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Original Articles

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NITRIC OXIDE SYNTHASE ACTIVITY IN BLOOD VESSELS OF SPONTANEOUSLY HYPERTENSIVE RATS: ANTIOXIDANT PROTECTION BY γ-TOCOTRIENOL

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Involvement of free radicals and nitric oxide (NO) has long been implicated to the pathogenesis of essential hypertension. Several studies using antioxidants as the radical scavenger have shown to confer protection against free radical mediated diseases. This study is designed to investigate the role of antioxidant γ-tocotrienol on endothelial nitric oxide synthase (NOS) activity in spontaneously hypertensive rats (SHR). SHR's were divided into four groups namely untreated SHR (HC), treatment with 15mg γ-tocotrienol/kg diet (γl), 30mg γ-tocotrienol/kg diet (γ2) and 150mg γ-tocotrienol/kg diet (γ3) and studied for three months. Wister Kyoto (WKY) rats were used as the control (C). Blood pressure was recorded every fortnightly by tail plethysmography. Animals were sacrificed and NOS activity in blood vessels was measured by [^{3}H]arginine radioactive assay. Nitrite concentration in plasma was determined by Greis assay and lipid peroxides in the blood vessels by spectrofluorometry. This study showed that γ-tocotrienol significantly reduced systolic blood pressure (SBP) in SHRs with a maximum reduction in group treated with γ-tocotrienol 15 mg/kg diet (HC: 210±9 mmHg, γl:123±19 mmHg). Blood vessels from untreated SHR showed a reduced NOS activity compare to that of WKY rats (C: 1.54±0.26 pmol/mg protein, HC: 0.87+0.23pmol/mg protein; p,0.001). γ-tocotrienol improves NOS activity in all the groups with more significance in group γ2 ($p<0.001$) and γ3 ($p<0.05$). Plasma level of nitrite was reduced in SHR from 55±3 μM/ml in WKY to 26±2 μM/ml ($p<0.001$). Plasma nitrite level was reversed by treatment with γ-tocotrienol. (γl: $p<0.001$, γ2: $p<0.005$, γ3: $p<0.001$, respectively). In all the treatment groups, NOS activity showed significant negative correlation with blood pressure (γl: $r=-0.716$, $p<0.05$; γ2: $r=-0.709$, $p<0.05$; γ3: $r=-0.789$, $p<0.05$). For plasma nitrite, although it shows a negative correlation with blood pressure it was significant only in γl ($r=-0.676$, $p<0.05$) and γ2 ($r=-0.721$, $p<0.05$). From this study we found that compared to WKY rats, SHR has lower NOS activity in blood vessels, which upon treatment with antioxidant γ-tocotrienol increased the NO activity and concomitantly reduced the blood pressure. These findings further strengthen the hypothesis that free radicals and NO play critical role in pathogenesis of essential hypertension.

Key Words: *free radical, nitric oxide synthase, nitric oxide, blood pressure, antioxidant, SHR*

INTRODUCTION

In the spontaneously hypertensive rats (SHR), a model of genetic hypertension we have shown a reduced endothelial NOS activity and reduced availability of NO together with an increased free radical generation as evident by increased production of lipid peroxides and reduced total antioxidant status (1,2). Endothelial nitric oxide synthase (NOS) produces (NO) from L-arginine in a calcium and calmodulin dependent process. NO is responsible for the acetylcholine mediated vascular relaxation through increased cGMP (3). cGMP inhibits calcium influx and mobilization from intracellular stores (4) thus facilitating vascular relaxation. Reduced activity of NOS or an accelerated degradation of NO by oxygen free radical may lead to an impaired vasodilatation, hence raised blood pressure.

An exaggerated production of superoxide anion ($^{\cdot}O_2^-$) by the vascular wall has been observed in different animal model of hypertension, including SHR (5). In the majority of cases source of $^{\cdot}O_2^-$ is uncertain although involvement of endothelial NOS and xanthine oxidase (6,7) have been suggested. A growing amount of evidence supports the possibility that increased oxidative inactivation of NO by an excess of ($^{\cdot}O_2^-$) may account for the decrease in available NO and endothelial dysfunction seen in SHR (8).

