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Implications of oxidative stress in the pathophysiology of obstructive uropathy

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Abstract Although the functional and clinical alterations occurring in patients with obstructive uropathy are not well understood, it has been suggested that oxidative stress could contribute in the mechanism responsible for the impairment of sodium and water balance. This study aimed to test the hypothesis that red wine administration causes an amelioration of both the renal damage and impairment of renal Na^+, K^+-ATPase activity occurring after ureteral obstruction in the rat. Twenty-four male Wistar adult rats weighting 200–250 g were used. Half of them received a 10-week treatment with wine as the sole fluid source, while the other group received water. Both groups were subjected to 24-h unilateral ureteral obstruction (UUO). Kidney tissue was collected following the relief of the ligature to perform the biochemical assessments. Urine and blood samples were taken at baseline and after the relief. Results show that the treatment with red wine significantly enhances the activity of antioxidant enzymes, and thus reduces renal lipid peroxidation secondary to UUO, which correlated negatively with Na^+, K^+-ATPase activity. Based on this and other previous data, it could be suggested that red wine administration may prevent renal damage secondary to UUO by inducing enhanced antioxidant potential.

Keywords Unilateral ureteral obstruction (UUO) · Oxidative stress · Antioxidant enzymes · Lipid peroxidation · Na^+, K^+-ATPase · Ischemia-reperfusion (IR)

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Introduction

Obstructive uropathy is a blockage of the flow of urine making it to back up and may damage the kidneys. An obstruction may occur at any level along the urinary tract, resulting in increased intraluminal pressure and urinary stasis with increased risk of urinary tract infections and lithiasis. The blockage may compromise one or both kidneys, resulting in bilateral or unilateral ureteral obstruction (UUO), respectively. In adult humans, the most common cause of UUO is renal lithiasis [1], which typically causes a sudden blockage of one ureter and leads to an acute obstruction. UUO in rats is a well-established experimental model of renal injury that reproduces human effects of an obstructive uropathy [2, 3].

The renal capsule is a fixed container, so even a little change in intrarenal volume may affect dramatically the pressure within the collecting system proximal to the point of obstruction. These mechanisms lead to renal tissue ischemia and activation of inflammation. Following the release of the obstruction, a renal dysfunction is evidenced by a progressive decrease in glomerular filtration rate (GFR) and in renal blood flow (RBF), due to arteriolar vasoconstriction and impairment of water and sodium balance [4, 5], and increased pelvic pressure [6]. Consequently, a marked compensatory increase in renal excretion of sodium and water occurs, thereby contributing to maintain fluid and salt homeostasis [7]. The molecular mechanisms accounting for these physiological response have not been well elucidated, but oxidative stress could play a role in the impairment of the activity of Na^+, K^+-ATPase. Increased reactive oxygen species (ROS) results in damage to cellular biomolecules involving polyunsaturated fatty acid residues of phospholipids, which are abundant in kidney and are extremely sensitive to oxidation [8–10]. Na^+, K^+-ATPase activity along

the renal tubule is diminished following ureteral obstruction, especially in the outer medulla [11].

Over the last decade, considerable experimental evidence has supported the view that ROS play a key role in numerous mechanisms of seemingly unrelated nephropathies [12]. They may have an important role in the tubulointerstitial inflammation associated with obstructive nephropathy [13], due to the tubular injury caused by mechanical disturbance, which leads to a proinflammatory state and tubulointerstitial fibrosis [14]. After releasing the obstruction, an increased renal ROS production [15] causes overexpression of fibrogenic cytokines and chemoattractants [14], which could be at least partly explained because of the occurrence of renal oxidative stress.

Many molecular and cellular mechanisms have been described to explain this injury, such as the mechanical disturbance resulting from ureteral ligation (tension stress) [16, 17], hypoxia induced by a marked decline in plasma flow, macrophage influx into interstitium [18, 19] and production of cytokines [20, 21]. All these mechanisms may affect the redox state, increasing ROS levels, which coincides with findings in rat models [22, 23].

