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Garlic prevents the oxidizing and inflammatory effects of sepsis induced by bacterial lipopolysaccharide at the systemic and aortic level in the rat. Role of trpv1

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ABSTRACT

Garlic (*Allium sativum*) possesses healing properties for diseases like systemic arterial hypertension, cancer and diabetes, among others. Its main component, allicin, binds to the Transient Receptor Potential Vanilloid Type 1 (TRPV1). In this study, we investigated TRPV1's involvement in the regulation of various molecules at the systemic and aortic levels in Wistar rats treated with bacterial lipopolysaccharide (LPS) and garlic to activate the receptor. The experimental groups were as follows: 1) Control, 2) LPS, 3) Garlic, and 4) LPS + Garlic.

Using Uv–visible spectrophotometry and capillary zone electrophoresis, we measured the levels of nitric oxide (NO), biopterins BH2 and BH4, total antioxidant capacity (TAC) and oxidizing capacity (OXCA). We also analyzed molecules related to vascular homeostasis such as angiotensin Ang 1–7 and Ang II, as well as endothelin ET-1. In addition, we assessed the inflammatory response by determining the levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), and galectin-3 (GTN-3). For cell damage assessment, we measured levels of malondialdehyde (MDA), malonate (MTO) and 8-hydroxy-2-deoxyguanosine (8HO2dG).

The results showed that LPS influenced the NO pathway at both systemic and aortic levels by increasing OXCA and reducing TAC. It also disrupted vascular homeostasis by increasing Ang-II and ET-1, while decreasing Ang1-7 levels. IL-6, TNFα, GTN-3, as well as MDA, MTO, and 8HO2dG were significantly elevated compared to the control group. The expression of iNOS was

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increased, but TRPV1 remained unaffected by LPS. However, garlic treatment effectively mitigated the effects of LPS and significantly increased TRPV1 expression.

Furthermore, LPS caused a significant decrease in calcitonin gene-related peptide (CGRP) in the aorta, which was counteracted by garlic treatment. Overall, TRPV1 appears to play a crucial role in regulating oxidative stress and the molecules involved in damage and inflammation induced by LPS. Thus, studying TRPV1, CGRP, and allicin may offer a potential strategy for mitigating inflammatory and oxidative stress in sepsis.

1. Introduction

Curing diseases has been a fundamental human necessity since ancient times which gave rise to herbalism or traditional medicine in Early History. Presently, we have the capability to identify chemical compounds found in each medicinal plant and study which specific components are responsible for observed healing effects described by our ancestors. As an example, garlic (Allium sativum) is one of the medicinal plants most used by Asian and Eastern cultures, and also by the pre- Hispanic cultures of America. In recent decades, the curative properties and beneficial effects against hypertension, cancer [1] and diabetes among others [2,3] have been widely studied.

Garlic has components such as amino acids, fatty acids, phenols and sulfur compounds such as allicin, alliin and ajoene [1,4]. Allicin and its derivative S-allyl-L-cysteine are sulfur compounds known for their potent antibacterial and antioxidant properties. They also inhibit the production of inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin-6 (IL-6), interleukin-1alpha (IL-1α), and interferon-6. In 2012 Lee et al. reported that the sulfur components of garlic suppressed the expression of inducible nitric oxide synthase (iNOS). Additionally, they found that S-allyl-L-cysteine has an inhibitory effect on IL-1β in macrophages stimulated with bacterial lipopolysaccharide (LPS) [5,6]. In a recent work [7] we found that, garlic generated important antioxidant and cardioprotective effects which are attributed mainly to the action of hydrogen sulfide (H_2S).

On the other hand, in our laboratory we have studied the functional characteristics of a membrane receptor that belongs to the family of receptors TRP (Transient Receptor Potential) and to Vanilloid subfamily. TRPV1 (Transient Receptor Potential Vanilloid Type 1) has specific binding sites for different molecules, among which is the C157 site to which allicin binds in amino terminal chain of subunit 1 [8]. TRPV1 is located in neurons and several non-neuronal cell types, including endothelium, vascular smooth muscle, myocytes, among others, and in organelles such as mitochondria and Golgi apparatus. TRPV1 can be activated by physical stimuli such as shear stress and also by chemical stimuli, among which, molecules found in plants and spicy foods such as capsaicin from chili peppers and allicin from garlic stand out [9–12]. TRPV1 has very important cardiovascular and cardioprotective actions through activation of the nitric oxide (NO) pathway and the calcitonin gene-related peptide (CGRP) [13].

We have recently published the effects of activation of TRPV1 with capsaicin on olecules that conform the NO pathway, molecules inducing oxidative stress and molecules that produce cell damage in rat aorta and in heart [14,15]. Here we study whether garlic pretreatment (to activate TRPV1) can counteract the inflammatory and oxidative effects of *Escherichia coli* LPS-induced sepsis at the systemic and aortic level in Wistar rats.

Sepsis or Systemic inflammatory response syndrome (SIRS) as it is also now known, is a microbial infection that has a mortality rate of 15–40 % in humans. The patient with sepsis responds to infection with a systemic inflammatory state characterized by multiorgan dysfunction. The circulatory failure that occurs is characterized by arterial hypotension and endothelial damage. Under these conditions, iNOS is overexpressed and it is responsible for the vascular hyporeactivity that leads the septic patient to a difficult to counteract critical state of health [16–18].

S-allyl-L-cysteine inhibits the iNOS/NO pathway and reduces the expression of the contractile gene Notch3 in the aorta of mice with sepsis [5,19]. In this work we study the participation of the activated TRPV1 by a pre-treatment with garlic on the regulation of some molecules of oxidative stress, molecules of the inflammatory pathway, molecules reflecting cell damage and molecules of the blood pressure regulatory system during sepsis induced with LPS.

The studies were carried out at the systemic and thoracic aortic levels in rats through the quantification of the following parameters: dihydrobiopterin (BH2), tetrahydrobiopterin (BH4), total antioxidant capacity (TAC), oxidant capacity (OXCA), Angiotensins Ang 1–7, Ang II, and endothelin ET-1, proinflammatory molecules such as IL-6, TNFα, and Galectin-3 (GTN-3) we evaluate cell damage molecules such as malondialdehyde (MDA), malonate (MTO) and 8-hydroxy-2- deoxyguanosine (8HO2dG).

