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Endothelium-Dependent Vasodilation by Ferulic Acid in Aorta from Chronic Renal Hypertensive Rats

朝鮮大學校 大學院
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신성고혈압쥐에서 Ferulic Acid에 의한 내피의존 혈관 이완반응

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신성고혈압쥐에서 Ferulic Acid에 의한 내피의존 혈관 이완반응

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Ferulic Acid (FA)는 혈압 하강효과가 있음이 보고되어 있으나 혈관의 기능에 대한 작용은 명확히 알려져 있지 않다. FA가 two-kidney, one clip (2K1C) 신성고혈압 모델에서 혈관 반응에 미치는 영향을 검토하고 정상혈압동물과 차이가 있는지 구명하고자 본 연구를 시행하였다.

흰쥐 일측 신동맥에 clip을 장치한 고혈압군과 대조군의 적출 흉부 대동맥 표본에서 FA의 이완반응을 확인하고 N^G-nitro-L-arginine methyl ester (L-NAME)에 의한 수축반응 및 acetylcholine에 의한 이완반응에 미치는 FA의 영향을 양군에서 비교 검토하였다.

FA에 의한 이완반응은 2K1C 고혈압군에서 대조군에 비해 항진되었다. L-NAME에 의한 수축반응은 FA에 의해 2K1C 고혈압군에서 증가되었으나 대조군은 차이가 없었다. FA는 2K1C 고혈압군에서 acetylcholine의 이완반응을 증가시켰으며 FA에 의해 항진된 이완효과는 hydroxyhydroquinone에 의해 소실되었다.

이상의 실험결과는 FA가 신성고혈압 상태에서 산화질소 (nitric oxide) 활성 변화를 통한 혈관내피층의 기능을 개선시켜 혈관 이완효과를 나타냄을 시사한다.

I . INTRODUCTION

Ferulic acid (FA; 4-hydroxy-3-methoxycinnamic acid) is an ubiquitous phenolic compound of plant tissues and thus constitutes a bioactive ingredient of many foods. FA is rich in rice bran, whole grain food, citrus fruits, banana, beet root, cabbage, spinach and broccoli[1]. Various studies have indicated that FA has antioxidant effects, antitumor activity, antihyperlipidemic and radioprotective properties[2-4]. Observational epidemiologic studies have shown that dietary consumption of fruits and vegetables is associated with a lower incidence of and mortality from cardiovascular disease[5]. It has been demonstrated that FA has an antihypertensive action when administered intravenously in spontaneously hypertensive rats and the blood pressure lowering effect of FA is blocked by pretreatment with a nitric oxide synthase (NOS) inhibitor[6], suggesting that NO-dependent vascular response is involved in this effect. The evidence has been accumulated by the results that FA restores endothelial function through enhancing the bioavailavility of basal and stimulated NO in hypertensive rat aortas[7].

The endothelium plays a pivotal role in the maintenance of vascular tone and blood pressure by regulating the release of several vasoactive susbstances, including NO[8]. Its functional changes in pathological conditions are characterized by impaired

endothelium-dependent relaxation. A deficient production of endothelium-derived NO results in diminished vasodilator tone, allowing vascular resistance to increase, and this contributes to the elevated blood pressure[9-11]. Therefore, an altered role of NO may be critical in the pathogenesis of hypertension. Indeed, the endothelium-dependent vasodilation is impaired in a number of experimental models, including two-kidney, one clip (2K1C) renovascular, aortic coarctation, Dahl salt-sensitive, deoxycorticosterone acetate-salt and spontaneous hypertension[10-13]. We have also been observed that the endothelium plays an inhibitory role against contractions in rat aorta by releasing NO, and the role of endothelium is altered in 2K1C hypertension[14]. Although an improved endothelium-dependent vasodilation by FA has been demonstrated in genetically hypertensive rat aortas[7], the effects of FA on vascular function in 2K1C hypertension, remains unclear.

The present study was designed to examine the effects of FA on the involvement of NO and endothelium-dependent and -independent vasoreactivity in chronic 2K1C renal hypertensive rats. The thoracic aorta was isolated and the changes in isometric tension were recorded.

Ⅱ. METHODS

1. Development of 2K1C hypertension

Male Sprague-Dawley rats, weighing 160 to 180 g, were anesthetized with intraperitoneal injections of thiopental sodium (40 mg/kg). An incision was made on the left flank to provide access to the left renal artery which was separated from the renal vein and cleaned of the connective tissue. A U-shaped solid silver clip with an opening of 0.2 mm was applied on the exposed renal artery, resulting in partial occlusion of renal perfusion. The contralateral kidney remained untouched and the wound was closed. A group of age-matched rats received a sham treatment: they were operated as in 2K1C rats, except for that no clip was made. All animals were fed normal chow and were given tap water. They were used at 10 weeks after the clipping, since the endothelial dysfunction is associated with a duration of hypertension[15].

Hypertensive rats were selected on the basis of the systolic blood pressure measured in a conscious state by use of tail cuff method.

2. Tissue preparation

The thoracic aorta between the aortic arch and diaphragm was rapidly excised, cleared of adherent connective tissue and cut into rings 2 to 3 mm in length under a dissection microscope. In some preparations, the endothelium was removed by gentle rubbing of the intimal surface with a moistened cotton swab. Successful removal of endothelial cells from aortic rings was confirmed by the inability of acetylcholine to induce relaxation.

The rings were mounted by means of two triangle shaped stainless steel holders in the vessel lumen in organ baths containing 15 mL of physiological salt solution (PSS) of the following composition (in mM): NaCl 118.3, KCl 4.7, $NaHCO_3$ 25, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5 and glucose 11.1 at 37℃ and bubbled with a mixture of 95 % O_2 and 5 % CO_2. One of the holders was fixed at the bottom of the chambers and the other was connected to a force displacement transducer (Grass FTO3) for measurement of isometric tension (Fig. 1). Before initiating specific experimental protocols, the aortic rings were equilibrated under a resting tension of 2 g for at least 90 min. During this period, the incubation medium was changed at 15 min intervals.

