CHAPTER V
DISCUSSION

Discussion is expressed in relation to the heading topics as follow.

Oxidant and Antioxidant Imbalance Induce Endothelial Cell Cytotoxicity

Reactive oxygen species namely superoxide anion, hydrogen peroxide, peroxynitrate etc are implicated in cell signaling, gene transcription, mitosis, apoptosis and vasoconstriction[55,56]. The cellular sources of reactive oxygen species are multiple namely NADPH oxidase, lipoxygenases, cyclooxygenase from plasma membrane, electron transport system from mitochondria, xanthine oxidase, hemoglobin, transition metals ($FE^{2+\ 3+}$, $Cu^{1+\ 2+}$) from cytosol and cytochrome P-450 from endoplasmic reticulum[57]. Increased cellular metabolism with enhanced production of reactive oxygen species have been delineated in a variety of glomerulonephropathies and renal failure[58-59]. This study indicates that both increased reactive oxygen species and decreased antioxidant defense have been substantiated in our renal patients. Increased plasma MDA and erythrocyte MDA in conjunction with a depleted plasma glutathione and vitamin C and a reverse ratio of oxidant and antioxidant imply

that there is an oxidant/antioxidant imbalanced state. Similar observation in chronic renal failure have also been reported[58-60]. In excess, reactive oxygen species and their by products are capable of causing oxidative damage and cytotoxicity to cells. This results in increased oxidized LDL, oxidative metabolic products of carbohydrates, fat and protein[61], as well as liberation of eisosanoids[62], chemokine, cytokine, growth factor[63], intercellular adhesion molecule (ICAM)[64] and activation of transcription factor nuclear factor kappa B[65]. In the presence of antioxidant imbalance, the defective antioxidant would allow the excessively generated reactive oxygen species to induce a sustained oxidative damage to cells in particular the endothelial cells which are optimally situated at the interface between the circulating blood and the vessel wall to serve as a sensor and tranducer of signals within the circulatory microenvironment. The oxidant and antioxidant imbalance observed in this study is likely to explain the invitro increased endothelial cell cytotoxicity induced by sera of nephrotic patients[66-67]. Increased oxidative stress to the glomerular endothelial cell would induce dysfunctioning of the endothelial cell and in excessive amount incriminate in endothelial cell death.

Glomerular Endothelial Dysfunction, Hemodynamic Maladjustment and Renal Tissue Injury

In response to such oxidative injury, the glomerular endothelial cell would increase productions of vasoconstrictive substances namely angiotensin II, endothelin and thromboxane A_2 whereas the production of endothelium dependent vasodilator such as nitric oxide is defective as well as being neutralized by the excessive amount of reactive oxygen species. The intrarenal hemodynamic study which revealed an elevated renal arteriolar resistance in these nephrotic patients does support this preceding notation. Such a provasoconstrictive state would induce a hemodynamic maladjustment with a predominant vasoconstriction at the efferent arteriole (increased filtration fraction ratio between GFR and RPF to above 0.2). The preponderant vasoconstriction at the efferent arteriole not only increases the intraglomerular hydrostatic pressure but also exaggeratedly reduces the peritubular capillary flow which supplies the tubulointerstitial compartment.(Figures1,2) The hemodynamic study indicates that there is indeed an increase in intraglomerular hydrostatic pressure to 55±2 mm Hg (normal ≤ 53 mm Hg) implicating an intraglomerular hypertension The presence of intraglomerular hypertension in conjunction with the reduction in renal plasma flow (mean 178±73 ml/min/1.73m^2) and with the hemorheologic alteration secondary to the additive effect of oxidative

stress to the endothelial cell inducing vascular inflammatory gene expression such as vascular adhesion molecule, mononuclear cell infiltration and procoagulant surface expression; would culminate in the glomerular cells injury inducing glomerulosclerosis[32,68-70].

