응용약물학회지, The Journal of Applied Pharmacology 2, 131-135(1994)

Effect of Ginsenoside on Basal and Nitro-L-Arginine Suppressed Nitric Oxide Production in Rat Kidney

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(Received May 25, 1994; accepted June 29, 1994)

Abstract—The effect of ginsenoside (GS) from *Panax ginseng* on basal and nitro-L-arginine suppressed nitric oxide (NO) production was studied in rat kidney. NO production was determined by conversion to [^{14}C]-L-citrulline from [^{14}C]-L-arginine both in whole kidney and three renal segments; glomerulus, cortex excluding glomerulus (cortex-) and medulla. Nitro-L-arginine (total dose of 30 mg/kg/3 days, *i.p.*) significantly reduced NO production in whole kidney, which was prevented by GS pretreatment (30 mg/kg/3 days, *i.p.*). Relative high dose of GS (120 mg/kg/4 days, *i.p.*) selectively increased NO production in glomerulus and cortex-. Protein content, on wet weight basis, in cortex- and glomerular DNA content were significantly reduced by GS. Our results confirm the existence of constitutive nitric oxide synthase in kidney and it seems that target nephron segment for volume expansion due to GS' NO-mediated vasodilation and for NO production stimulated by GS is cortex including glomerulus.

Kcywords □ ginsenoside, nitro-L-arginine, nitric oxide, kidney, glomerulus, cortex, medulla.

Nitric oxide (NO) exerts a wide variety of effects on renal blood flow, acetylcholine-induced vasodilation of afferent and efferent arterioles within glomerulus (Edwards, 1985) and inhibits sodium transport by cortical collecting tubule cells (Stoos *et al.*, 1992). NO decreases renal vascular responsiveness to vasoconstrictors (Ito *et al.*, 1991) while inhibitors of NO synthesis reduce renal blood flow (Lahera *et al.*, 1991). The source of NO in the kidney has been assumed to be endothelial cells, but constitutive forms of NO synthase was identified in tubule epithelial cells (Markewitz *et al.*, 1993) and macula densa at the junction of the ascending limb of the loop of Henle and the distal convuluted tubule. During tubular-fluid reabsorption in kidney, arginine-derived NO is generated in macula densa as an intercellular signalling molecule and mediates a vasodilating component to the tubuloglomerular feedback response by countering the vasoconstrction of the afferent arteriole (Wilcox *et al.*, 1992).

Ginsenoside (GS), a mixture saponin from *Panax ginseng*, protected renal function from ischemia and contralateral nephrectomy in rabbits (Zhang, 1992) and exerted antinephritic action by preventing urinary protein excretion, platelet aggregation and hypercellularity via increased renal blood flow in rats (Hattori *et al.*, 1991). For vascular tone, GS reversed norephinephrine or prostanglandin $F_{2\alpha}$-induced contraction of rabbit pulmonary and intrapulmonary arteries (Chen *et al.*, 19 84). Our previous study showed that GS promoted release of NO in pulmonary vascular endothelium and in clutured aortic endothelial cells (Kim *et al.*, 1992).

The purpose of the present study was twofold. Firstly, we evaluated whether GS prevents the suppressive effect of nitro-L-arginine, an inhibitor of nitric oxide synthase, on NO production in rat kidney by measuring plasma cGMP levels and NO production in whole kidney. Secondly, we compared basal NO production in three renal segments, glomerulus, cortex excluding glomerulus (cortex-) and medulla. After treatment of GS, changes in NO produced in renal segments were observed. Kidney DNA and protein contents, on wet weight basis, were determined as an index of cellular hypertrophy or hyperplasia.

Materials and Methods

Materials

Chemicals were from the following sources: [^{14}C]-

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L-arginine monohydrochloride (300 mCi/mmol) and cGMP assay kit, Amersham International plc (Buckinghamshire, England); Dowex AG 50WX-8, Bio-Rad Laboratories (Richmond, CA); β-Nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt (NADPH), and all other chemicals, Sigma Chemical Co. (St. Louis, MO). Ginsenoside (GS), purified from *Panax ginseng* C. A. Meyer, was provided from the Korea Ginseng & Tobacco Research Institute.