Recent studies showed that in hypertension, endothelium dependent vascular relaxation was impaired (9). So it was postulated that increased free radical generation would lead to a reduced activity of NOS or an increased removal of NO in blood vessels, which results in impairment of vascular relaxation. In addition several studies have demonstrated that ($^{\cdot}O_2^-$) can also act as a vasoconstriction (10). In the mean time, it is reported that L-arginine reduced blood pressure in experimental animal (11) and superoxide dismutase (SOD) a potent free radical scavenger, also reduce the blood pressure by increasing the availability of NO (12).

Dietary modulation of endothelial function by antioxidant vitamin E and C has long been studied (13). Vitamin E reduces superoxide generation and lipid peroxidation (14). In our previous studies we have shown α-tocopherol, an active component of vitamin E reduced systolic blood pressure and lipid peroxidation in SHR (1,2). In the mean time, study shows a significant up-regulation of endothelial and inducible NOS activity after antioxidant therapy (15). Carr and Frei also reported that natural antioxidant can preserve the biological activity of endothelium-derived NO (14). Tocotrienol, another component of vitamin E has long been identified as a potent antioxidant (16) and recent studies have utilized tocotrienol in the treatment and / or prevention of cardiovascular diseases and cancer (17). In a similar study γ-tocotrienol has shown antioxidant protection in SHR by reducing lipid peroxidation and enhancing total antioxidant status. We hypothesize that γ-tocotrienol, being more potent than α-tocopherol, may protect NOS / NO from the free radical attack thus, may play a beneficial role in the SHR.

In This study we investigate the role of γ-tocotrienol in the prevention of free radical mediated attenuation of NOS and NO in the blood vessels from SHR.

MATERIALS AND METHODS

Chemicals. γ-Tocotrienol (90% purity) used in this study was supplied by Palm Oil Research Institute of Malaysia (PORIM), Bangi, Malaysia. [H] L-arginine (Amersham, UK), leupeptin, pepstatin and pefabloc (Boehringer Mannheim) and all other reagents used were the highest grade commercially available and obtained from Sigma Chemical Co. (St Louis, MO) unless otherwise specified.

Animal treatment. Thirty-three male SHR, 150-200 gm, 8-10 weeks old, were caged individually and maintained on normal or treated rat chow and water *ad libitiim* for the duration of three month (12 week). The rats were divided into 4 groups consisting of the SHR control (HC), γ-tocotrienol treatment with doses 15 mg/kg diet (γl), 30 mg/kg diet (γ2) and 150 mg/kg diet (γ3). Dose of γ-tocotrienol (γl) has been chosen based on the Recommended Daily Allowance (RDA) of 200 mg vitamin E / day for a 70 kg adult. Subsequent doses were γ2 (γl x 2) and γ3 (γl x l0). Seventeen age and weight matched Wister Kyoto (WKY) rats were used as the normal control (C). This investigation conforms with the institutional guideline for the care and use of laboratory animals.

At the end of the study period the rats were killed by cervical dislocation. Blood was taken immediately from the heart. Animals were dissected and approximately 3 cm of aorta with intact endothelium were collected in liquid nitrogen.

Blood pressure monitoring. Blood pressure was measured every fortnightly in all animals using standard tail cuff method. Tails of the animal were occluded with an appropriate size metal tubular tail cuff (7/16 inch) and pulse was detected as the cuff pressure was lowered. The pressure at which the first pulse appears was the measurement of systolic blood pressure (SBP). The occluding tubular cuff together with pneumatic pulse transducer (Narco Bio Systems, USA) was connected to electrophysiograph (Narco Bio Systems, USA) cuff outlet. Rat tail was pre-warmed by lamp and the average of three readings was taken as the final reading.

