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CLINICAL STUDY

PERITUBULAR CAPILLARY FLOW DETERMINES TUBULOINTERSTITIAL DISEASE IN IDIOPATHIC NEPHROTIC SYNDROME

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ABSTRACT

The spatial relationship between renal perfusion and nephronal structure was determined in 51 nephrotic patients consisting of 11 patients with steroid sensitive, minimal change (MC) nephrosis, 12 patients with steroid resistant, mesangial proliferative (MesP) nephrosis and without tubulointerstitial fibrosis (TIF), 11 patients with steroid resistant, MesP nephrosis and with low grade TIF and 17 patients with focal segmental glomerulosclerosis (FSGS). The intrarenal hemodynamic study revealed a unique correlation between renal perfusion and nephronal structure. A normal or slight reduction in peritubular capillary flow observed in MC or mild MesP nephrosis correlates with an intact tubulointerstitial structure. A moderate reduction in peritubular capillary flow observed in steroid resistant, MesP nephrosis induces a low incidence of TIF. A severe reduction in peritubular capillary flow denotes a higher incidence of TIF as that observed in nephrosis with FSGS. Thus, it is of notion that the reduction in renal perfusion precedes the development of tubulo-

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$$\text{Body surface area} = \frac{\text{body weight (kg)} \times 4 + 7}{90 + \text{body weight (kg)}}$$

Vascular Function (Hemodynamic Assessment)

Simultaneous assessments of effective renal plasma flow (RPF) using ^{131}I-labeled orthoiodohippuric acid (hippuran) and of glomerular filtration rate (GFR) using ^{99m}Tc-labeled DTPA were determined by injecting the labeled materials intravenously into the left antecubital vein at zero time. A peritubular capillary flow (PTCF) is derived from the substraction of glomerular filtration rate from renal plasma flow.

Renal Histopathologic Study

The morphometric analysis was performed on the renal biopsied specimens by the method described elsewhere[4]. In brief, the kidney tissue was fixed in 4% buffered formalin and embedded in paraffin. Sections (2μm) were prepared and stained with haematoxylin-eosin, periodic acid Schiff reagent (PAS), silver methenamine and Massons Trichrome. At least eight serial sections were prepared from each case and examined. The number of glomeruli varied from 10 to 35. The tubulointerstitial fibrosis (TIF) was quantitated in a single blind fashion by a pathologist who had no information regarding the hemodynamic value of each individual. The widening of interstitium was assessed by a point-count technique using a counting grid. This manner would cover most area in the renal cortex which was ascertained by determining at ×100 magnification and the result obtained was expressed as percent.

STATISTICAL ANALYSIS

Comparison of the sample mean of two quantitative variables was determined by the non-parametric method using the Mann-Whitney test. The difference between groups was performed by Student's unpaired t-test. The linear regression analysis was used to correlate two quantitative variables. The scatter plot was the first step to correlate between two continuous variables. The Pearson correlation coefficient (r) was used to quantify the strength of the linear relationship. The method of least square was calculated to estimate the regression equation ($y = a \pm bx$) if the scatter plot seemed to be linear. Some relationship of the scatter plot data which had shown to be curvilinear, was further analysed to meet the criteria of straight line. P values below 0.05 were considered to be significant.

Table 1. Table 1 depicts the correlation between renal perfusion and tubulointerstitial structure. GFR = glomerular filtration rate, RPF = renal plasma flow, PTCF = peritubular capillary flow, TIF = tubulointerstitial fibrosis, MC-NS = minimal change nephrosis, MesP-NS = mesangial proliferative nephrosis, NS-FSGS = nephrosis associated with focal segmental glomerulosclerosis

Patients	RPF mL/min/ 1.73m^2	PTCF mL/min/ 1.73m^2	TIF %	GFR mL/min/ 1.73m^2
Normal	600	480	0	120
Group 1:MC-NS (steroid-sensitiveness)	711 ± 92	584 ± 85	0	127 ± 26
Group 2:MesP-NS with no TIF (steroid-resistance)	491 ± 52	392 ± 50	0	99 ± 14
Group 3:MesP-NS with TIF (steroid-resistance)	311 ± 67	235 ± 49	5 ± 2	78 ± 36
Group 4:NS-FSGS (severe)	179 ± 80	142 ± 69	58 ± 14	36 ± 18

documented in the steroid-resistant, mesangial proliferature nephrosis (group 2) and such a mild reduction in renal perfusion is generally unable to induce tubulointerstitial fibrosis. With a greater reduction in peritubular capillary flow (mean 235 ± 49 mL/min/1.73 m^2) in this severe category, such degree of perfusion deficit is then capable of inducing tubulointerstitial fibrosis. The above evidence simply implies that a substantial reduction in renal perfusion is prerequisited and precedes the development of nephronal death or tubulointerstitial fibrosis. Such a conceptual view of renal perfusion deficit inducing tubulointerstitial fibrosis is well illustrated by the multiple regression analysis approach in which the peritubular capillary flow is inversely proportional to the incidence of tubulointerstitial fibrosis (Figure 1). The development of tubulointerstitial fibrosis would occur as the reduction in peritubular capillary flow approaching the fifty per cent area of normal value. As the reduction in peritubular capillary flow becomes progressive, there is also a steady increase in the incidence of tubulo-interstitial fibrosis. Such reduction in renal perfusion has been uniquely observed in a variety of chronic glomerulonephritides[5].

