eNOS/eNOS ratio

Compared to the WC group, the SC group showed a lower level of phospho-eNOS/eNOS ratio ($p < 0.05$, Fig. 6B). Long-term treatment with alendronate notably increased the eNOS phosphorylation in a dose-dependent manner.

3.5. Alendronate increased serum NO end products

The basal serum NO end products, nitrate and nitrite, were significantly higher in WC than in SC groups ($p < 0.05$). Alendronate increased the serum NO end products in SHR in a dose-dependent manner after 12 weeks (Fig. 7). The serum nitrate

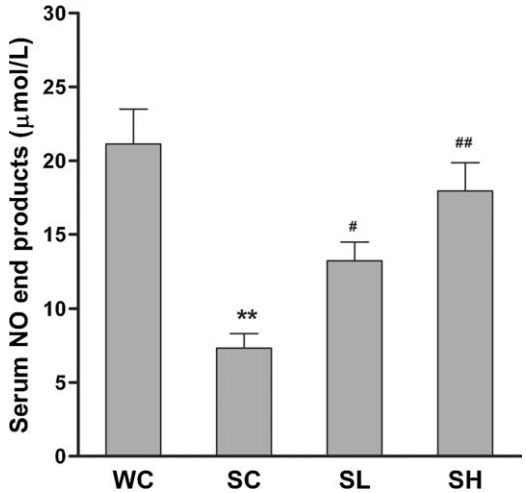

Fig. 7. Effects of alendronate treatment on serum NO end products. Serum nitrite and nitrate as a measure of NO were determined using a NO assay kit. SHRs were gavaged daily with distilled water (SC, $n = 6$), 1 mg/kg alendronate (SL, $n = 6$), or 10 mg/kg alendronate (SH, $n = 6$), and WKY rats were gavaged with distilled water as control (WC, $n = 6$). Data are expressed as mean ± SEM. $^{**}p < 0.01$ versus WC group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus SC group.

and nitrite concentrations of SHR were as follows: SC group (7.33 ± 0.98 μmol/l), SL group (13.25 ± 1.24 μmol/l, $p < 0.05$ versus SC group), and SH group (17.96 ± 1.90 μmol/l, $p < 0.01$ versus SC group).

4. Discussion

We herein demonstrated that chronic FPPS inhibition in SHR improved the impaired endothelium-dependent vasodilation of aortic rings ex vivo, and the mechanism primarily involves inhibiting RhoA activity and increasing phospho-eNOS/eNOS ratio as well as enhancing NO bioavailability.

It is well established that endothelium-dependent vasodilation relies almost entirely on the NO [26]. ACh binds to an endothelial cell, causes a rise of cytosolic Ca^{2+}, and activates eNOS. Then, eNOS catalyzed NO diffuses into the adjacent smooth muscle cells and stimulates guanylyl cyclase, the enzyme that synthesizes cyclic GMP. Cyclic GMP leads to a decrease in cytosolic Ca^{2+}, which causes relaxation of the muscle cell and dilation of the blood vessel. In our experiment, an improvement of ACh-induced endothelium-dependent relaxation was observed by treatment with alendronate at a high dosage. Additionally the present study shows that the endothelium-independent relaxation induced by SNP was similar in SHR and WKY and was unaffected by alendronate treatment. These data indicated that the improvement of endothelial function by FPPS inhibitor is due to the changes in endothelium-derived NO availability rather than the downstream effects on vascular smooth muscle. Moreover, the differences of endothelium-dependent relaxation between alendronate-treated and -untreated SHR were abolished in the presence of L-NAME, suggesting that enhanced NO bioavailability is the cause of the improved relaxation response.

GGOH, when incubated in vitro, is used as an isoprenoid intermediate in mevalonate pathway [27,28]. In our study, incubation with GGOH could partially reverse the alendronate-induced endothelial improvement in isolated aortic rings in SHR received high-dose alendronate treatment, suggesting that the protective effect of FPPS inhibition on endothelial function was mediated at least partly, if not all, by the inhibition of the mevalonate pathway, especially by the suppression of isoprenoid intermediates. Therefore, from vasomotor studies ex vivo, we have shown that FPPS inhibition improved endothelial function in SHR via NO and mevalonate pathway. In general, FPPS catalyzed isoprenoid intermediates are necessary for proper function of RhoA [12,13], which negatively regulated the expression and phosphorylation of eNOS [10,29,30]. A hallmark of endothelial dysfunction is the reduced NO bioavailability, which could be caused by decreased expression of eNOS or impairment of eNOS activation. In order to get a better understanding of the mechanisms for the endothelial protective effects of FPPS inhibition, we detected RhoA, eNOS, and NO end products.

