Two different approaches to restore renal nitric oxide and prevent hypertension in young spontaneously hypertensive rats: L-citrulline and nitrate

SHAO-JU CHIEN, KUAN-MIAO LIN, HSUAN-CHANG KUO, CHIEN-FU HUANG, YING-JUI LIN, LI-TUNG HUANG, and YOU-LIN TAIN

KAOHSIUNG AND LINKOW, TAIWAN

Nitric oxide (NO) deficiency mediates oxidative stress in the kidney and is involved in the development of hypertension. NO synthesis occurs via 2 pathways: nitric oxide synthase (NOS) dependent and NOS-independent. We tested whether the development of hypertension is prevented by restoration of NO by dietary L-citrulline or nitrate supplementation in young spontaneously hypertensive rats (SHRs). Male SHRs and normotensive Wistar Kyoto control rats (WKYs)s age 4 weeks were assigned to 4 groups: untreated SHRs and WKYs, and SHRs and WKYs that received 0.25% L-citrulline for 8 weeks. In our second series of studies, we replaced L-citrulline with 1 mmol/kg/d sodium nitrate. All rats were sacrificed at age 12 weeks. We found an increase in the blood pressure of SHRs was prevented by dietary supplementation of L-citrulline or nitrate. Both treatments restored NO bioavailability and reduced oxidative stress in SHR kidneys. L-Citrulline therapy reduced levels of L-arginine and asymmetric dimethylarginine (ADMA)—an endogenous inhibitor of NOS—and increased the L-arginine-to-ADMA ratio in SHR kidneys. Nitrate treatment reduced plasma levels of L-arginine and ADMA concurrently in SHRs. Our findings suggest that both NOS-dependent and -independent approaches in the prehypertensive stage toward augmentation of NO can prevent the development of hypertension in young SHRs. (Translational Research 2014;163:43–52)

Abbreviations: ADMA = asymmetric dimethylarginine; ASL = argininosuccinate lyase; ASS = argininosuccinate synthetase; BP = blood pressure; CAT = cationic amino acid transporter; DDAH = dimethylarginine dimethylaminohydrolase; eNOS = endothelial nitric oxide synthase; EPR = electron paramagnetic resonance; IOD = integrated optical density; NO = nitric oxide; NOS = nitric oxide synthase; nNOS = neuronal nitric oxide synthase; OHdG = 8-hydroxydeoxyguanosine; ROS = reactive oxygen species; SHR = spontaneously hypertensive rat; WKY = Wistar Kyoto rat

From the Department of Pediatrics, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University, College of Medicine, Kaohsiung, Taiwan; Department of Traditional Chinese Medicine, Chang Gung University, Linkow, Taiwan; Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University, College of Medicine, Kaohsiung, Taiwan.

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Shao-Ju Chien and Kuan-Miao Lin are co-first authors.

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Reprint requests: You-Lin Tain, MD, PhD, Department of Pediatrics, Kaohsiung Chang Gung Memorial Hospital, 123 Dabi Road, Niausung, Kaohsiung 833, Taiwan; e-mail: tainyl@hotmail.com.

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AT A GLANCE COMMENTARY

Chien S-J et al.

Background

Nitric oxide (NO) deficiency mediates oxidative stress in the kidney and contributes to hypertension. We tested whether the development of hypertension is prevented by restoration of NO by nitric oxide synthase (NOS)-dependent (L-citrulline) or NOS-independent (nitrate) therapy in young spontaneously hypertensive rats (SHRs).

Translational Significance

Both early supplementations of L-citrulline and nitrate are able to increase NO and reduce oxidative stress to prevent the development of hypertension in young SHRs. Our findings support the consideration of both NOS-dependent and -independent approaches as viable therapies to restore a disturbed ROS/NO balance to prevent the transition from prehypertension to hypertension.

Nitric oxide (NO), a vasodilator controls local and systemic blood flow. NO deficiency mediates oxidative stress in the kidney and has been documented in experimental and human hypertension.[1-3] We and others have shown that renal NO deficiency contributes to the development of hypertension in young spontaneously hypertensive rats (SHRs).[4,5] Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS) can reduce NO production and induction of oxidative stress.[6,7] We recently showed that 4-week-old SHRs develop increased levels of ADMA and had a decreased L-arginine-to-ADMA ratio in the plasma and kidneys early, even before the onset of hypertension.[8] In addition, our previous studies demonstrated that therapeutic approaches shifting disturbed reactive oxygen species (ROS)/NO balance in prehypertensive stage toward increase of NO can lead to blood pressure (BP) lowering in young SHRs.[4,9]

NO synthesis occurs via 2 physiological pathways: NOS dependent and NOS independent.[10] In the former, L-arginine is converted by NOS to generate NO and L-citrulline. In the latter, nitrate and nitrite are the main substrates to produce NO via the NOS-independent pathway. L-Arginine supplementation is the most common approach to increase NO via the NOS-dependent pathway. Although L-arginine has been shown to reduce systemic BP in some forms of experimental hypertension, L-citrulline supplementation enhances NO production more than L-arginine because it bypasses splanchnic extraction and it is not a substrate for arginase.[11,12] Thus, supplemental L-citrulline has been used therapeutically to treat cardiovascular disease involving NO deficiency, including hypertension.[12,13] On the other hand, organic nitrates (eg, nitroglycerine) have been long-term used to cause vasodilatation in clinical practice. However, nitrate tolerance and increased formation of peroxynitrite under oxidative stress limits their application. Recent evidence demonstrated that dietary nitrate attenuates oxidative stress and reduces BP in experimental hypertension.[14]

In this study, we intended to restore NO bioavailability by NOS-dependent (L-citrulline supplementation) and NOS-independent (nitrate) approaches to examine whether the transition from prehypertension to hypertension can be prevented in young SHRs and whether this is ADMA pathway dependent.

METHODS

Animals. This study was carried out in strict accordance of the care and use of laboratory animals by the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital. All efforts were made to minimize suffering. Male SHRs and control normotensive Wistar Kyoto rats (WKYs) at 3 weeks of age were obtained (BioLASCO Taiwan Co., Ltd., Taipei, Taiwan) and maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility with free access to tap water and standard rat chow.

The aim of the first series of experiments was to assess whether L-citrulline supplementation prevents the transition from prehypertension to hypertension in young SHRs. At the age of 4 weeks, the rats were assigned into 4 groups (n = 6 for each group): group 1, WKYs without treatment; group 2, SHRs without treatment; group 3, WKYs treated with 0.25% L-citrulline (Sigma-Aldrich, Steinheim, Germany) dissolved in drinking water as described previously[15]; and group 4, SHRs treated with 0.25% L-citrulline in drinking water.

Our second series of experiments assessed the effects of dietary nitrate supplementation on the protection of hypertension development in young SHRs. Four-week-old SHRs and WKYs were assigned into 4 groups (n = 6 for each group): group 1, WKYs; group 2, SHRs; group 3, WKYs that received 1 mmol/kg/d sodium nitrate (Sigma-Aldrich) in drinking water; and group 4, SHRs treated with 1 mmol/kg/d sodium nitrate in