3. Experimental Protocols

In the first set of experiments, to confirm the vasorelaxant

ISOLATED TISSUE BATH

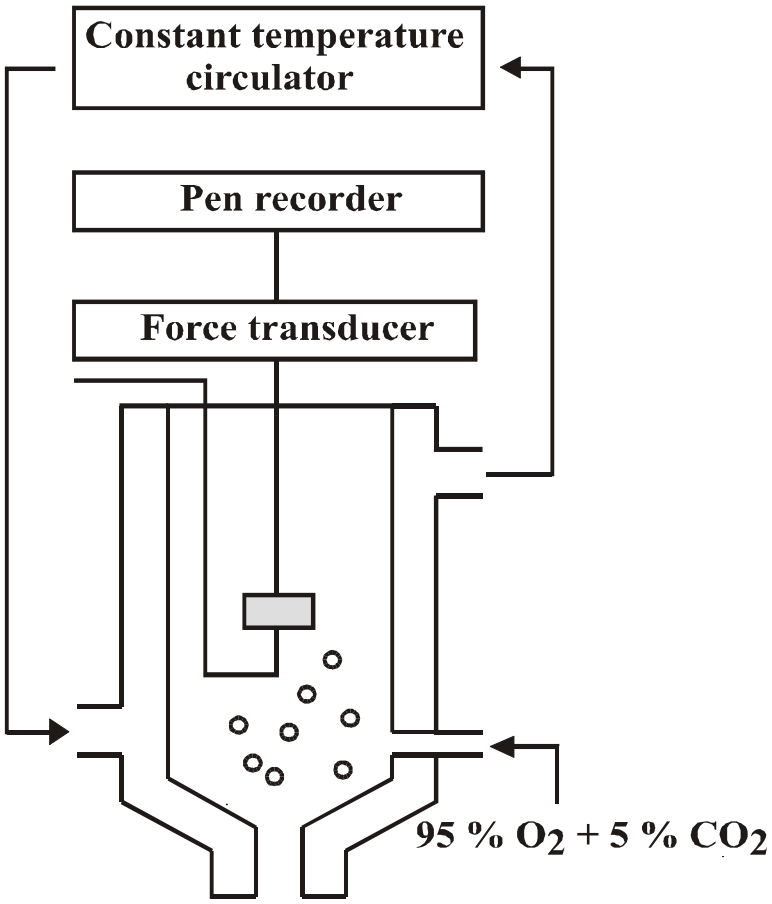

Fig. 1. A schematic representation of the recording system for isometric contraction with 15 mL tissue bath.

activity of FA, the aortic rings from 2K1C and sham rats were precontracted to 50 % effective concentration (EC_{50}) with phenylephrine (3×10^{-7} M in sham and 4×10^{-8} M in 2K1C), which were obtained in preliminary experiments. When the contractile response achieved a steady state, concentration-response curves to the cumulative addition of FA (10^{-5} to 10^{-3} M) were determined in aortic rings with or without a functional endothelium. To verify the participation of endothelium-derived products in the relaxant effects of FA, experiments were performed in the presence of N^{G}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), a nonselective NOS inhibitor; indomethacin (10^{-5} M), a nonselective cyclooxygenase inhibitor; thapsigargin (10^{-5} M), a sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitor, which were added to the bath 20 min before the addition of phenylephrine.

In the second set of experiments, after an incubation period of 45 min in 25 mM KCl (obtained by equimolar replacement of NaCl by KCl in the PSS), L-NAME (10^{-4} M) was added in aortic rings with intact endothelium from 2K1C and sham rats. The contraction in rings from rats that had been pretreated with FA (10^{-5} to 10^{-3} M) for 30 min was measured. The resulting contraction as a percent change of the 25 mM KCl, was taken as a measure of the basal bioavailability of NO[7,16].

In the third set of experiments, relaxation responses to acetylcholine (10^{-9} to 10^{-5} M) were performed in the presence or absence of L-NAME (10^{-4} M) in aortic rings with endothelium

precontracted with phenylephrine from 2K1C and sham rats. Acetylcholine-induced relaxations were also examined pretreated for 30 min with FA (10^{-4} M) before the addition of phenylephrine, the resulting relaxation was taken as a measure of the stimulating bioavailability of NO[7]. In addition, an aortic ring preparation from which the functional endothelium had been mechanically removed was pretreated for 30 min with FA (10^{-4} M) before the addition of phenylephrine. Endothelium-independent vasodilation was induced by treatment with sodium nitroprusside (SNP, 10^{-10} to $10^{-6.5}$ M) and 3-morpholino-sydnonimine (SIN-1, 10^{-10} to $10^{-6.5}$ M) in aortic rings from 2K1C rats. The SNP has been used as a NO donor and SIN-1 is known to release both NO and superoxide anions (O_2^-)[17]. Superoxide dismutase (SOD, 150 U/mL) was added 5 min before the addition of phenylephrine in the case of treatment with SIN-1.

In the fourth set of experiments, aortic rings with endothelium from 2K1C rats were treated with FA (10^{-4} M) for 30 min before the addition of phenylephrine. Hydroxyhydroquinone(HHQ, 10^{-7} M), a generator of O_2^-[18], was added, followed by contraction by phenylephrine after 5 min, and the extent of acetylcholine (4×10^{-8} M)-induced vasodilation was measured. SOD (150 U/mL) or catalase (1000 U/mL) was added 5 min before the addition of HHQ.

4. Drugs

Drugs used were FA, L-NAME, phenylephrine, acetylcholine, indomethacin, thapsigargin, SNP, SIN-1, SOD, HHQ and catalase. HHQ was purchased from Wako Pure chemical Industries, Ltd. (Osaka, Japan) and the other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo). FA and indomethacin were dissolved in dimethylsulfoxide (DMSO) and the others were prepared in distilled water. Final bath concentrations of DMSO were less than 0.05 %, which did not alter contraction or relaxation responses.

5. Analysis and statistics

Values presented in the figures are expressed as the means and standard error of the means. Relaxant responses are given as the percent change in phenylephrine-induced contractile tension. Statistical comparisons were performed by Student's t-test or analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons. Probability values of <0.05 were considered statistically significant.

Ⅲ. RESULTS

Ten weeks after the operative intervention, the systolic blood pressure were 193±4 mmHg (n=43, $P<0.05$) and 138±5 mmHg (n=36) in 2K1C hypertensive and sham-clipped control rats, respectively (Fig. 2).

1. Vasorelaxant responses to ferulic acid

The tension induced by phenylephrine was enhanced in aortic rings from 2K1C rats (1.38±0.09 g, $P<0.05$) than in those from sham rats (1.05±0.08 g). In aortic rings from 2K1C rats, FA relaxed phenylephrine-induced contraction in concentration dependent manner. Relaxation was also induced in sham rats, but the degree was significantly
lower than that for the 2K1C aortic rings (Fig. 3). The relaxation induced by FA was markedly inhibited by removing the endothelium or by pretreatment of the aorta with L-NAME in 2K1C rats (Fig. 4). The treatment with indomethacin or thapsigargin did not affect FA-induced vasorelaxation (data not shown).

