Nitric Oxide 74 (2018) 65–73

Contents lists available at ScienceDirect

Nitric Oxide

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

Contrasting effects of low *versus* high ascorbate doses on blood pressure responses to oral nitrite in L-NAME-induced hypertension

Lucas C. Pinheiro[a], Graziele C. Ferreira[a], Kelvin H. Vilalva[a], José C. Toledo Jr.[b], Jose E. Tanus-Santos[a,*]

[a] Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirao Preto, SP, Brazil
[b] Department of Chemisty, Faculty of Phylosophy, Sciences and Languages of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes 3900, 14040-901, Ribeirao Preto, SP, Brazil

ARTICLE INFO

Keywords:
Nitrite
Ascorbate
S-nitrosothiols

ABSTRACT

Nitrite reduces blood pressure (BP) in both clinical and experimental hypertension. This effect is attributable to the formation of nitric oxide (NO) and other NO-related species, which may be improved by ascorbate or other antioxidants. However, the BP responses to oral nitrite result, at least in part, of increased gastric S-nitrosothiol formation. This study tested the hypothesis that ascorbate may destroy S-nitrosothiols and therefore not all doses of ascorbate enhance the BP responses to oral nitrite. We assessed the BP responses to oral sodim nitrite (0.2 mmol/kg) in L-NAME hypertensive rats pretreated with ascorbate (0, 0.02, 0.2, or 2 mmol/kg). Plasma and gastric wall concentrations of nitrite and nitroso compounds concentrations were determined using an ozone-based reductive chemiluminescence assay. Nitrate concentrations were determined using the Griess reaction. Free thiol concentrations were determined by a colorimetric assay. The BP responses to nitrite exhibited a bell-shape profile as they were not modified by ascorbate 0.02 mmol/l, whereas the 0.2 mmol/kg dose enhanced and the 2 mmol/kg dose attenuated BP responses. In parallel with BP responses, nitrite-induced increases in plasma nitrite and RSNO species were not modified by ascorbate 0.02 mmol/l, whereas the 0.2 mmol/kg dose enhanced and the 2 mmol/kg dose attenuated them. Similar experiments were carried out with an equimolar dose of S-nitrosogluthathione. Ascorbate dose-dependently impaired the BP responses to S-nitrosogluthathione, and the corresponding increases in plasma RSNO, but not in plasma nitrite concentrations. This is the first study to show that while ascorbate dose-dependently impairs the BP responses to oral S-nitrosogluthathione, there are contrasting effects when low *versus* high ascorbate doses are compared with respect to its effects on the blood pressure responses to oral nitrite administration. Our findings may have special implications to patients taking ascorbate, as high doses of this vitamin may impair protective mechanisms associated with nitrite or nitrate from dietary sources.

1. Introduction

Many experimental studies have consistently shown that treatment with oral nitrate (NO_3^-) or nitrite (NO_2^-) reduce blood pressure in a variety of hypertension animal models [1–6]. This effect was shown in humans more than two decades ago [7], and later elegant studies confirmed blood pressure reductions by nitrate-rich beetroot juice [8]. It is now becoming clear that this effect may depend, at least in part, on nitrite-derived formation of S-nitrosothiols in acid environment of stomach [5,6,9]. However, although a previous study showed that esomeprazole blunted the acute responses to oral nitrite in normotensive volunteers [10], acute increases in gastric pH impaired the formation S-nitrosothiols in the stomach without completely blunting the blood pressure responses to oral nitrite in hypertensive animals [9]. This finding probably results of increased circulating nitrite concentrations after nitrite administration [9,11], thus stimulating nitric oxide (NO) generation by enzymes with nitrite-reductase activity [12].

Lundberg et al. showed in a seminal study that ingestion of lettuce increases gastric nonenzymatic NO production, which is critically dependent on pH to generate different nitrogen oxides in the stomach [13]. However, the redox state of the gastric environment also affects the formation of particular vasoactive mediators that improve nitrite-mediated antihypertensive effects [11,14]. In fact, previous studies have shown that a variety of antioxidants increase NO formation in the stomach when nitrite or nitrate are administered [11,14,15]. Interestingly, the nitroxyl tempol facilitated nitrite-derived NO formation in a

* Corresponding author.
E-mail addresses: tanus@fmrp.usp.br, tanussantos@yahoo.com (J.E. Tanus-Santos).

https://doi.org/10.1016/j.niox.2018.01.006
Received 22 September 2017; Received in revised form 10 January 2018; Accepted 15 January 2018

