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Quercetin decreases the activity of matrix metalloproteinase-2 and ameliorates vascular remodeling in renovascular hypertension

Sherliane C. Pereira [a, 1], Juliana M. Parente [a, 1], Vanessa A. Belo [a], Atlante S. Mendes [a], Natália A. Gonzaga [a, b], Gabriel T. do Vale [a, b], Carla S. Ceron [a, c], José Eduardo Tanus-Santos [a], Carlos R. Tirapelli [a, b], Michele M. Castro [a, *]

[a] Department of Pharmacology, Ribeirao Preto Medical School, University of Sao, Paulo, Av. Bandeirantes, 3900, 14049-900, Ribeirao Preto, SP, Brazil
[b] Laboratory of Pharmacology, DEPCH, College of Nursing of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, 14040-902, Ribeirao Preto, Brazil
[c] Faculty of Pharmaceutical Sciences, Federal University of Alfenas, Gabriel Monteiro da Silva, 700, 37130001, Alfenas, MG, Brazil

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ABSTRACT

Background and aims: Increased activity of matrix metalloproteinase (MMP)-2 is observed in aortas of different models of hypertension, and its activation is directly mediated by oxidative stress. As quercetin is an important flavonoid with significant antioxidant effects, the hypothesis here is that quercetin will reduce increased MMP-2 activity by decreasing oxidative stress in aortas of hypertensive rats and then ameliorate hypertension-induced vascular remodeling.

Methods: Male two-kidney one-clip (2K1C) hypertensive Wistar rats and controls were treated with quercetin (10 mg/kg/day) or its vehicle for three weeks by gavage. Rats were then analyzed at five weeks of hypertension. Systolic blood pressure (SBP) was determined by tail-cuff plethysmography. Aortas were used to determine MMP activity by *in situ* zymography and reactive oxygen species (ROS) levels by dihydroethidium. Western blot was performed to detect focal adhesion kinase (FAK) and phosphorylated-FAK levels.

Results: SBP was increased in 2K1C rats and only a borderline reduction in SBP was observed after treating 2K1C rats with quercetin. Cross-sectional area and the number of vascular smooth muscle cells were significantly increased in aortas of hypertensive rats, and quercetin reduced them. Quercetin reduced ROS levels in aortas of 2K1C rats and the increased activity of gelatinases *in situ*. However, quercetin did not affect the levels of tissue inhibitor of MMP (TIMP)-2 and did not interfere with FAK and p-FAK levels in aortas of hypertensive rats. Furthermore, different concentrations of quercetin did not directly reduce the activity of human recombinant MMP-2 *in vitro*.

Conclusions: Quercetin reduces hypertension-induced vascular remodeling, oxidative stress and MMP-2 activity in aortas.

1. Introduction

Endothelial dysfunction and chronic vascular remodeling are general hallmarks of hypertension, which is a leading cause of stroke and heart failure worldwide. Increased activity of superoxide-producing nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase and oxidative stress contribute to hypertension-induced arterial contractility, hypertrophy and endothelial dysfunction [1–3]. Increased levels of reactive oxygen species (ROS) in vascular smooth muscle cells (VSMC) trigger proliferative signaling pathways that notably contribute to proliferation and maladaptive remodeling in hypertension [4–8]. In fact, oxidative stress increases the phosphorylation of focal adhesion kinase (FAK) in endothelial and VSMC cells to contribute to migration [9,10]. Treating hypertensive rats with antioxidants decrease NAD(P)H oxidase activity and excessive levels of ROS, which ameliorate vascular dysfunction and remodeling [2,11–13]. Increased ROS regulate gene expression and activation of matrix metalloproteinases (MMPs) [12,14–16], which are responsible for

* Corresponding author.
E-mail address: castro@fmrp.usp.br (M.M. Castro).
[1] These authors contributed equally to this work.

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the proteolysis of extra- and intracellular proteins, thus resulting in progressive changes in vascular structure and function [17]. Increased activity of MMP-2 is observed in both conductance and resistance arteries in different models of hypertension, which contributes to vascular hypertrophic and eutrophic remodeling [18–22]. Treating two kidney-one clip (2K1C) hypertensive rats with tempol reduced the increased levels of ROS and the activity of MMP-2 in aorta and cardiac ventricle, which reduced hypertension-induced hypertrophic remodeling [12,23]. The hypertrophic vascular remodeling is characterized per hypertrophy and hyperplasia of VSMC in the aortic media to compensate for hypertension-induced arterial stretch. As a result, there are significant increase in the arterial cross-sectional area (CSA) and media-to-lumen (M/L) ratio, which are important indicators of chronic remodeling [17,24]. Therefore, it would be useful to decrease local oxidative stress and MMP activity to ameliorate hypertension-induced cardiovascular complications. Among several synthetic antioxidants that are used to reduce oxidative stress, the natural polyphenols, which are normally obtained in a healthy diet, do not have severe adverse effects and are also very effective in reducing oxidative stress in experimental models of hypertension.