In addition, antioxidant defenses block radical processes and eliminate harmful ROS. Superoxide dismutases (SOD) and catalase (CAT), two antioxidant enzymes from tubular cells, have shown a downregulation in the obstructed kidney, decreasing the antioxidant defenses and this way contributing to the development of oxidative stress [24]. In turn, antioxidant substances, such as those produced endogenously or provided exogenously by the diet or supplements, can neutralize free radicals by accepting or donating an electron. There are many exogenous diet antioxidants, which can be incorporated as supplements [25]. Polyphenols have shown a powerful antioxidant effect, based on properties such as metal chelation, ROS scavenging and reinforcement of antioxidant response [26]. They are particularly abundant in Chilean red wine [27]. The ethanol contained in red wine, also has antioxidant properties, inducing antioxidant enzymes and improving the bioavailability of polyphenols. In addition, it was reported that a chronic wine treatment results in upregulation of the antioxidant enzymes CAT, SOD and glutathione peroxidase (GSH-Px) and attenuation of damage to biomolecules such as lipid peroxidation [28, 29].

From these data it seems reasonable to suggest that the administration of antioxidants may prevent both structural and functional renal damage produced during and after UUO, which may be associated with diminution of Na^+, K^+-ATPase activity.

Calcium oxalate urolithiasis constitutes a frequent cause of obstructive uropathy. Together with the pathogenic role of oxalate in the formation of stones, it was reported earlier that it has the ability to generate free radicals, and thus lipid peroxidation. Studies in animal models are in agreement with these data, as shown by the association of hyperoxaluria-induced lipid peroxidation accompanied by a diminution of antioxidant enzyme levels [30]. The aim of the present study was to test the hypothesis that red wine administration causes an amelioration of the renal damage and impairment of Na^+, K^+-ATPase activity occurring by ureteral obstruction in the rat.

Materials and methods

Animals

Twenty-four male Wistar adult rats weighting 250–300 g provided by the Nutrition Department, School of Medicine, University of Chile were used. The animals were housed in individual cages under habitual conditions in a temperature-controlled room (24°C) and were randomly assigned into two groups, control and case, each of twelve rats. The animals received a balanced diet plus free access to either water in the control group or red wine in the case group, for a period of 10 weeks, as published previously [31]. Daily fluid intake was measured with graduated Richter tubes, whereas food intake was measured gravimetrically. The animals received human care compliance with internationally accepted ethic procedures, and a minimal number was used to achieve statistical significance. The study protocol was approved by the local bioethics committee.

Wine

The red wine used in the present study was previously chosen after analyzing 25 samples of Chilean Cabernet Sauvignon and assessing the concentration of flavonols, as reported in a previous publication [28]. We used Cabernet Sauvignon from Lomas de Cauquenes, Cauquenes Valley, Chile, harvest from year 2004.

Preparation of samples for biochemical assessment

To ensure expansion of extracellular fluid volume, a salt loading was given the day before the experiment, by intragastric tubing, in a dose of 1.25 mmol/NaCl per 100 g body weight, in 1 mL of hypertonic solution. Thereafter, the animals were maintained for 17 h with free access to water and solid diet. Lithium chloride (0.12 mmol/100 g body weight) was given in a solution containing 60 mM LiCl and 5% glucose 24 h before the surgery.

Unilateral ureteral obstruction model

All the animals were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally). An abdominal midline

incision was made in each animal and the left ureter was ligated, keeping its structure unaffected. This procedure yielded complete ureteral obstruction with subsequent uniform hydronephrosis in the affected kidney. Blood samples from ophthalmic artery were also taken from the anesthetized animals.

