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Review

Biochemical-molecular markers in unilateral ureteral obstruction

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ABSTRACT: Congenital obstructive nephropathy is the primary cause of end-stage renal disease in children. Rapid diagnosis and initiation of the treatment are vital to preserve function and/or to slow down renal injury. Obstructive uropathy effects -decline in the plasmatic renal flow and glomerular filtration rate, interstitial infiltrate of leukocytes, significant decrease of the urine concentration, loss of the capacity to concentrate urine as well as fibrosis and apoptosis- are a consequence of a variety of factors that work in complex ways and are still not fully understood. Mediators as angiotensin II, transforming growth factor-β (TGF-β) and nitric oxide (NO) have been implicated in congenital obstructive nephropathy.

The renin-angiotensin system is regulated in different ways, affecting both renal structure and function, and that it in turn depends upon the duration of the obstruction. On the other hand, the role of nitric oxide in renal injury remains somewhat controversial due to the fact that it can exert opposite effects such as cytoprotective and prooxidant / proapoptotic efects as well as proinflammatory and anti-inflammatory effects. In addition**,** reactive oxidative species (ROS) might contribute to the progression of renal disease.

During unilateral ureteral obstruction induced uncoordinated and aberrant growth may lead to the loss of cellular phenotype and apoptosis. Promoting inflammatory responses, the oxidizers can regulate the adherence of certain molecules and proinflammatory mediators, transcription factors and fibrogenic cytokines, that are clearly involved in the progression of renal disease.

The congenital obstructive nephropathy is characterized by tubular atrophy, cellular proliferation, apoptosis and fibrosis; immature kidney is more susceptible than adult kidney to showing the above mentioned alterations. Apoptosis seems to be the principal mechanism that leads to tubular atrophy during the neonatal unilateral ureteral obstruction (UUO). Considering the significant role of the apoptosis in UUO, we believe of big interest the study of the regulatory factors of apoptosis in the renal obstruction neonatal.

The complex biochemical and molecular events during the development, maintenance and progression of the renal injury in unilateral ureteral obstruction require further major studies to better understand the alterations mentioned above.

Introduction

Congenital obstructive nephropathy is the primary cause of end-stage renal disease in children. Kidney and urinary tract malformations are associated with this pathology; nevertheless, the therapeutic conduct of intervening surgically or it does not turn out to be complicated by unpredictable consequences. The etiology is variable and wideranging. Classification is according to cause, degree and duration. Most commonly, obstruction occurs at the ureteropelvic junction. The incidence of unilateral ureteral obstruction (UUO) is about 1/1,000 in adults. The obstructive congenital nephropathy is characterized by tubular atrophy, cellular prolif-

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eration, apoptosis and fibrosis; the immature kidney is more sensitive than the adult kidney to the alterations mentioned above (Cachat *et al*., 2003). Obstruction of the urinary tract in both human beings and experimental animals produces diverse effects on kidney tubular and glomerular function.

There is a pronounced reduction in plasmatic renal flow and glomerular filtration rate with significant changes in tubular function (Klahr *et al*., 1988). If the obstruction persists, it can cause slow and progressive cellular atrophy with nephron destruction, leading to chronic renal insufficiency. Since obstruction is in general a cause of reversible renal malfunction, rapid diagnosis and initiation of treatment are vital to preserve function and/or to slow down renal injury.

The effects of obstructive uropathy on renal function are a consequence of a variety of factors that work in complex ways and are still not fully understood. After three hours of unilateral obstruction, glomerular filtration begins to diminish in spite of compensation for the decrease in proximal duct pressure. This has been linked to vasoconstriction of afferents arteriole by thromboxane A_2, the renin-angiotensin system and other intra-renal mechanisms. The direct consequence is a rapid decline in the plasmatic renal flow and glomerular filtration rate that, after 24 hours of obstruction, falls to about 25% of normal in every case. In our laboratory, we showed a significant reduction in inulin clearance to 14% in the obstructed kidney compared to control, as well as a significant increase in the intact contralateral kidney of around 25% after 24 hours of obstruction (Vallés *et al*., 1999). Global renal haemodynamic responses to acute unilateral ureteral obstruction have been extensively studied in animals; however little is known about the concurrent changes in haemodynamics and tubular fluid dynamics that occur within the distinct regions of the kidney during UUO. Pelaez *et al*. (2005) demonstrated decreased cortical, but not medullary perfusion of the ipsilateral kidney, and an increase of the concurrent intratubular fluid concentrated in most tubular segments, suggesting increased tubular reabsorption that may have helped to maintain glomerular filtration rate and tubular fluid flow (Pelaez *et al*., 2005).

After 4 hours of unilateral obstruction, an interstitial infiltrate of leukocytes appears and reaches a maximum at 12 hours, thereafter it becomes stable. This infiltrate is located in cortex and medulla ducts, especially in the distal ducts. The infiltrate helps to reduce plasmatic renal flow and the glomerular filtration rate, probably by the production of vasoactive prostanoids such as thromboxane A_2 (Harris *et al*., 1993). If the obstruction persists, the cellular infiltrate will cause irreversible damage to the renal parenchyma through induction of fibroblast proliferation that produces interstitial fibrosis and then segmentary focal glomerulosclerosis. These alterations might stem from the production of cytokines (interleukin 1 and transforming growth factor), proteolytic enzymes, reactive oxygen species (ROS), products of enzyme activity such as cycloxygenases and lipoxygenases, thrombocyte-derived growth factor, coagulation factors and thrombocyte activating factors (Harris and Martínez-Maldonado, 1995; Harris *et al*., 1993).

