นิพนธ์ต้นฉบับ . Original Article

โนบิเลตินบรรเทาความผิดปกติของการทำงานและโครงสร้างของหลอดเลือดในหนูแรทความดันเลือดสูงจากหลอดเลือดแดงไตตีบ

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Nobiletin Alleviates Vascular Functional and Structural Alterations in Renovascular Hypertensive Rats

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บทคัดย่อ

หลักการและวัตถุประสงค์: ภาวะความดันเลือดสูงจากหลอดเลือดแดงไตตีบซึ่งก่อให้เกิดความผิดปกติของระบบหัวใจและหลอดเลือดมีความสัมพันธ์กับภาวะเครียดออกซิเดชัน และการลดลงของชีวปริมาณออกฤทธิ์ของไนตริกออกไซด์ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของโนบิเลติน ซึ่งเป็นฟลาโวนชนิดโพลิเมทอกซีเลต ที่พบในเปลือกผลไม้ตระกูลส้ม ต่อการทำงานและการเปลี่ยนแปลงโครงสร้างของหลอดเลือดในหนูแรทที่ถูกเหนี่ยวนำให้เกิดความดันเลือดสูงจากหลอดเลือดแดงไตตีบโดยวิธี two-kidney, one-clip (2K-1C)

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley (น้ำหนัก 160-180 กรัม) ได้รับการผ่าตัดแบบ 2K-1C เพื่อเหนี่ยวนำให้เกิดภาวะความดันเลือดสูง สามสัปดาห์ต่อมาหนูแรทที่มีความดันเลือดสูงได้รับการรักษาด้วยโนบิเลติน (15 หรือ 30 มก./กก./วัน) หรือลอซาร์แทน (10 มก./กก./วัน) กลุ่มละ 6 ตัว เป็นระยะเวลา 4 สัปดาห์ เมื่อสิ้นสุดการทดลองการเปลี่ยนแปลงด้านการทำงานและโครงสร้างของหลอดเลือด รวมถึงระดับไนไตรต์ในพลาสมาถูกวัด

ผลการศึกษา: โนบิเลติน 30 มก./กก./วัน ฟื้นฟูการคลายตัวของหลอดเลือดที่บกพร่องต่อการตอบสนองต่ออะเซทิลโคลีนทั้งในหลอดเลือดเอออร์ตา และหลอดเลือดมีเซนเทอริกของหนูแรทความดันเลือดสูง ($p<0.05$) นอกจากนี้ โนบิเลตินยังลดการหดตัวของหลอดเลือดมีเซนเทอริกที่ตอบสนองต่อการกระตุ้นด้วยสนามไฟฟ้าได้อย่างมีนัยสำคัญ ($p<0.05$) ที่สำคัญการให้โนบิเลตินขนาด 30 มก./กก./วัน อย่างต่อเนื่องช่วยบรรเทาการเปลี่ยนแปลงทางสัณฐานวิทยาของหลอดเลือดเอออร์ตา และเพิ่มการสร้างไนไตรต์ในหนูแรทความดันเลือดสูงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

สรุป: โนบิเลตินบรรเทาความผิดปกติด้านการทำงานและการเปลี่ยนแปลงทางสัณฐานวิทยาของหลอดเลือดในหนูแรทความดันเลือดสูงแบบ 2K-1C ซึ่งผลดังกล่าวมีความสัมพันธ์กับการเพิ่มขึ้นของชีวปริมาณออกฤทธิ์ของไนตริกออกไซด์

คำสำคัญ: โนบิเลติน, ความดันเลือดสูง, สัณฐานวิทยาหลอดเลือด, การทำงานของหลอดเลือด, ไนตริกออกไซด์

Abstract

Background and Objectives: Renovascular hypertension-induced cardiovascular impairments are associated with oxidative stress and reduction of nitric oxide bioavailability. This study investigated the effects of nobiletin, a polymethoxylated flavone found in citrus fruit peel, on vascular function and morphological changes in two-kidney, one-clip (2K-1C)-induced renovascular hypertensive rats.

Method: 2K-1C operation was conducted in male Sprague-Dawley rats (160 -180 g) to induce renovascular hypertension. Three weeks later, hypertensive rats were treated with nobiletin (15 or 30 mg/kg/day) or losartan (10 mg/kg/day) each group n=6 for 4 weeks. At the end of the experiment, vascular function, histomorphology, and plasma nitric oxide levels were measured.

Results: Nobiletin at 30 mg/kg/day significantly restored the impaired vasorelaxation responses to acetylcholine (ACh) in both the aorta and mesenteric vascular beds of 2K-1C hypertensive rats ($p<0.05$). In addition, nobiletin markedly decreased mesenteric contractile responses to electrical field stimulation ($p<0.05$). Notably, daily administration of 30 mg/kg nobiletin alleviated aortic remodeling and significantly increased nitrite production in renovascular hypertensive rats ($p<0.05$).

