Redox Biology 9 (2016) 134–143

Contents lists available at ScienceDirect

Redox Biology

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

Omeprazole impairs vascular redox biology and causes xanthine oxidoreductase-mediated endothelial dysfunction

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ARTICLE INFO

Article history:
Received 4 July 2016
Received in revised form
29 July 2016
Accepted 3 August 2016
Available online 4 August 2016

Keywords:
Endothelial dysfunction
Omeprazole
Oxidative stress
Proton pump inhibitors

ABSTRACT

Proton pump inhibitors (PPIs) are widely used drugs that may increase the cardiovascular risk by mechanisms not entirely known. While PPIs increase asymmetric dimethylarginine (ADMA) levels and inhibit nitric oxide production, it is unknown whether impaired vascular redox biology resulting of increased xanthine oxidoreductase (XOR) activity mediates PPIs-induced endothelial dysfunction (ED). We examined whether increased XOR activity impairs vascular redox biology and causes ED in rats treated with omeprazole. We also examined whether omeprazole aggravates the ED found in hypertension. Treatment with omeprazole reduced endothelium-dependent aortic responses to acetylcholine without causing hypertension. However, omeprazole did not aggravate two-kidney, one-clip (2K1C) hypertension, nor hypertension-induced ED. Omeprazole and 2K1C increased vascular oxidative stress as assessed with dihydroethidium (DHE), which reacts with superoxide, and by the lucigenin chemiluminescence assay. The selective XOR inhibitor febuxostat blunted both effects induced by omeprazole. Treatment with omeprazole increased plasma ADMA concentrations, XOR activity and systemic markers of oxidative stress. Incubation of aortic rings with ADMA increased XOR activity, DHE fluorescence and lucigenin chemiluminescence signals, and febuxostat blunted these effects. Providing functional evidence that omeprazole causes ED by XOR-mediated mechanisms, we found that febuxostat blunted the ED caused by omeprazole treatment. This study shows that treatment with omeprazole impairs the vascular redox biology by XOR-mediated mechanisms leading to ED. While omeprazole did not further impair hypertension-induced ED, further studies in less severe animal models are warranted. Our findings may have major relevance, particularly to patients with cardiovascular diseases taking PPIs.

1. Introduction

Proton pump inhibitors (PPIs) are over-the-counter drugs widely used in gastroenterology because they inhibit gastric acid secretion. While PPIs have usually been considered relatively safe drugs, recent studies suggest that the use of omeprazole increases the risk of acute myocardial infarction [1–3]. At least two mechanisms involving impaired nitric oxide (NO) activity have been implicated in the increased cardiovascular risk associated with the use of PPIs. First, these drugs were shown to elevate the concentrations of a NO synthase (NOS) inhibitor, asymmetric dimethylarginine (ADMA) [4], which inhibits NOS. Second, interference with gastric formation of protective NO-related species from dietary nitrite and nitrate may also impair non-enzymatic formation of NO and normal cardiovascular function [5–8].

Although these mechanisms disturbing NO biology may impair vascular redox biology and cause endothelial dysfunction [9], no previous study has examined the possibility that omeprazole causes endothelial dysfunction by activating other critical mechanisms leading to endothelial dysfunction. Indeed, while omeprazole and other PPIs increase ADMA concentrations [4], no previous study has examined the possibility that PPIs may impair vascular redox biology by mechanisms increasing the formation of reactive oxygen species (ROS), thus promoting endothelial dysfunction. Examining this possibility is important because PPIs used to be accepted as a safe therapy without major adverse effects on the cardiovascular system. However, mounting clinical evidence now challenges this view [1–3]. Astonishingly, some studies suggest that omeprazole exerts antioxidant effects [10] and may induce vascular relaxation that is partially NO-mediated [11,12], and

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http://dx.doi.org/10.1016/j.redox.2016.08.001
2213-2317/

therefore no deleterious effects would be expected on the cardiovascular system. However, these previous studies have not assessed *in vivo* effects of omeprazole on the vascular function, and it is possible that increased ADMA concentrations after treatment with omeprazole [4] decrease NO activity and promote prooxidant mechanisms and vascular dysfunction.

Because NO down-regulates xanthine oxidoreductase (XOR) activity [13], a major contributor to oxidative stress in many cardiovascular diseases [13,14], we hypothesized that omeprazole increases ADMA concentrations, which impair NO formation and cause endothelial dysfunction by increasing XOR activity and impairing vascular redox biology. While previous studies showed that ADMA promotes tissue oxidative stress [15], a direct relationship between PPIs-induced increases in ADMA concentrations and vascular oxidative stress has not been shown. This mechanism possibly activated by PPIs may be critically involved in the vascular dysfunction and enhanced cardiovascular risk of patients taking PPIs. In addition, given that omeprazole is widely prescribed to hypertensive subjects, we examined whether treatment with omeprazole further impairs hypertension-induced vascular dysfunction.