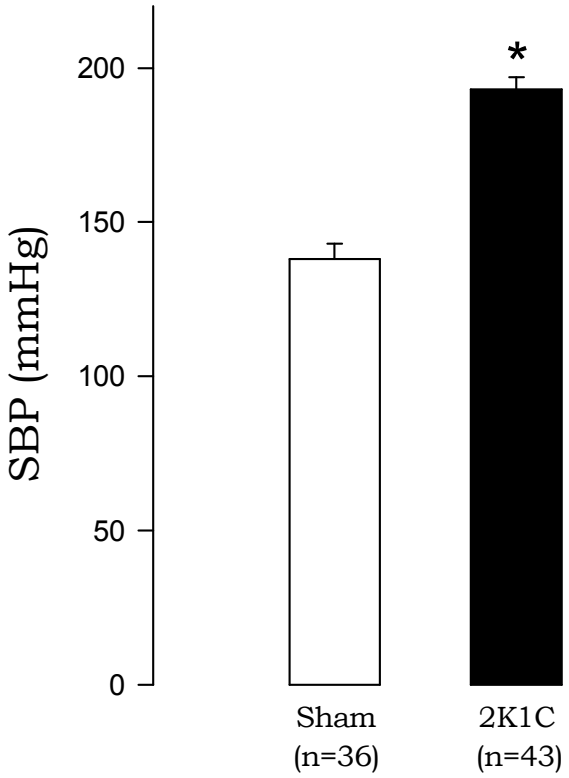

Fig. 2. Systolic blood pressure (SBP) in 2K1C hypertensive and sham-operated control rats. *P<0.05, compared with the sham value.

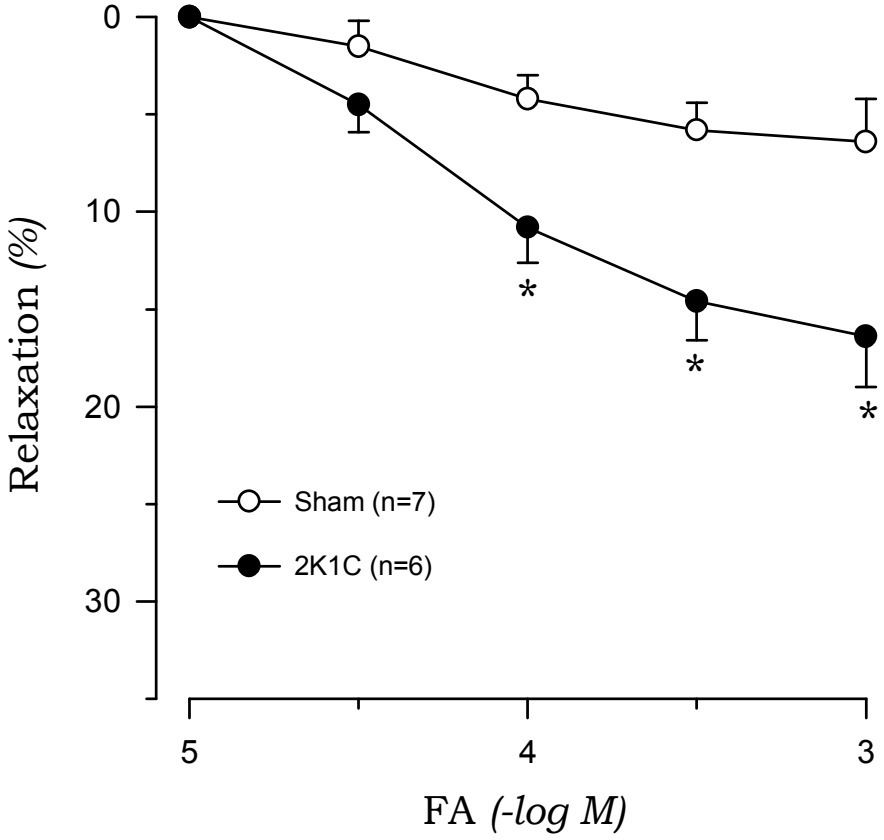

Fig. 3. Ferulic acid (FA)-induced vasorelaxation in phenylephrine-precontracted aortic rings from 2K1C hypertensive and sham-clipped control rats. Points represent means±SE for number(n) of experiments in parentheses. * P <0.05, compared with sham values.

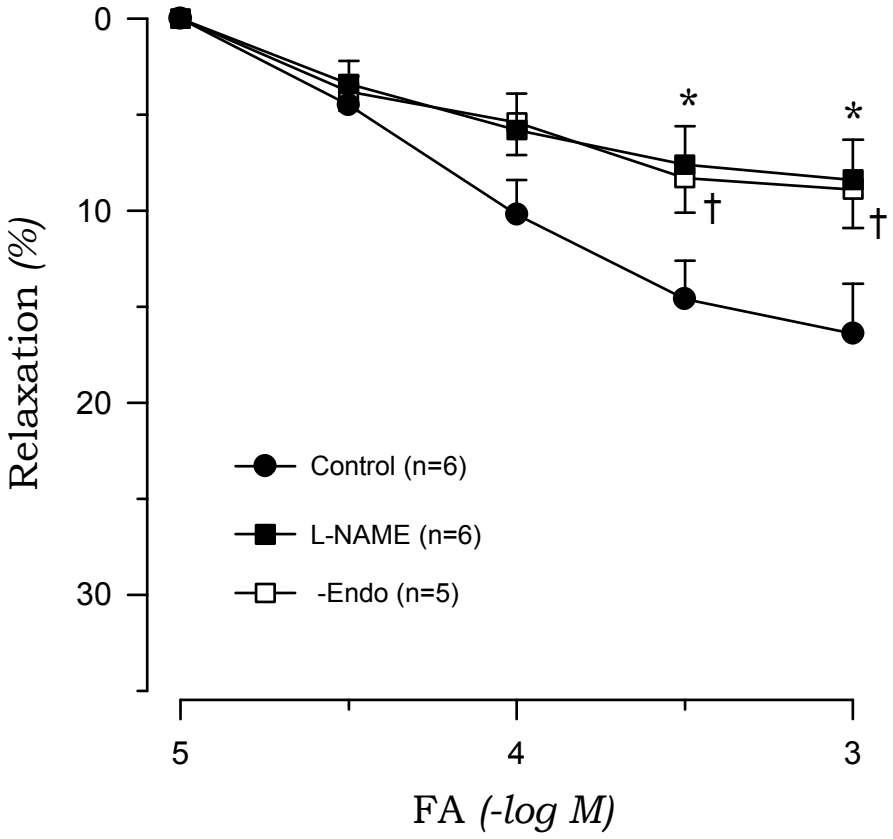

Fig. 4. Ferulic acid (FA)-induced vasorelaxation in phenylephrine-precontracted aortic rings without endothelium (-Endo) from 2K1C hypertensive rats. The results obtained by treatment with N^G-nitro-L-arginine methyl ester (L-NAME) are also shown. *, † P <0.05, compared with control values, respectively.

