INTERACTION OF SYMPATHETIC NERVOUS SYSTEM AND RENIN ANGIOTENSIN SYSTEM IN RENAL HAEMODYNAMICS OF RENAL FAILURE, HYPERTENSIVE AND RENAL FAILURE HYPERTENSIVE RATS

FATHIHAH BINTI BASRI

UNIVERSITI SAINS MALAYSIA

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by

FATHIHAH BINTI BASRI

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for the degree of
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INTERAKSI ANTARA SISTEM SARAF SIMPATETIK DAN RENIN ANGIOTENSIN KE ATAS HEMODINAMIK GINJAL DALAM KEGAGALAN GINJAL, HIPERTENSI DAN GABUNGAN KEGAGALAN GINJAL DAN HIPERTENSI

oleh

FATHIHAH BINTI BASRI

Tesis yang diserahkan untuk memenuhi keperluan bagi Ijazah Sarjana Sains

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Fathihah Basri
March 09'

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LIST OF SYMBOLS AND ABREVIATIONS

%	percent
%age	percentage
6OHDA	6-hydroxydopamine
α	alpha
β	beta
µg	microgram
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
Ang	angiotensin
Ang I	angiotensin I
Ang II	angiotensin II
ACE	angiotensin converting enzyme
ACEi	angiotensin converting enzyme inhibitor
ACTH	adrenocorticothropic hormone
AT_1	angiotensin II receptor subtype type 1
ATN	acute tubular necrosis
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ARB	angiotensin receptor blocker
ARF	acute renal failure
BP	blood pressure
BW	body weight
cAMP	cyclic adenosine monophosphate
CEC	chloroethylclonidine
cGMP	cyclic guanine monophosphate
Ccr	creatinine clearance
CHF	congestive heart failure
CNS	central nervous system
CRF	chronic renal failure
Ctr1	copper transport system
ECF	extracellular fluid
eNOS	endothelial nitric oxide synthase
ESKD	end stage kidney disease
ESRF	end stage renal failure
et al.	and others
FE_{NA}	fractional excretion of sodium
g	gram

GFR	glomerular filtration rate
IL-1	interleukin-1
iNOS	inducible nitric oxide synthase
i.p.	intraperitoneally
i.v.	intravenously
IGF	insulin-like growth factor
IP_3	inositol triphosphate
JGC	juxtaglomerular cell
kg	kilogram
L-NAME	N(G)-nitro-L-arginine methyl ester
L/min	liter per minute
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase
MBF	medullary blood flow
MC	metabolic cage
ME	methoxamine
mg	milligram
$mg.dl^{-1}$	milligram per deciliter
$mg.kg^{-1}$	milligram per kilogram
ml	Milliliter
$ml.min^{-1}.kg^{-1}$	milliliter per minute per kilogram
mmHg	millimeter mercury
MR	myogenic response
mRNA	messenger ribonucleic acid
n	number of animals
NaCl	sodium chloride
NA	noradrenaline
NEP	neutral-endopeptidase
ng	nanogram
NO	nitric oxide
NOS	nitric oxide synthase
NRFSHRCONTROL	non-renal failure SHR (control)
NRFSHR6OHDA	6OHDA treated non-renal failure SHR
NRFSHRLOS	losartan treated non-renal failure SHR
NRFSHR6OHDALOS	6OHDA and losartan combination treated non-renal failure SHR
NRFWKYCONTROL	non-renal failure WKY (control)
NRFWKY6OHDA	6OHDA treated non-renal failure WKY
NRFWKYLOS	losartan treated non-renal failure WKY
NRFWKY6OHDALOS	6OHDA and losartan combination treated non-renal failure WKY

PE	phenylephrine
PNa	plasma sodium
PNS	peripheral nervous system
RAP	renal arterial pressure
RAS	renin angiotensin system
RBF	renal blood flow
RIFLE	Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage kidney disease
RF	Renal failure
RFSHRCONTROL	renal failure SHR (control)
RFSHR6OHDA	6OHDA treated renal failure SHR
RFSHRLOS	losartan treated renal failure SHR
RFSHR6OHDALOS	6OHDA and losartan combination treated renal failure SHR
RFWKYCONTROL	renal failure WKY (control)
RFWKY6OHDA	6OHDA treated renal failure WKY
RFWKYLOS	losartan treated renal failure WKY
RFWKY6OHDALOS	6OHDA and losartan combination treated renal failure WKY
RGD	Arg-Gly-Asp sequence
RNS	renal nerve stimulation
ROS	reactive oxygen species
RPP	renal perfusion pressure
RRT	renal replacement therapy
RSNA	renal sympathetic nerve activity
RT-PCR	reverse transcriptase-polymerase chain reaction
RVLM	rostral excitory region of the ventrolateral medulla
RVR	renal vascular resistance
S_3	segment of the proximal tubule and the thick ascending limb of the Henle loop
SCreat	serum creatinine
SHR	spontaneously hypertensive rat
SNS	sympathetic nervous system
TGF	tubuloglomerular feedback
TGF	tumor growth factor
UNa	urinary sodium
UO	urine output
WB 4101	2-(2,6-Dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxa ne hydrochloride
WI	water intake
WKY	Wistar Kyoto

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ABSTRAK

Adrenoseptor α_1 dan reseptor subjenis angiotensin jenis 1 (AT_1) memainkan peranan penting dalam pengawalaturan hemodinamik. Aktiviti simpatetik yang melampau ialah punca penyakit yang selalu menyebabkan kegagalan ginjal kepada komplikasi yang seterusnya. Kajian ini membincangkan peranan fungsi adrenoseptor α_1 dan reseptor subjenis angiotensin jenis 1 (AT_1) dalam pengantaraan pengecutan salur darah berintangan di dalam hemodinamik ginjal untuk penyakit kegagalan ginjal, hipertensi dan gabungan kedua-dua keadaan patologikal dengan penumpuan kepada interaksi antara sistem saraf simpatetik (SNS) dan sistem renin angiotensin (RAS). Tikus normotensif WKY dan hipertensi SHR telah digunakan. Kegagalan ginjal diaruhkan dengan cisplatin (5 mg/kg i.p). Tikus-tikus WKY dan SHR tanpa kegagalan ginjal dan kegagalan ginjal dibahagikan mengikut rawatan awalan masing-masing iaitu kawalan, denervasi oleh 6OHDA, losartan (10 mg/kg/day) (LOS) dan gabungan 6OHDA dan losartan (6OHDALOS). Losartan diberikan secara oral selama 7 hari sebelum kajian akut. Berat badan, jumlah air yang diminum, jumlah pengeluaran air kencing, kandungan natrium dan kreatinin dalam air kencing dan plasma, penyingkiran kreatinin, perkumuhan terpecah natrium dan indeks bacaan ginjal diukur. Dalam kajian akut, tikus-tikus ini dibiuskan dengan menggunakan natrium pentobarbiton (60 mg/kg i.p) untuk pengukuran tekanan darah dan pengaliran darah ginjal. Pengurangan pengaliran darah ginjal terhadap rangsangan elektrik saraf ginjal, pemberian noradrenalina, fenilefrina, metoksamina dan angiotensin II secara intra ginjal ditentukan. Data dirakam dengan menggunakan sistem perolehan data berkomputer dan diekspresikan sebagai purata ± s.e.m serta