One of the tubular consequences of obstruction is the loss of the capacity of the kidney to concentrate urine. In agreement with other authors, we demonstrated a significant decrease of the urine concentration in the obstructed kidney with respect to the intact contralateral after 24 hours of obstruction (Vallés *et al*., 1999). On this matter; it has been recently established that major sodium transporters and aquaporins in the obstructed kidney are down-regulated in response to neonatally induced UUO, which indicates that these transporters may play a crucial role in the persistent reduction in renal handling of sodium and water in response to UUO (Shi *et al*., 2004). Another important disorder during obstruction is the modification of acidification mechanisms. Experiments in animals and humans have demonstrated that acid excretion is affected after either unilateral or bilateral obstruction (Klahr *et al*., 1988). In obstruction, abnormal acidification exists at the cortical collecting duct level, but the greatest shortcoming of acidification is located at the medullary collecting duct level. Related to this, we demonstrated in microdissected segments a significant decrease in the ATPase activity of 37% in the medullary collecting ducts of the obstructed kidneys and 14% in the cortical collecting ducts. A reduced number of H^+-ATPase pumps in cell's apical surfaces might be causing the attenuated acidification that follows ureteral obstruction. Indeed, we have demonstrated recovery of the ATPase activity in medullary collecting ducts upon release in kidneys after obstruction for thirty days (Vallés *et al*., 1999).

The reduced activity of Na-K ATPase can be explained by a decrease of the enzyme protein. However, the discordant reports related to Na-K ATPase subunits protein amounts and mRNA changes suggest that renal Na-K ATPase subunits are ubder different cellular regulatory processes during obstruction; transcriptional, translational and even intra-cytoplasmic processing may be involved (Hwang *et al*., 2002).

The renin-angiotensin system and obstruction

Studies performed during the last decades have demonstrated the existence of numerous, powerful vasoactive substances (angiotensin II, thromboxane A_2, prostaglandins PGE_2 and PGI_2, bradykinin, atrial natriuretic factor, nitric oxide, etc.) and their probable link with changes in renal function during obstruction (Klotman *et al*., 1986; Yarger *et al*., 1980; Gadnapaphornchai *et al*., 1978; Blackshear and Wathen, 1978).

In the kidney, angiotensin II has been implicated in the regulation of important physiological functions such as electrolyte balance and the control of the blood volume (Saavedra, 1992); it producer multiple effects including hemodynamic regulation, renin-angiotensin system inhibition and stimulation of proximal tubular ion transport (Sexton *et al*., 1992). The majority of the well-known actions of angiotensin II on water regulation and saline metabolism are dependent on AT_1 receptor stimulation (Matsusaka and Ichikawa, 1997), including sodium retention in the tubules, vasoconstriction of renal arteries, and also the fall in glomerular filtration rate (Ardaillou, 1999).

Several studies have shown that unilateral ureteral obstruction (UUO) is accompanied by complex changes in renovascular response and function during the first 24 hours of its course. The increase in sensitivity to angiotensin II conduce to progressive vasoconstriction in cortex and medulla after 2 hours of obstruction. The demonstration that inhibition of the renin-angiotensin system reiterates the hemodynamic changes seen after unilateral ureteral obstruction, especially with enzyme conversion inhibitors of angiotensin (ECA), and more specifically the non-peptide blockers of the angiotensin II AT_1 receptors, such as losartan, has been very important in this context (Pimentel *et al*., 1993). Also, enalapril inhibits the interstitial fibrosis in UUO kidneys. It also suggests a beneficial and unforeseen effect of enalapril on the obstructed kidney by potentially stimulating the production of nitric oxide through an increased expression of the endothelin ET (B) receptor (Moridaira *et al*., 2003).

Nevertheless, complete recovery of the pathological alterations has not been achieved in spite of using these blockers. This suggests that the stimulation of the renin-angiotensin system persists and overcomes the effect of the pharmacological agents and/or that other vasoconstrictors might be equally involved and might be responsible for the residual functional changes. On this matter, we have recently published that losartan protects against the progression of fibrosis through an anti-inflammatory effect that is independent of blood pressure (Manucha *et al*., 2004; Fig. 1) and (Manucha *et al*., 2005b; Fig. 2). These results suggest a potential clinical application in the prevention of fibrosis during obstruction.

All these studies indicate that, in UUO, the renin-angiotensin system is regulated in a distinctive way, affecting both renal structure and function, and that it in turn depends upon the duration of the obstruction.

Nitric oxide (NO) and obstruction

The role of NO in renal injury remains controversial due to the fact that it can exert both cytoprotective and prooxidant/proapoptotic efects as well as proinflammatory and anti-inflammatory effects (Goligorsky *et al*., 2002).