After 24 h, the rats were operated again to relieve the ligated ureters. Only those rats in which adequate release could be achieved as judged by lack of dilatation of the proximal ureter were used for further experimentation. Urine was collected in metabolic cages for 1 h and then both urine and blood samples from the ophthalmic artery were obtained. Then, the left kidney was perfused with Earle's balanced salt solution of pH 7.40 (Sigma Chemical Co., St Louis, MO) for 15 min in situ and then the anesthetized rats were sacrificed and both kidneys were quickly removed and homogenized [32]. For determinations performed in kidneys, we took the values of the contralateral kidneys as basal levels.

Lipid peroxidation

Lipid peroxidation was determined in the kidney and also in erythrocytes to measure the systemic effect. The assay for lipid peroxide products was performed spectrophotometrically at 532 nm by the thiobarbituric acid reaction at pH 3.5, followed by solvent extraction with a mixture of *n*-butanol/pyridine (15/1, v/v) [33]. Tetramethoxy-propane was used as the external standard and the level of lipid peroxides was expressed as nanomolar malondialdehyde (MDA) equivalent/mg protein. To determine lipid peroxidation in erythrocytes membranes, the cells were previously subjected to hypotonic shock with distilled water to cause hemolysis.

Antioxidant enzymes

Homogenates of the tissues in 0.25 M sucrose were used for the determination of SOD activity; whereas 1.15% KCl–0.010 M Tris pH 7.40 buffer was used for the homogenates to determine the activities of both CAT and GSH-Px. The SOD assay is based on the SOD-mediated increase in the rate of auto-oxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo(c)fluorene in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm [34]. One SOD unit is defined as the activity that doubles the auto-oxidation background. The results were expressed as U/mg protein. CAT activity was assayed from the kinetic of breakdown of hydrogen peroxide at 240 nm [35] by the supernatant of 2,400*g*; it was expressed on the basis of the rate constant of the first order reaction (*k*)/mg protein. Soluble GSH-Px activity was measured in the cytosolic fraction (supernatant of 100,000*g*) by a spectrophotometric method based on the reduction of glutathione disulfide coupled to the NADPH oxidation by glutathione reductase [36]. One GSH-Px unit is defined as the activity that oxidizes 1 μmol NADPH per minute. The activity of GSH-Px was expressed as U/mg protein.

Na^+, K^+-ATPase activity

The activity of this membrane-bound enzyme was determined by a method based on the measurement of ATP hydrolysis [37]. Total protein content was determined by the method of Lowry et al. [38], and enzyme activity was expressed as micromoles of inorganic phosphate per milligram protein per hour.

Sodium reabsorption

As stated previously, the fraction of filtered sodium delivered out of the proximal tubule and the distal straight tubule (Henle's thick ascending limb) were estimated using the fractional excretion of lithium (FELi) [39].

Materials

The reagents were purchased from Sigma-Aldrich (St. Lois, MO, USA), Merck (Darmstadt, Germany), and Riedel-de Häen (Germany), and were of the highest commercial grade available.

Statistical analyses

Results were expressed as means ± standard deviation (SD). Both groups were compared by Student's *t* test and significant differences were established for $P < 0.05$. To analyze the association between different variables, the Pearson correlation coefficient was used. All statistical analyses were computed using GraphPad Prism™ version 4.03 (GraphPad Software Inc., San Diego, CA, USA).

Results

Intake and weight gain

The daily energy intake and the body-weight gain during the experimental period were not significantly influenced in the wine group. Both groups had free access to either water or wine, showing no significant difference in the amount of fluid ingested (Table 1). The energy supplied by ethanol corresponded to 28% of the energy intake in the wine group, but as have been found in a previous experiment, there was no significant difference between total energy intake in both groups [31].