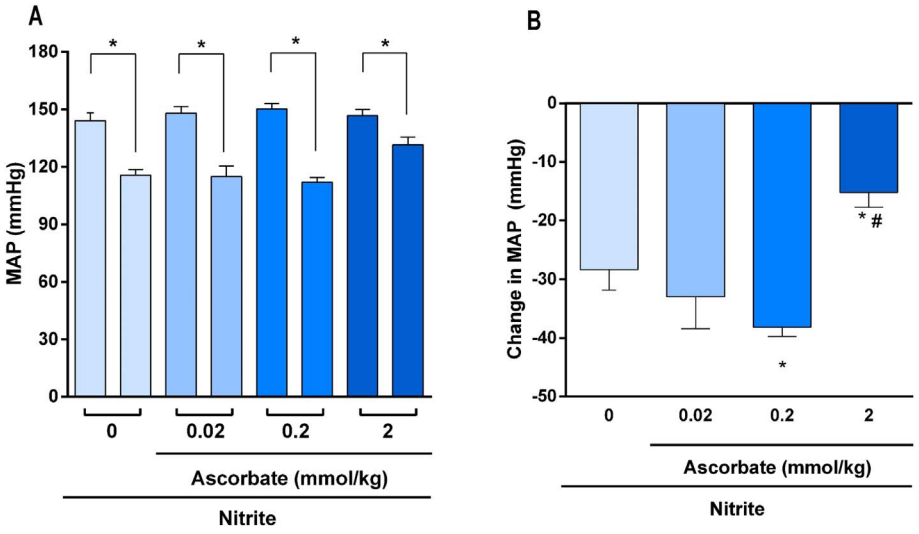

Fig. 1. Effects of ascorbate on the blood pressure responses to sodium nitrite in hypertensive rats. (a) Each pair of bars shows invasive mean arterial pressure (MAP) before and after treatment with nitrite (0.2 mmol/kg by gavage) in L-NAME hypertensive rats pretreated with ascorbate (0, 0.02, 0.2, or 2 mmol/kg by gavage) 5 min before nitrite administration. **(b)** Change in MAP after sodium nitrite administration as described in (a)Data are shown as mean ± S.E.M. (n = 5–8 per group).*P < 0.05 *versus* vehicle (control).#P < 0.05 *versus* Ascorbate 0.2 mmol/kg.

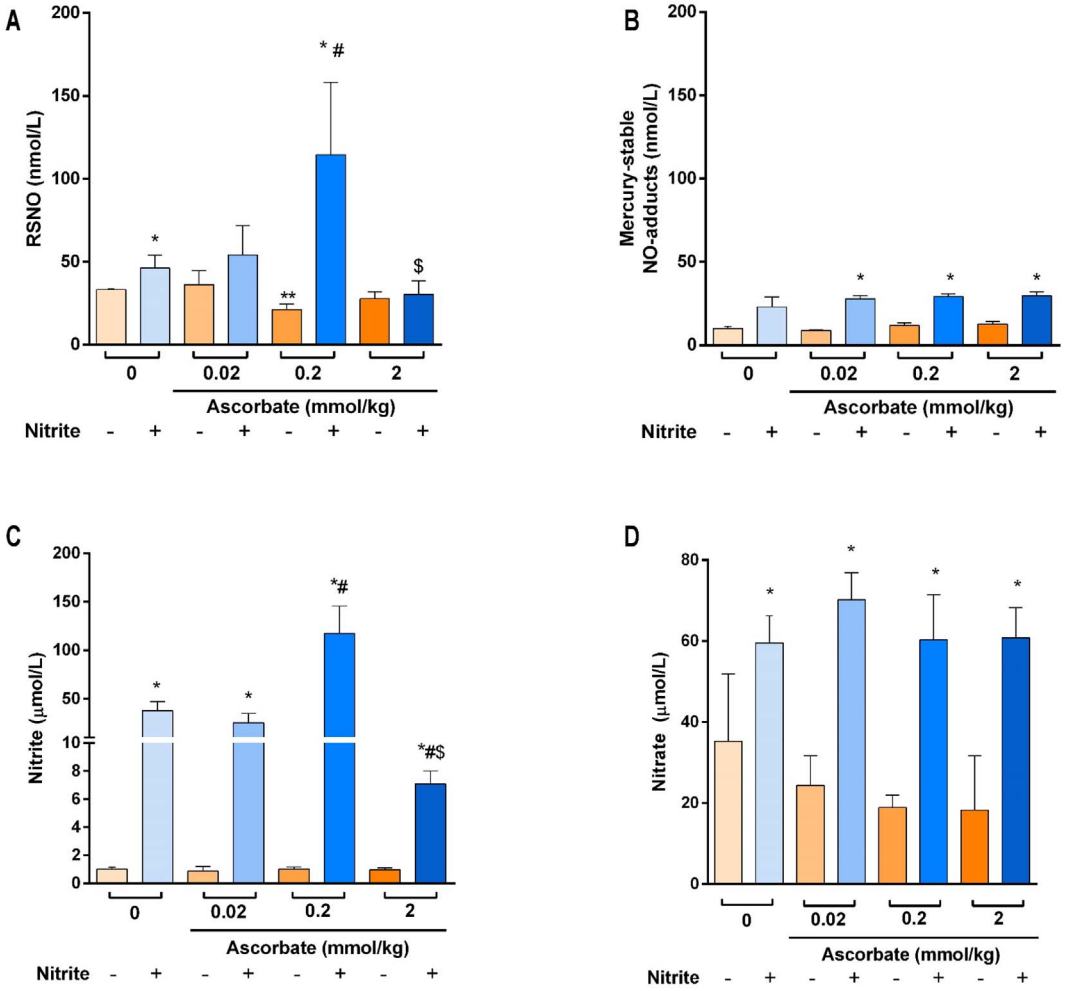

Fig. 2. Plasma concentrations of S-nitrosothiols (RSNO). (a), mercury stable NO-adducts (b), nitrite (c), and nitrate (d) in L-NAME hypertensive rats pretreated with ascorbate (0, 0.02, 0.2, or 2 mmol/kg by gavage) 5 min before nitrite (+, blue bars) or vehicle (-, orange bars) administration. Data are shown as mean ± S.E.M. (n = 5–8 per group). *P < 0.05 *versus* respective vehicle treated control group. **P < 0.05 *versus* ascorbate 0 + vehicle control group. #P < 0.05 *versus* ascorbate 0 + nitrite group. $ P < 0.05 *versus* ascorbate 0.2 mmol/kg + nitrite group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

concentration-dependent manner, and this effect was significantly enhanced at low pH [11]. Correspondingly, blood pressure responses to nitrite increased when tempol was also administered orally, but not intravenously, strongly suggesting that the interaction between tempol and nitrite under acidic conditions promotes blood pressure lowering effects of nitrite [11]. However, the possibility that some antioxidant drugs may dose-dependently enhance the blood pressure responses to oral nitrite has not been explored, particularly with respect to