

**Inducible Nitric Oxide Synthase (iNOS) and Renal Obstruction Research Literatures**Ma Hongbao<sup>1</sup>, Margaret Young<sup>2</sup><sup>1</sup> Brookdale Hospital, Brooklyn, New York 11212, USA; <sup>2</sup> Cambridge, MA 02138, USA  
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**Abstract:** Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. Nitric oxide synthases (NOSs, EC 1.14.13.39) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule that helps to modulate the vascular tone, insulin secretion, airway tone and peristalsis, etc, and NO is involved in angiogenesis and neural development. It may function as a retrograde neurotransmitter. NO is mediated in mammals by the calcium-calmodulin controlled isoenzymes eNOS (endothelial NOS) and nNOS (neuronal NOS).

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**Key words:** inducible nitric oxide synthase (iNOS); life; obstruction; renal

**Introduction**

Nitric oxide synthases (NOSs, EC 1.14.13.39) are a family of enzymes catalyzing the production of nitric oxide (NO) from L-arginine. NO is an important cellular signaling molecule that helps to modulate the vascular tone, insulin secretion, airway tone and peristalsis, etc, and NO is involved in angiogenesis and neural development. It may function as a retrograde neurotransmitter. NO is mediated in mammals by the calcium-calmodulin controlled isoenzymes eNOS (endothelial NOS) and nNOS (neuronal NOS). The inducible isoform, iNOS, is involved in immune response, binds calmodulin at physiologically relevant concentrations, and produces NO as an immune defense mechanism, as NO is a free radical with an unpaired electron. It is the proximate cause of septic shock and may function in autoimmune disease.

NOS isoforms catalyze other leak and side reactions, such as superoxide production at the expense of NADPH. As such, this stoichiometry is not generally observed, and reflects the three electrons supplied per NO by NADPH. NOS catalyzes the reaction:

L-arginine + 3/2 NADPH + H<sup>+</sup> + 2 O<sub>2</sub> → citrulline + nitric oxide + 3/2 NADP<sup>+</sup>

NOSs are unusual in that they require five cofactors. Eukaryotic NOS isozymes are catalytically self-sufficient. The electron flow in the NO synthase reaction is: NADPH → FAD → FMN → heme → O<sub>2</sub>. Tetrahydrobiopterin provides an additional electron during the catalytic cycle which is replaced during turnover. NOS is the only known enzyme that binds flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH<sub>4</sub>) and calmodulin.

Arginine-derived NO synthesis has been identified in mammals, fish, birds, invertebrates, and bacteria. Best studied are mammals, where three distinct genes encode NOS isozymes: neuronal (nNOS or NOS-1), cytokine-inducible (iNOS or NOS-2) and endothelial (eNOS or NOS-3). iNOS and nNOS are soluble and found predominantly in the cytosol, while eNOS is membrane associated. Evidence has been found for NO signaling in plants, but plant genomes are devoid of homologs to the superfamily which generates NO in other kingdoms.

The neuronal isoform is involved in the development of nervous system. It functions as a retrograde neurotransmitter important in long term potentiation and hence is likely to be important in memory and learning. nNOS has many other physiological functions, including regulation of cardiac function and peristalsis and sexual arousal in males and females. An alternatively spliced form of nNOS is a major muscle protein that produces signals in response to calcium release from the SR. nNOS in the heart protects against cardiac arrhythmia induced by myocardial infarction. The primary receiver for NO produced by eNOS and nNOS is soluble guanylate cyclase, but many secondary targets have been identified. S-nitrosylation appears to be an important mode of action. The inducible isoform iNOS produces large amounts of NO as a defense mechanism. It is synthesized by many cell types in response to cytokines and is an important factor in the response of the body to attack by parasites, bacterial infection, and tumor growth. It is also the cause of septic shock and may play a role in many diseases with an autoimmune etiology.

NOS signaling is involved in development and in fertilization in vertebrates. It has been

implicated in transitions between vegetative and reproductive states in invertebrates, and in differentiation leading to spore formation in slime molds. NO produced by bacterial NOS is protective against oxidative damage.

Different members of the NOS family are encoded by separate genes. There are three known isoforms in mammals, two are constitutive (cNOS) and the third is inducible (iNOS). Cloning of NOS enzymes indicates that cNOS include both brain constitutive (NOS1) and endothelial constitutive (NOS3); the third is the inducible (NOS2) gene. Recently, NOS activity has been demonstrated in several bacterial species, including notorious pathogens *Bacillus anthracis* and *Staphylococcus aureus*.

As opposed to the critical calcium-dependent regulation of constitutive NOS enzymes (nNOS and eNOS), iNOS has been described as calcium-insensitive, likely due to its tight non-covalent interaction with calmodulin (CaM) and  $Ca^{2+}$ . The gene coding for iNOS is located on Chromosome 17. While evidence for 'baseline' iNOS expression has been elusive, IRF1 and NF- $\kappa$ B-dependent activation of the inducible NOS promoter supports an inflammation mediated stimulation of this transcript. iNOS produces large quantities of NO upon stimulation, such as by proinflammatory cytokines (e.g. Interleukin-1, Tumor necrosis factor alpha and Interferon gamma).

Induction of the high-output iNOS usually occurs in an oxidative environment, and thus high levels of NO have the opportunity to react with superoxide leading to peroxynitrite formation and cell toxicity. These properties may define the roles of iNOS in host immunity, enabling its participation in anti-microbial and anti-tumor activities as part of the oxidative burst of macrophages.

It has been suggested that pathologic generation of nitric oxide through increased iNOS production may decrease tubal ciliary beats and smooth muscle contractions and thus affect embryo transport, which may consequently result in ectopic pregnancy.

The enzymes exist as homodimers. In eukaryotes, each monomer consisting of two major regions: an N-terminal oxygenase domain, which belongs to the class of heme-thiolate proteins, and a multi-domain C-terminal reductase, which is homologous to NADPH:cytochrome P450 reductase (EC 1.6.2.4) and other flavoproteins. The FMN binding domain is homologous to flavodoxins, and the two domain fragment containing the FAD and NADPH binding sites is homologous to flavodoxin-NADPH reductases. The interdomain linker between the oxygenase and reductase domains contains a calmodulin-binding sequence. The oxygenase domain

is a unique extended beta sheet cage with binding sites for heme and pterin. NOSs can be dimeric, calmodulin-dependent or calmodulin-containing cytochrome p450-like hemoprotein that combines reductase and oxygenase catalytic domains in one dimer, bear both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and carry out a 5'-electron oxidation of non-aromatic amino acid arginine with the aid of tetrahydrobiopterin.

All three isoforms (each of which is presumed to function as a homodimer during activation) share a carboxyl-terminal reductase domain homologous to the cytochrome P450 reductase. They also share an amino-terminal oxygenase domain containing a heme prosthetic group, which is linked in the middle of the protein to a calmodulin-binding domain. Binding of calmodulin appears to act as a "molecular switch" to enable electron flow from flavin prosthetic groups in the reductase domain to heme. This facilitates the conversion of O<sub>2</sub> and L-arginine to NO and L-citrulline. The oxygenase domain of each NOS isoform also contains an BH<sub>4</sub> prosthetic group, which is required for the efficient generation of NO. Unlike other enzymes where BH<sub>4</sub> is used as a source of reducing equivalents and is recycled by dihydrobiopterin reductase (EC 1.5.1.33), BH<sub>4</sub> activates heme-bound O<sub>2</sub> by donating a single electron, which is then recaptured to enable nitric oxide release.

The first nitric oxide synthase to be identified was found in neuronal tissue (NOS1 or nNOS); the endothelial NOS (eNOS or NOS3) was the third to be identified. They were originally classified as "constitutively expressed" and "Ca<sup>2+</sup> sensitive" but it is now known that they are present in many different cell types and that expression is regulated under specific physiological conditions. In NOS1 and NOS3, physiological concentrations of Ca<sup>2+</sup> in cells regulate the binding of calmodulin to the "latch domains", thereby initiating electron transfer from the flavins to the heme moieties. In contrast, calmodulin remains tightly bound to the inducible and Ca<sup>2+</sup>-insensitive isoform (iNOS or NOS2) even at a low intracellular Ca<sup>2+</sup> activity, acting essentially as a subunit of this isoform.

Nitric oxide may itself regulate NOS expression and activity. Specifically, NO has been shown to play an important negative feedback regulatory role on NOS3, and therefore vascular endothelial cell function. This process, known formally as S-nitrosation, has been shown to reversibly inhibit NOS3 activity in vascular endothelial cells. This process may be important because it is regulated by cellular redox conditions and may thereby provide a mechanism for the association between "oxidative stress" and endothelial dysfunction. In addition to NOS3, both NOS1 and NOS2 have been found to be

S-nitrosated, but the evidence for dynamic regulation of those NOS isoforms by this process is less complete. In addition, both NOS1 and NOS2 have been shown to form ferrous-nitrosyl complexes in their heme prosthetic groups that may act partially to self-inactivate these enzymes under certain conditions. The rate-limiting step for the production of nitric oxide may well be the availability of L-arginine in some cell types. This may be particularly important after the induction of NOS2.

Obstructive uropathy is a condition in which the flow of urine is blocked. This causes the urine to back up and injure one or both kidneys. Obstructive uropathy occurs when urine cannot drain through a ureter. Urine backs up into the kidney and causes it to become hydronephrosis. It can occur suddenly, or be a long-term problem. If the blockage comes on suddenly, kidney damage is less likely if the problem is detected and treated promptly, and the damage to the kidneys goes away normally. Long-term damage to the kidneys may occur if the blockage has been present for a long time. If the problem is caused by a blockage in the bladder, the bladder may have long-term damage, which may lead to problems emptying the bladder or leakage of urine.

The following introduces recent reports as references in the related studies.

Akgul, T., E. Huri, et al. "Phosphodiesterase 5 inhibitors attenuate renal tubular apoptosis after partial unilateral ureteral obstruction: an experimental study." Kaohsiung J Med Sci. 2011 Jan;27(1):15-9. doi: [10.1016/j.kjms.2010.03.001](https://doi.org/10.1016/j.kjms.2010.03.001). Epub 2011 Jan 28.