Table 1 Weight gain and fluid consumption in water and wine groups

	Water	Wine
Weight gain (g/day per 100 g rat)	2.7 ± 1.3	2.9 ± 0.5
Fluid consumption (mL/day per 100 g rat)	8.5 ± 2.6	7.9 ± 2.0

There were no significant differences in weight or fluid consumption between both groups. Values are expressed as mean ± SD (n = 24)

Renal lipid peroxidation

The results obtained for renal and erythrocyte lipid peroxidation (nmol MDA/mg protein or nmol MDA/g hemoglobin, respectively) are shown in Fig. 1. The basal levels of MDA both in kidney and erythrocytes showed no significant difference between water and red wine groups. After 24 h of complete UUO and an accurate release, the MDA levels both in kidney and erythrocytes were significantly higher in the water group. In red wine group this change occurred only in erythrocytes. After the UUO, the levels of MDA in both kidney and erythrocytes were significantly lower in the red wine group.

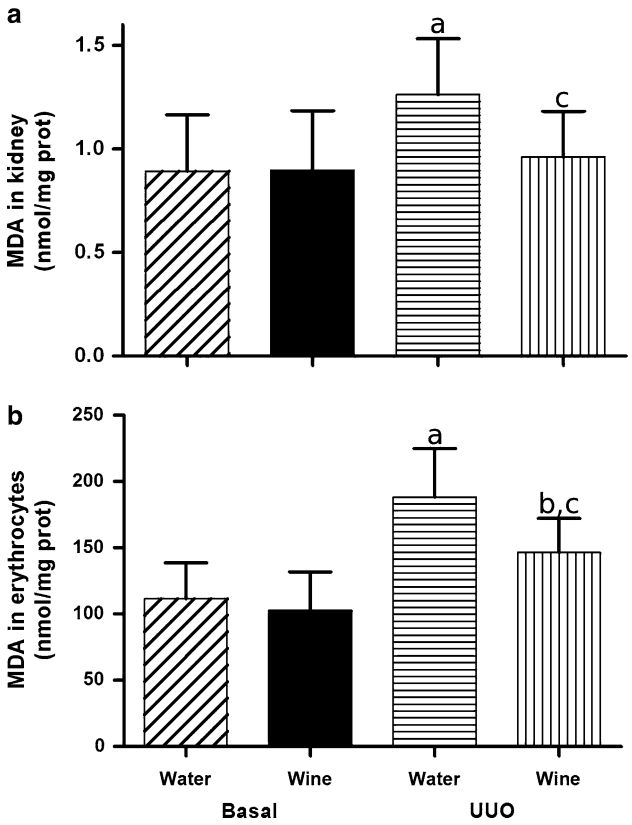

Fig. 1 Effects of 24 h of UUO in lipid peroxidation assessed through MDA levels in kidney (**a**) and erythrocytes (**b**) of rats treated with wine for 10 weeks (wine group) or water (water group). Values are means ± SD (n = 24). Statistically significant differences, at $P < 0.05$, are indicated as letters on the bar: *a* versus basal water group, *b* versus basal wine group, *c* versus UUO water group. *UUO* unilateral ureteral obstruction, *MDA* malondialdehyde

Renal antioxidant enzymes

The activities of antioxidant enzymes (CAT, SOD and GSH-Px) are shown in Fig. 2. Basal levels of activity of these enzymes were significantly higher in wine group. Both groups suffered a significant decrease on the activity of these enzymes after 24 h of UUO. However, after the UUO only SOD showed a significantly lower activity level in wine group compared to water group.

Na^+, K^+-ATPase activity

The activity of renal Na^+, K^+-ATPase of both groups is shown in Fig. 3. Both groups showed no significant differences in basal activity. After UUO, water group showed a significant diminution of this activity, whereas the red wine group did not show significant changes.

Correlation between renal MDA levels and Na^+, K^+-ATPase activity and sodium distal reabsorption

Malondialdehyde levels in kidney correlated negatively with both Na^+, K^+-ATPase activity (Fig. 4a) and distal sodium reabsorption (Fig. 4b) of both water and wine groups.

Discussion

The present study shows that UUO causes increased lipid peroxidation in kidney, that is associated with diminution in Na^+, K^+-ATPase activity, a key enzyme of tubular sodium transport.

