DOI:10.1021/jf1006819

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Aqueous Garlic Extracts Prevent Oxidative Stress and Vascular Remodeling in an Experimental Model of Metabolic Syndrome

Marcela Alejandra Vazquez-Prieto,[†,‡] Roxana Elizabeth González,[§] Nicolás Federico Renna,[†,‡] Claudio Rómulo Galmarini,[§,∥,⊥] and Roberto Miguel Miatello*[,†,‡]

[†]Área de Fisiología Patológica, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Av. Libertador 80 (5500), Mendoza, Argentina, [‡]Instituto de Medicina y Biología Experimental de Cuyo (IMBECU), Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Av. Ruiz Leal s/n (5500), Mendoza, Argentina, [§]Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, (5505) Chacras de Coria, Luján, Mendoza, Argentina, [∥]EEA INTA La Consulta, CC8 (5567), La Consulta, Mendoza, Argentina, and [⊥]CONICET, Argentina

The organosulfur profile and the effect on oxidative stress and vascular remodeling in fructose-fed rats (FFR) were evaluated in Fuego INTA and Morado INTA garlic cultivars. Wistar rats were fed either normal rat chow (control) or the same diet plus 10% fructose in drinking water. During the last 6 weeks of a 12 week period of the corresponding diet, a subgroup of control and FFR received an aqueous extract of Fuego INTA and Morado INTA. Fuego INTA showed higher levels of total thiosulfinates, allicin, and pungency than Morado INTA. FFR showed an increase of systolic blood pressure, aortic NAD(P)H oxidase activity, plasma thiobarbituric acid reactive substances, and vascular remodeling that was significantly reduced after both garlic administrations. The beneficial effect was slightly higher when Fuego INTA was administered. Both aqueous garlic extracts prevent oxidative stress and vascular remodeling in rats with metabolic syndrome, suggesting the existence of slight differences among cultivars.

KEYWORDS: ***Allium sativum*; metabolic syndrome; oxidative stress; vascular remodeling**

INTRODUCTION

Cardiovascular disease is the leading cause of mortality in western countries and is responsible for 30% of deaths worldwide (*1*). Metabolic syndrome (MS) characterized by hyperglycemia, hyperlipidemia, and hypertension is a significant risk factor for cardiovascular disease where insulin resistance is the underlying factor that links atherosclerosis to MS (*2*). Fructose-fed rats (FFRs) provide a model of dietary-induced insulin resistance, which has been used to assess the pathophysiological mechanisms involved in metabolic and cardiovascular changes associated with MS (*3*, *4*). The hypertensive and metabolic effect of a fructose diet is associated with oxidative stress (*5*), but the precise mechanism is not fully understood. Reactive oxygen species (ROS) play a physiological role in the vessel wall. ROS, especially superoxide anion ($^{\bullet}O_2^-$), inactivate nitric oxide ($NO^{\bullet}$) forming peroxynitrite, which constitutes a strong oxidant molecule that affects proteins, lipids, and nucleic acids. The $NO^{\bullet}$ bioavailability is critical in the modulation of vascular remodeling. The major sources of ROS in vascular tissue are membrane-associated NAD(P)H-oxidase (*6*). In this model of MS, our previous studies showed an enhanced aortic NAD(P)H-oxidase activity (*4*) and lipid peroxidation (*7*). More recently, we demonstrated for the first time that FFRs developed vascular remodeling. This remodeling was reverted by chronic aspirin administration (*8*), supporting the hypothesis that vascular inflammation contributed to arterial vascular remodeling (*9*).

Dietary factors play a key role in the development or prevention of various human diseases, including cardiovascular disease and MS (*10*). Garlic (*Allium sativum* L.) is now used all over the world as a spice and has played an important medical as well as dietary role in human history (*11*). Subsequent studies found the efficacy of garlic as a cardioprotectant and in the regulation of blood pressure and lowering blood sugar and cholesterol levels (*12*). It is also effective against bacterial, viral, fungal, and parasitic infections, enhancing the immune system and having antitumoral and antioxidant features (*13*). Many of the physiological effects are attributed to volatile sulfur-containing flavor compounds, which are responsible for its pungent aroma (*14*). These compounds are alk(en)yl-thiosulfinates formed by the action of allinase (EC 4.4.1.4), an odorless and nonvolatile cysteine-derived substance; *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs) are released when garlic tissue is damaged (*15*). Allicin (allyl-2-propenethiosulfinate), formed from the precursor alliin (*S*-2-propenyl-L-cysteine sulfoxide), is the predominant garlic thiosulfinate and a potent platelet inhibitor (*16*). Although several

*To whom correspondence should be addressed. Tel: 54 261 4135000 ext. 2686. Fax: 54 261 4135242. E-mail: rmiatell@fcm.uncu.edu.ar.