Measurement of NOS and NO. Nitric oxide synthase activity was measured in blood vessel as described earlier (2) where production of ^{3}[H]-citrulline from ^{3}[H]-arginine was measured by β-scintillation counter. Aorta was cleaned off its surrounding fat and collected in I ml freshly prepared homogenization mixture (HEPES 20 mM, EDTA 0.05 mM, DTT I mM, leupeptin 0.5 [μg/ml, pepstatin 0.7 [μg/ml, pefabloc 0.5 mM, pH 7.2). Samples were homogenized by Ultra Turrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, UK) and then sonicated three times for 20 second in Sonicator XL (Heat systems, NY). The homogenized samples were ultracentrifuged at 20,000 x g for 25 minutes. The supernatant (0.34 ml) was mixed with L-[2,3,4,5-^{3}H]-arginine (0.6μci), L-arginine 50 μm, NADPH 2mM, $CaCI_2$ 0.45 mM and calmodulin (10μg/ml) in a final volume of 0.4ml and incubated at 37°C for 45 minutes. N^{G}-Nitro-L-arginine methyl ester (LNAME, 1mM) was used for the determination of blank activity. Immediately after the incubation, assay was terminated by I ml of stop buffer (20 mM IIEPES, 2 mM EDTA, pH 5.5) and the samples were applied to I ml column of DOWEX (50WX-8, 200-400 dry mash, H^{+} form) which were eluted with 2 mls of water. ^{3}[H] was quantified by liquid scintillation in a β-counter. NOS activity were expressed as pmol ^{3}[H]-citrulline /mg protein.

Nitric oxide was measured as the breakdown product, $N0_2^-$. Nitrite levels in the plasma were determined by reacting it with Greiss reagent (2). 500 μl of plasma was mixed with 50 μl of 6.5M HCI and 50 μl of 37.5 mM Sulfanilic acid and incubated for 10 minutes in room temperature. Napthyl ethyline diamine of volume 50 μl (12.5 mM) were added and incubated again for 30 mins at room temperature. Samples were centrifuged at 1000 x g for 10 minutes and the nitrite was quantified spectrophotometrically at wavelength 540 nm against the standards.

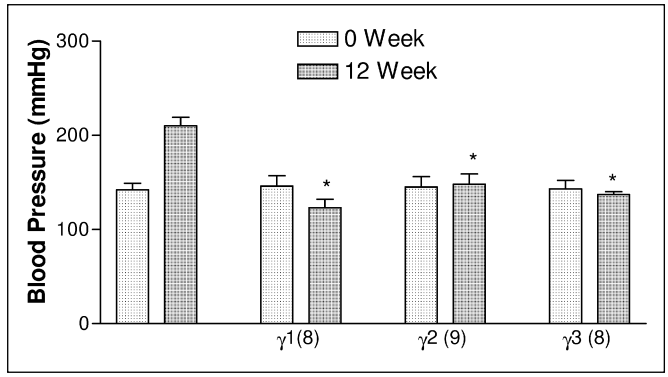

Fig.1. Comparison of blood pressure between SHR and SHR treated with different doses of γ Tocotrienol. SHR = SHR control, γ1= γ-Tocotrienol 15 mg/kg diet, γ2= γ-Tocotrienol 30 mg/kg diet, γ3= γ-Tocotrienol 150 mg/kg diet. Values are mean ± SD. *p<0.05 versus HC. Values in the parentheses are the number of experiments.

Lipid peroxides. Lipid peroxides in blood vessels were measured as thiobarbituric acid reaction product by spectrofluorometry following well established procedure (1) and were expressed as nmol MDA (Melondialdehyde) equivalent /mg protein.

Statistical Analysis. Results obtained from different treatment groups were expressed as Mean ± SEM and compared by ANOVA for significant difference. A value of $p<0.05$ was considered as significant.

RESULTS

Results from this study shows that γ-tocotrienol significantly reduced age related development of blood pressure in all the treated groups (*Fig.1*). The best reduction of blood pressure of 42% was achieved in groups treated with γ-tocotrienol 15 mg/kg diet. γ tocotrienol increased NOS activity in the blood vessels which was significant in groups γ2 and γ3 where increase of NOS was 81% ($p<0.01$) and 21% ($p<0.05$) respectively (*Fg.2*). Plasma nitrite level was significantly reduced in SHR from 54.62±2.96 to 26.24±2.14 µmole/ml.