It has been long and widely described that any obstruction in urine flow may lead to a long-term impairment in the ability to regulate urinary excretion of both water and sodium. It reduces the ability to concentrate and dilute urine, and also the transport of Na^+, K^+, and H^+ in renal tubules [40–45]. Releasing the obstruction produces a dramatic increase in sodium and water excretion [46–48], which explains polyuria and sodium depletion shown in these type of patients. The mechanisms responsible for these alterations are not entirely clear, but recent studies have demonstrated a lower expression of aquaporins 1–4 and all major renal sodium transporters, such as type 3 Na^+/H^+ exchanger, type 2 Na^+, Pi cotransporter, Na^+, K^+, $2Cl^-$ cotransporter, Na^+, Cl^- cotransporter and specially Na^+, K^+-ATPase, during and after relief of the obstruction [44, 49–51]. All these alterations may contribute to the impairment of urine concentration and salt excretion in response to urinary tract obstruction.

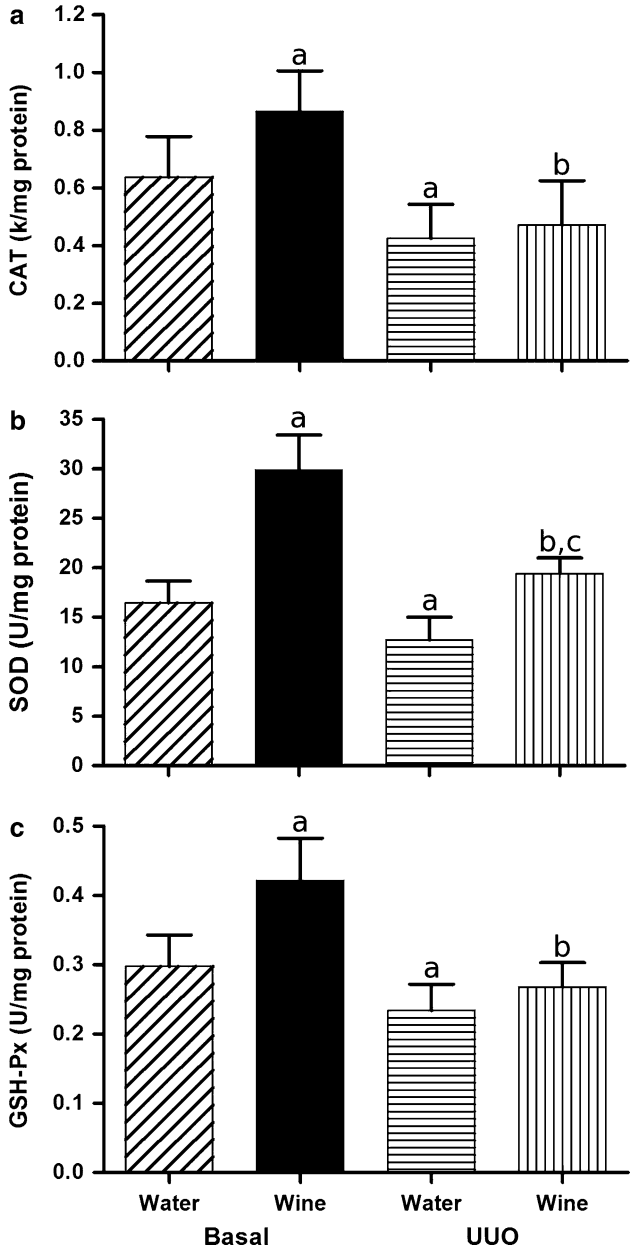

Fig. 2 Effects of 24 h of UUO on the activity of the antioxidant enzymes catalase (CAT, **a**), superoxide dismutase (SOD, **b**) and glutathione peroxidase (GSH-Px, **c**) in kidney of rats treated with wine for 10 weeks (wine group) or water (water group). Values are means ± SD ($n = 24$). Statistically significant differences, at $P < 0.05$, are indicated as letters on the bar: *a* versus basal water group, *b* versus basal wine group, *c* versus UUO water group. *UUO* unilateral ureteral obstruction, *k* catalase first order kinetic constant for breakdown of hydrogen peroxide