Conclusion: Nobiletin alleviates vascular dysfunction and morphological alterations in 2K-1C hypertensive rats. These effects are associated with enhanced nitric oxide bioavailability

Keywords: nobiletin, hypertension, vascular morphology, vascular function, nitric oxide

Introduction

Renovascular hypertension is one of the progressive conditions that can lead to declining renal function and damage to other vital organs.[1,2] Goldblatt and coworkers developed an experimental animal model representing renovascular hypertension, known as the two-kidney, one-clip (2K-1C) model.[3] Previous studies reported that the progressive increase in blood pressure in 2K-1C rats is primarily mediated by both intra-renal and systemic activation of the renin-angiotensin system (RAS) which is involved in elevated oxidative stress.[4-6] Furthermore, several studies demonstrated that the vascular endothelial dysfunction in renovascular hypertensive rats may result from either excessive reactive oxygen species (ROS) and declined nitric oxide (NO) bioavailability.[7,8] Notably, ROS such as superoxide ($O_2 \cdot^-$) can reduce NO bioavailability by forming peroxynitrite ($ONOO^-$), contributing to vascular dysfunction in hypertension.[9,10] Casto and coworker found that increased superoxide in the aortic endothelium impairs vascular relaxation in 2K-1C rats.[11]

For the pharmacological management of hypertension losartan, an angiotensin II (Ang II) receptor blockers (ARBs) have been suggested as one of first line drugs. Losartan is a selective antagonist of Ang II type 1 receptor (AT1R).[12] Previous evidence demonstrated that losartan ameliorated renal and cardiovascular impairments in hypertensive patients.[13-15] Nowadays, besides pharmacological treatment and lifestyle modifications, functional foods derived from medicinal herbs or plants are gaining more attention as the alternative treatment of cardiovascular disease. Nobiletin, a polymethoxylated flavone mostly found in citrus peel, is one of the bioactive compounds in citrus fruits that has been proven to have cardiovascular protective effects. It exhibited a wide range of pharmacological effects, including antioxidative property, anti-apoptotic factors, anti-inflammation and cardiovascular protection.[16,17] Potue and coworkers reported that nobiletin potentially alleviated L-N^G-Nitro arginine methyl ester (L-NAME)-induced hypertension through the modulation of Nrf-2/HO-1 and Matrix Metalloproteinases (MMP) pathways in rats.[18] Bunbupha and coworkers have demonstrated that Nobiletin (NB) attenuated aortic remodeling and renal fibrosis through inhibition of oxidative stress markers and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase protein expression in high-fat diet- induced metabolic syndrome rats.[19,20] Moreover, NB had RAS inhibitory and antioxidant effects and attenuated left venticular dysfunction and remodelling via restoration of the AT1R/ Janus Kinase/Signal Transducers And Activators Of Transcription (JAK/STAT) pathway. NB also resolved renal damage that was related to modulation of the AT1R/Nox4 cascade in 2K-1C hypertension[21].

However, the effects of NB on vascular dysfunction and morphology alterations related with the NO bioavailability in 2K-1C hypertensive model are still unclear. Therefore, the recent study aimed to examine the effects of NB on the vascular function and morphology, and alterations of NO bioavailability in 2K-1C hypertensive rats.

Material and Methods

Chemicals

Nobiletin was obtained from INDOFINE Chemical Company, Inc. (NJ, USA). Losartan (Cozaar 50 mg) was obtained from MSD. Merck & Co., Inc., (NJ, USA). Nitric oxide assay kit was obtained from Sigma-Aldrich (Saint Louis, MO, USA). All the other chemicals used in this study were obtained from standard companies.

Animals

In the present study, male Sprague Dawley rats weighing 160-180 g were obtained from Nomura Siam International Co, Ltd., Bangkok, Thailand. All the rats were housed in the HVAC (heating, ventilation, and air-conditioning) system (23±2°C). This study complied with the standard for care and use of experimental animals that was approved by the Animal Ethic Committee of Khon Kaen University, Khon Kaen, Thailand (Permit No. IACUC-KKU-73/63).

Induction of 2K-1C hypertension

After one week of acclimation period, the rats were induced to renovascular hypertension by restriction of renal blood flow. The 2K-1C procedure was performed under anesthesia with an intraperitoneal injection of xylazine (5 mg/kg) and zoletil (25 mg/kg). Thereafter, a silver clip (0.2 mm inner diameter) was placed on the left renal artery, while the sham rats were operated without the arterial clip.