To explore the role of TRPV1 in the regulation of these parameters, we evaluated its expression and CGRP levels in aortic tissue [13,20].

The aim of this work was to study, at a systemic level and in the aorta, whether the activation of TRPV1 by a food supplement with a high allicin content participates in the regulation of the NO pathway and of molecules involved in oxidative stress, molecules of tissue damage and proinflammatory molecules.

2. Methods

2.1. Rats

The Laboratory Animal Care Ethics Committee of the National Institute of Cardiology Ignacio Chávez in Mexico City approved the

experimental design, and experiments were done in accordance with the Guide for the Care and Use of Laboratory Animals.

The experimental animals were male Wistar rats of 300–350 g body weight and were kept under normal light/dark conditions (12 h light/dark), with controlled temperature (25 ± 3 °C) and humidity (50 ± 10 %); food and drinking water were ad libitum (certified diet for rat, LabDiet 5026, PMI Nutrition International, Richmond, IN, USA). The procedures were performed under the guidelines of the Federal Regulation for Experimentation and Animal Care (SAGARPA, NOM-062-ZOO- 1999, Mexico).

It is important to mention that these animals were used for different protocols, and it was interesting for us to focus the study at the systemic level and on the thoracic aorta in this paper.

2.2. Experimental groups

The animals were randomly divided into 4 groups of 6 animals each as follows: 1) Control and with the treatments 2) garlic, 3) LPS and 4) garlic + LPS.

Treatments were applied as follows; LPS (Lipopolysaccharide from *Escherichia coli*. Urbana, IL, USA CATALOGO L3129) (15 mg/kg ip) 3 h before the experiment. Chinese garlic tablets (Ajolín Forte® plus, Deodorized Garlic) were given in the drinking water (Ajolin Forte ® plus) 500 mg/L. The tablets are added with 600 μg of sodium, 750 mg of fat, 20 g of carbohydrates and 0 g of protein. The solution was changed every 12 h for 1 month [7].

Sodium pentobarbital was used as anesthetic at a dose of 60 mg/kg body weight. Blood samples were taken from each group and the thoracic aorta was removed and stored. The blood was centrifuged at 2500–3500 rpm for 15 min to obtain the sera and frozen at −70 °C until the day of metabolite quantification. Traces of blood and connective tissue were carefully removed from the thoracic aorta to then freeze them at −70 °C until the day of the experimental tests.

2.3. Sample treatments

2.3.1. Serum

500 μL of the sample were deproteinized 1:1 with analytical grade cold methanol. They were centrifuged at 16,000×*g* (Specrafuge 24D, Labnet International Inc., Edison, NJ, USA) for 15 min at 4 °C. The precipitate was removed and the supernatant was stored at −70 °C for later analysis.

2.3.2. Aortic tissue homogenate

From 20 to 50 mg of tissue were homogenized in 1000 μL of cold 100 mM phosphate buffer at pH 7.4. They were centrifuged at 16,000×*g* (Specrafuge 24D, Labnet International Inc., Edison, NJ, USA) for 15 min at 4 °C. The precipitate was removed, and the supernatant was stored at −70 °C for later analysis.

2.3.3. Measurement of metabolites at the systemic level (serum) and aortic tissue

Once the samples were treated and gradually thawed for analysis, the methodologies used for metabolite quantification were the same for both serum samples and aortic tissue homogenate.

2.4. Nitric oxide

To 20 μL of the treated sample, 100 μL of 0.8 % vanadium III chloride in 1 M phosphoric acid was added. Samples were homogenized and 50 μL of 2 % sulfanilamide in 5 % phosphoric acid were added, vigorously homogenized and mixed. 50 μL of 0.2 % N-(1-naphthyl)-ethylenediamine in distilled water were then added. Once again, samples were gently homogenized and allowed to stand for 50 min protected from light. After the incubation time, the samples were analyzed spectrophotometrically at 572 nm and later at 587 nm (UV-1800 spectrophotometer, Shimadzu LA, S.A. Buenos Aires, Argentina). The concentration of nitric oxide was obtained indirectly by preparing a moisture-free HPLC-grade sodium nitrite standard curve, in a range of 0–1000 pmol/mL. The difference in absorbances (572 nm–587 nm) was considered for the calculations [21].

2.5. Dihydrobiopterin and tetrahydrobiopterin

BH2 and BH4 were determined simultaneously in the processed samples by capillary zone electrophoresis, with fluorescence detection by laser excitation. For this, the sample was diluted 1:1 with 0.1 M sodium hydroxide (Sigma Chemical Co., St. Louis, MI, USA), passed through a Sep-Pak Classic NH2 cartridge (Waters, Urbana, IL, USA) and directly analyzed by capillary electrophoresis P/ACETM MDQ System (Beckman Coulter, Redlands, CA, USA) to which the capillary was preconditioned by passing a 1.0 M solution of sodium hydroxide (Sigma Chemical Co., St. Louis, MI, USA) for 30 min, then deionized water (Hycel Reactivos Químicos, S.A. de C.V., Zapopan, Jalisco, México) for 30 min and finally the running buffer (0.1 M Tris – 0.1 M boric acid – 2 mM EDTA at pH 8.75/Sigma Chemical Co., St. Louis, MI, USA) for 30 min. The samples were injected under hydrodynamic pressure at 0.5 psi/10 s. Separation was performed at 20 kV for 10 min at 448 nm laser excitation and 435 nm detection. The capillary was washed between runs with 1.0 M NaOH (Sigma Chemical Co., St. Louis, MI, USA) for 2 min and deionized water (Hycel Reactivos Químicos, S.A. de C.V., Zapopan, Jalisco, México) for 2 min. Results are expressed in pmoles/mL. The concentrations of BH2 and BH4 are determined using a separate standard curve for dihydro and tetrahydrobiopterin (Sigma Chemical Co., St. Louis, MI, USA) [22].

