Toxicology Letters 241 (2016) 152–158

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Alpha lipoic acid supplementation attenuates reactive oxygen species in hypothalamic paraventricular nucleus and sympathoexcitation in high salt-induced hypertension

Qing Su[a,1], Jin-Jun Liu[b,1], Wei Cui[c,1], Xiao-Lian Shi[d], Jing Guo[a], Hong-Bao Li[a], Chan-Juan Huo[a], Yu-Wang Miao[a], Meng Zhang[a], Qing Yang[a], Yu-Ming Kang[a,*]

[a] Department of Physiology and Pathophysiology, Key Laboratory of Environment and Genes Related to Diseases, Xi'an Jiaotong University School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an Jiaotong University Cardiovascular Research Center, Xi'an 710061, China
[b] Department of Physiology and Pathophysiology, Xi'an Jiaotong University School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an 710061, China
[c] Department of Endocrinology and Metabolism, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an Jiaotong University Health Science Center, Xi'an 710061, China
[d] Department of Pharmacology, Xi'an Jiaotong University School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an 710061, China

HIGHLIGHTS

- The effect of chronic ALA supplementation on salt-induced hypertension is reported.
- High-salt diet induced increased proinflammatory cytokines and superoxide in PVN.
- High-salt diet induced oxidative stress in PVN, sympathoexcitation and hypertension.
- Chronic ALA supplementation attenuates ROS in PVN and sympathoexcitation.
- Chronic inhibiting superoxide attenuates RAS and cytokines in PVN in hypertension.

ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form 6 October 2015
Accepted 21 October 2015
Available online 27 October 2015

Keywords:
Alpha lipoic acid
Oxidative stress
Hypothalamic paraventricular nucleus
Sympathoexcitation
High salt-induced hypertension

ABSTRACT

Aims: High salt-induced oxidative stress plays an important role in the development of hypertension. Alpha lipoic acid (ALA) is extensively recognized as having a powerful superoxide inhibitory property. In this study, we determined whether ALA supplementation attenuates oxidative stress in hypothalamic paraventricular nucleus (PVN), decreases the sympathetic activity and arterial pressure in high salt-induced hypertension by cross-talking with renin–angiotensin system (RAS) and pro-inflammatory cytokines (PICs).

Methods: Male Wistar rats were administered a normal-salt diet (NS, 0.3% NaCl) or a high-salt diet (HS, 8.0% NaCl) for 8 weeks. These rats received ALA (60 mg/kg) dissolved in vehicle (0.9% saline) or an equal voleme of vehicle, by gastric perfusion for 9 weeks.

Results: High salt intake resulted in higher renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP). These rats also had higher levels of superoxide, $gp91^{phox}$, $gp47^{phox}$ (subunits of NAD(P)H oxidase), angiotensin-converting enzyme (ACE), angiotensin II type1 receptor (AT1-R), interleukin-1beta (IL-1β), interleukin-6 (IL-6), and lower levels of interleukin-10 (IL-10) and copper/zinc superoxide dismutase (Cu/Zn-SOD) than control animals. Treatment with ALA significantly attenuated the levels of superoxide, $gp91^{phox}$, $gp47^{phox}$, ACE, AT1-R, IL-1β and IL-6, increased the levels of IL-10 and Cu/Zn-SOD, and decreased MAP and RSNA compared with high-salt induced hypertensive rats. The mRNA expression of $gp47^{phox}$ and $gp91^{phox}$ are in accordance with their protein expression.

* Corresponding author at: Department of Physiology & Pathophysiology, Xi'an Jiaotong University School of Basic Medical Sciences, Xi'an 710061, China. Fax: +86 2982657677.
E-mail address: ykang@mail.xjtu.edu.cn (Y.-M. Kang).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.toxlet.2015.10.019
0378-4274/

Conclusion: These findings suggest that supplementation of ALA obviously decreases the sympathetic activity and arterial pressure in high salt-induced hypertension by improving the superoxide inhibitory property, suppressing the activation of RAS and restoring the balance between pro- and anti-inflammatory cytokines in the PVN.

1. Introduction

Oxidative stress is one of the important mechanisms responsible for the development of hypertension, which has been shown to be triggered by the imbalance between reactive oxygen species (ROS) and antioxidant defenses system due to overproduction of ROS (Datla and Griendling, 2010; Rodrigo et al., 2011). Studies have demonstrated that ROS in the central nervous system is extremely critical for arterial pressure regulation by modulating renal sympathetic nerve activity (RSNA) (Fujita et al., 2007; Nagae et al., 2009). NAD(P)H oxidase family enzymes composed of seven members gp91^{phox}, gp47^{phox}, NOX-1, NOX-3, NOX-5, DUOX1 (dual oxidase-1) and DUOX2 (dual oxidase-2), are one of the main sources of ROS. Among NOX enzymes, gp91^{phox} and gp47^{phox} play an important role in the ROS overproduction in the progression of hypertension (Datla and Griendling, 2010). There are accumulating evidences suggesting that salt-induced hypertension is also closely related to the NOX-derived ROS (Akasaki et al., 2006; Iwai et al., 2006).