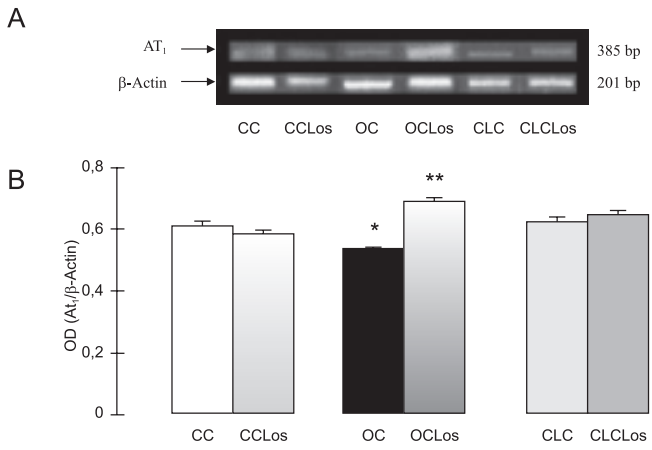

FIGURE 1. A. ***Expression of angiotensin II AT_1 receptor in renal cortex 24 hours after UUO. Effect of Losartan.*** RT-PCR for AT_1 receptor (385 bp product) was performed ussing RNA isolated from homogenates of obstructed, control and contralateral kidney cortex.
Top: Representative gel of AT_1 cDNA in control cortex (CC), obstructed cortex (OC) and contralateral cortex (CLC) before and after Losartan treatment (Los).
Bottom: Amplification of corresponding β-actin mRNA. These results are representative of five independent observations.
B. ***Semiquantitative PCR analysis of the angiotensin II AT_1 receptor.*** Densitometric analysis of the angiotensin II AT_1 receptor and β-actin shows a decreased ratio of OC compared to control cortex (*p< 0.05, n:5). Increased AT_1 receptor/β-actin mRNA ratio in obstructed cortex with Losartan compared to obstructed cortex (**p<0.01, n:5). Values are means ± SEM

The gas NO is a seccond messenger of simple structure that is generated in situ and diffuses easily across the biological membranes.

During UUO, an increasing influence of NO has been demonstrated on cortical circulation maintaining suitable plasmatic renal flow in angiotensin II-treated animals. These observations indicate that NO serves as a compensating vasodilator system because of its influence on the specific vasoconstrictor action of angiotensin II. Also, dietary arginine supplementation during UUO relief significantly improved the renal damage including fibrosis, apoptosis, and macrophage infiltration. This suggests that increasing NO availability could be beneficial in the setting of UUO relief (Ito *et al*., 2005).

Considering the diversity of NO actions and the multiplicity of cellular phenotypes in the kidney, it is not surprising that NO has been chosen as a candidate to try to explain the complex functions of the kidney. To date for example, it has been implicated in the paracrine control of renal hemodynamics (Navar *et al*., 1996), tubulointerstitial balance (Thorup and Persson, 1994), natriuresis (Fenoy *et al*., 1995), immune response, and in the sequelae of hypoxia-reoxygenation in renal injury.

The location of NO sources in the kidney grants an ideal handle to determine the ways in which it acts to control renal function. The different isoforms of nitric oxide synthase (NOS) are available for rapid activation to produce NO in response to physical stimuli such as stress from stretching (shear stress) and soluble factors such as acetylcholine and bradykinin.

In the kidney, the inducible nitric oxide synthase (iNOS) can act constitutively at very low levels in certain areas where its mRNA is found but, after induction, it increases considerably the NO levels. Recent studies indicate that iNOS mRNA is differentially expressed in cell types along the nephron under basal conditions and after immuno-activation (Ahn *et al*., 1994). Studies of rat kidney homogenates *in vitro* verified that the iNOS specific activity in the medulla was 3 times greater than in the cortex. The significance of the high iNOS expression in control kidneys is not completely

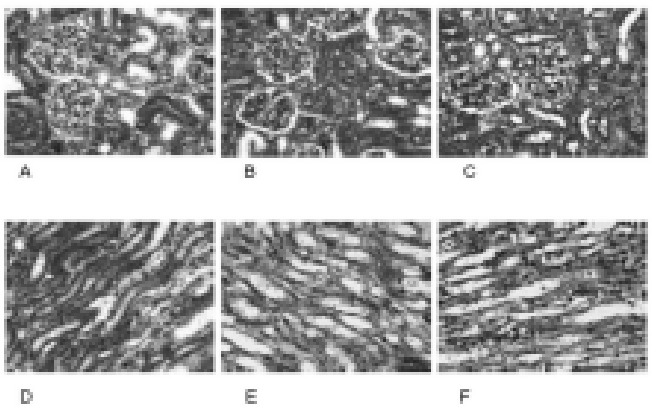

FIGURE 2. *Trichromic-Masson stained sections of rat kidney cortex and medulla.*
(**A**) Sham operated kidney cortex or (**B**) untreated ureter-obstructed kidney cortex or (**C**) Losartan-treated ureter-obstructed kidney cortex. (**D**) Sham operated kidney medulla or (**E**) untreated ureter-obstructed kidney medulla or (**F**) Losartan-treated ureter-obstructed kidney medulla. Magnification X 400.

understood. It has been proposed that the iNOS-generated NO serves to support and regulate blood flow and oxygenation (Ito, 1995). Additionally, bearing in mind that the loop of Henle is the synthesis site of the Tamm-Horsfall protein, known for linking and deactivating several cytokines, it may be postulated that cytokines sequestered by above glycoprotein, acting in synergy, might be responsible for the basal induction of iNOS (Morrisey *et al*., 1994).

Evidence also exists that NO might induce natriuresis in opposition to the effects of angiotensin II on proximal tubular sodium reabsorption (Ito, 1995), and by directly inhibiting Na^+/K^+ adenosine triphosphatase (ATPase) activity at the tubular level (Guzman *et al*., 1995). In studies of H^+-ATPase activity carried out in our laboratory, we demonstrated that endogenous NO could be involved in the inhibition of H^+-ATPase activity in microdissected collector duct segments during UUO (Vallés and Manucha, 2000).

At present, there are two iNOS sub-isoforms described in kidney, the macrophage-type iNOS that exists in the glomerulus, proximal ducts and cortical collecting ducts, and the smooth vascular muscle-type iNOS, which has been decript in arcuate arteries, interlobular arteries and mesangial cells (Mohaupt *et al*., 1994). In the renal cortex, neuronal nitric oxide synthase (nNOS) exists only in the dense macule and in minor proportion in the internal nephrons (Beierwaltes, 1995).