The result of this intrarenal hemodynamic study appears to be somewhat contradictory to the general belief that the nephronal death such as tubulointerstitial fibrosis is the cause rather than the effect of the renal perfusion deficit. A similar observation to us was also remarked by Bohle and associates[6] who had denoted that the degree of tubulointerstitial fibrosis correlated inversely with the intensity of postglomerular capillary patency. In addition, Truong and Farhood[7] demonstrated in their experimental model

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Letter to the Editor

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Endothelial Cell Cytotoxicity and Renal Hypoperfusion in Idiopathic Nephrotic Syndrome

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Dear Sir,

The importance of the hemodynamically mediated renal disease progression has emerged inasmuch as the therapeutic intervention with immunosuppressive agents fails to counteract the accepted concept of immunologic mechanism as a determinant of disease progression and to prevent the severe form of glomerulonephropathy such as nephrosis associated with focal-segmental glomerulosclerosis from progressing to end stage renal failure [1]. In this regard, it has recently been demonstrated by intrarenal hemodynamics that a normal renal perfusion is usually associated with an intact nephronal structure and function, whereas renal hypoperfusion is a unique characteristic observation documented in severe forms of idiopathic nephrosis associated with nephronal death such as glomerulosclerosis and tubulointerstitial fibrosis [2]. In addition, it is of notion that there is a progressive reduction in renal perfusion, as the disease severity progresses [3]. This view is delineated by multiple regression analysis which demonstrates that the reduction in renal perfusion is inversely proportional to the intensity of tubulointerstitial disease [4]. Furthermore, Bohle et al. [5] also noted that there was an inverse correlation between the relative volume of the intertubular capillaries and the serum creatinine concentration.

The preceding information would raise an interesting issue as to whether there is any factor in the serum of the nephrotic patient associated with clinical severity capable of inducing such a phenomenon of progressive reduction in renal perfusion. In order to address this specific issue, we performed an endothelial cell cytotoxicity test using sera from nephrotic patients as previously described [6]. In brief, the human endothelial cell line ECV 304 (American Tissue Culture Collection) in medium 199 with 10% fetal bovine serum, approximately 2×10^4 cells/well of 96-well tissue culture plates, was incubated overnight at 37°C in a 5% CO_2 atmosphere. Sera from nephrotic patients were added in duplicate wells. The culture medium and 10% Triton X were used as controls that showed no cell lysis and 100% cell lysis, respectively. The testing cultures were incubated as above for an additional 48 h. After incubation, each well was washed with phosphate-buffered saline and then stained with crystal violet. The stained cells were lysed with acid alcohol solution, and the optical density (OD) was determined by using a microtiter plate reader (model 3550; Biorad) at 550 nm. The percentage of cytotoxicity was calculated by:

$$\text{Percent cytotoxicity} = \frac{[1 - (OD_{testing} - OD_{Triton\ x})]}{OD_{control} - OD_{Triton\ x}} \times 100$$

The results of the endothelial cell cytotoxicity test using sera from nephrotic patients revealed (1) that endothelial cell cytotoxicity could be enhanced by sera from patients with both mild nephrosis associated with mesangial proliferation (mean 18 ± 9% versus control 1.8 ± 0.8%) and severe nephrosis associated with focal-segmental glomerulosclerosis (mean 40 ± 9%) – the differences were statistically significant ($p < 0.001$) and (2) that sera from patients with nephrosis associated with focal-segmental glomerulosclerosis induced a higher degree of endothelial cell cytotoxicity. Thus, the endothelial cell cytotoxicity test renders a supportive view that endothelial cell injury in vivo is likely to be spontaneously induced in the clinical setting of nephrosis.

In accordance with the higher incidence of endothelial cell cytotoxicity induced by sera from patients with nephrosis associated with focal-segmental glomerulosclerosis, it is likely to imply that a significant endothelial cell death would occur in the renal microcirculation in such severe disease and that this spontaneous endothelial cell cytotoxicity would explain the phenomenon of progressive reduction in renal perfusion uniquely observed in the severe form of nephrosis. The nature of such a factor that spontaneously induced endothelial cell cytotoxicity remains to be further elucidated. However, an immunocirculatory imbalance with predominant Th-1 cell activity has recently been proposed [7].