Animals

Male Sprague-Dawley rats (110~140 g) were housed two or three to a cage at an ambient temperature of 25℃, humidity of 55℃ and light/dark cycles of 12hr. They had free access to standard animal chow and to tap water throughout the study.

GS Treatment and Kidney Tissue Preparation

To examine the effect of ginsenoside (GS) on renal NO production, the study was performed by two ways. In study I, nitro-L-arginine (10 mg/kg/day), an inhibitor of nitric oxide synthase, was administered with or without GS (10 mg/kg/day) for 3 days intraperitoneally to determine the preventive effect of GS on nitro-L-arginine suppressed NO production in rat kidney. At sacrifice, whole kidney was removed, decapsulated and homogenized without perfusion. In study II, GS (30 mg/kg/day) was injected intraperitoneally for 4 days to evaluate the effect of GS on three renal segments; glomerulus, cortex excluding glomerulus (cortex-) and medulla. Rats were anesthetized with pentobarbital and a midline incision was performed to expose the aorta at the level of the renal arteries. A 22-gauge needle was placed in the aorta with the tip positioned between the left and right renal artery. The aorta was clamped above the level of the renal arteries and both renal veins were cut to facilitate drainage. The kidneys were perfused gently with phosphate buffered saline until they blanched and were then removed. One kidney from each rat was sliced and glomerulus, cortex excluding glomerulus (cortex-) and medulla were isolated by sieving technique from decapsulated kidneys (Shultz *et al.*, 1987). GS and nitro-L-arginine were dissolved in saline and control rats were received saline alone.

Renal DNA, Protein Contents and Plasma cGMP Assay

Each kidney tissue was homogenized in 50 m*l* of buffer containing 0.32 M sucrose, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4), 0.5 mM EDTA, 1 mM dithiothreitol and 5 mg of phenylmethylsulfonyl fluoride. Tissue protein was determined by the method of Lowry *et al.* (1951). Kidney DNA contents were measured by fluorophotometric assay using Hoechst 33258 according to Labarca and Paigen's method (1980). Plasma cGMP was assessed by radioimmunoassay kit commercially available.

Assay of NO Synthase

NO synthase was measured by determining the production of [^{14}C]-L-citrulline, which is a coproduct of the enzyme reaction from [^{14}C]-L-arginine. The reaction mixture (1 m*l*) containing 0.8 m*l* of homogenate, 0.45 mM calcium, 1 mM NADPH and [^{14}C]-L-arginine (20 nCi/m*l*) was incubated at 37℃ for 30 min. The reaction was stopped by addition of 1.5 m*l* of ice-cold Hepes/EDTA buffer (20 mM/2 mM, pH 6.0), which applied to a 1 m*l* Dowex 50 cation exchange column. [^{14}C]-L-citrulline in the effluent and a subsequent 1 m*l* water wash was quantified by liquid scintillation spectrometry (Kim *et al.*, 1992).

Statistics

All values represent mean± S. E. M. Differences among groups were determined by one-way ANOVA with Newman-Keuls test (Zar, 1984). Values were considered significantly different if $P<0.05$.

Results

Effect of GS on nitro-L-arginine suppressed NO production in whole kidney

We monitored the effect of 3 day treatment of nitro-L-arginine (10 mg/kg/day) on NO production in whole kidney and plasma cGMP level (Fig. 1). As an index of NO production, [^{14}C]-L-citrulline conversion from [^{14}C]-L-arginine was determined in kidney homogenate. Nitro-L-arginine, a competitive inhibitor of NO