The reduction in peritubular capillary flow secondary to the hemodynamic maladjustment at the efferent arteriole exerts a significant hemodynamic impact upon the tubulointerstitial structure. It is of notion that a simulated ischemia in flow-adapted endothelial cells lead to generation of reactive oxygen species and cell signaling through the NADPH oxidase pathway[69]. This is followed by an increased production of nuclear factor-kappa B with then upregulates the inflammatory gene expressions namely cytokines, growth factors and adhesion molecules[68-70]. A sustained reduction in peritubular capillary flow in conjunction with the oxidative stress would therefore, induce an ischemic injury to the tubulointerstitial structure and the development of tubulointerstitial fibrosis. In this regard, increased production of nuclear factor-kappa B was detected in nephrosis associated with focal glomerulosclerosis[71] and increased chemokine expression was also demonstrated in puromycin aminonucleoside nephrosis[72]. It has also recently been demonstrated that (1) there is an inverse correlation between the reducton in peritubular capillary flow and the incidence of tubulointerstitial fibrosis. That (2) the reduction in peritubular capillary flow precedes the development of

tubulointerstitial fibrosis[39]. In addition, we have recently demonstrated that there is also a correlation between the renal perfusion and the tubular function determined by the fractional excretion of filtered magnesium (FE Mg)[73]. Thus, this preceding information has emphasized the important role of glomerular endothelial injury and its hemodynamic impact that induces a secondarily ischemic injury to the tubulointerstitial structure and function. This in fact forms a concept of hemodynamically mediated mechanism of renal tissue injury and progression of renal disease which is quite contrast to the general consensus which believes in the primary trigger of injury to the tubulointerstitial structure and therefore the glomerular endothelial and microvascular injuries are the secondary event[74].

Therapy with Antioxidant and Vasodilator

The preceding information renders a supportive view that the oxidative stress and antioxidant defect is likely to be responsible for the endothelial cell cytotoxicity and a spontaneous glomerular endothelial cell dysfunction with impaired release of vasodilator and subsequent reduction in renal perfusion observed in nephrotic patient by which it induces nephronal damage and renal disease progression. In accordance with the therapeutic strategy, a correction of antioxidant defect in conjunction with the administration

of vasodilator[75] to correct the hemodynamic maladjustment would likely improve the renal perfusion and prevent the progression of renal disease. Based upon this therapeutically strategic approach, the administration of antioxidants namely vitamin C and vitamin E restores the antioxidant status toward normal. It has been a general consensus that vitamin C is capable of neutralizing superoxide anion, reactive oxygen species such as peroxynitrite, nitrogen dioxide and also acts as a coantioxidant by regenerating α-tocopherol (vitamin E) from the α-tocopherol radical[76-78]. Vitamin C has also been shown to regenerate glutathione and β-carotene in vitro from respective one-electron oxidation product[75-79]. Another major property that makes vitamin C such an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species[80]. The impaired endothelium dependent vasodilation was markedly improve by vitamin C in essential hypertension[81].

In respect to vitamin E, a combined vitamin E and selenium or glutathione deficiency leads to pronounced and progressive oxidative damage to renal structure and function[82-84]. Increasing dietary vitamin E level significantly attenuates renal oxidative damage in the puromycin nephrotoxicity model of focal segmental glomerulosclerosis in the rat[85-87]. Therefore, both vitamins C and E administration would assist in neutralizing the reactive oxygen

species and thereby minimizing the tissue damage by oxidative stress. Such an event would spare the vasodilating status of nitric oxide (NO). Increased available NO would exert a cellular protection to the nephronal structure as well as to the glomerular endothelial cell. A decreased endothelial cell cytotoxicity was demonstrated following the therapeutic administration of antioxidants and vasodilator. (Table 8 and Figure 4). In respect to the renal function, the therapeutic regimen reduced the renal arteriolar resistance. The relaxation of efferent arteriole not only reduced the intraglomerular hydrostatic pressure (PG 51±1 mm Hg), but also enhanced the peritubular capillary flow. The peritubular capillary flow increased from 144±67 ml/min/1.73m^2 to 284±63 ml/min/1.73m^2 following treatment (Table 5 and Figure 2). The improvement in renal perfusion correlated with the glomerular function as well as the tubular function. The creatinine clearance increased from 44.8±24 ml/min/1.73m^2 to 60.6±34 ml/min/1.73m^2 , the glomerular filtration rate increased from 34±13 ml/min/1.73m^2 to 62±16 ml/min/1.73m^2 and the FE Mg significantly reduced from 7±2% to 5.7±3 following treatment; $p < 0.05$. Therefore, the success of this specific treatment with antioxidant and vasodilator in relevant to the improvement in renal functions (vascular + glomerular + tubular) is quite contrast to the natural course of this disease in severe nephrosis under conventional therapy wherein there is a progressive reduction in renal perfusion, a progressive decline in creatinine clearance and a progressive increase in FE Mg.[88-92]