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Book Review

G.G. Fogazzi, C. Ponticelli, E. Ritz

The Urinary Sediment: An Integrated View, ed 2

Oxford University Press, Oxford, 1999
ISBN 0–19–263074–1

This is a beautifully illustrated 186-page book on the urinary sediment. It has the most excellent phase contrast illustrations of urinary sediment I have ever seen in print, with some interference, fluorescent, plain and polarized microscopy photographs. It is a most practical work with detailed instructions on the preparation of the sediment, and even how one sets up a phase contrast microscope. The integration of a pleasant and amusing chapter on the history of urine microscopy by Cameron, and a chapter on the findings of the sediment in various diseases is well illustrated by superb glomerular histology. It is debatable whether the history and renal histology should be included in the book which is otherwise a most excellent manual and atlas of the urinary sediment. This is of course purely a matter of personal preference for the integrated approach, depending on the reader. However, this is still an extraordinary book, and the best buy on the market. It should be owned by every nephrologist, and those internists who still examine urine sediments. It is essential for nephrologists in training. The beauty of its presentation will give pleasure to all who read it, and the Oxford University Press is to be congratulated on the finished product, and the authors for their writing this fine book.

G.M. Berlyne

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Endothelial Cell Cytotoxicity Induced by Nephrotic Serum

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Summary

An endothelial cell cytotoxicity (ECC) could be enhanced in accord with the clinical severity in vitro by sera of both mild and severe forms of nephrosis. Such finding correlated with the intrarenal hemodynamics which demonstrated a mild reduction in renal plasma flow in mesangial proliferative nephrosis and a severe renal perfusion deficit in nephrosis with focal segmental glomerulosclerosis. Thus, the magnitude of ECC observed in vitro study is likely to reflect the in vivo reduction in renal perfusion in these nephrotic patients.

Introduction

A glomerular endothelial cell dysfunction has been delineated in glomerular inflammation such as idiopathic nephrotic syndrome [1]. By virtue of its vicinity with the inflammatory site in the glomerulus, the glomerular endothelium is likely to be perturbed by the process of inflammation. In this regard, a glomerular endothelial dysfunction has been supported in active nephrosis by 2 lines of evidence. First, the glomerular endothelial phenotype expression of normal anticoagulant activity is altered in nephrosis and becomes procoagulant. Such a procoagulant activity is associated with blood hypercoagulability, blood hyperviscosity and local intravascular coagulation which is reflected by altered kinetic studies of platelet and fibrinogen and elevated level of fibrin degradation product in the

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serum and urine of nephrotic patient [2-5]. Second, the glomerular endothelial phenotype expression of normal vasodilation is altered and becomes provasoconstrictive. Such a provasoconstrictive activity is substantiated by the intrarenal hemodynamic study of nephrotic patients in which a characteristic reduction in renal perfusion is generally observed [6,7].

The significance of glomerular endothelial dysfunction and its hemodynamic impact has recently been related to the pathogenesis of renal disease progression. It is of notion that (1) a mild reduction in renal perfusion as that observed in mesangial proliferative nephrosis is usually associated with an intact structure and function of the kidney (2) the reduction in renal perfusion in the severe category of nephrosis usually *precedes* the development of tubulointerstitial fibrosis or glomerulosclerosis and (3) the magnitude of damage to the nephronal structure increases as the renal perfusion becomes progressively decreased [8].

The slight reduction in renal perfusion observed in mesangial proliferative nephrosis and the progressive reduction in renal perfusion documented in nephrosis associated with focal segmental glomerulosclerosis are indeed interesting phenomena. The difference in the amplitude of renal perfusion deficit between the two subsets of nephrosis (mild and severe) would likely reflect the difference in the underlying severity of glomerular endothelial injury. In addition, the spontaneous pattern of glomerular endothelial injury observed in the clinical setting of human nephrosis renders a strong suspicion that plasma or serum would be the good candidate for such source of endothelial injury. It is therefore, interesting to know whether an injury to the endothelial cell can plausibly be induced in vitro by the patient's serum.

Material and Method

1. Endothelial Cell Cytotoxicity Test

Human endothelial cell line (ECV 304, ATCC : American tissue culture collection) in M199 with 10% FBS approximately 2 x 10^4 cells per well of 96-well tissue culture plate were incubated overnight at 37°C with 5% CO_2 atmosphere by the previously described method [9]. Sera from nephrotic patients (11 patients with mesangial proliferation and 8 patients with focal segmental glomerulosclerosis) were added in duplicate wells. The culture medium and 10% tritonX were used as controls that showed no cell lysis and 100% cell lysis, respectively. The testing cultures were incubated as above for an additional 48 h. After the incubation, each well was washed with PBS and then stained with crystal violet. The stained cells were lysed with acid alcohol solution and the optical density was determined by a microtiter plate reader (Model 3550, Biorad) at 550 nm.

The percentage of cytotoxicity was calculated by the equation as follow:

$$\text{Percent cytotoxicity} = [\,1 - \frac{(OD_{testing} - OD_{triton}\ X)}{OD_{control} - OD_{triton}\ X}\,] \times 100$$

2. Glomerular Endothelial Function Determined by Intrarenal Hemodynamic Study

The hemodynamic aspect of glomerular endothelial function was assessed in nephrotic patient by the method of simultaneous determinations of renal plasma flow using ^{131}I – labeled orthoiodohippuric acid (hippuran) and of glomerular filtration rate using ^{99m}Tc – labeled diethylenetriamine pentaacetic acid (DTPA) as previously described [6].