The entire vasculature of the kidney contains endothelial nitric oxide synthase (eNOS). Renal vascular resistance is tonically influenced by NO production and diffusion from endothelials cells. The NO is rapidly deactivated in the blood if it does not enter vascular smooth muscle cells by diffusion, where it produces relaxation. Some studies suggest that NO may be a critical factor in the maintenance of renal vascular resistance under normal conditions (King and Brenner, 1991). The renal vasoconstriction induced by NOS inhibition is probably related to the influence of a variety of endogenous systemic vasoconstrictors. Some of these factors include endothelium-derived vasoconstrictors such as endothelin or thromboxane endoperoxide. The literature suggests that angiotensin exercises its greatest influence on renal hemodynamics in the absence of NO, especially under circumstances where renin and angiotensin are increased.

Since it has been described that endothelium-derived NO regulates renal vascular resistance, and that neuronal NOS has a discrete cortical location in the dense macule, it was suggested that NO in this region could be involved in aspects of renal function controlled by the dense macule, from distal tubules to aferent arterioles. The apparent indirect renin stimulation produced by NO through an autocrine route is directly opposed to the paracrine effect mediated by cGMP-dependent renin inhibition in juxtamedular cells by NO synthesized in the renal vascular endothelium.

The concept that NOS activity changes according to the type of obstruction is very important. Whereas in unilateral obstruction NO would be increased to limit vasoconstriction (Chevalier *et al.*, 1992), in bilateral obstruction the NOS activity is diminished (Reyes *et al*., 1994). Miyajima *et al*. (2001) demostrated the importance of the NO system in the maintenance of tubular integrity during UUO were the obstructed kidneys exhibit less NOS activity in the chronic phase. Supporting these results, Ito and collaborators (2004) demonstrated the feasibility of liposome-mediated iNOS gene transfer into the UUO kidney. Furthermore, the improvement of renal function in UUO demonstrates that the transfected iNOS gene is active and suggests that decreased iNOS activity contributes to the decreased renal function in UUO. This iNOS construct may have therapeutic utility in the pathophysiologic sequelae of UUO and other renal diseases (Ito *et al*., 2004).

We examined nitric oxide synthase (NOS) activity and expression in kidneys from children who underwent surgery release of unilateral ureteropelvic junction (UPJ) obstruction in relation to clinical and histologic parameters. In children, a positive linear relationship was found between cNOS activity in cortex and creatinine clearance. The degree of interstitial fibrosis correlated negatively with cNOS activity in cortex. In kidneys from children with UPJ obstruction, an increased activity and expression of iNOS in medulla and cNOS-dependent in cortex were also demonstrated; therefore and we infer that a role of cNOS in modulating GFR and interstitial fibrosis can be suggested. Prolonged UPJ obstruction might lead to a worsened prognosis on renal injury (Vallés *et al*., 2003).

NO produces cytotoxicity at high concentrations, whereas NO at lower concentrations may have the opposite effect and protect against apoptotic cell death from various stimuli. DNA damage triggers p53 protein accumulation which produces growth arrest and/or apoptosis. Supporting these affirmations, there is evidence of a NO-p53 regulatory loop whereby increased production of NO induces an increased level of p53 protein, which then down-regulates iNOS expression (Ambs *et al*., 1998). Furthermore, Docherty *et al*. (2006) demostrated that inhibition of tubular cell apoptosis

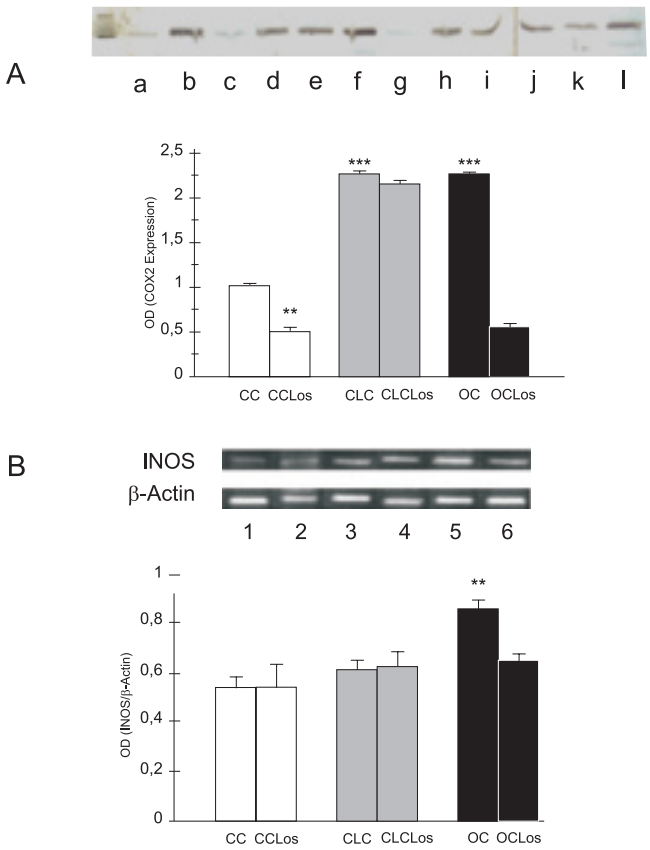

FIGURE 3. A. ***Effect of Losartan on in vivo Renal COX-2 Protein Expression.*** Immunoblot analysis of COX-2 from cortex and medulla of rats treated with Losartan (10 mg/Kg) and Densitometric analysis of COX-2 protein abundance in medulla of UUO. Effect of Losartan. **Lanes a and b:** Control medulla and cortex respectively. **Lanes c and d:** Losartan-treated in control medulla and cortex, respectively. **Lanes e and f:** obstructed medulla and cortex, respectively. **Lanes g and h:** Losartan-treated in obstructed medulla and cortex, respectively. **Lanes i and j:** Contralateral medulla and cortex, respectively. **Lanes k and l:** Losartan-treated in contralateral medulla and cortex, respectively. The first non-lettered lane shows the protein standards (COX-2: 72 kDa). Immunoblots were quantified for COX-2 expression. The relative amount of COX-2 protein was determined after normalization of the level of COX-2 protein to the appropriate control without treatment.

