

Soluble silica and coral sand suppress high blood pressure and improve the related aortic gene expressions in spontaneously hypertensive rats

Fusako Maehira^{a,*}, Kyoko Motomura^a, Nau Ishimine^a, Ikuko Miyagi^a,
Yukinori Eguchi^b, Shoei Teruya^c

^aDepartment of Biometabolic Chemistry, School of Health Sciences, Faculty of Medicine, University of the Ryukyus, Nishihara, Okinawa 903-0215, Japan

^bResearch Laboratory Center, Faculty of Medicine, University of the Ryukyus, Nishihara, Okinawa 903-0215, Japan

^cOkinawa Industrial Technology Center, Gushikawa, Okinawa 904-2234, Japan

Received 26 August 2010; revised 2 December 2010; accepted 7 December 2010

Abstract

Silicon is rich in the normal human aorta but decreases with age and the development of atherosclerosis. We hypothesized that soluble silica (Si) and coral sand (CS), as a natural Si-containing material, would suppress high blood pressure (BP) in spontaneously hypertensive rats (SHRs), and clarify the observed antihypertensive mechanism by cell cultures by quantifying messenger RNA expressions in the aorta. In SHR fed diets containing 1% Ca supplemented with CaCO₃ as the control (CT) and CS in a Ca-deficient diet and containing 50 mg/kg Si in the CT diet for 8 weeks, systolic BP was significantly ($P < .05$) lowered by 18 mm Hg for the Si group and 16 mm Hg for the CS group compared with the control CT group with 207 mm Hg. Magnesium (Mg) uptake by rat aortic smooth muscle cells significantly increased (177%, $P < .005$) in cells cultured with a physiologic Mg level plus Si compared with those with no Si addition. Furthermore, the increase of systolic BP by the CT diet was significantly suppressed by 17 mm Hg ($P < .001$) in SHR fed the diet containing Mg along with Si, but not by the Mg-deficient diet with or without Si. Soluble silica and CS treatments suppressed the aortic gene expressions of angiotensinogen and growth factors related to vascular remodeling, whereas, Si stimulated the expression of peroxisome proliferator-activated receptor- γ , the activation of which has anti-inflammatory and antihypertensive effects on vascular cells. These findings suggest that Si reduces hypertension in SHR by stimulating the intracellular Mg uptake and related gene expression in the aorta.

© 2011 Elsevier Inc. All rights reserved.

Keywords:

Antihypertension; Soluble silica; Intracellular magnesium; Aortic gene expressions; Spontaneously hypertensive rats

Abbreviations:

ACE, angiotensin-converting enzyme; AGN, angiotensinogen; Ang II, angiotensin II; ASMC, aortic smooth muscle cells; AT1A, angiotensin II type 1A receptor; bFGF, basic fibroblast growth factor; BP, blood pressure; CS, coral sand; DMEM, dulbecco's modified eagle's medium; eNOS, endothelial nitric oxide synthetase; Mg, magnesium; PDGF-A, platelet-derived growth factor A chain; PPAR, peroxisome proliferator-activated receptor; PCR, polymerase chain reaction; RAS, renin-angiotensin system; SHR, spontaneously hypertensive rats; Si, soluble silica; TGF- β 1, transforming growth factor β 1.

1. Introduction

Next to oxygen, silicon is the most abundant (29.5%) element of the Earth's crust. The most commonly occurring

mineral is quartz, SiO₂, which is a major constituent of igneous and sedimentary rocks. The biologically important form is silicic acid, Si(OH)₄, which is referred to as "a soluble silica (Si)" and freely diffusible across cell walls and membranes. Silicic acid is not found naturally in large quantities; at concentrations above 2 mmol/L (56 mg/L),

* Corresponding author. Tel.: +81 98 895 1277; fax: +81 98 895 1277.

E-mail address: fmaehira@med.u-ryukyu.ac.jp (F. Maehira).

silicic acid molecules undergo polymerization to form insoluble polysilicic acid [1]. Bioavailable soluble silicate (monosilicic acid) reacts with molybdate reagent to give a yellow color, whereas, polysilicic acid does not react with this reagent [1,2]. Silicon levels in human serum have been estimated at an average of about 0.5 mg/L in the soluble form [1,3], and this range is similar to those found for most of the other well-recognized trace elements in human nutrition. Connective tissues such as the aorta, trachea, tendon, bone, skin, and appendages are unusually rich in Si, containing 4 to 5 times more Si than the liver, heart, and muscles in the rat [3]. The Si content of the normal human aorta decreases considerably with age; furthermore, the level of silicon in the arterial wall decreases with the development of atherosclerosis [4]. The potential involvement of Si in atherosclerosis has been suggested by others [5,6], but so far, to our knowledge, no attention has been paid to the possible effects of Si intake on blood pressure (BP).

Elevated BP is a major contributor in cardiovascular and renal diseases and stroke worldwide [7]. Although the multigenic nature of essential hypertension is widely accepted, the importance of dietary factors including minerals in the primary prevention and control of high BP has been emphasized for normotensive and hypertensive individuals [8]. An inverse relationship between dietary magnesium (Mg) intakes and BP has been reported [9,10]. A large volume of evidence [11] has accumulated showing that Mg, a natural Ca antagonist [12,13], modulates vasomotor tone, BP, and peripheral blood flow through the regulation of intracellular Ca ions. Low serum Mg levels have been involved in various events of atherosclerosis in basic [14] and clinical studies [15].

Because the key role of genetic factors in regulating the multiple pathways in neural, endocrinal, and vascular systems is proposed to be related to the pathogenesis of essential hypertension [16], alterations of gene expression can affect hypertension. In this study, we hypothesized that Si may improve high BP in spontaneously hypertensive rat (SHR), a model used to study multiple factors in the etiology of essential hypertension, and test in vivo the mechanism of the suppressive effect of Si on the elevation of BP by culturing rat aortic smooth muscle cells (ASMCs). This will be done by quantifying messenger RNA (mRNA) expressions related to hypertension in the SHR aorta. We also examined the antihypertensive effects of coral sand (CS) as a natural Si-containing material, together with strontium (Sr), which is a constituent of CS, to demonstrate similar antidiabetic effects for Si and CS, including genetic expressions in our previous study [17].

