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Curcumin reverses glomerular hemodynamic alterations and oxidant stress in 5/6 nephrectomized rats

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ABSTRACT

The administration of curcumin before and throughout the study attenuates oxidant stress and glomerular hemodynamic alterations induced by 5/6 nephrectomy (5/6NX). The purpose of this work was to study if curcumin is able to reverse established glomerular hemodynamic alterations (e.g. hyperfiltration and glomerular hypertension) and oxidant stress in rats with 5/6NX. Curcumin (120 mg/kg) was given to rats with established renal injury (30 days after surgery) and continued for 30 days (days 31–60 of the study). All rats were studied on day 60 after surgery. Curcumin was able (a) to reverse 5/6NX-induced glomerular hypertension and hyperfiltration, (b) to induce cell proliferation and nuclear translocation of Nrf2 and (c) to reverse 5/6NX-induced oxidant stress and decrease in antioxidant enzymes. These beneficial effects of curcumin were associated with the ability of this antioxidant to reverse renal structural alterations, proteinuria, hypertension, interstitial fibrosis, fibrotic glomeruli, tubular atrophy and mesangial expansion. It has been shown for the first time that curcumin is able to reverse established oxidants stress glomerular hypertension and hyperfiltration in rats with 5/6NX. These novel findings may play a key role in the attenuation of proteinuria and progression of renal damage in rats with 5/6NX. These data suggest that curcumin may be useful to reverse established hemodynamic alterations and renal injury in patients with chronic renal failure.

Introduction

The powerful antioxidant curcumin (diferuloyl methane) is a component of turmeric found in the Curcuma longa plant (Jurenka 2009). This compound has been widely used in traditional Ayurvedic and Chinese medicine (Singh 2007). The therapeutic potential has been explored in inflammatory, neoplastic, renal and neurodegenerative diseases, diabetes and other disorders (Jurenka 2009; Ghosh et al. 2009; Gupta et al. 2011; Calabrese et al. 2008). Curcumin is considered a bifunctional antioxidant (Dinkova-Kostova and Talalay 2008) based on the fact that it exerts both direct and indirect antioxidant effects by scavenging reactive oxygen species (ROS) (Sreejayan 1997) and inducing the expression of cytoprotective proteins in an Nrf2-dependent way (Dinkova-Kostova and Talalay 2008), respectively.

Furthermore, the remnant kidney induced by 5/6 nephrectomy (5/6NX) is a widely used model to study the progression of renal disease (Tapia et al. 2003). The damage in rats with 5/6NX is characterized by structural and functional hyperthropy, glomerulosclerosis, tubulointerstitial injury, alterations in renal hemodynamics including glomerular hypertension and hyperfiltration, fibrosis, oxidant stress, and progression to end stage renal disease (Tapia et al. 2012a). Hyperfiltration in these nephrons results form elevations in the mean glomerular transcapillary hydraulic pressure gradient (ΔP) and plasma flow rate (Qa). In the remnant kidneys of rats subjected to extensive renal ablation, these elevated glomerular capillary pressures and flows are associated with progressive proteinuria and eventual glomerular sclerosis (Anderson et al. 1986). Oxidant stress may induce inflammation and renal fibrosis by direct effects of reactive oxygen species (ROS) (Small et al. 2012).

Interestingly, the administration of curcumin before and throughout the study exerts protective effects against renal damage

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and inflammation induced by 5/6NX in rats (Tapia et al. 2012b). In addition, curcumin was able to protect rats against 5/6NX-induced oxidant stress, decrease in antioxidant enzymes and glomerular hemodynamic alterations including glomerular hypertension and hyperfiltration. This protection was associated with the increase in nuclear translocation of Nrf2 (Tapia et al. 2012b). The present work was designed to study if curcumin is able to reverse established glomerular hemodynamic alterations (e.g., glomerular hypertension and hyperfiltration) and structural damage in rats with 5/6NX and if this may be associated to Nrf2 nuclear translocation and to the attenuation of both oxidant stress and decrease in the activity of antioxidant enzymes.

Materials and methods

Chemicals

Curcumin, N-acetylcysteine (NAC), and periodic acid Schiff (PAS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Enalapril was from Merck Sharp & Dohme (Mexico city). Sodium pentobarbital was from Holland of México (Mexico city). Antibodies against Nrf2, proliferating cell nuclear antigen (PCNA) and malondialdehyde (MDA) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), Abcam (Cambridge, MA, USA) and EMD Millipore (Billerica, MA, USA), respectively.