2.6. Total antioxidant capacity

35 μL of the treated sample were placed in a 96-well plate. Subsequently, 145 μL of 0.1 M phosphate buffer at pH 7.5 were added and homogenized at 500 rpm for 200 s. Immediately afterwards, 100 μL of the diluted sample was transferred to the adjacent well and 50 μL of 0.01 M CuCl2 was added and homogenized at 500 rpm for 200 s. After this time, 50 μL of 0.01 M batocuproin were added and the mixture was homogenized again at 500 rpm for 200s. Both samples (diluted sample and treated sample) were analyzed spectrophotometrically at a wavelength of 490 nm, previously adjusting the equipment with a phosphate buffer blank. The total antioxidant capacity was expressed in μmol/L and was calculated using a standard curve using a normal control serum (8002101 Normal Control Serum N, Valtek, Nuñoa, Metropolitan Region, Chile) [23].

2.7. Oxidizing capacity

In a 96-well plate, 50 μL of the treated sample and 50 μL of isotonic saline solution at 37 °C were added, gently homogenized for 1 min, and 100 μL of freshly prepared Mito-ID MP reagent (Enzo Life Sciences Farmingdale, NY, USA), was gently homogenized for 1 min and incubated at room temperature for 30 min, protected from light. 100 μL of 50 mM phosphate buffer were added and gently homogenized for 1 min. 20 μL of CCCP reagent (Carbonyl Cyanide *m*-Chlorophenylhydrazone, Sigma-Aldrich, Urbana, IL, USA) prepared in 50 mM Citrate buffer at a concentration of 2–4 μM were added, gently homogenized and fluorescence was monitored (disappearance of the orange color) at an excitation wavelength of 590 nm and an emission wavelength of 620 nm for 15 min. A control was run without CCCP reagent. The greater the potential for cytotoxicity, the greater the consumption of Mito-ID MP and the less intense the orange color of the reaction. It is expressed in pmol/L [24].

2.8. Angiotensin II and angiotensin 1-7

Angiotensin II and Angiotensin 1-7 were determined simultaneously in the processed sample by capillary zone electrophoresis, under UV–Visible detection by diode array. The treated sample was mixed in a 1:1 proportion with cold 20 % trichloroacetic acid. It was centrifuged at 16000×*g* for 15 min at 10 °C and filtered with 0.22 μm nitrocellulose membrane filters, diluted 1:2 with cold 0.1 M sodium hydroxide, the sample was passed through a Sep-Pak Classic C- 18 cold (Waters Corporation, Milford, MA, USA) and analyzed directly with the Beckman Coulter P/ACETM MDQ system, to which the capillary was preconditioned by passing a 1.0 M solution of sodium hydroxide for 30 min, then water deionized for 30 min and finally the running buffer (100 mM boric acid + 3 mM tartaric acid + 10 fM gold III chloride at pH 9.8) for 30 min. The sample was injected under hydrodynamic pressure at 0.5 psi/10s. Separation was performed at 30 kV for 10 min at 200 nm at 20 °C. The capillary was washed between runs with 1.0 M sodium hydroxide for 2 min, deionized water for 2 min, and running buffer for 4 min. The results were expressed in pmoles/mL. Angiotensin II and Angiotensin 1-7 concentrations were determined using a standard curve [25].

2.9. Endothelin-1

Endothelin-1 was determined in the treated samples by high pressure liquid chromatography (ACQUITY UPLC H-Class PLUS System, Waters Corporation, Urbana, IL. USA). For which a reverse phase Supelcosil LC-318 column (25 cm long, 4.6 mm id, 5 μm particle size, 300 Å pore size; Supelco, Oakville, ON, Canada) with fluorescence detection was used. (490 nm Ex. and 512 nm Em). The injection volume was 20 μL. The mobile phase consisted of two solvent mixtures: Mix A, 30 % acetonitrile in water (0.1 % TFA); Mix B, 90 % acetonitrile in water (0.1 % TFA). Entothelin-1 concentration was determined using a standard curve [26].

2.10. Interleukin-6

Interleukin-6 determination was performed on the treated samples using a commercial kit (IL-6 Rat ELISA Kit, Invitrogen, Thermo Fisher Scientific, Cat.: BMS625, Palo Alto, CA, USA).

2.11. Tumor necrosis factor α

The determination of tumor necrosis factor alpha was performed in the treated samples (serum, tissue homogenate), using a commercial kit (Rat TNF alpha ELISA Kit, Abcam, Cat.: ab100785, Ontario, Canada).

2.12. Galectin-3

Galectin-3 determination was performed on the treated samples using a commercial kit (Galectin-3 LGALS3 Rat ELISA Kit, Invitrogen, Thermo Fisher Scientific, Cat.: ERLGALS3, Palo Alto, CA, USA).

2.13. Malondialdehyde and malonate

Malondialdehyde and malonate were determined simultaneously in the treated samples by capillary zone electrophoresis. The treated sample is diluted 1:2 with cold 0.1 M sodium hydroxide and analyzed directly. For this purpose, the P/ACETM MDQ system

from Beckman Coulter (Urbana, IL, USA) was used, in which the capillary was preconditioned by passing a 0.1 M sodium hydroxide solution at 20 psi for 10 min, followed by water distilled for 10 min and finally the running buffer (10 mM borates + 0.5 mM CTAB at pH 9.0) for 10 min. The samples were injected under hydrodynamic pressure at 0.5 psi/10 s. Separation was performed at −25 KV for 4 min at 267 nm. The capillary was flushed between runs at 20 psi with 0.1 M NaOH for 2 min, distilled water for 2 min, and buffer for 4 min. Malondialdehyde and malonate concentrations are expressed in pmoles/mL and determined separately using a standard curve [27].

2.14. 8-Hydroxy-2-deoxyguanosine

8-Hydroxy-2-deoxyguanosine was determined in the treated samples by capillary zone electrophoresis, by UV detection by diode array. The sample was deproteinized with 20 % trichloroacetic acid, in a 10:1 ratio. It was centrifuged at 16′000×*g* for 15 min and filtered with 0.22 μm nitrocellulose membrane filters, diluted 1:10 with 2 M sodium hydroxide and analyzed directly with the Beckman Coulter P/ACETM MDQ system (Urbana, IL, USA), to which the capillary was preconditioned by passing a 2 M solution of sodium hydroxide for 30 min, then deionized water for 30 min and finally the running buffer (10 mM borates at pH 9.0] for 30 min). The sample was injected under hydrodynamic pressure at 0.5 psi/10s, the separation was performed at 20 kV for 8 min at 200 nm. The capillary was washed between runs with 2 M sodium hydroxide for 2 min and deionized water for 2 min The results are expressed in pmoles/mL The concentration of 8-hydroxy-2-deoxyguanosine was determined using a standard curve [28].