Quercetin is a polyphenol that is considerably present in green vegetables and fruits, and possesses a significant antioxidant and anti-inflammatory effects that facilitate its use for preventing cardiovascular diseases [25]. Quercetin inhibits the activity of NAD(P)H oxidase in addition to decrease p47phox levels, and exerts oxygen radical-scavenging properties [26,27]. Treating hypertensive rats with quercetin reduced oxidative stress, improved nitric oxide bioavailability and reduced arterial endothelial dysfunction and blood pressure [27,28]. Quercetin also decreased the accentuated activity of MMP-2 in a mouse aorta with aneurysm [29] and in the heart of doxorubicin-treated rats, thus recovering cardiac function during post-ischemia [30]. Reduced levels of MMP-2 and others circulating biomarkers of cardiovascular diseases were also observed in plasma of healthy vegetarian men [31], which suggest that a diet rich in polyphenols may help to prevent from cardiovascular diseases. Although studies have investigated the effects of quercetin in ameliorating vascular dysfunction in hypertension, none investigated whether quercetin reduces VSMC proliferation and vascular remodeling or MMP activity. Here we investigated for the first time whether treatment with quercetin decreases hypertrophic vascular remodeling in 2K1C hypertension, accompanied by reduced oxidative stress and proliferative signaling molecules that regulate VSMC proliferation, including MMP-2.

2. Materials and methods

2.1. Rats and ethics statement

Male Wistar rats (180–200 g) were taken from the University of Sao Paulo and were maintained on 12 h light/dark cycle at 25 °C, with free access to chow and water. This study was approved by the Ethics Committee on Animal Research of Ribeirao Preto Medical School (protocol n°0146/2016) and was in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH) and Conselho Nacional de Controle de Experimentação Animal (CONCEA).

2.2. Two kidney-one clip (2K1C) model of hypertension

Rats were anesthetized with ketamine/xylazine (100/10 mg/kg) intraperitoneally to undergo two kidney-one clip (2K1C) hypertension surgery, where a silver clip with 0.2 mm internal gap was placed in the left renal artery. For sham, surgery was performed without clip placement. Systolic blood pressure (SBP) was weekly measured by tail-cuff plethysmography and rats were considered hypertensive when SBP had increased up to 30 mmHg after two weeks of surgery compared to baseline. Rats were daily treated with quercetin at 10 mg/kg/day [28] or vehicle (carboxymethylcellulose, CMC, 0.05%) by gavage. Treatment started at the second-week post-surgery and was maintained for three weeks. Rats were randomly allocated to sham and 2K1C receiving CMC, and sham and 2K1C receiving quercetin. Body weight was weekly measured.

2.3. Morphometric analysis and collagen deposition in aortas

Thoracic aortas were removed and fixed in 10% paraformaldehyde. Aortas were embedded in paraffin, cut at 5 μm and stained using hematoxylin and eosin to next determine the morphometric parameters CSA and M/L using ImageJ from NIH, as previously described [18]. The number of VSMC in aortic media was calculated using the tri-dimensional dissector method on two consecutive sections as described by Dao et al. [32], which is independent of the nuclei orientation and size. Furthermore, some sections were stained with Picrosirius Red to determine collagen levels in aortic media. Sections were then captured at x25 by a light microscopy, and the area of collagen deposition was defined by subtraction of the lumen area from the area of the outer elastic lamina by ImageJ.

2.4. Detection of superoxide in aortas by lucigenin chemiluminescence and dihydroethidium (DHE) fluorescence in situ

The generation of superoxide anion was determined in aortas using the lucigenin-derived chemiluminescence technique, as previously described [33]. Luminescence was read in a luminometer (Orion II luminometer, Berthold Detection Systems, Pforzheim, Germany) and results were expressed as relative light units (RLU)/mg protein. Furthermore, DHE probe was used in Tissue-tek frozen aortas to evaluate *in situ* production of ROS. For details, see Supplementary Data.

2.5. Western blot to detect the aortic levels of total and phosphorylated FAK

Aortas were crushed in liquid nitrogen and lysed in RIPA-buffer (Sigma, #R0278) with protease inhibitors (1x, Sigma, #S8820) and 1 mM sodium orthovanadate (Sigma, #S6508) and 10 mM sodium fluoride (Sigma, #201154). Homogenates were centrifuged at 10,000 RPM at 4 °C and supernatants were collected for analysis. Protein was quantified by Bradford. Western blots for FAK and p-FAK were then performed in aorta homogenates. For details, see Supplementary Data. We also performed some Western blots of total stress-activated protein kinase/c-Jun N-terminal kinases (SAPK/JNK) and total p38MAPK (both 1:500 dilution, Cell Signaling #9252S and #9212S), but no alterations were observed in their aortic levels between groups, at five weeks of hypertension (data not shown).

2.6. MMP activity by in situ zymography followed by immunofluorescence of MMP-2

Aortas were frozen and cryosectioned at 5 μm, and then dye-quenched (DQ) gelatin was used *in situ* to analyze MMP activity. For details, see Supplementary Data.

2.7. Immunohistochemistry of TIMP-2 in the aortic tissues

TIMP-2 was examined in aortic sections by immunohistochemistry. It was visualized after counterstained the aortic sections with hematoxylin and 3,3′-diaminobenzidine (DAB) substrate. For