B. ***Expression of iNOS and β-actin mRNAs in kidney cortex after UUO.*** Representative gel of iNOS mRNA obtained from control (lane 1), contralateral (lane 3) and obstructed (lane 5) cortex 24 hours following unilateral ureteral obstruction. After AT_1 receptor blockade with the inhibitor Losartan, products of RT-PCR for iNOS are shown in lane 2 (control cortex), lane 4 (contralateral cortex) and lane 6 (obstructed cortex). Expression of the housekeeping gene β-actin is included below the iNOS expression. These results are representative of six independent blots. Semiquantitative PCR analysis of the iNOS isoform in renal cortex of obstructed, contralateral and control kidneys. Effect of Losartan. Increased iNOS/β-actin mRNA ratio in obstructed cortex OC (n:6) compared to control cortex CC (**P<0.01, n:6) and to contralateral cortex CLC (*P<0.05). Increased iNOS/β-actin mRNA ratio in OC (n:6) compared to OC with Losartan treatment (*P<0.05, n:6). Values are means ±SEM.

protects against renal damage and development of fibrosis following ureteric obstruction.

NOS shares important characteristics with cyclooxygenase (COX). The inducible isoform of COX (COX_2) is present in endothelial cells (Maier *et al*., 1990), fibroblasts (Raz *et al*., 1988) and macrophages (Masferrer *et al*., 1992) after treatment with pro-inflammatory agents such as lipopolysaccharides (LPS) as well as interleukin 1β (IL 1β).

Anti-inflammatory steroids such as dexamethasone, inhibit iNOS *in vitro* and *in vivo*; they lack effect on constitutive NOS expression (Moncada *et al*., 1991). Dexamethasone also inhibits cyclooxygenase (COX) protein synthesis stimulated by lipopolysaccharides (LPS) and Interleukin-1 (IL1β), *in vitro* (Fu *et al*., 1990) and *in vivo* (Masferrer *et al*., 1992); however, has not effect on the constitutive COX isoform. On the other hand, numerous NO effectors can be simultaneously recruited by products of COX activation [Prostaglandin E_2 (PGE_2) and Prostaglandin I_2 (PGI_2)]. This is true in the rapid agonist action of, for example, bradykinin (De Nuci *et al*., 1988) and in the long lasting action of agents like LPS or IL1β (Stadler *et al*., 1991).

It is known that NO, PGI_2 and PGE_2 increase the levels of cyclic guanosyl monophosphate (cGMP) or cyclic adenosine monophosphate (cAMP) in target cells. This synergistic effect may be the mechanism through which NOS and COX act in a cooperative fashion. Another possibility to consided is the that COX might be a target for NO, because it possesses an active heme-Fe center. In fact, most of the effects mediated by NO are a consequence of its interaction with iron or with enzymes that contain it. This makes possible an interaction with NO and consequent modulation of the COX activity. The regulation of COX activity by NO might represent an important mechanism by which the inflammatory response would increase or attenuate, as suggested by our results in the context of the unilateral ureteral obstruction model (Manucha *et al*., 2004) (Fig. 3).

Oxidative stress in obstruction

All the cells that shape renal structure, at the vascular (endothelial and smooth muscular cells), glomerular (endothelial and mesangial cells), or tubular

(proximal, distal and collecting cells) levels, are capable of producing and liberating reactive oxygen species (ROS) in response to specific stimuli - certain drugs, hypertension, radiation, high oxygen pressure or hypoxia, among others. Infiltrates of circulating cells (granulocytes, monocyte-macrophages and thrombocytes), present in numerous renal inflammatory processes (glomerulonephritis, vasculitis, pyelonephritis), are also capable of producing large quantities of ROS; however, it is practically impossible to discriminate the role of these ROS species from these produced by resident cells to finally evaluate their action on renal pathology (Stratta *et al*., 1991).

The stimuli that favor neutrophil ROS production include bacteria, immunocomplexes, complement C5 fraction, platelet activating factor (PAF), and interleukin-1. In macrophages as well as in neutrophils the Tamm-Horsfall protein can induce ROS liberation. In thrombocytes, ROS production can take place during the metabolism of arachidonic acid and those stimuli that augment this process also favor ROS production.

Several ROS-dependent mechanisms might contribute to the progression of renal disease. The above mentioned mechanisms induce uncoordinated and aberrant growth, which may lead to the loss of cellular phenotype and apoptosis. Promoting inflammatory responses, the oxidizers can regulate the adherence of certain molecules and proinflammatory mediators, including transcription factors and fibrogenic cytokines that are clearly involved in the progression of renal disease (Haugen and Nath, 1999).

After lowing renal mass, consumption of O_2 increases in the surviving nephrons, which implies an increase in potentially harmful oxidative stress for these nephrons. The work of the surviving ducts also increases, in parallel with O_2 consumption as well as ROS production. This implies that under basal conditions, there can be ROS-induced damage that, in certain circumstances, might be aggravated by an increase in local metabolism and ROS production. This might be the case with a protein overload, since a major increase in oxygen consumption is seen in the remaining nephrons, increasing ROS production and accelerating renal deterioration (Haugen and Nath, 1999).