2. Methods and materials

2.1. Animal experiments

The experimental protocols of this study were approved by the Animal Experiment Ethics Committee of the University of the Ryukyus. Male SHR, aged 4 weeks, were

purchased from Japan SLC, Inc (Shizuoka, Japan). The rats were divided into 4 groups of 8 each and housed 2 per cage at 24°C with a 12-hour light-dark cycle. Before the start of the experiment, the rats were given free access to a commercial diet (MF; Oriental Yeast Co, Tokyo, Japan) and tap water. They were also acclimated to the measurement of systolic BP by the tail-cuff method using an automatic BP analyzer (model BP-98A; Softron Co, Tokyo, Japan) for 3 weeks according to the manufacturer's instructions. The rats at 7 weeks of age were maintained for 8 weeks on an ad libitum semisolid experimental diet of 34% tap water and 66% the experimental powder diet (or powder diet), containing 1% Ca supplemented with CaCO₃ as the control (CT) and CS in Ca-deficient purified diet (Oriental Yeast Co) and diet with 50 mg/kg Si or 750 mg/kg Sr added to the CT diet (Tables 1 and 2); these doses have been used in previous studies without toxic effects [3,5,17,18]. Systolic BP was measured twice a week, and the mean of 3 repeated measurements was used for each datum. Available soluble silicon compound, sodium metasilicate Na₂SiO₃·9H₂O, and strontium chloride SrCl₂·6H₂O were used in the animal studies. The CS used was defined in detail previously [18]. In brief, CS smaller than approximately 1 cm is collected from the sea floor at 55- to 60-m depth in the designated sea region by using a sand pump with a mesh filter, and larger CS is returned to the sea. Crude CS smaller than approximately 2 mm is disinfected at 120 to 200°C and ground into a fine powder less than 22 μm.

At the end of the 8-week-experiment, rats from each group were euthanized by ether anesthesia at regular intervals after 6-hour fasting, and heparinized blood was obtained from each rat by heart puncture. Blood samples were centrifuged, and plasma was divided into aliquots and stored at -80°C until analysis. The immediately excised thoracic-to-abdominal aorta (from which other tissues were removed) was homogenized for RNA isolation. The results

Table 1
Ingredient composition of the experimental diet^a

Ingredient (g/kg diet)	Basal diet
Cornstarch	380
Casein	250
α-Cornstarch	100
Cellulose	80
Soybean oil	60
Oriental mineral mix ^b	60
Oriental vitamin mix ^c	20
Sucrose	50

^a Purified basal diet was prepared by Oriental Yeast Co.

^b Oriental mineral mix composition (g/kg): CaHPO₄·2H₂O, 145.6; Ca lactate, 350.9; KH₂PO₄, 257.2; NaHPO₄ 93.5; NaCl, 46.6; Fe citrate, 31.8; MgSO₄, 71.7; ZnCO₃, 1.1; MnSO₄·4-5H₂O, 1.2; CuSO₄·5H₂O, 0.3; KI, 0.1.

^c Oriental vitamin mix composition (g/kg): thiamin HCl, 1.2; riboflavin, 1.2; pyridoxine HCl, 0.8; nicotinic acid, 6.0; inositol, 6.0; Ca pantothenate, 5.0; *p*-aminobenzoic acid, 5.0; folic acid, 0.2; D-biotin, 0.02; vitamin B₁₂, 0.0005; vitamin C, 30; vitamin A acetate, 500 000 IU; vitamin D₃, 100 000 IU; vitamin E acetate, 5.0; vitamin K₃, 5.2.

Table 2
Mineral composition of the diets

	Minerals in basal diet and CS		Minerals in the experimental diets			
	Basal diet ^a	CS	CT	Si	CS	Sr
Ca (g/kg)	11.1 ^b	361.0	11.1	11.1	11.1	11.1
Mg (g/kg)	2.4 ^c	23.0	2.3	2.3	3.0	2.3
Na (g/kg)	2.4	3.2	2.3	2.3	2.4	2.3
K (g/kg)	8.7	0.2	8.5	8.5	8.4	8.5
P (g/kg)	8.3	0.0	8.1	8.1	8.1	8.1
Sr (mg/kg)	29.5	2800.0	28.7	28.7	113.0 ^c	758.0 ^c
Si (mg/kg/ ^d)	0.2	9.8	0.2	50.2 ^c	0.5 ^c	0.2
Fe (mg/kg)	320.0	390.0	311.1	311.1	331.7	311.1
Zn (mg/kg)	51.0	4.7	49.6	49.6	49.6	49.6
Cu (mg/kg)	7.5	2.3	7.3	7.3	7.3	7.3
Mn (mg/kg)	53.2	170.0	51.7	51.7	56.7	51.7

Ca indicates calcium; CS, coral sand; CT, calcium carbonate (control); Cu, copper; Fe, iron; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; P, phosphorus; Si, silicon; Sr, strontium; Zn, zinc.

^a All basal diets were prepared by Oriental Yeast Co.

^b Ca was eliminated for Ca-deficient basal diet, and calcium carbonate or CS was used for its replacement.

^c Mg was eliminated for Mg-deficient basal diet, and magnesium sulfate was used for its replacement.

^d Soluble Si was measured by micromolybdenum blue method [16].

^e The mineral levels are more than twice that of CT.

observed in the cell culture study were confirmed by the additional animal experiment of 4 groups of 8 rats each fed with the Mg-deficient purified diet (Table 2) with or without Mg as magnesium sulfate and with or without 50 mg/kg Si for 7 weeks. At the end of the 7-week-experiment, the excised thoracic aorta was kept at -80°C until assay for the Si, Mg, and Ca contents. The NaCl contents of all diets were adjusted to 1%.

2.2. Biochemical measurements

The soluble silica content in powder diets was measured by the micromolybdenum blue method [1,2,18]. Plasma angiotensin II (Ang II) was determined using a sandwich ELISA kit (Phoenix Pharmaceuticals, Burlingame, CA) in duplicate in single batches and averaged. The Si, Mg, and Ca contents were analyzed for the aorta. Aorta samples (63–133 mg) were digested in 1 mL of nitric acid in acid-cleaned Teflon vessels in a microwave digestion apparatus (MULTI-WAVE; Anton Paar GmbH, Graz, Austria-Europe). Blanks of acid without sample were also prepared and digested. The digested samples and blanks were transferred into an acid-cleaned polypropylene flask containing 1 mL of 0.05 mg/L $\text{Y}(\text{NO}_3)_3$ in 1 mol/L HNO_3 as an internal standard, and then diluted with 0.01N- HNO_3 to 10 mL. Ultrahigh purity grade nitric acid (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and ultrahigh purity water (Kanto Chemical Co, Inc, Tokyo, Japan) were used for the sample preparations. Digested samples and blanks were analyzed for Si at 251.611 nm, Mg at 280.271 nm, and Ca at 317.933 nm by an inductively coupled plasma atomic

emission spectrometer (Optima 4300 DV; PerkinElmer, Inc, Waltham, MA) in a single batch. Samples were analyzed with sample-based standards prepared by diluting aliquots of the stock element standard solutions (1000 mg/L; Wako Pure Chemical Industries).