dianalisakan dengan ANOVA dua hala diikuti dengan post-hoc Bonferroni pada tahap signifikasi 5 %. Pengurangan secara signifikan dalam berat badan dan jumlah air yang diminum, peningkatan dalam jumlah pengeluaran air kencing dan perkumuhan terpecah natrium serta pengurangan terhadap penyingkiran kreatinin telah didapati dalam tikus dengan kegagalan ginjal. Dari respon vasokonstriktor ginjal, saraf ginjal yang sempurna sangat penting dalam pengawalaturan hemodinamik ginjal. Didapati bahawa fungsi adrenoseptor α_{1B} / α_{1D} dalam interaksi silang positif dengan reseptor AT_1 dipengaruhi dengan kuat oleh simpatektomi dan losartan. Tambahan lagi, sekatan terhadap RAS telah menghasilkan interaksi positif dengan SNS. Kajian ini mengukuhkan lagi fakta yang menyatakan aktiviti simpatetik yang terlampau yang berlaku dalam tikus yang berpenyakit dan keadaan ini lebih teruk dalam tikus yang mempunyai penyakit berganda. Lagipun di bawah pengaruh kegagalan ginjal dan hipertensi, terdapat perubahan sumbangan fungsi subjenis adrenoseptor α_1 dalam keadaan aktiviti simpatetik yang terlampau. Kesimpulannya, kajian ini mencadangkan kemungkinan wujud hubungan silang yang positif antara adrenoseptor α_1 dan reseptor AT_1 dan hubungan ini amat dipengaruhi oleh keadaan patologi kegagalan ginjal dan hipertensi.

INTERACTION OF SYMPATHETIC NERVOUS SYSTEM AND RENIN ANGIOTENSIN SYSTEM IN RENAL HAEMODYNAMICS OF RENAL FAILURE, HYPERTENSIVE AND RENAL FAILURE HYPERTENSIVE RATS

ABSTRACT

α_1-adrenoceptors and angiotensin type 1 (AT_1) receptor subtypes play a key role in the regulation of renal haemodynamics. Sympathetic overactivity is the common pathogenesis that aggravates renal failure into further complications. This study discusses the functional role of α_1-adrenoceptors and AT_1 receptor in mediating the vasoconstriction of renal resistance vessels in renal haemodynamics of renal failure, hypertension and combination of both pathological states emphasizing on the interaction between sympathetic nervous system (SNS) and renin-angiotensin system (RAS). Normotensive WKY and hypertensive SHR were utilized. Cisplatin (5 mg/kg i.p) was used to induce renal failure. Non-renal failure and renal failure WKY and SHR were grouped according to their pre-treament which were control, denervation by 6-hydroxydopamine (6OHDA), losartan (10 mg/kg/day) (LOS) and a combination of losartan and 6OHDA (6OHDALOS). Losartan was given orally for 7 days prior to the acute study. Body weight, water intake, urine output, urine and plasma sodium, creatinine clearance, fractional excretion of sodium and kidney index were measured. In acute study, the animals were anesthetized (60 mg/kg i.p. sodium pentobarbitone) for blood pressure and renal blood flow (RBF) measurements. Reductions in RBF to electrical stimulation of renal nerve and intrarenal administration of noradrenaline, phenylephrine, methoxamine and angiotensin II were determined. Data were recorded using a computerized data acquisition system and expressed as mean ± s.e.m and analysed by 2-way ANOVA followed by Bonferronni post-hoc test with a significance level at 5%. Significant reductions in the body weight and water intake,

increased urine output and fractional excretion of sodium as well as a marked decrease in the creatinine clearance were observed in the renal failure rats. From the renal vasoconstrictor responses, an intact renal nerve is very important in regulation of renal haemodynamics. It seems that functionality of α_{1B} / α_{1D} adrenoceptors in the positive crosstalk with AT_1 receptor was greatly influenced by sympathectomy and losartan. Furthermore blockade of RAS produced a positive interaction with SNS. This study further supported that the fact there is exaggerated sympathetic activity in diseased animals and its severity increases in multiple diseased states. Moreover, under the influence of renal failure and hypertensive conditions, there was a shift in functional contributions of α_1 adrenoceptors subtype in enhanced sympathetic conditions. Collectively it is suggested that there was a positive crosstalk relationship between α_1- adrenoceptors and AT_1 receptors, and this is greatly influenced by the pathological conditions of renal failure and hypertension.

CHAPTER 1

LITERATURE REVIEW

1.1 The kidney

1.1.1 Basic anatomy

The kidneys are retroperitoneal organs, meaning they lie against the posterior abdominal wall just behind the peritoneum and can be found on each side of the spine, specifically located between 12^{th} thoracic and 3^{rd} lumbar vertebrae. They are partially secured by the lower ribs, closely held by a connective tissue renal fascia and is surrounded by a thick layer of adipose tissue called perirenal fat. The kidneys are roughly bean shape with an indentation called the hilum, where the renal artery enters while the renal vein and ureter leave the kidney. Kidney has a granular appearance. Each kidney is enclosed in a fibrous capsule and composed of cortex and inner medulla. The cortex and medulla form the parenchyma, which is the functional tissue of the kidney (Applegate, 2000).

The functional units in the kidney are nephrons. There are over a million nephrons in each kidney. Each nephron consists of a renal corpuscle, an initial filtering component and renal tubule. Kidneys' granular-like appearance is due to the renal corpucles that are located in the cortex of the kidney. The renal corpuscle filters blood free from cells and proteins by its component, the glomerulus, a compact tuft of interconnected capillary. The glomeruli are surrounded by a fluid-filled capsule called Bowman's capsule. Blood enters the glomerulus via afferent arterioles and leaves through efferent arterioles. As blood flows throughout the glomerulus, a portion of plasma is filtered into Bowman's capsule, which is continuous with the renal tubule. Reabsorbtion or excretion process occurs as the filtrate flows all the

way through the tubules. Ultimately, all the fluid from all the nephrons exit the kidneys as urine. The very narrow hollow renal tubules consist of three different regions which are proximal convoluted tubule, loop of Henle and distal convoluted tubule. Drains from the Bowman's capsule are the highly coiled proximal convoluted tubule. The next portion is loop of Henle which is the sharp hairpin-like loop consisting of a descending and ascending limb leading to the next tubular segment, the distal convoluted tubule (Applegate, 2000; Vander et al., 2001).