The aim of the present study was to evaluate the effects of phosphodiesterase 5 inhibitors on renal tubular apoptosis and also on expressions of endothelial and inducible nitric oxide synthases (eNOS and iNOS) in the ipsilateral kidney after partial unilateral ureteral obstruction (PUUO) in a rat model. 40 Wistar albino rats were divided into 5 groups. In Groups 1-4, left experimental PUUO was created. Sildenafil, vardenafil, and tadalafil were administered to the rats of Groups 2-4, respectively. The pills were orally given to the rats for 30 days. Group 5 was defined as sham. After 30 days, all rats were sacrificed, and nephrectomy was performed. The renal specimens were examined histopathologically. Left hydronephrosis was observed in Groups 1-4. Mean apoptotic cell count and eNOS and iNOS levels were significantly increased in Group 1 when compared with the other groups. The rats in Groups 2-4 showed significantly decreased apoptotic cell count and eNOS and iNOS values in the renal tubular tissue in accordance with Group 1 ( $p < 0.05$ ). There were significant differences in apoptotic cell counts between

sildenafil group and the other two study groups. The sildenafil group demonstrated lesser apoptotic cell count than the vardenafil ( $p=0.021$ ) and tadalafil ( $p=0.009$ ) groups. PUUO increases the renal tubular apoptosis and elevates NOS concentrations in renal tubular tissue after PUUO. Phosphodiesterase 5 inhibitors have a protective effect against the tubular apoptosis.

Broadbelt, N. V., P. J. Stahl, et al. "Early upregulation of iNOS mRNA expression and increase in NO metabolites in pressurized renal epithelial cells." Am J Physiol Renal Physiol. 2007 Dec;293(6):F1877-88. Epub 2007 Sep 19.

Pressure is an important physiological regulator, but under abnormal conditions it may be a critical factor in the onset and progression of disease in many organs. In vivo, proximal tubular epithelial cells are subjected to pressure as a result of ureteral obstruction, which may influence the production of nitric oxide (NO), a ubiquitous multifunctional cytokine. To directly explore the effect of pressure on the expression and activity of NO synthase (NOS) in cultured proximal tubular epithelial cells, a novel pressure apparatus was developed. Cells were subjected to pressures of 20-120 mmHg over time (5 min-72 h). RT-PCR demonstrated an increase in inducible NOS (iNOS) and sGC, while endothelial NOS remained unchanged. Real-time PCR (qPCR) confirmed an earlier induction of iNOS transcript subjected to 60 mmHg compared with cytokine mix. iNOS protein expression was significantly increased following 60 mmHg of pressure for 24 h. Use of nuclear factor-kappaB inhibitors was shown to prevent the increase in iNOS expression following 60 mmHg for 2 h. NO and cGMP were increased with the application of pressure. The addition of the irreversible iNOS inhibitor (1400W) was shown to prevent this increase. We demonstrate that with the use of a simply designed apparatus, pressure led to an extremely early induction of iNOS and a rapid activation of NOS activity to increase NO and cGMP in proximal tubule epithelial cells. The rapid effects of pressure on iNOS may have important implications in the obstructed kidney.

Chade, A. R., M. Rodriguez-Porcel, et al. "Antioxidant intervention blunts renal injury in experimental renovascular disease." J Am Soc Nephrol. 2004 Apr;15(4):958-66.

Atherosclerotic renovascular disease (RVD) amplifies damage in a stenotic kidney by inducing pro-inflammatory mechanisms and disrupting tissue remodeling. Oxidative stress is increased in RVD, but its direct contribution to renal injury has not been fully established. The authors hypothesized that chronic

antioxidant intervention in RVD would improve renal function and attenuate tissue injury. Single-kidney hemodynamics and function at baseline and during vasoactive challenge were quantified using electron-beam computed tomography in pigs after 12 wk of experimental RVD (simulated by concurrent hypercholesterolemia and renal artery stenosis,  $n = 7$ ), RVD daily supplemented with antioxidant vitamins C (1 g), and E (100 IU/kg) (RVD+Vitamins,  $n = 7$ ), or controls (normal,  $n = 7$ ). Renal tissue was studied *ex vivo* using Western blot analysis and immunohistochemistry. Basal renal blood flow (RBF) and glomerular filtration rate (GFR) were similarly decreased in the stenotic kidney of both RVD groups. RBF and GFR response to acetylcholine was blunted in RVD, but significantly improved in RVD+Vitamins ( $P < 0.05$  versus RVD). RVD+Vitamins also showed increased renal expression of endothelial nitric oxide synthase (eNOS) and decreased expression of NAD(P)H-oxidase, nitrotyrosine, inducible-NOS, and NF-kappaB, suggesting decreased superoxide abundance and inflammation. Furthermore, decreased expression of pro-fibrotic factors in RVD+Vitamins was accompanied by augmented expression of extracellular (matrix metalloproteinase-2) and intracellular (ubiquitin) protein degradation systems, resulting in significantly attenuated glomerulosclerosis and renal fibrosis. In conclusion, chronic antioxidant intervention in early experimental RVD improved renal functional responses, enhanced tissue remodeling, and decreased structural injury. This study supports critical pathogenic contribution of increased oxidative stress to renal injury and scarring in RVD and suggests a role for antioxidant strategies in preserving the atherosclerotic and ischemic kidney.

Cherla, G. and E. A. Jaimes "Role of L-arginine in the pathogenesis and treatment of renal disease." J Nutr. 2004 Oct;134(10 Suppl):2801S-2806S; discussion 2818S-2819S.

L-arginine is a semi essential amino acid and also a substrate for the synthesis of nitric oxide (NO), polyamines, and agmatine. These L-arginine metabolites may participate in the pathogenesis of renal disease and constitute the rationale for manipulating L-arginine metabolism as a strategy to ameliorate kidney disease. Modification of dietary L-arginine intake in experimental models of kidney diseases has been shown to have both beneficial as well as deleterious effects depending on the specific model studied. L-arginine supplementation in animal models of glomerulonephritis has been shown to be detrimental, probably by increasing the production of NO from increased local expression of inducible NO synthase (iNOS). L-arginine supplementation does not modify the course of renal disease in humans with

chronic glomerular diseases. However, beneficial effects of L-arginine supplementation have been reported in several models of chronic kidney disease including renal ablation, ureteral obstruction, nephropathy secondary to diabetes, and salt-sensitive hypertension. L-arginine is reduced in preeclampsia and recent experimental studies indicate that L-arginine supplementation may be beneficial in attenuating the symptoms of preeclampsia. Administration of exogenous L-arginine has been shown to be protective in ischemic acute renal failure. In summary, the role of L-arginine in the pathogenesis and treatment of renal disease is not completely understood and remains to be established.

Chiang, C. W., H. T. Lee, et al. "Genetic deletion of soluble epoxide hydrolase attenuates inflammation and fibrosis in experimental obstructive nephropathy." Mediators Inflamm. 2015;2015:693260. doi: 10.1155/2015/693260. Epub 2015 Jan 22.

Soluble epoxide hydrolase (sEH) is abundantly expressed in kidney and plays a potent role in regulating inflammatory response in inflammatory diseases. However, the role of sEH in progression of chronic kidney diseases such as obstructive nephropathy is still elusive. In current study, wild-type (WT) and sEH deficient (sEH (-/-)) mice were subjected to the unilateral ureteral obstruction (UUO) surgery and the kidney injury was evaluated by histological examination, western blotting, and ELISA. The protein level of sEH in kidney was increased in UUO-treated mice group compared to nonobstructed group. Additionally, UUO-induced hydronephrosis, renal tubular injury, inflammation, and fibrosis were ameliorated in sEH (-/-) mice with the exception of glomerulosclerosis. Moreover, sEH (-/-) mice with UUO showed lower levels of inflammation-related and fibrosis-related protein such as monocyte chemoattractant protein-1, macrophage inflammatory protein-2, interleukin-1beta (IL-1beta), IL-6, inducible nitric oxide synthase, collagen 1A1, and alpha-actin. The levels of superoxide anion radical and hydrogen peroxide as well as NADPH oxidase activity were also decreased in UUO kidneys of sEH (-/-) mice compared to that observed in WT mice. Collectively, our findings suggest that sEH plays an important role in the pathogenesis of experimental obstructive nephropathy and may be a therapeutic target for the treatment of obstructive nephropathy-related diseases.

Chow, B. S., M. Kocan, et al. "Relaxin requires the angiotensin II type 2 receptor to abrogate renal interstitial fibrosis." Kidney Int. 2014 Jul;86(1):75-85. doi: 10.1038/ki.2013.518. Epub 2014 Jan 15.



Fibrosis is a hallmark of chronic kidney disease, for which there is currently no effective cure. The hormone relaxin is emerging as an effective antifibrotic therapy; however, its mechanism of action is poorly understood. Recent studies have shown that relaxin disrupts the profibrotic actions of transforming growth factor-beta1 (TGF-beta1) by its cognate receptor, relaxin family peptide receptor 1 (RXFP1), extracellular signal-regulated kinase phosphorylation, and a neuronal nitric oxide synthase-dependent pathway to abrogate Smad2 phosphorylation. Since angiotensin II also inhibits TGF-beta1 activity through its AT2 receptor (AT2R), we investigated the extent to which relaxin interacts with the AT2R. The effects of the AT2R antagonist, PD123319, on relaxin activity were examined in primary rat kidney myofibroblasts, and in kidney tissue from relaxin-treated male wild-type and AT2R-knockout mice subjected to unilateral ureteric obstruction. Relaxin's antifibrotic actions were significantly blocked by PD123319 in vitro and in vivo, or when relaxin was administered to AT2R-knockout mice. While heterodimer complexes were formed between RXFP1 and AT2Rs independent of ligand binding, relaxin did not directly bind to AT2Rs but signaled through RXFP1-AT2R heterodimers to induce its antifibrotic actions. These findings highlight a hitherto unrecognized interaction that may be targeted to control fibrosis progression.

Dikmen, B., H. Yagmurdur, et al. "Preventive effects of propofol and ketamine on renal injury in unilateral ureteral obstruction." *J Anesth.* 2010 Feb;24(1):73-80. doi: 10.1007/s00540-009-0861-1.

**PURPOSE:** The aim of the present study was to investigate the preventive effects of propofol and ketamine as anesthetics on renal injury in unilateral ureteral obstruction (UO). **METHODS:** Twenty-four male New Zealand white rabbits were randomly assigned to four groups of six rabbits each. Anesthesia was induced and maintained with propofol in groups 1 and 2 and with ketamine in groups 3 and 4. Groups 2 and 4 received complete left ureteral ligation. Groups 1 and 3 (control groups) underwent an identical surgical procedure without ureteral ligation. At 14 days of obstruction, animals were sacrificed and ipsilateral kidneys were removed for determination of tissue nitric oxide (NO) levels and immunohistochemical evaluation of endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), and apoptosis protease-activating factor 1 (APAF-1). **RESULTS:** Between groups 1 and 3, there were no differences in tissue NO levels and eNOS, iNOS, and APAF-1 expressions. iNOS and APAF-1 expressions were at the mild to moderate levels in group 2, but these parameters were markedly increased in group 4 at 14 days of obstruction. Also, elevated expression of

iNOS was accompanied by a high NO production in group 4 compared with group 2. Although eNOS expressions were increased in both groups 2 and 4, there were no significant differences between these groups. **CONCLUSIONS:** Propofol as an anesthetic agent may attenuate NO-induced renal tubular cell apoptosis by downregulating the expression of iNOS in an animal model of unilateral UO.

Ebrahimi, B., Z. Li, et al. "Addition of endothelial progenitor cells to renal revascularization restores medullary tubular oxygen consumption in swine renal artery stenosis." *Am J Physiol Renal Physiol.* 2012 Jun 1;302(11):F1478-85. doi: 10.1152/ajprenal.00563.2011. Epub 2012 Mar 14.

Renal artery stenosis (RAS) promotes microvascular rarefaction and fibrogenesis, which may eventuate in irreversible kidney injury. We have shown that percutaneous transluminal renal angioplasty (PTRA) or endothelial progenitor cells (EPC) improve renal cortical hemodynamics and function in the poststenotic kidney. The renal medulla is particularly sensitive to hypoxia, yet little is known about reversibility of medullary injury on restoration of renal blood flow. This study was designed to test the hypothesis that PTRA, with or without adjunct EPC delivery to the stenotic kidney, may improve medullary remodeling and tubular function. RAS was induced in 21 pigs using implantation of irritant coils, while another group served as normal controls (n = 7 each). Two RAS groups were then treated 6 wk later with PTRA or both PTRA and EPC. Four weeks later, medullary hemodynamics, microvascular architecture, and oxygen-dependent tubular function of the stenotic kidneys were examined using multidetector computed tomography, microcomputed tomography, and blood oxygenation level-dependent MRI, respectively. Medullary protein expression of vascular endothelial growth factor, endothelial nitric oxide synthase, hypoxia-inducible factor-1alpha, and NAD(P)H oxidase p47 were determined. All RAS groups showed decreased medullary vascular density and blood flow. However, in RAS+PTRA+EPC animals, EPC were engrafted in tubular structures, oxygen-dependent tubular function was normalized, and fibrosis attenuated, despite elevated expression of hypoxia-inducible factor-1alpha and sustained downregulation of vascular endothelial growth factor. In conclusion, EPC delivery, in addition to PTRA, restores medullary oxygen-dependent tubular function, despite impaired medullary blood and oxygen supply. These results support further development of cell-based therapy as an adjunct to revascularization of RAS.

Eskild-Jensen, A., K. Thomsen, et al. "Glomerular and tubular function during AT1 receptor blockade in pigs

with neonatal induced partial ureteropelvic obstruction." *Am J Physiol Renal Physiol.* 2007 Mar;292(3):F921-9. Epub 2006 Nov 28.

Previously, we showed that neonatal induced chronic partial unilateral ureteral obstruction (PUUO) of the multipapillary pig kidney decreased glomerular filtration rate (GFR) of the obstructed kidney. We hypothesized that ANG II and nitric oxide (NO) are important for the changes in renal function and in the present study we examined the effects of chronic AT1 receptor blockade using CV-11974 (0.12 mg/h candesartan from age 23 to 30 days) on kidney function development after PUUO was induced in 2-day-old piglets. Moreover, the effect of superimposed acute NO inhibition using N(G)-nitro-L-arginine methyl ester (L-NAME; 15 mg/kg) was examined to identify if this has diagnostic potential. PUUO significantly increased GFR in the nonobstructed contralateral kidney independent of candesartan. In candesartan-treated piglets, the L-NAME-induced GFR reduction seen in normal and nonobstructed kidneys was absent in the partial obstructed kidneys. Urine output and fractional excretion of water were increased from the partial obstructed kidneys. Consistent with this immunohistochemical analyses showed a reduced aquaporin-2 labeling in the collecting duct principal cells. Moreover, renal sodium handling was compromised by PUUO evidenced by an increased fractional excretion of sodium which was enhanced by candesartan treatment. In conclusion, our findings suggest that the counterbalance between AT1 receptor-mediated vasoconstriction and NO-mediated vasodilatation which maintain GFR in normal young porcine kidneys is changed by neonatal induced chronic PUUO. This may have diagnostic potential in children with suspected congenital obstruction. Our results also demonstrate compromised tubular functions in response to chronic PUUO despite preservation of glomerular function.

Felsen, D., D. Schulsinger, et al. "Renal hemodynamic and ureteral pressure changes in response to ureteral obstruction: the role of nitric oxide." *J Urol.* 2003 Jan;169(1):373-6.

**PURPOSE:** Triphasic changes in renal blood flow and ureteral pressure after unilateral ureteral obstruction have long been known. The contribution of nitric oxide to the decline in renal blood flow and ureteral pressure in unilateral ureteral obstruction was studied in this model using arginine infusion and by studying the effect of 2 inhibitors of nitric oxide synthase (NOS). **MATERIALS AND METHODS:** Left ureteral obstruction was created in dogs. Renal blood flow and ureteral pressure were monitored. Groups 1 to 4 underwent unilateral ureteral obstruction and group 5 dogs underwent sham operation. Groups 2

to 5 received an infusion of arginine at hour 18 of obstruction that was sustained for 1 hour. In addition, NOS inhibitors were administered to dogs in groups 3 (N-monomethyl-L-arginine) and 4 (triamcinolone diacetate). **RESULTS:** Arginine administration at 18 hours of obstruction caused a significant increase in renal blood flow and ureteral pressure compared to sham operated animals. Triamcinolone diacetate eliminated the increase in renal blood flow and ureteral pressure, whereas N-monomethyl-L-arginine did not, reflecting the competitive nature of its inhibition of NOS. **CONCLUSIONS:** Arginine infusion 18 hours after unilateral ureteral obstruction led to increases in renal blood flow and ureteral pressure that were not seen in control animals. These results suggest that the nitric oxide system of the kidney is activated in unilateral ureteral obstruction. Since the addition of arginine is accompanied by an increase in renal blood flow and ureteral pressure, it further suggests that a lack of availability of substrate for NOS may explain the decrease in renal blood flow and ureteral pressure in obstruction. Providing substrate may be a way of maintaining renal blood flow in unilateral ureteral obstruction.

Fitzgerald, J., S. Y. Chou, et al. "Regional expression of inducible nitric oxide synthase in the kidney in dogs with unilateral ureteral obstruction." *J Urol.* 2001 Oct;166(4):1524-9.

**PURPOSE:** In the early stage of unilateral ureteral obstruction total renal blood flow increases but medullary blood flow decreases, exacerbating medullary tissue hypoxia. We examined the expression of inducible nitric oxide synthase, a product of a hypoxia sensitive gene, in the cortex and medulla in dogs with unilateral ureteral obstruction for 21 hours. **MATERIALS AND METHODS:** Hemodynamic and clearance experiments were performed after release of ureteral obstruction in 6 dogs with unilateral ureteral obstruction, followed by Western blot analysis of nitric oxide synthase and immunohistochemistry. **RESULTS:** Ureteral obstruction raised mean ureteral pressure plus or minus standard error to 35.0 +/- 7.2 mm. Hg. In dogs with unilateral ureteral obstruction mean renal blood flow was 116 +/- 10 ml. per minute, lower than the 213 +/- 22 ml. per minute in sham operated dogs (p <0.01). After unilateral ureteral obstruction release the mean glomerular filtration rate was 9.5 +/- 2.1 ml. per minute, lower than the 27.3 +/- 1.8 ml. per minute in the contralateral unobstructed kidney (p <0.01). Western blot analysis showed that mean nitric oxide synthase/beta-actin in the cortex of the obstructed kidney was 0.04 +/- 0.01 densitometry units, lower than 0.11 +/- 0.02 densitometry units in the unobstructed contralateral kidney (p <0.05). In contrast, mean nitric oxide synthase/beta-actin in the

medulla of the obstructed kidney was 1.29 +/- 0.33 densitometry units, greater than the 0.34 +/- 0.03 densitometry units in the unobstructed kidney ( $p < 0.05$ ). Immunohistochemistry revealed that the increased expression of nitric oxide synthase protein was localized to the endothelium of the vasa recta. CONCLUSIONS: Unilateral ureteral obstruction enhances nitric oxide synthase expression in the medulla but not in the cortex. This increased expression in the medulla may be the result of increased medullary hypoxia in unilateral ureteral obstruction, possibly contributing to medullary hyperemia after unilateral ureteral obstruction release.

Glynne, P. A., J. Picot, et al. "Coexpressed nitric oxide synthase and apical beta(1) integrins influence tubule cell adhesion after cytokine-induced injury." *J Am Soc Nephrol*. 2001 Nov;12(11):2370-83.

In sepsis-induced acute renal failure, actin cytoskeletal alterations result in shedding of proximal tubule epithelial cells (PTEC) and tubular obstruction. This study examined the hypothesis that inflammatory cytokines, released early in sepsis, cause PTEC cytoskeletal damage and alter integrin-dependent cell-matrix adhesion. The question of whether the intermediate nitric oxide (NO) modulates these cytokine effects was also examined. After exposure of human PTEC to tumor necrosis factor-alpha, interleukin-1 alpha, and interferon-gamma, the actin cytoskeleton was disrupted and cells became elongated, with extension of long filopodial processes. Cytokines induced shedding of viable, apoptotic, and necrotic PTEC, which was dependent on NO synthesized by inducible NO synthase (iNOS) produced as a result of cytokine actions on PTEC. Basolateral exposure of polarized PTEC monolayers to cytokines induced maximal NO-dependent cell shedding, mediated in part through NO effects on cGMP. Cell shedding was accompanied by dispersal of basolateral beta(1) integrins and E-cadherin, with corresponding upregulation of integrin expression in clusters of cells elevated above the epithelial monolayer. These cells demonstrated coexpression of iNOS and apically redistributed beta(1) integrins. Attachment studies demonstrated that the major ligand involved in cell anchorage was laminin, probably through interactions with the integrin alpha(3)beta(1). This interaction was downregulated by cytokines but was not dependent on NO. These studies provide a mechanism by which inflammatory cytokines induce PTEC damage in sepsis, in the absence of hypotension and ischemia. Future therapeutic strategies aimed at specific iNOS inhibition might inhibit PTEC shedding after cytokine-induced injury and delay the onset of acute renal failure in sepsis.

Grau, V., O. Stehling, et al. "Accumulating monocytes in the vasculature of rat renal allografts: phenotype, cytokine, inducible NO synthase, and tissue factor mRNA expression." *Transplantation*. 2001 Jan 15;71(1):37-46.

Necrotic patches and hemorrhagic lesions develop in the renal tissue between day 4 and day 5 after transplantation of fully allogeneic DA rat kidneys to LEW recipients. These lesions are at least in part due to destruction and obstruction of blood vessels. Damage of graft endothelial cells and blood coagulation are likely to be mediated by intravascular graft leukocytes. However, this cell population has not been thoroughly characterized before. We perfused untreated control kidneys, renal isografts, and allografts on day 4 after transplantation with phosphate-buffered saline/ethylenediaminetetraacetic acid to harvest leukocytes from both the blood stream as well as from the marginal intravascular pool. The mRNA expression of typical products of activated monocytes was analyzed in reverse-transcriptase polymerase chain reaction experiments. Graft monocytes were purified and their immunophenotype was investigated by flow cytometry. RESULTS: Allograft rejection led to a 10-fold increase in the number of intravascular graft leukocytes compared to isografts. A mean number of about  $100 \times 10^6$  leukocytes was harvested from a single allogeneic kidney, about 73% of these cells were monocytes and most of them displayed an activated phenotype. Compared to isografts, intravascular allograft leukocytes displayed an increased expression of tumor necrosis factor-alpha, inducible NO synthase and tissue factor. Our study shows that large numbers of activated monocytes accumulate inside allograft vessels. As they express genes the products of which might damage the allograft by inducing cell death or thrombosis, we speculate that they directly participate in allograft destruction.

Gueler, F., S. Rong, et al. "Postischemic acute renal failure is reduced by short-term statin treatment in a rat model." *J Am Soc Nephrol*. 2002 Sep;13(9):2288-98.

Postischemic acute renal failure (ARF) is common and often fatal. Cellular mechanisms include cell adhesion, cell infiltration and generation of oxygen free radicals, and inflammatory cytokine production. Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors ("statins") directly influence inflammatory mechanisms. The hypothesis that ischemia-induced ARF could be ameliorated with statin treatment was investigated and possible molecular mechanisms were analyzed in a uninephrectomized rat model. Male Sprague-Dawley rats were pretreated with cerivastatin (0.5 mg/kg) or vehicle for 3 d. Ischemic ARF was induced by left

renal artery clipping for 45 min, while the right kidney was being removed. After 24 h of ARF, serum creatinine levels were increased 7.5-fold in vehicle-treated control animals with ARF, compared with sham-operated animals ( $P < 0.005$ ). Statin treatment reduced the creatinine level elevation by 40% ( $P < 0.005$ ). Simultaneously, ischemia-induced severe decreases in GFR were significantly ameliorated by statin treatment (sham operation,  $0.95 \pm 0.09$  ml/min,  $n = 13$ ; ischemia without treatment,  $0.06 \pm 0.02$  ml/min,  $n = 9$ ; ischemia with statin pretreatment,  $0.21 \pm 0.03$  ml/min,  $n = 11$ ;  $P < 0.001$ ). Furthermore, statin pretreatment prevented the occurrence of tubular necrosis, with marked loss of the brush border, tubular epithelial cell detachment, and tubular obstruction in the S3 segment of the outer medullary stripe. In addition, monocyte and macrophage infiltration was almost completely prevented, intercellular adhesion molecule-1 upregulation was greatly decreased, and inducible nitric oxide synthase expression was reduced. Fibronectin and collagen IV expression was reduced, approaching levels observed in sham-operated animals. In vehicle-treated rats with ARF, mitogen-activated protein kinase extracellular activated kinase-1/2 activity was increased and the transcription factors nuclear factor-kappaB and activator protein-1 were activated. Statin treatment reduced this activation toward levels observed in sham-operated rats. The data suggest that hydroxy-3-methylglutaryl coenzyme A reductase inhibition protects renal tissue from the effects of ischemia-reperfusion injury and thus reduces the severity of ARF. The chain of events may involve anti-inflammatory effects, with inhibition of mitogen-activated protein kinase activation and the redox-sensitive transcription factors nuclear factor-kappaB and activator protein-1.

Hanatani, S., Y. Izumiya, et al. "Akt1-mediated fast/glycolytic skeletal muscle growth attenuates renal damage in experimental kidney disease." *J Am Soc Nephrol.* 2014 Dec;25(12):2800-11. doi: 10.1681/ASN.2013091025. Epub 2014 Jul 10.

Muscle wasting is frequently observed in patients with kidney disease, and low muscle strength is associated with poor outcomes in these patients. However, little is known about the effects of skeletal muscle growth per se on kidney diseases. In this study, we utilized a skeletal muscle-specific, inducible Akt1 transgenic (Akt1 TG) mouse model that promotes the growth of functional skeletal muscle independent of exercise to investigate the effects of muscle growth on kidney diseases. Seven days after Akt1 activation in skeletal muscle, renal injury was induced by unilateral ureteral obstruction (UUO) in Akt1 TG and wild-type (WT) control mice. The expression of atrogen-1, an

atrophy-inducing gene in skeletal muscle, was upregulated 7 days after UUO in WT mice but not in Akt1 TG mice. UUO-induced renal interstitial fibrosis, tubular injury, apoptosis, and increased expression of inflammatory, fibrosis-related, and adhesion molecule genes were significantly diminished in Akt1 TG mice compared with WT mice. An increase in the activating phosphorylation of eNOS in the kidney accompanied the attenuation of renal damage by myogenic Akt1 activation. Treatment with the NOS inhibitor L-NAME abolished the protective effect of skeletal muscle Akt activation on obstructive kidney disease. In conclusion, Akt1-mediated muscle growth reduces renal damage in a model of obstructive kidney disease. This improvement appears to be mediated by an increase in eNOS signaling in the kidney. Our data support the concept that loss of muscle mass during kidney disease can contribute to renal failure, and maintaining muscle mass may improve clinical outcome.

Hegarty, N. J., L. S. Young, et al. "Nitric oxide in unilateral ureteral obstruction: effect on regional renal blood flow." *Kidney Int.* 2001 Mar;59(3):1059-65.

Ureteral obstruction (UO) is characterized by reduced blood flow and loss of tissue mass in the involved kidney(s). Vasoactive mediators interact to produce an initial hyperemia, followed by a sustained decrease in renal blood flow in the obstructed kidney. Nitric oxide (NO) has been shown to play a central role in the acute hyperemic response to UO. Its role in the reduced perfusion of prolonged UO is less studied. Ureteral obstruction was achieved by ligation of the distal left ureter and maintained for 24 hours. Blood flow was studied in untreated animals and after the administration of the NO synthase (NOS) inhibitor N-mono-methyl L-arginine and the NO donor sodium nitroprusside. Tissue was collected for localization and quantitation of NOS. Serum and renal tissue L-arginine levels were measured in control and UO settings. RESULTS: Blood flow in the obstructed kidney diminished to approximately 50% of control values after 24 hours of UO. NOS blockade led to a further decrease in blood flow. Supplementation with exogenous nitrates restored renal blood flow to levels approaching control values. Serum and tissue L-arginine levels did not change with UO. NOS expression was seen to increase with increasing duration of obstruction, with staining most pronounced in the renal tubules. NO plays a vasodilatory role even in the hypoperfusion of prolonged UO. The administration of exogenous nitrates has a restorative effect on blood flow, suggesting therapeutic potential in UO.



Hewitson, T. D., M. G. Tait, et al. "Dipyridamole inhibits in vitro renal fibroblast proliferation and collagen synthesis." J Lab Clin Med. 2002 Sep;140(3):199-208.

Fibroblasts are universally recognized in situations of tubulointerstitial injury, where their presence has been shown to be a marker of disease progression. The objective of this study was to determine whether the functions of fibroblasts relevant to fibrogenesis can be modified in vitro with dipyridamole. Cells were obtained from obstructed rat renal tissue and characterized on the basis of immunohistochemical findings. Fibroblasts constituted all of the cells from passage 3. Functional parameters were measured in cells cultured with 1, 5, and 50 micromol/L dipyridamole and compared to basal parameters of cells grown in Dulbecco's modified Eagle's medium plus 10% fetal calf serum (control). Northern-blot analysis indicated that dipyridamole decreased procollagen alpha1(I) messenger ribonucleic acid expression ( $P < .05$ , 50 micromol/L vs control), results that were reflected in a reduction in total collagen secretion as measured on the basis of hydroxyproline incorporation ( $P < .001$ , 50 micromol/L vs control). Mitogenesis, as measured on the basis of incorporation of tritiated thymidine, was decreased in a dose-dependent fashion by dipyridamole. Likewise, 50 micromol/L dipyridamole reduced cell-population growth to 16.8% +/- 2.1% of basal growth over 3 days ( $P < .001$  vs control). Effects of dipyridamole on population growth were prevented by coincubation with a protein kinase G inhibitor peptide ( $P < .001$  vs 50 micromol/L dipyridamole;  $P =$  not significant vs control). No such effect was observed with inhibitors for protein kinase A (H-89) and protein kinase C (bisindolylmaleimide I). Consistent with a protein kinase G-dependent mechanism, immunofluorescence staining indicated that dipyridamole increased basal expression of the inducible form of nitric oxide synthase. In conclusion, the results of this study demonstrate that at clinically relevant concentrations, dipyridamole inhibits profibrotic activities of renal fibroblasts. Effects on mitogenesis are mediated through a cyclic guanosine monophosphate-protein kinase G effector pathway.

Hochberg, D., C. W. Johnson, et al. "Interstitial fibrosis of unilateral ureteral obstruction is exacerbated in kidneys of mice lacking the gene for inducible nitric oxide synthase." Lab Invest. 2000 Nov;80(11):1721-8.

Unilateral ureteral obstruction (UVO) is characterized by decreases in renal function and increases in interstitial fibrosis. Previous studies have indicated that pharmacologic manipulations that increase nitric oxide (NO) are beneficial to the

obstructed kidneys. NO is produced from arginine by nitric oxide synthase (NOS), an enzyme that exists in both constitutive and inducible (iNOS) forms. To determine the role of the inducible form of NOS in UVO, we used mice with a targeted deletion of iNOS (iNOS -/- mice) and compared them with wild-type (WT) mice. Kidneys were obstructed for 2 weeks in both WT and iNOS -/- mice, and were then removed and bisected. Half of the kidney was embedded in paraffin and tissue sections were examined for interstitial volume or the presence of macrophages. The remainder was flash-frozen and samples were used to measure tissue collagen (hydroxyproline) or transforming growth factor-beta (TGF-beta). This study demonstrates that both cortex and medulla of obstructed kidneys of iNOS -/- mice exhibit significantly increased interstitial volume and interstitial macrophages as compared with their WT counterparts. Furthermore tissue collagen was increased to 9.2 +/- 1.3 microg/mg tissue in WT obstructed kidneys, whereas in iNOS -/- kidneys, collagen was increased to 13.2 +/- 0.8 microg/mg tissue. The profibrotic cytokine TGF-beta was also significantly increased in obstructed kidneys of iNOS -/- mice, as compared with WT mice. No differences were noted between the unobstructed kidneys of iNOS -/- mice compared with WT mice in any of the parameters examined. These results demonstrate that targeted deletion of the iNOS results in exacerbation of fibrotic events in the obstructed kidney. These results confirm previous pharmacologic studies, and suggest that NO produced via the inducible NOS normally serves a protective function in UVO.

Ito, K., H. Yoshii, et al. "Adrenomedullin increases renal nitric oxide production and ameliorates renal injury in mice with unilateral ureteral obstruction." J Urol. 2010 Apr;183(4):1630-5. doi: 10.1016/j.juro.2009.12.002. Epub 2010 Feb 20.

PURPOSE: We evaluated the effects of adrenomedullin (Peptide Institute, Minoh-shi, Osaka, Japan) on mediators, including nitric oxide and transforming growth factor-beta, and parameters of renal injury in a murine unilateral ureteral obstruction model. MATERIALS AND METHODS: Three study groups of control, adrenomedullin treated and adrenomedullin plus L-NAME treated BALB/C mice, respectively, underwent left unilateral ureteral obstruction. A 24-hour urine sample was collected to measure urinary NO(2)/NO(3) 1 day before unilateral ureteral obstruction and kidneys were harvested on postoperative day 14. Tubulointerstitial damage markers were evaluated by immunohistochemistry. Tissue transforming growth factor-beta was determined by enzyme-linked immunosorbent assay. Endothelial and inducible nitric oxide synthase

immunolocalization was also determined. RESULTS: Urinary NO(2)/NO(3) was significantly higher in the adrenomedullin group than in controls, confirming increased renal nitric oxide production. Immunohistochemistry showed increased endothelial nitric oxide synthase in vascular endothelial cells in the adrenomedullin group but tissue transforming growth factor-beta did not significantly differ in controls vs the adrenomedullin group. Interstitial collagen deposition and fibroblasts in the obstructed kidney were significantly decreased in the adrenomedullin group. The number of leukocytes and apoptotic cells in the obstructed kidney were significantly decreased by adrenomedullin. Renal injury amelioration resulting from adrenomedullin was blunted by the nitric oxide synthase inhibitor L-NAME. CONCLUSIONS: Adrenomedullin increased renal nitric oxide, and suppressed tubular apoptosis, interstitial fibrosis and inflammatory cell infiltration in mice with unilateral ureteral obstruction. The renoprotective peptide adrenomedullin may be useful for that condition.

Kim, J. and B. J. Padanilam "Loss of poly(ADP-ribose) polymerase 1 attenuates renal fibrosis and inflammation during unilateral ureteral obstruction." Am J Physiol Renal Physiol. 2011 Aug;301(2):F450-9. doi: 10.1152/ajprenal.00059.2011. Epub 2011 May 25.

Poly(ADP-ribose) polymerase 1 (PARP1) contributes to necrotic cell death and inflammation in several disease models; however, the role of PARP1 in fibrogenesis remains to be defined. Here, we tested whether PARP1 was involved in the pathogenesis of renal fibrosis using the unilateral ureteral obstruction (UUO) mouse model. UUO was performed by ligation of the left ureter near the renal pelvis in Parp1-knockout (KO) and wild-type (WT) male mice. After 10 days of UUO, renal PARP1 expression and activation were strongly increased by 6- and 13-fold, respectively. Interstitial fibrosis induced by UUO was significantly attenuated in Parp1-KO kidneys compared with that in WT kidneys at 10 days, but not at 3 days, based on collagen deposition, alpha-smooth muscle actin (alpha-SMA), and fibronectin expression. Intriguingly, the UUO kidneys in Parp1-KO mice showed a dramatic decrease in infiltration of neutrophil and reduction in expression of proinflammatory proteins including intercellular adhesion molecule-1, tumor necrosis factor-alpha, inducible nitric oxide synthase, and toll-like receptor 4 as well as phosphorylation of nuclear factor-kappaB p65, but not transforming growth factor-beta1 (TGF-beta1) at both 3 and 10 days. Pharmacological inhibition of PARP1 in rat renal interstitial fibroblast (NRK-49F) cell line or genetic ablation in primary mouse embryonic fibroblast cells did not affect TGF-

beta1-induced de novo alpha-SMA expression. Parp1 deficiency significantly attenuated UUO-induced histological damage in the kidney tubular cells, but not apoptosis. These data suggest that PARP1 induces necrotic cell death and contributes to inflammatory signaling pathways that trigger fibrogenesis in obstructive nephropathy.

Kipari, T., J. F. Cailhier, et al. "Nitric oxide is an important mediator of renal tubular epithelial cell death in vitro and in murine experimental hydronephrosis." Am J Pathol. 2006 Aug;169(2):388-99.

Macrophages play a pivotal role in tissue injury and fibrosis during renal inflammation. Although macrophages may induce apoptosis of renal tubular epithelial cells, the mechanisms involved are unclear. We used a microscopically quantifiable coculture assay to dissect the cytotoxic interaction between murine bone marrow-derived macrophages and Madin-Darby canine kidney cells and primary murine renal tubular epithelial cells. The induction of tubular cell apoptosis by cytokine-activated macrophages was reduced by inhibitors of nitric oxide synthase whereas tubular cell proliferation was unaffected. Furthermore, cytokine-activated macrophages derived from mice targeted for the deletion of inducible nitric oxide synthase were noncytotoxic. We then examined the role of nitric oxide in vivo by inhibiting inducible nitric oxide synthase in the model of murine experimental hydronephrosis. l-N(6)-(1-iminoethyl)-lysine was administered in the drinking water between days 5 and 7 after ureteric obstruction. Macrophage infiltration was comparable between groups, but treatment significantly inhibited tubular cell apoptosis at day 7. Tubular cell proliferation was unaffected. Inducible nitric oxide synthase blockade also reduced interstitial cell apoptosis and increased collagen III deposition. These data indicate that nitric oxide is a key mediator of macrophage-directed tubular cell apoptosis in vitro and in vivo and also modulates tubulointerstitial fibrosis.

Knerr, I., K. Dittrich, et al. "Alteration of neuronal and endothelial nitric oxide synthase and neuropeptide Y in congenital ureteropelvic junction obstruction." Urol Res. 2001 Apr;29(2):134-40.

PROBLEM: We investigated whether deranged nitric oxide synthase (NOS) and neuropeptide Y (NPY) expression is detectable in the stenotic segments of patients with congenital ureteropelvic junction obstruction. METHODS: Using real-time reverse transcription-polymerase chain reaction (RT-PCR), we quantified mRNA amounts of NPY, neuronal (n), endothelial (e) and inducible (i)

NOS in the stenotic segments of 20 patients with congenital ureteropelvic junction obstruction (aged 5.1+/-7.0 years) and of 21 unaffected controls (aged 23.5+/-24.2 years). Additionally, mRNAs of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), smooth muscle alpha-actin (Smactin), endothelial cell marker (CD31), and protein gene product 9.5 (PGP 9.5) were evaluated. Immunohistochemistry was made for NPY, nNOS, eNOS, iNOS, PGP 9.5, and CD 31. RESULTS: The mRNA of nNOS was significantly reduced in the obstructed junctions when related to the mRNAs of Smactin (P < 0.001) or GAPDH (P < 0.05), respectively. A significant reduction was also obtained for eNOS mRNA when standardized to CD31 (P < 0.05), GAPDH or Smactin mRNA (P < 0.05, and P < 0.001, respectively). NPY, PGP 9.5 and iNOS mRNAs were found in comparable quantities in both groups. In the stenotic segments, Smactin mRNA level was about twofold higher than in our control specimens, as shown by the lower CT values for the patients in real-time PCR (16.9+/-2.0 vs 17.9+/-2.6, P < 0.05). Furthermore, Smactin, nNOS, iNOS, eNOS, and NPY mRNA levels in specimens of unaffected ureteropelvic junctions were independent of age. Major differences between control and stenotic tissues were detected by immunohistochemistry: There was a dramatic reduction of innervation density as evidenced by nNOS and NPY labeling. CONCLUSION: Taken together, we found alterations in NOS gene expression and NPY innervation in tissue specimens of patients with congenital ureteropelvic junction obstruction.

Lo, T. H., K. Y. Tseng, et al. "TREM-1 regulates macrophage polarization in ureteral obstruction." Kidney Int. 2014 Dec;86(6):1174-86. doi: 10.1038/ki.2014.205. Epub 2014 Jun 11.

Chronic kidney disease (CKD) is an emerging worldwide public health problem. Inflammatory cell infiltration and activation during the early stages in injured kidneys is a common pathologic feature of CKD. Here, we determined whether an important inflammatory regulator, triggering receptor expressed on myeloid cells (TREM)-1, is upregulated in renal tissues collected from mouse ureteral obstruction-induced nephritis. TREM-1 is crucial for modulating macrophage polarization, and has a pivotal role in mediating tubular injury and interstitial collagen deposition in obstructive nephritis. Lysates from nephritic kidneys triggered a TREM-1-dependent M1 polarization *ex vivo*, consistent with the observation that granulocyte-macrophage colony-stimulating factor (GM-CSF)-derived M1 macrophages express higher levels of TREM-1 in comparison with M-CSF-derived cells. Moreover, agonistic TREM-1 cross-link significantly strengthens

the inductions of iNOS and GM-CSF in M1 cells. These observations are validated by a strong clinical correlation between infiltrating TREM-1-expressing/iNOS-positive macrophages and renal injury in human obstructive nephropathy. Thus, TREM-1 may be a potential diagnostic and therapeutic target in human kidney disease.

Manucha, W. and P. G. Valles "Cytoprotective role of nitric oxide associated with Hsp70 expression in neonatal obstructive nephropathy." Nitric Oxide. 2008 May;18(3):204-15. doi: 10.1016/j.niox.2008.01.005. Epub 2008 Feb 1.

Nitric oxide (NO) has emerged as an important endogenous inhibitor of apoptosis. In this study, we postulated that the mechanism of apoptosis inhibition by NO would include stimulation of heat shock protein 70 (Hsp70) expression. Rats were subjected to unilateral ureteral obstruction (UUO) or sham operation, and kidneys were harvested 5 and 14 days after obstruction. After 14 days of obstruction, decreased endogenous NO and lower inducible nitric oxide synthase (iNOS) expression at mRNA and protein levels associated with downregulation of Hsp70 protein expression were shown in apoptosis induction, regulated by mitochondrial signal pathway, through the increased pro-apoptotic ratio Bax/BcL(2) and consequently caspase 3 activity. Conversely, 5 days after kidney obstruction, increased Hsp70 expression linked to increase NO and iNOS expression at transcriptional and post-transcriptional levels with absence of apoptotic response, were demonstrated. In obstructed neonatal rats, *in vivo* administration of L-Arginine induced heat shock protein 70 (Hsp70) expression, which was associated with cytoprotection from apoptosis and transiently decreased nicotinamide adenine dinucleotide phosphate reduced form (NADPH) oxidase activity. Opposite effects were obtained after nitro L-Arginine methyl ester (L-NAME) treatment. The interaction between B-cell lymphoma 2 anti-apoptotic members (BcL(2)) and Hsp70 in the presence of L-Arginine and L-NAME, was determined by coimmunoprecipitation. Binding of BcL(2) and Hsp70 increased after L-Arginine administration. These findings suggest that NO can produce resistance to obstruction-induced cell death by mitochondrial apoptotic pathway, through the induction of Hsp70 expression, in neonatal unilateral ureteral obstruction.

Mazzei, L., I. M. Garcia, et al. "WT-1 mRNA expression is modulated by nitric oxide availability and Hsp70 interaction after neonatal unilateral ureteral obstruction." Biocell. 2010 Dec;34(3):121-32.

Wilms tumor gene 1 (wt-1), a key regulator of mesenchymal-epithelial transformation, is

downregulated during congenital obstructive nephropathy, leading to apoptosis. There is a functional interaction between WT-1 and inducible nitric oxide synthase (iNOS). In this regard, we reported that after neonatal unilateral ureteral obstruction, rosuvastatin prevents apoptosis through an increase in nitric oxide bioavailability, which in turn is linked to higher Hsp70 expression. Hence, the goal of this study was to determine whether a nitric oxide/Hsp70 interaction is involved in changes in WT-1 mRNA expression after ureteral obstruction. Neonatal rats submitted to experimental ureteral obstruction were treated with either vehicle or rosuvastatin for 14 days. Decreased nitric oxide and iNOS/Hsp70 expression associated with WT-1 low expression was shown in obstructed kidneys. Apoptosis was induced and it was associated with an increased Bax/Bcl2 ratio. Conversely, iNOS/Hsp70 upregulation and an increased WT-1 mRNA expression, without an apoptotic response, were observed in the cortex of obstructed kidneys of rosuvastatin-treated rats. Nitric oxide also modulated Hsp70 and WT-1 mRNA expression in MDCK cells. Finally, *in vivo* experiments with nitric oxide modulators support our hypothesis that WT-1 mRNA expression is associated with nitric oxide level. Results suggest that rosuvastatin may modulate WT-1 mRNA expression through renal nitric oxide bioavailability, preventing neonatal obstruction-induced apoptosis associated with Hsp70 interaction.

Miyajima, A., J. Chen, et al. "Interaction of nitric oxide and transforming growth factor-beta1 induced by angiotensin II and mechanical stretch in rat renal tubular epithelial cells." *J Urol.* 2000 Nov;164(5):1729-34.

**PURPOSE:** Changes in intrarenal pressure accompanying unilateral ureteral obstruction can result in tubular mechanical stretch and mediator release from renal tubules. Therefore, we examined the synthesis of nitric oxide and transforming growth factor-beta (TGF-beta), and their interaction in rat renal epithelial cells (NRK-52E) exposed to either angiotensin II or mechanical stretch. **MATERIALS AND METHODS:** NRK-52E were exposed to either angiotensin II or mechanical stretch. Nitrite and TGF-beta in the supernatant were assessed by the Greiss reaction and bioassay, respectively. The level of cell hypertrophy and intracellular TGF-beta protein was determined by flow cytometry. TGF-beta messenger RNA and inducible nitric oxide synthase protein were detected by reverse transcriptase polymerase chain reaction and Western blot, respectively. **RESULTS:** Angiotensin II stimulated TGF-beta1 and nitric oxide. The nitric oxide synthase inhibitor, N-nitro-L-arginine (L-NAME) or angiotensin II type I receptor blocker,

losartan, inhibited nitric oxide and TGF-beta1 induced by angiotensin II. Flow cytometry showed that either L-NAME or losartan inhibited angiotensin II induced cell hypertrophy. TGF-beta1 inhibited iNOS protein and nitric oxide, whereas an anti-TGF-beta antibody enhanced iNOS. Mechanical stretch induced TGF-beta, inducible NOS protein and nitric oxide. However, TGF-beta expression was not affected by L-arginine or L-NAME when cells were exposed to mechanical stretch. **CONCLUSIONS:** These results demonstrate that nitric oxide is an intermediate in angiotensin II stimulated TGF-beta1 expression but not in stretch induced TGF-beta expression, and that TGF-beta1 is a negative regulator of nitric oxide in rat renal epithelial cells. The complex interaction of these cytokines may be a target for intervention in the fibrotic and apoptotic processes in the obstructed kidney.

Miyajima, A., J. Chen, et al. "Role of nitric oxide in renal tubular apoptosis of unilateral ureteral obstruction." *Kidney Int.* 2001 Apr;59(4):1290-303.

**BACKGROUND:** The obstructed kidney in unilateral ureteral obstruction (UUO) is characterized by renal atrophy and tissue loss, which is mediated by renal tubular apoptosis. We sought to determine whether NO is involved in renal tubular apoptosis *in vitro* and *in vivo*. **METHODS:** Rat renal tubular epithelial cells (NRK-52E) were subjected to mechanical stretch, and apoptosis and cell size were analyzed by flow cytometry. Furthermore, we studied UUO in mice lacking the gene for inducible nitric oxide synthase (iNOS<sup>-/-</sup>) and their wild-type littermates. Tubular apoptosis and proliferation were detected by immunostaining. NOS activity and NOS expression were assessed by a citrulline assay and Western blot, respectively. **RESULTS:** Stretching-induced apoptosis in NRK-52E, which was reduced when NO was increased; conversely, stretch-induced apoptosis was increased when a NOS inhibitor was added to the cells. Stretched cells are larger and more apoptotic than unstretched cells. In UUO, the obstructed kidney of iNOS<sup>-/-</sup> mice exhibited more apoptotic renal tubules than the wild-type mice through 14 days of UUO. The obstructed kidney of iNOS<sup>-/-</sup> mice at day 3 showed more proliferative tubules compared with wild type. The obstructed kidney of wild-type mice exhibited higher total NOS activity until day 7 after UUO compared with iNOS<sup>-/-</sup> mice. However, the obstructed kidney of day 14 wild-type mice exhibited significantly lower iNOS activity and protein compared with the day 0 kidney. **CONCLUSION:** These results suggest that mechanical stretch is related to renal tubular apoptosis and that NO plays a protective role in this system in UUO.



Moosavi, S. M., Z. Bagheri, et al. "Pre- or post-treatment with aminoguanidine attenuates a renal distal acidification defect induced by acute ureteral obstruction in rats." Can J Physiol Pharmacol. 2013 Nov;91(11):920-8. doi: 10.1139/cjpp-2013-0059. Epub 2013 Jun 20.

Acute unilateral ureteral obstruction (UUO) impairs distal nephron acid secretion and stimulates expression of inducible nitric oxide synthase (iNOS) in post-obstructed kidney (POK). This study investigated the influence of pre- or post-treatment with aminoguanidine as a selective iNOS inhibitor on UUO-induced renal functional disturbances. To induce acute UUO, the left ureter in rats was ligated and released after 24 h. Then, a 3 h clearance period followed by bicarbonate loading and thereafter a 30 min clearance period were allocated. Aminoguanidine was administered either prior to the UUO induction or after release of the obstruction in the different rat groups, while untreated and sham groups received normal saline. During the first clearance period, fractional bicarbonate excretion and urinary pH increased markedly in the POK of the untreated group compared with the left kidney of sham group, and a large drop in the difference between urine and blood pCO<sub>2</sub> (U-B pCO<sub>2</sub>) was observed after bicarbonate loading; all of these parameters were ameliorated in the pre-treated and post-treated groups. However, the UUO-induced decreases in creatinine clearance, sodium reabsorption, urine osmolality, and free-water reabsorption in the POK were attenuated only in the post-treated group. Therefore, the in vivo application of a selective iNOS inhibitor partially improved the acute UUO-induced distal nephron acidification defect, while post-treatment but not pre-treatment with aminoguanidine ameliorated decrements of glomerular filtration, sodium reabsorption, and urine-concentrating ability.

Moridaira, K., H. Yanagisawa, et al. "Enhanced expression of vsmNOS mRNA in glomeruli from rats with unilateral ureteral obstruction." Kidney Int. 2000 Apr;57(4):1502-11.

**BACKGROUND:** The vasodilatory/cytotoxic gas, nitric oxide (NO), is associated with an alteration in glomerular hemodynamics seen after the induction of ureteral ligation. As yet the type of nitric oxide synthase (NOS) protein involved in the mechanism has not been clearly established in the unilateral ureteral obstruction (UUO) model. **METHODS:** Using reverse transcription (RT)-polymerase chain reaction (PCR), the expression and localization of vascular smooth muscle-derived nitric oxide synthase (vsmNOS) mRNA were examined in glomeruli from sham-operated control (SOC) rats and rats with UUO of three hours duration. Moreover, the effect of

endogenous angiotensin II on the expression of vsmNOS mRNA in glomeruli was explored using SOC rats and rats with UUO that were pretreated or not with enalapril, an angiotensin-converting enzyme inhibitor. **RESULTS:** The expression of vsmNOS mRNA was significantly greater in glomeruli of rats with UUO than in those of SOC rats. In rats with UUO, the expression of vsmNOS mRNA was substantially increased in glomeruli of the obstructed kidney (OK) compared to the contralateral, nonobstructed kidney (CLK). Suppression of angiotensin II production in vivo with enalapril restored the expression of vsmNOS mRNA in glomeruli of the CLK and OK from rats with UUO to levels comparable to that seen in glomeruli from SOC rats. In addition, the in situ RT-PCR analysis, a novel method for mRNA identification in cells and tissue, revealed that vsmNOS mRNA was expressed in the cytoplasm of glomerular mesangial and epithelial cells in SOC rats and rats with UUO. **CONCLUSIONS:** An increase in vsmNOS mRNA expression in glomeruli of the CLK and OK from rats with UUO may be mediated by increased action of endogenous angiotensin II that occurs after the onset of ureteral obstruction. Enhanced expression of vsmNOS mRNA in glomeruli of the OK compared to the CLK may be due to differences in levels of angiotensin II acting on the two kidneys in vivo. Additionally, the expression of vsmNOS mRNA in glomeruli originates in mesangial and epithelial cells in SOC rats and rats with UUO.

Morisada, N., M. Nomura, et al. "Complete disruption of all nitric oxide synthase genes causes markedly accelerated renal lesion formation following unilateral ureteral obstruction in mice in vivo." J Pharmacol Sci. 2010;114(4):379-89. Epub 2010 Nov 9.

The role of nitric oxide (NO) derived from all three NO synthases (NOSs) in renal lesion formation remains to be fully elucidated. We addressed this point in mice lacking all NOSs. Renal injury was induced by unilateral ureteral obstruction (UUO). UUO caused significant renal lesion formation (tubular apoptosis, interstitial fibrosis, and glomerulosclerosis) in wild-type, singly, and triply NOS(-/-) mice. However, the extents of renal lesion formation were markedly and most accelerated in the triply NOS(-/-) genotype. UUO also elicited the infiltration of inflammatory macrophages, up-regulation of transforming growth factor (TGF)- $\beta$ 1, and induction of epithelial mesenchymal transition (EMT) in all of the genotypes; however, the extents were again largest by far in the triply NOS(-/-) genotype. Importantly, long-term treatment with the angiotensin II type 1 (AT(1))-receptor blocker olmesartan significantly prevented the exacerbation of those renal structural changes after

UUO in the triply NOS(-/-) genotype, along with amelioration of the macrophage infiltration, TGF-beta1 levels, and EMT. These results provide the first evidence that the complete disruption of all NOS genes results in markedly accelerated renal lesion formation in response to UUO in mice in vivo through the AT(1)-receptor pathway, demonstrating the critical renoprotective role of all NOSs-derived NO against pathological renal remodeling.

Nguyen, V. T., A. Ndoye, et al. "Classification, clinical manifestations, and immunopathological mechanisms of the epithelial variant of paraneoplastic autoimmune multiorgan syndrome: a reappraisal of paraneoplastic pemphigus." *Arch Dermatol.* 2001 Feb;137(2):193-206.

**BACKGROUND:** Recent studies suggest that paraneoplastic pemphigus (PNP) is a heterogeneous autoimmune syndrome involving several internal organs and that the pathophysiological mechanisms mediating cutaneous, mucosal, and internal lesions are not limited to autoantibodies targeting adhesion molecules. **OBJECTIVE:** To classify the diverse mucocutaneous and respiratory presentations of PNP and characterize the effectors of humoral and cellular autoimmunity mediating epithelial tissue damage. **METHODS:** We examined 3 patients manifesting the lichen planus pemphigoideslike subtype of PNP. A combination of standard immunohistochemical techniques, enzyme-linked immunosorbent assay with desmoglein (DSG) baculoproteins, and an immunoprecipitation assay were used to characterize effectors of humoral and cellular autoimmunity in patients with PNP and in neonatal wild-type and DSG3-knockout mice with PNP phenotype induced by passive transfer of patients' IgGs. **RESULTS:** In addition to the known "PNP antigenic complex," epithelial targets recognized by PNP antibodies included 240-, 150-, 130-, 95-, 80-, 70-, 66-, and 40/42-kd proteins but excluded DSG1 and DSG3. In addition to skin and the epithelium lining upper digestive and respiratory tract mucosa, deposits of autoantibodies were found in kidney, urinary bladder, and smooth as well as striated muscle. Autoreactive cellular cytotoxicity was mediated by CD8(+) cytotoxic T lymphocytes, CD56(+) natural killer cells, and CD68(+) monocytes/macrophages. Inducible nitric oxide synthase was visualized both in activated effectors of cellular cytotoxicity and their targets. Keratin 14-positive basal epithelial cells sloughed from the large airways and obstructed small airways. **CONCLUSIONS:** The paraneoplastic disease of epithelial adhesion known as PNP in fact represents only 1 manifestation of a heterogeneous autoimmune syndrome in which patients, in addition to small airway occlusion and deposition of autoantibodies in

different organs, may display a spectrum of at least 5 different clinical and immunopathological mucocutaneous variants (ie, pemphiguslike, pemphigoidlike, erythema multiforme-like, graft-vs-host disease-like, and lichen planus-like). We suggest that the more encompassing term "paraneoplastic autoimmune multiorgan syndrome," or PAMS, be applied. The pathophysiological mechanisms of PAMS involve both humoral and cellular autoimmunity responses. Epithelial cell membrane antigens other than DSG1 or DSG3 are targeted by effectors of PAMS autoimmunity. Apoptosis of damaged basal cells mediates epithelial clefting, and respiratory failure results possibly from obstruction of small airways with sloughed epithelial cells.

Nishida, M., Y. Okumura, et al. "The role of apelin on the alleviative effect of Angiotensin receptor blocker in unilateral ureteral obstruction-induced renal fibrosis." *Nephron Extra.* 2012 Jan;2(1):39-47. doi: 10.1159/000337091. Epub 2012 Mar 7.

**BACKGROUND:** Apelin is a selective endogenous ligand of the APJ receptor, which genetically has closest identity to the angiotensin II type 1 receptor (AT-1). The effects of the apelin/APJ system on renal fibrosis still remain unclear. **METHODS:** We examined the effects of the apelin/APJ system on renal fibrosis during AT-1 blockade in a mouse unilateral ureteral obstruction (UUO) model. **RESULTS:** WE OBTAINED THE FOLLOWING RESULTS: (1) At UUO day 7, mRNA expressions of apelin/APJ and phosphorylations of Akt/endothelial nitric oxide synthase (eNOS) in the UUO kidney were increased compared to those in the nonobstructed kidney. (2) AT-1 blockade by the treatment with losartan resulted in a further increase of apelin mRNA as well as phosphorylations of Akt/eNOS proteins, and this was accompanied by alleviated renal interstitial fibrosis, decreased myofibroblast accumulation, and a decreased number of interstitial macrophages. (3) Blockade of the APJ receptor by the treatment with F13A during losartan administration completely abrogated the effects of losartan in the activation of the Akt/eNOS pathway and the amelioration of renal fibrosis. (4) Inhibition of NOS by the treatment with L-NAME also resulted in a further increase in renal fibrosis compared to the control group. **CONCLUSION:** These results suggest that increased nitric oxide production through the apelin/APJ/Akt/eNOS pathway may, at least in part, contribute to the alleviative effect of losartan in UUO-induced renal fibrosis.

Ozbek, E., Y. O. Ilbey, et al. "Melatonin attenuates unilateral ureteral obstruction-induced renal injury by reducing oxidative stress, iNOS, MAPK, and NF-kB

expression." *J Endourol.* 2009 Jul;23(7):1165-73. doi: 10.1089/end.2009.0035.

**PURPOSE:** To investigate whether melatonin (MLT) treatment has any protective effect on unilateral ureteral obstruction (UUO)-induced kidney injury in rats. **MATERIALS AND METHODS:** Six animals were included in each of the following five groups: group 1, sham operation but no treatment; group 2, unilateral ureteral ligation but no treatment; group 3, sham operation + MLT; group 4, unilateral ureteral ligation + MLT; group 5, unilateral ureteral ligation +5% ethanol (the vehicle of MLT). The injected dose of MLT was 1 mg/kg/day (intraperitoneal). MLT and vehicle were injected daily, beginning 5 days before the unilateral ureteral ligation or sham operation and until 10 days after it. At 10 days after UUO, all rats were sacrificed with high-dose ketamine. Malondialdehyde, glutathione, nitric oxide (NO), and 8-hydroxydeoxyguanosine levels and inducible NO synthase (iNOS), p38-mitogen-activated protein kinase (p38-MAPK), and nuclear factor kappa B (NF- $\kappa$ B) expression were studied. Histopathological examination of the obstructed kidney was also performed. **RESULTS:** UUO was accompanied by a significant increase in malondialdehyde, NO, and 8-hydroxydeoxyguanosine along with a significant decrease in glutathione levels in the kidney tissue, as well as a significant elevation in iNOS, p38-MAPK, and NF- $\kappa$ B expression. MLT treatment resulted in reduction of the parameters of oxidative stress and the iNOS, p38-MAPK, and NF- $\kappa$ B expression. MLT treatment also reduced the development of leukocyte infiltration and interstitial fibrosis in UUO rats. **CONCLUSIONS:** MLT may prevent UUO-induced kidney damage in rats by reducing oxidative stress. The mechanism for this is likely mediated via reduction in the expression of iNOS, p38-MAPK, and NF- $\kappa$ B, since MLT reduces the activation of these pathways.

Rivera-Huizar, S., A. R. Rincon-Sanchez, et al. "Renal dysfunction as a consequence of acute liver damage by bile duct ligation in cirrhotic rats." *Exp Toxicol Pathol.* 2006 Nov;58(2-3):185-95. Epub 2006 Jul 7.

Renal failure is a common complication in patients with alcohol-induced cirrhosis who undergo a superimposed severe alcoholic hepatitis. **AIM:** Our aim was to evaluate renal dysfunction established as a consequence of acute liver damage (ALD) induced by bile duct ligation (BDL) in cirrhotic rats. Hepatic and renal functional assays were performed. **RESULTS:** Hyperbilirubinemia and increased alanine aminotransferase and aspartate aminotransferase ( $p < 0.05$ ) in rats with BDL were observed since the first day of bile obstruction in cirrhotic rats. Urinary volume and urinary sodium concentration showed a

significant reduction ( $p < 0.05$ ) on days 3 and 5 after BDL. Plasma renin activity, plasma renin concentration, serum creatinine, and BUN values increased ( $p < 0.05$ ) from day 1 to day 7 after BDL. Glomerular filtration rate was substantially decreased from day 1 to day 7. Histological changes became apparent since day 3 after BDL in which glomeruli with mesangial hypercellularity took place in the absence of tubular necrosis; with portal inflammation and proliferation of biliar conduits. Results of the present work demonstrate that ALD induced by BDL in cirrhotic rats produces changes in renal function. In conclusion, this experimental model demonstrates that an ALD of variable etiology, either surgical or induced by CCI(4), can cause important damage that eventually results in renal function deterioration. This experimental model may be suitable, to study the physiopathology of this syndrome, as well as for the evaluation of different pharmacological therapies.

Stern, J. M., J. Chen, et al. "Effect of UUO on D1aR expression reveals a link among dopamine, transforming growth factor-beta, and nitric oxide." *Am J Physiol Renal Physiol.* 2004 Mar;286(3):F509-15. Epub 2003 Nov 11.

Interactions between transforming growth factor-beta (TGF-beta) and nitric oxide (NO) are important in the pathophysiology of unilateral ureteral obstruction (UUO). Dopamine (DA) is a vasoactive renal mediator active at the D(1A) receptor (D(1A)R), which has not been studied in UUO; therefore, we examined the interactions among DA, TGF-beta, and NO in UUO. In vivo, UUO was carried out in rats with or without concurrent treatment with 1D11, a monoclonal antibody to TGF-beta, for 14 days. In vitro, NRK-52E cells (normal rat kidney tubules) were treated with DA, and NO and TGF-beta release were examined. UUO resulted in a 70% decrease in the expression of renal D(1A)R, confirmed by both Western blot analysis and immunohistochemistry. 1D11 treatment restored expression to 60% of control values. DA treatment decreased NRK-52E release of TGF-beta by 80%; conversely, DA significantly increased NO release from NRK-52E cells. These results suggest that DA modulates the release of cytokines, which are involved in the fibrotic and apoptotic sequelae of UUO, and that these effects are independent of DA's known vasoactive properties.

Sun, D., Y. Wang, et al. "Effects of nitric oxide on renal interstitial fibrosis in rats with unilateral ureteral obstruction." *Life Sci.* 2012 Jun 14;90(23-24):900-9. doi: 10.1016/j.lfs.2012.04.018. Epub 2012 Apr 30.

**AIMS:** It is well recognized that microvascular injury is a major determinant of renal fibrosis. Mounting evidence shows that nitric oxide

(NO) plays an important role in angiogenesis. Therefore, we investigated to the effects of NO on kidney angiogenesis and renal fibrosis. **METHODS:** In the present study, a unilateral ureteral obstruction (UUO) model was established with L-arginine (L-Arg, 1 g/dl) and N-nitro-L-arginine methyl ester (L-NAME, 5 mg/dl) serving as interference factors. We investigated the alteration of NO concentration with spectrophotometry, peritubular capillary (PTC) density with aminopeptidase P (JG12) immunohistochemical staining, and the expression of vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), hypoxia inducible factor-1alpha (HIF-1alpha) and transforming growth factor-beta1 (TGF-beta1) with immunohistochemical staining and Western blotting at weeks 2, 3 and 4. **KEY FINDINGS:** Our findings showed that the expressions of VEGF, eNOS and PTC density were significantly decreased in rats with UUO, which was accompanied by a progressive increase in HIF-1alpha, TGF-beta1 and an area of renal interstitial fibrosis. The administration of L-Arg promoted the synthesis of NO and significantly elevated the expressions of VEGF, eNOS and PTC density with the conspicuous loss of HIF-1alpha and TGF-beta1 expressions and ultimately ameliorated renal fibrosis, which was markedly aggravated by L-NAME administration.

Valles, P. G., L. Pascual, et al. "Role of endogenous nitric oxide in unilateral ureteropelvic junction obstruction in children." Kidney Int. 2003 Mar;63(3):1104-15.

**BACKGROUND:** Obstructive nephropathy leads to tubulointerstitial fibrosis and loss of renal function. Nitric oxide has been shown to have antifibrotic properties. 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. **METHODS:** NOS activity and the expression of NOS isoforms measured at the mRNA level by reverse transcription-polymerase chain reaction (RT-PCR) assay were determined in tissue obtained by biopsy from obstructed kidneys of 18 children at the time of pyeloplasty. Tissue from kidneys removed because of various malignancies were issued as control. **RESULTS:** A significant increase in calcium/calmodulin-independent NOS activity (iNOS) and iNOS mRNA expression was found in the medulla of obstructed kidneys. Calcium/calmodulin-dependent NOS activity (cNOS) and endothelial (eNOS) mRNA, by contrast, were increased in the cortex from obstructed kidneys. A role of tumor necrosis factor-alpha (TNF-alpha) on enhanced iNOS was suggested by the finding of increased urine levels in obstructed

pelvis. Increased interstitium macrophage number, by immunolabeling of CD68, was related to the delay in obstruction release and to decreased glomerular filtration rate (GFR) at surgery. 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. **CONCLUSION:** In kidneys from children with UPJ obstruction an increased activity and expression of iNOS in medulla and cNOS-dependent eNOS in cortex were demonstrated. A role of cNOS in modulating GFR and interstitial fibrosis can be suggested. Prolonged UPJ obstruction would lead to a worsened prognosis on renal injury.

Vaughan, E. D., Jr., D. Marion, et al. "Pathophysiology of unilateral ureteral obstruction: studies from Charlottesville to New York." J Urol. 2004 Dec;172(6 Pt 2):2563-9.

**PURPOSE:** More than 30 years ago the decreased renal blood flow and glomerular filtration rate characteristic of unilateral ureteral obstruction (UUO) was described. In the ensuing time, much has been learned about the involvement of nitric oxide (NO) and transforming growth factor-beta (TGF-beta) in the pathophysiology of UUO. **MATERIALS AND METHODS:** We measured renal blood flow and glomerular filtration rate in dogs and rats, and assessed the effect of altering the availability of NO on these parameters. In rats and mice we used an antibody to TGF-beta, NO synthase gene deletion and inducible nitric oxide synthase gene therapy to assess the role of TGF-beta and NO in renal fibrosis. **RESULTS:** Results of our studies suggest 2 strategies that have the potential to be incorporated into clinical trials. The first would be replenishment of the NO system with arginine (or a NO donor) to decrease renovascular resistance and increase renal nitric oxide. Either in addition to the first strategy or separately, interstitial fibrosis could be targeted. Strategies for inhibiting fibrosis include antibody to TGF-beta, use of antisense oligonucleotides to TGF-beta, use of drugs that inhibit other pro-fibrotic mediators or gene therapy to inhibit fibrosis.

Yoo, K. H., B. A. Thornhill, et al. "Inducible nitric oxide synthase modulates hydronephrosis following partial or complete unilateral ureteral obstruction in the neonatal mouse." Am J Physiol Renal Physiol. 2010 Jan;298(1):F62-71. doi: 10.1152/ajprenal.00234.2009. Epub 2009 Nov 4.

To investigate the role of endogenous inducible nitric oxide synthase (iNOS) in the response of the developing kidney to unilateral ureteral obstruction (UUO), neonatal iNOS null mutant (-/-) and wild-type (WT) mice were subjected to partial or



complete UO. At 7 and 21 days of age, apoptosis, renin, vascular endothelial growth factor (VEGF), fibroblasts (anti-fibroblast-specific peptide 1), myofibroblasts (alpha-smooth muscle actin), macrophages (F4/80), and collagen were measured in kidney tissue. Compared with WT, renal parenchymal thickness was increased, with preservation of the papilla, in *-/-* mice with partial UO, but decreased in *-/-* mice with complete UO. Ureteral peristalsis increased with severity of pelvic dilatation in WT, and increased further in *-/-* mice with partial UO. Apoptosis, fibroblasts, and macrophages were increased in *-/-* mice with complete UO, but there was no effect of iNOS on other histological parameters following complete UO. Renin was decreased in *-/-* mice with partial UO. There was no effect of iNOS genotype on renal collagen accumulation at either 7 or 21 days of age. These results are consistent with an injurious role for endogenous iNOS following partial UO by inhibiting ureteral peristalsis and increasing renal renin although renal fibrosis is not affected. In contrast, in mice with complete UO, iNOS attenuates apoptosis and enhances renal parenchymal thickness. Alterations in the severity of ureteral obstruction may therefore influence the effect of iNOS on long-term renal injury.

Zebger-Gong, H., J. Kampmann, et al. "Decreased transplant arteriosclerosis in endothelial nitric oxide synthase-deficient mice." *Transplantation*. 2010 Mar 15;89(5):518-26. doi: 10.1097/TP.0b013e3181c7dce4.

Occlusive vascular changes, characterized by the formation of a neointima with lumen obstruction, are key histologic findings of allograft arteriosclerosis. Vascular integrity of the graft is critically dependent on nitric oxide (NO), synthesized by NO synthases (NOS), of which three isoforms have been located in the arterial wall: endothelial NOS (eNOS), inducible NOS, and neuronal NOS (nNOS). We have studied the role of NOS in a murine model of aortic allograft rejection. METHODS: The descending thoracic aorta of donor mice (BALB/c mice) was transplanted into two groups of recipients: (a) C57BL/6J and (b) C57BL/6J mice homozygous (*-/-*) for a knockout of the eNOS gene (eNOS(*-/-*)). RESULTS: After 4 weeks, pronounced neointima formation, upregulated expression of adhesion molecules, and increased infiltration by inflammatory cells were demonstrated in wild-type recipient mice, whereas eNOS(*-/-*) recipient mice were protected from neointima development by a significantly increased synthesis of NO, as shown by increased formation of cGMP; this was mainly explained by upregulation of inducible NOS and nNOS. CONCLUSIONS: Upregulation of inducible NOS and nNOS isoforms may be beneficial

in preventing allograft arteriosclerosis in the early posttransplant period.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

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