2.3. Cell cultures

Rat ASMC (A7r5 from ATCC, Rockville, MD) were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS), 40 mmol/L sodium bicarbonate, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin, at 37°C in 5% $\text{CO}_2/95\%$ air. Aortic smooth muscle cells (1×10^6 cells/dish with 6-cm diameter) at 5-day subculture were made "quiescent" by another 3-day incubation in DMEM with 0.4% FCS and followed by a 5-day incubation in the same media containing 1 or 4 mmol/L Mg with 3 different concentrations of 0, 5, and 50 mg/L Si for the analysis of Mg uptake by ASMC. Aortic smooth muscle cells were rinsed, scraped, and suspended in ice-cold saline. The cells were centrifuged to a pellet at $700 \times g$, 4°C for 2 minutes, washed 2 times with cold saline, and then dissolved with 0.1 mol/L perchloric acid. The supernatant after centrifugation at $10\,000 \times g$, 4°C for 10 minutes, was subjected to measurement of Mg by atomic absorption spectrophotometer (model 518; Hitachi Co, Tokyo, Japan), and the mean of duplicate analyses was used. For the measurement of calcium (Ca) uptake by ASMC, after 1×10^6 ASMC were cultured in DMEM with 0.4% FCS for 3 days to establish quiescence, the cultured media were switched to identical fresh media with 0.1 $\mu\text{Ci}/\text{mL}$ $^{45}\text{CaCl}_2$ (2.4 mCi/mL; Amersham Biosciences, Piscataway, NJ) containing 1 or 4 mmol/L Mg with 3 different concentrations of 0, 5, and 50 mg/L Si as sodium metasilicate $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, and cells were incubated for 5 more days. Cells were then washed 3 times with ice-cold Krebs-Ringer (118 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl_2 , 1.2 mmol/L KH_2PO_4 , 1.2 mmol/L MgSO_4 , 10 mmol/L glucose, 25 mmol/L NaHCO_3) and scraped in 0.05% Triton X-100. After centrifugation at $10\,000 \times g$, 4°C for 5 minutes, aliquots of the supernatant were measured for ^{45}Ca radioactivity using a scintillation counter.

2.4. RNA isolation and gene expression analysis

Total RNA was isolated and purified using an RNeasy fibrous tissue midi kit (QIAGEN KK, Tokyo, Japan), including proteinase K digestion according to the manufacturer's protocol. The excised thoracic aorta was immediately homogenized using a rotor-stator homogenizer (Polytron, model PT1200; Kinematica AG, Westbury, NY); approximately 30 mg of tissue was immersed in 500 μL of lysing buffer containing guanidine thiocyanate and β -mercaptoethanol (QIAGEN) in a microcentrifuge tube and homogenized at 25 000 rpm for 2 minutes. Proteinase K digestion on the homogenate and the remaining procedures followed the protocol provided by the manufacturer. Two

micrograms of RNA was used for complementary DNA synthesis by reverse transcription with an Omniscript RT kit (QIAGEN). Quantitative real-time polymerase chain reaction (PCR) was performed using the Mx3000P real-time PCR system (Stratagene, La Jolla, CA), and fluorescent dye SYBR Green (Brilliant SYBR Green QPCR Master Mix; Stratagene) was used to detect a double-stranded DNA amplicon [17,18].

The mRNA expression of hypertension-related genes such as angiotensinogen (AGN), angiotensin-converting enzyme (ACE), angiotensin II type 1A receptor (AT1AR), platelet-derived growth factor A chain (PDGF-A), basic fibroblast growth factor (bFGF), transforming growth factor β 1 (TGF- β 1), peroxisome proliferator-activated receptor- γ and peroxisome proliferator-activated receptor- α (PPAR γ and PPAR α), adiponectin, and endothelial nitric oxide synthetase (eNOS) were analyzed using the pairs of primers summarized in Table 3. All reverse transcription PCR reactions contained first-strand complementary DNA corresponding to 1–10 ng of RNA. The PCR protocol included the following cycling conditions: 95°C denaturation for 10 minutes, then 45 cycles of 95°C denaturation for 30 seconds followed by 60°C annealing for 1 minute and 72°C extension for 30 seconds. The fluorescent amplicon was primarily detected at the end of the 60°C annealing period. Polymerase chain reaction products were subjected to melting curve analysis and quantified with Mx3000P software version 1.20c (Stratagene) [17,18]. Polymerase chain reaction results were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase in the same samples. Triplicate analyses were performed for each sample.

2.5. Statistical analyses

Statistical analysis of results was conducted by comparing each group with the control group using 1-way analysis of variance (SAS Institute Inc, Cary, NC). Statistical differences were analyzed by the 2-tailed Student *t* test (Microsoft Excel; Microsoft, Redmond, WA) and considered significant at $P < .05$. Individual differences between groups for gene expression were separately assessed using Duncan multiple range test, and means with different letters were significant at $P < .05$. Experimental results are reported as the means \pm SD of 8 rats, unless otherwise indicated. Data of the cell culture experiments are presented as the means \pm SEM of 3 dishes.

3. Results

All animals appeared healthy during the study period, and there were no significant differences in diet intake and weight gain among groups at the end of the experiment. The average daily intake of minerals per rat adjusted for the average dietary intake through the experimental period, together with plasma Ang II levels at the end of the experiment, is presented in Table 4. The plasma Ang II level was significantly suppressed to 65% and 70% of the control in the Si and CS groups, respectively. Increased Si intake was determined to be 267-fold in the Si group and 2.6-fold in the CS group, whereas Sr intake was 27-fold in the Sr group and 4-fold in the CS group compared with the CT group because of the rapid elimination of Si from the circulation into urine [19].

Table 3
Primer sequences for real-time PCR

Target gene	Primers	Product size (base pair)	
GAPDH	F	5'-GAGTCTACTGGCGTCTCAC-3'	281
	R	5'-CCATCCACAGTCTTCTGAGT-3'	
ACE	F	5'-AGAAGGCCAAGGAGCTGTATG-3'	201
	R	5'-AGCAGGTGGCAGTCTTGTTG-3'	
AGN	F	5'-CCTCGCTCTCTGGACTTATC-3'	226
	R	5'-CAGACACTGAGGTGCTGTTG-3'	
AT1AR	F	5'-TCTCAATCTCGCCTGGCTGACT-3'	155
	R	5'-AAGGAACACACTGGCGTAGAGGTT-3'	
PDGF-A	F	5'-CTTGAGACAAACCTGAGAG-3'	143
	R	5'-GTATCTCGTAAATGACCGTC-3'	
bFGF	F	5'-AAGCGGCTCTACTGCAAG-3'	372
	R	5'-AGCAGACATTGGAAGAAACA-3'	
TGF- β 1	F	5'-GCCCTGGATACCACTACTGCT-3'	161
	R	5'-AGGCTCCAAATGTAGGGGCAAG-3'	
PPAR- γ	F	5'-CGAGCCCTGGCAAAGCATTGTAT-3'	90
	R	5'-TGTCTTTCCTGTCAAGATCGCCCT-3'	
Adiponectin	F	5'-CATTATGACGGCAGCACTGGCAAA-3'	123
	R	5'-AGAGAACGGCCTTGTCTTCTTGA-3'	
PPAR- α	F	5'-AGACCTGTGCATGGCTGAGAAGA-3'	163
	R	5'-TTTGCAAAGCCTGGGATAGCCTTG-3'	
eNOS	F	5'-TCACTGTAGCTGTGCTGGCATAACA-3'	162
	R	5'-AAGGCAAGTTAGGATCAGGTGGCA-3'	

GAPDH indicates glyceraldehyde-3-phosphate dehydrogenase.