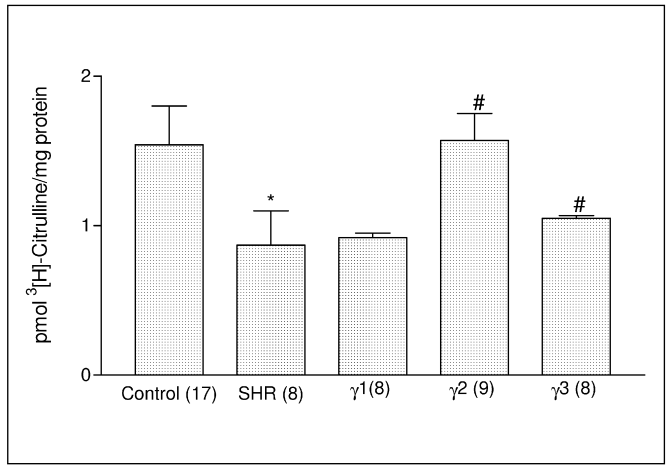

Fig.2. Comparison of nitric oxide synthase activity (NOS) in the blood vessels between WKY rats, SHR and SHR treated with g-tocotrienol. SHR = SHR control, γ1= γ-Tocotrienol 15 mg/kg diet, γ2= γ-Tocotrienol 30 mg/kg diet, γ3= γ-Tocotrienol 150 mg/kg diet. Values are mean ± SD. *p<0.05 versus control, #p<0.05 versus SHR. Values in the parentheses are the number of experiments.

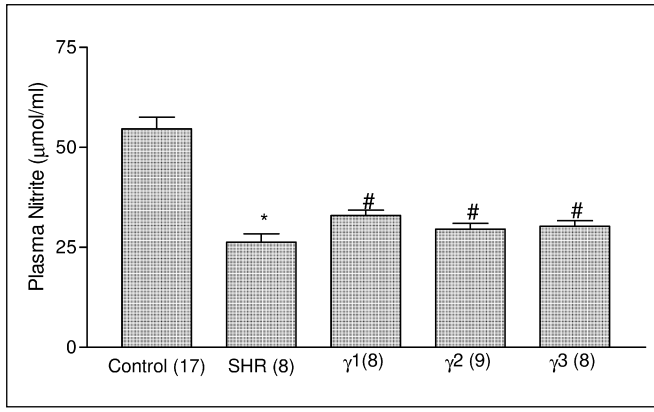

Fig.3. Plasma nitrite levels compared between control (WKY), SHR and SHR treated with γ-tocotrienol. SHR = SHR control, γ1=γ-Tocotrienol 15 mg/kg diet, γ2=γ-Tocotrienol 30 mg/kg diet, γ3=γ-Tocotrienol 150 mg/kg diet. Values are mean ± SD. *p<0.05 versus control, #-p<0.05 versus SHR. Values in the parentheses are the number of experiments.

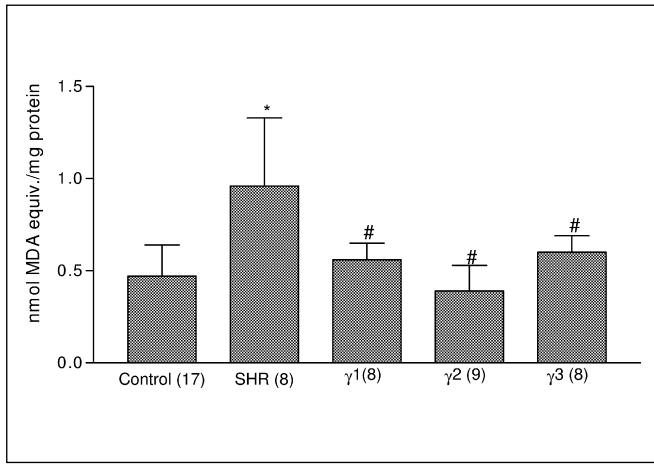

Fig.4. Generation of lipid peroxide in the blood vessels from control (WKY) rats, SHR and SHR treated with γ-tocotrienol. SHR = SHR control, γ1= γ-Tocotrienol 15 mg/kg diet, γ2=γ-Tocotrienol 30 mg/kg diet, γ3=γ-Tocotrienol 150 mg/kg diet. Values are mean ± SD. *p<0.05 versus control, #p<0.05 versus SHR. Number in the parentheses is the number of experiments.