2. Materials and methods

2.1. Animals, treatment with omeprazole and hypertension model

This study followed the guidelines of the Ribeirao Preto Medical School, University of Sao Paulo, and the animals were handled according to the guiding principles published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Wistar rats (180–200 g) from the colony at University of São Paulo were maintained at room temperature (22–25 °C) on light/dark cycle (12 h) and had free access to standard rat chow and water.

To assess the cardiovascular effects of omeprazole, the rats were treated with omeprazole 10 mg/kg i.p. (or vehicle) daily [5,6] for four weeks. This dose significantly impaired antihypertensive effects associated with increased nitric oxide formation [5–7] . Moreover, to examine the possibility that treatment with omeprazole further impairs the cardiovascular alterations of hypertension, the same treatment with omeprazole (or vehicle) was administered to two kidney, one clip (2K1C)-hypertensive rats. Treatment with omeprazole started after two weeks of hypertension, when the animals were randomly allocated to treatment with omeprazole or control group.

2K1C hypertension was induced as previously described [16,17]. Systolic blood pressure (SBP) was assessed weekly by tail-cuff plethysmography [18]. By the end of the sixth week of study, the rats were anesthetized with tribromoethanol (250 mg/kg), and arterial blood samples were collected into tubes containing heparin for further biochemical determinations. The thoracic aorta was carefully excised, cleaned of adherent connective tissues and fat, and cut into 4 mm rings for biochemical determinations and vascular reactivity assessment. One ring was embedded in tissue-tek® and used later to prepare cryosections.

2.2. Assessment of changes in vascular reactivity associated with omeprazole treatment and/or hypertension

To assess the effects of omeprazole treatment and/or hypertension on vascular function, the thoracic aorta was carefully excised as described above, and cut into 4 mm rings. The rings were studied as previously detailed [19]. Endothelial integrity was examined by assessing the relaxation in response to acetylcholine (10^{-6} mol/L) under contractile tone induced by phenylephrine (10^{-7} mol/L). Thereafter, the aortic rings with intact, functional endothelium were precontracted with phenylephrine (10^{-7} mol/L) and the relaxing responses to cumulative concentrations (from 10^{-10} to 10^{-5} mol/L) of acetylcholine were measured to construct concentration-response curves. These experiments were carried out using aortas from normotensive (or 2K1C hypertensive) rats treated with omeprazole (or vehicle).

2.3. Assessment of gastric washing pH

The effects of omeprazole on gastric pH were assessed by measuring gastric washing pH as previously detailed [6].

2.4. Assessment of vascular reactive oxygen species production

To assess vascular oxidative stress, two independent biochemical assays were used to assess reactive oxygen species (ROS) production. First, superoxide production by the aortas was measured by dihydroethidium (DHE), as previously described [20]. Aortic cryosections (5 μm thick) were incubated with DHE (10 μmol/l) for 30 min and examined by fluorescence microscopy (Leica Imaging Systems Ltd., Cambridge, England) at × 400 using λ=525 nm excitation and λ=605 nm emission, which is not specific to detect only superoxide [21].

In some experiments, the aortas were pretreated for 1 h with Tiron (1 mmol/L, a superoxide scavenger) [22], diphenyl iodonium (DPI 10 μmol/L, a flavoprotein inhibitor) [22], tempol (100 μmol/L, a superoxide dismutase analogue) [23], oxypurinol (300 μmol/L, a XOR inhibitor) [24], febuxostat (50 nmol/L, a selective XOR inhibitor) [25] or asymmetric dimethylarginine (ADMA, 1 μmol/L).

Second, to further explore the effects of omeprazole and/or hypertension on vascular ROS production, NADPH-dependent superoxide production by aortic tissue was measured as previously described [26]. Some experiments were performed in the absence or presence of febuxostat (50 nmol/L) or DPI (10 μmol/L).

2.5. Assessment of vascular and plasma xanthine oxidase activity

Aortic and plasma xanthine oxidoreductase activity were measured as previously described [27], with a commercial fluorometric assay kit (Cayman Chemical Co., Ann Arbor, MI, USA, item number 10010895), following the manufacturer's instructions. Some experiments were performed in presence of febuxostat (50 nmol/L), a selective inhibitor or xanthine oxide reductase [25].

2.6. Assessment of circulating markers of oxidative stress (plasma lipid peroxide and 8-isoprostanes)

To examine whether treatment with omeprazole affects the circulating levels of markers of oxidative stress, plasma lipid peroxide (determined by measuring thiobarbituric acid reactive substances; TBARS) and 8-isoprostanes concentrations were determined. TBARS were measured by a fluorometric method, as previously detailed [28].

2.7. Assessment of plasma asymmetric dimethylarginine (ADMA) concentrations

Because treatment with omeprazole or other PPIs increases the formation of ADMA, the plasma ADMA concentrations were measured both in normotensive and hypertensive rats treated with omeprazole (or vehicle) with a commercially available enzyme-linked immunosorbent assay kit (MyBioSource, San Diego, CA, USA, item number: MBS703256).