Table 4

Body weight; average daily intakes of diet, Si, Sr, and Mg per rat; and plasma angiotensin II

	CT group	Si group	CS group	Sr group
Body weight (g) at the end of experiment	234.4 ± 51.7	223.4 ± 51.2	239.1 ± 52.8	224.3 ± 50.1
% Increase	133 *	142 *	143 *	142 *
% Body weight	100	95	102	96
Average daily intakes				
Diet ^a (g)	24.2 ± 2.0	25.2 ± 0.8	24.7 ± 1.6	24.4 ± 0.9
% Intake	100	104	102	101
Si (μg)	4.7 ± 0.4	1265.0 ± 40.2	12.4 ± 0.8	4.9 ± 0.2
% Intake	100	26661 ***	260 ***	103
Sr (mg)	0.7 ± 0.1	0.7 ± 0.02	2.8 ± 0.2	18.5 ± 0.7
% Intake	100	100	402 ***	2665 ***
Mg (mg)	56.5 ± 4.7	58.8 ± 1.9	74.5 ± 4.8	56.9 ± 2.1
% Intake	100	104	132 ***	101
Plasma angiotensin II (μg/L)	2.76 ± 0.54	1.84 ± 0.49	1.93 ± 0.35	2.50 ± 0.42
% Level	100	65 **	70 **	88

CS, coral sand; CT, calcium carbonate (control); Mg, magnesium; Si, silicon; Sr, strontium. Values are expressed as mean ± SD (n = 8).

^a Powder diet = solid diet × 0.66.

* P < .001, significantly different from values at the start of experiment.

** P < .05, significantly different from the CT group.

*** P < .001, significantly different from the CT group.

The systolic BP changes in SHR during the 8-week-experiment are shown in Fig. 1. Systolic BP of all groups was elevated to about 190 mm Hg by 5 weeks after the start of feeding, and those of the CT and Sr groups continued to rise in parallel, showing approximately a 9-mm Hg difference until the end of experiment, whereas BPs of the Si and CS groups were significantly reduced after 6 weeks. At the end of the experiment, the mean systolic BPs of the Si, CS, and Sr groups were 18, 16, and

9 mm Hg lower than 207 mm Hg of the CT group, respectively, and values of the former 2 groups were significantly different. Mean systolic BP increases during the 8-week feeding period were 59 mm Hg (100%) in CT, 33 mm Hg (56%) in Si, 39 mm Hg (66%) in CS, and 53 mm Hg (90%) in Sr, respectively, and BP increases of the Si and CS groups were significantly suppressed compared with that of the CT group.

The study by culturing rat ASMC for 5 days at physiologic 1 mmol/L Mg in medium revealed that additions of 5 and 50 mg/L Si significantly increased the intracellular Mg content to 177% and 135%, respectively, as compared with no Si addition (Table 5). High concentrations of 4 mmol/L Mg and 50 mg/L Si in the medium reduced the intracellular Mg of rat ASMC. On the other hand, no differences were observed in Ca uptake by rat ASMC under the same conditions as for the Mg uptake cultures (Table 6).

The results observed in the cell culture study (Table 5) were confirmed by feeding rats for 7 weeks with the Mg-deficient diets with or without Mg as magnesium sulfate and with or without 50 mg/kg Si (Table 7, Fig. 2). As the average

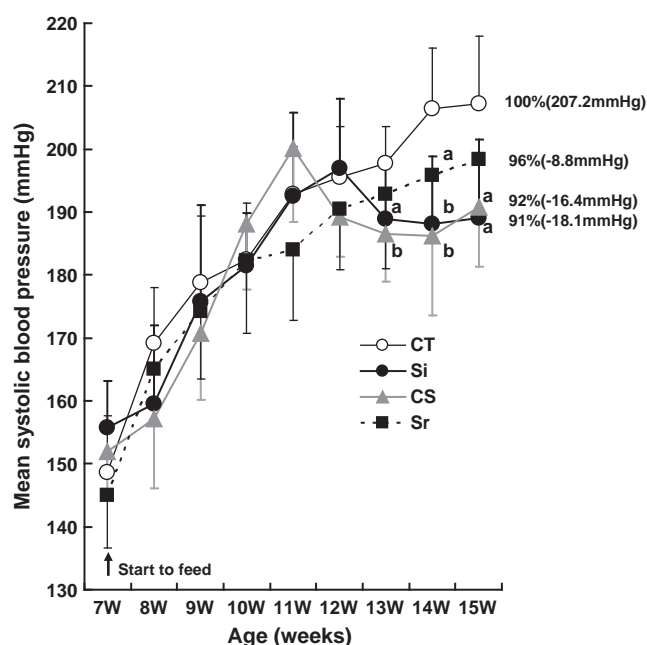


Fig. 1. Effects of silicon, strontium, and CS on mean systolic BP. CT indicates control group; Si, silicon group; CS, coral sand group. Values are means ± SD (n = 8). ^aP < .05, ^bP < .005, significantly different from the CT group.

Table 5
Effects of silicon on uptake of Mg by rat aortic smooth muscle cells

Periods (d)	Mg in medium (mmol/L)	Si in medium (mg/L)	Cellular Mg ^a (ng/dish)	%
5	1	0	34 ± 3	100
		5	60 ± 4	177 **
		50	46 ± 4	135 *
5	4	0	55 ± 4	100
		5	63 ± 4	115
		50	47 ± 4	86 **

^a Values are expressed as mean ± SEM of 3 dishes.

* P < .05, significantly different from the medium without Si.

** P < .005, significantly different from the medium without Si.

Table 6
Effects of silicon on uptake of ^{45}Ca by rat aortic smooth muscle cells

Periods (d)	Mg in medium (mmol/L)	Si in medium (mg/L)	Cellular Ca ^a ($\mu\text{g}/\text{mg}$ protein)	%
5	1	0	570 ± 14	100
		5	560 ± 14	99
		50	620 ± 14	109
5	4	0	630 ± 15	100
		5	610 ± 14	96
		50	610 ± 14	96

^a Values are expressed as mean ± SEM of 3 dishes.