Treatment with γ-tocotrienol enhanced nitrite level in groups γ1, γ2 and γ3 by 25% (p<o.oo1), 13% (p<0.05) and 17% (p<0.05) respectively (*Fig.3*). On the other hand lipid peroxide generation was higher in SHR compare to the control which was significantly reduced by 42% (p<0.05), 59% (p<0.001) and 48% (p<0.05) respectively in groups γ1, γ2 and γ3 where SHR were treated with different doses of γ-tocotrienol (*Fig.4*).

Correlation analysis was done between blood pressure at 12th week and the parameters in all the groups, treated and untreated (*Table 1*). Results showed that NOS activity and NO have a negative correlation with blood pressure. For NOS, it was significant for groups HC (p=0.038), γl (p=0.046), γ2 (p=0.032) and γ3 (p=0.020). For NO, significant correlation was observed in groups γl (p=0.044) and γ2 (0.046). On the other hand, lipid peroxide in blood vessel showed a significant positive correlation with blood pressure in groups HC (p=0.003), γl (p=0.039), γ2 (p=0.000) and γ3 (p=0.002).

Table 1. Correlation of NOS, NO and lipid peroxides with blood pressure in experimental groups after 12 weeks of treatment with different doses of γ-tocotrienol on SHR. Values are mean ±SD.

Parameters	SHR		γ1		γ2		γ3	
	r value	p value	r value	p value	r value	p value	r value	p value
NOS (BV)	-0.735	0.038*	-0.716	0.046*	-0.709	0.032*	-0.789	0.020*
NO	-0.622	0.100	-0.721	0.044*	-0.676	0.046*	-0.595	0.120
LP	0.892	0.003*	0.731	0.039*	0.919	0.0008	0.898	0.002*

N.B: NOS=Nitric oxide synthase, NO=Nitric oxide, LP=Lipid peroxide, BV=Blood vessel, SHR=SHR control, γ1=γ-Tocotrienol 1-5 mg/kg diet, γ2=γ-Tocotrienol 30 mg/kg diet, γ3=γ-Tocotrienol 150 mg /kg diet. *p<0.05 versus SHR.

DISCUSSION

During the recent years, involvement of free radicals in the pathogenesis of essential hypertension became a major point of research. As the vascular endothelium is the major site for the regulation of vessel tone and NO can be scavenged by increased endothelial production of free radicals (18), study of endothelial dysfunction in relation to increased free radicals may provide more in sight to the mechanism of this disease process. Impaired NOS activity in the experimental hypertensions reported by previous studies correlate with our findings of increased lipid peroxides in the blood vessels of SHR and further supports the hypothesis of free radical involvement in SHR.

Acetylcholine mediated vascular relaxation is manifested through the release of NO in the vascular endothelium which elevates cGMP (3). It was reported earlier that free radical can reduce the NOS activity thus, decreasing NO (19). The exact mechanism is not clear, but it may be via direct reduction of NO synthesis from NOS (20) or by interruption of endothelial receptor signal transduction (21). Beside this, free radical can act directly on NO and make it less available. In agreement with these suggestions, a reduced NOS activity in the blood vessels of SHR was observed in this study together with reduced NO in plasma. Our finding of a negative correlation between lipid peroxides in blood vessel with the NOS activity and NO further strengthen the role of free radicals in the activity and availability of NOS and NO in this experimental hypertension.

It has been reported earlier that, NOS also can generate $\cdot O_2^-$ (19), which may be the reason in young SHR where there is an upregulation of NOS and the increased radical generation can be overcome by L-NAME (22). In the mean time, other studies hypothesize that increased NOS activity in SHR is the compensatory response to increased free radicals that leads to the development of hypertension (23). On the contrary, suppression of development of hypertension have been achieved by inhibiting nitric oxide synthase (24), whereas, Lin and co-workers reported that prolonged reduction of blood pressure can be achieved by using NOS gene delivery (25). This confusion, whether increased NOS or

decreased NOS is involved in this model of hypertension have been resolved by the study of Chou et. al. where they reported that, reduced eNOS (endothelial NOS) is involved in the development of hypertension, whereas increased iNOS (inducible NOS) is the consequences of pathological state of vessels associated with hypertension in SHR (26). Findings from this study also correlate with this hypothesis that reduced activity of endothelial NOS system is involved in the process of developing blood pressure in SHR which can be reversed by administering antioxidant.