2. Effect of ferulic acid on L-NAME-induced contraction

The magnitude of 25 mM KCl-induced contraction was comparable in between aortic rings from 2K1C (342±47 mg, n=19) and sham rats (327±33 mg, n=22). When L-NAME was added to the rings at 25 mM KCl, the resulting contraction was greater in sham rats (375±22 %, $P<0.05$, n=6) compared to 2K1C rats (185±19 %, n=5). In addition, the contraction in rings from 2K1C rats that had been treated with FA (10^{-4} and 10^{-3} M) was significantly greater than the values for vehicle-treated aorta, while no significant differences were shown in sham rats (Fig. 5, Fig. 6).

3. Acetylcholine-induced or nitric oxide donor-induced vasodilation

Acetylcholine-induced vasodilation was significantly attenuated in aortic rings from 2K1C rats compared to those from sham rats. Treatment with L-NAME (10^{-4} M) completely inhibited acetylcholine-induced vasodilatory effect in both 2K1C and sham groups (data not shown). FA (10^{-4} M) had no effect on acetylcholine-induced vasodilation in sham rats, while it significantly potentiated the acetylcholine-induced vascular response in aortic rings from 2K1C rats (Fig. 7). In aortic rings without endothelium from 2K1C rats that had been precontracted

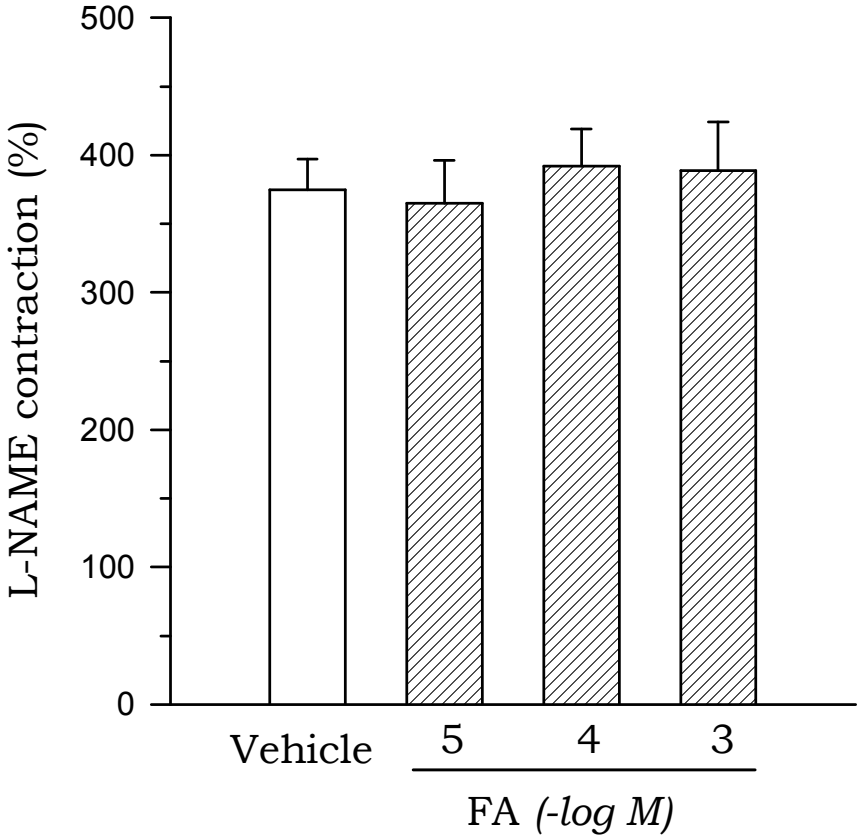

Fig. 5. Effects of ferulic acid (FA) on the N^G-nitro-L-arginine methyl ester (L-NAME)-induced contraction in aortic rings from sham-clipped control rats. The contractile responses to L-NAME were obtained in rings submaximally precontracted with KCl (25 mM). Data are attained from 5 to 8 experiments.

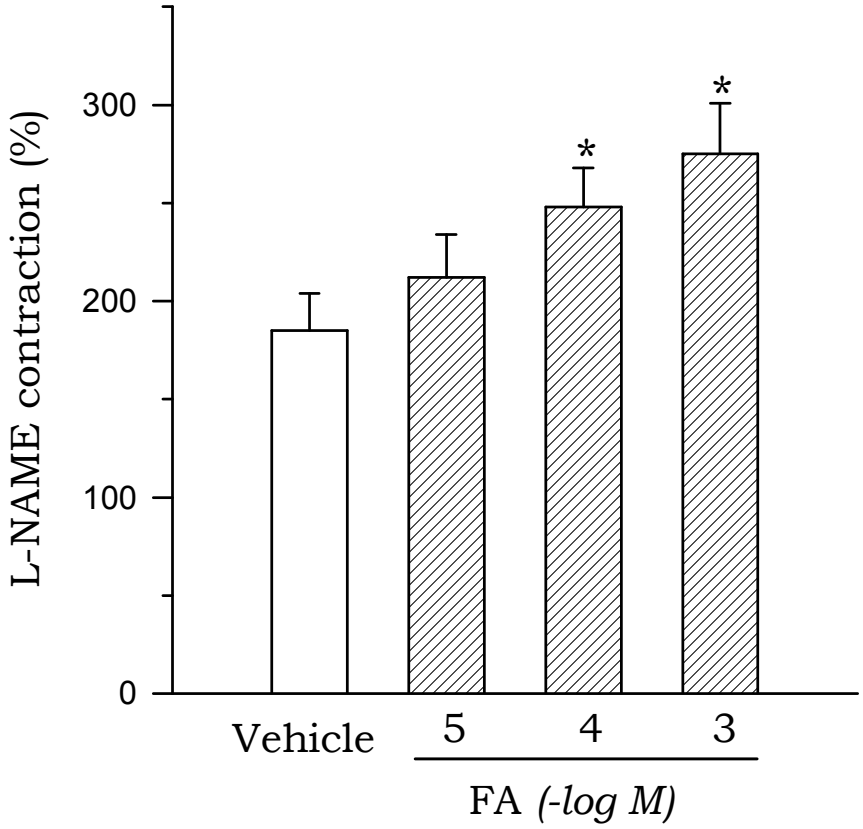

Fig. 6. Effects of ferulic acid (FA) on the N^G-nitro-L-arginine methyl ester (L-NAME)-induced contraction in aortic rings from 2K1C hypertensive rats. * P <0.05, compared with the vehicle value. Other legends as in Fig. 5.