The successful rate of the induction procedure is around 75-80%

Thereafter, hypertension was presented in the 2K-1C operated rats at the 3rd week after operation followed our previous finding.[21]

Experimental protocols

After 3 weeks of surgery, the rats that had a systolic blood pressure (SP) > 160 mmHg were included as 2K-1C hypertensive rats[22]. The rats were randomly divided into 5 groups (n = 6 /group) including sham operated group received vehicle (propylene glycol, 1.5 mL/kg/day), 2K-1C untreated group received vehicle, 2K-1C treated with NB at dose 15 or 30 mg/kg/day, and 2K-1C treated with losartan (Los) at dose 10 mg/kg/day. The dose of NB and Los was followed by previous study[21]. The treatment period was conducted for 4 weeks. However, after the induction process and completion of the experimental period, the survival rate of rats was approximately 80%. At the end of the experiment, vascular function, vascular morphology study and plasma NO2- were evaluated.

Vascular function study

After isolation, the mesenteric vascular bed was perfused with oxygenated Krebs solution at 37°C to ensure tissue viability. Vasoconstrictive responses were assessed using electrical field stimulation (EFS) (5–40 Hz; 90 V, 1-ms pulse, 30 s) and by bolus injection of norepinephrine (NE) (0.15–15 nmol). For vasorelaxation studies, the tissue was precontracted with methoxamine (5–7 μM), followed by administration of either acetylcholine (ACh) or sodium nitroprusside (SNP) (both 0.1–10 nmol). Changes in mean perfusion pressure were continuously measured with a pressure transducer and recorded via a BIOPAC acquisition system (BIOPAC Systems Inc., California, USA). To assess vasorelaxation in conduit arteries, thoracic aortic rings (2–3 mm) were precontracted with phenylephrine (10 μM). Relaxation responses to increasing concentrations of either acetylcholine or sodium nitroprusside (both 0.01–3 μM) were then monitored in organ baths filled with oxygenated Krebs solution. Isometric tension was continuously measured using force transducers connected to a four-channel bridge amplifier and recorded via a PowerLab system (ADInstruments, Australia with Chart v5 software).

Plasma nitrate/nitrite level

Evaluation of systemic NO bioavailability was represented by the plasma levels of nitrite. The measurement investigated the conversion of nitrate to nitrite in plasma using nitric oxide assay kit (MAK454-1KT, Sigma-Aldrich Crop., Saint Louis, MO, USA).

Vascular histological and morphology analysis

The aortic tissues were fixed in 4% formalin for 48 h and then processed as previous described.[23] The aortic tissues were paraffin embedded and cut into 5 μm-thick sections. The morphometric changes in thoracic aorta were evaluated using the hematoxylin and eosin (H&E) staining techniques. The cross-sectional area (CSA), aortic wall thickness, lumen diameter, and wall/lumen ratio were investigated under a light microscope (Nikon digital sight10, Tokyo, Japan).

Statistical analysis

The data were presented as mean ± standard error of the mean (SEM). The statistical analysis was performed using GraphPad prism (version 8.3) with a one-way analysis of variance (ANOVA), followed by Turkey's post hoc test. A p-value of <0.05 was considered as statistical significance.

Results

Effect of nobiletin or losartan on vascular contractile responses to electrical field stimulation (EFS) and exogenous norepinephrine (NE) in 2K-1C hypertensive rats

The contractile responses to EFS significantly increased in the mesenteric vascular beds isolated from 2K-1C induced hypertensive group compared to the sham-operated group ($p<0.05$; Figure 1A). After 4 weeks of 30 mg/kg of nobiletin or losartan treatment, the responses significantly reduced, compared to the 2K-1C untreated rats. However, the contractile responses to exogenous NE were not different among groups (Figure 1B).