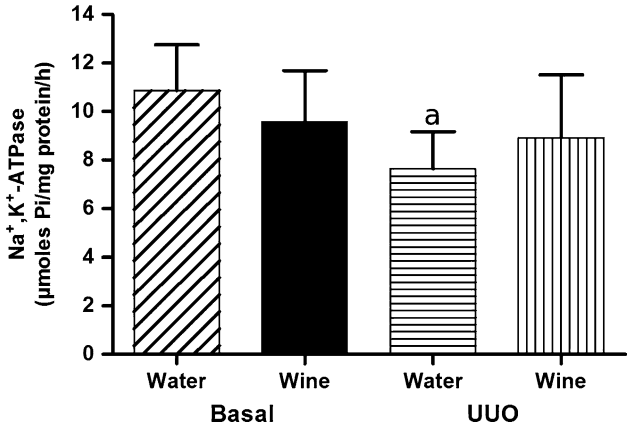

Fig. 3 Effects of 24 h of UUO in Na^+, K^+-ATPase activity in rats treated with wine for 10 weeks (wine group) or water (water group). Values are means ± SD ($n = 24$). Statistically significant differences, at $P < 0.05$, are indicated as letters on the bar: *a* versus basal water group. *UUO* unilateral ureteral obstruction

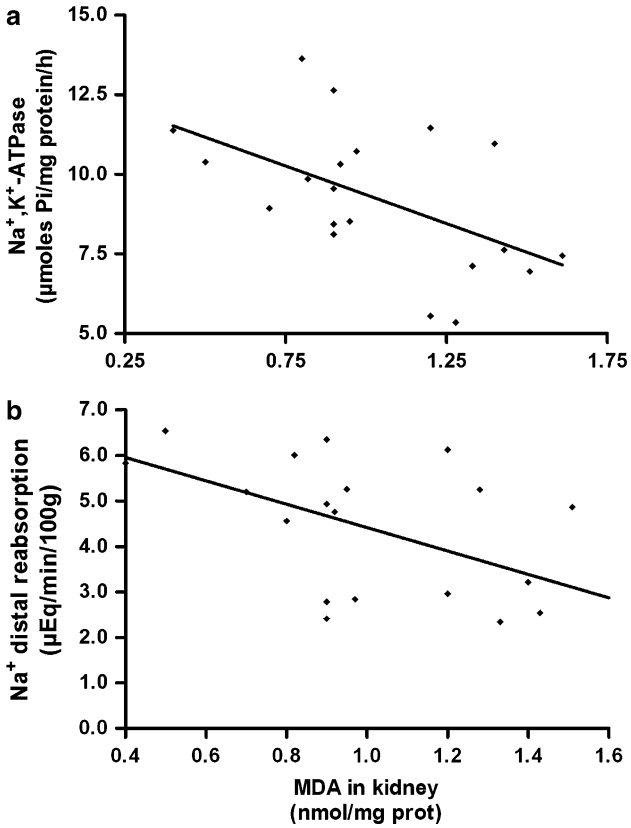

Fig. 4 Correlation between renal MDA levels of rats treated with wine or water for 10 weeks and an UUO for 24 h and either Na^+, K^+-ATPase activity (**a**) ($r = 0.28$, $P = 0.02$, $n = 20$) or distal Na^+ reabsorption (**b**) ($r = 0.3$, $P = 0.01$, $n = 20$). *UUO* unilateral ureteral obstruction, *MDA* malondialdehyde

It has been demonstrated that Na^+, K^+-ATPase, as a membrane protein, goes through conformational changes due to lipid peroxidation [52]. On this basis, it could be suggested that oxidative stress could act as a trigger of these alterations, based on the inverse correlation found between Na^+, K^+-ATPase activity and lipid peroxidation in kidney (Fig. 4a), thus supporting a functional involvement of oxidative stress in UUO.