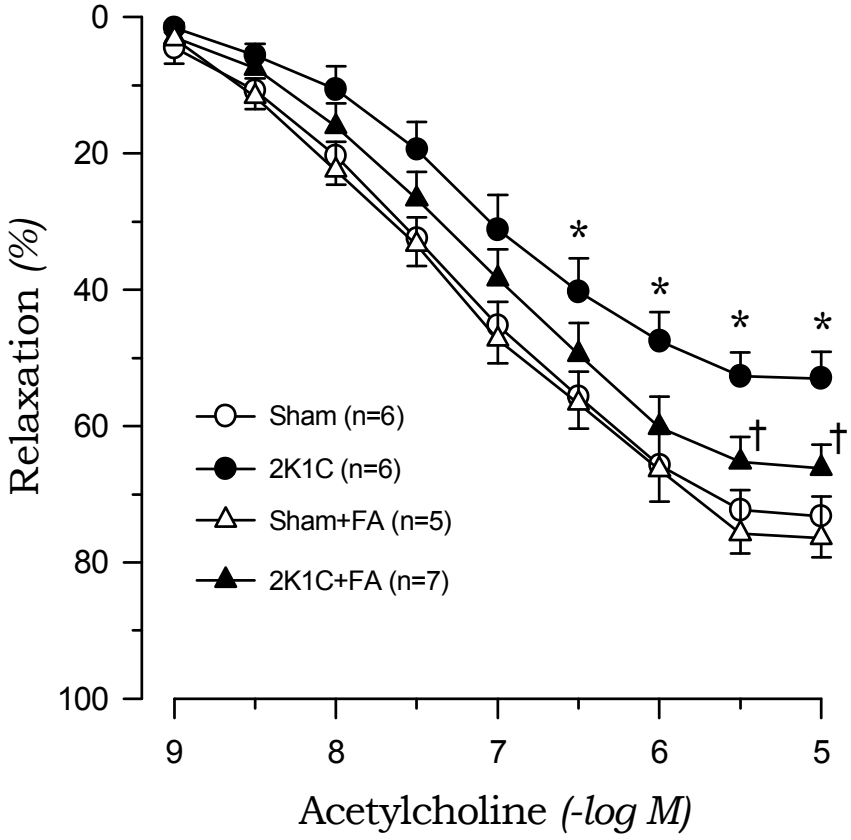

Fig. 7. Effects of ferulic acid (FA) on vasodilatory responses induced by acetylcholine in aortic rings with endothelium from 2K1C hypertensive and sham-clipped control rats. * P <0.05, compared with corresponding sham values. † <0.05, compared with corresponding 2K1C values.

with phenylephrine, SNP or SIN-1 produced a concentration-dependent vasodilation. FA had no effect on SNP-induced vasodilation. FA also did not affect SIN-1-induced vasodilation, and SOD enhanced the vasodilatory effect of SIN-1 (Fig. 8, Fig. 9).

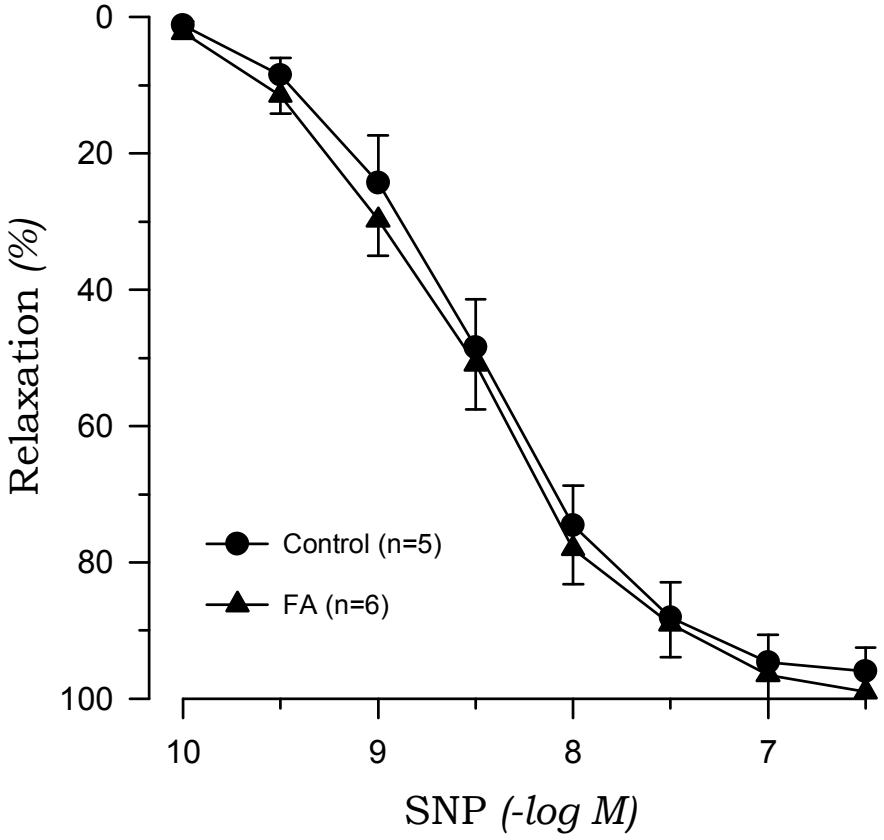

Fig. 8. Effects of ferulic acid (FA) on vasodilatory responses induced by sodium nitroprusside (SNP) in aortic rings without endothelium from 2K1C hypertensive rats.