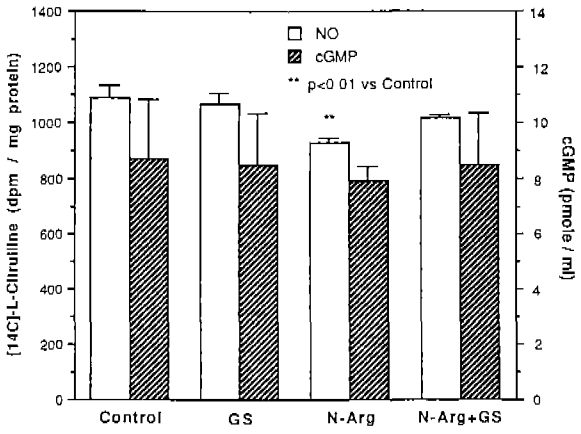

Fig. 1. [^{14}C]-L-citrulline conversion from [^{14}C]-L-arginine in whole kidney and plasma cGMP levels in basal and nitro-L-arginine suppressed rats with or without treatment of ginsenoside (30 mg/kg/3 days, *i.p.*). Significant decrease in renal NO production was observed in nitro-L-arginine treated rats ($n=5$, $P<0.01$).

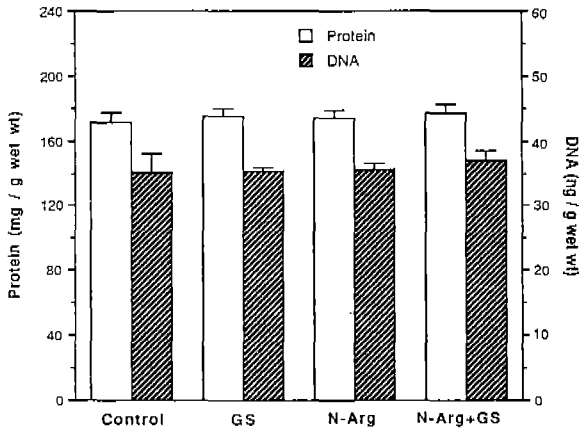

Fig. 2. Kidney DNA and protein contents in basal and nitro-L-arginine suppressed rats with or without treatment of ginsenosides (30 mg/kg/3 days, *i.p.*). Neither ginsenosides (GS) nor nitro-L-arginine showed any effects on DNA and protein contents in whole kidney.

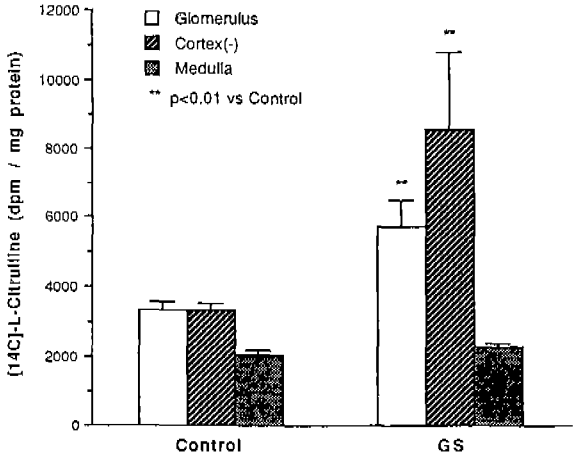

Fig. 3. Effect of ginsenoside on NO production in three nephron segments of rats. In control rats, NO production was similar in glomerulus and cortex- while medulla produced relatively small amount of NO. Four day treatment of ginsenoside, daily dose of 30 mg/kg body weight, significantly increased NO production in glomerulus and cortex- of kidney (n=5, P<0.01).

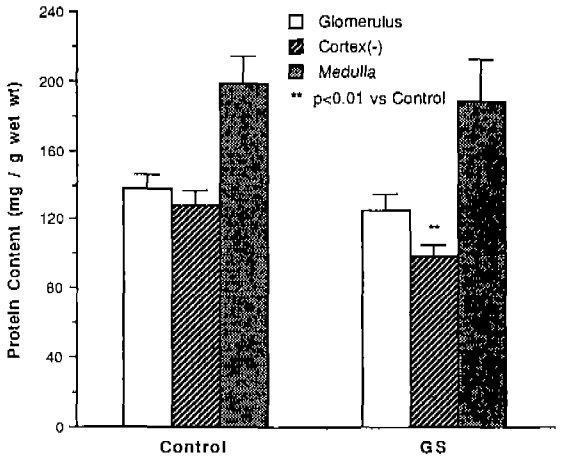

Fig. 4. Effect of ginsenoside on kidney protein content. Protein content in medulla was higher than that in glomerulus or cortex- in control rats. Ginsenoside significantly reduced protein content in cortex-.