In renal tubules, we can observe edema, tearing of the basal membrane and lysis. Changes in function consist of an increased permeability, transmembrane potential alteration and a proliferative response. Toxic damage (gentamycin, cisplatin) and pigment cylinders are observed in reperfusion situations. Numerous clinical and experimental studies have clearly demonstrated that one of the initial events in progressive renal disease is macrophage infiltration into the tubulointerstitial and glomerular compartments. Macrophages are liberated in both pro-inflammatory and pro-fibrogenic situations, leading to the production of reactive oxygen species, cytokines, growth factors, proteases and proteins of the extracellular matrix.

Unilateral ureteral obstruction (UUO) shows the characteristics of an early progressive interstitial fibrosis related to macrophage infiltration of the cortical tubulointerstitium.

In 1968, Risdon *et al*. demonstrated a positive correlation between the histology of tubular atrophy and the decrease in glomerular filtration rate. Later, other investigators also observed that renal function was altered, although it correlated better with structural aberrations in the tubular renal interstitium. Bearing in mind that approximately 80% of renal volume is occupied by tubules, it should not be surprising that the chronic tubular damage associated with interstitial fibrosis might be tightly linked to global renal disfunction.

Scars development in the renal cortex as a consequence of obstruction of the urinary tract is the principal result of interstitial fibrosis.

Macrophage recruitment in the medullar interstitium is dependent, at least partly, on the production of chemoattractive proteins and adhesion molecules. After a few hours of mechanical disturbance by the ureteral obstruction, a dramatic upregulation is seen of intercellular adhesion molecules (ICAM)-1, monocyte chemoattractive protein (MCP)-1 and osteopontin (Ricardo *et al*., 1996; Diamond *et al*., 1995; Diamond *et al*., 1994). Along the same lines, increased expression of vascular cells adhesion molecule (VCAM)-1 mRNA has also been described after three days of unilateral ureteral obstruction, which is significantly slowed down by the administration of enalapril (Morrisey and Klahr, 1998).

ICAM-1 is a cell surface glycoprotein that can be upregulated by pro-inflammatory cytokines as well as by ROS. Tumor necrosis factor (TNF-α), interleukin 1 and gamma interferon (IFN-γ) have all been described as excellent *in vitro* inducers of ICAM-1 synthesis in renal tubule epithelial cells, mesangial cells and glomerular epithelial cells (Springer, 1990). ROS generation can activate ICAM-1 expression, and also that of other adhesion molecules and chemoattractants, including monocyte chemoattractant protein-1 (MCP-1) and Platelet-derived chemokine (RANTES) in cultured mesangial cells (Schlondorff, 1995). MCP-1 is a member of a new family of small cytokines produced by a variety of im-

mune and non-immune cells such as mononuclear cells, endothelial cells, fibroblasts and smooth muscle cells. MCP-1 activity is increased by stimulation with specific pro-inflammatory cytokines like interleukin 1, TNF-α and IFN-γ (Larsen *et al*., 1989). In addition, Robin *et al*. (1990) demonstrated that UUO produces a specific, lipidic chemoattractive molecule. MCP-1 upregulation is clearly seen in the tubule apical membrane 12 hours after UUO. An increase in MCP-1 mRNA expression has been demonstrated by Northern blot analysis in obstructed kidneys compared to intact contralateral control (Diamond *et al*., 1994).

Increased osteopontin expression at the renal tubular cortical epithelium has been described, as a marker for macrophage infiltration into the interstitium, leading to fibrogenesis culminating in interstitial fibrosis (Diamond *et al*., 1995). Osteopontin is a glycosylated phosphoprotein isolated originally from bone matrix; usually it is absent in renal cortex, with the exception of the parietal epithelium and Bowman's capsule. Augmented osteopontin mRNA has been described in the proximal tubular epithelium of obstructed kidneys after four hours of initiating the obstruction (Diamond *et al*., 1995).

ROS can play a vital role in tubulointerstitial inflammation associated with obstructive nephropathy. ROS derived from initial vasoconstriction during UUO as a result of modifications in renal plasmatic flow, due to a progressive vasoconstriction at the expense of local vasoactive compound production -including angiotensin II (Pimentel *et al*., 1995)- with subsequent reperfusion and deregulation of tubular antioxidant enzymes, might irritate the pro-inflammatory state post-unilateral ureteral obstruction. Nevertheless, they might produce by themselves disturbances in proximal tubular enzymes during ischemia. In this context, Modi *et*