3. Statistical analysis

The Data are presented as mean ± standard error. Statistical significance was determined with student's ***t*** one-way ANOVA. At $p \leq 0.05$ it was considered significant.

4. Results

4.1. Systemic effect of garlic on LPS-induced oxidative stress

Table 1 shows the results obtained in serum. There were changes in the levels of some of the biomarkers involved in oxidative stress (NO, BH4, BH2, TAC and CAOX) due to the effect of LPS and garlic treatments.

In the animals treated with LPS oxidative stress increased, which was evidenced by a decrease in TAC and BH4 with the consequent increase in BH2 and OXCA with respect to the control group. NO increased in an unregulated and significant way in the LPS group when compared with the control group (7.5 ± 1 to 57.1 ± 5.5 pmol/mL).

In the animals treated with garlic there were no important differences in the levels of BH4, BH2, although the TAC increased significantly from 466.4 ± 0.02 to 628.5 ± 23 mmol/L with respect to the control group.

The systemic levels of BH4 in the control group (7.8 ± 0.5 pmol/mL) and in the garlic group (7.0 ± 0.8 pmol/mL) did not present significant differences. Compared with control group, BH4 was significantly decreased in the LPS group from 7.8 ± 0.5 to 3.8 ± 0.4 pmol/mL respectively. In the LPS + garlic group, BH4 levels were restored from 3.8 ± 0.4 to 9.1 ± 0.7 pmol/mL with respect to the LPS group.

On the other hand, with respect to the control group, the levels of BH2 increased from 5.6 ± 0.3 to 18.0 ± 2.0 in LPS group, to 9.0 ± 0.7 in garlic group and to 12.4 ± 1.1 pmol/mL in LPS ± garlic group.

4.2. Systemic effect of garlic on LPS-induced vasodilation-vasoconstriction

The data in Table 2 show that the endotoxicity of LPS generated a significant decrease in the systemic levels of the vasodilator Ang 1–7 (0.57 ± 0.05 to 0.30 ± 0.02 pmol/mL) when compared to the control group. With respect to its own controls, treatment with LPS elevated vasoconstrictor biomarkers such as Ang II (0.40 ± 0.03 to 1.8 ± 0.15 pmol/mL) and ET-1 (0.30 ± 0.014 to 0.82 ± 0.086 pg/mL).

Treatment with garlic inhibited the effects of LPS, restoring the levels of Ang 1–7 to 0.059 ± 0.06 pmol/mL and significantly

Table 1
Effect of Garlic on LPS-induced oxidative stress.

	CONTROL	LPS	GARLIC	LPS + GARLIC
NO (pmol/mL)	7.5 ± 1.0	57.1 ± 5.5[a]	7.5 ± 0.5 β	16.2 ± 1.4 b
BH4 (pmol/mL)	7.8 ± 0.5	3.8 ± 0.4[a]	7.0 ± 0.8 β	9.1 ± 0.7 b
BH2 (pmol/mL)	5.6 ± 0.3	18.0 ± 2.0[a]	9.0 ± 0.7 β	12.4 ± 1.1 b
TAC (mmol/L)	466.4 ± 40.1	120.9 ± 20.0[a]	628.5 ± 22.5 β	601.7 ± 17.0 b
OXCA (pmol/L)	0.14 ± 0.02	0.61 ± 0.08[a]	0.08 ± 0.01 β	0.12 ± 0.01 b

$n = 6$; $p \leq 0.05$.
[a] Control *vs* LPS; β LPS *vs* GARLIC.
b LPS *vs* LPS + GARLIC.

Table 2
Effect of Garlic on LPS-induced vasoconstriction-vasodilation.

	CONTROL	LPS	GARLIC	LPS + GARLIC
Ang 1–7 (pmol/mL)	0.57 ± 0.05	0.30 ± 0.02[a]	0.37 ± 0.02	0.59 ± 0.06 [b]
Ang II (pmol/mL)	0.40 ± 0.03	1.8 ± 0.15[a]	0.4 ± 0.02 β	1.0 ± 0.03 [b]
ET-1 (pg/mL)	0.30 ± 0.014	0.82 ± 0.086[a]	0.33 ± 0.011 β	0.11 ± 0.06 [b]

$n = 6$; $p \leq 0.05$.
[a] Control *vs* LPS; β LPS *vs* GARLIC.
b LPS *vs* LPS + GARLIC.

reducing the levels of Ang II (1.8 ± 0.15 to 1.0 ± 0.03 pmol/mL and ET-1 (0.30 ± 0.014 to 0.11 ± 0.06 pg/mL) with respect to its own controls.

4.3. Inflammatory response

Table 3 shows that the endotoxicity induced by LPS resulted in an inflammatory response, which is reflected by an increase in the systemic levels of IL-6 (109.4 ± 3.8 to 417.5 ± 17.7 pg/mL) and TNFα (12.15 ± 2.1 to 78.1 ± 6.2 pg/mL) with respect to control animals. For its part, regard to control group, GTNA-3 increased in the presence of LPS (1272.1 ± 94.9 to 2918.5 ± 222.2 pg/mL). These LPS effects are significantly decreased in the LPS + garlic group as follows; IL-6 (417.5 ± to 296.3 ± 17.3 pg/mL) and GTNA-3 (2918.5 ± 222.2 to 17066 ± 59.9 pg/mL).

4.4. Effect of garlic on LPS-induced cell damage

As can be seen in Table 4, systemic levels of MDA (0.2 ± 0.02 pmol/mL), MTO (0.07 ± 0.007 pmol/mL), and 8HO2dG (4.9 ± 0.7 pM) were significantly increased in the LPS-treated group to 0.4 ± 0.03 pmol/mL, 0.58 ± 0.051 pmol/mL and 15.5 ± 1.5 pM respectively. Garlic treatment produces significantly diminished of MDA (0.4 ± 0.03 to 0.2 ± 0.01 pmol/mL), MTO (0.58 ± 0.051 to 0.07 ± 0.004 pmol/mL), and 8HO2dG (15.5 ± 1.5 to 10.4 ± 1.1 pM) with respect to LPS group.