synthase, significantly reduced NO production (928± 17 dpm/mg protein, n=5, P<0.01) than control (1085± 46). GS treatment (30 mg/kg/3 days) prevented the suppressed effect of nitro-L-arginine on renal NO production (1013± 12.5) and GS itself had no effect on NO production (1065± 41). Plasma cGMP levels tended to be lowered in nitro-L-arginine treated rats but there were no significant differences among groups. As shown in Fig. 2, neither GS nor nitro-L-arginine showed any effects on DNA and protein contents in whole kidney. Total dose of 30 mg GS/kg body weight did not induce cellular hypertrophy or hyperplasia in whole kidney.

Effect of GS on basal NO production in renal segments

We compared NO production in three renal segments; glomerulus, cortex excluding glomerulus (cortex-) and medulla (Fig. 3). In control rats, NO production was similar in glomerulus (3355± 235) and cortex (3344± 215) while medulla produced relatively small amounts of NO (2032± 178). Four day treatment of GS (30 mg/kg/day) significantly increased NO production in glomerulus (5741± 757 dpm/mg protein), and cortex- (8535± 292, n=5, P<0.01) while medulla (2286 ± 119) produced similar NO as that produced by saline-injected control rats. In control rats, weighing 110~140 g, protein content in medulla (198± 6.8 mg/g wet weight) was higher than that in glomerulus (137± 3.8) or cortex- (128± 3.6). GS significantly reduced protein content in cortex- (78± 2.8, n=5, P<0.05) while protein contents in glomerulus (124± 4.4) and medulla (187± 10.5) were not significantly different from control rats (Fig. 4). DNA content in medulla (1152± 125 ng/g wet weight) was the highest and then those in cortex- (578± 35) and in glomerulus (437± 26) in reducing order. GS significantly reduced glomerular DNA content (322± 33). DNA contents in cortex- (373 ± 100) and in medulla (969± 96) tended to be decreased but there was no significant difference between DNA contents in cortex- and in medulla.

Discussion

Endothelium plays an important role in the regula-

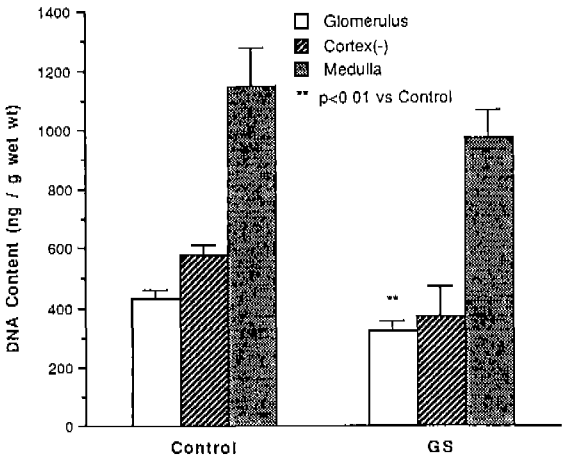

Fig. 5. Effect of ginsenoside on DNA content of kidney. DNA content in medulla was the highest and then those in cortex- and in glomerulus in reducing order. Glomerular DNA content, on wet weight basis, was significantly decreased by treatment of ginsenoside (120 mg/kg/4 days, *i.p.*).