In our study, we found a higher level of GTP-bound active RhoA but only a comparable level of total RhoA in aortas from SHR than those from WKY, and these results were consistent with several recent investigations [31,32]. Furthermore, chronic administration of alendronate decreased the level of active RhoA in a dose-dependent manner in SHR but without effect on total RhoA expression. Thus, our results demonstrated that FPPS inhibitor only affects the activated process of RhoA, possibly by inhibition of RhoA isoprenylation. Consequently, we found a lower phospho-eNOS/eNOS ratio in aortas in SHR compared to WKY. Chronic FPPS inhibition increased phospho-eNOS/eNOS ratio and NO bioavailability in SHR in a dose-dependent manner. These results, together with previous reports [9,10,33], supported the viewpoint that alterations in activity of RhoA make a great contribution to the regulation of eNOS activation and NO bioavailability. Furthermore, isoprenoid intermediates–RhoA–eNOS interaction may be an important mechanism by which FPPS inhibition improved endothelial function in SHR. As a result, by improving the impaired endothelial function, alendronate could slightly decrease blood pressure in SHR.

3-Hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase is a rate-limiting enzyme located upstream of FPPS. Theoretically, HMG-CoA reductase inhibitors, statins, can also inhibit the synthesis of isoprenoid intermediates, suppress the activation of RhoA, upregulate the expression of eNOS, and finally improve the endothelial function. Indeed, statins have been shown to improve endothelium-dependent vasorelaxation in both human and animal studies [34–37]. Therefore, this beyond lipid-lowering effect of statins also supported our assumption about FPPS.

In conclusion, we herein provide the experimental evidence that chronic FPPS inhibition improved the endothelial function in SHR, and the upregulation of phospho-eNOS/eNOS ratio with inhibition of RhoA activation may be an important mechanism underlying the endothelial protection of FPPS inhibition. Therefore, FPPS plays an important role in regulating the endothelial function.

5. Limitations

Previous reports [18,38] have demonstrated that endothelium-dependent vasodilation was impaired in SHR compared with WKY. The mechanisms were speculated to involve not only the impaired formation of NO but also the elevated products of cyclooxygenase (COX). Although this study proposed several possible reasons why chronic FPPS inhibition improved the endothelial function in SHR, other mechanisms such as oxidative stress should be explored in further study.

Conflict of interest

None.

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References

[1] Shimokawa H. Primary endothelial dysfunction: atherosclerosis. J Mol Cell Cardiol 1999;31:23–37.
[2] Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:III27–32.
[3] Landmesser U, Drexler H. Endothelial function and hypertension. Curr Opin Cardiol 2007;22:316–20.
[4] Boulanger CM. Secondary endothelial dysfunction: hypertension and heart failure. J Mol Cell Cardiol 1999;31:39–49.
[5] Gokce N. L-arginine and hypertension. J Nutr 2004;134:2807s–11s.
[6] Massion PB, Feron O, Dessy C, Balligand JL. Nitric oxide and cardiac function: ten years after, and continuing. Circ Res 2003;93:388–98.
[7] Yetik-Anacak G, Catravas JD. Nitric oxide and the endothelium: history and impact on cardiovascular disease. Vascul Pharmacol 2006;45:268–76.
[8] Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J Biol Chem 1998;273:24266–71.
[9] Ming XF, Viswambharan H, Barandier C, Buffieux J, Kaibuchi K, Rusconi S, et al. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. Mol Cell Biol 2002;22:8467–77.

