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Curcumin maintains cardiac and mitochondrial function in chronic kidney disease

Francisco Correa [a,b,1], Mabel Buelna-Chontal [a,b,1], Sauri Hernández-Reséndiz [b], Wylly R. García-Niño [c], Francisco J. Roldán [d], Virgilia Soto [e], Alejandro Silva-Palacios [a], Alejandra Amador [a], José Pedraza-Chaverrí [f], Edilia Tapia [c], Cecilia Zazueta [a,b,*]

[a] Department of Cardiovascular Biomedicine, National Institute of Cardiology Ignacio Chavez, Mexico City, 14080 DF, Mexico
[b] Department of Biochemistry, National Institute of Cardiology Ignacio Chavez, Mexico City, 14080 DF, Mexico
[c] Renal Pathophysiology Laboratory, Department of Nephrology, National Institute of Cardiology Ignacio Chavez, Mexico City, 14080 DF, Mexico
[d] Department of Echocardiography, National Institute of Cardiology Ignacio Chavez, Mexico City, 14080 DF, Mexico
[e] Department of Pathology, National Institute of Cardiology Ignacio Chavez, Mexico City, 14080, DF, Mexico
[f] Department of Biology, Faculty of Chemistry, UNAM, Mexico City, Mexico

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ABSTRACT

Curcumin, a natural pigment with antioxidant activity obtained from turmeric and largely used in traditional medicine, is currently being studied in the chemoprevention of several diseases for its pleiotropic effects and nontoxicity. In chronic renal failure, the pathogenic mechanisms leading to cardiovascular disorders have been associated with increased oxidative stress, a process inevitably linked with mitochondrial dysfunction. Thus, in this study we aimed at investigating if curcumin pretreatment exerts cardioprotective effects in a rat model of subtotal nephrectomy (5/6Nx) and its impact on mitochondrial homeostasis. Curcumin was orally administered (120 mg/kg) to Wistar rats 7 days before nephrectomy and after surgery for 60 days (5/6Nx+curc). Renal dysfunction was detected a few days after nephrectomy, whereas changes in cardiac function were observed until the end of the protocol. Our results indicate that curcumin treatment protects against pathological remodeling, diminishes ischemic events, and preserves cardiac function in uremic rats. Cardioprotection was related to diminished reactive oxygen species production, decreased oxidative stress markers, increased antioxidant response, and diminution of active metalloproteinase-2. We also observed that curcumin's cardioprotective effects were related to maintaining mitochondrial function. Aconitase activity was significantly higher in the 5/6Nx + curc (408.5 ± 68.7 nmol/min/mg protein) than in the 5/6Nx group (104.4 ± 52.3 nmol/min/mg protein, $P < 0.05$), and mitochondria from curcumin-treated rats showed enhanced oxidative phosphorylation capacities with both NADH-linked substrates and succinate plus rotenone (3.6 ± 1 vs 1.1 ± 0.9 and 3.1 ± 0.7 vs 1.2 ± 0.8, respectively, $P < 0.05$). The mechanisms involved in cardioprotection included both direct antioxidant effects and indirect strategies that could be related to protein kinase C-activated downstream signaling.

Introduction

Chronic renal failure (CRF) is associated with a high incidence of cardiovascular complications. In fact, CRF diagnosis is so clinically relevant that it places the patient into the highest cardiovascular risk level, irrespective of stratification, according to traditional cardiovascular risk factors [1]. Strikingly, the high mortality in CRF patients is mainly due to cardiovascular events, rather than to advanced kidney dysfunction, and may occur even before the need for renal replacement therapy [2]. In CRF patients, it has been suggested that the high prevalence of acute-phase inflammation and oxidative stress could account for the high rate of cardiovascular morbidity and mortality [3].

A fundamental response to intrinsic and biomechanical stress in CRF is cardiomyocyte and chamber hypertrophy, which at first stages may be successfully compensatory, but inevitably will progress to dilation and heart failure. In this sense, the observation that over-expression of the intrinsic antioxidant enzyme glutathione peroxidase ameliorates post-myocardial infarction remodeling [4] supports the relationship between oxidative stress and pathological hypertrophy. In cardiovascular diseases, oxidative stress is related to mitochondrial dysfunction and, furthermore the antiremodeling effects of some compounds have been partially associated with improved mitochondrial function [5]; thus, a

* Corresponding author at: National Institute of Cardiology I. Ch., Department of Cardiovascular Biomedicine, Juan Badiano No. 1, Colonia Sección XVI, 14080 D.F. MEXICO, Mexico. Fax: +52 555573 2911.
E-mail address: azazuetam@yahoo.com (C. Zazueta).
[1] These authors contributed equally to this work.

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major challenge to protect against heart damage is to develop successful antioxidant therapeutics targeting mitochondria.

Curcumin is the major active component of turmeric, a yellow compound isolated from the plant *Curcuma longa,* used for centuries in traditional medicine [6]. This molecule has therapeutic potential against a wide range of diseases, such as cancer, lung diseases, renal diseases, neurological diseases, liver diseases, metabolic diseases, and cardiovascular diseases [6,7], mainly due to its anti-inflammatory [8], hypoglycemic [9], antimicrobial [10], and antioxidant effects [11–13]. Curcumin exerts both direct and indirect antioxidant effects by scavenging reactive oxygen species (ROS) [14,15] of both cytosolic [16] and mitochondrial origin [12] and by inducing the expression of nuclear factor erythroid 2-related factor-2 (Nrf2)-dependent cytoprotective proteins [17]. Also, curcumin deactivates some hypertrophic signals in the heart, including the Akt/glycogen synthase kinase-3β (GSK-3β)/nuclear factor of activated T cells (NFAT) pathway [18].