 Published on Web 05/07/2010

studies relate garlic consumption to the control of cardiovascular disorders, many of them are controversial, mainly due to methodological differences (*17*). Furthermore, there is no study that compares the beneficial effects exerted by different cultivars of garlic on an MS experimental model in rats.

The main objective was to study the effect of chronic administration of different garlic cultivars on oxidative stress and vascular remodeling in FFRs, an experimental model of MS. Furthermore, we analyzed the organosulfur profile from garlic cultivars.

MATERIALS AND METHODS

Plant Material. Two garlic cultivars, Fuego INTA and Morado INTA, belonging to different ecophysiological groups (*18*), from the germplasm collection of INTA La Consulta, Mendoza, Argentina, were used in all experiments. These cultivars are extensively cultivated and consumed in Argentina. Fuego INTA is a red type garlic clone, with a relatively mild pyruvate content and high antiplatelet activity, while Morado INTA is a purple garlic clone, which has a low pyruvate content and relatively mild antiplatelet activity (*18*). Garlic cultivars were field-grown at the INTA La Consulta experimental station in Mendoza in 2006. Mature bulbs were harvested and fully cured and were then stored at 0 ± 3 °C. When most of the unsprouted bulbs reached 40−60% visual index overcame (*19*), 10 bulbs of each cultivar were sampled.

Characterization of Garlic Cultivars. The characterization of garlic cultivars was carried out according to their flavor precursor levels, thiosulfinates, allicin content, and pungency. Flavor precursor levels and allicin contents were determined by a Konik KNK-500 series system liquid chromatograph (Barcelona, Spain) coupled with an UV−vis 200 detector (Nevada; scan 190−380 nm) equipped with a 5 μm Waters Spherisorb ODS2 RP-C18 column (250 mm × 4.6 mm). The quantitative analysis of precursors was performed according to refs *20* and *21*. Garlic powder (1 g) was added to 30 mL of 90% methanol solution containing 0.01 N HCl, and the mixture was shaken for 30 min using a magnetic stirrer. An additional 90% methanol solution containing 0.01 HCl was added to the mixture to obtain 50 mL. The resulting mixture was centrifuged at 14000 rpm for 5 min. The supernatant was analyzed through high-performance liquid chromatography (HPLC).

The allicin content was determined as previously described (*22*), and distilled water was added to 1 g of garlic powder (30 mL per g), mixed, kept for 10 min at room temperature, and then centrifuged at 14000 rpm for 5 min. After that, 600 μL of supernatant was added to methanol (1:1 v/v). Dried garlic powder, with a standardized quantity of allicin, was used as a secondary standard for allicin quantification.

For pungency determination, garlic juice was prepared from garlic cloves that were blended for 1 min in distilled water (1:10 w/v). The juice was collected, filtered, and kept at room temperature for 15 min to allow enzymatic hydrolysis of the flavor precursors to occur. A Beckman DU 520 spectrophotometer (General Purpose UV−vis; Fullerton, CA) was used to analyze the total contents of thiosulfinates and pyruvate. The pyruvate content was determined according to ref *23*. A juice aliquot was added to an equal volume of 5% trichloroacetic acid and centrifuged for 10 min at 10000 rpm. One milliliter of 0.0125% 2.4-DNPH in 2 N HCl was added to 2 mL of juice/TCA diluted (1:20 v/v). The tubes were incubated at 37 °C for 10 min in a thermostatized bath, and then, 5 mL of 0.6 N NaOH was added. The absorbance was measured at 420 nm. The pyruvic acid concentration was determined using a standard curve of reference developed with known concentrations of pyruvate. Values were expressed as mM % g of fresh garlic weight.