daily intake of diet per rat during the experimental period was reduced to 80% in SHR fed with the Mg-deficient diet, the intake of NaCl and body weight were also significantly decreased to 80% and to 85% to 88%, respectively, as compared with those of rats fed with the Mg-supplemented diet (Table 7). There were no differences in water intake among the 4 groups. The average daily intake of Si per rat was determined to be 232-fold and 203-fold in both Si-supplemented groups, with or without Mg supplementation, when compared with the Mg but no Si-supplemented group, respectively, whereas it was 0.8-fold in the group fed the Mg-deficient diet. The increase of systolic BP in the basal Mg-containing diet to 190 mm Hg after 7 weeks of feeding was significantly suppressed to 17 mm Hg (91%, $P < .001$) in SHR fed the diet containing Mg along with Si, but not the Mg-deficient diet with or without Si addition (Fig. 2). Although an association of Mg deficiency with hypertension has been previously reported, the Mg-deficient diet with or without Si addition showed only a 6- to 8-mm Hg reduction from BP of the basal Mg diet. This was probably related to the significant reduction in food intake resulting in significant decreases of NaCl intake and body weight

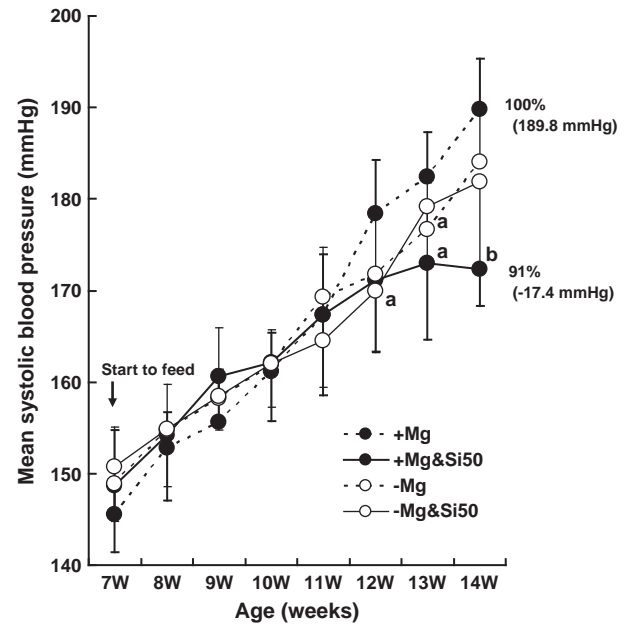


Fig. 2. Effects of silicon on BP of SHR fed diets with or without magnesium. Definitions of each group refer to “Methods and materials” and footnote of Table 7. Values are means ± SD (n = 8). ^a $P < .05$, ^b $P < .005$, significantly different from the +Mg group.

(Table 7). Aortic Si (122%) and Mg (112%) contents were significantly higher in SHR fed the diet containing Mg along with Si compared with those fed the basal Mg-containing diet, whereas a significantly reduced Mg (86%) content was observed in the group fed the Mg-deficient diet (Table 8). There were no significant differences observed in the aortic Ca contents among the groups.

Table 7
Body weight and average daily intakes of diet, NaCl, water, and Si per SHR fed diets with and without magnesium

Groups (8 rats/group)	+Mg ^a	+Mg ^a and 50 mg/kg Si	-Mg ^b	-Mg ^b and 50 mg/kg Si
Body weight (g) at the end of experiment	298.4 ± 11.0	304.4 ± 20.3	262.7 ± 10.0	253.0 ± 11.1
% Increase	140 ^d	145*	122*	121*
% Body weight	100	102	88**	85**
Average daily intakes per rat				
Diet ^c (g)	29.0 ± 3.8	26.8 ± 3.2	23.2 ± 3.8	23.5 ± 5.4
% Intake	100	93	80***	81***
NaCl (mg)	343.8 ± 44.4	381.1 ± 38.5	274.7 ± 44.7	278.9 ± 63.7
% Intake	100	93	80***	81***
Water (mL)	29.1 ± 4.4	30.0 ± 6.2	32.2 ± 5.2	29.3 ± 6.6
% Intake	100	103	111	101
Si (μg)	5.8 ± 0.8	1345.4 ± 160.6	4.6 ± 0.8	1179.7 ± 271.1
% Intake	100	23196**	80***	20340**

Values are expressed as mean ± SD (n = 8).

^a Magnesium sulfate was added to Mg-deficient diet as shown in Table 2.

^b Mg-deficient purified basal diet (Oriental Yeast, Co).

^c Powder diet = solid diet × 0.66.

* $P < .001$, significantly different from values at the start of experiment.

** $P < .05$, significantly different from the +Mg group.

*** $P < .001$, significantly different from the +Mg group.

Table 8
Aortic contents of Si, Mg, and Ca of SHR fed diets with and without magnesium

Groups (8 rats/group)	+Mg ^a	+Mg ^a and 50 mg/kg Si	-Mg ^b	-Mg ^b and 50 mg/kg Si
Aortic wet weight (mg)	103 ± 9	99 ± 21	95 ± 15	93 ± 22
% Weight	100	95	90	92
Mineral contents (μg/g tissues)				
Si	7.4 ± 1.2	9.0 ± 1.4	7.1 ± 1.1	8.3 ± 1.5
% Content	100	122*	96	112
Mg	40.4 ± 4.1	45.3 ± 4.4	34.8 ± 5.4	39.0 ± 6.3
% Content	100	112*	86*	96
Ca	252.0 ± 15.8	255.2 ± 16.7	264.6 ± 22.4	273.1 ± 27.1
% Content	100	101	105	108

Values are expressed as mean ± SD (n = 8).

^a Magnesium sulfate was added to Mg-deficient diet as shown in Table 2.

^b Mg-deficient purified basal diet (Oriental Yeast, Co).

* P < .05, significantly different from the +Mg group.

Changes in mRNA expressions related to hypertension in the SHR aorta are summarized in Fig. 3. Among members of the renin-angiotensin system (RAS) (Fig. 3A-C), mRNA expression of AGN (Fig. 3B) was suppressed to 76% and 70% in the Si and CS groups when compared with the control group, respectively; there were no differences in ACE and ATIAR expressions (Fig. 3A, C) among these 3 groups. The mRNA expressions of growth factors related to vascular remodeling (Fig. 3D-F) were significantly decreased to 71% and 76% in Si and CS groups for bFGF (Fig. 3E) and to 72% and 80% in Si and CS groups for TGF-β1 (Fig. 3F) when compared with the control group, respectively; there was no change in the PDGF-A expression among the 3 groups (Fig. 3D). Other mRNA expressions related to anti-inflammatory and antihypertensive effects on the vascular cells (Fig. 3G-J) were significantly increased in

the Si group to 135%, 154%, and 175% for PPARγ (Fig. 3G), adiponectin (Fig. 3H), and eNOS (Fig. 3J), respectively, whereas there were slight increases of 120% to 112% in the PPARα expression (Fig. 3I) of the Si and CS groups without statistical significance.