Treatment with γ-tocotrienol increased the NOS activity in all the treated groups in a variable manner together with increased NO availability. This explains how the γ tocotrienol prevent the age related development of raised blood pressure observed in our study. From this observation it may be hypothesized that in SHR there is a progressive increase in free radical generation which reduces NOS activity and NO, either directly or via producing lipid peroxides which contributes to the development of high blood pressure. Antioxidant γ-tocotrienol scavenges this free radical, maintains the NOS activity and availability of NO and reduces the lipid peroxide formation, thus preventing the development of raised blood pressure. This is also in accordance with our previous study where we reported prevention of development of raised blood pressure in SHR by γ-tocotrienol via reducing lipid peroxidation and by enhancing total antioxidant status in SHR (27).

Correlation that observed for NOS and NO with blood pressure in this study gave an insight into their complex role in the regulation of blood pressure. It is postulated that lipid peroxides have an effect on NOS and NO level that influenced the blood pressure. The effect of lipid peroxide on blood pressure was reflected as a positive correlation between the two parameters observed in this study. Peroxynitrite ($ONOO^-$) generated from NO by $^{\cdot}O_2^-$ can produce peroxynitrous acid, which is one of the most potent reactive oxygen species in the biological system (28). In this context, further elucidation is required to ascertain whether this influence of lipid peroxides on blood pressure is a direct effect on the vessel or mediated through NOS and NO.

In this study low dose of γ-tocotrienol (15 mg/kg diet) has shown better protection than the higher doses although higher doses have also shown the antioxidant protection. This increased efficacy of low dose of γ-tocotrienol over the higher dose in reducing blood pressure is not unusual. It may be attributed to the pro-oxidant activity of this vitamin in higher dose. There are reports showing that under certain condition vitamin E may act as a pro-oxidant and actually enhance rather than retard lipid peroxidation (29).

In conclusion, from this study we found that the age related development of raised blood pressure in SHR could be attributed to reduced NOS activity with a decreased NO availability due to increased production of free radicals. γ-tocotrienol prevented this development of age related raised pressure in SHR by increasing the NOS activity in blood vessel and increasing NO availability. This

improvement of NOS and NO are mediated through the antioxidant properties of γ-tocotrienol where it effectively scavenges the free radicals. As a positive response was obtained with γ-tocotrienol treatment of SHR, the involvement of free radicals in the pathogenesis of essential hypertension was further ascertained.

REFERENCES.

1. Newaz MA, Nawal NN. Effect of a-tocopherol on lipid peroxidation and total antioxidant status in spontaneously hypertensive rats. *Am J Hypertens* 1998; 11: 1480-1485.
2. Newaz MA, Nawal NN. Rohaizan CH, Muslim N, Gapor A. α-tocopherol increased nitric oxide synthase activity in blood vessels of spontaneously hypertensive rats. *Am J Hypertens* 1999; 12: 839-844.
3. Rapoport RM, Draznin MB & Murad F. Endothelium dependent relaxation in rat aorta may be mediated through cyclic GMP dependent protein phosphorylation. *Nature* 1983; 306: 174-176.
4. Henderson AH, Morgan RO & Newby AC. The inhibition by sodium nitropruside of ADP induced calcium influx and calcium mobilisation in human platelets. *J Physiol*. 1987; 387: 89P.
5. Suzuki H, Swei A, Zweifach BW, Schmid-Schonbein GW. In vivo evidence for microvascular oxidative stress in spontaneously hypertensive rats. *Hypertension* 1995; 25:1083-1089.
6. Cosentino F, Patton S, D'Uscio LV, Werner ER, Werner-Felmayer G, Moreau P, Malinski T, Luscher TF. Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. *J Clin Invest* 1998; 101: 1530-1537.
7. Miyamoto Y, Akaike T, Yoshida M, Goto S, Horie H, Maeda H. potentiation of nitric oxide-mediated vasorelaxation by xanthine oxidase inhibitors. *Proc Soc Exp Biol Med* 1996; 21:366373.
8. Schnackenberg CG, Welch WJ, Wilcox CS> Normalization of blood pressure and renal vascular resistance In SHR and with a membrane-permeable superoxide dismutase mimetic: role of nitric oxide. *Hypertension* 1998; 32: 59-64.
9. Lickette W, Otsuka Y, Carretero U. The loss of endothelium dependent vascular relaxation in hypertension. *Hypertension* 1986; 8(suppl 11): 1161-1166.
10. Auch-Schelk W, Katusic ZS, Vanhoutte PM. Contraction to oxygen-derive free radicals are augmented in aorta of the spontaneously hypertensive rats. *Hypertension* 1989; 13:859-864.
11. Laurant P, Demolombe B, Berthelol A. Dietary L-arginine attenuate blood pressure in mineralocorticoid-salt hypertensive rats. *Clin and Exp Hypertension* 1995; 17; 1009-1024.
12. Rubanyi GM. Vascular effects of oxygen derived free radicals. *Free Rad Biol. Med.* 1988;4: 107-120.
13. Brown AA, Hu FB. Dietary modulation of endothelial function: implications of cardiovascular diseases. *Am J Clin Nutr* 2001; 73: 673-686.
14. Carr A, Frei B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Radic Biol Med* 2000;28:1806-1814.
15. Vaziri ND, Ni Z, Oveisi F, Trnavsky-Hobbs DL. Effect of antioxidant therapy on blood pressure and NO synthase expressiin in hypertensive rats. *Hypertension* 2000; 36: 957-964.
16. Hamilton CA, Brosnan MJ, McIntyre M, Graham D, Dominiczak. Superoxide excess in hypertension and aging. A common cause of endothelial dysfunction. Hypertension 2001; 37:529.
17. Koba K, Abe K, Ikeda I, Sugano M. Effects of alpha-tocopherol and tocotrienols on blood pressure and linoleic acid metabolism in the spontaneously hypertensive rat. *Biosci Biotechnol Biochem* 1992; 56:1420-1423.