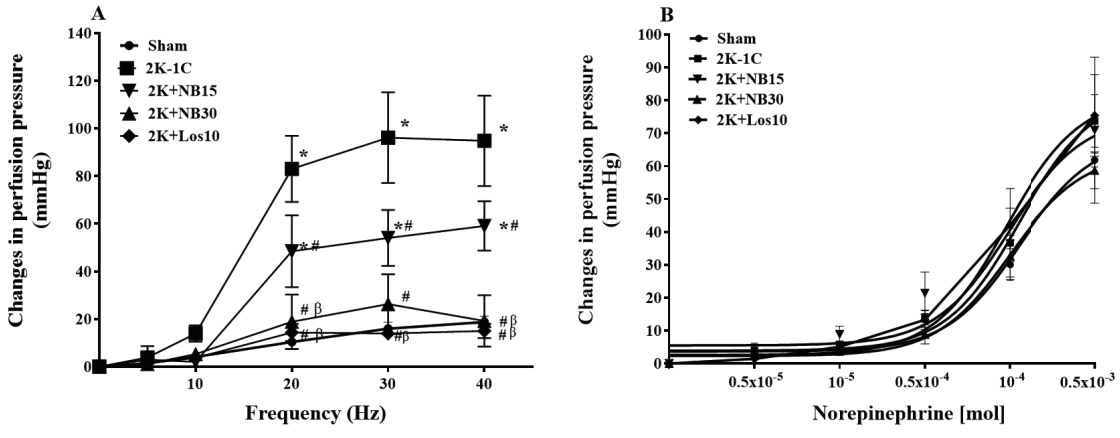


Figure 1 Effect of nobiletin or losartan on contractile responses to electrical field stimulation (A), and exogenous norepinephrine (B) in 2K-1C hypertensive rats. Data are expressed as mean ± S.E.M. (n =6/group). *: $p<0.05$ vs Sham, #: $p<0.05$ vs 2K-1C. 2K-1C is 2K-1C received vehicle, 2K+NB15 is 2K-1C received nobiletin 15mg/kg, 2K+NB30 is 2K-1C received nobiletin 30mg/kg, and 2K+Los10 is 2K-1C received losartan 10 mg/kg.

Effect of nobiletin or losartan on vascular relaxation responses to vasoactive agents in the mesenteric in 2K-1C hypertensive rats

The hypertensive untreated rats exhibited the significant decrease in relaxation responses of mesenteric vascular beds and aortic rings to ACh compared to the sham-operated rats ($p<0.05$; Figure 2A and 2C, respectively). After thoracic oral administration of 30 mg/kg of nobiletin or losartan, the relaxation responses to ACh of both types of vascular were significantly improved, when compared to hypertensive untreated rats ($p<0.05$; Figure 2A and 2C, respectively). However, the relaxation responses to sodium nitroprusside (SNP) in both vascular set ups did not differ among groups (Figure 2A and 2D).

Effect of nobiletin or losartan on systemic NO bioavailability in 2K-1C hypertensive rats

Circulating NO bioavailability indicated by plasma nitrite levels were significantly reduced in the 2K-1C untreated group compared to the sham-operated group ($p<0.05$; Figure 3). However, 30 mg/kg of nobiletin or losartan significantly upregulated the levels of plasma nitrite in hypertensive rats induced by 2K-1C operation. ($p<0.05$).

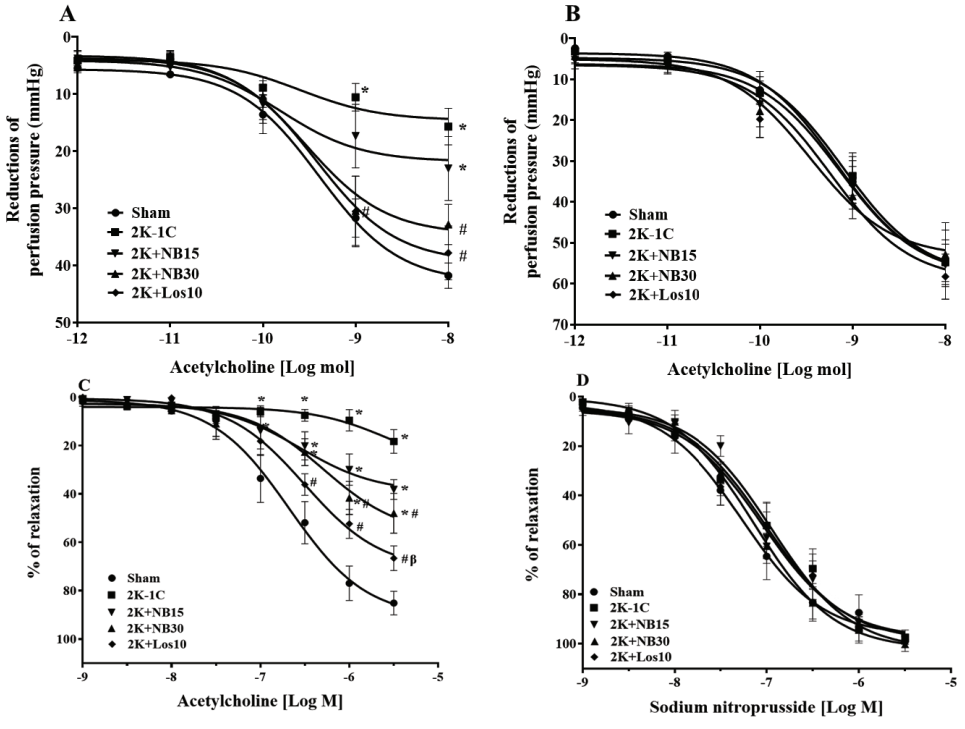


Figure 2 Effect of nobiletin or losartan on vascular responses to exogenous acetylcholine in mesenteric vascular beds (A) and thoracic aorta (C), and responses to sodium nitroprusside in mesenteric vascular beds (B) and thoracic aorta (D) in 2K-1C hypertensive rats. Data are expressed as mean ± S.E.M. (n =6/group). *: p<0.05 vs Sham, $^{\#}$: p<0.05 vs 2K-1C, $^{\beta}$: p<0.05 vs 2K+NB15. 2K-1C is 2K-1C received vehicle, 2K+NB15 is 2K-1C received nobiletin 15mg/kg, 2K+NB30 is 2K-1C received nobiletin 30mg/kg, and 2K+Los10 is 2K-1C received losartan 10 mg/kg.