The thiosulfinate content was quantified as previously described (*24*). One gram of freeze-dried garlic powder was shaken for 30 min with 30 mL of distilled water at room temperature. Filtered and distilled water was then added to the mixture to obtain 100 mL exactly. Aliquots of 625 μL of 0.8 mM cysteine solution were added to an aliquot of 375 μL of diluted garlic extract and a similar aliquot of distilled water (reference test tube). After they were shaken, the solutions were kept for 10 min at room temperature. An aliquot of 200 μL of garlic/cysteine solution or water/cysteine solution was added to the 800 μL of 200 μM DTNB, which was prepared with 50 mM Hepes buffer (pH 7.5). A blank of 200 μL of water was added to the DTNB tube. After the test tubes were shaken, the test tubes were left for 10 min to allow color development. The absorbance was measured at 412 nm.

Animals and Experimental Design. All procedures were performed according to institutional guidelines for animal experimentation. Thirty day old male Wistar rats, weighing 90−130 g, were fed a standard commercial chow diet ad libitum and housed during the experimental period of 12 weeks in a room under conditions of controlled temperature (20 °C), humidity, and a 12 h light/dark cycle.

The administration of 10% (w/v) fructose solution as drinking water was used for 6 weeks to achieve the pathological model (FFR). At the beginning of the study, 60 rats were randomly distributed in two groups for 6 weeks: one control group ($n = 30$) and one experimental group ($n = 30$). After 6 weeks, the control and experimental groups were further divided into six groups ($n = 10$). For the subsequent 6 weeks, the groups received the following treatment: (I) control, rats had free access to tap water; (II) control + garlic Fuego INTA; (III) control + garlic Morado INTA; (IV) FFRs; (V) FFR + garlic Fuego INTA; and (VI) FFR + garlic Morado INTA.

Aqueous garlic extracts from bulbs of Fuego INTA and Morado INTA cultivars were peeled, and 75 g was diluted in 500 mL of distilled water, homogenized, and filtered to obtain a final dilution of 150 mg/mL. Solutions were then aliquoted and stored (−70 °C) until administered. Aqueous garlic was orally administered (150 mg/kg/day) to FFR and control groups.

At the end of the experimental period, and after an overnight fast, the rats were anesthetized with ketamine (50 mg/kg) and acepromacine (1 mg/kg). Blood was collected from the abdominal aorta into heparinized tubes. Plasma obtained after centrifugation (3000 rpm, 15 min at 4 °C) was aliquoted, frozen, and later assayed for glucose, insulin, triglycerides, and thiobarbituric acid reactive substances (TBARS). The aorta from six rats of each group was excised aseptically to measure NAD(P)H oxidase. The remaining four rats of each group were kept for histological observations.

Biochemical Determinations. Plasma glucose and triglyceride concentrations were determined by enzymatic colorimetric methods using commercial kits (GTLab, Buenos Aires, Argentina). Plasma insulin was measured by radioimmunoassay (Coat-A-Count, Siemens, CA), and insulin resistance was assessed using the homeostasis model assessment (HOMA-IR) described by Mathew et al. (*25*). HOMA-IR was calculated using the following formula: HOMA-IR (mmol/L × μU/mL) = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5. The systolic blood pressure (SBP) was monitored indirectly once a week in conscious, prewarmed, slightly restrained rats by the tail-cuff method and recorded on a Grass model 7 polygraph (Grass Instruments Co., Quincy, MA).

Markers of Oxidative Stress. The vascular NAD(P)H-oxidase activity in the aorta was measured by the lucigenin-derived chemiluminiscence assay as previously described (*8*). A 2 cm length segment of thoracic aorta was transferred to a tube with 2 mL of Krebs buffer and lucigenin (5 μM). This concentration of lucigenin does not appear to be involved in redox cycling and specifically detects superoxide anion. To assess NAD(P)H-oxidase activity, βNAD(P)H (500 μM) was added, and chemiluminiscence was immediately measured for 3 min in a liquid scintillation counter (LKB Wallac model 1219 Rack-Beta Scintillation Counter, Finland) set in the out-of-coincidence mode. Time-adjusted and normalized to tissue weight, scintillation counts were used for calculations. To specify the oxidase activity, measurements were repeated in the absence and presence of diphenylene iodinium (10^{-6} mol/L), which inhibits flavin-containing enzymes, including NAD(P)H oxidase.