4.5. Results in aortic tissue

Under septic conditions, NO (Fig. 1A) increased significantly from its control from 17 ± 1 to 177 ± 9 pmol/mL. The NO excess was not correlated with the elevation of BH4, the cofactor of NO synthesis. On the other hand, the garlic treatment decreased the effect of LPS by 70 %, but NO levels were still elevated compared to their control (17 ± 1 pmol/mL), in the garlic treatment group (56 ± 17 pmol/mL) and in the group treated with LPS ± garlic (48 ± 13 pmol/mL).

BH2 levels increased significantly in the LPS group with respect to the control from 4 ± 0.1 to 7 ± 0.7 pmol/mL and this effect was diminished by the garlic treatment in the LPS + garlic group to 5 ± 0.6 pmol/mL.

Fig. 1B compares the levels of Ang 1–7, Ang-II and ET-1 in the different experimental groups. The levels of Ang 1–7 in the aortic tissue in the group treated with LPS decreased from 0.15 ± 0.006 to 0.13 ± 0.002 and this effect was eliminated by the action of garlic. Therefore, garlic restored the levels of this vasodilator to 0.16 ± 0.01 pmol/mL.

In the LPS group, the levels of Ang-II and ET-1 were increased in the aortic tissue from 0.1 ± 0.004 to 0.3 ± 0.02 and from 0.02 ± 0.001 to 0.05 ± 0.006 pmol/mL, respectively.

The treatment with garlic restored Ang 1–7 levels in the LPS + garlic group. ET-1 levels were restored with garlic treatment in the LPS + garlic group.

Inflammatory factors were significantly increased in the LPS group compared to the corresponding control (Fig. 2A). IL-6 increased from 9 ± 0.5 to 20 ± 3.4 pg/mL, TNFα increased from 16 ± 1 to 28 ± 3 pg/mL, GTNA-3 increased from 73 ± 3 to 129 ± 15 pg/mL. In the LPS + garlic group, the effects of LPS on IL-6 and GTNA-3 were significantly diminished by garlic treatment compared to the LPS group. IL-6 decreased from 20 ± 3.4 to 14 ± 2.8 pg/mL, GTNA-3 decreased from 129 ± 15 to 93 ± 7 pg/mL, TNFα did not change with respect to the LPS group (28 ± 3 to 27 ± 6 pg/mL).

Damage factors increased their levels in the aortic tissue of the LPS group compared to the control group (Fig. 2B). MDA increased from 0.012 ± 0.001 to 0.017 ± 0.001 pmol/mL, MTO increased from 0.0013 ± 9.6-5 to 0.0016 ± 9.2–5 pmol/mL, and 8HO2dG

Table 3
Effect of Garlic on the inflammatory response induced by LPS.

	CONTROL	LPS	GARLIC	LPS + GARLIC
IL-6 (pg/mL)	**109.4 ± 3.8**	**417.5 ± 17.7[a]**	**96.6 ± 3.1 β**	**296.3 ± 17.3 [b]**
TNFα (pg/mL)	**12.15 ± 2.1**	**78.1 ± 6.2[a]**	**11.4 ± 2.4 β**	**34.7 ± 2.2 [b]**
GTNA-3 (pg/mL)	**1272.1 ± 94.9**	**2918.5 ± 222.2[a]**	**1193.0 ± 42.5 β**	**17066 ± 59.9 [b]**

$n = 6$; $p \leq 0.05$.
[a] Control *vs* LPS; β LPS *vs* GARLIC.
b LPS *vs* LPS + GARLIC.

Table 4
Effect of Garlic on LPS-induced cell damage.

	CONTROL	LPS	GARLIC	LPS + GARLIC
MDA (pmol/mL)	0.2 ± 0.02	0.4 ± 0.03[a]	0.2 ± 0.01 β	0.2 ± 0.01 [b]
MTO (pmol/mL)	0.07 ± 0.007	0.58 ± 0.051[a]	0.06 ± 0.003 β	0.07 ± 0.004 [b]
8HO2dG (pM)	4.9 ± 0.7	15.5 ± 1.5[a]	5.0 ± 0.27 β	10.4 ± 1.1 [b]

$n = 6$; $p \leq 0.05$.
[a] Control *vs* LPS; β LPS *vs* GARLIC.
[b] LPS *vs* LPS + GARLIC.

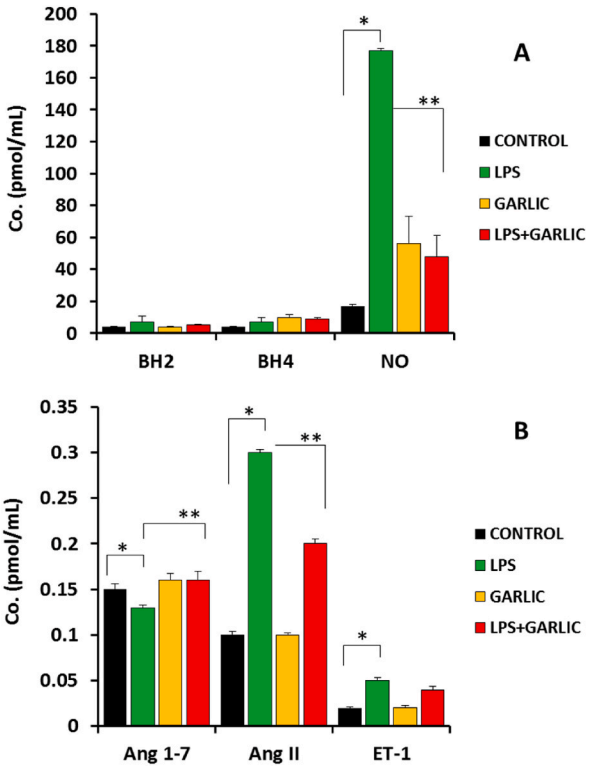

Fig. 1. Shows levels of biomarkers that are part of NO synthesis and vasodilation (A), such as BH2, BH4, and NO. In (B) the changes in the systemic levels of Ang 1–7, Ang-II and ET-1 are shown. Control (black bar), LPS-treated (green bar), Garlic-treated (yellow bar) and LPS + garlic-treated (red bar) rats. n = 6 p ≤ 0.05.
*Control *vs* LPS; βLPS *vs* Garlic; LPS + Garlic. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

increased from 0.1 ± 0.02 to 0.33 ± 0.03 pM. These increases generated in the LPS group were decreased by treatment with garlic in the LPS + garlic group. MDA decreased from 0.017 ± 0.001 to 0.004 ± 0 pmol/mL, MTO decreased from 0.0016 ± 9.2-5 to 0.0013 ± 2.3–4 pmol/mL, and 8HO2dG

decreased from 0.33 ± 0.03 to 0.17 ± 0.04 pM.