Result

As depicted in Table 1, the endothelial cell cytotoxicity induced by sera from mesangial proliferative nephrosis was 18±9 % as compared to 40±9 % induced by sera from nephrosis associated with focal segmental glomerulosclerosis, the difference between the two groups was statistically significant; $p < .001$.

The intrarenal hemodynamic study revealed a mild reduction in renal plasma flow (mean 491±71 ml/min/1.73 m^2) in mesangial proliferative nephrosis and a moderately severe reduction in renal plasma flow (mean 285±89 ml/min/1.73 m^2) in nephrosis with focal segmental glomerulosclerosis.

Table 1 illustrated the endothelial cell cytotoxicity and renal plasma flow in idiopathic nephrotic syndrome
EC = endothelial cell, MesP-NS = mesangial proliferative nephrosis, NS-FSGS = nephrosis with focal segmental glomerulosclerosis

		EC Cytotoxicity %	Renal Plasma Flow ml/min/1.73 m^2
Normal Control (n = 5)		1.8±0.8	598±80
	$p < .001$		
MesP-NS (n = 11)		18±9	491±71
	$p < .001$		
NS-FSGS (n = 8)		40±9	285±89

Discussion

The result of this in vitro study indicates that serum from the nephrotic patient can induce endothelial cell cytotoxicity. The serum derived from mild case of nephrosis associated with mesangial proliferation significantly enhances a greater index of endothelial cell cytotoxicity than the control (18±9 % versus 1.8±0.8 %). The induction of endothelial cell cytotoxicity is even greater by the serum derived from the severe form of nephrosis associated with focal segmental glomerulosclerosis than that of the mesangial proliferative nephrosis (40±9 % versus 18±9 %). This in vitro study does infact support of what one would expect the glomerular endothelial cell injury to naturally occur in the clinical setting of nephrosis in vivo.

The magnitude of endothelial cell cytotoxicity observed in these two subsets of nephrosis also concurs with the glomerular endothelial cell function determined by an intrarenal hemodynamic study. In mild form of nephrosis associated with mesangial proliferation, the mild reduction in renal plasma flow (491±71 ml/min/1.73 m^2; control 598±80 ml/min/1.73 m^2) observed agrees with the low incidence of endothelial cell cytotoxicity. Similarly, there is a greater degree of reduction in renal plasma flow (285±89 ml/min/1.73 m^2) documented in severe nephrosis associated with focal segmental glomerulosclerosis of which it is in accord with the higher endothelial cell cytotoxicity index.

The preceding information of endothelial cell cytotoxicity induced by the nephrotic serum would address to a specific issue relating to the mechanism of renal disease progression or damage to the nephronal structure. Inasmuch as the glomerular endothelial function associated with its hemodynamic impact or renal perfusion has been proven to be the determinant of nephronal structure and funciton, a further exploration as to what would be the likely trigger of endothelial cell cytotoxicity in severe form of nephrosis and if so, how to prevent it from such injury would be the appropriate issues that need to be addressed in future.

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Letter to the Editor

NEPHRON

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Peritubular Capillary Flow and Tubular Function in Idiopathic Nephrotic Syndrome

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Dear Sir,

The correlation between renal perfusion and nephronal structure has recently been proposed [1]. In essence, a normal or balanced renal perfusion, as observed in minimal change disease, is usually associated with an intact nephronal structure, absence of glomerulosclerosis or tubulointerstitial fibrosis, and a benign clinical course. On the contrary, nephronal damage such as glomerulosclerosis and tubulointerstitial fibrosis is usually encountered in patients having a renal perfusion deficit or renal microvascular disease [2–4]. Such a correlation between renal perfusion and nephronal structure does indeed have a clinical impact on therapeutic as well as preventive aspects, and thus the hemodynamic approach is regarded as a clinically useful, noninvasive instrument to serve the therapeutic purpose.

A correlation between tubulointerstitial structure and tubular function by means of determining the fractional excretion of filtered solutes, namely sodium, calcium, magnesium, phosphate, and uric acid, has also recently been implicated [5]. The study indicates that a normal tubular transport, which is reflected by a low fractional excretion of filtered solutes, implies an intact tubular or tubulointerstitial structure and that an abnormal tubular transport, which is reflected by a high fractional excretion of filtered solutes, suggests tubulointerstitial fibrosis. In this regard, there is also a linear correlation between tubular function and nephronal structure by multiple regression analysis. Thus, the tubular function would provide another noninvasive diagnostic approach for the clinical practice.