[10] Brandes RP. Statin-mediated inhibition of Rho: only to get more NO? Circ Res 2005;96:927–9.
[11] Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. Circ Res 2005; 97:1232–5.
[12] Roskoski Jr R. Protein prenylation: a pivotal posttranslational process. Biochem Biophys Res Commun 2003;303:1–7.
[13] Casey PJ. Protein lipidation in cell signaling. Science 1995;268:221–5.
[14] Chen GP, Yao L, Lu X, Li L, Hu SJ. Tissue-specific effects of atorvastatin on 3-hydroxy-3-methylglutarylcoenzyme A reductase expression and activity in spontaneously hypertensive rats. Acta Pharmacol Sin 2008;29:1181–6.
[15] Li L, Hu SJ, Dong HT, Kang L, Chen NY, Fang YQ. Alterations in gene expression of series key enzymes in mevalonic acid pathway detected by RNA array in spontaneously hypertensive rats. Chin J Pathophysiol 2008;24:54–9.
[16] Li L, Chen GP, Yang Y, Ye Y, Yao L, Hu SJ. Chronic inhibition of farnesyl pyrophosphate synthase attenuates cardiac hypertrophy and fibrosis in spontaneously hypertensive rats. Biochem Pharmacol 2010;79:399–406.
[17] Szkopińska A, Płochocka D. Farnesyl diphosphate synthase; regulation of product specificity. Acta Biochim Pol 2005;52:45–55.
[18] Vanhoutte PM, Feletou M, Taddei S. Endothelium-dependent contractions in hypertension. Br J Pharmacol 2005;144:449–58.
[19] Chou TC, Yen MH, Li CY, Ding YA. Alterations of nitric oxide synthase expression with aging and hypertension in rats. Hypertension 1998;31:643–8.
[20] Bergstrom JD, Bostedor RG, Masarachia PJ, Reszka AA, Rodan G. Alendronate is a specific, nanomolar inhibitor of farnesyl diphosphate synthase. Arch Biochem Biophys 2000;373:231–41.
[21] Roelofs AJ, Thompson K, Gordon S, Rogers MJ. Molecular mechanisms of action of bisphosphonates: current status. Clin Cancer Res 2006;12(20 Suppl): 6222s–6230s.
[22] Rogers MJ, Gordon S, Benford HL, Coxon FP, Luckman SP, Mönkkönen J, et al. Cellular and molecular mechanisms of action of bisphosphonates. Cancer 2000;88(12 Suppl):2961–78.
[23] Kubota Y, Umegaki K, Kagota S, Tanaka N, Nakamura K, Kunitomo M, et al. Evaluation of blood pressure measured by Tail-Cuff methods (without heating) in spontaneously hypertensive rats. Biol Pharm Bull 2006;29:1756–8.
[24] Williams JR, Harrison TR, Grollman A. A simple method for determining the systolic blood pressure of the un-anaesthetized rat. J Clin Invest 1939;18:373–6.
[25] Roullet JB, Xue H, Chapman J, McDougal P, Roullet CM, McCarron DA. Farnesyl analogues inhibit vasoconstriction in animal and human arteries. J Clin Invest 1996;97:2384–90.
[26] Nagao T, Illiano S, Vanhoutte PM. Heterogeneous distribution of endothelium-dependent relaxations resistant to NG-nitro-l-arginine in rats. Am J Physiol 1992;263:H1090–4.
[27] Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, Masarachia PJ. Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci USA 1999;96: 133–138.
[28] Virtanen SS, Väänänen HK, Härkönen PL, Lakkakorpi PT. Alendronate inhibits invasion of PC-3 prostate cancer cells by affecting the mevalonate pathway. Cancer Res 2002;62:2708–14.
[29] Mount PF, Kemp BE, Power DA. Regulation of endothelial and myocardial NO synthesis by multi-site eNOS phosphorylation. J Mol Cell Cardiol 2007;42: 271–279.
[30] Shiga N, Hirano K, Hirano M, Hishimura J, Nawata H, Kanaide H. Long-term inhibition of RhoA attenuates vascular contractility by enhancing endothelial NO production in an intact rabbit mesenteric artery. Circ Res 2005;96: 1014–21.
[31] Moriki N, Ito M, Seko T, Kureishi Y, Okamoto R, Nakakuki T, et al. RhoA activation in vascular smooth muscle cells from stroke-prone spontaneously hypertensive rats. Hypertens Res 2004;27:263–70.
[32] Seko T, Ito M, Kureishi Y, Okamato R, Moriki N, Onishi K, et al. Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. Circ Res 2003;92:411–8.
[33] Laufs U, Endres M, Stagliano N, Amin-Hanjani S, Chui DS, Yang SX, et al. Neuroprotection mediated by changes in the endothelial actin cytoskeleton. J Clin Invest 2000;106:15–24.
[34] Bellosta S, Ferri N, Bernini F, Paoletti R, Corsini A. Non-lipid-related effects of statins. Ann Med 2000;32:164–76.
[35] Laufs U, Liao JK. Direct vascular effects of HMG-CoA reductase inhibitors. Trends Cardiovasc Med 2000;10:143–8.
[36] Lefer AM, Scalia R, Lefer DJ. Vascular effects of HMG CoA-reductase inhibitors (statins) unrelated to cholesterol lowering: new concepts for cardiovascular disease. Cardiovasc Res 2001;49:281–7.
[37] Liao JK. Beyond lipid lowering: the role of statins in vascular protection. Int J Cardiol 2002;86:5–18.
[38] Yang D, Feletou M, Levens N, Zhang JN, Vanhoutte PM. A diffusible substance(s) mediates endothelium-dependent contractions in the aorta of SHR. Hypertension 2003;41:143–8.