Urol Res (2009) 37:211–220
DOI 10.1007/s00240-009-0197-1

ORIGINAL PAPER

Taurine protected kidney from oxidative injury through mitochondrial-linked pathway in a rat model of nephrolithiasis

Cheng Yang Li · Yao Liang Deng · Bing Hua Sun

Received: 6 November 2008 / Accepted: 21 May 2009 / Published online: 10 June 2009

Abstract Hyperoxaluria and crystal deposition induce oxidative stress (OS) and renal epithelial cells injury, both mitochondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase are considered as the main sources of reactive oxygen species (ROS). Taurine is known to have antioxidant activity and shows renoprotective effect. We investigate the effect of taurine treatment on renal protection, and the putative source of ROS, in a rat model of calcium oxalate nephrolithiasis. Rats were administered with 2.5% (V/V) ethylene glycol + 2.5% (W/V) ammonium chloride (4 ml/day), with restriction on intake of drinking water (20 ml/day) for 4 weeks. Simultaneous treatment with taurine (2% W/W, mixed with the chow) was performed. At the end of the study, indexes of OS and renal injury were assessed. Renal tubular ultrastructure changes were analyzed under transmission electron microscopy. Crystal deposition in kidney was scored under light microscopy. Angiotensin II in kidney homogenates was determined by radioimmunoassay. Expression of NADPH oxidase subunits p47phox and Nox-4 mRNAs in kidney was evaluated by real time-polymerase chain reaction. The data showed that oxidative injury of the kidney occurred in nephrolithiasis-induced rats. Hyperplasia of mitochondria developed in renal tubular epithelium. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in mitochondria decreased and the mitochondrial membrane showed oxidative injury. Taurine treatment alleviated the oxidative injury of the kidney, improved SOD and GSH-Px activities, as well as the mitochondrial membrane injury, with lesser crystal depositions in the kidney. We could not detect statistical changes in the renal angiotensin II level, and the renal p47phox and Nox-4 mRNAs expression in those rats. The results suggest that mitochondria but not NADPH oxidase may account for the OS and taurine protected kidney from oxidative injury through mitochondrial-linked pathway in this rat model.

Keywords Taurine · Nephrolithiasis · Calcium oxalate · Oxidative stress · Mitochondria · NADPH oxidase

C. Y. Li · Y. L. Deng (✉) · B. H. Sun
Department of Urology, The First Affiliated Hospital of Guangxi Medical University, 530021 Nanning, China
e-mail: dylkf317@163.com

Introduction

Calcium oxalate (CaOx) is the most common type of human kidney stone, of which hyperoxaluria is the major risk factor [1]. The mechanism by which a CaOx stone is formed is complex, and many factors are believed to be involved. The key role of renal epithelial cells injury in the development of CaOx renal stone has been deeply investigated and discussed elsewhere [2, 3]. Tissue culture and animal model studies have provided evidence that renal epithelial cells are injured in the presence of high levels of oxalate (Ox) and CaOx crystals [4–8]. It has been proposed that cellular injury promotes crystal retention within the renal tubules through promotion of nucleation, aggregation and attachment of crystals to the renal epithelium, a cascade of events, which is important in the pathogenesis of nephrolithiasis [2, 3].

It has been confirmed that injury of renal epithelial cells is mediated by the overproduction of reactive oxygen species (ROS) [7–10], produced mostly from mitochondria [11, 12], or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [9, 13]. The principal ROS's are superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl

radical (OH^-); they are highly reactive and can damage lipids, proteins, carbohydrates and nucleotides. The balance between ROS production and the antioxidant defense system (including antioxidants, such as glutathione (GSH), and antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase) determines the degree of oxidative stress (OS). Treatment with this kind of antioxidants provided protection from the generation of ROS and associated lipid peroxidation and injury in vitro [7, 9, 10]. Moreover, treatment with other antioxidant agents, such as vitamin E [14], methionine [15], GSH monoester [16], green tea [17], etc., resulted in a decrease in crystal deposition in the kidneys of hyperoxaluric rats. Since ROS appears responsible for cellular injury, therapeutic utilization of antioxidants to control renal OS may prove an effective therapy in renal protection and control of stone recurrence [18–20], especially in view of the recent finding that total antioxidant status is frequently deficient in stone-forming individuals [21].

Taurine is a ß-amino acid naturally found in kidney. Previous studies have shown that taurine has a protective effect in kidney [22–25], and serves as an antioxidant agent [26]. Moreover, taurine was found to prevent tamoxifen-induced mitochondrial oxidative injury in mice and the protection is afforded either by reversing the decline of antioxidants or by the direct free radical-scavenging activity [27]. In addition, taurine is an effective inhibitor of angiotensin II action [28], and hyperoxaluria was proved to activate the renal renin-angiotensin system (RAS) [29–31]. It has been shown that angiotensin II is implicated in causing OS by stimulating NADPH oxidase leading to increased generation of superoxide [32]. Based on those findings, we hypothesized that taurine would be beneficial in the treatment of renal oxidative injury induced by hyperoxaluria and crystal deposition, through the mitochondrial and/or NADPH oxidase-linked pathway. Therefore, this study was performed to investigate the effect of taurine treatment on renal protection in a rat model of CaOx nephrolithiasis, the putative source of ROS was explored as well.

Materials and methods

Animals and experimental protocols

Animal model of CaOx nephrolithiasis was established in adult male Sprague–Dawley rats (190–200 g) by intragastric administration of 2.5% (V/V) ethylene glycol (EG) + 2.5% (W/V) ammonium chloride (AC) 2 ml twice daily, with restriction on intake of drinking water (20 ml/day) for 4 weeks. EG and AC were dissolved in water and given through a stomach tube. Four groups of eight rats each were studied: group A, untreated control animals; group B, nephrolithiasis without treatment; group C, nephrolithiasis with taurine (2%, W/W, mixed with the chow); group D, only taurine (2%, W/W). Rats were kept under a controlled 12 h light/dark cycle at 22 ± 2°C. All rats had free access to chow. Taurine (purity > 99.5%) was purchased from Shanghai Seebio Biotech, Inc. Rats were purchased from Shanghai SLAC Laboratory Animal CO. Ltd. All experimental protocols were conducted in accordance with the guidelines of the Guangxi Medical University Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Urine collection and analysis

At the end of the study, 24-h urine collection was made with 0.02% sodium azide to prevent bacterial growth. Rats were placed in metabolic cages for the collection of urine. After determining urinary volume, urine was aliquoted for various assays. For the determination of Ox, an aliquot of 24-h urine samples was acidified by the addition of 1 M HCl (urine:HCl = 20:1). Analyses were performed by ion chromatography method using a Dionex DX100 gradient ion chromatography system equipped with a 0.4 × 25 cm AS10A anion exchange analytical column containing an AG10A guard column. Urinary creatinine was determined by enzymatic method (automatic biochemistry analyzer, HITACHI, 7170A). Crystalluria was examined by light microscopy.