4. Discussion

Earlier studies suggested the potential involvement of Si in atherosclerosis based on observations in which the Si content in connective tissues of the aorta is richer than in other tissues but decreased with aging and the development of atherosclerosis [3-6]. One of those studies found an inverse relation between silicic acid in drinking water and the high mortality rate from coronary heart disease in Finland [5], and another epidemiologic study reported significant

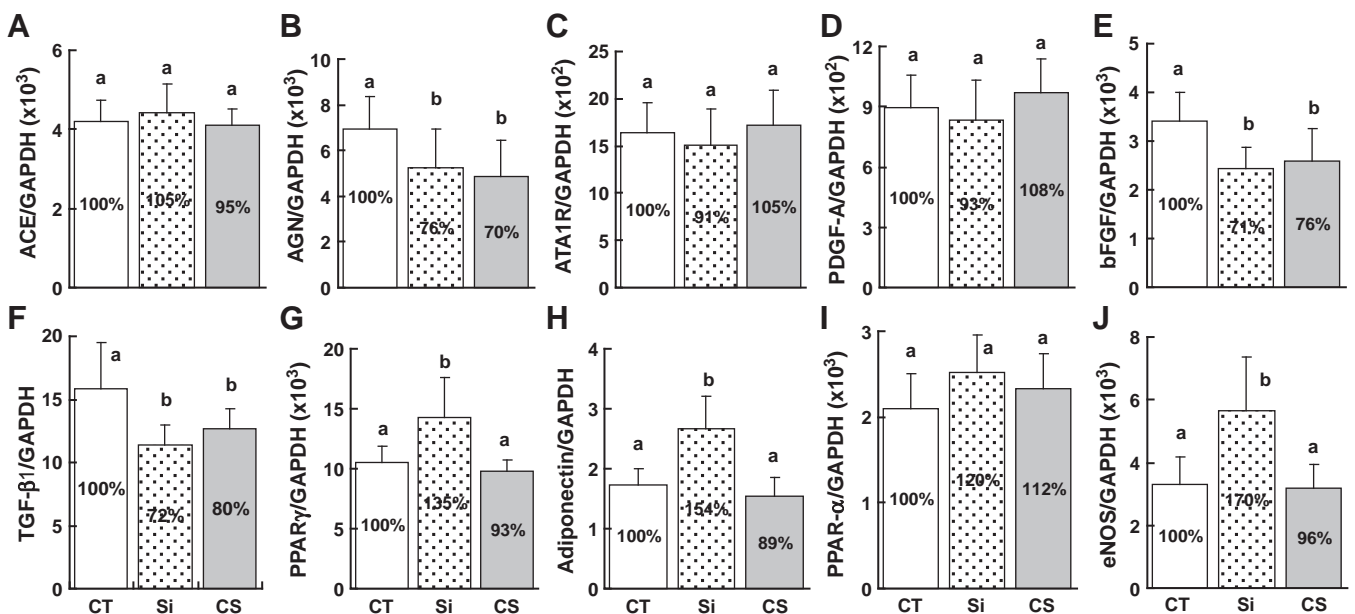


Fig. 3. Effects of silicon and CS on the gene expressions in aorta of SHR. CT indicates control group; Si, silicon group; CS, coral sand group. Values are means ± SD for 8 animals in each group. Means with different letters are significantly different at P < .05 (Duncan new multiple range test).

inverse correlations between drinking water levels of metals such as Ca, Mg, lithium, Sr, and Si and vascular disease mortality rates in Texas [6]. In the latter study, a significant inverse relationship of Si and Sr levels was seen with mortality rates due to hypertension with heart disease. In our findings, Si and natural Si-containing CS added to the control diet significantly prevented elevation of systolic BP by 18 mm Hg (9%) and 16 mm Hg (8%) below the control SHR, respectively, whereas Sr added to the control diet reduced systolic BP by only 9 mm Hg from 207 mm Hg of the control group. In a cell culture study conducted to elucidate the mechanism behind the observed BP-lowering effect of Si, Mg uptake by rat ASMC increased 1.8-fold when the cells were cultured in the presence of 5 mg/L Si and physiologic Mg levels compared with no Si addition to the medium, whereas Ca uptake by cells was not affected under the same conditions. The results of the cell culture were consistent with the hypotensive effect of Mg in the presence of Si observed in the additional animal study, probably due to the stimulation of Mg uptake by SHR aorta as found in the cell culture.

Magnesium acts extracellularly by blocking transmembrane Ca transport in vascular smooth muscle [11,13,20] and neural cells [11,21] by inhibiting norepinephrine release and intracellularly, as a Ca antagonist [12,13], thereby modulating the vasoconstrictor actions of increased intracellular Ca. Because the deficiency of intracellular Mg, the physiologically active form of the element has been implicated in hypertension [22,23], and intracellular Mg homeostasis in cardiovascular cells is regulated by various hormones and vasoactive agents [11], intracellular Mg levels may play a key role in modulating vascular tone and BP. However, the therapeutic value of Mg in the prevention and management of essential hypertension is inconsistent with data from clinical trials [10]. Identification and control of various factors influencing intracellular Mg homeostasis may contribute to an improvement of the inconsistency in results from clinical trials. In a study that focused on the influence of excessive oral inorganic silicon administration in the form of sodium metasilicate on the levels of Ca and Mg in serum and several tissues in Wistar rats, a decrease of serum Mg concentration was observed with an accompanying elevation of registered calcemia, whereas a reduction of aortic Ca levels was found with a simultaneous increase of aortic Mg pool [24], hence providing evidence for silicon involvement in the Ca and Mg metabolisms without clarification of its physiologic significance. In our present study with 1/20 less Si intake than the study discussed above, a similar result of a significant increase of aortic Mg content in SHR was observed when compared with the controls.

The contributions of the circulating RAS to regulation of BP, as well as electrolyte balance, are well known [25]. In addition to the circulating RAS, local angiotensin production is an important modulator of tissue function, and the RAS components are expressed and differentially regulated in multiple tissues, including those of the cardiovascular

system [26,27]. Among mRNA expressions of the RAS components in the aorta of SHR in the present study, AGN mRNA expression was suppressed in the Si and CS groups with no differences in ACE and AT1AR expressions, accompanied with significant suppressions of plasma Ang II levels. Aortic AGN mRNA expressions are positively regulated by hormonal stimuli including dexamethasone, estradiol, and thyroid hormone [25]; bacterial lipopolysaccharide [27]; and Ang II [28]. Agents known to decrease intracellular cyclic adenosine monophosphate (cAMP) such as Ang II or the cAMP-antagonists stimulated ANG synthesis, whereas cAMP-stimulating agents such as catecholamines, glucagon, or the cAMP-agonists inhibited ANG synthesis [28]. The rate-limiting step in the activation of adenylyl cyclase is conversion of the regulatory component from its inactive to active form, and both the rate and extent of this activation are regulated by the intracellular Mg ion, for which other divalent cations, including Ca^{2+} , did not substitute [29]. In addition, glucagon and arginine vasopressin, which increase cellular cAMP concentrations, have been reported to stimulate Mg^{2+} uptake in mouse distal convoluted tubule cells [30]. In an early study of Si-gonadal interaction [31], Ca-Mg imbalance caused by hormonal imbalances, which were themselves caused by natural (aging) and experimental (ovariectomy) factors, was improved by administration of Si and an involvement of intracellular cAMP in the mechanism was suggested without further clarification.