The hypothalamic paraventricular nucleus (PVN) is a central integration site for the regulation of cardiovascular functions, including sympathetic nerve activity and blood pressure control, through coordination of neuroendocrine and autonomic (Benarroch, 2005; Ferguson et al., 2008; Sriramula et al., 2011). As an important intracellular messenger, the overproduction of ROS in PVN increases the RSNA and arterial pressure in the development of cardiovascular diseases (Kang et al., 2009b). Angiotensin II (ANG II) is a principal and biologically active component of the renin–angiotensin system (RAS). The binding of ANG II to angiotensin II type 1 receptor (AT1-R) in the PVN modulates sympathetic outflow, which could be a trigger of the hypertensive responses. It has been shown that increased inflammatory cytokines (PICs), such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and the interleukin-6 (IL-6), in the PVN have been associated with a higher cardiovascular risk in hypertension (Kang et al., 2014; Li et al., 2014; Song et al., 2014). Therefore, ROS, RAS, and PICs are all involved in the pathogenesis of hypertension. However, little is known about the cross-talk between these factors during the development of hypertension.

Alpha lipoic acid (ALA) is an organosulfur compound found naturally in our diets, which could be soluble in both aqueous and lipid portions of the cell. It has been reported that ALA is a powerful antioxidant which is more efficient than vitamin C, vitamin E and glutathione against oxidative stress (Shay et al., 2009; Wollin and Jones, 2003). A previous study has shown that chronic treatment with ALA reduces hypertension in rats with renovascular hypertension (Queiroz et al., 2012). So ALA has been recognized as a potential therapeutic for the treatment of high salt-induced hypertension. Therefore, the present study was aimed to determine whether ALA treatment could attenuate high salt-induced hypertension by cross-talking with RAS and PICs.

Table 1
Rat primers used for real-time PCR.

Rat genes	Forward (5′–3′)	Reverse (5′–3′)
gp91^{phox}	CTGCCAGTGTGTCGGAATCT	TGTGAATGGCCGTGTGAAGT
gp47^{phox}	GGATCACAGAAGGTCCCTAGC	AGAAGTTCAGGGCGTTCACC
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT

2. Material and methods

2.1. Animals

Six-week-old male Wistar rats weighing 100–120 g were purchased from the experimental animal center of Xi'an Jiaotong University. All rats were housed in a room maintained at 23–25 °C with a 12:12 h light:dark cycle and allowed access to tap water *ad libitum*. All experimental procedures were reviewed and approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (the US National Institutes of Health Publication No. 85-23, revised 1996). All of the animal procedures were conducted according to the Animal Care and Use Committees of Xi'an Jiaotong University.

2.2. General experimental protocol

Rats were fed on high-salt diet containing 8% NaCl for 8 weeks to induce hypertension or the normal salt diet containing 0.3% NaCl (NS) as control. After 8 weeks, NS group and HS group were administered ALA (60 mg/kg/day; Sigma) dissolved in 0.9% saline or an equal voleme of vehicle for 9 weeks by intragastric administration (i.g.) respectively. Therefore, 4 groups were included in this study: (1) Wistar normal salt diet (0.3% NaCl) + vehicle (NS + vehicle), (2) Wistar normal salt diet (0.3% NaCl) + alpha lipoic acid (NS + ALA), (3) Wistar high salt diet (8% NaCl) + vehicle (HS + vehicle), (4) Wistar high salt diet (8% NaCl) + alpha lipoic acid (HS + ALA).

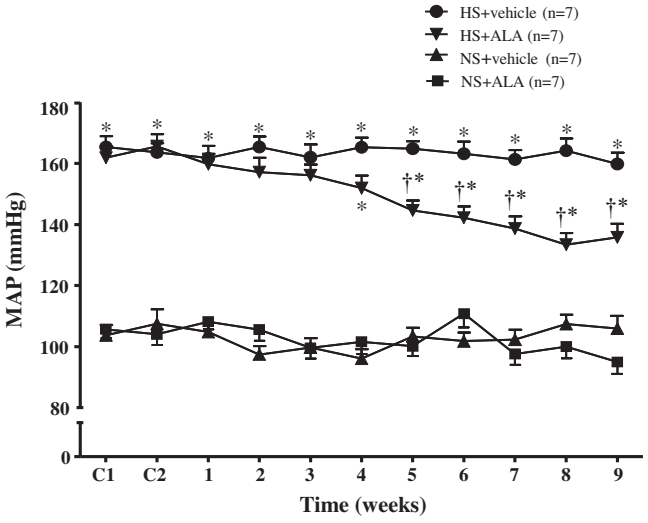

Fig. 1. Effects of ALA on mean arterial pressure (MAP) in salt-induced hypertensive rats. MAP is increased in high salt (HS) intake rats. ALA administration for 9 weeks attenuated high salt-induced presser response. Values are expressed as means ± SME. *$P < 0.05$ *versus* NS groups (NS + ALA or NS + vehicle); †$P < 0.05$ HS + ALA *versus* HS + vehicle.