To investigate the development of OS, we determined urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ (8-IP), a product of lipid peroxidation, by enzyme-linked immunosorbent assay (ELISA) using a kit from ADL (ADL, USA.Cat:552014) according to the manufacturer's protocol [33].

Kidney tissue processing and morphologic study

The rats were euthanized intraperitoneally with sodium pentobarbital; kidneys were taken immediately after blood extraction via vena cava for serum creatinine measurement (enzymatic method). The details of kidney tissue preparation for light microscopy and transmission electron microscopy (TEM) analyses are described in publications [34]. The numbers of crystal depositions (percentage in total tubules) in renal tubules were determined by assessing randomly selected 20 fields per kidney (200×) [29].

To validate that p47phox and Nox-4 express in kidney of normal and EG + AC-treated rats, the two proteins were localized immunohistochemically using specific antibodies (Anti-p47phox, Upstate Inc, Cat 07-497; Anti-Nox-4, Santa Cruz Biotechnology, Cat sc-21860).

Preparation of renal mitochondria

Renal mitochondria were prepared by conventional methods of differential centrifugation using a kit from GENMED (GENMED Scientifics INC. USA. Cat: GMS10006.2). Details of preparation for renal mitochondria are described in publications [35]. Mitochondrial protein was determined using the BioRad Protein Assay kit (BioRad Laboratories, Hercules, CA, USA).

Determination of enzymatic activities of SOD and GSH-Px in renal mitochondria

The activity of antioxidant enzymes SOD and GSH-Px in the renal mitochondria samples were determined spectrophotometrically [22] using two kits from the Nanjing Jiancheng Biochemistry (China, Cat: A001 and A005) according to the manufacturer's protocol.

Assessment of mitochondrial oxidative injury

Oxidative injury of mitochondria were assessed by fluorescence spectrophotometrical analysis using a kit from GENMED (GENMED Scientifics INC. USA. Cat:GMS10017.2) according to the manufacturer's protocol. The levels of injury were assessed by measuring the relative fluorescence unit (RFU) of sample, which represents the quantities of cardiolipin, the main component of the mitochondrial membrane. Cardiolipin was degraded in the state of OS (lipid peroxidation) and the RFU decreased.

Measurement of angiotensin II in kidney homogenate

Four rats of each group were killed to take the whole kidney for angiotensin II measurement. Kidney was homogenized with three volumes of cold PBS and centrifuged at 8,000×g for 10 min. The supernatant of kidney homogenate were assayed for total protein using the Bio-Rad Protein Assay kit (BioRad Laboratories, Hercules, CA, USA) and the concentration of angiotensin II was measured by radioimmunoassay method [29] using a kit from the North Biochemistry Institute(China).

Real-time polymerase chain reaction for NADPH oxidase subunits p47phox and Nox-4 mRNAs in kidney

The mRNA levels of p47phox, Nox-4 and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) in whole kidney were determined by real-time polymerase chain reaction (RT-PCR). Total RNA was isolated from kidney using TRIZOL Reagent (Takara, Japan) according to the manufacturer's protocol. One microgram of total RNA was reverse-transcribed to cDNA using a kit from Takara (Cat: DRR063A). In brief, 20-μl reactions contained total RNA 1.0 μg, oligo dT Primer 1 μl, Random 6 mers 1 μl, PrimeScript™ RT Enzyme Mix I 1 μl, 5 × PrimeScript™ Buffer 4 μl. This mixture was incubated 15 min at 37°C, and then reaction mixture was heated to 85°C for 5 s to stop the reaction.

Primers for real-time PCR

Primers were designed using Primer Express software (PE Applied Biosystems, Foster City, CA, USA) as follows: GAPDH (accession no. NM_017008), 5′-ggcacagtcaaggc tgagaatg-3′ (forward primer) and 5′-atggtggtgaagacgc cagta-3′ (reverse primer), amplifying a 143-bp product; p47phox (accession no. NM_053734) 5′-ccacgggtattgc taggatgaga-3′ (forward primer) and 5′-agactaaggcagcggg taatcaga-3′ (reverse primer), amplifying a 91-bp product; Nox-4(accession no. NM_053734) 5′-actgcctccatcaagcc aaga-3′ (forward primer) and 5′-cttccaaatgggccatcaatgta-3′ (reverse primer), amplifying a 90-bp product.

Real-time quantitative PCR

Polymerase chain reaction product was directly monitored by measuring the increase in fluorescence of dye (SYBR green; Takara) bound to the amplified double-stranded DNA. The parameter of threshold cycle (CT) was defined as the fractional cycle number at which fluorescence exceeds a threshold level. The comparative CT method quantifies the amount of mRNA relative to that of a reference sample, termed the calibrator, for comparison of the expression level of every unknown sample. For normalizing the relative amount of p47phox and Nox-4 mRNAs, GAPDH mRNA was chosen as an internal reference. The changes in expression are given by unknown samples of interest. All PCR reactions were performed using an iCycler Sequence Detection System (iCycler Biosystems). For each PCR run, a master mix was prepared: 2 μl cDNA, 0.4 μl (10 μM) each of the forward and reverse primer set for GAPDH, p47phox or Nox-4,2 × SYBR Premix Ex Taq™ 10 μl, dH_2O 7.2 μl (all from a kit of Takara, Cat: DRR063A).After an initial 10-s denaturation at 95°C, the thermal cycling comprised 40 cycles of denaturation at 95°C for 10 s and annealing and extension at 60°C for 20 s. After the end of reaction, the melting curve analysis was made to validate the specificity of the PCR products by increase the temperature from 55 to 95°C at the speed of 0.5°C per cycle.

Statistical analysis

The data are presented as mean ± SD. We used one-way factorial ANOVA to test for overall differences among the

groups, followed by Student–Newman–Keuls to compare the separate groups. P values <0.05 denoted the presence of a statistically significant difference.

Results

Urinary oxalate, creatinine clearance, 8-IP and kidney/body weight

As expected, there was a marked increase in urinary excretion of oxalate by rats in groups B and C. Rate of creatinine clearance was lower in EG + AC received rats of group B compared with the normal rats of group A. The clearance rate, however, improved significantly when rats were given taurine as seen in group C rats. Compared to the control, the concentration of urinary 8-IP elevated significantly in group B ($P < 0.01$), demonstrating the development of OS after induction of nephrolithiasis. In contrast, when treated with taurine, urinary 8-IP decreased significantly (group B vs group C, $P < 0.05$). There were no significant differences in kidney/body weight between group A and D, but rate of that increased significantly after induction of nephrolithiasis by EG + AC in group B (group A vs. group B, $P < 0.01$). Taurine treatment also reduced the rate of kidney/body weight (group B vs. group C, $P < 0.05$). (All data are summarized in Table 1).