From the nephrons, urine flow into collecting duct. The distal convoluted tubules from various nephrons join with each collecting duct. All the way from Bowman's capsule to the collecting duct system, each nephron is completely separate from the others. This separation ends when multiple collecting ducts merge into the minor calyces that surround the renal papillae. Then the completed urine draws off into the kidney's central cavity, the renal pelvis that is continuous with ureter draining the contents into the bladder (Applegate, 2000; Vander et al., 2001).

1.1.1a Juxtaglomerular apparatus

Areas near the end of ascending limb of nephron loop that are continuous with distal convoluted tubule converge with glomerular afferent arterioles of that nephron. At this point the cells of the ascending limb are modified into macula densa and those in the wall of the afferent arteriole are modified to form the juxtaglomerular cells. The combination of both cells makes up the juxtaglomerular apparatus where their function is to secrete renin and monitors blood pressure (Applegate, 2000).

1.1.2 Renal functions

First and foremost, the kidneys are one of the decisive organs of fluid balance. This is because they precisely regulate very hard to keep the balance among the water concentration, inorganic-ion composition and volume of the internal environment in the body. Secondly, the kidney is responsible for removal of metabolic waste products such as urea, uric acid, creatinine and many others from the blood into the urine as quickly as they are produced. This is to prevent the toxic waste products from being accumulated in the body. Next is the elimination of the foreign chemicals like drugs, pesticides, food additives and their metabolites from the blood and urine. Fourth, kidneys are accountable for gluconeogenesis. Gluconeogenesis is a biochemical process where glucose, the source of energy synthesised from non-carbohydrate source like amino acids. Ultimately, the kidneys function as endocrine glands. They secrete three hormones. These are erythropoietin, renin and 1, 25-dihydroxyvitamin D_3 (Vander et al., 2001).

1.1.3 Renal haemodynamics

Blood flows through the kidney at an approximate rate of 1200 millimeters per minute. Renal arteries are branched from abdominal aorta. They carry about 20-25% of total resting cardiac output (5 L/min) into the kidney which is about 1.2 L/min (Murphy and Robinson, 2006). At the hilum, the renal arteries are divided into segmental arteries that pass through the renal sinus. Segmental arteries are bifurcated into interlobular arteries, traverse the renal column and split to form arcuate arteries, which pass over the base of the pyramids. Subsequently, arcuate arteries divide into interlobular arteries that extend to the cortex of the kidney and ascend to the afferent arterioles. From the afferent arterioles, the blood enters the capillaries in the

glomerulus and finally exit through efferent arterioles. The renal circulation consist of two sets of arterioles (afferent and efferent arterioles) and two sets of capillaries that are glomerulus and peritubular capillaries. The afferent arterioles are also known as preglomerular arterioles that divide into the tangled capillary network, glomerulus, which then coalesce to form the efferent arterioles (postglomerular arterioles). Next, each of efferent arteriole diverges to develop an extensive capillary network, called peritubular capillaries around the tubular region (proximal and distal convoluted tubules) of the nephron or form a long hairpin loop of capillaries, known as vasa rectae. Vasa rectae is another part of peritubular capillaries that loop deep down to medulla alongside the loop of Henle. Eventually, the peritubular capillaries reunite to form interlobular veins. From there, the blood flows through the arcuate veins, interlobular veins, segmental veins and into renal veins, which return the blood to the inferior vena cava (Ganong, 1999; Applegate, 2000). Approximately, 90% of the renal blood flow remains in the renal cortex and perfuses the peritubular capillaries. The remaining 10% of the blood flow perfuses the renal medulla via vasa rectae (Kriz, 1981; Zimmerhackl *et al*., 1987; Cupples *et al*., 1988).

Intrarenal haemodynamics determine the construction of glomerular filtrate, the reabsorbtion of fluid by peritubular capillaries and the maintenance of a hyperosmotic medullary environment (Arendshorst and Navar, 2001). There are several factors either intrinsic or extrinsic to the kidneys that regulate the renal blood flow. Those major mechanisms intrinsic to kidney are the autoregulatory mechanism, intrarenal renin-angiotensin mechanism, renal prostaglandin or eicosanoids and kinins while the extrinsic factors are such as circulating vasoactive agents namely nitric oxides (NO), purinergic agents such as ATP and adenosine, other peptide

hormones including endothelin and atrial natriuretic peptide (ANP) and renal sympathetic nerve activity (Arendshorst and Navar, 2001).

1.1.4 Renal autoregulation

Autoregulation can be experimentally defined by a rapidly-acting mechanism by the vascular bed to functionally maintain its perfusion constant from perturbation of blood pressure that may cause an acute change in renal blood flow (RBF) and glomerular filtration rate (GFR) (Loutzenhiser *et al*., 2006). This function is present in almost all tissues but is particularly pronounced in some organs, such as brain and kidney (Just, 2007). One of the most striking criteria of the renal circulation is the competency of the kidney to maintain a constant RBF and GFR as perfusion pressure fluctuates (Loutzenhiser *et al*., 2006). There is accrued evidence, which indicates that autoregulatory response plays a concurrent role in protecting the kidney from hypertensive injury (Bidani and Griffin, 2004; 2002). Evidence suggests that in the presence of intact autoregulation, less injury happens in spite of substantial hypertension. Conversely, susceptibility to hypertensive renal damage is greatly increased and injury with even moderate hypertension is seen when there is diminished autoregulatory response. Moreover, when the renal autoregulation is impaired, there is no evidence of disturbed volume regulation. This shows that intact renal autoregulation is not an obligate requirement for adequate volume control but it is important for normal renal protection (Loutzenhiser *et al*., 2006).

Autoregulation of renal blood flow (RBF) has long been recognized. RBF autoregulation is believed to be mediated by two mechanisms, the renal myogenic response (MR) and the tubulo-glomerular feedback (TGF). These two mechanisms

either act in concert or differently in their primary role to stabilize renal function by preventing pressure-induced fluctuations in RBF, GFR and the delivery of filtrate to the distal tubule (distal delivery). The current view is that when accentuation of blood pressure occurs, these two mechanisms act parri passu to achieve a precise regulation of GFR and RBF (Loutzenhiser *et al*., 2006).

Myogenic response (MR) involves a direct vasoconstriction of the afferent arteriole in response to stretching forces. A rise in intra-luminal pressure induces vasoconstriction in vascular smooth muscle. This effect prevails over the passive distension of the elastic vascular wall and also reduces the diameter of small resistance vessels below the one at lower pressure. This causes an increased vascular resistance at higher pressure, thus allowing autoregulation to take place (Just, 2007). It has been suggested that the primary purpose of MR is to protect the kidney against the damaging effects of hypertension. This is due to its kinetic attributes which allow the afferent arterioles to sense elevations in the rapidly oscillating systolic BP and adjust tone to this signal. Nevertheless this postulation deserves critical evaluation (Loutzenhiser *et al*., 2006).

