Aurantio-obtusin relaxes systemic arteries through endothelial PI3K/AKT/eNOS-dependent signaling pathway in rats

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Aurantio-obtusin is a natural effective compound isolated from Semen Cassiae, which possesses hypotensive and hypolipidemic effects. Although its hypotensive effect have been clarified, mechanisms of Aurantio-obtusin on isolated mesenteric arteries remain unclear. This study was to investigate effects and mechanisms of Aurantio-obtusin on isolated mesenteric arteries (MAs). We examined MAs relaxation induced by Aurantio-obtusin on rat isolated MAs, expression and activity of endothelial nitric oxide synthase (eNOS) and protein kinase B (AKT), and nitric oxide (NO) production in bovine artery endothelial cells (BAECs). Findings showed Aurantio-obtusin elicited dose-dependent vasorelaxation with phenylephrine (PE) precontracted rat MA rings (diameter: 200–300 mm), which can be diminished by denudation of endothelium and inhibition of eNOS activity, while having no effect on rat isolated pulmonary artery (PA) rings. Aurantio-obtusin increased NO production by promoting phosphorylations of eNOS at Ser-1177 and Thr-495 in endothelial cells. Aurantio-obtusin also promoted phosphorylations of Akt at Ser-473. PI3K inhibitor LY290042 could diminish vasorelaxation induced by Aurantio-obtusin. Moreover Aurantio-obtusin also elicited dose-dependent vasorelaxation effect with PE precontracted MA rings (diameter: 100–150 mm). Therefore, vasorelaxation induced by Aurantio-obtusin was dependent on endothelium integrity and NO production, which mediated by endothelial PI3K/Akt/eNOS pathway. Results suggest Aurantio-obtusin may offer therapeutic effects in hypertension, as a new potential vasodilator.

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1. Introduction

Cardiovascular diseases remain the leading cause of death in the under-developed country, with increasing rapidly in developing countries. And incidence of death is 30% worldwide (1). The applications of most clinical medications were limited because patients have serious adverse reactions and high medical bills, especially after being given continuously for a prolonged period, which limit their applications. Therefore, specific natural drugs have been searched for the therapy of hypertension.

Aurantio-obtusin, 1, 3, 7-Trihydroxy-2, 8-diMethoxy-6-Methyl-9, 10-anthracenedione (Fig. 1), the major bio-activity compound of Semen Cassiae (2), has kinds of biological properties, such as antioxidative, anti-coagulating and anti-hypertension. It has been reported that Semen Cassiae could have significant anti-hypertension effect (3,4). However whether Aurantio-obtusin plays a pivotal role in anti-hypertension effects of Semen Cassiae is still unclear. And the underlying intracellular mechanisms of Aurantio-obtusin induced anti-hypertension effect also should be further explored.

The development of cardiovascular disease has related with vascular endothelium, which was confirmed in previous research.
indicates that PI3K/Akt/eNOS pathway has the important role in protein kinase B (Akt) is activated by eNOS. Accumulating evidence artery rings through PI3K/Akt/eNOS signaling pathway (10,11). polypeptide has vasorelaxation effect on rat isolated pulmonary (NICPBP, Beijing, China). Phenylephrine, N

2.2. Drugs and chemicals reagents

Aurantio-obtusin (99% purity) was purchased from National Institute for the control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). Phenylephrine, NO-nitro-L-arginine methyl ester (l-NAME) and Wortmannin were obtained Sigma Chemical Co (St Louis, MO). Cell-permeable fluorescent indicator 3-amino, 4-aminomethyl-2, 7-difluorofluorescein diacetate (DAF-FMMA) was from Calbiochem (San Diego, CA, USA), protein kinase B (Akt) antibody, phosphorylated Akt at Ser-473 (p-Akt-Ser-473) antibody, phosphorylated endothelial NO synthase (eNOS) at Ser-1177 (p-eNOS-Ser-1177) and Thr-495 (p-eNOS-Thr-495) and total eNOS antibody were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). All other reagents were from common commercial sources.