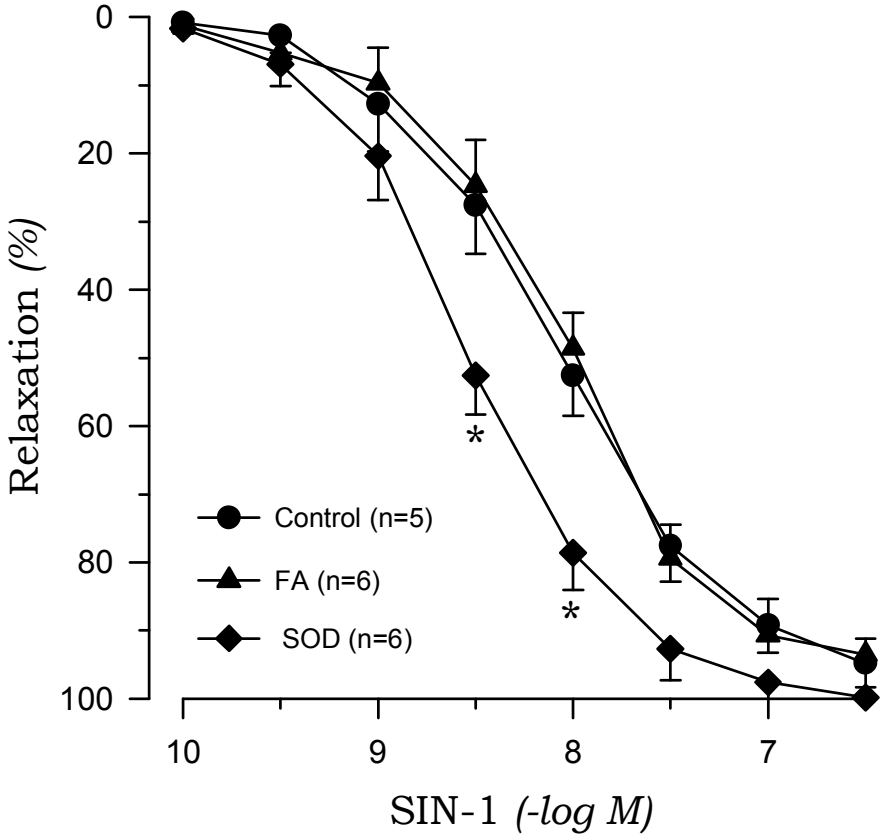

Fig. 9. Effects of ferulic acid (FA) on vasodilatory responses induced by 3-morpholino-sydnonimine (SIN-1) in aortic rings without endothelium from 2K1C hypertensive rats. The results obtained by pretreatment with superoxide dismutase (SOD). * P <0.05, compared with 2K1C values.

4. Effect of hydroxyhydroquinone on acetylcholine-induced vasodilation

HHQ (10^{-7} M) alone had no effect on phenylephrine-induced contraction in aortic rings with endothelium from 2K1C rats. FA augmented acetylcholine-induced vasodilation, and HHQ inhibited the FA-induced improvement in acetylcholine reactivity. The effect of FA on acetylcholine-induced vasodilation in the presence of HHQ was recovered by the addition of SOD, while catalase did not affect acetylcholine-induced vasorelaxation (Fig. 10).

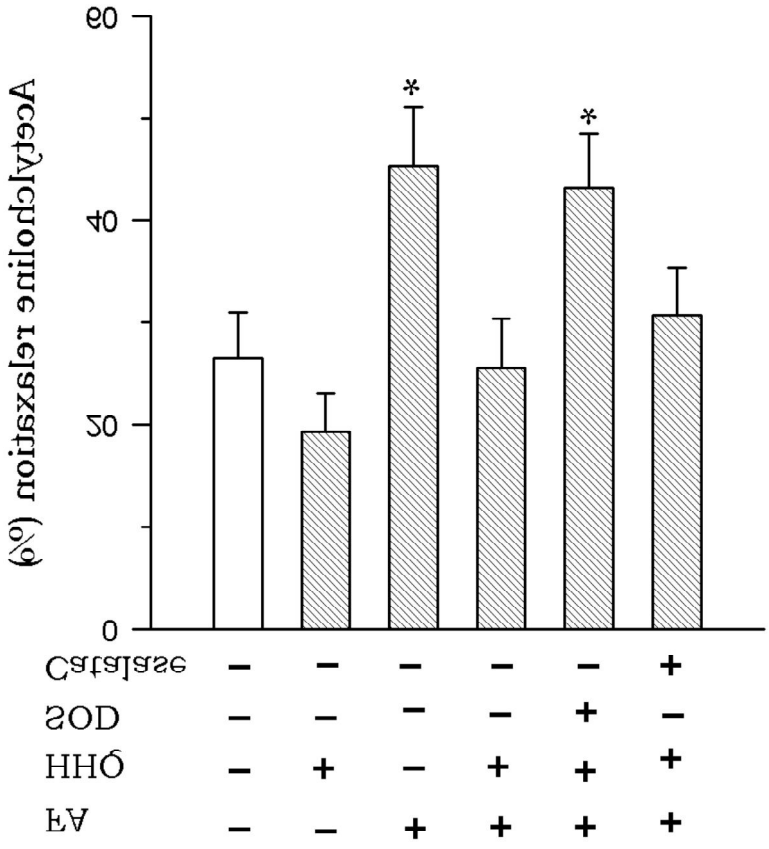

Fig. 10. Effects of hydroxyhydroquinone (HHQ) on vasodilatory responses induced by acetylcholine in aortic rings with endothelium from 2K1C hypertensive rats. The effects of HHQ on the ferulic acid (FA)-induced enhancement in the acetylcholine-stimulated vasodilatory response after pretreatment with superoxide dismutase (SOD) or catalase are also shown. Data are attained from 5 to 8 experiments. * P <0.05, compared with the vehicle-treated value.

Ⅳ. DISCUSSION

It has been shown that vascular reactivity to contractile agonist is enhanced in disease states such as hypertension[19]. Comparable to our previous findings[14], the contractile response to phenylephrine was augmented in 2K1C hypertensive rats as compared to sham-clipped control rats. In the present study, FA caused a relaxation in phenylephrine-precontracted aortic ring preparations isolated from 2K1C rats with an intact endothelium, while the relaxant effect of FA was negligible in sham rats. Similar results were also observed previously in spontaneously hypertensive rats.[7] The FA-induced endothelium-dependent relaxation was significantly inhibited by L-NAME, whereas the blocking of cyclooxygenase activity by indomethacin or SERCA activity by thapsigargin had no effects on the relaxation in aortic rings from 2K1C rats. The results suggest that FA evoked relaxation is NO-dependent.