Tubuloglomerular feedback (TGF) is a more complicated mechanism specific to the kidney. Macula densa senses a flow-dependent signal and alters tone in the adjacent segment of afferent arteriole via a mechanism that likely involves adenosine and/or ATP (Castrop *et al*., 2004(a); Insco *et al*., 2003; Sun *et al*., 2001). This leads the afferent arterioles to contract in response to an increase in the sodium chloride (NaCl) concentration at the macula densa in the early distal tubule (Komlosi *et al*., 2005; Schnermann *et al*., 1998). At the ascending part of Henle's Loop, the

reabsorption of salts is active and is a rate-limited process. The concentration of NaCl reaching the macula densa is dependent on the tubular flow rate. Enhanced tubular flow in response to an increase in arterial pressure raises the NaCl concentration at macula densa, thus causing vasoconstriction of afferent arteriole. These actions provide–restoration of filtration and autoregulation of RBF (Just, 2007).

It is thought today, perhaps a third regulatory mechanism, that is independent of TGF but slower than MR, also contributes to a smaller extent of role in mediating renal autoregulation (Just, 2007). However, much less is known about the contribution of the third additional mechanism. Since the detection of the third regulating mechanism has been complicated due to difficulty in eliminating MR without affecting TGF, Just and Arendshorst (2003) suggested an alternative approach, that is, by distinguishing between the responsible mechanisms based on their dynamics and relative contributions. In the experimental setup, continuous inhibition of TGF by furosemide, it can be seen that based on the response times, the primary increase in renal vascular resistance (RVR) is due to action of MR and secondary to TGF. It also establishes evidence for a slow third regulatory component (Just, 2007; Just and Arendshorst, 2003). However, the underlying nature of the third mechanism awaits further investigation although possibilities may include angiotensin II (Ang II) (Cupples, 1993), ATP acting on P2X1 and slow TGF-independent regulation reflecting a slow component of MR (Just, 2007). Several reports have suggested that the third regulatory element is more readily noticeable and more significant at lower pressures than at resting pressure levels which are relatively small and depending on the complete inhibition of TGF. This element may represent either a fading

vasodilator or an accumulating constricting substance with rising renal arterial pressure (RAP) (Just and Arendshorst, 2003). Other important findings were, in the absence of TGF, both the strength and speed of the MR were augmented indicating a negative interaction linking TGF and MR. The enhancement and its acceleration lead to a more rapid achievement of the steady-state level of autoregulation (Just and Arendshorst, 2003).

Other regulatory mechanisms may slightly play essential roles in RBF autoregulation which might impair rather than support the maintenance of a constant flow of blood by vascular bed. They also may impinge on overall renal function through modulation of MR or TGF (Just, 2007).

The relative contribution of the responsible mechanisms was estimated based on initial changes in renal vascular resistance (RVR) which represent that MR is considerably faster than TGF and the third regulatory mechanism (Just and Arendshorst, 2003). In the kidney vasculature, MR requires less than 10 seconds for completion of autoregulatory action. The total response time of TGF is 30-60 seconds and the third regulatory component may contribute 30-60 seconds for the overall autoregulation of RBF (Just, 2007). Consequently in the study by Just and Arendshorst (2003), they conclude RBF autoregulation is mediated by each of the triumvirate: MR, TGF, and the ill-defined third regulatory mechanism are 55%, 35-45% and 0-12% respectively in response to a rise in renal arterial pressure (RAP) whilst 73%, 18-27% and 0-9% correspondingly during response to falling RAP in euvolemic rat. The rationale behind this remains open for exploration. Due to differences in their response time (kinetic differences), the speed of the overall

autoregulation can get affected. Their relative contributions determine the size and range of pressure fluctuation reaching glomeruli, peritubular capillaries and medullary perfusion, which therefore will impact on filtration, reabsorption, and hypertensive renal damage (Just and Arendshorst, 2003).

The balance among the triumvirate contributing to RBF autoregulation can be influenced by the interaction between them and subjected to modulation, most likely by Ang II and nitric oxide (NO). A positive interaction has been observed where activation of TGF causes vasoconstriction on its own and induces the autoregulatory vasoconstrictor response of MR (Schnermann and Briggs, 1989). Whereas, inhibition of TGF might not only impair the autoregulation in the segment immediately affected by TGF but also in upstream part of the vascular tree (Moore and Casellas, 1990).

Ang II is a strong modulator of TGF. It is a very potent vasoconstrictor and has a strong effect on the baseline level of RBF. Yet the steady-state autoregulation is neither affected by Ang II infusion (Kiil *et al*., 1969) and antagonism by angiotensin converting enzyme inhibitor (ACEi) (Arendshorst and Finn, 1977; Hall *et al*., 1979; Persson *et al*., 1988) nor Ang II receptor antagonists (Hall *et al*., 1979; Persson *et al*., 1988). It also does not influence the balance of each triumvirate contribution of MR, TGF and the third regulatory mechanism (Just, 2007).

Nitric oxide (NO) strongly modulates TGF by its attenuating influence on the vasoconstrictor response elicited by high tubular perfusion rates or NaCl concentration. Nevertheless it does not affect TGF at low perfusion rates (Ito and

Ren, 1993). There is evidence that the speed of MR is accelerated in the absence of NO (Just and Arendshorst, 2005). The nature of this underlying mechanism is still in ambiguity. For the predominance of MR during inhibition of NO, it is suggested that MR might naturally dominate the autoregulatory function due to the speed and upstream location to TGF and the third regulatory mechanism. Therefore, it can minimize any error signal from reaching the latter mechanisms (Just, 2007). Despite a strong and continuous vasodilator influence of endogenous NO on baseline RBF, steady-state autoregulation is typically not affected by inhibition of NO production (Baumann *et al*., 1992; Beierwaltes *et al*., 1992; Majid and Navar, 1992).

In summary, RBF autoregulation is primarily mediated by rapid MR and TGF, contributing ~50% and 35-50% respectively and even more sluggish third regulatory mechanism appears to contribute < 15% at resting arterial pressure (Just, 2007; Just and Arendshorst, 2003).

1.2 Acute renal failure

Acute renal failure (ARF) can be characterized as an abrupt deterioration of the glomerular filtration rate (GFR) over a period of hours to days resulting in the failure of the kidney to excrete neither nitrogenous (urea and creatinine) nor non-nitrogenous waste products and to maintain fluid and electrolytes homeostasis (Lameire *et al*., 2005).

Acute renal failure (ARF) is a common complication of critical illness and occurs anywhere depending on the population being studied and the criteria used to define its presence. Due to extensively contrasting definitions of ARF, trials of prevention and therapies are not parallel and hence, have complicated the research about ARF (Bellomo *et al*., 2004; Mehta *et al*., 2007). It is increasingly recognized to be a broad clinical entity rather than a specific diagnosis (Murphy and Robinson, 2006). There are more than 30 separate definition of ARF found in the literature that is objectionable. ARF definition can range from severe to slight increases in serum creatinine concentration. Therefore a practical definition of ARF is acute and sustained increase in serum creatinine concentration of 44.2 μmol/ L if the baseline is less than 221 μmol/ L, or an increase in serum creatinine concentration of more than 20% if the baseline is more than 221 μmol/ L (Singri *et al*., 2003).