Fig. 3 shows the changes in the levels of TAC, OXCA and CGRP in the aortic tissue with the pre-treatments of LPS and garlic. The TAC (Fig. 3A) decreased due to the effect of LPS from 444 ± 12 to 221 ± 34 mmol/L. Garlic treatment increased the antioxidant capacity from 444 ± 12 to 493 ± 54 mmol/L. In the case of the LPS + garlic group, the effects of LPS were diminished and the TAC (349 ± 36 mmol/L) was significantly improved compared to the LPS group (221 ± 34 mmol/L). On the other hand, the oxidizing capacity was doubled due to the effect of LPS (Fig. 3 B) compared to the control group from 0.1 ± 0.01 to 0.2 ± 0.03 pmol/L and in this case, garlic did not reduce the effects of LPS.

To explore the possible role of TRPV1 as a regulator of the parameters studied, CGRP levels in aortic tissue were quantified (Fig. 3C). They were decreased as a result of LPS from 0.046 ± 0.002 to 0.028 ± 0.002 fmol/mL with respect to control group. Garlic treatment decreased the effects of LPS and improved CGRP levels in the LPS + garlic group compared to the LPS group from 0.028 ± 0.002 to 0.034 ± 0.001 fmol/mL.

We also analyzed iNOS and TRPV1 expression in aortic tissue and the expression of TRPV1 was increased in the group treated with garlic (Fig. 4A). The expression of iNOS was increased due to the effect of LPS. Garlic maintained expression levels as in control (see

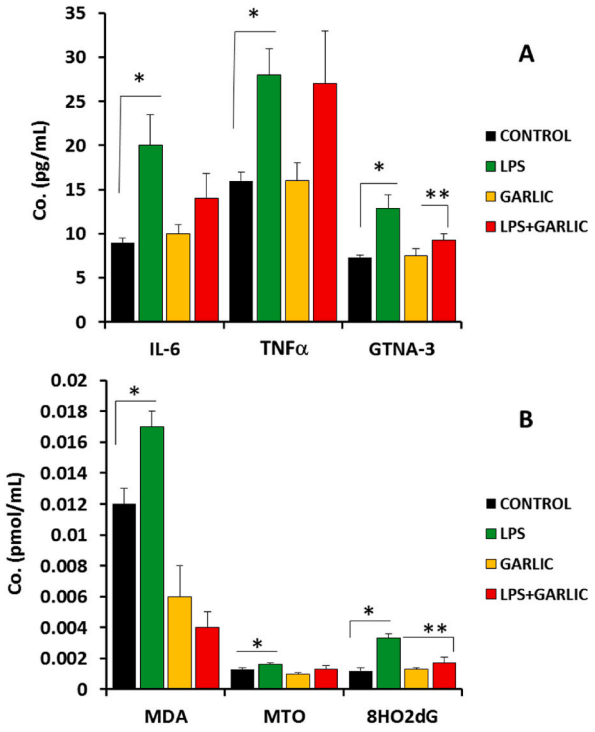

Fig. 2. It shows the results obtained from levels of inflammatory biomarkers (A) IL-6, TNFα and GTNA-3. In (B) the systemic levels of biomarkers of cell damage MDA, MTO and 8HO2dG are represented.
Control (black bar), LPS-treated (green bar), Garlic (yellow bar) and LPS + garlic-treated (red bar) rats. n = 6 $p \leq 0.05$
Control *vs* LPS; βLPS *vs* Garlic; LPS + garlic. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5).

5. Discussion

The role of aorta is to supply oxygenated blood to organs, muscles, and tissues through a branched conduction system. In this system there is a regional variation in bioarchitecture, determined by distribution of smooth muscle cells, which is greater in areas distal to heart [29,30]. Depending on the aortic segment, cellular components are: smooth muscle and endothelial cells, which with proper functioning ensure the transport of oxygenated blood, nutrients, immune system molecules, etc., throughout the body [29].

TRPV1 is expressed in endothelial and smooth muscle cells in aorta and for this reason we explored the effects of its stimulation at the systemic level and in rat thoracic aorta [12]. The participation of TRPV1 in the regulation of some biomarkers of vasodilation, vasoconstriction, inflammation, as well as tissue damage and nuclear DNA was evaluated under conditions of experimental infection with LPS [11].

We chose as a strategy a treatment with a food supplement based on garlic since TRPV1 has a binding site for allicin. We had previously analyzed this food supplement in our laboratory using HPLC (High-Performance Liquid Chromatography), and specific functional reactivity and we verified that Allicin is its main component.

The studies were carried out at systemic and thoracic aorta level in rats. We measured BH2, which is precursor for the formation of BH4 which has biological activity as a precursor of some neurotransmitters and, it catalyzes the synthesis reaction of NO [31]. We evaluated the content of antioxidant factors such as TAC and determined the content of oxidant factors through OXCA [32] Ang 1–7, Ang II and endothelin ET-1 were evaluated due to their importance for the regulation of vascular homeostasis [33,34] IL-6 and TNFα, which are biomarkers of the inflammatory response and the onset of vasculopathies, were also evaluated [35] GTN-3 secreted by macrophages which is a biomarker of the evolution of inflammation, tissue fibrosis and cardiovascular diseases was also quantified [36,37] Our results show that elevation of TNFα and IL-6 was induced by LPS. Further research is required to explore possible mechanisms involved in regulation of TNFα and IL-6 through TRPV1.