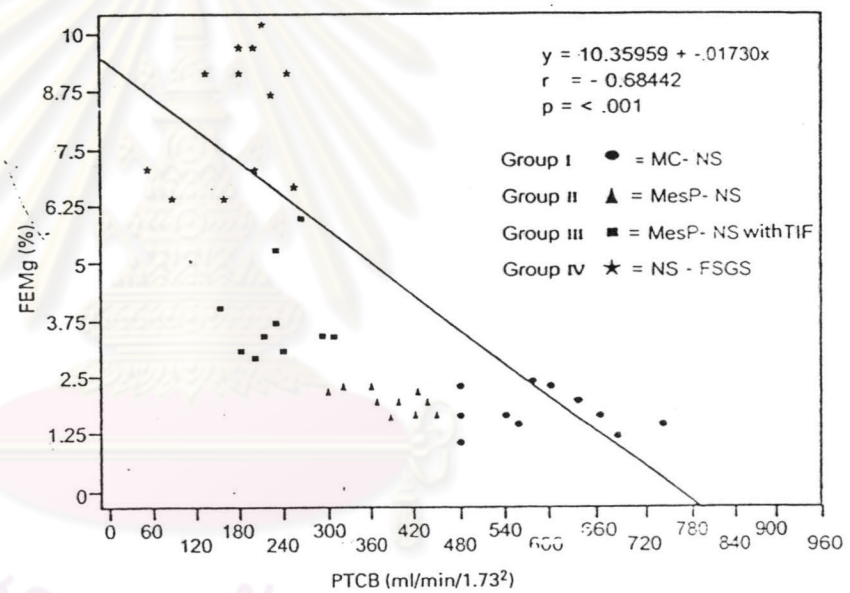

Fig. 1. Correlation between peritubular capillary flow (PTCB) and fractional excretion of filtered magnesium (FEMg). MC-NS = Minimal change steroid-sensitive nephrosis; MesP-NS = mesangial proliferative nephrosis; MesP-NS with TIF = mesangial proliferative nephrosis with tubulointerstitial fibrosis; NS-FSGS = nephrosis with focal segmental glomerulosclerosis.

Tubular preceding information regarding both correlations, one between renal perfusion and nephronal structure and the other between tubulointerstitial disease and tubular function, would render a suggestive view that there should also be a correlation between renal perfusion and nephronal function. In this regard, such a correlation be-

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tween peritubular capillary flow and tubular function has been calculated in various clinical subsettings of glomerulonephropathies, the results indicating that there is an inverse linear correlation between peritubular capillary flow and tubular function as determined by fractional excretion of filtered magnesium (FEMg). FEMg is always normal in the presence of normal peritubular capillary flow. As the peritubular capillary flow declines and approaches 50% of normal, there is an early detection of tubular dysfunction expressed as increasing FEMg. With a greater reduction in peritubular capillary flow, there is a higher amplitude of FEMg, as shown in figure 1.

Inasmuch as both peritubular capillary flow and tubular function are noninvasive diagnostic parameters, the significance of such tools is of clinical relevance. The tubular function can be easily determined and the result obtained can assist in differentiating the presence or absence of tubulointerstitial involvement. This should be helpful in avoiding an unnecessary renal biopsy in those nephrotic patients having normal tubular function. Similarly, information regarding renal perfusion also assists in therapeutic and preventive planning for those patients having a renal perfusion deficit.

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RENAL FAILURE

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Oxidative Stress and Hemodynamic Maladjustment in Chronic Renal Disease : A Therapeutic Implication

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ABSTRACT

Hemodynamic maladjustment with predominant constriction at the efferent arteriole has been encountered in a variety of clinical settings of glomerulonephropathies. In essence, it induces not only intraglomerular hypertension but also exaggeratedly reduces the peritubular capillary flow which supplies the tubulointerstitial compartment. The hemodynamic maladjustment is believed to reflect a glomerular endothelial cell dysfunction. In this regard, oxidative stress and antioxidant defect are likely responsible for the glomerular endothelial dysfunction. Improvement in renal function was accomplished following the correction of oxidant and antioxidant imbalance with antioxidant therapy and vasodilators. Following such therapy, there was a correction in hemodynamic maladjustment with a decline in intraglomerular hydrostatic pressure and an increase in renal perfusion with a subsequent increase in renal functions namely creatinine clearance, glomerular filtration rate and a decline in FEMg.

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Key Words : Hemodynamic, Chronic renal failure, Intraglomerular hypertension, Oxidant, Antioxidant, Vasodilators, Glomerular endothelial dysfunction.

INTRODUCTION

The pathogenetic mechanism of renal disease progression with particularly relevant to the development of tubulointerstitial fibrosis in chronic renal disease is complex which implicates both hemodynamic and non-hemodynamic (immunologic) factors(1). Due to the unsuccessful attempt of immunosuppressant in preventing the progression of renal disease, the focus of interest has switched toward the issue of hemodynamic impact upon the mechanism of renal disease progression. Within this context, it has recently been demonstrated that there is a spatial relationship between renal perfusion and nephronal structure. A normal renal perfusion is usually associated with an intact nephronal structure with no tubulointerstitial disease such as that observed in steroid-sensitive, minimal change or mild mesangial proliferative nephrosis. In contrast, a reduction in renal perfusion such as peritubular capillary flow observed in nephrosis associated with focal segmental glomerulosclerosis generally encounters nephronal damage such as tubulointerstitial fibrosis(2). Furthermore, Bohle and associates(3) also denoted that the degree of tubulointerstitial fibrosis correlated inversely with the intensity of postglomerular capillary patency. The cause-and-effect relationship between renal perfusion and nephronal structure has been implicated by the observation in steroid-resistant mesangial proliferative nephrosis that the reduction in peribubular capillary flow precedes the development of tubulointerstitial fibrosis(4).