The plasma TBARS concentration was determined according to a previously described method (*26*). This method is based on the reaction between plasma malondialdehyde, a product of lipid peroxidation, and thiobarbituric acid. The chromogen was read spectrophotometrically at 532 nm, and the results were expressed in μmol/L of TBARS, using a standard curve as a reference with a known quantity of malondialdehyde.

Vascular Histological Analysis. Tissue samples from four rats of each group were used for the histological vascular remodeling study as previously described (*8*). The kidney was preserved by in vivo perfusion through the abdominal aorta with a sucrose washing solution and then fixed with 4% paraformaldehyde. The tissues were kept in 10% formaldehyde solution until dehydration and then embedded in paraffin. Slices of 5 μm thickness were cut transversely on a microtome (Microm HM325, Walldorf, Germany), stained with Masson's trichrome and examined

under a light microscope (Nikon Optiphot-2, Kanagawa, Japan). To evaluate the renal arterial wall thickening, images of kidney arcuate arteries were identified along the cortical−medullar junction and surrounded by tubules. Photos were taken with a digital camera (Panasonic GP-KR222 color CCD, Panasonic, Osaka, Japan) and processed with a two-dimensional analysis system Scion Image 4.01 (Scion, Bethesda, MD). Arteries that were not sectioned transversely were excluded from the study. The lumen-to-wall media ratio (the internal diameter to the medial thickness) (L/M ratio) was then calculated. Four slices from each rat kidney were processed, and 8−10 arteries of each slice were analyzed to obtain an average value for each rat. These average values were then used for final analysis.

Reagents. Unless otherwise noted, all reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical and Data Analysis. Data are expressed as means ± standard deviations (SDs). The statistical significance was assessed by one-way analysis of variance followed by Student−Newman−Keuls and Tukey post-tests. A $p < 0.05$ was considered significant.

RESULTS

Characterization of Garlic Cultivars. Alliin was the most abundant ACSO in both garlic cultivars, representing more than 50% of the total content of ACSOs, whereas the methiin content represented less than 10%. Fuego INTA showed significantly higher levels of thiosulfinates, allicin, and pungency than Morado INTA (**Table 1**).

Body Weight, SBP, and Metabolic Variables. The body weight of animals did not differ between the control group (I) and the experimental groups (IV, V, and VI). The body weights of the control rats fed with both garlic cultivars (groups II and III) were lower than those from the experimental groups (IV, V, and VI) but did not differ from control animals (group I) (**Table 2**). At the end of the study, SBP was significantly higher in FFRs (group IV) as compared with control animals (groups I, II, and III). Chronic administration of Fuego INTA and Morado INTA garlic extracts to the experimental groups (V and VI) significantly reduced the SBP as compared with FFRs (group IV). No differences in SBP were observed between control animals (groups I, II, and III). FFRs (group IV) showed an increase in fasting levels of plasma glucose and insulin as compared to control animals (groups I, II, and III). FFRs treated with both garlic cultivars (groups V and VI) showed glucose and insulin levels between FFR (group IV) and control animal (groups I, II, and III) values, without significant differences. When we estimated the index of insulin resistance, HOMA-IR, we found higher levels in FFRs (group IV) as compared with control rats (groups I, II, and III); the administration with Fuego and Morado INTA garlic extracts (groups V and VI) significantly reduced the HOMA-IR index as compared with FFRs (group IV) but did not reach control animal levels. The triglyceride levels were higher in FFRs (group IV, V, and VI) as compared with control animals (groups I, II, and III).

Markers of Oxidative Stress. The NAD(P)H-oxidase activity in aortic tissue and lipid peroxidation, evaluated through plasma TBARS concentration, were higher in FFRs (group IV) than in control animals (groups I, II, and III) (**Figure 1A**,**B**, respectively). The administration of both aqueous garlic extracts to FFRs (groups V and VI) significantly reduced the NAD(P)H-oxidase aorta activity and plasma lipid peroxidation as compared to group IV. Moreover, extracts of Fuego INTA significantly reduced the activity of NAD(P)H-oxidase as compared to Morado INTA.

Vascular Remodeling. The FFRs (group IV) significantly decreased the L/M ratio, as compared with control groups (I, II, and III) (**Figure 2**). Prolonged administration of both garlic cultivars to FFRs (groups V and VI) significantly increased the L/M ratio close to control groups (I, II, and III).