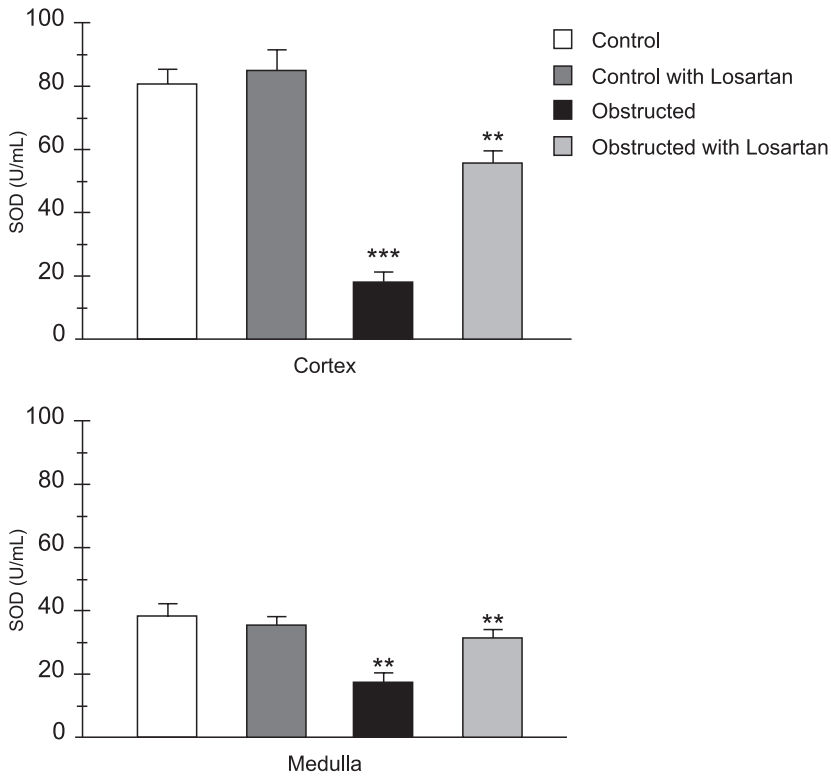

FIGURE 4. ***Total superoxide dismutase activity (SOD) in kidney cortex 24 hours after UUO. Effect of Losartan.*** Top. SOD activity in control and obstructed kidney cortex before and after Losartan administration (10mg/Kg/day). Decreased SOD activity in non-treated OC compared to non-treated control cortex, *** p<0.001. Recovery of SOD activity in treated obstructed cortex compared to non-treated OC,** p < 0.01. Bottom. SOD activity in control and obstructed kidney medulla before and after Losartan administration (10mg/Kg). Decreased SOD activity in non-treated obstructed medulla (OM) compared to non-treated control medulla (CM), ** p<0.01. Recovery of SOD activity in treated obstructed medulla (OM) compared to non-treated OM,** p < 0.01.

al. (1990) investigated the effect of Probucol, a synthetic antioxidant, on unilaterally and bilaterally obstructed rats over 24 hours. They demonstrated that in the rats with bilateral obstruction treated with Probucol caused a pronounced and significant decrease in the renal levels of malonyldialdehyde, a lipidic peroxidation product, in addition to the decrease in leukocyte infiltration and renal levels of oxidized glutathione in comparison with bilateral obstruction without Probucol.

Decreased expression of antioxidant enzyme mRNA proteins, and increased ROS generation might contribute together to development of the tubulointerstitial injury associated with experimental hydronephrosis (Ricardo *et al*., 1997).

The development of interstitial fibrosis is characterized by a progressive accumulation of connective tissue due to a complex pathophysiological sequence of cellular and molecular events. Oxidative stress and/or an alteration in the cellular oxidative state might contribute to the development of interstitial fibrosis, through an increase of the fibrogenic cytokine expression, transcription and extracellular matrix protein synthesis (Poli and Parola, 1997).

Several studies have revealed the importance of oxidative stress and lipid peroxidation products in a wide variety of experimental and clinical models with fibrotic disorders.

ROS may be involved in the activation of MCP1 and ICAM-1 genes, adhesion molecules that take part in the infiltration of inflammatory cells, cellular proliferation and extracellular matrix accumulation, all important events in the development of tubulointerstitial fibrosis (Schlondorff, 1995). Oxidative stress disturbs the adhesion of renal tubular epithelial cells to extracellular matrix proteins by altering integrins, disrupting the interaction between cells, modified by oxidation, and the adjacent matrix (Gailit *et al*., 1993). Andreoli and Mc Ateer (1990) observed that proximal tubules are more susceptible to oxidative injury than distal ducts. In turn, ROS increases the synthesis of collagenase type I by fibroblasts. Related to this, it has been suggested that oxidative stress stimulates the expression of collagen type I gene in human fibroblast cultures (Houglam *et al*., 1991). The addition of á-tocopherol to fibroblast cultures diminishes transcription of collagen gene and protein synthesis. Changes in the redox state of the cells can also influence the synthesis and accumulation of collagen. Shan *et al*. demonstrated in 1994 that high glutathione levels increase collagen secretion and collagenase types I and IV mRNA levels.

On the other hand, numerous experiments support the participation of hydrogen peroxide and other ROS in the activation of signaling pathways and transcription factors (Palmer and Paulson, 1997) such as nuclear factor kappa-B (NF-κB) and activating protein-1 (AP-1) (Meyer *et al*., 1993). Subunits of the NF-κB family form dimeric protein complexes in the cytosol of cells which activate genes that contribute to tissue inflammation. The AP-1 sequence is present in numerous eukaryotic genes and is activated by a family of nuclear proteins (jun-fos) (Angel and Karin, 1991). In this context, an increase in NF-κB expression in rat cortical tubular cell nuclei during UUO has been demonstrated by immunohistochemistry (Morrissey and Klahr, 1997). The same authors thought that the anti-inflammatory effect of the enalapril, an angiotensin converting enzyme (ACE) inhibitor, might be due to a decrease in NF-κB activation. ROS, acting directly or through aldehydes that are the final product of lipid peroxidation, might stimulate the expression of fibrogenic cytokines during inflammation by NF-κB and AP-1 activation.

In lipid peroxidation induced by carbon tetrachloride, transforming growth factor β (TGF-β) and extracellular matrix protein expression were completely abolished by vitamin E supplements (Parola *et al*., 1992), as seen for other pro-inflammatories such as TNF-α induced by ROS (Poli *et al*., 1990).

The maintenance of homeostasis in normal tissue reflects a balance between proliferation and cellular death. Apoptosis plays an important role in morphogenesis and in the survival of normal cells in adult tissue.

Renal illness can appear in response to stimuli such as exogenous toxins or ROS, which disrupt the normal control mechanisms of numerous cells by altering the cellular cytoskeletal or adhesion receptors between cells, generating apoptosis (Korsmeyer *et al*., 1995).

From the morphological point of view, apoptosis is characterized by cell shrinkage, loss of contacts between cells, chromatin aggregation, and the shedding of apoptotic bodies linked to the membrane, which are rapidly consumed by macrophages (Kerr *et al*., 1972).