In addition, there has been proposed that oxidized low-density lipoprotein contributes to increasing lipid peroxida-

tion in UUO [53] and that it acts as a chemoattractant to macrophages [54, 55]. Thus an interstitial inflammation occurs as a consequence of UUO [56], in which ROS may have an important role [13] through activation of transcription factors and molecules involved in apoptosis or cellular survival. Manucha [57] has recently reviewed different markers in congenital UUO and found apoptosis as a central mechanism of tubular atrophy. It seems reasonable to assume a similar role in acquired UUO, thus making promoters of apoptosis a possible therapeutic target.

It is well known that the urine backflow due to UUO increases the intrarenal pressure because of its capsule, which leads to ischemia in the most active areas of the kidney. In conditions of prolonged ischemia functional changes occur that include a decrease of oxidative phosphorylation and impairment in function of ATP dependent membrane pumps, with a consequent entry of calcium, sodium and water to the cell and degradation of the ATP that leads to accumulation of hypoxanthine with generation of ROS [58]. Therefore, ischemia induces a proinflammatory state which increases the tissue vulnerability during reperfusion [59]. The antioxidant enzymes CAT, GSH-Px, and SOD from tubular cells from the obstructed kidney show downregulation, which increases the vulnerability of the kidney to oxidative damage [24], a process exacerbated by sodium depletion [60]. We propose that additional to these phenomenons, after the release of the obstructed ureter, ischemia-reperfusion (IR) renal injury is likely to occur. The mechanisms of this phenomenon may include, among others, ROS and reactive nitrogen species generation, microcirculatory changes, overexpression of fibrogenic cytokines like transforming growth factor $\beta 1$ [53], loss of calcium homeostasis, complement activation [58], chemoattractants and the activation of redox-sensitive transcriptional factors like NFκB and AP1, with the consequent gene activation of mediators of cell death (Mn SOD, iNOS and antiapoptotic proteins) or cellular survival signals, as had been described in other organs [58, 61]. It is possible that the administration of alcohol contained in wine corresponds to a kind of non-ischemic preconditioning in kidney, inducing ischemic tolerance as seen in other tissues [62, 63].

Considering this data, a chronic or acute antioxidant therapy may prevent or diminish the structural and functional damage occurring in obstructive uropathy. The significant decrease in lipid peroxidation after a chronic treatment with red wine (Fig. 1), the enhancement found in the activity of antioxidant enzymes (Fig. 2) and the inverse correlation between lipid peroxidation and both Na^+, K^+-ATPase activity and Na^+ excretion (Fig. 4) support this hypothesis. If an IR injury occurs as suggested by the authors, it may also be possible to prevent renal damage secondary to UUO with ischemic preconditioning. The alternatives may include among others, transient ischemia [58], hypothermia and hyperthermia [64, 65], inflammation [66] and hyperbaric oxygen [67, 68], in addition to an antioxidant therapy with both antioxidants and antioxidant enzymes enhancers.

We did not make different measures for renal cortex and medulla because a previous study made in our laboratory showed no significant differences between them in terms of what we evaluated [28]. However, some authors demonstrated regional differences, such as a decreased cortical but not medullar perfusion in the obstructed kidney of rats with UUO [69] and an accumulation of lipids in the renal medulla of the obstructed kidney [70–72]. This may constitute a limitation of the present study.

In summary, these data are consistent with an involvement of oxidative stress as a pathophysiologic mechanism after UUO following ureteral obstruction and provide a mechanistic basis to suggest the preventive use of antioxidants in obstructive uropathy to attenuate damage derived from an increase of ROS in the kidney. Probably an IR cycle may be associated with renal damage after releasing the obstruction. However, further studies are needed to establish this type of treatment in humans.

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