2.3. PAs and MAs preparation

The experiments were carried out according to published protocols. Briefly, rats were anesthetized with Chloral hydrate (6%, 0.5 ml/100 g). Dissected out lungs and mesenteries from thoracic and abdominal cavities respectively, and immersed into cold oxygenated Krebs solution (In mM: NaCl116, KCl4.2, CaCl2.5, NaH2PO41.6, MgSO41.2, NaHCO322, and D-glucose11, PH7.4). Both PAs and MAs were carefully dissected from lungs and mesenteries. The rings (diameter: 200–300 μm) were cut into 2 mm–3 mm length rings under the dissecting microscope and inserted with the tungsten wires (diameter: 100 μm). And they were mounted in the tension-detecting device (ALC-MPA, Shanghai Alcbio Biology Technology Co, Ltd, China) and submerged in a water-jacketed organ bath with 3 ml oxygenated Krebs solution. The second- or third-order branches from the superior mesenteries arteries (diameter: 100–150 μm) were isolated and cut into 1.5 mm length rings. They were inserted on two stainless steel wires (diameter: 40 μm) and mounted in four-chamber wire myograph (model 620M, Danish Myo Technology, Gainesville, FL) with 3 ml oxygenated Krebs solution. And they were sparged with 95% O2/5% CO2 and maintained at 37°C continuously. The endothelium of rings was removed by rubbing with the micro-tweezers. And the activity of rings were confirmed with acetylcholine (Ach 10⁻⁶ M) as described previously (12).

2.4. Tension studies of PA and MA rings

Aurantio-obtusin was freshly dissolved in alcohol and stored at 4°C in dark. As previously described, both of PAs and MAs (diameter: 200–300 μm) were initially equilibrated with 0.3–0.4 g preload respectively for 30–40 min before the studies started. And MAs (diameter: 100–150 μm) were initially equilibrated with 0.05–0.1 g preload. The rings were incubated with phenylephrine (10⁻⁶ M) in order to study the vasorelaxation induced by Aurantio-obtusin. When the contraction of rings reached a steady state, they were treated with Aurantio-obtusin (10⁻⁹ M to 10⁻⁵ M). To ensure the volume of alcohol added had no effect on the precontracted tone, Aurantio-obtusin solution was added into the tissue bath with.less than 0.1% volume. The cumulative concentration-response curves were reported showing the ring tension at 5 min intervals. On the other hand, the rings with no endothelium were also treated with Aurantio-obtusin (10⁻⁹ M to 10⁻⁵ M). And the rings precontracted were incubated with l-NAME (10⁻⁴ M) or LY290042 (3 × 10⁻⁵ M) respectively for 30 min then exposed to Aurantio-obtusin again. All curves were recorded. At the end of studies, all rings were treated with carbachol (10⁻⁶ M) in order to ensure the rings vitality. Both the basal tension of 0.3 g and stable tension of rings induced by phenylephrine (10⁻⁶ M) were regarded as 100%.

2.5. Cell culture

Primary cultured bovine artery endothelial cells (BAECs) were prepared from neonatal bovine. In vitro experiment, the hearts of neonatal bovine were offered by a local abattoir. The thoracic arteries were separated and washed with HEPEs buffer (In mM: NaCl145, KCl5.4, D-glucose10, HEPEs10, PH7.3) to remove blood. The fat and excess adventitial were removed, and the endothelial cells were scraped gently from thoracic arteries, then cultured according to previous published manners (13,14). In this study, the approval about use of neonatal bovine was obtained from the Ethics Committee of Laboratory Animals at Harbin Medical University.
2.6. Measurement of NO production in BAECs

NO production in cultured BAECs was evaluated using DAF-FM DA fluorescence indicator by detecting endogenous NO. The BAECs were grown on a micro cover glass of the 100 mm culture dish to 80% confluence. The cells were treated with Aurantio-obtusin (10⁻⁵ M) alone, L-NAME (10⁻⁴ M) alone, Aurantio-obtusin (10⁻⁵ M) plus L-NAME (10⁻⁴ M) immediately and respectively in serum-free conditions. Then the cells were incubated with 5 × 10⁻⁶ M (final concentration) DAF-FM DA (Calbiochem, San Diego, CA, USA) for 1 h at 37 °C and rinsed three times with phosphate-buffered saline (PBS pH 7.4). The cells were imaged using Nikon fluorescent microscopy with 495-nm excitation and 515-nm emission wavelengths.