18. Rubanyi GM, Vanhoutte PM. Superoxide anion and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 1986; 250: H822-H827.
19. Kerr S, Brosnan MJ, McIntyre M, Reid JL. Superoxide anion production is increased in a model of genetic hypertension. Role of the endothelium. *Hypertension* 1999; 33: 1353-1358.
20. Chin JH, Azhar S & Hoffman BB. Inactivation of endothelium derived relaxing factor by oxidized lipoproteins. *J. Clin. Invest.* 1992; 89: 10-18.
21. Inoue N, Hirata K, Yamada M, Hamamori Y, Matsuda Y, Akita H & Yokoyama M. Lysophosphatidylcholine inhibits bradykinin induced phosphoinositide hydrolysis and calcium transients in cultured bovine aortic endothelial cell. *Circ. Res.* 1992; 71: 1410-1421.
22. Pou S, Pou WS, Bredt DS, Snyder SH, Rosen GM. Generation of superoxide by purified brain nitric oxide synthase. *J Bio Chem* 1992; 267:24173-24176.
23. Akiba Y, Yamaguchi N, Amano H, Fujii T, Fujimoto K, Suzuki T, Kawashima K. Role of nitric oxide in control of blood pressure in young and adult spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol Suppl* 1995; 1: S142-143.
24. Hong HJ, Loh SH, Yen MH. Suppression of the development of hypertension by the inhibitor of inducible nitric oxide synthase. *Br J Pharmacol* 2000; 131: 631-637.
25. Lin K, Chao L, Chao J. Prolonged reduction of hogh blood pressure with human nitric oxide synthase gene delivery. *Hypertension* 1997; 30:307-313.
26. Chou T, Yen M, Li C, Ding Y. Alterations of nitric oxide synthase expression with aging and hypertension in rats. *Hypertension* 1998; 31:643-648
27. Newaz MA, Nawal NN. Effect of γ-tocotrienol on blood pressure, lipid peroxidation and total antioxidant status in spontaneously hypertensive rats (SHR). *Clin Exp Hypertens* 1999; 21: 1297-1313.
28. Beckman JS, Chen J, Ischiropoulos H, Crow JP. Oxydative chemistry of peroxynitrite. In: Packer L ed. Methods of Enzymology. San Diego, Califf: Academic Press Inc; 1994: 229-240.
29. Bowry VW, Stocker R. Tocopherol mediated peroxidation. The pro-oxidant effect of vitamin E on the radical initiated oxidation of human low density lipoprotein. *J Am. Chem. Soc.* 1993; 115: 6029-6044.

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