Enzymatic activities of SOD and GSH-Px in mitochondria

As shown in Fig. 1, both enzymatic activities of SOD and GSH-Px were significantly decreased in the renal mitochondria of rats received EG + AC (group B vs. group A, $P < 0.01$). However, when treated simultaneously along with taurine in rats of group C, the SOD and GSH-Px activities improved significantly (group C vs. group B, $P < 0.05$).

The relative fluorescence unit of mitochondria

Figure 2 shows that compared to the control, the RFU of mitochondria samples decreased significantly after induction of nephrolithiasis by EG + AC in group B ($P < 0.01$), but when treated simultaneously along with taurine in group C, the RFU improved significantly (group B vs. group C, $P < 0.05$).

Angiotensin II in kidney homogenate

The concentration of angiotensin II in kidney homogenates was normalized by that of protein. As shown in Fig. 3, there were no statistically significant differences in the concentration of angiotensin II in kidney homogenates among the four groups ($P > 0.05$).

Urinary crystals

Urinary crystals appeared distinctly during treatment with EG + AC in the group B and C. We found no differences in the shape and quantities of crystals between the two groups (Fig. 4).

Renal tubular ultrastructure changes under TEM

As shown in Fig. 5, hyperplasia of mitochondria developed notably in renal tubular epithelium of the EG + AC-received rats in group B (Fig. 5b, c); mitochondria swelling and nuclei disfiguration of the tubular epithelial cells were also apparently (Fig. 5c); while ultrastructure changes

Table 1 Urinary oxalate, creatinine clearance, 8-IP and kidney/body weight ($\bar{x} + s$)

Group	n	Urinary oxalate (mg/24 h)	Creatinine clearance (ml/min/100 g body weight)	Urinary 8-IP (pg/ml)	Kidney/body weight (mg/g)
A	8	1.75 ± 0.65	0.42 ± 0.02	2.38 ± 0.44	3.22 ± 0.31
B	8	3.50 ± 0.96[a]	0.36 ± 0.03[a]	5.00 ± 0.74[a]	4.93 ± 0.43[a]
C	8	3.27 ± 0.95[b]	0.40 ± 0.03[d]	4.13 ± 0.87[f]	4.38 ± 0.75[f]
D	8	1.51 ± 0.42[c]	0.44 ± 0.02[e]	2.31 ± 0.53[c]	3.47 ± 0.30[c]

Values represent mean ± SD

A represents control, B represents EG + AC-treated rats, C represents EG + AC + taurine-treated rats, D represents only taurine-treated rats

[a] $P < 0.01$ vs group A

[b] $P > 0.05$ vs group B

[c] $P < 0.01$ vs group C

[d] $P < 0.01$ vs group B

[e] $P < 0.05$ vs group C

[f] $P < 0.05$ vs group B

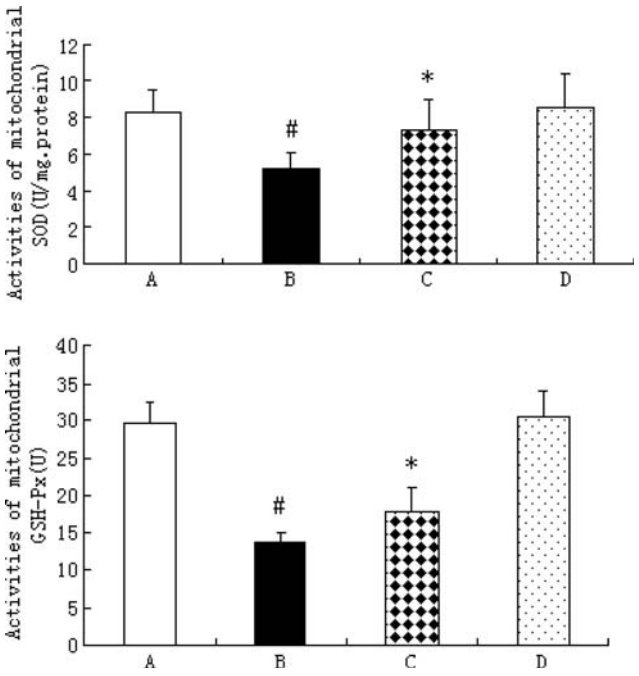

Fig. 1 Enzymatic activities of SOD and GSH-Px in mitochondria from kidney homogenates ($n = 8$). A represents control, B represents EG + AC-treated rats, C represents EG + AC + taurine-treated rats, D represents only taurine-treated rats. Compared with group A $^{\#}P < 0.01$, compared with group B $^{*}P < 0.05$

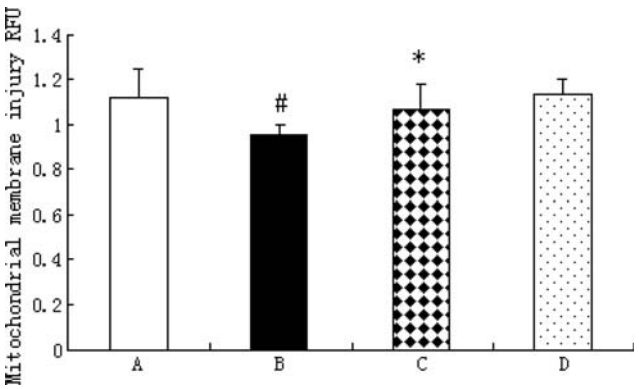

Fig. 2 The relative fluorescence unit (RFU) of mitochondria from kidney homogenates ($n = 8$). A represents control, B represents EG + AC-treated rats, C represents EG + AC + taurine-treated rats, D represents only taurine-treated rats. Compared with group A $^{\#}P < 0.01$, compared with group B $^{*}P < 0.05$

were relatively slight in kidney of EG + AC + taurine-treated rats from group C (Fig. 5d).