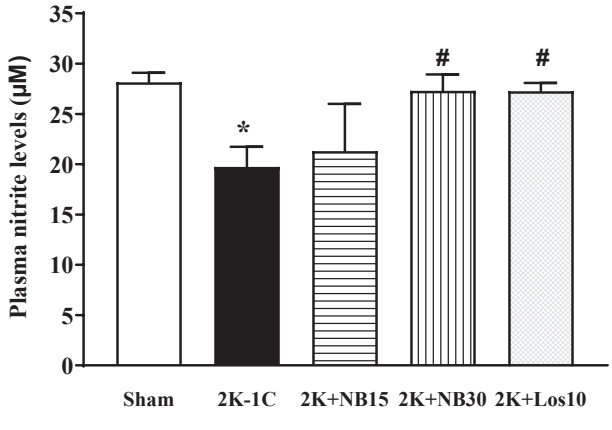
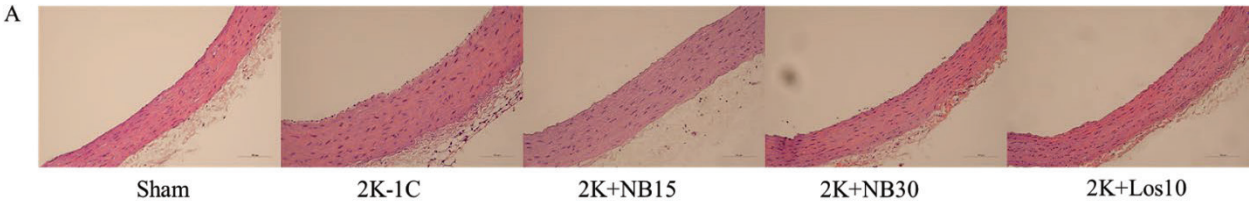


Figure 3 Effect of nobiletin or losartan on plasma nitrite level. Data are expressed as mean ± S.E.M. (n =6/group). *: p<0.05 vs Sham, $^{\#}$: p<0.05 vs 2K-1C. 2K-1C is 2K-1C received vehicle, 2K+NB15 is 2K-1C received nobiletin 15mg/kg, 2K+NB30 is 2K-1C received nobiletin 30mg/kg, and 2K+Los10 is 2K-1C received losartan 10 mg/kg.

Effect of nobiletin or losartan on vascular morphology changes in 2K-1C hypertensive rats



According to the H&E staining in the aortic sections, the aortic wall thickness, cross sectional area, and wall to lumen ratios of 2K-1C untreated group significantly rises, while the lumen diameter was not different, when compared to sham-operated group ($p<0.05$). However, oral administration of nobiletin at dose 30 mg/kg or losartan for 4 weeks considerably suppressed the aortic morphology alterations more than nobiletin at dose 15 mg/kg respectively ($p<0.05$; Figure 4 A-E).

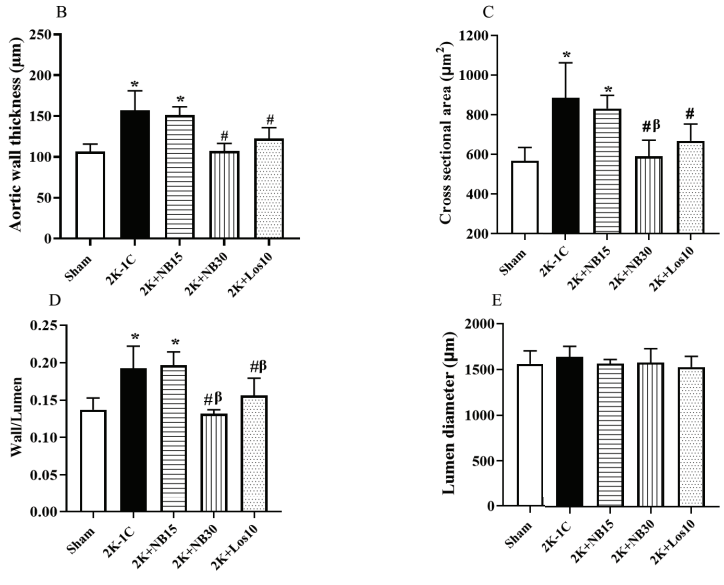


Figure 4 Effect of nobiletin or losartan on the vascular morphology changes in 2K-1C hypertensive rats. Representative figures of aortic sections stained with H&E (200X magnification, scale bar = 100 μm, A). Semi-quantitative analyses of vascular morphology are represented in aortic wall thickness (B), lumen diameter (C), ratios of the wall to lumen (D), and cross-sectional area (E). Data are expressed as mean ± S.E.M. (n =6/ group). *: $p<0.05$ vs Sham, $^{\#}$: $p<0.05$ vs 2K-1C, $^{\beta}$: $p<0.05$ vs 2K+NB15. 2K-1C is 2K-1C received vehicle, 2K+NB15 is 2K-1C received nobiletin 15mg/kg, 2K+NB30 is 2K-1C received nobiletin 30mg/kg, and 2K+Los10 is 2K-1C received losartan 10 mg/kg.