The reduction in renal perfusion is likely to be a reflection of glomerular endothelial dysfunction. The endothelial cell in nephrosis expresses procoagulant instead of anticoagulant surface and provasoconstrictive instead of vasodilating activity, thereby reducing the renal perfusion(2). In this regard, an experimental study mimicing glomerular endothelial cell injury has recently been demonstrated that serum of nephrotic patient is capable of inducing endothelial cell cytotoxicity in vitro. That the greater incidence of endothelial cell cytotoxicity is derived from the serum of severe nephrosis associated with focal segmental glomerulosclerosis whereas the serum from mesangial proliferative nephrosis induces a low incidence of endothelial cell cytotoxicity. Furthermore, it is also denoted that the degree of in vitro endothelial cell cytotoxicity correlates with the magnitude of reduction in renal plasma flow in vivo determined by the intrarenal hemodynamic study(5).

The preceding information has raised an interesting issue as to what would be the factor responsible for the induction of such endothelial cell cytotoxicity and dysfunction. Inasmuch as reactive oxygen species is capable of not only inducing proteinuria and nephronal damage in experimental model of puromycin aminonucleoside in animal(6), but also participating in a variety of clinical setting of glomerulonephritides(7-11), it is therefore of interest to perform in this study an assessment of oxidant and antioxidant status in nephrotic patients as well as in a group of patients associated with chronic renal failure who are likely to progress to an end-stage renal disease. If there would be any evidence of oxidative stress, it would also be interested to see whether a

correction of oxidant / antioxidant imbalance would improve the renal function and retard the progression of renal disease in these patients.

MATERIAL AND METHODS

Fifteen patients associated with idiopathic nephrotic syndrome (8 patients with focal segmental glomerulosclerosis and 7 patients with mesangial proliferation) and 9 patients associated with chronic renal failure were included and subject to the following studies.

I. Oxidant and Antioxidant Study

Ten milliliters of blood was drawn from the vein. Blood was placed into heparinized tube and centrifuged at 1500 g. for 15 minutes to separate plasma and RBCs. The RBCs were washed three times with cold saline, and the erythrocyte pellets were frozen at –20°C until further analysis.

A. Lipid Peroxidation (MDA)

MDA was assessed by thiobarbituric acid (TBA) colorimetric assay of hydroperoxides. The TBA assay was performed using a modification of the technique described by Askawa and Matsushita (12).

B. Glutathione (GSH)

Determination of glutathione in the erythrocytes was made by colorimetric methods of Beutler E et al(13), using the glutathione disulfide reductase-DTNB [5-5'-dithiobis (2-nitrobenzoic acid)] to react with sulfhydril compound and yield a stable yellow color. GSH concentration in the erythrocytes was expressed as μ mol/g hemoglobin(14). The hemoglobin concentration was assayed by using a cyanmethemoglobin technique.

C. Vitamin C

Ascorbic acid in serum and plasma was determined by specific enzymatic spectrophotometric method. Samples were analysed indirectly by measuring the absorbance at 593 nm(15).

D. Vitamin E

Vitamin E was assayed by the modified Emmeric and Engle's method(16). The oxidation of xylene-extracted tocopherols from the blood sample by ferric chloride and the pink complex of ferrous ions with bathophenanthroline was measured colorimetrically at 536 nm.

E. Glutathione peroxidase (GSH-Px)

GSH-Px activity was determined according to Gunzler et al(17), with modification by following oxidation of the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) measured at 340 nm.

II. Renal Function Studies

A. Glomerular Function

A glomerular filtration rate was performed by measuring the 10-hour endogenous creatinine clearance (CCr) or glomerular filtration rate (GFR) by the radioisotope technique using ^{99m}Tc-labeled diethylene triamine pentaacetic acid (DTPA) and the value was converted to the body surface area of 1.73 m^2 by the method of calculation :

$$\text{Body surface area} = \frac{\text{body weight (kg)} \times 4 + 7}{90 + \text{body weight (kg)}}$$

Intraglomerular pressure (PG mm Hg) was assessed by the method of calculation as previously described(18).

B. Tubular Function

Tubular transport was assessed by a 10-hour urinary collection during fasting. Blood drawn at the end of the test and urine were analysed for creatinine, magnesium and protein. A reflection of tubular transport was derived from the determination of fractional excretion (FE) of filtered solute namely magnesium (Mg). The FE Mg was calculated through the formula.

$$\text{FE Mg} = \frac{{}^{U}/_{P}\ \text{Mg}}{{}^{U}/_{P}\ \text{Creatinine}} \times 100\%$$

The normal value by FE Mg is $\leq 2.2\%$

C. Vascular Function

The renal plasma flow (RPF) value using ^{131}I-labeled orthoiodohippuric acid (hippuran) and the renal afferent (RA) and efferent arteriolar resistance (RE) were determined by the previously described method(19). A peritubular capillary flow (PTCF) is derived from the substraction of glomerular filtration rate from renal plasma flow.

