ORIGINAL ARTICLES

Metformin reduces asymmetric dimethylarginine and prevents hypertension in spontaneously hypertensive rats

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Elevated asymmetric dimethylarginine (ADMA) levels and nitric oxide (NO) deficiency are associated with the development of hypertension. Metformin, an antidiabetic agent, is a structural analog of ADMA. We examined whether metformin can prevent the development of hypertension in spontaneously hypertensive rats (SHRs) by restoration of ADMA-NO balance. SHRs and control normotensive Wistar-Kyoto (WKY) rats were assigned to 4 groups (N = 8 for each group): untreated SHRs and WKY rats, metformin-treated SHRs and WKY rats. Metformin-treated rats received metformin 500 mg/kg per day via oral gavage for 8 weeks. All rats were sacrificed at the age of 12 weeks. We found an increase in the blood pressure of SHRs was prevented by metformin. ADMA levels in the plasma and lung were elevated in SHRs, which metformin prevented. Lung dimethylarginine dimethylaminohydrolase (DDAH, ADMA-metabolizing enzyme) activity was lower in SHRs than WKY rats. Next, metformin had no effect on protein arginine methyltransferase 1 (ADMA-synthesizing enzyme), DDAH-1, DDAH-2, NO synthase enzymes, and DDAH activity in the kidney. Moreover, metformin increased the levels of NO in kidney. Conclusively, the observed antihypertensive effect of metformin in SHRs is because of the restoration of the ADMA-NO pathway. Our findings support the consideration of metformin as an antihypertensive agent for diabetic patients with prehypertension. (Translational Research 2014;164:452–459)

Abbreviations: AAR = L-Arginine-to-ADMA ratio; ADMA = asymmetric dimethylarginine; ASR = ADMA-to-SDMA ratio; CAT = cationic amino acid transporter; DDAH = dimethylarginine dimethylaminohydrolase; NOS = nitric oxide synthase; PRMT = protein arginine methyltransferase; SDMA = symmetric dimethylarginine; SHR = spontaneously hypertensive rat; WKY = Wistar Kyoto

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AT A GLANCE COMMENTARY

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Background

The imbalance between asymmetric dimethylarginine (ADMA) and nitric oxide (NO) is associated with the development of hypertension. Metformin is a structural analog of ADMA. We examined whether metformin can restore ADMA-NO to prevent hypertension in spontaneously hypertensive rats.

Translational Significance

Metformin blocks the development of hypertension in spontaneously hypertensive rats by reduction in plasma ADMA and increases in renal NO production. Our findings support the consideration of metformin as an antihypertensive agent for diabetic patients with prehypertension and highlight that the restoration of ADMA-NO pathway might be a therapeutic target for prehypertension.

INTRODUCTION

Hypertension is a highly prevalent disease. Emerging evidence indicates nitric oxide (NO)-reactive oxygen species (ROS) imbalance in the kidney is involved in the development of hypertension.[1,2] Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOS), which competes with L-arginine to inhibit NO production and induction of ROS.[3] Balance between L-arginine and ADMA maintains NO homeostasis. Elevated ADMA levels have been shown in various experimental hypertensive models and human hypertension.[4-7] We recently showed that ADMA level increase and an L-arginine-to-ADMA ratio (AAR) decrease in plasma and kidneys develop early on, even before the onset of hypertension in young spontaneously hypertensive rat (SHR).[8] Additionally, our studies demonstrated that therapeutic approaches shifting disturbed ROS/NO balance in prehypertensive stage toward increase in NO lead to blood pressure (BP) lowering in young SHRs.[9-11] These observations support that the restoration of ADMA-NO balance might be a therapeutic target for prehypertension.

Metformin (dimethylbiguanide) is a first-line drug in the treatment of type 2 diabetic patients by its effect on insulin resistance.[12] In addition to lowering blood glucose, metformin has been shown to have platelet antiaggregating effects to reduce advanced glycation end products and to decrease the cellular oxidative reactions, thus demonstrating its broad set of pharmacologic properties.[13] Previous human studies on the effect of metformin on BP have been varied,[14,15] finding decreased or unaltered BP. Metformin has been reported to lower BP in SHRs.[16-18] The protective effect of metformin on the development of hypertension in SHRs is unclear, as are the underlying mechanisms of its antihypertensive effect.

Metformin and ADMA are structural analogs; it has been proposed that they have opposite effects at multiple signaling pathways.[19] ADMA is mainly generated by type I protein arginine methyltransferase (PRMT) and metabolized by dimethylarginine dimethylaminohydrolase (DDAH).[4] Plasma levels of ADMA are elevated in diabetic patients and positively correlated with insulin resistance.[20] Given that metformin reduces ADMA levels in patients with diabetes[21] and that ADMA-NO pathway is involved in the development of hypertension,[6-9] we examined whether metformin reduces ADMA to prevent the development of hypertension in SHRs in this study. The second aim of this study was to elucidate whether an ADMA-lowering effect of metformin is associated with increased DDAH expression and activity, decreased PRMT-1 expression, or its transport.

METHODS

Experimental design. This experiment was approved and performed under the Guidelines for Animal Experiments of Chang Gung Memorial Hospital and Chang Gung University. The treatment of animals conformed to the US National Institutes of Health guidelines. Male SHRs and control Wistar-Kyoto (WKY) rats at the age of 3 weeks 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.

Rats aged 4 weeks were randomly assigned to 4 groups (N = 8 for each group): group 1, WKY rats without treatment; group 2, SHRs without treatment; group 3, WKY rats received metformin 500 mg/kg per day via oral gavage (WKY + M); and group 4, SHRs received metformin treatment (SHR + M). The dose of metformin used here was based on the previous study conducted on SHRs.[16] BP was measured in conscious rats by an indirect tail-cuff method (BP-2000; Visitech Systems, Inc, Apex, NC) after systematically trained at the of age 4, 6, 8, 10, and 12 weeks. To ensure accuracy and reproducibility, the rats were acclimated to restraint and tail-cuff inflation for 1 week before the experiment, and measurements were taken at 1:00 PM to 5:00 PM each day. Rats were placed on the specimen platform, and their tails were passed through tail cuffs