Table 1. ACSOs, Thiosulfinates, Allicin, and Pungency of Garlic Cultivars[a]

variables		cultivar: Fuego INTA	cultivar: Morado INTA
ACSOs (mM % g fw)	alliin	8.00 ± 0.60 a	8.60 ± 0.26 a
	methiin	0.60 ± 0.01 a	0.50 ± 0.03 b
	isoalliin	3.00 ± 0.03 a	2.90 ± 0.14 a
	total	11.60 ± 0.58 a	12.00 ± 0.60 a
total thiosulfinates (mM % g fw)		3.92 ± 0.01 a	2.49 ± 0.02 b
allicin (mM % g fw)		2.70 ± 0.01 a	1.7 ± 0.02 b
pungency (mM % g fw)		7.27 ± 1.92 a	5.17 ± 2.44 b

[a] Each value is the mean ± SD of fresh material ($n = 5$). Values of each variable followed by the same letter were not significantly different according to the Tukey test ($P < 0.05$).

DISCUSSION

In this study, we found that aqueous garlic extract prevents the increase of SBP and the development of insulin resistance, oxidative stress, and vascular remodeling in an experimental model of MS induced by fructose administration. Insulin resistance has been implicated as a central pathogenic feature of MS in human and animal models (*2*). Consistent with previous studies reported by our group (*4*, *7*, *8*), FFRs developed insulin resistance and increased blood pressure, oxidative stress, and vascular remodeling. Chronic administration of aqueous garlic extracts from Fuego INTA and Morado INTA cultivars not only prevented the increase in SBP and oxidative stress but also reduced vascular remodeling in FFRs.

Garlic's SBP-lowering effects have been widely studied in human and animal models (*27*, *28*). Administration of allicin 8 mg/kg to rats with an enriched fructose diet has been found to decrease SBP, triglycerides, and insulin (*29*). We observed that both aqueous garlic cultivars extracts decreased SBP and HOMA-IR index in FFR but did not significantly modify the plasma triglycerides and insulin levels. These differences may be

Table 2. Body Weight, SBP, and Metabolic Parameters Measured from Control (I), C + Fuego INTA (II), C + Morado INTA (III), FFRs (IV), F + Fuego INTA (V), and F + Morado INTA (VI)[a]

variables	groups: I	II	III	IV	V	VI
body weight (g)	334 ± 22 ac	300 ± 16 bc	303 ± 26 bc	354 ± 20 a	361 ± 20 a	361 ± 20 a
final SBP (mmHg)	126.9 ± 5.9 b	123.0 ± 4.5 b	123.0 ± 4.5 b	133.1 ± 3.7 a	126.3 ± 5.2 b	124.4 ± 3.2 b
glucose (mM)	5.5 ± 0.8 b	5.2 ± 1.0 b	5.5 ± 1.0 b	7.3 ± 0.8 a	6.8 ± 1.0 ab	6.8 ± 1.0 ab
insulin (μIU/mL)	20.7 ± 2.3 b	22.0 ± 3.0 b	20.0 ± 3.4 b	27.4 ± 3.0 a	23.0 ± 5.0 ab	25.0 ± 3.6 ab
HOMA-IR (mmol/L × μU/mL)	5.2 ± 1.0 c	5.0 ± 0.8 c	4.9 ± 1.2 c	10.5 ± 1.6 a	7.1 ± 1.5 b	7.5 ± 1.5 b
triglycerides (mM)	0.71 ± 0.20 b	0.70 ± 0.08 b	0.60 ± 0.18 b	1.11 ± 0.32 a	1.22 ± 0.22 a	1.09 ± 0.18 a

[a] Each value is the mean ± SD of fresh material. For body weight and SBP, $n = 10$; for the rest of the variables, $n = 6$. Values of each variable followed by the same letter were not significantly different according to the Newman−Keuls test ($P < 0.05$). HOMA-IR, homeostatic model assessment−insulin resistance.