2.7. Western blotting

BAECs were grown in serum-free DMEM medium for 12 h before they were treated with Aurantio-obtusin (10⁻⁵ M) alone, Wortmannin (10⁻⁷ M) alone, Aurantio-obtusin plus Wortmannin (10⁻⁷ M), L-NAME (10⁻⁴ M) alone, Aurantio-obtusin (10⁻⁵ M) plus L-NAME (10⁻⁴ M) for indicated time respectively. The proteins were extracted from BAECs as previously reported (15). Equal amounts of protein (20–50 μg) from each sample were subjected to electro- phoresis on the 10% SDS-polyacrylamide gel and transferred onto nitrocellulose membrane. After 2 h incubation in a blocking buffer (Tris 20 mM, pH 7.6, NaCl 150 mM, and Tween20 0.1%) containing 5% nonfat dry milk powder, the membranes were reacted with the particular antibodies at suitable concentrations overnight 4 °C, followed by reaction with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reagents, with β-actin as an internal control.

2.8. Statistical analysis

The composite data are expressed as means ± SEM. Statistical analysis was performed with one-way ANOVA followed by Dunnett’s test. Differences were considered to be significant at P < 0.05.

3. Results

3.1. Vascular effect of Aurantio-obtusin on isolated rat PA and MA rings

The MA (diameter: 100–150 μm) and PA rings preloaded with 0.4 g and 0.3 g respectively were equilibration for 30–40 min, and then they were exposed to phenylephrine (10⁻⁶ M), which produced rapidly contraction of MA rings (0.7 g–1.0 g) and PA rings (0.4–0.5 g). The peak contraction of MA rings were reached in 10 min and maintained for at least 20 min without evident decline. During the vasorelaxation, administration of Aurantio-obtusin (10⁻⁹–10⁻⁵ M) produced concentration dependent relaxation of MA rings (n = 8, P < 0.01, Fig. 2A and C). At the maximum effect, Aurantio-obtusin (10⁻⁵ M) relaxed the MAs by 32.85 ± 1.32%. Next we explored whether similar results would be obtained with rat PA rings. Results showed that Aurantio-obtusin had no effects on rat precontracted PA rings (n = 8, Fig. 2B and C). These data suggested that concentration-dependent vasorelaxation induced by Aurantio-obtusin was specific on rat MAs, but not on rat PAs.

3.2. Role of endothelium and L-Name in MAs vasorelaxation induced by Aurantio-obtusin

To investigate the role of endothelium in the vasorelaxation induced by Aurantio-obtusin, we pretreated MA rings with or without endothelium respectively with phenylephrine (10⁻⁶ M), then added Aurantio-obtusin (10⁻⁵ M), the relaxation induced by Aurantio-obtusin in MA rings without endothelium were diminished markedly compared with the endothelium intact control (n = 12, Fig. 3A). Aurantio-obtusin (10⁻⁵ M) reduced the vascular tension to 8.97 ± 2.38% in the endothelium denuded MA rings (n = 12, Fig. 3C).

NO is a major vasorelaxant in endothelium and the effect of NO was studied with NG-nitro-L-arginine methyl ester (L-NAME, eNOS inhibitor). To test whether endothelium NO involved in the MAs relaxation induced by Aurantio-obtusin, MA rings were pretreated with L-NAME (10⁻⁴ M) for 30 min before precontracted with phenylephrine (10⁻⁶ M). The MAs vasorelaxation of Aurantio-obtusin was significantly diminished (n = 12, Fig. 3B). Aurantio-obtusin (10⁻⁵ M) reduced the vascular tension to 10.93 ± 1.61% in the presence of L-NAME, which is 67% less as compared to that without L-NAME (n = 12, Fig. 3D).

3.3. The increase of NO production induced by Aurantio-obtusin

To determine whether NO release indeed is regulated by Aurantio-obtusin (10⁻⁵ M), the intracellular NO production in cultured BAECs were measured using DAF-FM DA fluorescence indicator. Aurantio-obtusin increased NO production in BAECs, meanwhile the eNOS inhibitor L-NAME (10⁻⁴ M) diminished this effect (Fig. 4A). The relative intensity of DAF-FM DA fluorescence suggested that Aurantio-obtusin stimulated NO production rapidly in BAECs (n = cell number, P < 0.01, Fig. 4B).

3.4. The activity of eNOS enhanced by Aurantio-obtusin in cultured BAECs

Which mechanisms underlie the NO production by Aurantio-obtusin, and which intra-endothelial signaling systems play a role? To address these questions, we studied the expression and activity of eNOS in cultured BAECs.