A multilevel definition and classification in a recent attempt at defining renal failure has been advocated by the Acute Dialysis Quality Initiative group viz. the RIFLE system (http://www.ccforum.com/content/8/4/R204) (Figure 1.1), which uses a combination of creatinine and urine output compared with baseline measurements. These measurements define three severity categories whether the patient is at risk,

has an injury, has failure and two clinical outcomes loss of function, or is at end-stage kidney disease (ESKD). The purpose of this is to acknowledge the important adaptations that happen in ESKD but not in persistent ARF. Persistent ARF (loss) mean necessitation for renal replacement therapy (RRT) for more than 4 weeks whilst ESKD is defined by dialysis requirement for longer than 3 months (Bellomo *et al*., 2004).

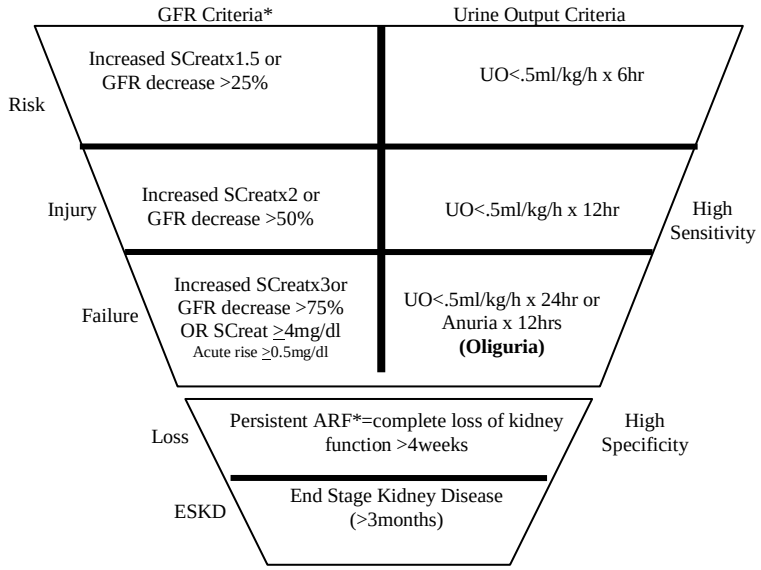

Figure 1.1: Proposed classification scheme for acute renal failure (ARF). The classification system includes separate criteria for creatinine and urine output (UO). A patient can fulfill the criteria through changes in serum creatinine (SCreat) or changes in UO, or both. The criteria that lead to the worst possible classification should be used. Note that the F component of RIFLE (Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage kidney disease) is present even if the increase in SCreat is under threefold as long as the new SCreat is greater than 4.0 mg/dl (350 μmol/l) in the setting of an acute increase of at least 0.5 mg/dl (44 μmol/l). The designation RIFLE-F_C should be used in this case to denote 'acute-on chronic' disease. Similarly, when the RIFLE-F classification is achieved by UO criteria, a designation of RIFLE-F_O should be used to denote oliguria. The shape of the figure denotes the fact that more patients (high sensitivity) will be included in the mild category, including some without actually having renal failure (less specificity). In contrast, at the bottom of the figure the criteria are strict and therefore specific, but some patients will be missed. *GFR = Glomerular Filtration Rate; ARF= Acute Renal Failure (Adapted from Bellomo *et al*., 2004).

Traditionally, classifications of ARF aetiologies have been identified as prerenal, postrenal and intrinsic renal azotaemia (Figure 1.2). Prerenal azotaemia (acute prerenal failure) is a physiological response to renal hypoperfusion which leads to a reduction in GFR. In the prerenal form, there is a reversible increase in serum creatinine and blood urea concentrations. It contributes 30-60% of all cases of ARF and is frequently community-acquired especially affecting aged population. In this acute prerenal failure, the integrity of the renal tissue is preserved. However it can complicate any disease characterized by either true hypovolaemia or a reduction in the effective circulating volume viz. low cardiac output, systemic vasodilation or intrarenal vasoconstriction (Lameire *et al*., 2005). Persistent renal hypoperfusion may progress into ischemic acute tubular necrosis. Prerenal azotaemia and ischemic acute tubular necrosis are part of a continuum of renal hypoperfusion and together account for 75% of the cases of acute renal failure (Lameire *et al*., 2005; 2004).

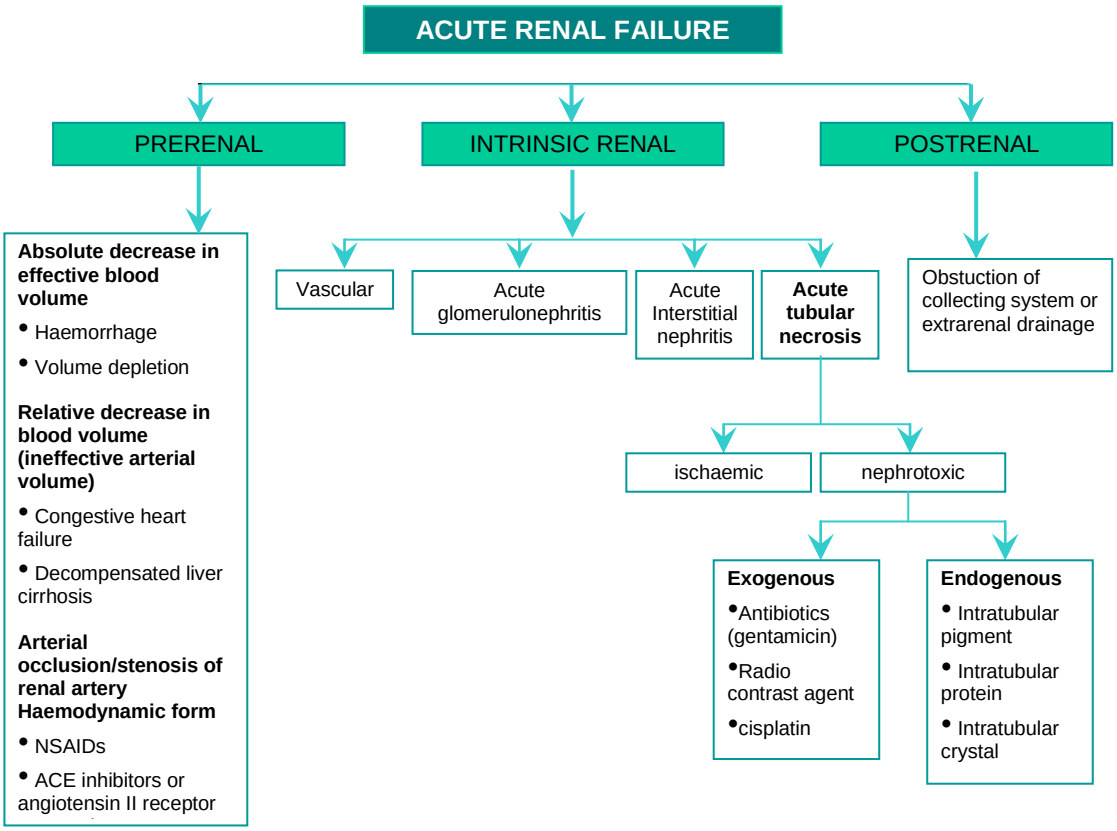

Figure 1.2: Classification and major causes of acute renal failure. NSAIDs = non-steroidal anti-inflammatory agents, ACE = angiotensin converting enzymes (Adapted from Lameire *et al*., 2005).