III. Mode of Therapy

All of these 24 patients were treated with enhanced renal perfusion therapy, which consisted of (a) angiotension converting enzyme inhibitor such as cilazapril 1.25-10 mg/day or enalapril (0.25-1 mg/kg/day) or AII receptor antagonist 50-100 mg/day (b) calcium channel blocker isradipine 2.5-10 mg/day and (c) antiplatelet agent dipyridamole 3-5 mg/kg/day. In addition, all patients received antioxidants as a combination of vitamin E (800 units) and vitamin C (1000-3000 milligrams) daily. The nephrotic patients had been on a regular protein, non added salt diet and the patients in chronic renal failure group; in addition to the general supportive treatment such as correction of electrolyte and acid base imbalance, had been placed on a low protein, low cholesterol diet and adequate hydration.

STATISTICAL ANALYSIS

Values in text and tables are expressed as mean $\pm$ SEM. Non parametric Mann-Whitney was used to establish the significance of between group differences. The differences between pre-and post-

treatment values between each treatment group was performed by Student's paired t-test. The difference was statistically significant when the P value was less than 0.05.

RESULTS

The initial assesement of oxidant revealed an elevated level of plasma MDA in nephrotic patients (3.4±0.3 μ molar versus 2.6±1 μ molar of controls) as well as in patients with chronic renal failure (3.2±0.6 μ molar). The initial level of erythrocyte MDA in both nephrotic (11.5±2 n mol/L versus 7.6±0.9 n mol/L of control) and chronic renal failure patients (10.8±1 n mol/L) were also elevated as depicted in Table 1. In respect to the antioxidant study, there was a significant depletion in GSH concentration in nephrotic (6.8±1 μ mol/g Hb versus 9±1 μ mol/g Hb of control) and in chronic renal failure patients (6.3±1 μ mol/g Hb). The concentration of vitamin C was also found to be significantly depleted in nephrotic (1±0.8 mg/L versus 5.7±4 mg/L of control) and in chronic renal failure patients (1±1 mg/L). The level of vitamin E in plasma in both nephrotic and chronic renal failure patients were not significantly different from the controls. The level of glutathione peroxidase in nephrosis and chronic renal failure patients were 0.2±0.2 n mol/10^6 cells and 0.2±0.18 n mol/10^6 cells respectively which were not significantly different from the control value of 0.28±0.4 n mol/10^6 cells. The oxidant and antioxidant (OA) ratio in both groups of patients were significantly different from the control (Table 1).

The result of renal function study in the nephrosis group revealed a significant impairment. Of the glomerular function, the initial creatinine clearance (CCr) value was 44.8±24 ml/min/1.73 m^2. The tubular function revealed a significant elevation of FE Mg (7.2±2% versus 2.2% of control). In the chronic renal failure (CRF) patients, the mean CCr was 17±10 ml/min/1.73m^2 and the FE Mg was 13±6.3%. The intrarenal hemodynamic study revealed a significant reduction in renal plasma flow (RPF) 219±84 ml/min/1.73 m^2 (normal 600 ml/min/1.73 m^2), a low peritubular capillary flow (PTCF) 167±67 ml/min/1.73 m^2 (normal 480 ml/min/1.73 m^2), an elevated efferent arteriolar resistance (RE) 19442±5206 dyne.s.cm^{-5} (normal 3000 dyne.s.cm^{-5}) and an elevated intraglomerular hydrostatic pressure (PG) 56±1 mm Hg versus 53 mm Hg of control.

Following the therapy, there had been a significant improvement in oxidant and antioxidant status. The plasma and erythrocyte MDA declined to normal level whereas there had been a steady increase in the concentration of GSH, vitamin C and E as depicted in Table 2. In accordance with this improvement in antioxidant status, there was also a significant improvement in renal function. The creatinine clearance in nephrosis increased to 60±34 ml/min/1.73 m^2 whereas the FE Mg declined to 5.7±3% (Table 3). In the CRF patients, the CCr rose to 23±12 ml/min/1.73 m^2 and the FE Mg declined to 11.4±6%. The intrarenal hemodynamic study showed a significant

improvement in RPF (391±133 ml/min/1.73 m^2), PTCF (314±120 ml/min/1.73 m^2), a reduction in RE (3918±2040 dyne.s.cm^{-5}) and PG (51±0.8 mm Hg).

DISSCUSSION

Reactive oxygen species are implicated in cell signaling, gene transcription, mitosis, apoptosis and vasoconstriction(20,21). 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(22). Increased cellular metabolism with enhanced production of reactive oxygen species have been delineated in a variety of glomerulonephropathies and renal failure(23,24). 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 vitamin C and a reverse ratio of oxidant and antioxidant imply that there is an oxidant/antioxidant imbalanced state. Similar observation have also been reported(23,24,25). In excess, reactive oxygen species and their byproducts are capable of causing oxidative damage and cytotoxicity to cells. This results in increased oxidized LDL, advanced glycation end products of carbohydrates, fat and protein(26). 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 is optimally situated at the interface between the circulating blood and the vessel wall to serve as a sensor and transducer of signals within the circulatory microenvironment. The oxidant and antioxidant imbalance observed in this study is likely to explain the in vitro increased endothelial cell cytotoxicity induced by sera of nephrotic patients(5). Increased oxidative stress to the glomerular endothelial cell would induce dysfunctioning of the endothelial cell and in excessive amount incriminate in endothelial cell death. In respone to such oxidative injury, the endothelial cell would increase productions of vasoconstrictive substances namely angiotensin II, endothelin and thromboxaneA_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. Such a provasoconstrictive state would induce a hemodynamic maladjustment with a predominant vasoconstriction at the efferent arteriole. 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. The hemodynamic study indicates that there is indeed an increase in intraglomerular hydrostatic pressure to 56±1 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 219±84 ml/min/1.73 m^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 injury to the glomerular cell inducing glomerulosclerosis(27-30).