Hematoxylin and eosin-stained and Von Kossa-stained kidney sections

To examine the presence of CaOx crystal in kidney, hematoxylin and eosin (H&E)-stained and Von Kossa-stained sections of kidney were examined under light microscopy. Compared with control kidneys, where tubules were

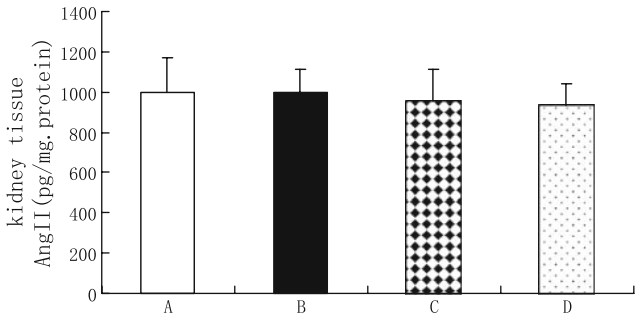

Fig. 3 Angiotensin II in kidney homogenates ($n = 4$). A represents control, B represents EG + AC-treated rats, C EG + AC + taurine-treated rats, D represents only taurine-treated rats. Compared with the control $P > 0.05$

compact and at a higher density (Fig. 6a), histological examination of kidneys of rats received EG + AC in group B revealed major changes. Crystals were observed in the lumen (Fig. 6b, c, respectively, black asterisk), as were distended distal tubules and tubular cells injury (Fig. 6b, c, black arrows). Compared with kidneys of rats received EG + AC, histological examination of the EG + AC + taurine-treated rats showed lesser crystal depositions in kidney. The crystal deposition numbers were 17.7 ± 3.5% of group B vs 14.0 ± 3.1% of group C, $P < 0.05$, with relatively slight morphological changes (Fig. 6d).

Immunostaining localization for p47phox and Nox-4 in kidney

Light microscopic observation of 2-μm wax sections of kidneys from the control and the rats received EG + AC demonstrated that p47phox and Nox-4 express widely in kidney, including renal cortex, inner medulla and outer medulla, as shown in Fig. 7.

Quantification of p47phox and Nox-4 mRNAs in kidney

The specificity of the PCR products had been confirmed by 2% agarose electrophoresis and by melting curve analysis. The standard, amplification and melting curves for GAD-PH, p47phox and Nox-4 indicated that assays were done in optimum conditions. Figure 8 shows that there were no statistically significant differences in the quantities of p47phox/GAPDH and Nox-4/GAPDH mRNAs among the four groups ($P > 0.05$).

Discussion

Experimental hyperoxaluria and crystal deposition are always associated with OS and cell injury [18]. Clinic

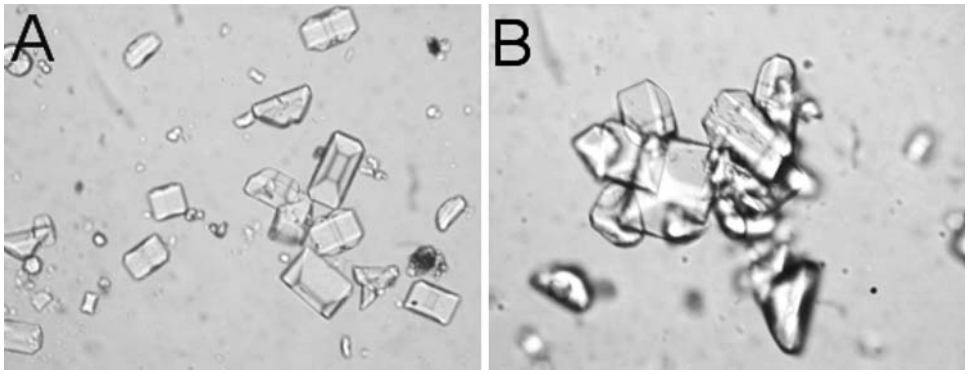

Fig. 4 Photomicrographs of crystalluria of EG + AC-treated rats (magnification A: ×100, B: ×400)

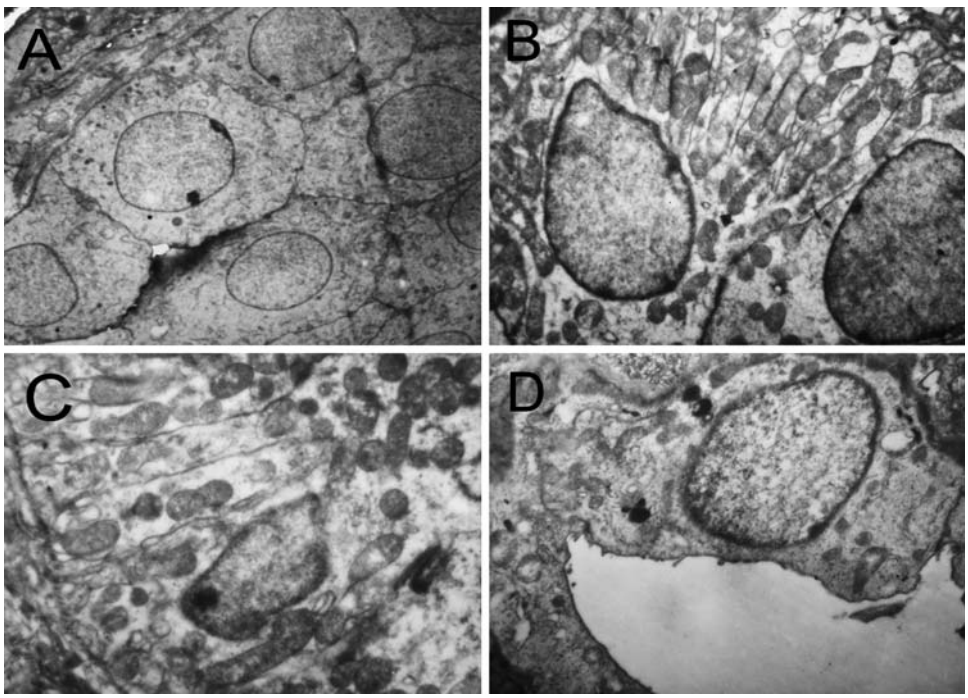

Fig. 5 Photomicrographs of kidney sections under TEM. Kidneys of control (a), and EG + AC-treated rats (b, c) and EG + AC + taurine-treated rats (d) are shown in representative photomicrographs, demonstrating the hyperplasia of mitochondria, mitochondria swelling and nuclei disfiguration of the tubular epithelial cell (b, c); ultrastructure changes were relatively slight in kidney of EG + AC + taurine-treated rats (d). Magnification ×2,800

studies have also provided evidence for the development of OS and renal injury in kidney stone patients [20, 36]. In this rat model of CaOx nephrolithiasis, development of OS and renal injury are shown evidently as the elevation of urinary 8-IP, the decrease of creatinine clearance, the increase of kidney/body weight, and the morphological changes of kidney in rats induced nephrolithiasis by EG + AC.