Discussion

Our findings demonstrated that partial restriction of left renal arterial blood flow induced by the 2K-1C operation impairs endothelium-dependent vasorelaxation, as evidenced by reduced responses to ACh in both resistance and conduit arteries. Notably, the vascular responses to SNP remained unaffected, indicating that the impairment is specific to endothelial function. Additionally, increased vascular contractile responses to EFS in 2K-1C rats suggest heightened perivascular sympathetic nerve activity under hypertensive conditions. From a morphological perspective, seven weeks of 2K-1C induction resulted in vascular remodeling, as shown by increased aortic wall thickness, cross-sectional area, and wall-to-lumen ratio. This structural alteration

was accompanied by reduced NO bioavailability, evidenced by lower plasma nitrite levels in untreated hypertensive rats. Importantly, treatment with a higher dose of nobiletin or losartan for four weeks significantly improved endothelial-dependent vasorelaxation, suppressed sympathetic overactivity, attenuated aortic remodeling, and restored NO bioavailability in 2K-1C hypertensive rats.

In the 2K-1C animal model, numerous studies have documented progressive deterioration in vascular function and structure. The narrowing of the renal artery leads to reduced renal perfusion pressure, which is sensed by baroreceptors in the afferent arteriole. Concurrently, decreased sodium delivery to the distal tubule is detected by macula densa (MD) cells. These stimuli collectively activate the RAS, a key driver of blood pressure elevation in this model. Ang II, a key effector of the RAS, exerts its actions primarily through binding to AT1R, resulting in enhanced sodium and water reabsorption, increased sympathetic tone, and systemic vasoconstriction — all of which contribute to sustained hypertension. Overactivation of RAS components such as ACE, Ang II, and AT1R has been associated with elevated blood pressure and impaired cardiovascular function.[24,25] In line with our previous findings, 2K-1C rats exhibited systemic ACE overactivity, increased Ang II levels, and upregulated AT1R expression in the cardiac tissue, which were attenuated by nobiletin administration.[21] Relatively, the present study showed that an impairment of vascular endothelial-dependent relaxation response in 2K-1C rats was alleviated by nobiletin treatment.

Besides functional impairment, structural alterations of the aorta were also observed in renovascular hypertensive rats. Previous studies have demonstrated that Ang II, acting via AT1R, stimulates NADPH oxidase (Nox) activity, leading to increased production of superoxide anions ($O_2^{\cdot -}$). Supportively, Guo reported that 2K-1C rats exhibited elevated blood pressure and cardiac remodeling, attributed to overactivation of the RAS and enhanced ROS production.[26] Similarly, Maneesai et al. found that seven weeks of 2K-1C induction led to vascular dysfunction and cardiac hypertrophy, associated with upregulation of RAS activity, cardiac $O_2^{\cdot -}$ production, and increased TGF-β1 expression.[27] Elevated superoxide levels in vascular endothelial cells react with NO, resulting in peroxynitrite ($ONOO^-$) formation, which decreases NO bioavailability a key contributor to both vascular functional and structural deterioration.[28, 29] Wunpathe and coworkers further demonstrated that overexpression of Nox subunits (p47 phox and gp91 phox) in aortic tissue was associated with increased vascular $O_2^{\cdot -}$ production, elevated circulating malondialdehyde (MDA) and NE levels, and reduced NO bioavailability in 2K-1C rats.[30] However, our current findings show that oral administration of nobiletin at 30 mg/kg/day for four weeks significantly improved vascular endothelial function, attenuated aortic structural remodeling, and suppressed sympathetic nerve overactivity in 2K-1C rats. These effects were accompanied by enhanced NO bioavailability. The vascular protective effects of nobiletin are likely mediated through its antioxidant and anti-inflammatory properties, consistent with our earlier findings showing that nobiletin restored oxidative/antioxidative imbalance and downregulated inflammatory cytokine expression in cardiac tissue of 2K-1C rats.[21]

Losartan, an ARB, was used as positive control in this study. It demonstrated vascular protective effects in both conduit and resistance arteries, improving both vascular function and structure. In addition, treatment with losartan significantly enhanced NO bioavailability in 2K-1C hypertensive rats. These beneficial effects are consistent with its well-established role in inhibiting the RAS, effectively reducing Ang II-induced blood pressure elevation, oxidative stress, inflammatory responses, and associated cardiovascular abnormalities in animal models.[21,31,32]