On the other hand, multi-organ damage occurs under sepsis conditions due to an important production of reactive oxygen species (ROS), and therefore it is important to evaluate cell damage molecules such as MDA, which is an indicator of lipid peroxidation in cell membranes and MTO which is an inhibitor of cellular respiration, blocking the binding site of succinate dehydrogenase. Finally, we quantified levels of 8HO2dG which is a marker of oxidative stress and direct damage to nuclear DNA [38–40] To explore the role of TRPV1 in the regulation of these parameters, we evaluated its expression and CGRP levels in aortic tissue [13,20].

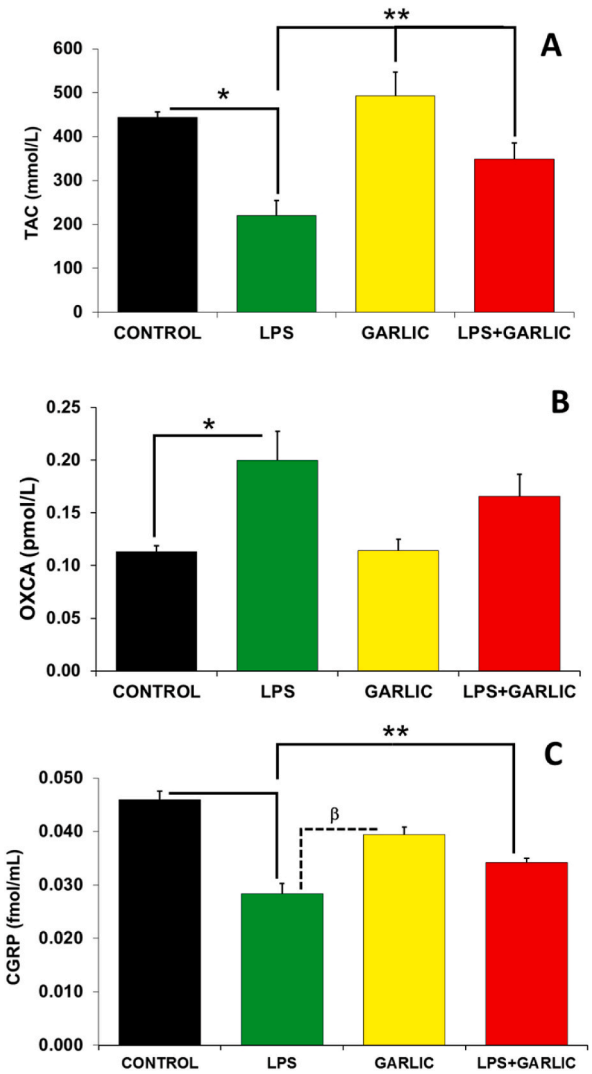

Fig. 3. This figure shows the results obtained for the levels of TAC (A), OXCA (B) and CGRP (C) in the control, LPS, Garlic and LPS + garlic groups in the ascending aortic tissue.
Control rats (black bar), LPS-treated (green bar), Garlic (yellow bar) and LPS + garlic-treated rats (red bar). $n = 6$ $p \leq 0.05$
*Control *vs* LPS; βLPS *vs* Garlic; LPS + garlic. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

From changes observed in the levels of different biomarkers, we deduce that the increase in oxidative stress was favored by oxidation of BH4 to BH2 and consequently by enzymatic uncoupling of BH4 and endothelial nitric oxide synthase (eNOS), altering the bioavailability of NO by this route.

Under conditions of infection induced by LPS, the excess in NO levels is attributed to iNOS, which, once activated, produces NO in greater quantity and for a longer time period than its isoforms, increasing possible interactions with reactive oxygen and nitrogen species. Under these conditions, the role of NO as a vasodilator was modified to that of a generator of oxidative stress [6].

Garlic, modulated the alterations induced by LPS in the group (LPS + garlic), by reducing endotoxic effect. This was evidenced by the decrease in OXCA, BH2 and NO levels, and by the increase in systemic and aortic tissue levels of TAC and BH4.

Regarding variations of the damage indicator molecules, their increase by LPS treatment is directly related to the level of oxidative stress. In this condition, production of MDA was very high, leading to an increase in MTO levels as the MDA is degraded. The direct effect of this alteration is damage to mitochondrial respiration by hypoxia and a decrease in the production of ATP [39].

When there is an increase in 8HO2dG induced by LPS endotoxicity, there is nuclear damage at the level of DNA. Garlic reduced the damage generated by LPS, lowering the levels of the damage markers MDA, MTO and 8HO2dG. As can be seen in Fig. 2B, the predominant cell damage process is lipoperoxidation. It is possible that with a longer exposure to LPS treatment, damage would also occur at the cell nucleus and DNA levels with greater increases in MTO and 8HO2dG [41]. On the other hand, the increase in GTNA-3 in presence of LPS denotes a possible phase of sepsis in which the process that leads to myocardial and vascular fibrosis begins. The effects

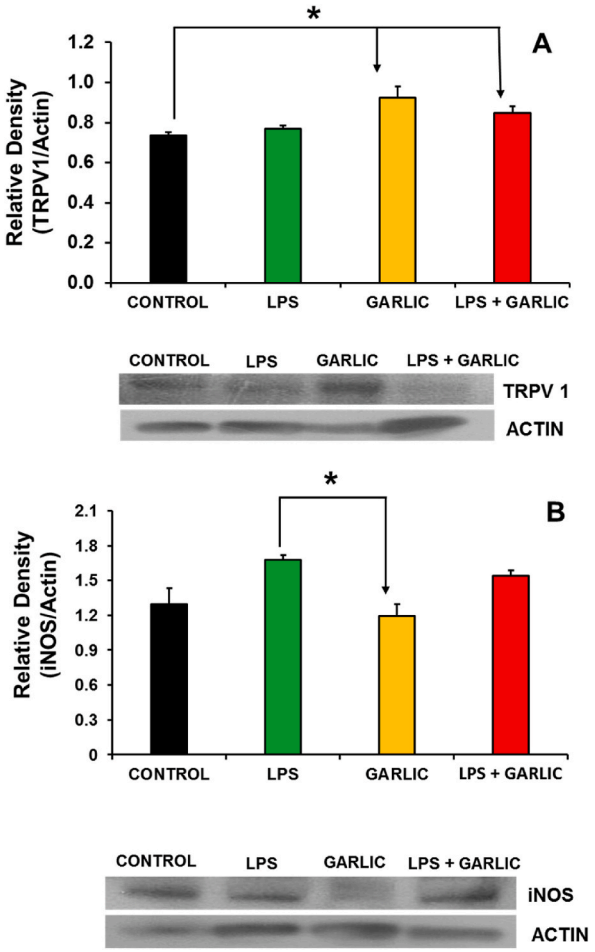

Fig. 4. Garlic treatment increased TRPV1 expression and prevented the effect of LPS (A). The expression of iNOS (B) was increased by the effect of LPS and treatment with garlic prevented the effect of LPS.
***Control *vs* Garlic; LPS + garlic**; ****LPS *vs* LPS + garlic.**

produced by LPS are significantly diminished due in part to TRPV1 activation with garlic treatment in the LPS + garlic group.