It is known that the protein phosphorylation at residue Ser-1177 increases eNOS activation, while phosphorylation at Thr-495 results in eNOS inhibition (16). Therefore, we examined phosphorylation levels at the two sites using phosphorylation-specific antibodies. The BAECs were treated with Aurantio-obtusin (10⁻⁵ M). We found the density band of Ser-1177 treated by Aurantio-obtusin was stronger than that of control. On the contrary, the density band of Thr-495 treated with Aurantio-obtusin was decreased as compared with control group. The eNOS inhibitor L-NAME (10⁻⁴ M) significantly diminished the level of eNOS phosphorylation at Ser-1177 and increased the level of eNOS phosphorylation at Thr-495 as compared with BAECs treated by Aurantio-obtusin. The expression of total eNOS had no change (n = 3, Fig. 5). These results indicated that Aurantio-obtusin transiently enhanced the activity of eNOS by increasing the level of eNOS phosphorylation at Ser-1177 and decreasing the level of eNOS phosphorylation at Thr-495.

3.5. Relaxation induced by Aurantio-obtusin through the PI3K/Akt signaling pathway

Subsequently, we examined the role of PI3K/Akt signaling pathway in vasorelaxation induced by Aurantio-obtusin. MAs were pretreated with the PI3K/Akt inhibitor LY290042 (3 × 10⁻⁵ M). Then, added Aurantio-obtusin (10⁻⁵ M) after a precontraction with phenylephrine (10⁻⁶ M). Relaxation induced by Aurantio-obtusin was diminished markedly after a treatment with LY290042 (3 × 10⁻⁵ M) (n = 12, 5.16 ± 0.65%), which was significantly lower than the control rings (n = 12, P < 0.01, Fig. 6).
Fig. 2. Effect of Aurantio-obtusin on rats MA and PA rings precontracted with 10^{-6} M phenylephrine. (A) Cumulative dosing of Aurantio-obtusin had dilatation effect on precontracted rat MA rings at concentration 10^{-5} M. (B) Cumulative dosing of Aurantio-obtusin had no effect on precontracted rat PA rings at concentration 10^{-5} M. (C) Concentration-response curves of Aurantio-obtusin in rats MA and PA rings. Results are expressed as mean ± S.E.M. (n = 8) **P < 0.01 versus control group, “AO” means Aurantio-obtusin, “PE” means phenylephrine, “CARB” means carbachol.

Fig. 3. Effect of endothelium and NO in Aurantio-obtusin induced Rats MAs relaxation. (A) Endothelium denudation diminished the vasorelaxation elicited by cumulative dosing of Aurantio-obtusin. (C) Concentration-response curves of endothelium intact and denuded of Aurantio-obtusin on rats MA rings. (B) Inhibition of eNOS by i-NAME (10^{-4} M) on endothelium intact MA rings significantly diminished vasorelaxation effect induced by cumulative dosing of Aurantio-obtusin. (D) Concentration-response curves of i-NAME on endothelium intact MA rings. Results are expressed as mean ± S.E.M. (n = 8) **P < 0.01 versus control group, “AO” means Aurantio-obtusin, “PE” means phenylephrine, “CARB” means Carbachol, “EI” means endothelium intact, “ED” means endothelium denuded.

Fig. 4. Effects of Aurantio-obtusin on NO production in BAECs. Basal NO production is visualized with DAF-FM. BAECs were treated with Aurantio-obtusin. Evident increase of DAF-FM fluorescent intensity was seen. (Ab) Aurantio-obtusin stimulated the release of NO production. (Ad) The eNOS inhibitor i-NAME (10^{-4} M) diminished this effect. (B) Densitometric analysis of DAF-FM assays. Results are expressed as mean ± S.E.M. (n = 3) **P < 0.01 compared with others groups. “NC” means negative control, “AO” means Aurantio-obtusin, “i-N” means eNOS inhibitor i-NAME.
3.6. Involvement of PI3K/Akt signaling pathway in Aurantio-obtusin induced eNOS activation

The eNOS is known to bind with Akt after the Akt is phosphorylated at Ser-473, leading to Akt activation (17). To explore whether Aurantio-obtusin affects on PI3K/Akt signaling pathway, the activity and expression of Akt were examined in BAECs treated with Aurantio-obtusin. Similar to eNOS phosphorylation at Ser-1177, Aurantio-obtusin enhanced the Akt phosphorylation at Ser-473, not affected the expression of total Akt (n = 3, P < 0.01, Fig. 7). The results suggested that Aurantio-obtusin also transiently enhanced the activity of Akt.