Conclusion

In conclusion, nobiletin attenuated vascular remodeling and endothelial dysfunction and reduced sympathetic nerve overactivity in 2K-1C renovascular hypertensive rats. These vascular protective effects were associated with improved nitric oxide (NO) bioavailability, suggesting a potential therapeutic role for nobiletin in managing hypertension-related vascular complications

Acknowledgments

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References

1. Mennuni S, Rubattu S, Pierelli G, Tocci G, Fofi C, Volpe M. Hypertension and kidneys: unraveling complex molecular mechanisms underlying hypertensive renal damage. J Hum Hypertens 2014;28(2):74-9. doi:10.1038/jhh.2013.55.
2. Textor SC. Renal arterial disease and hypertension. Medical Clinics 2017;101(1):65-79. doi:10.1016/j.mcna.2016.08.010.
3. Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies on experimental hypertension: I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J Exp Med 1934;59(3):347-79. doi:10.1084/jem.59.3.347.
4. Fahning BM, Potje SR, Paula TD, Grando MD, Bendhack LM. Ang II-induced contraction is impaired in the aortas of renovascular hypertensive animal model. Vasc Biol 2024;6(1):e230021. doi:10.1530/VB-23-0021.
5. Morishita R, Higaki J, Miyazaki M, Ogihara T. Possible role of the vascular renin-angiotensin system in hypertension and vascular hypertrophy. Hypertension 1992;19(2 Suppl):II62-7. doi:10.1161/01.HYP.19.2_Suppl.II62.
6. Bivol L, Berge R, Iversen B. Differential effect of tetradecythioacetic acid on the renin-angiotensin system and blood pressure in SHR and 2-kidney, 1-clip hypertension. Am J Physiol Renal Physiol 2007;293:F839-45. doi:10.1152/ajprenal.00140.2007.
7. Boonla O, Kukongviriyapan U, Pakdeechote P, Kukongviriyapan V, Pannangpetch P, Thawornchinsombut S. Peptides-derived from Thai rice bran improves endothelial function in 2K-1C renovascular hypertensive rats. Nutrients 2015;7(7):5783-99. doi:10.3390/nu7075252.
8. Martins-Oliveira A, Castro MM, Oliveira DM, Rizzi E, Ceron CS, Guimaraes D, et al. Contrasting effects of aliskiren versus losartan on hypertensive vascular remodeling. Int J Cardiol 2013;167(4):1199-205. doi:10.1016/j.ijcard.2012.03.137.
9. Touyz RM, Briones AM. "Reactive oxygen species and vascular biology: implications in human hypertension. Hypertension research 2011;34(1):5-14. doi:10.1038/hr.2010.201.
10. Griendling KK, FitzGerald GA. "Oxidative stress and cardiovascular injury: part II: animal and human studies. Circulation 2003;108(17):2034-40. doi:10.1161/01.CIR.0000093661.90582.c4.
11. Castro MM, Rizzi E, Ceron CS, Guimaraes DA, Rodrigues GJ, Bendhack LM, et al. Doxycycline ameliorates 2K-1C hypertension-induced vascular dysfunction in rats by attenuating oxidative stress and improving nitric oxide bioavailability. Nitric Oxide 2012;26(3):162-8. doi:10.1016/j.niox.2012.01.009.
12. Israili Z. Clinical pharmacokinetics of angiotensin II (AT1) receptor blockers in hypertension. J Hum Hypertens 2000;14(1):S73-S86. doi:10.1038/sj.jhh.1000991.
13. Oparil S, Williams D, Chrysant SG, Marbury TC, Neutel J. Comparative efficacy of olmesartan, losartan, valsartan, and irbesartan in the control of essential hypertension. J Clin Hypertens 2001;3(5):283-318. doi:10.1111/j.1524-6175.2001.01136.x.
14. Munger MA. Use of Angiotensin receptor blockers in cardiovascular protection: current evidence and future directions. Pharm Ther 2011;36(1):22-40.