Immediately after UUO, there are a both decrease in the expression of antioxidant substances, such as the enzyme superoxide dismutase (SOD) (Fig. 4), and an also amplification in ROS generation (Fig. 5) (Manucha *et al*. 2005b), which might help to increase apoptosis. Thus, during renal tubular illness, apoptosis might contribute to tubular atrophy after obstruction (Truong *et al*., 1996) and tubular damage after injury by ischemia-reperfusion (Farber, 1992). It is currently that endonu-

clease activation is an early event in DNA damage, and cell death in renal tubular epithelial cells exposed to hydrogen peroxide. The peroxides have been implicated as mediators of apoptosis in several systems, supported by the high coefficient of diffusion of hydrogen peroxide and its capacity to generate more toxic oxygen intermediates such as the hydroxyl radical. Cellular oxidative status is also an important regulator of apoptosis due to ROS. Glutathione peroxidase expression, an enzyme that inhibits hydrogen peroxide-mediated lipid peroxidation, might also act as an apoptosis marker (Korsmeyer *et al*., 1995).

Morphological studies have demonstrated that atrophy of the renal parenchyma after obstruction is as extensive as the cell death caused by apoptosis (Gobe and Axelsen, 1995). To date there is suficient evidence suggesting that apoptosis contributes to tubular damage and interstitial fibrosis associated with UUO (Docherty *et al*., 2006).

It is also expected that molecular and cellular events that remodel the interstitial matrix during the pro-inflammatory state might also promote tubular damage. The tubular damage might develop as a consequence of peritubular capillary occlusion-generated ischemia, or through increased apoptosis due to ROS and permanent DNA damage.

The process of tubular damage is fundamental to the decline in renal function. The above mentioned process occurs in parallel with alterations in the integrity of cellular membranes, in particular, ROS-mediated lipoperoxidation. According to our results in obstructed cortex, the cholesterol content fell while the total phospholipid content did not change, suggesting modifications in membrane fluidity and, consequently, in membrane function (Manucha *et al*., 2005a).

Recently, genetically modified animals and the use of knockout mice have made a major contribution to our current understanding of the pathogenesis of obstructive nephropathy. These animals have yielded important information about the beneficial and deleterious roles of specific gene products including angiotensin II and TGF-β, among others (Bascands and Schanstra, 2006).

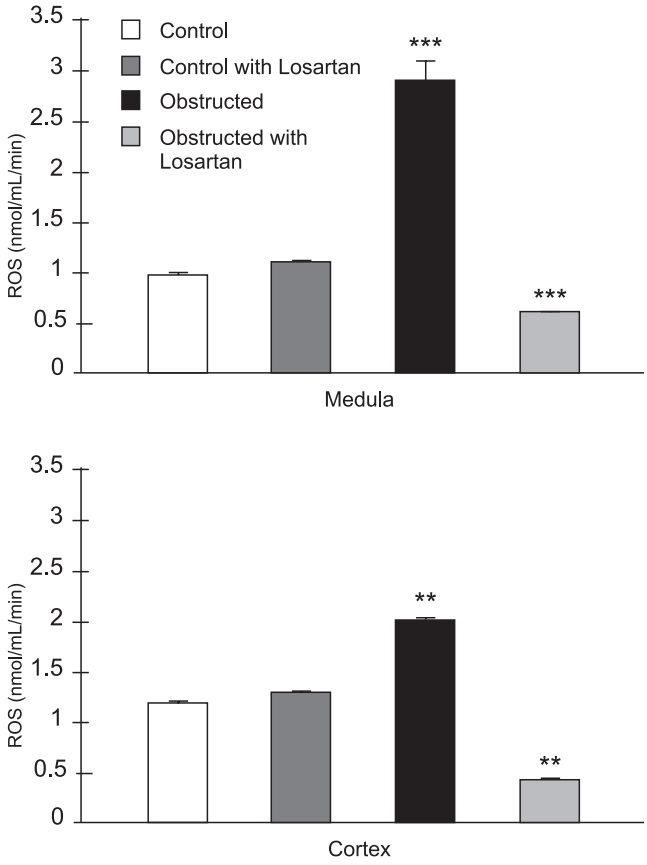

FIGURE 5. ***Effect of Losartan on superoxide anion O_2^- and hydroxyl radical (ROS) concentration.*** Top: Generation of hydroxyl radicals and O_2^- anion in medullar tissue from OUU cortex 24 hours after obstruction. Increase concentration of ROS (nmol/ml/min) was demonstrated in non-treated obstructed medulla (OM) compared to control medulla (CM) (***p<0.001, n:6). Losartan administration decreased ROS (nmol/ml/min) concentration in obstructed medulla (OM) compared to control medulla (CM), ***p<0.001. Bottom: Generation of O_2^- anion and hydroxyl radicals in tissue from OUU cortex 24 hours after obstruction. Increased concentration of ROS (nmol/ml/min) was demonstrated in non-treated OC compared to control cortex (**p<0.01, n:6). Losartan administration decreased ROS (nmol/ml/min) concentration in OC compared to control cortex (CC), **p<0.01

Conclusion

The congenital obstructive nephropathy is characterized by tubular atrophy, cellular proliferation, apoptosis and fibrosis; immature kidney is more susceptible than adult kidney to showing the above mentioned alterations. Apoptosis seems to be the principal mechanism that leads to tubular atrophy during the neonatal unilateral ureteral obstruction (UUO). Considering the significant role of the apoptosis in UUO, we believe of big interest the study of the regulatory factors of apoptosis in the renal obstruction neonatal.

Finally, the complex biochemical and molecular events involved in the development, maintenance and progression of the renal injury in unilateral ureteral obstruction require further major studies to better understand the alterations mentioned above.

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