Garlic treatment increased TRPV1 expression and CGRP levels in aortic tissue. This confirms that TRPV1 was mainly activated by allicin, as it is so far the only component of garlic that has a receptor binding site. On the other hand, CGRP can be considered as a biomarker of TRPV1 activation because there is evidence that it is not only a peptide that is released in neurons but also in other cell types [20].

More studies are required on the participation of TRPV1 in the regulation of the pathways explored in this work and to analyze them separately and specifically to elucidate each mechanism. In this work we proved that TRPV1 was activated by allicin since with treatment the expression of this receptor increased and CGRP levels were elevated participating in the decrease of effects of LPS.

Therefore, TRPV1 participates in an important way in the regulation of the markers studied in this work. The study of regulatory actions of TRPV1 at cardiovascular level remains to be continued. It is important to mention that consumption of garlic or a garlic-based food supplement, as well as the consumption of other foods or medicines, must be controlled and balanced, since administration of unnecessary elements to healthy subjects or patients with diseases can alter vascular homeostasis or magnify a disease, respectively.

Although more studies are required; we can speculate that the benefit of garlic treatment may also occur in other organs as deduced from experimental results at the systemic level. Therefore, in disease conditions such as sepsis, TRPV1 and allicin can help lessen the effects of infection, especially at an early stage.

6. Conclusions

TRPV1 plays an important role in the regulation of oxidative stress, expression of molecular markers of damage and inflammatory molecules generated in a state of sepsis induced by LPS. TRPV1, CGRP and allicin constitute a therapeutic target to counteract the inflammatory state and oxidative stress.

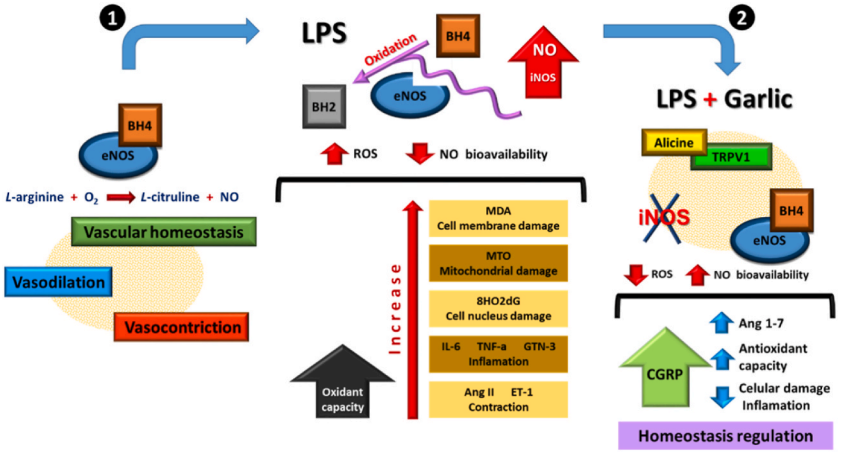

Fig. 5. This figure shows a summary of the effect of LPS and garlic on some factors acting on vasodilation, vasoconstriction, on factors of cell damage and of inflammation.
In control conditions, NO synthesis is catalyzed by an enzymatic coupling between eNOS and BH4 and vascular homeostasis is maintained. In infection caused by *E. coli* LPS, this coupling is broken by oxidation of BH4 to BH2. NO is increased by activation of iNOS, which promotes an increase in ROS and, as a result of this oxidative stress, NO bioavailability decreases. The increase in the oxidant capacity generates damage to the cell membrane, mitochondrial function, and the cell nucleus due to an increase in the levels of MDA, MTO and 8HO2dG. Inflammatory molecules such as IL-6, TNF-α and GTN-3, as well as regulators of contractile molecules such as Ang-II and ET-1 are also significantly increased in response to infection.
In animals treated with LPS, administration of garlic decreased the effects of infection and this is attributed to the binding of allicin to TRPV1 as one of the mechanisms involved in the regulation of oxidative stress, decreasing ROS levels and restoring bioavailability of NO by enzymatic coupling of BH4 and eNOS. In addition, CGRP and Ang 1–7 levels were increased to restore antioxidant capacity and to maintain homeostasis of inflammatory and cell damage molecules.

Data availability

All data generated or analyzed during this study are included in this published article and its additional information files.

Additional information

No additional information is available for this paper.

Author contributions

Juan Carlos Torres-Narváez: Conceived and designed the experiments, performed the experiments and wrote the paper. ***Israel Pérez-Torres:*** Analyzed and interpreted the data and contributed reagents, materials, analysis tools or data. ***Leonardo del Valle-Mondragón:*** Analyzed and interpreted the data and contributed reagents, materials, analysis tools or data. ***Vicente Castrejón-Tellez:*** Analyzed and interpreted the data and contributed reagents, materials, analysis tools or data. ***Verónica Guarner-Lans:*** Analyzed and interpreted the data and wrote the paper. ***María Sánchez-Aguilar:*** Analyzed and interpreted the data. ***Elvira Varela-López:*** Analyzed and interpreted the data. ***Alvaro Vargas-González:*** Analyzed and interpreted the data. ***Gustavo Pastelín-Hernández:*** Analyzed and interpreted the data and contributed reagents, materials, analysis tools or data. ***Julieta Anabell Díaz-Juárez:*** Conceived and designed the experiments, performed the experiments and wrote the paper.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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To Instituto Nacional de Cardiología Ignacio Chávez.

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