Postrenal azotaemia (postrenal acute renal failure) occurs when there is obstruction of the urinary collection system or extrarenal drainage by either intrinsic or extrinsic masses. Obstructive uropathy particularly affects older men with prostatic disease and patients with a single kidney or intra-abdominal cancer particularly pelvic cancer (Bhandari *et al*., 1995; Chapman *et al*., 1991). The vital sequelae of postrenal acute renal failure are the post obstructive diuresis and the presence of hyperkalaemic renal tubular acidosis (Yarger, 1992). Moreover, postrenal ARF only account 1-10% of hospital-acquired ARF (Lameire *et al*., 2005; 2004).

The major cause of intrinsic renal azotaemia is acute tubular necrosis (ATN) although acute vascular, glomerular and interstitial processes may also cause intrinsic

ARF. ATN is caused by ischemic or nephrotoxic injury to the kidney in 50% and 35% of all hospital- acquired ARF respectively. There is 30-50% established decrease of RBF in clinical ATN. The pathophysiology of ATN involves the vascular and tubular components (Lameire, 2005).

1.2.1 Vascular component

In most of experimental animals, acute ischemic injury has been shown to be associated with a loss of renal autoregulation (Conger *et al*., 1988). In normal autoregulatory response, renal vasodilatation by the vasodilating product of arachidonic acid (prostaglandin) and nitric oxide will try to counterattack with the decrease in renal perfusion pressure. However the ischemic kidney is associated with renal vasoconstriction (Schrier, 2004). Renal vasoconstriction occurs as a result of an increase in afferent and efferent arteriolar vascular resistance, reduced glomerular plasma flow and a decrease in glomerular hydrostatic pressure (Conger, 2001). Enhanced renal sympathetic tone has been observed in the setting of ischemic and nephrotoxic ATN. Moreover, the vasoconstrictor response to exogenous norepinephrine and circulating vasoconstrictors such as catecholamine, angiotensin II and endothelin has been shown to be augmented in the acute ischemic insult. These renal vascular abnormalities are related to the resultant increase in cytosolic calcium observed in the afferent arterioles of the glomerulus. Therefore, administration of calcium channel blockers may reverse the loss of autoregulation, thus reducing the renal dysfunction of the acute ischemic kidney (Conger *et al*., 1988).

Outer medullary congestion of the kidney is another vascular hallmark of acute renal ischaemia by worsening the relative hypoxia in the outer medulla ($S_{3,}$ segment of the

proximal tubule and the thick ascending limb of the loop of Henle) (Mason *et al*., 1984). Endothelial damage due to increase oxidant injury is also associated with acute renal ischemia which eventually may enhance the renal vasoconstrictor effect of circulating pressor agents present in ARF. Oxidant injury results in decreased endothelial nitric oxide synthase (eNOS) and prostaglandins but leads to increase in endothelin (Molitoris *et al*., 2002). The defence action by vasodilating pharmacological manoeuvres returns the renal blood flow to normal level but GFR continues to fall (Lameire and Vanholder, 2004; Schrier *et al*., 2004).

1.2.2 Tubular component

There are three aspects of tubular abnormalities: structural changes, tubular obstruction and tubuloglomerular balance and tubular fluid backleak in ischemic acute renal failure. As for structural changes, ARF that is characterized by tubular dysfunction with impaired sodium and water reabsorption is correlated with the shedding and excretion of proximal tubule brush border membranes and epithelial tubule cells into the urine (Thadani *et al*., 1996). Abnormalities in the proximal tubule cytoskeleton are associated with translocation of Na+/K+-ATPase from the basolateral to the apical membrane. This has been shown by *in vitro* studies using chemical anoxia (Molitoris *et al*., 1989). Na+/K+-ATPase facilitates vectorial sodium transport, thus its translocation by hypoxia or ischemia impedes the tubular sodium reabsorption in ARF (Schrier *et al*., 2004).

There are potential pathways through which the loss of brush border membranes, loss of viable and non-viable proximal tubule cells and less proximal tubule sodium reabsorption may lead to diminished GFR during ARF. Brush borders that detach

from the basement membrane and the cellular debris may contribute to the intraluminal aggregation of cells and protein resulting in tubular obstruction (Thadani *et al*., 1996) (Figure 1.3). The occurrence of the obstructing cast may explain the dilation of the tubules including collecting duct that have been revealed upon the renal biopsy of ARF kidneys albeit GFR is less than 10% of normal (Schrier *et al*., 2004). However, this remains open for discussion whether tubular obstruction by cast is alone sufficient in reducing GRF correlated with clinical ARF. Moreover, some micropuncture studies have shown that with normal tubular and glomerular pressure, formerly obstructing luminal cast can be dislodged by the proximal tubular flow rate in a single nephron whilst improved GFR in the same nephron (Conger *et al*., 1984). Besides, during ischemic insult the cellular adhesions of viable cells into the other tubular cells and extracellular matrix also happen to cause tubular obstruction. This adherence involving integrin-mediated adhesion molecules via binding to Arg-Gly-Asp (RGD) sequences. Thereby lesser tubular obstruction was seen and increase in proximal tubular pressure has been reversed in reperfusion period after synthetic cyclical RGD being induced (Noiri *et al*., 1994).