In this work, we sought to explore the mechanisms by which curcumin exerts therapeutic effects on heart dysfunction secondary to CRF, and its impact on mitochondrial function, aimed at suggesting alternative therapeutic interventions that may alleviate cardiovascular complications confronted by patients with chronic kidney disease.

Material and methods

Ethical approval

This investigation was performed in accordance with the *Guide for the Care and Use of Laboratory Animals,* published by the U.S. National Institutes of Health and approved by the Ethics Committee of the National Institute of Cardiology I. Ch. Experimental work followed the guidelines of the Norma Oficial Mexicana guide for the use and care of laboratory animals (NOM-062-ZOO-1999) and for the disposal of biological residues (NOM-087-ECOL-1995).

Reagents

Curcumin and other chemicals were of reagent or higher grade from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise specified. Polyclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and monoclonal anti-phosphorylated (PHO) Nrf2 (Ser40) were purchased from Abcam (San Francisco, CA, USA); anti-Nrf2 polyclonal antibody, anti-catalase, anti-glutathione *S*-transferase, and anti-endothelial nitric oxide synthase (eNOS) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-PHO-eNOS (Ser1177) was from Cell Signaling (Danvers, MA, USA). Secondary antibodies conjugated with alkaline phosphatase or horseradish peroxidase were from Santa Cruz Biotechnology.

Subtotal nephrectomy

Male Wistar rats weighing 280–300 g were used for renal ablation. The animals were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg) and subtotal nephrectomy (5/6Nx) was performed by removal of the right kidney and selective infarction of approximately two-thirds of the left kidney by ligation of two or three branches of the renal artery.

Experimental groups

Three groups of rats were studied: (1) the sham group ($n=20$) was subjected to ventral laparotomy and manipulation of kidneys and renal pedicle without destruction of renal tissue [19]; (2) the 5/6Nx group ($n=20$) was subjected to subtotal nephrectomy and euthanized after 60 days; and (3) the 5/6Nx+curcumin group ($n=20$) received 120 mg/kg/day of curcumin dissolved in 0.05% carboxymethylcellulose via oral gavage for 7 days before and 60 days after 5/6Nx. We had observed that 60 mg/kg curcumin, but not lower doses, effectively ameliorated proteinuria and hypertension in our rats [16]; however, as we expected more damage under this protocol, we decided to increase the dose to 120 mg/kg. Functional analyses of heart and kidney were also performed in a group of sham rats treated with curcumin ($n=9$); it is worth mentioning that sham rats subjected to vehicle administration during the whole protocol yielded results identical to those obtained in sham rats that did not receive carboxymethylcellulose.

Twenty-four-hour urine collections were obtained by placing the rats in metabolic cages at baseline and every 2 weeks during the study. Proteinuria was measured under basal conditions and on days 15, 30, 45, and 60. Plasma creatinine and blood urea nitrogen were measured on day 60 in all groups.

Pressure overload and cardiac remodeling in 5/6Nx rats

Systolic blood pressure (SBP) was measured under basal conditions and every 15 days by connecting the tail cuff to a pneumatic pulse transducer and a programmed electrosphygmomanometer (Narco Biosystems, Austin, TX, USA) as described previously [20]. Recordings were made in triplicate by means of a Grass polygraph (Grass Medical Instruments, Quincy, MA, USA). Echocardiographic images were obtained using a Sonos 5500 echocardiographer (Koninlijke Philips Electronics, Eindhoven, The Netherlands) with a 12-MHz transducer as previously described [21]. Parasternal long- and short-axis views were analyzed in the anesthetized rats. Two-dimensional-guided M-mode echocardiography was performed and determinations were made from at least three beats in each rat. Left-ventricular (LV) cavity and wall thickness were measured to calculate the ejection fraction (EF) and fraction shortening (FS) as follows: $\%EF=[(EDV-ESV/EDV)\times 100]$, where EDV is end-diastolic volume and ESV is end-systolic volume, and $\%FS=[(LVDd-LVSd/LVDd)\times 100]$, where LVDd is LV dimension at end diastole and LVSd is LV dimension at end systole. EDV and ESV were calculated as $1.047\times LVDd^3$ and $1.047\times LVSd^3$, respectively, and stroke volume was calculated as EDV−EDS, according to Wandt et al. [22].

Frozen cardiac tissue from all groups was powdered with a prechilled pestle in a frozen mortar and dissolved in ice-cold buffer containing 50 mM Tris–HCl, 120 mM NaCl, 0.5% Igepal, 100 μM NaF, 200 μM $NaVO_3$, pH 8.0, and centrifuged at 4000*g* for 10 min. The proteins in the homogenates were separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), transferred to polyvinylidene difluoride (PVDF) membranes, and evaluated for remodeling by determining metalloproteinase-2 (MMP-2) activation, using 1:1000 MMP-2 polyclonal antibodies (Merck Millipore Corp., Billerica, MA, USA). Enhanced chemiluminescence was used as the detection system, and control loading was determined by incubating the membranes against anti-GAPDH (1:2500).

Cardiac function evaluated in isolated heart system

Some rats were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg) and complete lack of pain response was assessed by determining pedal withdrawal reflex. Then, sodium heparin was injected (1000 U/kg) and 5 min later a midsternal thoracotomy was performed. Hearts were rapidly excised and placed in ice-cold Krebs–Henseleit buffer solution of pH 7.4, containing 118 mM NaCl, 4.75 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4\cdot 7H_2O$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 5 mM glucose, and 0.1 mM sodium octanoate, and mounted onto a Langendorff heart