In order to examine whether the basal bioavailability of NO is altered by FA in hypertension, L-NAME was added in aortic rings with intact endothelium, which were precontracted with 25 mM KCl[16,20]. L-NAME-induced contractions were attenuated in 2K1C rats as compared with sham rats. In agreement with these observations, it has been demonstrated that the basal release of NO is less pronounced in the resistance and conduit arteries of hypertensive when compared to normotensive

rats[16,21]. We have also confirmed previously that the contractile response to N^{ω}-nitro-L-arginine (L-NNA), another NOS inhibitor, is impaired in KCl-precontracted aortic rings from 2K1C rats as compared with those from sham rats[20]. Furthermore, the magnitude of L-NAME-induced contractions in rings from 2K1C rats that had been treated with FA was significantly enhanced than that for vehicle-treated aorta. The results demonstrate that FA increases basal NO bioavailability in the L-NAME-induced contractile response in 2K1C hypertensive rats, as has been shown previously in genetically hypertensive rats[7]. These findings suggest that the vasorelaxant effect of FA on phenylephrine-induced contractions is partially mediated by endothelial NO in aortic rings from 2K1C rats. On the other hand, the FA-induced relaxation persisted, even after the removal of the intact endothelium or treatment with L-NAME. It can not be ruled out the possibility that FA has a direct effect on vascular smooth muscle cells in addition to its effects on endothelial cells.

The present study confirmed earlier observations[13,14,22], in that endothelium-dependent relaxations to acetylcholine are markedly depressed in 2K1C hypertensive rats as compared with sham-clipped control rats. Treatment with L-NAME completely inhibited the acetylcholine-induced vasodilatory effect in both groups, suggesting that the acetylcholine-induced vasodilation is largely due to NOS-derived NO. FA potentiated acetylcholine-induced vasorelaxation in aortic rings from 2K1C

rats, while it did not affect the acetylcholine-induced vasodilation in sham rats. Acetylcholine causes NO release through activation of specific endothelial receptors, resulting in activation of endothelial NOS[23]. Therefore, the results imply that FA may stimulate the release of NO in this experimental condition in 2K1C hypertensive rats. In association with these observations, it has been suggested that FA enhances the stimulating bioavailability of NO in aortic rings from spontaneous hypertensive rats[7]. In addition, in the experiment using an NO donor in aortic rings without endothelium from 2K1C rats, FA had no effect on SNP-induced endothelium-independent vasodilation. These results suggest that FA at this case does not affect the NO-dependent pathway in vascular smooth muscle in 2K1C hypertensive rats.

Impaired endothelium-mediated vasodilation in hypertension has been linked to decreased NO bioavailability. This may be secondary to decreased NO synthesis or to increased NO degradation because of its interaction with O_2^-[24]. It has been demonstrated that NO can be scavenged by O_2^- to form peroxynitrite ($ONOO^-$)[24,25], effectively reducing endothelium-derived NO. Indeed, it has been observed that O_2^- generation is increased in hypertension[26,27]. In the present study, the report that FA scavenges O_2^- derived from xanthine and xanthine oxidase[28] led us to hypothesize that the scavenging ability of FA might explain the improvement in NO bioavailability in aortic rings from 2K1C rats. To investigate

this issue, the effects of FA on the SIN-1-induced vasodilation were examined, since SIN-1 is known to release both NO and O_2^-[17]. Contrary to our expectations, FA failed to augment SIN-1-induced endothelium-independent vasodilation, whereas SOD potentiated the SIN-1-induced vasorelaxation. Based on this findings, it appears unlikely that FA scavenges O_2^- derived from SIN-1 in this experimental condition.

The major finding in this study is that the L-NAME-induced vasoconstrictions and the acetylcholine-induced endothelium-dependent vasorelaxations are augmented by FA in aortic rings from 2K1C hypertensive rats, while FA has no effect in sham-clipped control rats. These results suggest that FA may alter the bioavailability of NO in the vascular endothelium in 2K1C rats. The issue of how NO bioavailability is stimulated by FA in endothelial cells is unclear based on the findings in this study. The vascular effects induced by FA in 2K1C hypertensive rats should not be assumed to reflect the increase in NO production in the endothelium, since the NO system is overactive in hypertension[10]. Several studies have reported that excessive O_2^- reacts with NO, which decreases the bioavailability of NO, thereby impairing endothelial function in spontaneous and experimental hypertension[26,27,29]. Therefore, one possible explanation may be attributed to the regulation of O_2^- by FA in endothelial cells in aortic rings from 2K1C rats, although FA did not affect the SIN-1-induced endothelium-independent vasodilation. We found in the present

study that HHQ, a generator of O_2^-[18], inhibited the FA-induced improvement in endothelium-dependent vasodilation by acetylcholine in an O_2^--dependent manner in aortic rings with endothelium from 2K1C rats, as has been shown previously in genetically hypertensive rats[7]. The results imply that HHQ-derived O_2^- most likely interferes with the FA-induced increase in available NO in endothelial cells in 2K1C hypertensive rats.

In summary, FA restored endothelial function through an alteration of NO bioavailability in 2K1C hypertensive rats. The results explain, in part, the mechanism underlying the vascular effects of FA in 2K1C chronic renal hypertension.

V. SUMMARY

Ferulic acid (FA), a naturally occurring nutritional compound, has an antihypertensive effects, but a detailed understanding of its effects on vascular function has not been intensively established. Aim of the present study was to assess the vasoreactivity of FA in chronic two-kidney, one clip (2K1C) renal hypertensive rats. 2K1C hypertension was made by clipping the left renal artery and age-matched rats received a sham treatment served as control. Thoracic aortas were mounted in tissue baths for measurement of isometric tension. The effects of FA on vasodilatory responses were evaluated based on contractile responses induced by phenylephrine in aortic rings from 2K1C and sham rats. Basal nitric oxide (NO) bioavailability in the aorta was determined from the contractile response induced by NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). The impact of hydroxyhydroquinone (HHQ), a generator of superoxide anions (O_2^-), on the FA-induced enhancement in acetylcholine-stimulated vasodilation was also investigated. FA caused a concentration-dependent relaxation of which the responses were greater in 2K1C hypertensive rats than in sham-clipped control rats. The relaxation induced by FA was partially blocked by removal of endothelium or by pretreating with L-NAME. L-NAME-induced contractile responses were augmented by FA in 2K1C rats,

while no significant differences were noted in sham rats. FA improved acetylcholine-induced endothelium-dependent vasodilation in 2K1C rats, but not in sham rats. The simultaneous addition of HHQ significantly inhibited the increase in acetylcholine-induced vasodilation by FA. These results suggest that FA restored endothelial function through an alteration of NO bioavailability in 2K1C hypertensive rats. The results explain, in part, the mechanism underlying the vascular effects of FA in chronic renal hypertension.