To further confirm whether PI3K/Akt signaling pathway participates in Aurantio-obtusin-induced increase of eNOS phosphorylation at Ser-1177 and NO production, we blocked PI3K/Akt signaling pathway with Wortmannin (10⁻⁷ M). Wortmannin (10⁻⁷ M) significantly attenuated Aurantio-obtusin induced Akt phosphorylation at Ser-473 in BAECs (n = 3, P < 0.01, Fig. 7). All of these results indicated that activation of PI3K/Akt signaling pathway was necessary for eNOS activity and NO production induced by Aurantio-obtusin.

3.7. Vascular effect of Aurantio-obtusin on isolated rat MA (diameter: 100–150 μm) rings

Blood pressure levels are determined by vascular resistance. To examine the effect of Aurantio-obtusin on vascular reactivity of small resistance blood vessels, we isolated the second- or third-order branches from the superior mesentery arteries (diameter: 100–150 μm) and prepared the vascular rings. The rings (diameter: 100–150 μm) preloaded with 0.05–0.1 g were equilibration for 30–40 min, and then they were exposed to phenylephrine (10⁻⁶ M). The peak contraction of rings (diameter: 100–150 μm) were reached in 10 min and maintained for at least 20 min without

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**Fig. 5.** Effect of Aurantio-obtusin on the phosphorylation and protein of eNOS in BAECs. (A) Aurantio-obtusin up-regulated the level of phosphor-eNOS at Ser-1177 and down-regulated the level of phosphor-eNOS at Thr-495. But Aurantio-obtusin had almost no effect on the expression of total eNOS. The eNOS inhibitor i-NAME (10⁻⁴ M) blocked the eNOS phosphorylation at Ser-1177 and Thy-495 stimulated by Aurantio-obtusin, thus inhibited the activity of eNOS. (B, C, and D) Densitometric analysis of the Western blot assays. Results are expressed as mean ± S.E.M. (n = 3) **P < 0.01 compared with others groups. “NC” means negative control, “AO” means Aurantio-obtusin, “i-N” means eNOS inhibitor i-NAME.

**Fig. 6.** Effect of PI3K/Akt in Aurantio-obtusin induced Rats MAs relaxation. (A) LY290042 (3 × 10⁻⁵ M) (PI3K inhibitor) significantly diminished vasorelaxation effect induced by cumulative dosing of Aurantio-obtusin. (B) Concentration-response curves of LY290042 (3 × 10⁻⁵ M) on endothelium intact MA rings. Results are expressed as mean ± S.E.M. (n = 12) **P < 0.01 versus control group, “AO” means Aurantio-obtusin, “PE” means phenylephrine, “CARB” means carbachol, “EI” means endothelium intact.
During the vasorelaxation, administration of Aurantio-obtusin (10⁻⁶ M) produced concentration dependent relaxation of rings (diameter: 100–150 μm) (n = 7, P < 0.05, Fig. 8). At the maximum effect, Aurantio-obtusin (10⁻⁵ M) relaxed the MAs by 92.14 ± 0.03%. These data suggested that Aurantio-obtusin induced the relaxation of rat MA small resistance blood vessels on concentration-dependent way. Moreover, the relaxation range was bigger than MA rings (diameter: 200–300 μm).

4. Discussion

This is the first systematic study of cellular mechanisms for vasorelaxation by Aurantio-obtusin. Aurantio-obtusin relaxed precontracted rat MAs (200–300 and 100–150 μm respectively) in a concentration-dependent manner (10⁻⁶ M to 10⁻⁵ M), whereas there is no effect on rat PAs in our study. Moreover, the vascular relaxation effect of Aurantio-obtusin on rat MAs depended on the functional integrity of the endothelium and NO production. Our results indicated that Aurantio-obtusin acted on the PI3K/AKT signaling pathway to enhance eNOS activity. Aurantio-obtusin (10⁻⁵ M) activated Akt through phosphorylation at Ser-473 in BAECs, enhanced eNOS activation via phosphorylation at Ser-1177 and Thr-495, and stimulated endothelial NO production, leading to MAs relaxation in 5 min. The PI3K inhibitor LY290042 (3 × 10⁻⁵ M) and Wortmannin (10⁻⁷ M) diminished these effects. These results suggested Aurantio-obtusin relaxed systemic arteries through endothelial PI3K/AKT/eNOS/NO signaling pathway, as a potent systemic arteries vasodilator.