Experimental design

Male Wistar rats with an initial body weight of 280–300 g were used. Experimental protocol was approved by Bioethics and Investigation Committees of Instituto Nacional de Cardiología Ignacio Chávez. Under anesthesia with sodium pentobarbital (60 mg/kg), renal ablation 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. Curcumin was given daily by gavage using two different schemes: (1) complete treatment (60 and 120 mg/kg bw 7 days before and 60 days after 5/6NX and (2) posttreatment (120 mg/kg bw for 30 days starting on day 30 after 5/6NX). All animals were sacrificed 60 days after 5/6NX or laparotomy. Seven groups of rats were studied ($n = 7$–9/group): (1) control group, treated daily via oral gavage with carboxymethylcellulose 0.05% (curcumin vehicle) 7 days before laparatomy and along all the study. (2) 5/6NX group. (3) 5/6NX-CUR(120). Curcumin was given as a posttreatment for 30 days on days 31–60 of the study. (4) CUR (60)-5/6NX-CUR(60). Curcumin (60 mg/kg/day) was given as a complete treatment along all the study (7 days before and 60 days after 5/6NX). (5) CUR (120)-5/6NX-CUR(120). Curcumin (120 mg/kg/day) was given as a complete treatment along all the study (7 days before and 60 days after 5/6NX). (6) 5/6NX + NAC. Rats with 5/6NX were treated with NAC (600 mg/L in the drinking water) along all the study (by 60 days) starting the day of surgery (Shimizu et al. 2005). (7) 5/6NX + enalapril. Rats with 5/6NX were treated with enalapril (10 mg/L in the drinking water along all the study (by 60 days) starting the day of surgery (Witte and Lemmer 1999). Curcumin was dissolved in carboxymethylcellulose 0.05% and was given daily according to the above-described schemes. The doses of curcumin used (60 and 120 mg/kg/day) were chosen based on previous experiments in which the dose of 60 mg/kg was effective to prevent proteinuria, hypertension, renal injury and alterations in renal hemodynamics 30 days after 5/6NX. Systolic blood pressure (SBP) was measured by tail-cuff pletysmography at basal period and on days 15, 30, 45 and 60 of the study (Tapia et al. 2003). Twenty four-hour urine collections were obtained, placing the rats in metabolic cages, at baseline and on days 15, 30, 45 and 60 of the study.

Micropuncture studies to evaluate renal hemodynamics

Micropuncture studies were performed, as previously described in detail (Tapia et al. 2012a,b), 60 days after the surgical procedure (5/6NX or sham). One femoral artery catheter was used for monitoring mean arterial pressure (MAP). Samples of proximal tubule fluid were obtained from seven different nephrons for determination of flow rate and polyfructosan concentration to calculate single-nephron glomerular filtration rate (SNGFR). Polyfructosan was measured in plasma and urine samples to calculate whole-kidney glomerular filtration rate (GFR). Intratubular hydrostatic pressure (FF) was measured in additional proximal tubules. Colloid osmotic pressure (π) in glomerular capillaries was estimated from the protein concentration in blood taken from the femoral artery (Ca) and in blood obtained by puncturing surface efferent arterioles (Ce). The following variables were measured: MAP, stop flow pressure (SFP), transcapillary hydraulic pressure (ΔP), hematocrit (Hct), free flow pressure (FFP), capillary pressure (PC), and afferent (CA) and efferent (CE) protein concentration.

Analytical procedures

Proteinuria, polyfructosan concentrations in plasma, urine and tubular fluid, protein concentration in efferent samples and femoral arterial blood plasma were measured as previously described (Tapia et al. 2012b). GFR, SNGFR, glomerular capillary pressure (P_{GC}), single nephron plasma flow (Qa), single nephron filtration fraction (SNFF), afferent (AR) and efferent (ER) resistances, afferent (πA) and efferent (πE) oncotic pressure and ultrafiltration coefficient (Kf) were calculated.

Histological analysis

Kidney sections stained with hematoxylin/eosin (H/E), Masson trichrome and PAS were examined in a blinded fashion. Tubulointerstitial fibrosis was evaluated in Masson's trichrome sections. The blue stained areas (excluding glomeruli and vessels), which corresponded to fibrosis, were determined. Mesangial expansion was studied in PAS sections. For each analyzed glomerulus, its total area including the Bowman space was measured and the percentage area occupied by the mesangium (cells and matrix) was determined. Glomerulosclerosis was evaluated in hematoxylin/eosin and Masson trichrome stained sections. The number of glomeruli with total fibrosis was recorded in the whole section per rat kidney and reported as fibrotic glomeruli per kidney section. The tubular epithelium height as an indicator of tubular atrophy was evaluated in hematoxylin/eosin stained sections. Ten proximal tubules around glomerulus were analyzed using automated morphometry, the height of the epithelial cells was determined from the basal membrane to the lumen. For each section, a total of 100 tubules were measured and their heights averaged.

Immunohistochemical studies of Nrf2, MDA and PCNA

Immunohistochemical detection of Nrf2, PCNA (nuclear cell proliferation marker) and MDA (a marker of oxidant damage) was performed essentially as previously described (Tapia et al. 2012b). Distant to the surgical scar, in five random choice fields per section the number of immunostained nuclei or cells were determined and their percentage was determined.

Activity of antioxidant enzymes

The activity of the antioxidant enzymes catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx),