CHAPTER II

THEORY AND LITERATURE REVIEW

URETERAL OBSTRUCTION

Obstructive nephropathy is responsible for end-stage renal failure in approximately 4% of persons (Gloor and Torres, 1999). The frequency of urinary tract obstruction depends on many factors, including the patient's age, sex, and concurrent medical condition. No substantial sex differences are found until age of 20 years old. Between age of 20 and 60 years old, owing predominantly to pregnancy and uterine cancer, urinary tract obstruction is more common in women than in men. After the age of 60 years old, urinary tract obstruction is more common in men because of prostatic disease (Curhan et al., 2000).

Obstructive nephropathy refers to the renal disease resulting from impaired flow of urine or tubular fluid as a consequence of structural or functional abnormalities in the urinary tract (Klahr, 1991).

Acute Phase of Ureteral Obstruction

The acute phase of the obstructed kidney is characterized by dramatic decrease in glomerular filtration rate (GFR), renal blood flow (RBF) (Vaughan et al., 1970), interstitial edema, and influx of leukocytes into the kidney (Schreiner et al., 1988).

Phase I

During the first 2 to 3 hours after obstruction, blockade of antegrade urine flow markedly increases tubule hydrostatic pressure (P_T). Propagation of increased pressure back into Bowman space would be expected to reduce GFR (Gaedio et al., 1980), however, during the early phase of obstruction, glomerular capillary pressures (P_{GC}) also increases markedly and GFR is preserved. The increase in P_{GC} is caused by afferent arteriolar dilatation, leading to reduced afferent resistance (R_A) (Dal et al., 1977; Ichikawa, 1982; Gaedio et al., 1980) (Figure 1).

This afferent vasodilation is caused by several potential mechanisms, including increases in vasodilatory hormones [prostaglandin E_2 (PGE_2), prostacyclin, and nitric oxide (NO)], regulation by the macula densa, and a direct myogenic reflex (Wrigh and Briggs, 1979). Since this hyperemic response is not attenuated by renal nerve stimulation or infusion of catecholamines (Schramm and Carlson, 1975), it may be caused by increases in interstitial pressure, which reduces the transmural pressure gradient in the efferent arteriolar wall, leading to reduce contractility of the smooth muscle cells of the vessel wall (Francisco et al., 1980).

Obstruction reduces urine flow that passes the macula densa, leading to stimulation of afferent blood flow. The similar regulation occurs in the tubuloglomerular feedback response (Wrigh and Briggs, 1979) when decreasing rates of distal tubular flow passing the macula densa decrease R_A and increase P_{GC}, resulting in increased single-nephron glomerular filtration rate (SNGFR) (Wrigh and Briggs, 1979).

Phase II

After 3 hours, and through 12 to 24 hours of obstruction, RBF declines (Moody et al., 1975; Yarger and Griffith, 1974) (Figure 1). Initially, tubular pressures are elevated. However, the pressure will decline, by 24 hours. All RPF, GFR, and intratubular pressures have dropped below normal values (Dal et al., 1977; Gaedio et al., 1980; Prevoost and Molenaar, 1981). Thus, a major reason for reduced whole kidney GFR at this stage of obstruction is the nonperfusion of many glomeruli. At the single-nephron level, SNGFR is also markedly decreased, owing to afferent vasocostriction, which reduces P_{GC} (Dal et al., 1980; Dal et al., 1979) and then decreases RBF to 40% to 70% of normal. Reduction of P_{GC} lowers the driving pressure for filtration in the setting of nearly normal intratubular pressures (Dal et al., 1979). As the ureteral obstruction persists, activation of the renin-angiotensin system and increased production of thromboxane A_2 ($TBXA_2$) and endothelin result in progressive vasoconstriction, reductions in renal blood flow and glomerular capillary pressure. The glomerular filtration rate decreases to approximately 20% of baseline, despite normalization of the intratubular pressures (Gloor and Torres, 1999; Schulsinger et al., 1997).

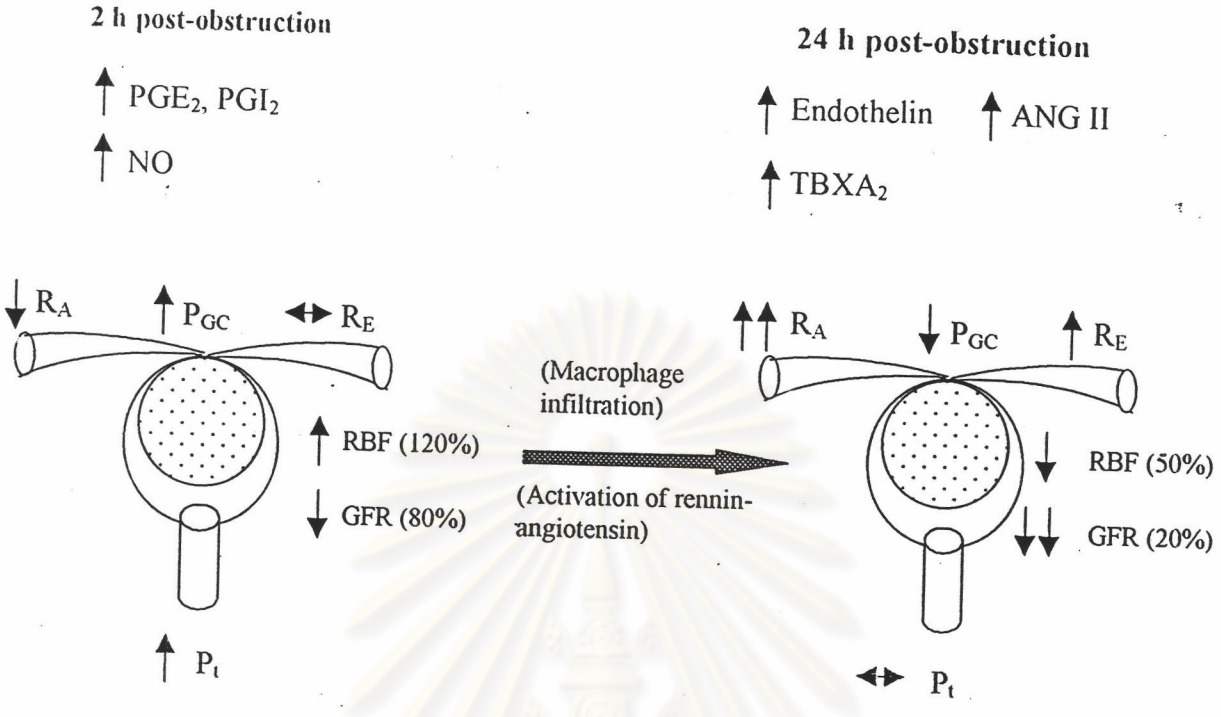

Figure 1 Acute renal hemodynamic response to ureteral obstruction. (Gloor and Torres, 1999). (ANG II = angiotensin II, R_A = afferent arteriolar resistance, R_E = efferent arteriolar resistance, P_{GC} = glomerular capillary hydraulic pressure, NO = nitric oxide, PGE_2 = prostaglandin E_2, PGI_2 = prostaglandin I_2, $TBXA_2$ = Thromboxane A_2, Pt = tubule hydrostatic pressure)

Chronic Phase of Ureteral Obstruction

On the other hand, the chronic phase (>24 hr.) of the obstructed kidney results in the increased intrarenal pressure leading to a decline in RBF with progressive ischemia (Sutherland et al., 2001), compression of the papillae with decreased glomerular filtration, and thining of the parenchyma secondary to loss of the nephrons. Significant cortical atrophy is associated with functional renal impairment (Sutherland et al., 2001) Moreover, the chronic ureteral obstruction causes the infiltration of the kidney by macrophages (Schreiner et al., 1988) and subsequently fibrosis of tubulointerstitium (Klahr and Purkerson, 1994). Individuals with

chronic obstructive uropathy may progress to end-stage renal disease (Curhan et al., 2000).

Effects of Ureteral Obstruction on Tubule Function

Ureteral obstruction disrupts the transport capacity of renal tubules for Na^+, K^+, and H^+, as well as reduces tubular ability to concentrate and dilute the urine (Yarger, 1991). The extent to which transepithelial transport is impaired depending on the severity and duration of the obstruction. With prolonged obstruction, profound tubular atrophy and chronic interstitial inflammatory changes are observed, whereas at earlier time points (24 hours), there are only modest structural and ultrastructural changes (Nagle et al., 1973; McDougal et al., 1976). These changes include slight mitochondrial swelling and a reduction in basolateral interdigitations in the proximal tubules and the medullary thick ascending limb of the loop of Henle (mTAL), and flattening of epithelium and some widening of intercellular spaces in the collecting ducts (Nagle et al., 1973; McDougal et al., 1976). Except at the very tip of the papilla, where there is focal necrosis of epithelial cells, there is little or no cell death (Nagle et al., 1973).

As above described, it has been shown that ureteral obstruction involves a complex inter-related sequence of events. The injury principally mediates many mechanisms as noted previously. Besides the loss of renal function, ureteral obstruction causes endothelial cell dysfunction. The endothelial cell is an important source of multiple vasoactive factors, widely recognized of which are ANG II and NO. Both could modulate vascular tone and have been postulated to interact closely in ureteral obstruction condition.

THE RENIN ANGIOTENSIN SYSTEM

The renin-angiotensin system, considered originally an endocrine system, has a pivotal role in the regulation of extracellular volume and systemic blood pressure (Kang, 2002). The renin-angiotensin system also functions at the intercrine, autocrine, and paracrine level (Kang, 2002). Components of this system have been found in many tissues, and the evidence for local biosynthesis of angiotensin II is substantial (Sutherland et al., 2001; Kang, 2002).

Renin

Renin is the key rate-limiting enzyme secreted predominantly by the juxtaglomerular cell in the kidney into the blood in response to a number of stimuli including a reduction in blood pressure and a reduction in extra cellular fluid volume (Levens et al., 1992). The active renin cleaves 10 amino acid from its α-2 globulin substrate angiotensinogen from the decapeptide angiotensin I (ANG I). ANG I is rapidly converted to be ANG II by the action of the endothelial enzyme, ACE (Gunning et al., 1996).

Angitensin Converting Enzyme (ACE)

ACE is a large acidic glycoprotein metalloenzyme composed of a single polypeptide chain. This enzyme is a rather nonspecific exopeptidase that sequentially cleaves dipeptides from the carboxy terminus of polypeptides (Gunning et al., 1996). ACE is distributed throughout the body, most abundantly in kidney, ileum, duodenum, and uterus. The

enzyme is thought to be expressed primarily by endothelial, epithelial, and neuroepithelial cells, and by some endocrine cells (Gunning et al., 1996). It is membrane bound with the active site directed outward, although in some cells such as sperm, ACE is found on the intracellular membrane. Endothelial cell ACE is positioned ideally to act on circulating ANG I to form ANG II. Although it was originally believed that pulmonary endothelium was the principle site of ANG I conversion to ANG II, it is now clear that ANG II formation in peripheral tissue may be equally important. In the kidney, ACE is localized on glomerular endothelial cells and on proximal tubule (PT) brush border menbrane (Gunning et al., 1996). ANG II exerts its biological actions by binding to its specific receptors on target cells (Arima and Ito, 2001).

Angiotensin II receptor

There are two major classes of ANG II receptors, type 1 (AT1) and type 2 (AT2) receptors (Matsubara, 1998). Most of the known effects of ANG II appear to be mediated through by the AT1 receptor (Horiushi et al., 1999; Ardaillou, 1999), which is predominantly found in tissue of adults and mediates ANG II-dependent vasoconstriction. AT2 receptor, on the other hand, is expressed in greater abundance in fetal tissues and declined after birth. The actions of the AT2 receptor are less well understood but may play a role in development (Allen et al., 1999).

Cellular Mechanism of Angiotensin II

AT1 receptor interacts with G-protein coupled receptor (Murphy et al., 1991). Stimulation of AT1 receptor activates phospholipase A2, C, and

D, resulting in increased intracellular Ca^{2+}, inositol 1,4,5-triphosphate (IP_3) concentration, and diacylglyceral (DAG) which stimulate protein kinase C (PKC) activity (Sasaki et al., 1991). ANG II binds to Gi-protein coupled receptor and then inhibits adenylate cyclase activity and reduces cyclic adenosine monophosphate (cAMP) levels (Inagami, 1999). The synthesis of PGs has been observed as well (Figure 2) (Gunning et al., 1996 Lassegue et al., 1991).

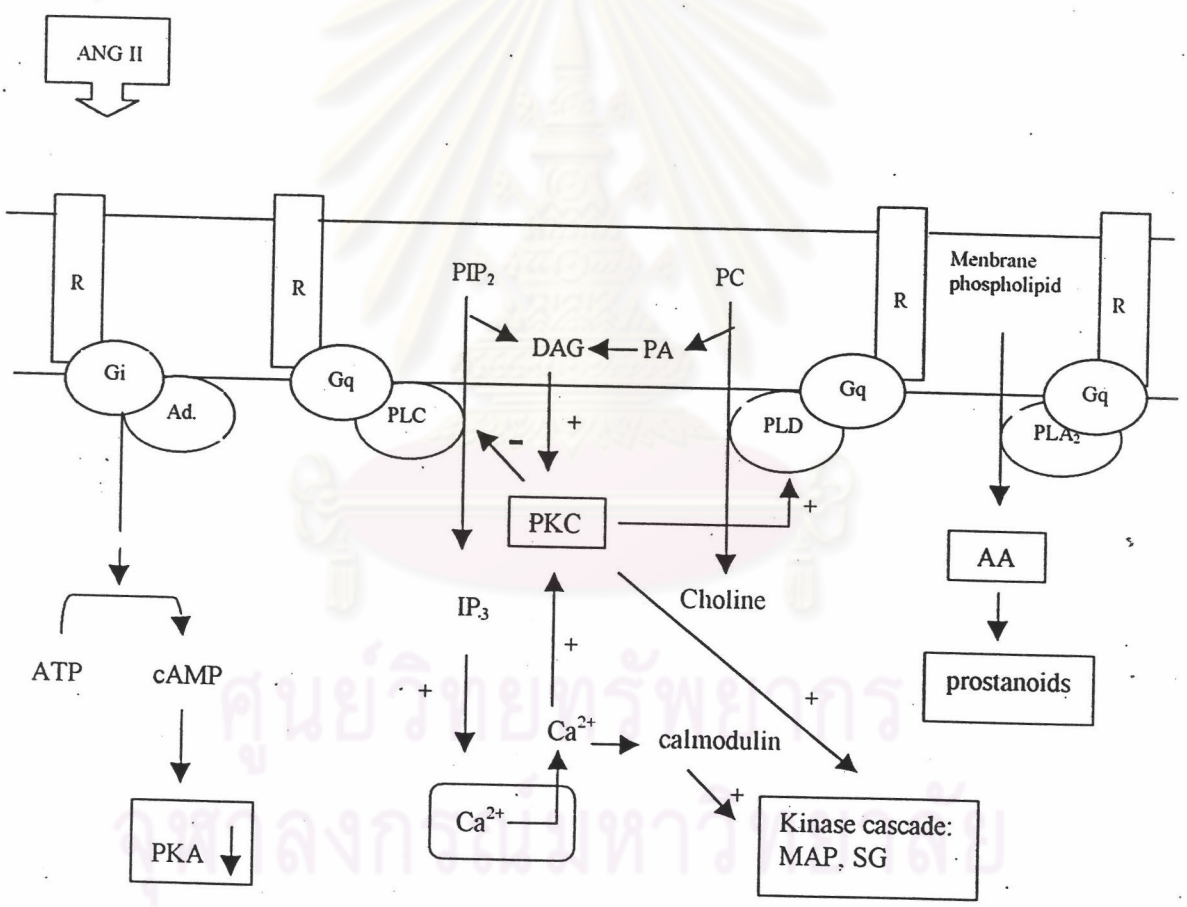

Figure 2 Linkage of angiotensin II receptor type 1 to biochemical processes (Levens et al., 1992). (Ad. = adenylate cyclase, R = angiotensin II receptor type 1, AA = arachidonic acid, DAG = diacylglyceral, IP_3 = inositol triphosphate, MAP = mitogen activated protein, SG = soluble guanylate cyclase, PA = phosphatidic acid, PC = phosphatidylcholine, PLP2 = phosphoinositoldiphosphate, PLA2 = phospholipase A2, PLC = phospholipase C, PLD = phospholipase D, PKC = protein kinase C)

The AT1 receptor has been shown to mediate most, if not all, of the known biological response secretion, cathecholamine release, secretion of pituitary peptides and hormones, dipsogenic effect and renal sodium reabsorption (Wong et al., 1990).

Intrarenal Action of Angiotensin II

ANG II is produced both systemically and locally in various tissues including the heart and blood vessel walls (Klahr, 1998). The intrarenal concentrations of ANG II are about 1,000-fold greater than the circulating levels of ANG II (Klahr, 1998). Within the kidney, AT1 receptor distribution is widespread. The AT1 receptors are present in the vasculature on both smooth muscle plasma membranes and endothelial cells including afferent and efferent arterioles (Ardaillou, 1999) as well as in the PT (Gunning et al., 1996).

Action of Angiotensin II on the Glomerular Circulation

The activation of AT1 receptor increases renal vascular resistance and reduces RBF (Arima and Ito, 2001). ANG II preferentially constricts efferent arterioles compared with afferent arteriole. As a result, the GFR is preserved (Yuan et al., 1990). AT1 receptor are present in abundance on mesangial cells, endothelial cells and podocytes (Sharma et al., 1998). In mesangial cells, ANG II causes contraction leading to decrease a capillary surface area and hence Kf (Arima and Ito, 2001). Under pathologic conditions, activation of AT1 receptor stimulates mesangial cell protein synthesis with enhanced production of extracellular matrix proteins and

transforming growth factor (TGF)-β (Kagami et al., 1994). In addition, ANG II, through the AT1 receptor, stimulates the mesangial cell to produce several growth factors [such as ET, interleukin (IL)-6 and platelet-dervied growth factor (PDGF)] which may contribute to proliferative responses in glomerular diseases (Bakris and Re, 1993; Moriyama et al., 1995). The function of AT1 receptors on podocytes remains unclear. However, Gloy et al. (1998) have suggested that stimulation of Ca^{2+} influx and reorganization of the actin cytoskeleton may involve in the induction of foot-process contractile responses.

Action of Angiotensin II on Renal Tubular Epithelial Transport

Tubular epithelial cells along the entire nephron also express AT1 receptors with a predominant expression in the PT on both apical and basolateral membrane (Li et al., 1994). In the luminal membrane of the PT, the concentration of ANG II is reported to be in the nanomolar range which is approximately, 1000 fold greater than that in plasma (Navar et al., 1994). ANG II, through AT1 receptor, affects the PT transport function in vitro with low concentrations ($<10^{-9}$ mol/L) stimulating, and high concentrations ($> 10^{-8}$ mol/L) inhibiting transepithelial Na^+ and bicarbonate (HCO_3^-) transport. These occur via both apical and basolateral Na^+-H^+ exchanger, (Arima and Ito, 2001), HCO_3^- trasportor and Na^+-K^+ ATPase activity (Thekkumkara et al., 1998). The biphasic effects of ANG II on PT transport may cause activation of PLA2 and subsequent arachidonic acid release at high concentrations. These lead to an inhibition of Na^+-H^+ exchanger (Li et al., 1994). By contrast, Gi-protein mediated inhibition of adenylate cyclase at low concentrations could decrease production of adenylate cyclase and

production of cAMP which, in turn, enhances the activity of Na^+-H^+ exchanger (Liu and Cogan, 1989; Thekkumkara et al., 1998).

AT1 receptors have also been detected on the macula densa cells and it may involve in the ANG II induced enhancement of tubuloglomerular feedback (TGF) (Schnerman and Briggs, 1990). These receptors in the distal tubule have been shown to be linked to the stimulation of unidirectional HCO_3^- flux, an effect that appears to be mediated by both apical and basolateral receptor activation (Levine et al., 1994). Finally, AT1 receptor has been identified in both the cortical collecting duct (CCD) and inner medullary collecting duct (IMCD) (Levine et al., 1996). In the CCD, AT1 receptors may regulate both Na^+ and HCO_3^- transport (Schlatter et al., 1995). In the MCD, the function of AT1 receptors is unclear, though its role in medullary water handling has been suggested (Oliverio et al., 2000). In addition, the expression of AT1 receptors in renal medullary interstitial cell situated between the vasa rectae and tubules has been shown, though the functions of these receptors are far unclear (Arima and Ito, 2001).

Angiotensin II and Unilateral Ureteral Obstruction

The renin-angiotensin system is up-regulated in the setting of ureteral obstruction (Frokiaer et al., 1992; Pimentel et al., 1993; Pimentel et al., 1995; Pimentel et al., 1994;). Generation of ANG II acts to rise perfusion pressure and to protect glomerular filtration by efferent arteriolar constriction (Lerman and Textor, 2001). These result in increased glomerular capillary hydraulic pressure but reduce RPF, GFR, Kf (Kubalak and Webb, 1993; Savin, 1986), and hydraulic conductivity (Li et al., 1993)

in the glomerulus. ANG II induces cellular hypertrophy and hyperplasia in several cell types, in addition to stimulating local hormone production and ion transport directly (Lerman and Textor, 2001; Purkerson and Klahr, 1989).

Angiotensin converting enzyme inhibitor (ACEI) and angiotensin receptor antagonist (ARA) in several experimental models have been demonstrated to inhibit the ANG II-induced alterations in renal hemodynamics and nephropathy in rat kidneys (Fontoura et al., 1991; Purkerson and Klahr, 1989).

In addition, increasing levels of ANG II in UUO in turn up-regulate the expression of several factors (Figure 3): tumor necrosis factor-α (TNF-α) (Gou et al., 2001), PDGF, nuclear factor kappa B (NF-kB) (Bander et al., 1985), insulin-like growth factor (IGF-1) (Klahr and Morrissey, 1998), monocyte chemoattractant peptide-1 (MCP-1) (Morrissey and Klahr, 1998), osteopontin (Ophascharoensuk et al., 1999), and TGF-β1 (Kaneto et al., 1993; Pimentel et al., 1995). TGF-β1 is an important mediator of fibrous tissue formation as part of the tissue repair response. Additionally, ANG II promotes conversion of latent TGF-β1 into its biologically active form (Kagami et al., 1994) and stimulates fibroblast collagen synthesis (Klahr et al., 1995). It has been suggested that the interaction between the renin-angiotensin system and TGF-β1 may explain the particular susceptibility of the kidney to fibrosis (Border and Noble, 1998).

Obstructive uropathy can cause major changes in the tubulointerstitial compartment of the kidney (Nath, 1992). Renal interstitial fibrosis is a common consequence of long standing obstructive uropathy.

The process of fibrosis, in part, represents an imbalance between extracellular matrix synthesis and deposition as well as matrix degradation. Several investigators have examined the mechanisms underlying the fibrogenesis of obstructive uropathy. Nagle et al. (1973) reported a widened interstitial space after 7 days of ureteral obstruction in rabbits, with an increase in collagen fibers and fibroblasts. Nagle and Bulger, (1978) also described a mononuclear cell infiltration and proliferation of interstitial cells in the renal parenchyma of rabbits with chronic UUO. Sharma et al. (1993) described interstitial fibrosis and thickening of the tubular basement membrane after UUO in the rabbit (Sharma et al., 1993). They found increased deposition of several extracellular matrix components (collagen types I, III, and IV), fibronectin, and heparin sulfate proteoglycans in the renal interstitium of rabbis with ureteral obstruction of 3 and 7 days duration (Nagle et al., 1973; Nagle and Bulger, 1978; Sharma et al., 1993)

An initial event in the interstitial fibrosis process is macrophage infiltration of the tubulointerstitial compartment (Sharma et al., 1993; Diamond et al., 1998). Renal proximal tubular cells and macrophages are potent sources of an array of growth factors, such as TGF-β1, IL-1, IL-6, fibroblast growth factor (FGF), TNF-α, and PDGF. These peptide growth factors are important regulators of cell growth and differentiation (Diamond et al., 1998) (Figure 3).

Additional studies have indicated that the monocyte/macrophage infiltration that occurs in the obstructed kidney is significantly decreased by treatment with ACEI as well as ARA (Ishidoya et al., 1995; Ishidoya et al., 1996; kento et al., 1994; Klahr and Morrissey, 1997; Morrissey and Klahr, 1998). This, in turn, would decrease interstitial cellularity and

contribute in part to the decreased volume of the tubulointerstitium seen after treatment with ACEI (Klahr and Morrissey, 1997). Interstitingly, a specific type 1 angiotensin II receptor (AT1 receptor) antagonist does not reduce the number of infiltrating monocytes/ macrophages or clusterin expression in obstructed kidney but reduces significantly other hallmarks of fibrosis, such as increases in interstitial volume, fibroblast proliferation, expression of TGF-β, and development of myofibroblast phenotype (Ishidoya et al., 1995; Klahr and Morrissey, 1997). Similarly, ANG II stimulates production of nitric oxide (Miyajima et al., 2000).

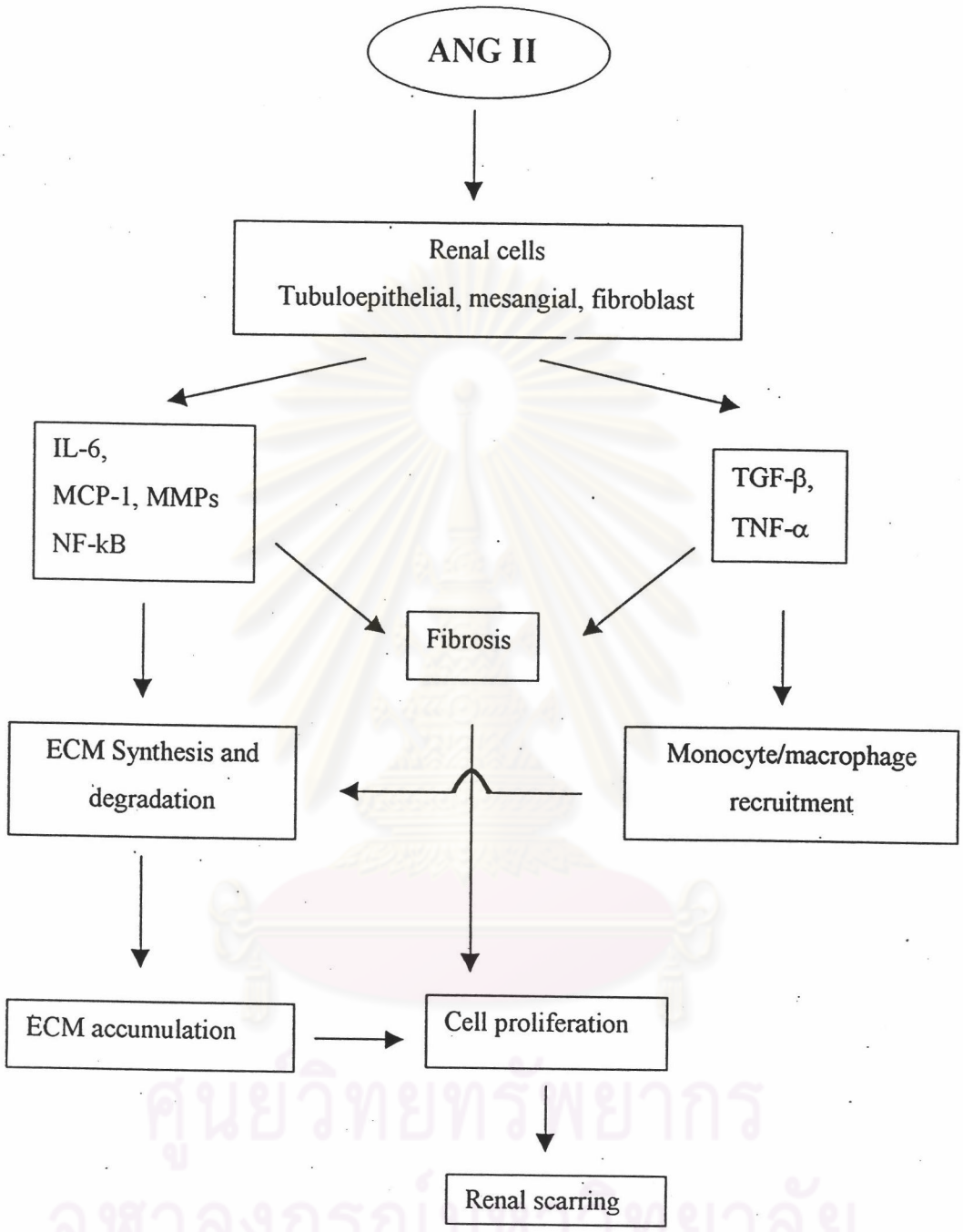

Figure 3 Events leading to tissue damage due to ANG II action (Klahr and Morrissey, 1998). (ECM = Extracellular matrix protein, IL-6 = interleukin 6, MMPs = metalloproteinases, MCP-1 = monocyte chemoattractant peptide-1, NF-kB = nuclear factor kappa B, TGF-β1 = transforming growth factor, TNF-α = tumor necrotic factor-α)

NITRIC OXIDE AND NITRIC OXIDE SYNTHASE

Nitric oxide (NO) is a lipophilic, highly reactive, free radical gas with diverse biomessenger functions (Lane and Gross, 1999). NO is produced by endothelial cells (Marsden et al., 2000). NO is generated by the stereo-specific oxidation of the terminal guanidio group of L-arginine in the presence of oxygen and NADPH by a family of enzymes known as NO synthase (NOS) (Vaziri, 2001).

Nitric Oxide Synthase

Three isoforms of nitric oxide synthase (NOS) have so far been identified: NOS I, also known as neuronal NOS (nNOS); NOS II, otherwise known as inducible NOS (iNOS); and NOS III also known as endothelial NOS (eNOS) (Vaziri 2001). The enzymatic activity of NOS is dependent on binding with calmodulin, which can only occur as a calcium-calmodulin complex with eNOS and nNOS (Vaziri 2001). Thus, the enzymatic activities of eNOS and nNOS are regulated by changes in cytoplasmic $[Ca^{2+}]$. In contrast, calmodulin binding to iNOS does not require Ca^{2+} and, as such, iNOS activity is calcium independent (Vaziri 2001).

Cellular Mechanism of Nitric Oxide

NO acting as a messenger molecule mediates vascular relaxation, inhibits platelet aggregation and adhesion to the endothelium and modulates leukocyte chemotaxis and adhesion (Moncada et al., 1991; Bolotina et al., 1994; Lander et al., 1993). All these effects of NO are mediated by activation of soluble guanylate cyclase after NO binding to its

heme iron, resulting in increased levels of cyclic guanosine, 3', 5', monophosphate (cGMP) (Moncada et al., 1991). NO also mediates relaxation of vascular smooth muscle by directly activating calcium-dependent potassium channels (Bolotina et al., 1994). In peripheral blood mononuclear cells NO may act as a signaling molecule by activating G proteins through a cGMP-independent pathway (Lander et al., 1993; Radomski et al., 1987; Mellion et al., 1981).

Intrarenal Action of Nitric Oxide

In the kidney cNOS, identified by immunohistochemical staining or by reverse transcription and polymerase chain reactions, has been found in glomeruli and vasculature as well as the macula densa, the collecting duct and the inner medullary thin limb (Mundel et al., 1992). In addition, iNOS occurs in vascular smooth muscle and granular cells at the juxtaglomerular apparatus (JGA) (Mundel et al., 1992). Cytokine induced iNOS has been reported in cultured proximal and collecting duct cells (Mundel et al., 1992; Markewitz et al., 1993).

Role of Nitric Oxide in the Regulation of Renal Blood Flow and Glomerular Filtration Rate

Evaluation of the role of NO in the regulation of RPF and GFR has heavily relied on the use of various NOS inhibitors with different degrees of specifity with respect to each one of NOS isoforms (Gabbai and Blantz, 1999). These nonspecific NOS inhibitors include N^G-Monomethyl-L-arginine (L-AMMA), L-NAME and N^w-nitro-L-arginine (L-NNA) (Gabbai and Blantz, 1999).

Acute administration of the NOS blocker, L-NMMA, to an awake conscious rat leads to increase in MAP and decrease in RBF and GFR. Large reductions in RBF relatively to GFR lead to significant increase in filtration fraction (Baylis et al., 1996). In anesthesized rats, a continuous intravenous infusion of NOS blockers increases systemic blood pressure and reduces RPF with variable changes in GFR (Tolins et al., 1990). At the single nephron level, the NOS blockade reduces nephron plasma flow because of increase in afferent arteriolar resistances (Zatz and De Nueci, 1991). Single nephron GFR is maintained or slightly reduced during L-NMMA infusion because of the opposing effects of an increased glomerular capillary hydrostatic pressure and transcapillary hydrostatic gradient, and the reduction of the Kf and nephron plasma flow (Zatz and De Nueci, 1991).

Experiments performed with renal artery infusion of NOS blockers have been shown that the NOS blockade has a preferential effect on the afferent arteriole and Kf with minimal imact on the efferent arteriole and glomerular capillary hydrostatic pressure (Deng and Baylis, 1993). In mesangial cell culture, the NOS blockade could produce contraction which constitutes a potential mechanism for the reduction of the Kf (Shultz et al., 1990).

Role of Nitric Oxide on Renal Tubular Epithelial Transport

Studies by several groups of investigator have found that NO inhibits the Na^+-H^+ exchanger and Na^+-K^+ ATPase activity in PT (Roczniak and Burna, 1996; Guzman et al., 1995). The NOS blocker is associated with reduced Na^+ and water excretion without affecting autoregulation of total

RBF and GFR (Lahera et al., 1991). NO also modulates tubular reabsorption in other tubular segments including cortical and inner medullary collecting duct (Rocziak et al., 1998). In these segments of the tubule, NO inhibits tubular reabsorption by inhibiting the Na^+-K^+ ATPase activity (Stoos et al., 1994).

Nitric Oxide and Unilateral Ureteral Obstruction

It has been shown that unilateral ureteral obstruction (UUO) enhances NO level in the obstructed kidney (Chevalier et al., 1992; Lanzone et al., 1995) as well as in urine (Morrissey et al., 1996). The non-specific NOS inhibitor, N^{ω}-monomethy-L-arginine (L-NMMA), shows its involvement in UUO both the initial hyperemic response (Lanzone et al., 1995) and during the hypoperfusion phase of prolonged obstruction (Hegarty et al., 2001). Efforts to supplement NO levels either with L-arginine (Morrissey et al., 1996) or sodium nitroprusside (SNP) (Hegarty et al., 2001) have been shown to afford protection to the obstructed kidney. This suggests that the kidney may maintain the necessary blood supply via the circulatory system by dilating arteries as a consequence of released NO during UUO.

Many studies demonstrate that both eNOS and iNOS proteins are increased in rats with UUO (Hegarty et al., 2001; Valles and Manucha, 2000). Furthermore, mechanical stretch in rat renal tubular epithelial cells significantly induce inducible nitric oxide synthase (Miyajima et al., 2000). It has been previously suggested that NO may play a protective role in the fibrosis of UUO. Morrissey et al demonstrated that an arginine-supplemented diet increases NO and acts as the antifibrosis in UUO

(Morrissey et al., 1996); conversely, treatment of UUO rats with L-NAME, a NOS inhibitor, increases the fibrosis. In confirmatory experiments, Huang et al (2000) demonstrated decrease fibrosis in UUO in iNOS-/- mice. This suggests that iNOS generated NO mediates damage in UUO injury (Moncada et al., 1991).

INTERACTION OF ANGIOTENSIN II AND NITRIC OXIDE

Ureteral obstruction increases many mediators including ANG II and NO. The loss of balance between ANG II and NO represents a disturbance of tissue homeostasis and may accelerate tissue damage (Lerman and Textor, 2001). NO participates in the regulation of kidney fuction by counteracting the vasoconstrictor effects of ANG II (Aki et al., 1997). A L-NAME administration significantly increases renal damage and MAP, while RBF and GFR are reduced (Kakoki et al., 2000). These are similar to those observed with increase ANG II (Blantz et al., 1976). In addition to effects on blood flow and tubular reabsorption of Na^+ (Bachmann and Mundel, 1994), NO could mediate inhibitions of fibroblast and vascular smooth muscle cell proliferation, matrix protein accumulatin, leukocyte adhesion, platelet adhesion and activation, and reduction of endothelial hyperpermeability (Moncada and Higgs, 1995). These effects occur, in part, by decreasing the expression of ACEI and AT1 receptors in cardiac myocytes (Raij, 1998).