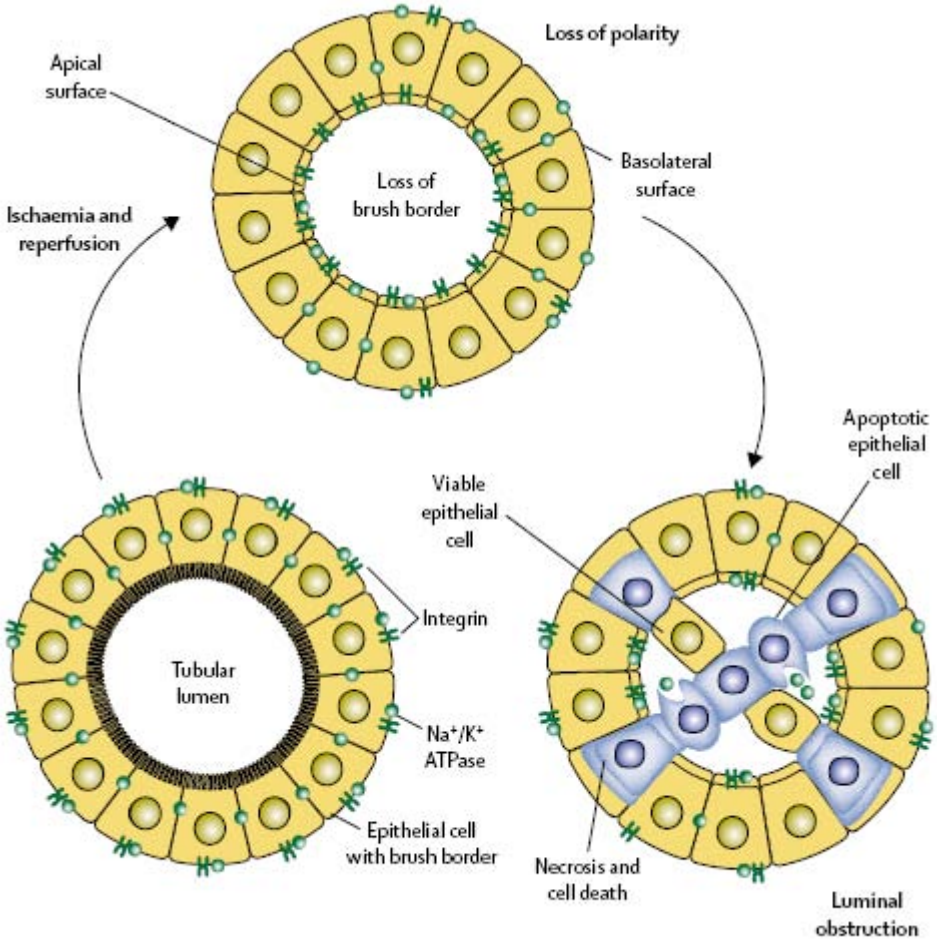

Figure 1.3: Tubular changes in the pathophysiology of ischaemic acute tubular necrosis. After ischaemia and reperfusion, morphological changes occur in the proximal tubules, including loss of polarity, loss of the brush border, and redistribution of integrins and sodium/potassium ATPase to the apical surface. Calcium and reactive oxygen species also have roles in these morphological changes, in addition to subsequent cell death resulting from necrosis and apoptosis. Both viable and non-viable cells are shed into the tubular lumen, resulting in the formation of casts and luminal obstruction and contributing to the reduction in the GFR (Reproduced with permission from Thadani *et al*., 1996).

Based on foregoing discussion of renal autoregulation, TGF will be activated when there is any alteration in the sodium chloride (NaCl) delivery at the macula densa. In acute ischemic kidney, less proximal tubular sodium reabsorption will increase the NaCl concentration to the macula densa, thus allowing the vasoconstriction of the glomerular arteriole which enhances the sensitivity of TGF and decreases GFR in patients with clinical ARF (Schnermann, 2003). An abrupt fall of GFR in ARF can

be explained by the combination of both tubular cast formation and activation of the tubuloglomerular feedback mechanism that is concurrent to the ARF-related decrease in proximal tubular sodium reabsorption. Positively, decrease in GFR while ischemic insult attenuates the demand for ATP-dependent tubular reabsorption due to less NaCl released to damage tubules (Schrier *et al*., 2004).

Tubular fluid backleak into the circulation can occur as a result of loss of tubular epithelial cell barrier and/or the tight junctions between viable cells in acutely ischemic kidney (Molitoris *et al*., 1989) thus providing false interpretation of low GFR especially to non-reabsorbable substances such as inulin in inulin clearance. However, glomerular filtrates seep out rarely observed with clinical ARF in human except in cadaveric transplanted kidneys with delayed graft function (Edelstein and Schrier, 2001).

Inflammation has a major role in the pathogenesis of decreased GFR associated with ischemic ARF (Lameire *et al*., 2005; Schrier *et al*., 2004). Contribution of inducible nitric oxide synthase (iNOS) in ARF has been experimentally approved in Western Blot analysis of ischemic kidney homogenates where there is a profound increase in the iNOS protein expression. Moreover blockage of the upregulation of iNOS by the antisense oligonucleotide was shown to protect the kidney from ischemic insult (Noiri *et al*., 1996). Peroxynitrite as a product of NO scavenging by oxygen radicals can cause tubular damages during ischemia (Noiri *et al*., 2001).

The other causes of acute renal failure include sepsis, hypovolaemia, pre-existing renal impairment, and nephrotoxins such as aminoglycoside antibiotics and radiological contrast agents (Uchino *et al*., 2005; 2004)

Acute renal failure is associated with a significant risk of mortality and morbidity. Due to modern renal replacement modalities, there is an insistent belief that acute renal failure (ARF) presents a rather harmless complication and that survival is determined by the severity of the underlying disease process/accompanying complications but not by renal dysfunction per se. However the evidence from the experimental and recent research shows the opposite outcomes where ARF presents a condition which exerts a fundamental impact on the course of the disease, the advancement of associated complications and on prognosis, independently from the type and severity of the underlying disease (Druml, 2004). ARF carries an independent risk of death that patients are rather dying "of" than "with" ARF (Kellum and Angus, 2002).

ARF is not restricted to kidney disease only, but it is a systemic disease that affects all physiologic functions and organ systems of the body (Druml, 2004). Systemic effects of ARF are manifolds. After several hour of ARF induction, there is increase in the gene expression in experimental animals, non-renal tissues and other organs e.g. lung, activation of circulating immunocompetent cells (Rabb *et al*., 2000) and increase in vascular-permeability for proteins and alveolar micro-haemorrhage mediated by neutrophils (Kramer *et al*., 1999). Furthermore, ARF can result in pulmonary oedema, increase levels of tumour necrosis factor (TNF)-alpha, interleukin-1 (IL-1) and intercellular adhesion molecule-1 mRNA in the heart

associated with functional changes in the heart 24 hours after renal ischemia such as increase in the left ventricular end-diastolic and systolic diameter. Even unilateral renal ischemia causes inflammation and injury in the contralateral kidney (Meldrum *et al*., 2002).

Table 1.1: Pathophysiologic consequences of acute renal failure.