Semen Cassiae is a well known traditional Chinese medicine that has been used to treat hypertension patient for hundreds of years. Aurantio-obtusin, an active and main content of Semen Cassiae, has been reported that it has the anti-hypertensive role derived from natural products. It also plays an important role in anti-oxidative, anti-dementia, anti-coagulating activity and cytotoxicity in tumor (18). However, limited information of Aurantio-obtusin is available in the effect on MAs and underlying mechanism, which is vital in the therapy of hypertension. The present study is the first to demonstrate that Aurantio-obtusin (10⁻⁵ M) relaxed isolated rat mesenteric arteries.
It is well known that the endothelium releases a series of endogenous vasodilative agents, such as NO, COX-derived products, and endothelium-derived hyperpolarization factors (EDHFs) which regulate vascular tone (19–21). NO is the major vasodilator in the endothelium-dependent vasorelaxation. The production of endothelial NO is tightly regulated by the level of its synthesis through the oxidation of L-arginine by NO synthases (eNOS) in pulmonary circulation (22).

Which underlying mechanism of Aurantio-obtusin mediated rat MAs relaxation? We found that denudation of endothelium and inhibition of NO production with L-NAME (10⁻⁴ M) diminished the vasodilation effect from Aurantio-obtusin, suggesting that vasodilation effect from Aurantio-obtusin relies on endothelium integrity and NO generation. And the effect was most possible from eNOS activation.

Phosphorylations at Ser-617, Ser-635, Ser-1177 and Ser-1179 are important for eNOS activation, whereas phosphorylations at Ser-116, Thr-495 and Thr-497 cause inhibition of eNOS activity (23). Our study showed that Aurantio-obtusin increased the level of phosphor-eNOS (Ser-1177) and inhibited the level of phosphor-eNOS (Thr-495), which means that Aurantio-obtusin activated eNOS by phosphorylation of Ser-1177 and phosphorylation of Thr-495. These observations indicated that Aurantio-obtusin stimulated NO production by eNOS in BAECs.

It was reported that eNOS is regulated by endothelial PI3K/akt signaling pathway (24). Akt kinase (protein kinase B) is the serine/threonine kinase activated by several phosphatidylinositol-dependent protein kinases. Phosphorylation of Ser-473 on Akt is coincident with Akt activation in vivo and vitro, which has been used as a marker for Akt activity (25,26). Activated Akt increased expression or activity of eNOS and NO production in endothelial cells by directly phosphorylation eNOS at Ser-1177 and Thr-495. Our results indicated that Aurantio-obtusin also played a role in the PI3K/AKT signaling pathway to elevate eNOS activity for several reasons: (1) Akt phosphorylation at Ser-473 was stimulated by Aurantio-obtusin and blocked by the PI3K inhibitor. (2) Enhanced eNOS phosphorylation at Ser-1177 was almost completely eliminated in the presence of PI3K/AKT inhibitor. (3) Akt phosphorylation at Ser-473 was consistent with eNOS phosphorylation at Ser-1177. Therefore, the activation of PI3K/AKT signaling pathway is crucial in the endothelial NO release system induced by Aurantio-obtusin.

We found that Aurantio-obtusin had modest vasorelaxation effect on rat MAs (systemic arterial system), but no effect on rat PAs (pulmonary arterial system). The reason may be related to different vascular structures and vasoactive mediators. It’s potential application for regulating differentiated tone of systemic circulation and pulmonary circulation.

In a word, Aurantio-obtusin, the natural product, contributed to a state of vasorelaxation on Rat MAs, but not PAs. Moreover, for the first time we found the vasorelaxation induced by Aurantio-obtusin on MAs was specific and NO dependent. The vasorelaxation induced by Aurantio-obtusin is mediated through endothelial intracellular PI3K/Akt/eNOS/NO signaling pathway in BAECs. This study provides a new potential vasodilator for the therapy of arterial hypertension.

Conflicts of Interest

The authors indicated no potential conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2015.05.006.

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