The source of ROS stimulated by Ox and CaOx has been particularly investigated. Mitochondria are generally the most common source of superoxide and hydrogen peroxide in most cells and tissues. It has been found in vitro that Ox or CaOx crystals increased significantly the mitochondrial superoxide production, with decreased GSH and increased oxidized thiol proteins in mitochondria [11, 12]. In a rat hyperoxaluria and crystalluria model, Meimaridou et al., [35] observed that the nephrolithic kidney underwent OS. The oxidative injury was manifest by a decrease in mitochondrial total GSH concentration, as well as increased activity of G6PDH, both important in maintaining cell redox. Severe kidney injury at the mitochondrial level was a key observation, indicated by the diminished oxygen consumption and decrease in mitochondrial cytochrome c. Recently, Veena et al., [37] reported that mitochondrial injury was evident by increased mitochondrial swelling in an animal model of hyperoxaluria. In the present study, with the establishment of hyperoxaluria, crystalluria and calcium crystal deposition in kidney, changes of the renal mitochondria were detected, including development of hyperplasia of mitochondria in renal tubular epithelium, decrease of enzymatic activities of SOD and GSH-Px in mitochondria, and oxidative injury of the mitochondrial membrane. Those results, consistent with the findings of others discussed above, suggest that mitochondrial changes are an important event in the formation of CaOx kidney stone.

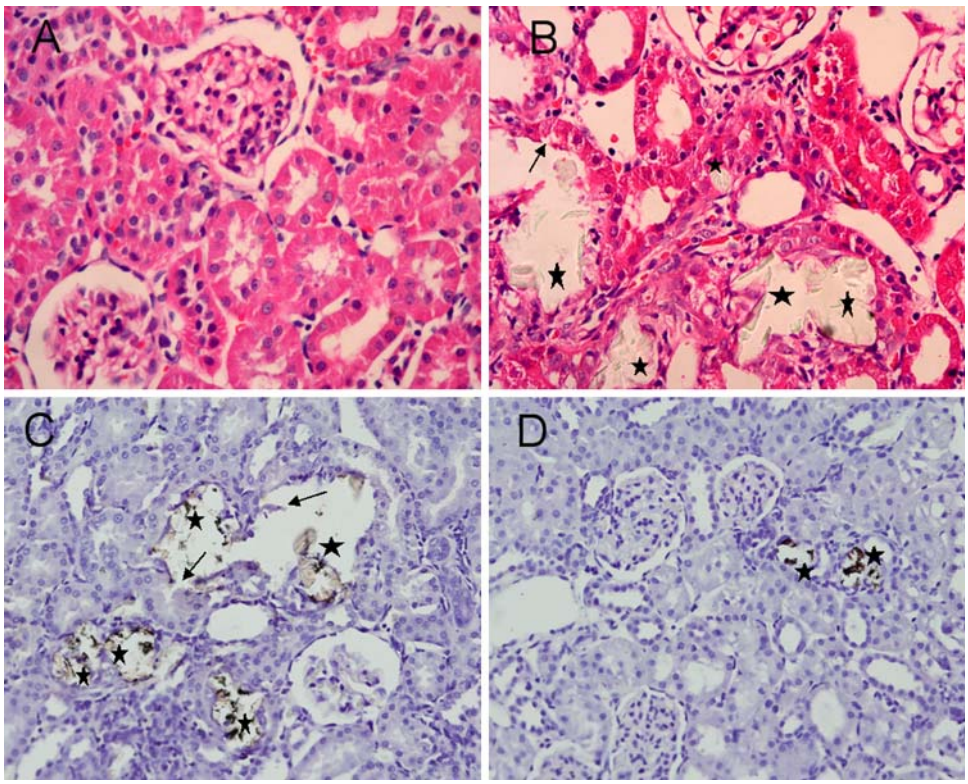

Fig. 6 Photomicrographs of H&E-stained and Von Kossa-stained kidney sections. Kidneys of control (a, H&E-stained), EG + AC-treated rats(b, H&E-stained; c Von Kossa-stained), EG + AC + taurine-treated rats(d, Von Kossa-stained) are shown in representative photomicrographs, demonstrating crystals deposit (*black asterisk*), distended tubules and tubular cells injury (*black arrows*); Lesser crystal depositions and relatively slight morphological changes were present in kidney of EG + AC + taurine-treated rats (magnification ×400)

NADPH oxidase was originally discovered in neutrophils, where it is a potent source of superoxide during phagocytosis [38]. It comprises a membrane-associated cytochrome b_{558}, composed of one gp91phox and one p22phox subunit and at least four cytosolic subunits (p47phox, p67phox, p40phox and rac1 or rac2). The expression and activity of NADPH oxidase have been detected in kidney cells such as proximal tubular epithelial cells, glomerular mesangial cells and cells of the thick ascending loops of Henle [39, 40]. NADPH oxidase has also been considered as a major source of ROS in kidney [40], particularly in the presence of angiotensin II [32]. Tissue culture studies found that Ox-induced injury of renal tubular cells were significantly reduced in the presence of DPI, an NADPH oxidase inhibitor [9, 13], indicated the involvement of NADPH oxidase in the forming of ROS. Furthermore, Hyperoxaluria induced OS, inflammation, fibrosis and CaOx crystal deposition in kidneys of rats was significantly reduced by the anti-RAS treatments [29–31].

P47phox is an important modulator of the activity of NADPH oxidase [41]. Expression of p47phox mRNA was high in kidney of the spontaneously hypertensive rat which has been found to has enhanced excretion of 8-IP [39]. Nox-4 was a gp91phox homologue and is highly expressed in kidneys, overexpression of Nox-4 in cultured cells leads to increased superoxide production and decreased rate of growth [40]. Therefore, in the present study, we focused on p47phox and Nox-4. Though similarly as reported in publications [39, 40], the two subunits express widely in the kidneys, we did not detect statistically significant changes in the expression of their mRNAs in kidney tissues among the four groups. Accordingly, there were no statistically significant differences in the concentration of angiotensin II in kidney homogenates too. This suggests that NADPH oxidase and RAS were not remarkably activated in this model.

Rat models of CaOx nephrolithiasis induced by either EG alone or in combination with other drugs such as AC, are often used to study the pathogenesis of renal crystal deposition [8, 42]. AC was used to accelerate the development of crystal deposition [43]. In this model, administration of EG + AC was limited to low doses so that exceeded intake of those renal toxicants can be avoided. Water restriction was aimed to increase urinary saturation. Depending on the hyperoxaluria, crystalluria and calcium crystal depositions in kidney, we are sure that this rat model of nephrolithiasis has been successfully established. But when compared with results of other researchers' studies, we found that the levels of hyperoxaluria and the crystal depositions numbers in kidney of this model are lower than that reported by Umekawa et al. [29], in that rats were treated with 1% EG in drinking water for 4 weeks. This may probably be the reasons for the inactivation of renal RAS and NADPH oxidase. Despite the absence of angiotensin II related-NADPH oxidase activation, oxidative injury of the kidneys developed

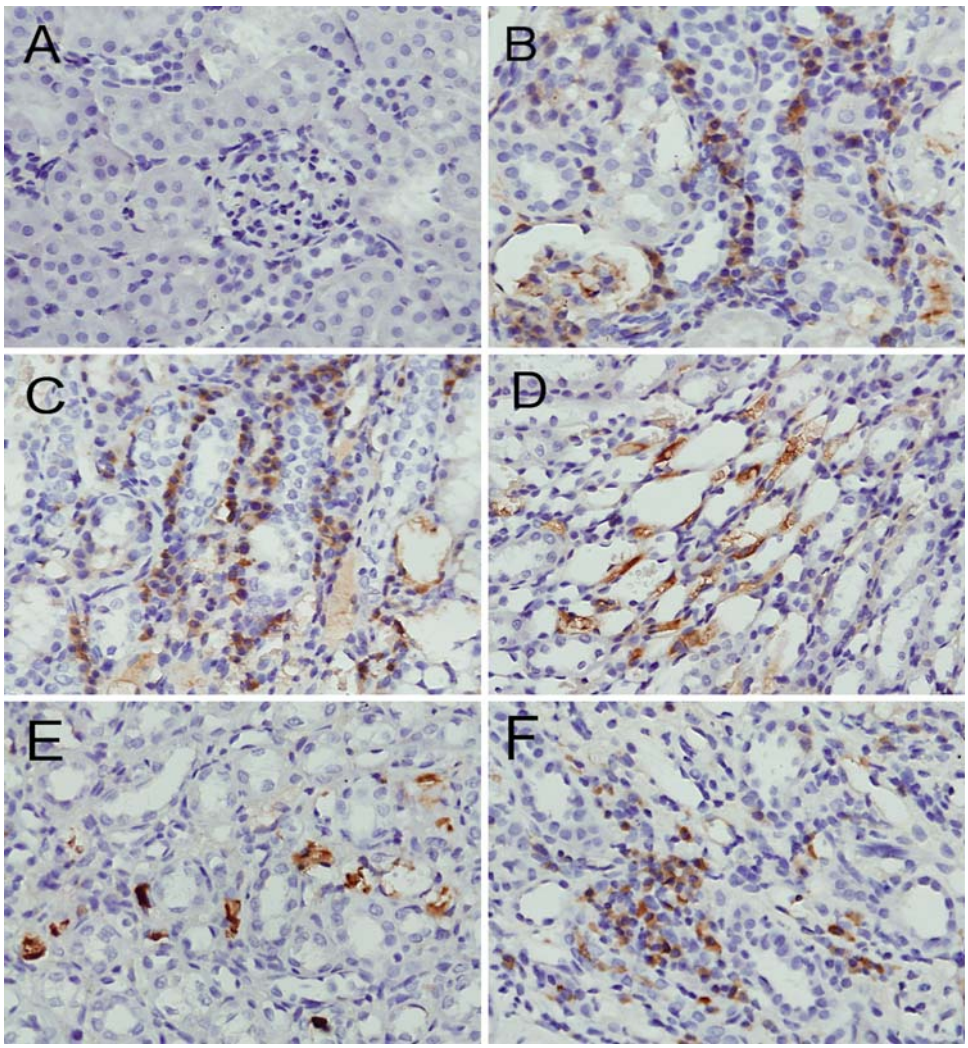

Fig. 7 Immunostaining localization for NADPH oxidase subunits p47phox (a–d) and Nox-4 (e, f) in kidney sections. a Negative, b renal cortex, c inner medulla, d outer medulla, e inner medulla, f outer medulla (magnification ×400)

apparently in this model, with changes of the mitochondria. Therefore, it can be highly suspected that mitochondrial changes preceded the activation of NADPH oxidase and mitochondria other than NADPH oxidase should be considered as the main sources of ROS in this model. However, as a shortage of this study, the activity of NADPH oxidase in kidney tissue cannot be determined directly.

The anti-oxidative role of taurine has been linked to the involvement in mitochondrial oxidation [44]. Taurine was found to localize in mitochondria and was suggested to serve as mitochondrial matrix buffer. It was proposed that by stabilizing the environment in the mitochondria, taurine would prevent leakage of the reactive compounds formed in the reactive mitochondrial environment and thus indirectly act as an antioxidant [44]. In this study, taurine showed to be effective in protecting the kidney from oxidative injury, and this may be the result of the mitochondrial-linked pathway. As shown in the results, taurine treatment improved the indexes of renal OS and injury (urinary 8-IP, creatinine clearance and kidney/body weight), as well as the mitochondrial changes which were caused by the induction of nephrolithiasis. SOD and GSH-Px are known as the major antioxidant enzymes in cells. Dismutation of superoxide by SOD produces hydrogen peroxide, which is subsequently metabolized to water via catalase or by GSH-Px [18]. The decrease in their enzymatic activities may contribute to the oxidative injury of mitochondrial membrane. Taurine may be beneficial in renal protection by reversing the decline of enzymatic activities and stabilizing the mitochondrial membrane, so that electronic leakage from the electron transport chain [11] was limited to low level. Again, improvement in the mitochondrial changes correlated with that in the renal OS and injury, indicated the important role of mitochondria in the development of renal OS in this model.

In addition, though the mechanism by which taurine treatment reduced the crystal deposition in this model needs to be studied further, the benefit of the protective effects of taurine against oxidative injury of renal tubular cells cannot be discounted.

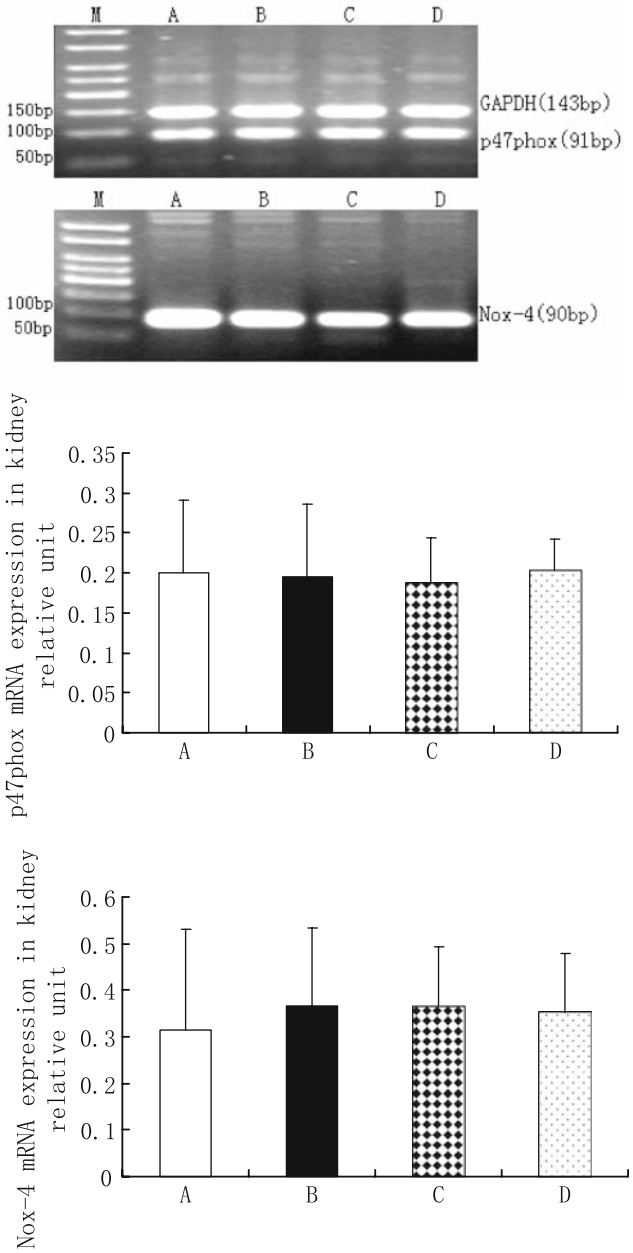

Fig. 8 Quantitative relative RT-PCR of p47phox and Nox-4 mRNAs expression in kidney among the four groups by comparison with GAPDH. A represents control, B represents EG + AC-treated rats, C represents EG + AC + taurine-treated rats, D represents only taurine-treated rats. Mean ± SD; n = 4. $P > 0.05$

With respect to the action of taurine as inhibitor of angiotensin II, since the mechanism that angiotensin II related-NADPH oxidase activation showed no evidence to work in this model, further studies will be needed to investigate the effects of taurine under the nephrolithic condition that renal RAS being activated and functioning. Interestingly, recently published data demonstrated the action of taurine on inhibition of NADPH oxidase in vitro [45].

In summary, our data suggests that mitochondria but not NADPH oxidase account for the OS in kidney in a rat model of CaOx nephrolithiasis. Taurine protected the kidney from oxidative injury through mitochondrial-linked pathway.

Acknowledgments This study was supported by the Major Program of Guangxi Zhuang Autonomous Region Bureau of Health (No. 200729), the Guangxi Science and Technology Development Program (No. 0816004-4), and the Guangxi Postgraduate Innovation Program (No. 2008105981002D31).

References

1. Asplin JR (2002) Hyperoxaluric calcium nephrolithiasis. Endocrinol Metab Clin North Am 31:927–949. doi:10.1016/S0889-8529(02)00030-0
2. Khan SR (2006) Renal tubular damage/dysfunction: key to the formation of kidney stones. Urol Res 34:86–91. doi:10.1007/s00240-005-0016-2
3. Tsujihata M (2008) Mechanism of calcium oxalate renal stone formation and renal tubular cell injury. Int J Urol 15:115–120. doi:10.1111/j.1442-2042.2007.01953.x
4. Scheid C, Koul H, Hill WA, Luber-Narod J, Jonassen J, Honeyman T, Kennington L, Kohli R, Hodapp J, Ayvazian P, Menon M (1996) Oxalate toxicity in LLC-PK1 cells, a line of renal epithelial cells. J Urol 155:1112–1116. doi:10.1016/S0022-5347(01)66402-4
5. Khan SR (1995) calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. Urol Res 23:71–79. doi:10.1007/BF00307936
6. Khan SR (2004) Role of renal epithelial cells in the initiation of calcium oxalate stones. Nephron Exp Nephrol 98:e55–e60. doi:10.1159/000080257
7. Thamilselvan S, Byer KJ, Hackett RL, Khan SR (2000) Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells. J Urol 164:230–236. doi:10.1016/S0022-5347(05)67499-X
8. Thamilselvan S, Hackett RL, Khan SR (1997) Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. J Urol 157:1059–1063. doi:10.1016/S0022-5347(01)65141-3
9. Rashed T, Menon M, Thamilselvan S (2004) Molecular mechanism of oxalate-induced free radical production and glutathione redox imbalance in renal epithelial cells: effect of antioxidants. Am J Nephrol 24:557–568. doi:10.1159/000082043
10. Thamilselvan S, Khan SR, Menon M (2003) Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. Urol Res 31:3–9
11. Khand FD, Gordge MP, Robertson WG, Noronha-Dutra AA, Hothersall JS (2002) Mitochondrial superoxide production during oxalate mediated oxidative stress in renal epithelial cells. Free Radic Biol Med 32:1339–1350. doi:10.1016/S0891-5849(02)00846-8
12. Cao LC, Honeyman TW, Cooney R, Kennington L, Scheid CR, Jonassen JA (2004) Mitochondrial dysfunction is a primary event in renal cell oxalate toxicity. Kidney Int 66:1890–1900. doi:10.1111/j.1523-1755.2004.00963.x
13. Umekawa T, Byer K, Uemura H, Khan SR (2005) Diphenyleneiodium (DPI) reduces oxalate ion- and calcium oxalate monohydrate and brushite crystal-induced upregulation of

MCP-1 in NRK 52E cells. Nephrol Dial Transplant 20:870–878. doi:10.1093/ndt/gfh750
14. Thamilselvan S, Menon M (2005) Vitamin E therapy prevents hyperoxaluria-induced calcium oxalate crystal deposition in the kidney by improving renal tissue antioxidant status. BJU Int 96:117–126. doi:10.1111/j.1464-410X.2005.05579.x
15. Selvam R, Ravichandran V (1993) Restoration of tissue antioxidants and prevention of renal stone deposition in vitamin B6 deficient rats fed with vitamin E or methionine. Indian J Exp Biol 31:882–887
16. Muthukumar A, Selvam R (1998) Role of glutathione on renal mitochondrial status in hyperoxaluria. Mol Cell Biochem 185:77–84. doi:10.1023/A:1006817319876
17. Itoh Y, Yasui T, Okada A, Tozawa K, Hayashi Y, Kohri K (2005) Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. J Urol 173:271–275
18. Khan SR (2005) Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res 33:349–357. doi:10.1007/s00240-005-0492-4
19. Selvam R (2002) Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. Urol Res 30:35–47. doi:10.1007/s00240-001-0228-z
20. Tungsanga K, Sriboonlue P, Futrakul P, Yachantha C, Tosukhowong P (2005) Renal tubular cell damage and oxidative stress in renal stone patients and the effect of potassium citrate treatment. Urol Res 33:65–69. doi:10.1007/s00240-004-0444-4
21. Schwille PO, Manoharan M, Schmiedl A (2005) Is idiopathic recurrent calcium urolithiasis in males a cellular disease? Laboratory findings in plasma, urine and erythrocytes, emphasizing the absence and presence of stones, oxidative and mineral metabolism: an observational study. Clin Chem Lab Med 43:590–600. doi:10.1515/CCLM.2005.103
22. Erdem A, Gündoğan NU, Usubütün A, Kilinç K, Erdem SR, Kara A, Bozkurt A (2000) The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. Nephrol Dial Transplant 15:1175–1182. doi:10.1093/ndt/15.8.1175
23. Wang L, Zhang L, Yu Y, Wang Y, Niu N (2008) The protective effects of taurine against early renal injury in STZ-induced diabetic rats, correlated with inhibition of renal LOX-1-mediated ICAM-1 expression. Ren Fail 30(8):763–771. doi:10.1080/08860220802272563
24. Manna P, Sinha M, Sil PC (2008) Taurine plays a beneficial role against cadmium-induced oxidative renal dysfunction. Amino Acids [Epub ahead of print]
25. Sener G, Sehirli O, Ipçi Y, Cetinel S, Cikler E, Gedik N, Alican I (2005) Protective effects of taurine against nicotine-induced oxidative damage of rat urinary bladder and kidney. Pharmacology 74:37–44. doi:10.1159/000083245
26. Schaffer S, Azuma J, Takahashi K, Mozaffari M (2003) Why is taurine cytoprotective? Adv Exp Med Biol 526:307–321
27. Parvez S, Tabassum H, Banerjee BD, Raisuddin S (2008) Taurine prevents tamoxifen-induced mitochondrial oxidative damage in mice. Basic Clin Pharmacol Toxicol 102:382–387. doi:10.1111/j.1742-7843.2008.00208.x
28. Takahahsi K, Azuma M, Baba A, Schaffer S, Azuma J (1998) Taurine improves angiotensin II-induced hypertrophy of cultured neonatal rat heart cells. Adv Exp Med Biol 442:129–135
29. Umekawa T, Hatanaka YJ, Kurita T, Khan SR (2004) Effect of Angiotensin II receptor blockage on osteopontin expression and calcium oxalate crystal deposition in rat kidneys. J Am Soc Nephrol 15:635–644. doi:10.1097/01.ASN.0000113321.49771.2D
30. Toblli JE, Ferder L, Stella I, Angerosa M, Inserra F (2001) Protective role of enalapril for chronic tubulointerstitial lesions of hyperoxaluria. J Urol 166:275–280. doi:10.1016/S0022-5347(05)66144-7
31. Toblli JE, Ferder L, Stella I, De Cavanagh MVE, Angerosa M, Inserra F (2002) Effects of angiotensin II subtype 1 receptor blockade by losartan on tubulointerstitial lesions caused by hyperoxaluria. J Urol 168:1550–1555. doi:10.1016/S0022-5347(05)64519-3
32. Hanna IR, Taniyama Y, Szocs K, Rocic P, Griendling KK (2002) NAD(P)H oxidase derived reactive oxygen species as mediators of angiotensin II signaling. Antioxid Redox Signal 4:899–914. doi:10.1089/152308602762197443
33. Aihara K, Byer KJ, Khan SR (2003) Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. Kidney Int 64:1283–1291. doi:10.1046/j.1523-1755.2003.00226.x
34. Gambaro G, Valente ML, Zanetti E, Della Barbera M, Del Prete D, D'Angelo A, Trevisan A (2006) Mild tubular damage induces calcium oxalate crystalluria in a model of subtle hyperoxaluria: evidence that a second hit is necessary for renal lithogenesis. J Am Soc Nephrol 17:2213–2219. doi:10.1681/ASN.2005121282
35. Meimaridou E, Lobos E, Hothersall JS (2006) Renal oxidative vulnerability due to changes in mitochondrial-glutathione and energy homeostasis in a rat model of calcium oxalate urolithiasis. Am J Physiol Renal Physiol 291:731–740. doi:10.1152/ajprenal.00024.2006
36. Huang H-S, Ma M-C, Chen C-F, Chen J (2003) Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology 62:1123–1128. doi:10.1016/S0090-4295(03)00764-7
37. Veena CK, Josephine A, Preetha SP, Rajesh NG, Varalakshmi P (2008) Mitochondrial dysfunction in an animal model of hyperoxaluria: a prophylactic approach with fucoidan. Eur J Pharmacol 579(1–3):330–336
38. Babior BM, Lambeth JD, Nauseef W (2002) The neutrophil NADPH oxidase. Arch Biochem Biophys 397:342–344. doi:10.1006/abbi.2001.2642
39. Chabrashvili T, Tojo A, Onozato ML, Kitiyakara C, Quinn MT, Fujita T, Welch WJ, Wilcox CS (2002) Expression and cellular localization of classic NADPH oxidase subunits in the spontaneously hypertensive rat kidney. Hypertension 39:269–274. doi:10.1161/hy0202.103264
40. Shiose A, Kuroda J, Tsuruya K (2001) A novel superoxide producing NADPH oxidase in kidney. J Biol Chem 276:1417–1423. doi:10.1074/jbc.M007597200
41. Li JM, Shah AM (2003) ROS generation by nonphagocytic NADPH oxidase: potential relevance in diabetic nephropathy. J Am Soc Nephrol 14:S221–S226. doi:10.1097/01.ASN.0000077406.67663.E7
42. de Water R, Noordermeer C, van der Kwast TH, Nizze H, Boevé ER, Kok DJ, Schröder FH (1999) Calcium oxalate nephrolithiasis: effect of renal crystal deposition on the cellular composition of the renal interstitium. Am J Kidney Dis 33(4):761–771. doi:10.1016/S0272-6386(99)70231-3
43. Khan SR, Glenton P (1995) Deposition of calcium phosphate and calcium oxalate crystals in the kidneys. J Urol 153:811–817. doi:10.1016/S0022-5347(01)67728-0
44. Hansen SH, Andersen ML, Birkedal H, Cornett C, Wibrand F (2006) The important role of taurine in oxidative metabolism. Adv Exp Med Biol 583:129–135. doi:10.1007/978-0-387-33504-9_13
45. Li Y, Arnold JM, Pampillo M, Babwah AV, Peng T (2009) Taurine prevents cardiomyocyte death by inhibiting NADPH oxidase-mediated calpain activation. Free Radic Biol Med 46:51–61. doi:10.1016/j.freeradbiomed.2008.09.025