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 leads to generation of reactive species and cell signaling through the NADPH oxidase pathway. This is followed by an increased production of nuclear factor-kappa B which then upregulates the inflammatory gene expressions namely cytokines, growth factors and adhesion molecules(27,28). 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(31) and increased chemokine expression was also demonstrated in puromycin aminonucleoside nephrosis(32). In this regard, it has recently been demonstrated that there is an inverse correlation between the peritubular capillary flow and the incidence of tubulointerstitial fibrosis(4). That the progressive reduction in peritubular capillary flow as the disease severity progresses inversely increases the magnitude of tubulointerstitial fibrosis. In addition, we have recently demonstrated that there is also a correlation between the renal perfusion and FE Mg(33).

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 endothelial cell dysfunction by which it induces a progressive reduction in renal perfusion as the disease severity progresses. In accordance with the therapeutic strategy, a correction of antioxidant defect in conjunction with the administration of vasodilators 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 concensus that vitamin C is capable of neutralizing super oxide anion, reactive nitrogen species such as peroxynitrite, nitrogen dioxide(34) and also acts as a coantioxidant by regenerating ∝-tocopherol (vitamin E) from the ∝-tocopherol radical(35-37). Vitamin C has also been shown to regenerate glutathione and B-carotene in vitro from their respective one-election oxidation product(34,38). 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(39). The impaired endothelium-dependent vasodilation was markedly improved by vitamin C in essential hypertension(40).

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(41-43). Increasing dietary vitamin E level significantly attenuates renal oxidative damage in the puromycin nephrotoxicity model of FSGS in the rat(44,45). 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 vasodilators.

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±0.8 mm Hg), but also enhanced the peritubular capillary flow. The peritubular capillary flow increased from 167±67 ml/min/1.73 m^2 to 314±120 ml/min/1.73 m^2 following treatment. The improvement in renal perfusion correlated with the glomerular function as well as the tubular function. The creatinine clearance in nephrosis increased from 44.8±24 ml/min/1.73 m^2 to 60.6±34 ml/min/1.73 m^2, the glomerular filtration rate increased from 52±21 ml/min/1.73 m^2 to 76±22 ml/min/1.73 m^2 and the FE Mg significantly reduced from 7±2% to 5.7±3% following treatment, $p < .05$. In chronic renal failure, the CCr rose from 17 ml/min/1.73 m^2 to 23±12 ml/min/1.73 m^2.

Thus the preceding information renders a supportive view that the oxidant and antioxidant imbalance is likely to induce the glomerular endothelial dysfunction with subsequent hemodynamic maladjustment by which the correction of such disorders by antioxidant therapy and vasodiltors can improve the renal function and prevent the renal disease progression.

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Table 1 Oxidant and antioxidant in nephrotic and chronic renal failure patients

	NS	p value	CONTROL	p value	CRF
Oxidant (O)					
P MDA μ molar	3.4 ± 0.3	< .05	2.6 ± 1	< .05	3.2 ± 0.6
R MDA n mol/L	11.5 ± 2	< .01	7.6 ± 0.9	< .01	10.8 ± 1
Antioxidant(A)					
GSH μ mol/gHb	6.8 ± 1	.001	9 ± 1	.001	6.3 ± 1
Vit C mg/L	1 ± 0.8	< .001	5.7 ± 4	< .001	1 ± 1
Vit E mM	0.17 ± 0.2	NS	0.17 ± 0.1	NS	0.16 ± 0.1
OA RATIO	1.9 ± 0.5	< .001	0.7 ± 0.2	< .001	1.9 ± 0.5

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Table 2 Initial and post treatment values of oxidant and antioxidant in nephrotic and CRF patients

	Initial	Post treatment	p value
GSH μ mol/gHb	6.8 ±1.5	8.2 ± 1.7	< .001
Vit C mg/L	1.2 ± 1.4	4.3 ± 1.9	< .001
Vit E mM	0.17± 0.1	0.29 ± 0.1	< .001
P-MDA μ molar	3.2 ± 0.9	1.5 ± 0.9	< .001
R-MDA n mol/L	11.2 ± 3	5.6 ± 3	< .001

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Table 3 Initial and post treatment values of renal function in nephrosis

	Initial	Post treatment	p value
CCr ml/ min/1.73m^3	44.8 $\pm$ 24	60.6 $\pm$ 34	<.05
FE Mg %	7.2 $\pm$ 2	5.7 $\pm$ 3	<.05

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