15. Zaiken K, Hudd TR, Cheng JW. A review of the use of angiotensin receptor blockers for the prevention of cardiovascular events in patients with essential hypertension without compelling indications. Ann Pharmacother 2013;47(5):686-93. doi:10.1345/aph.1R273.
16. Huang J, Chang Z, Lu Q, Chen X, Najafi M. Nobiletin as an inducer of programmed cell death in cancer: a review. Apoptosis 2022;27(5):297-310. doi:10.1007/s10495-022-01721-4.
17. Zhang N, Yang Z, Xiang S-Z, Jin Y-G, Wei W-Y, Bian Z-Y, et al. Nobiletin attenuates cardiac dysfunction, oxidative stress, and inflammatory in streptozotocin: induced diabetic cardiomyopathy. Mol Cell Biochem 2016;417(1-2):87-96. doi:10.1007/s11010-016-2716-z.
18. Potue P, Wunpathe C, Maneesai P, Kukongviriyapan U, Prachaney P, Pakdeechote P. Nobiletin alleviates vascular alterations through modulation of Nrf-2/HO-1 and MMP pathways in l-NAME induced hypertensive rats. Food Funt 2019;10(4):1880-92. doi:10.1039/C8FO02408A.
19. Bunbupha S, Apaijit K, Maneesai P, Prasarttong P, Pakdeechote P. Nobiletin ameliorates high-fat diet-induced vascular and renal changes by reducing inflammation with modulating AdipoR1 and TGF-β1 expression in rats. Life Sci 2020;260:118398. doi:10.1016/j.lfs.2020.118398.
20. Bunbupha S, Pakdeechote P, Maneesai P, Prasarttong P. Nobiletin alleviates high-fat diet-induced nonalcoholic fatty liver disease by modulating AdipoR1 and gp91phox expression in rats. J Nutr Biochem 2021;87:108526. doi:10.1016/j.jnutbio.2020.108526.
21. Iampanichakul M, Poasakate A, Potue P, Rattanakanokchai S, Maneesai P, Prachaney P, et al. Nobiletin resolves left ventricular and renal changes in 2K-1C hypertensive rats. Sci Rep 2022;12(1):9289. doi:10.1038/s41598-022-13513-6.
22. Babu CS, Kalaivani P, Ranju V, Sathiya S, Anbarasi C, Mahadevan MV, et al. A polyherbal Siddha medicine, alleviates hypertension via AT1R and eNOS signaling pathway in 2K1C hypertensive rats. Exp Biol Med 2014;239(6):758-69. doi:10.1177/1535370214525317.
23. Bunbupha S, Apaijit K, Potue P, Maneesai P, Pakdeechote P. Hesperidin inhibits L-NAME-induced vascular and renal alterations in rats by suppressing the renin-angiotensin system, transforming growth factor-β1, and oxidative stress. Clin Exp Pharmacol Physiol 2021;48(3):412-21. doi:10.1111/1440-1681.13438.
24. Valles P, Wysocki J, Batlle D. Angiotensin II and renal tubular ion transport. Sci World J 2005;5:680-90. doi:10.1100/tsw.2005.92.
25. Yang R, Smolders I, Dupont AG. Blood pressure and renal hemodynamic effects of angiotensin fragments. Hypertens Res 2011;34(6):674-83. doi:10.1038/hr.2011.24.
26. Guo K, Lan CZ, Yu TT, Huang LL, Wang XH, Pan C, et al. Effects of Xin-Ji-Er-Kang formula on 2K1C-induced hypertension and cardiovascular remodeling in rats. J Ethnopharmacol 2014;155(2):1227-35. doi:10.1016/j.jep.2014.07.006.
27. Maneesai P, Chaihongsa N, Iampanichakul M, Meephat S, Prasatthong P, Bunbupha S, et al. Clitoria ternatea (Linn.) flower extract attenuates vascular dysfunction and cardiac hypertrophy via modulation of Ang II/AT1R/TGF-β1 cascade in hypertensive rats. J Sci Food Agric 2022;102(6):2253-61. doi:10.1002/jsfa.11563.
28. Cavanagh E, Ferder M, Inserra F, Ferder L. Angiotensin II, mitochondria, cytoskeletal, and extracellular matrix connections: an integrating viewpoint. Am J Physiol Heart Circ Physiol 2009;296(3):H550-8. doi:10.1152/ajpheart.01176.2008.
29. Mitra AK, Gao L, Zucker IH. Angiotensin II-induced upregulation of AT(1) receptor expression: sequential activation of NF-kappaB and Elk-1 in neurons. Am J Physiol Cell Physiol 2010;299(3):C561-9. doi:10.1152/ajpcell.00127.2010.

30. Wunpathe C, Potue P, Maneesai P, Bunbupha S, Prachaney P, Kukongviriyapan U, et al. Hesperidin suppresses renin-angiotensin system mediated NOX2 over-expression and sympathoexcitation in 2K-1C hypertensive rats. Am J Chin Med 2018;46(4):751-67. doi:10.1142/S0192415X18500398.
31. Khattab M, Ahmad M, Al-Shabanah OA, Raza M. Effects of losartan on blood pressure, oxidative stress, and nitrate/nitrite levels in the nitric oxide deficient hypertensive rats. Recep Channels 2004;10(5-6):147–57. doi:10.3109/10606820490936141.
32. Vajic UJ, Mihailovic-Stanojevic N, Karanovic D, Zivotic M, Ivanov M, Jovovic D, et al. Urtica dioica L. leaf extract dose-dependently modulates oxidative stress in the kidney and exerts anti-fibrotic and anti-inflammatory properties by the molecular mechanisms independent of NRF-2 signalization mirroring the effects of losartan in SHR. Int J Mol Sci 2024;25(24):13272. doi:10.3390/ijms252413272.

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