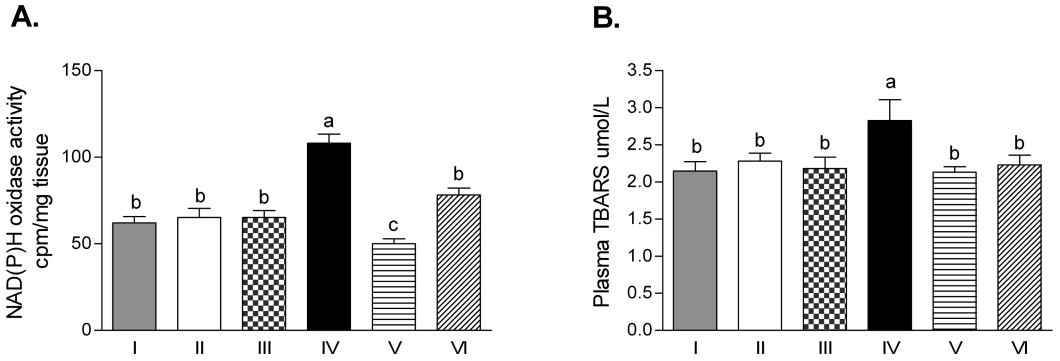

Figure 1. Markers of oxidative stress, NAD(P)H oxidase aorta activity (**A**), and TBARS plasma levels (**B**) of rats from control (I), C + Fuego INTA (II), C + Morado INTA (III), FFR (IV), FFR + Fuego INTA (V), and FFR + Morado INTA (VI). Values are means ± SDs; $n = 6$. Bars with different superscripts significantly differ ($P < 0.05$).

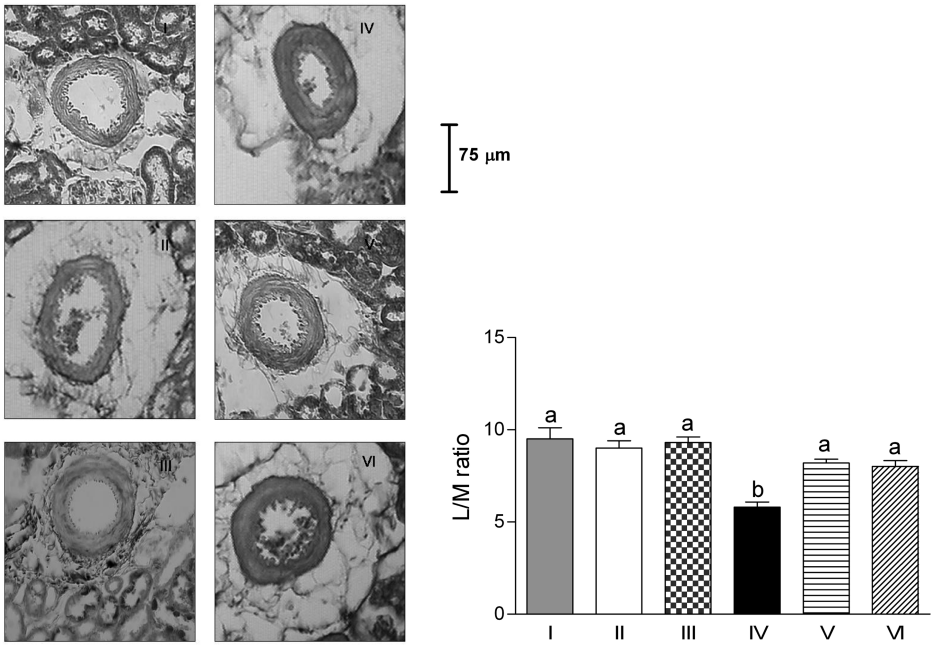

Figure 2. Vascular remodeling in kidney arcuate arteries described by microphotographs and analyzed in the bar graph. Groups are identified as follows: control (I), C + Fuego INTA (II), C + Morado INTA (III), FFR (IV), FFR + Fuego INTA (V), and FFR + Morado INTA (VI). Values are means ± SDs; $n = 4$ each. Bars with different superscripts significantly differ ($P < 0.05$).

related with the quantity and clone of garlic used. The antihypertensive mechanism of garlic is probably due to its prostaglandin-like effects, which decreases peripheral vascular resistance (*30*). Some authors have shown that garlic compounds inhibit angiotensin-converting enzyme in vitro (*31*), and others showed that garlic blocks the effect of L-nitro arginine methyl ester-induced hypertension (*32*).