The underlying mechanism in the present study for the observed increases of the intracellular Mg content in ASMC or aortic Mg content of SHR by Si addition to the media or to the diets is unclear, but the stimulation of adenylyl cyclase activity by increased Mg in ASMC or aorta of SHR, the increase in intracellular cAMP, and the subsequent decrease in ANG mRNA expression could be deduced. Studies of ANG-deficient mice with hypotension [32] and of transgenic mice carrying human ANG gene with hypertension [33] indicated the critical involvement of ANG in regulation of BP. Angiotensin II, an enzymatic product of renin and ACE, via its receptor, is an important regulator not only of vascular smooth muscle contraction but also of vascular SMC proliferation and migration both in vitro and in vivo [34]. The former regulation by Ang II is induced through an increase of the intracellular Ca^{2+} and a decrease of the intracellular Mg^{2+} [22,35,36]. For the latter regulation, Ang II directly causes cellular phenotypic changes and cell growth [37]; regulates the gene expressions of various bioactive substances including growth factors such as PDGF-A, bFGF, and TGF- β 1 [38]; and activates multiple intracellular signaling cascades in vascular smooth muscle cells [34]. Such regulatory actions by Ang II may be involved in the pathophysiologies of vascular thickening, atherosclerosis, and cardiac hypertrophy. The observed suppression of bFGF and TGF- β 1 mRNA expressions with no changes of PDGF-A in both Si and CS groups may be associated with significant suppressions of AGN mRNA expression and plasma Ang II levels in the 2 groups.

Evidence has accumulated that PPAR γ activation or its agonist ligands, thiozolidinediones, inhibit Ang II-induced proliferation and migration of vascular SMC and endothelial cells [39,40]. Peroxisome proliferator-activated receptor- γ agonists also inhibit migration of monocytes and their adhesion to endothelial cells, as well as the secretion and gene expression of proinflammatory cytokines, including tumor necrosis factor α , interleukin-1, and interleukin-6 [40]. Hence, PPAR γ activation may attenuate inflammation and thus atherosclerosis and hypertension. Moreover, PPAR γ promotes the gene expression of both adiponectin [41] and its receptors [42], which improve hypertension and coronary artery disease [40,43–45]. Endothelial production of nitric oxide leading to vasodilation is activated by adiponectin through stimulation of eNOS phosphorylation at Ser1179, which is linked to the increase of eNOS enzymatic activity [46]. Nitric oxide activates PPAR γ binding through the p38 mitogen-activated protein kinase signaling pathway [47], thus contributing to the anti-inflammatory and cytoprotective effects of adiponectin, nitric oxide, and PPAR γ in the vasculature [48]. Another isotype of nuclear receptors, PPAR α , regulates the expression of various genes related to lipid metabolism and associated with beneficial effects such as prevention of the development of hypertension and attenuation of atherosclerosis [49].

Our results showed that gene expressions of PPAR γ , adiponectin, and eNOS were significantly stimulated in the Si group, and slight increases in the PPAR α expression of the Si and CS groups were observed. The possible causes exhibiting a similar efficiency in suppressing elevation of BP in the CS group to the Si group without increases in the antiatherogenic gene expressions of PPAR γ , adiponectin, and eNOS may be associated with the higher dietary intakes of 132% for Mg and 402% for Sr compared with the Si group.

Further studies are necessary to elucidate the mechanism underlying the stimulations of aortic intracellular Mg uptake and PPAR γ gene expression by soluble silica observed in the present study. Research, including long-term trials, is also needed to assess the safety and potentially beneficial roles of Si and CS as its natural source material in the prevention and management of hypertension.

In conclusion, as discussed above, this study demonstrated a novel function of a trace mineral, Si, in reducing hypertension in SHR by stimulating the intracellular Mg uptake and the antihypertensive and antiatherogenic gene expressions in aorta. Coral sand as a natural Si-containing material exhibited a similar efficiency in suppressing elevation of BP in SHR without increases in the antiatherogenic gene expressions in aorta, suggesting the involvement of a different mechanism.

Acknowledgment

This work was conducted by the annual research fund, provided to national universities under the Ministry of

Education and Science in Japan. We are grateful for the gift of coral sand from Coral Biotech KK (Okinawa, Japan).

References

- [1] Iler RK. The chemistry of silica. New York: John Wiley & Sons; 1979.
- [2] Tarutani T. Polymerization of silicic acid. *Anal Sci* 1989;5:245-52.
- [3] Carlisle EM. Silicon. In: Frieden E, editor. *Biochemistry of the essential ultratrace elements*. New York: Plenum Press; 1984. p. 257-91.
- [4] Loeper J, Loeper J, Fragny M. The physiological role of silicon and its antiatheromatous action. In: Bendz G, Lindqvist I, editors. *Biochemistry of silicon and related problems*. New York: Plenum Press; 1978. p. 281-96.
- [5] Schwarz K. Significance and functions of silicon in warm-blooded animals. In: Bendz G, Lindqvist I, editors. *Biochemistry of silicon and related problems*. New York: Plenum Press; 1978. p. 207-30.
- [6] Dawson EB, Frey MJ, Moore TD, McGanity WJ. Relationship of metal metabolism to vascular disease mortality rates in Texas. *Am J Clin Nutr* 1978;31:1188-97.
- [7] Lawes CMM, Vander Hoorn S, Law MR, Elliot P, MacMahon S, Rodgers A. Blood pressure and the global burden of disease 2000: part I estimates of blood pressure levels; part II estimates of attributable burden. *J Hypertens* 2006;24:413-30.
- [8] Appel LJ, Brands MW, Daniels SR, Karanja N, Elmer PJ, Sacks FM. Dietary approaches to prevent and treat hypertension: a scientific statement from American Heart Association. *Hypertension* 2006;47:296-308.
- [9] Kawano Y, Matsuoka H, Takishita S, Omae T. Effects of magnesium supplementation in hypertensive patients. *Hypertension* 1998;32:260-5.
- [10] Jee SH, Miller III ER, Guallar E, Singh VK, Appel LJ, Klag MJ. The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. *Am J Hypertension* 2002;15:691-6.
- [11] Touyz RM. Role of magnesium in the pathogenesis of hypertension. *Mol Aspects Med* 2003;24:107-36.
- [12] Isari LT, French JH. Magnesium: nature's physiologic calcium blocker. *Am Heart J* 1984;108:188-93.
- [13] Altura BM, Altura BT. Magnesium-calcium interrelationship in vascular smooth muscle. *Mag-Bull* 1986;8:338-50.
- [14] King JL, Miller RJ, Blue JP, O'Brien WD, Erdman JW. Inadequate dietary magnesium intake increases atherosclerosis plaque development in rabbits. *Nutr Res* 2009;29:343-9.
- [15] Amighi J, Sabeti S, Schlager O, Mlekusch W, Exner M, Lalouschek W, et al. Low serum magnesium predicts neurological events in patients with advanced atherosclerosis. *Stroke* 2004;35:22-7.
- [16] Page IH. The mosaic theory of arterial hypertension: its interpretation. *Perspect Biol Med* 1967;10:325-33.
- [17] Maehira F, Ishimine N, Miyagi I, Eguchi Y, Shimada K, Kawaguchi D, Oshiro Y. Anti-diabetic effects including diabetic nephropathy, of anti-osteoporotic trace minerals on diabetic mice. In press *Nutrition* 2010 10.1016/j.nut.2010.04.007.
- [18] Maehira F, Miyagi I, Eguchi Y. Effects of calcium sources and soluble silicate on bone metabolism and the related gene expression in mice. *Nutrition* 2009;25:581-9.
- [19] Poppwell JF, King SJ, Day JP, Ackrill P, Fifield LK, Cresswell RG, et al. Kinetics of uptake and elimination of silicic acid by a human subject: a novel application of ^{32}Si and accelerator mass spectrometry. *J Inorg Biochem* 1998;69:177-80.
- [20] Nakajima T, Iwasawa K, Hazama H, Asano M, Okuda Y, Omata M. Extracellular Mg^{2+} inhibits receptor-mediated Ca^{2+} -permeable non-selective cation currents in aortic smooth muscle cells. *Eur J Pharmacol* 1997;320:81-6.