Cardiovascular	Hypercirculation, cardiomyopathy, pericarditis
Pulmonary	Lung edema, alveolitis, pneumonia, pulmonary hemorrhage
Gastrointestinal	Impairment of motility, erosions, ulcerations, hemorrhage, pancreatitis, colitis
Neuromuscular	Neuropathy, myopathy, encelopathy
Immunologic	Impairment of humoral and cellular immunity and immunocompetence
Hematologic	Anemia, thrombocytopenia hemorrhagic diathesis
Metabolic	Insulin resistance, hyperlipidaemia, activation of protein catabolism, depletion of antioxidants

(Adapted from Druml, 2004)

These multiple systemic consequences of ARF (Table 1.1) are mediated by the acutely uremic state per se ("uremic intoxication"), by immunomodulatory effects radiating from the injured organ kidney and by side effects associated with renal replacement therapies. The kidneys in ARF initially are mostly "victims" of a systemic disease process, such as a shock state or sepsis. Nevertheless, as the acutely uremic state induces negative repercussions on the organism, the kidneys become "offenders" (Druml, 2004).

1.2.3 Cisplatin induced acute renal failure

Cisplatin is one of the most remarkable successes in 'the war on cancer.' It is the most potent chemotherapeutic drug and the most widely used for the treatment of several human malignancies. It reveals one of the highest cure rates, over 90% in testicular cancers. Cisplatin-based combination chemotherapy regimens and related platinum-based therapeutics are currently used as front-line therapy in the treatment of testicular cancer, head and neck, ovarian, cervical, bladder, non-small cell lung carcinoma, and many other types of cancer (Pabla and Dong, 2008; Wang and Lippard, 2005; Cohen and Lippard, 2001; Arany and Safirstein, 2003; Siddik, 2003). Escalation of its dose significantly improves its therapeutic effects. However, to maximize its antineoplastic effects by using high-dose therapy with cisplatin is hindered due to its cumulative side effects in normal tissues and organs, notably its nephrotoxicity in the kidneys (O'Dwyer *et al*., 1999). Its use is mainly limited by two factors: acquired resistance to cisplatin and severe side effects in normal tissues, which include neurotoxicity, ototoxicity, nausea, hearing loss, vomiting, and nephrotoxicity (Loehrer and Einhorn, 1984; Ward and Fauvie, 1977; Pabla and Dong, 2008). Still, cisplatin is the drug of choice in many platinum-based therapy regimens and remains one of the most regularly used chemotherapeutic drugs (Hanigan and Devarajan, 2003).

It has long been recognized that nephrotoxicity induced by cisplatin can result in severe nephropathy leading to acute renal failure (Thadani *et al*., 1996; Kang *et al*., 2004; Kawai *et al*., 2006). Prevalence of cisplatin nephrotoxicity is high, occurring in about one-third of patient whereby having transient elevation of blood urea nitrogen levels or other evidence of kidney damage in the days following cisplatin treatment

(Meyer and Madias, 1994; Beyer *et al*., 1997; Arany and Safirstein, 2003). Within 48–72 hours of cisplatin administration, decreased glomerular filtration rate has been shown (Winston and Safirstein, 1985). Clinically, cisplatin nephrotoxicity is often seen after 10 days of cisplatin administration. It is manifested as severe reduction in the glomerular filtration rate, higher serum creatinine, severe reduction in the creatinine clearance, increased fractional excretion of sodium, increased kidney index, a variable fall in the renal blood flow and reduced serum magnesium and potassium levels, (Kang *et al*., 2004; Lameire, 2005; Arany and Safirstein, 2003). The long-term effects of cisplatin on renal function may lead to subclinical but permanent reduction in glomerular filtration rate (Brillet, 1994).

Cisplatin is known to accumulate in mitochondria of renal epithelial cells (Singh 1989; Gemba and Fukuishi, 1991). Consequently, complex signaling pathways will be activated when tubular cells are exposed to cisplatin, thus leading to tubular cell injury and death. Meanwhile, stimulated robust inflammatory response occurs, further exacerbating renal tissue damage. Cisplatin may also induce injury in renal vasculature and result in decreased blood flow and ischemic injury of the kidneys, contributing to a decline in glomerular filtration rate. These events act in concert, culminating in the loss of renal function during cisplatin nephrotoxicity and triggering acute renal failure (Figure 1.4).

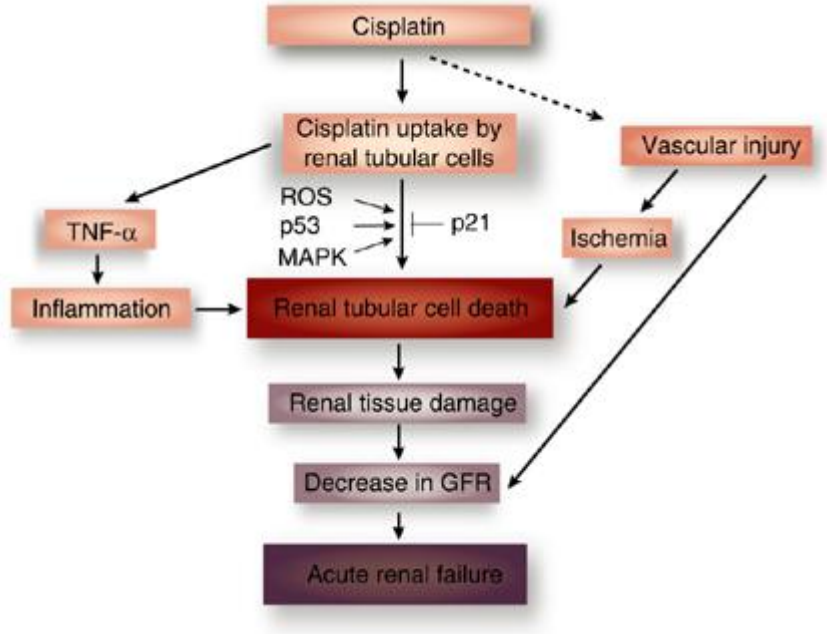

Figure 1.4: Overview of the pathophysiological events in cisplatin nephrotoxicity. Cisplatin enters renal cells by passive and/or facilitated mechanisms. Exposure of tubular cells to cisplatin activates signaling pathways that are cell death promoting (MAPK, p53, ROS, and so on) or cytoprotective (p21). Meanwhile, cisplatin induces TNF-α production in tubular cells, which triggers a robust inflammatory response, further contributing to tubular cell injury and death. Cisplatin may also induce injury in renal vasculature, leading to ischemic tubular cell death and decreased glomerular filtration rate (GFR). Together, these pathological events culminate in acute renal failure (Adapted from Pabla and Dong, 2008).

The acute renal failure caused by cisplatin in rat exhibits alterations in renal tubular epithelial structure. Renal tubular cells suffer a continuum of cytotoxic injuries, ranging from mild sublethal changes to a catastrophic necrotic death characterized by swelling and rupture of cells and activation of an inflammatory response (Thadani *et al*. 1996). There are at least two distinct mechanisms that may be responsible for renal tubular cell death: cell death in the form of both necrosis and apoptosis (Pabla and Dong, 2008). It is suggested that the dosage of cisplatin might determine whether the cells die due to necrosis or apoptosis. While extensive injury with a high concentration of cisplatin (millimolar) can lead to necrotic cell death, less severe renal injuries associated with lower concentrations of cisplatin (micromolar) leads to