There is strong evidence supporting an important role of vascular NAD(P)H oxidase-driven $^{\bullet}O_2^-$ production observed in experimental models of hypertension and insulin resistance (*33*). Our group previously described that angiotensin II-induced NAD(P)H-oxidase-driven ROS generation is enhanced in vascular smooth muscle cells from spontaneously hypertensive rat (SHR) during the development of hypertension (*34*). Our results clearly show an enhancement of NAD(P)H-oxidase activity in aorta from FFRs (group IV), associated with higher plasma lipid peroxidation measured through TBARS levels. The administration of Fuego INTA and Morado INTA garlic extracts to FFRs reduced both NAD(P)H-oxidase activity and plasma level TBARS. It is important to note that Fuego INTA had a greater effect in reducing the NAD(P)H-oxidase activity than Morado INTA; this fact may be related to the higher total thiosulfinates, allicin, and pungency present in Fuego INTA.

ROS also modulate vascular tone and structure, are proinflammatory, and stimulate monocyte migration and formation of oxidized low-density lipoproteins. Consequently, excessive ROS production may underlie pathologic processes associated with endothelial dysfunction and vascular remodeling, which are characteristic features in hypertension (*35*). Additionally, Zalba et al. (*36*) reported a vascular wall hypertrophy in SHR associated with an enhanced aortic NAD(P)H oxidase activity.

In this study, we found that the L/M ratio was reduced in kidney arcuate arteries from FFRs (group IV), as compared with arteries from the control group. Prolonged administration of both aqueous garlic extracts to experimental animals (groups V and VI) reduced the vascular structural changes in kidney arteries, indicating a close relationship between oxidative stress and vascular remodeling (*6*). The antioxidant and anti-inflammatory effects of garlic could be responsible for this relationship. Studies in vitro have shown that garlic and some major garlic compounds inhibit the expression of cell adhesion molecules through downregulation of intracellular transduction signal pathways like AP-1 and c-Jun NH2-terminal kinase (JNK) (*37*) or nuclear factor NF-κB (*38*).

Many pharmacological properties of garlic are attributed to organosulfur compounds like allicin and diallyl disulfide. Early studies using aqueous garlic extract in animal models indicated that allicin and allicin-derived compounds are rapidly metabolized to allyl mercaptan and diallyl disulfide, making these organosulfur compounds highly bioavailable (*40*). Although several studies regarding garlic health benefits have been carried out (*12*, *16*, *17*, *19*, *20*, *39*), in all of them, the profile of organosulfur compounds and several biological activities have been separately analyzed. This is the first study in which the principal organosulfur compounds, pungency, and biological activities were analyzed at the same time, in clones grown under controlled field conditions.

In our study, we considered the organosulfur profile of two garlic cultivars fed to rats with MS. The characterization of these two garlic cultivars showed significant differences in thiosulfinates, allicin contents, and pungency levels. We recently provided evidence of the correlation among organosulfur composition, pungency, and antiplatelet activity (*18*). The cultivar Fuego INTA showed the highest level of all variables analyzed. These higher levels could be responsible for slightly higher beneficial effects exerted by Fuego INTA, taking into account the possible correlations existing between organosulfur composition and biological variables studied in this work. However, further studies are needed to confirm this issue.

We administered aqueous garlic extract to rats with a dose of 150 mg/kg per day. Using an interspecies dose conversion factor based on equal body surface (7:1 for conversion from rat to human) (*41*), the doses of garlic used are equivalent to consuming 21.4 mg/kg per day for humans (1.5 g of garlic for a person weighing 70 kg). This quantity is consistent with other recent garlic studies (*12*, *39*) and also is in agreement with another well-known study that recommends consuming 1 clove of garlic daily, taking into consideration that a clove weights between 1.5 and 3 g (*42*).

In conclusion, we demonstrate that chronic administration of Fuego INTA and Morado INTA garlic aqueous extracts in rats with MS prevents oxidative stress. We also prove, for the first time, that garlic administration can prevent vascular remodeling, estimated through the L/M ratio. These results show that prolonged garlic consumption could be useful in the prevention of MS, and the results provide new insights in the understanding of the benefits of functional foods in cardiovascular diseases. Also, our findings show that there is great variability in the organosulfur composition between garlic cultivars. This variability should be further considered due to its potential in the prevention of cardiovascular diseases and also for the selection of garlic cultivars with better health-benefit properties.

ACKNOWLEDGMENT

We thank Susana Gonzalez and Cristina Lama for their technical assistance.

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Received for review November 10, 2009. Revised manuscript received April 21, 2010. Accepted April 26, 2010. This study was supported by PICTO 08-12902 and Program I+D 06/P01 of the National University of Cuyo.