tion of renal function, as has been demonstrated only recently (Baylis *et al.*, 1990; Lahera *et al.*, 1991a, b; Walder *et el.*, 1991; Welch *et al.*, 1991; Salom *et al.*, 1991). The capacity of the renal vasculature to respond to known endothelium-dependent vasodilators such as acethylcholine or bradykinin suggests that this vascular bed possesses the ability to produce large quantities of endothelium-derived nitric oxide (EDNO) (Lahera *et al.*, 1991b). It has been reported that basal production of EDNO is required to maintain the normal level of renal blood flow (RBF) and glomerular filtration rate (GFR) (Welch *et al.*, 1991; Walder *et al.*, 1991). The kidney seems to be more sensitive than other organs to the acute inhibition of EDNO synthesis because it has been demonstrated that RBF, GFR, natriuresis and diuresis were reduced during the intravenous infusion of N^G-nitro-L-arginine-methyl ester at a dose that did not modify arterial pressure (Lahera *et al.*, 1991a). Besides endothelial cells, L-arginine-derived nitric oxide is produced in glomerular mesangial cells (Schultz *et al.*, 1990), renal epithelial cells (Ishii *et al.*, 1989) and macula densa at the junction of the ascending limb of the loop of Henle and the distal convuluted tubule (Wilcox *et al.*, 1992), but its integral role in the control of renal function is not clear.

Ginsenoside (GS), saponin purified from *Panax ginseng*, provides different cardiovascular and hemodynamic effects depending on blood vessels; GS significantly reduced vetebral and femoral vascular resistance but increased renal vascular resistance by controlling blood flow (Chen *et al.*, 1984). For vascular endothelium, GS promoted NO release in pulmonary artery *in situ* and aortic endothelial cells *in vitro* (Kim *et al.*, 1992). Antinephritic action of GS came from increased renal blood flow in rats (Hattori *et al.*, 1991).

In present study, NO production in whole kidney was significantly reduced by nitro-L-arginine, a potent inhibitor of the enzymatic transformation of arginine to NO, which confirms the existence of constitutive NO synthase in kidney. Intrarenal infusion of N^G-nitro-L-arginine inhibited acetylcholine-induced renal vasodilation and reduced renal blood flow (Kiyomoto *et al.*, 1992; Naess *et al.*, 1992) and significantly enhanced the vasoconstrictor responses to sympathetic nerve stimulation and noradrenaline (Reid and Rand, 1992). These results suggest the contribution of NO in regulation of renal hemodynamics. NO activates soluble guanylate cyclase which converts GTP to cGMP. Thus, injection of acetylcholine, sodium nitroprusside and atrial natriuretic factor caused a marked release of cGMP into the venous effluent of isolated perfused rat kidneys (Heuze-Joubert *et al.*, 1992). Dose of nitro-L-arginine used in study I, 30 mg/kg/3 days, *i.p.*, was enough to inhibit NO production in kidney but not enough to suppress plasma reflection of cGMP. This dose of GS did not stimulate renal NO production but prevented nitro-L-arginine suppressed NO production in whole kidney, suggesting a possibility of stimulatory effect of GS on renal NO production by activating NO synthase.

To evaluate the stimulatory effect of GS on NO production in kidney, we compared basal NO production in three renal segments (glomerulus, cortex excluding glomerulus and medulla) after the administration of relative high dose of GS (120 mg/kg/4 days, *i.p.*) in study II. GS selectively increased NO production in glomerulus and cortex- of control rats. Glomerular DNA content was significantly reduced by GS, which may be explained by glomerular expansion by GS' vasodilator action. It seems that target segment of nephron for volume expansion due to GS' NO-mediated vasodilation and for NO producton stimulated by GS is cortex including glomerulus.

In our *in vitro* treatment of GS (2, 20, 200 μg/ml) showed different effect on NO synthesis in rat kidney; 20 μg/ml of GS stimulated NO production in glomerulus, cortex excluding glomerulus and medulla while lower (2 μg/ml) and higher (200 μg/ml) concentrations of GS had no effects on renal NO production (data not shown). This result supports present different effect of GS depending on dose injected.

For further study, nitro-L-arginine shall be applied

to animals treated with high dose of GS (120 mg/kg/4 days, *i.p.*) to compare the effect of low and high dose of GS on basal and nitro-L-arginine suppressed NO productin in kidney.

Acknowledgement

This study was partially supported by the grant from the Korea Ginseng and Tobacco Research Institute, Taejon, Korea.

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