- [21] Shimosawa T, Takano K, Ando K, Fujita T. Magnesium inhibits norepinephrine release by blocking N-type calcium channels at peripheral sympathetic nerve endings. *Hypertension* 2004;44:897-902.
- [22] Touyz RM, Schiffrin EL. The effect of angiotensin II on platelet intracellular free magnesium and calcium ionic concentrations in essential hypertension. *J Hypertens* 1993;11:551-8.
- [23] Resnick LM, Militianu D, Cunnings AJ, Pipe JG, Evelhoch JL, Soulen RL. Direct magnetic resonance determination of aortic distensibility in essential hypertension. Relation to age, abdominal visceral fat, and in situ intracellular magnesium. *Hypertension* 1997;30:654-9.
- [24] Najda J, Gmiński J, Drózd M, Danch A. The action of excessive, inorganic silicon (Si) on the mineral metabolism of calcium (Ca) and magnesium (Mg). *Biol Trace Elem Res* 1993;37:107-14.
- [25] Griendling KK, Murphy TJ, Alexander RW. Molecular biology of the renin-angiotensin system. *Circulation* 1993;87:1816-28.
- [26] Campbell DJ, Habener JR. Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. *J Clin Invest* 1986; 78:31-9.
- [27] Nyui N, Tamura K, Yamauchi S, Nakamaru M, Ishigami T, Yabana M, et al. Tissue angiotensinogen gene expression induced by lipopolysaccharide in hypertensive rats. *Hypertension* 1997;30:859-67.
- [28] Klett C, Nobiling R, Gierschik P, Hackenthal E. Angiotensin II stimulates the synthesis of angiotensinogen in hepatocytes by inhibiting adenylylase activity and stabilizing angiotensinogen mRNA. *J Biol Chem* 1993;268:25093-107.
- [29] Iyengar R. Hysteretic activation of adenylyl cyclases: II. Mg ion regulation of the activation of the regulatory component as analyzed by reconstitution. *J Biol Chem* 1981;256:11042-50.
- [30] Dai LJ, Bapty B, Ritchie G, Quamme GA. Glucagon and arginine vasopressin stimulate Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol* 1998;274:F328-35.
- [31] Charnot Y, Pérès G. Silicon, endocrine balance and mineral metabolism (calcium and magnesium). In: Bendz G, Lindqvist I, editors. *Biochemistry of silicon and related problems*. New York: Plenum Press; 1978. p. 269-80.
- [32] Tanimoto K, Sugiyama F, Goto Y, Ishida J, Takimoto E, Yagami K, et al. Angiotensinogen-deficient mice with hypotension. *J Biol Chem* 1994;269:31334-7.
- [33] Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, et al. Chimeric renin-angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. *J Biol Chem* 1993; 268:11617-21.
- [34] Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacol Rev* 2000;52:639-72.
- [35] Orlov SN, Resink TJ, Bernhardt J, Ferracin F, Buhler FR. Vascular smooth muscle cell calcium fluxes. Regulation by angiotensin II and lipoproteins. *Hypertension* 1993;21:195-203.
- [36] Touyz RM, Schiffrin EL. Activation of the Na^+H^+ exchanger modulates angiotensin II-stimulated Na^+ -dependent Mg^{2+} transport in vascular smooth muscle cells in genetic hypertension. *Hypertension* 1999;34:442-9.
- [37] Kubo A, Fukuda N, Teng J, Satoh C, Nakayama M, Kishioka H, et al. Angiotensin II regulates the cell cycle of smooth muscle cells from SHR. *Am J Hypertens* 2000;13:1117-24.
- [38] Satoh C, Fukuda N, Hu WY, Nakayama M, Kishioka H, Kanmatsuse K. Role of endogenous angiotensin II in the increased expression of growth factors in vascular smooth muscle cells from spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 2001;37:108-18.
- [39] Fukuda N, Hu WY, Teng J, Satoh C, Nakayama M, Kishioka H, et al. Troglitazone inhibits growth and improves insulin signaling by suppression of angiotensin II action in vascular smooth muscle cells from spontaneously hypertensive rats. *Atherosclerosis* 2002;163: 229-39.
- [40] Duan SZ, Usher MG, Mortensen RM. Peroxisome proliferator-activated receptor- γ -mediated effects in vasculature. *Circ Res* 2008; 102:283-94.
- [41] Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003;52:1655-63.
- [42] Sun X, Han R, Wang Z, Chen Y. Regulation of adiponectin receptors in hepatocytes by the peroxisome proliferator-activated receptor- γ agonist rosiglitazone. *Diabetologia* 2006;49:1303-10.
- [43] Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohashi K, Sugimoto K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension* 2004;43:1318-23.
- [44] Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, et al. Adiponectin replenishment ameliorates obesity-related hypertension. *Hypertension* 2006;47:1108-16.
- [45] Lu G, Chiem A, Anuurad E, Havel PJ, Pearson TA, Ormsby B, et al. Adiponectin levels are associated with coronary artery disease across Caucasian and African-American ethnicity. *Transl Res* 2007;149: 317-23.
- [46] Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003;278:45021-6.
- [47] Ptasinska A, Wang S, Zhang J, Wesley RA, Danner RL. Nitric oxide activation of peroxisome proliferator-activated receptor gamma through p38 MAPK signaling pathway. *FASEB J* 2007; 21:950-61.
- [48] Mathieu P, Poirier P, Pibarot P, Lemieux I, Despres JP. Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. *Hypertension* 2009;53:577-84.
- [49] Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: Regulators of gene expression in vascular cells. *Cir Res* 2004;94:1168-78.