As mentioned earlier, a NOS blockade induced in the anesthetized rat could change glomerular hemodynamics (increases in glomerular capillary hydrostatic pressure and transcapillary hydrostatic pressure

gradient, and reduction in nephron plasma flow and Kf) similarly to those observed with increases in ANG II (Blantz et al., 1976). Both ACEI and ARA are able to prevent most of the changes in renal and glomerular hemodynamics induced by NOS blockade (Sigmon and Beierwalts, 1993).

In vitro studies using afferent arteriolar vessel show that NOS blockade is associated with increased response to ANG II, as induced by larger and prolonged reduction of arteriolar lumen (Ito et al., 1993). This response predominates in the afferent arteriole because the change in efferent arteriolar tone induced by ANG II is not modified by NOS blockers (Gabbai and Blantz, 1999). The studies utilized anesthetized rats undergoing acute (3 hours) or chronic (2 weeks) administration of NOS blockers show that an acute infusion of NOS blocker L-NMMA increases systemic blood pressure, but do not modify GFR. Measurements of ANG II reveals a significant reduction in plasma ANG II levels in the absence of changes in kidney tissue ANG II (Garcia et al., 1997). Placement of an aortic snare to prevent increase in renal perfusion pressure during NOS blockade reveales similar changes in plasma and kidney tissue ANG II levels (Garcia et al., 1997). These imply that the inhibition of renin and plasma ANG II generation is independent of the changes in systemic blood pressure and that NOS blockers directly suppress renin secretion (Gabbai and Blantz, 1999).

Studies using isolated perfused rat kidney with perfusion pressure 100 mmHg, have shown that sodium nitroprusside (SNP; NO donor) 30 μmol/L induces 4.5 fold increase of basal renin secretion rate (Kurtz et al., 1998). However, the membrane permeable cGMP analogs 8-bromo-cGMP could inhibit the basal renin secretion and attenuate the stimulation

of renin secretion by SNP (Kurtz et al., 1998). If the perfusion pressure is lowered to 40 mmHg in the presence of 30 μmol/L SNP, the renin secretion rate is 12 fold of the basal value. In addition, NO has been shown to stimulate renin secretion in vivo from conscious (Persson et al., 1993) and anesthesized animal (Johnson and Freeman, 1994). Moreover, NO downregulates the synthesis of ACE in the endothelium and thus can affect ANG II production (Higashi et al., 1995). Furthermore, NO could downregulate AT1 receptor in vascular tissue and adrenal gland and thus mitigate the action of ANG II (Ichiki et al., 1998).

Besides the effect of NO production of renin, ANG II could increase NO production and renal NOS expression (Hennington et al., 1998). The study performed in an acute ANG II infusion (110 minutes) shows an increase eNOS mRNA by 70% without changing eNOS protein levels, whereas a chronic infusion of ANG II (10 days) increases eNOS protein by 90% without changing eNOS mRNA levels (Hennington et al., 1998). Thus, ANG II infusion time for only 110 minutes may not be long enough to allow translation of eNOS protein (Hennington et al., 1998). These data suggest that ANG II can increase NO production in the kidney (Hennington et al., 1998).

There are at least two mechanisms by which ANG II could increase renal NO production. First, ANG II could increase eNOS activity by causing an increase in intracellular Ca^{2+} concentration (Hennington et al., 1998). Secondly, ANG II may enhance eNOS synthesis either on a transcriptional level or on translational level (Hennington et al., 1998). In addition, it is possible that ANG II could casuse an increase in eNOS synthesis due to an increase in shear stress (Hennington et al., 1998). The

shear stress promotes eNOS gene transcription through increase activity of tyrosine kinase (in particular c-Src) (Davis et al., 2001; Ungvari et al., 2001). Schwobel et al. (2000) demonstrated that induced NO production is negatively controlled by AT2, whereas AT1 receptor stimulation enhances NO synthesis in mesangial cells. The overall NO availability depends on the onset of the inflammatory stimuli with respect to ANG II exposure and the available AT receptor.

As mentioned above, the collective data demonstrate rate that UUO causes increases both ANG II and NO. The interaction between ANG II and NO is more complex than just NO opposition of tonic effect of ANG II on the renal microcirculation (Gabbai and Blantz, 1999). The complex interaction between ANG II and NO is generated by the effect of NO on production of renin and ANG II. However, to date, there is no study of UUO related the role of angiotensin on renal NOS expression and nitric oxide production. Therefore, the present study aims to investigate this regard.

Research Questions

1. How angiotensin inhibition plays the role on such renal NOS protein expression, NO production and renal pathology?

2. Do the different duration of UUO have variant effect on renal NOS protein expression, NO production and renal pathology?

Objectives

1. To study NOS protein expression, serum nitrite level and renal pathology in rats with 1-day and 7-day UUO.

2. To study a role of angiotensin inhibition on NOS protein expression, serum nitrite level and renal pathology in rats with 1-day and 7-day UUO.

3. To compare the effect of ACEI and AT1 antagonist on NOS protein expression, serum nitrite level and renal pathology in rats with 1-day and 7-day UUO.