REFERENCES

1) Baskaran N, Manoharan S, Balakrishnan S, Pugalendhi P: Chemopreventive potential of ferulic acid in 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in Sprague-Dawley rats. *Eur J Pharmacol* 637:22-29, 2010

2) Saija A, Tomaino A, Trombetta D, De Pasquale A, Uccella N, Barbuzzi T, Paolino D, Bonina F: In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. *Int J Pharm* 199:39-47, 2000

3) Yan JJ, Cho JY, Kim HS, Kim KL, Jung JS, Huh SO, Suh HW, Kim YH, Song DK: Protection against beta-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. *Br J Pharmacol* 133:89-96, 2001

4) Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L, Whelton PK: Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. *Am J Clin Nutr* 76:93-99, 2002

5) Srinivasan M, Sudheer AR, Menon VP: Ferulic Acid: therapeutic potential through its antioxidant property. *J Clin Biochem Nutr* 40:92-100, 2007

6) Suzuki A, Kagawa D, Fujii A, Ochiai R, Tokimitsu I, Saito I: Short- and long-term effects of ferulic acid on blood

pressure in spontaneously hypertensive rats. *Am J Hypertens* 15:351-357, 2002

7) Suzuki A, Yamamoto M, Jokura H, Fujii A, Tokimitsu I, Hase T, Saito I: Ferulic acid restores endothelium-dependent vasodilation in aortas of spontaneously hypertensive rats. *Am J Hypertens* 20:508-513, 2007

8) Bayraktutan U: Free radicals, diabetes and endothelial dysfunction. *Diabetes Obes Metab* 4:224-238 2002

9) Lockette W, Otsuka Y, Carretero OA: The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension* 8(Suppl Ⅱ):61-66, 1986

10) Dominiczak AF, Bohr DF: Nitric oxide and its putative role in hypertension. *Hypertension* 25:1202-1211, 1995

11) Dohi Y, Kojima M, Sato K: Endothelial modulation of contractile responses in arteries from hypertensive rats. *Hypertension* 28:732-737, 1996

12) Luscher TF, Raij L, Vanhoutte PM: Endothelium-dependent responses in normotensive and hypertensive Dahl rats. *Hypertension* 9:157-163, 1987

13) Callera GE, Varanda WA, Bendhack LM: Impaired relaxation to acetylcholine in 2K-1C hypertensive rat aortas involves an abnormal contribution of endothelial factors. *Gen Pharmacol* 34:379-389, 2000

14) Jun JY, Yeum CH, Moon SH, Cho CH, Jun KB, Chung JH and Yoon PJ: Altered endothelial modulation of vasoconstriction in chronic two-kidney, one clip hypertensive

rats. *Kor J Nephrol* 20:381-392, 2001
15) Sigmon DH, Beierwaltes WH: Influence of nitric oxide in the chronic phase of two-kidney one clip renovascular hypertension. *Hypertension* 31:649-656, 1998
16) Gil-Longo J, Fernández-Grandal D, Alvarez M, Sieira M, Orallo F: Study of in vivo and in vitro resting vasodilator nitric oxide tone in normotensive and genetically hypertensive rats. *Eur J Pharmacol* 310:175-183, 1996
17) Andriantsitohaina R, Andriambeloson E, Stoclet JC: Pharmacological approaches of endothelial nitric oxide-dependent vasorelaxation induced by polyphenols from plant extracts. *Methods Enzymol* 301:522-532, 1999
18) Hiramoto K, Li X, Makimoto M, Kato T, Kikugawa K: Identification of hydroxyhydroquinone in coffee as a generator of reactive oxygen species that break DNA single strands. *Mutat Res* 419:43-51, 1998
19) Bohr DF, Dominiczak AF, Webb RC: Pathophysiology of the vasculature in hypertension. *Hypertension* 18(Suppl Ⅲ):69-75, 1991
20) Yeum CH, Oh HK, Jun JY, Jun KB, Hong SP, Yeum CH, Yoon PJ: Cardiovascular effects of nitric oxide synthesis inhibitor in renal hypertensive rats. *J Korean Soc Hypertens* 7:35-45, 2001
21) Pourageaud F, Freslon JL: Endothelium function in resistance and coronary arteries of spontaneously hypertensive compared to WKY rats.: effects of

nitro-L-arginine. *Gen Hypertens* 218:39-41, 1992
22) Choi S, Jun KB, Jun JY, Yoon PJ, Kim JS, Kim MY, Mun HS, Yeum CH: Effects of Aminoguanidine on Norepinephrine-Induced Vascular Contraction in Renovascular Hypertensive Rats. *Kor J Nephrol* 23:703-713, 2004
23) Forstermann U, Nakane M, Tracey WR, Pollock JS: Isoform of nitric oxide synthase: functions in the cardiovascular system. *Eur Heart J* 14(Suppl 1):10-15, 1993
24) Rubanyi GM, Vanhoutte PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 250:H822-H827, 1986
25) Touyz RM: Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? *Hypertension* 44:248-252, 2004
26) Park JB, Touyz RM, Chen X, Schiffrin EL: Chronic treatment with a superoxide dismutase mimetic prevents vascular remodeling and progression of hypertension in salt-loaded stroke-prone spontaneously hypertensive rats. *Am J Hypertens* 15:78-84, 2002
27) Tanito M, Nakamura H, Kwon YW, Teratani A, Masutani H, Shioji K, Kishimoto C, Ohira A, Horie R, Yodoi J: Enhanced oxidative stress and impaired thioredoxin expression in spontaneously hypertensive rats. *Antioxid Redox Signal* 6:89-97, 2004
28) Toda S, Kumura M, Ohnishi M: Effects of phenolcarboxylic

acids on superoxide anion and lipid peroxidation induced by superoxide anion. *Planta Med* 57:8-10, 1991

29) Rodriguez-Iturbe B, Zhan CD, Quiroz Y, Sindhu RK, Vaziri ND: Antioxidant-rich diet relieves hypertension and reduces renal immune infiltration in spontaneously hypertensive rats. *Hypertension* 41:341-346, 2003