The relationship between renal P-glycoprotein expression and response to steroid therapy in childhood nephrotic syndrome

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In this study, we aimed to investigate the relationship between renal P-glycoprotein (rP-gp) expression and response to corticosteroid therapy in childhood nephrotic syndrome (NS). Expression of rP-gp was evaluated prior to non-steroid immunosuppressive therapy in children with NS (Group 1), prior to any treatment in children with immunoglobulin (Ig)A nephropathy (Group 2), and during renal donation in healthy adults (Group 3); mesangial proliferation was evaluated in Groups 1 and 2. Total dosage of steroid was calculated in Group 1. The ratio of rP-gp (+) glomeruli was higher in Group 1 than Group 3 (33.8 ± 27.1% vs 4.7 ± 4.6%, p=0.000). There were no rP-gp (+) glomeruli in Group 2. The rate of mesangial proliferation was similar in Groups 1 and 2. There was no statistically significant correlation between rP-gp expression and total steroid dosage (r=0.455, p=0.160). Our findings showed that rP-gp expression is increased in patients receiving steroid therapy for NS regardless of the cumulative steroid dosage and mesangial cell proliferation.

Key words: renal P-glycoprotein expression, nephrotic syndrome, response to steroid.

Nephrotic syndrome (NS) is the most common primary glomerular disease in children. Immune mechanisms rather than primary structural defects of the filtration barrier are involved in the pathologic process[1,2]. Although the biochemical alterations and clinical signs are similar in all patients with NS, the response to steroid therapy differs1. About 60-80% of the patients respond to steroid therapy with relapses of proteinuria, while the remaining patients include those that are steroid-dependent and steroid-resistant despite an initial complete response[3]. Resistance to steroid therapy might result from various histological patterns ranging from minimal change lesion to mesangial nephropathy or focal segmental glomerulosclerosis (FSGS), mutations in the genes encoding structural components of the glomerular filtration barrier such as nephrin, podocin, CD2-associated protein, α-actinin-4 proteins, phospholipase C epsilon 1, Wilms' tumor 1, transient receptor potential cation channel 6, inverted formin 2, laminin beta 2, protein tyrosine phosphatase receptor type O, myosin 1E, and integrin alpha 3, or expression of P glycoprotein (P-gp), which is a product of the multidrug resistance-1 (MDR1) gene[2,4-10].

P-glycoprotein (P-gp) is an energy-dependent intracellular transport protein coded by the MDR1 gene located on chromosome 7q21, and functions to excrete and thus reduce the concentration of certain medications (xenobiotics or MDR-associated medications including anticancer medications, immunosuppressives, digoxin), proteins, steroids, and phospholipids[11,12]. P-gp is extensively expressed in various tissues including the blood-brain barrier, liver, kidney, intestines, and lymphocytes in peripheral blood[13,14]. It is located in the proximal tubules, thick arm of the loop of Henle, collecting ducts, and mesangium in kidneys[15].

The relationship between clinical response to

steroid therapy and lymphocyte P-gp expression; expression of P-gp at three different times (prior to therapy, during therapy and post-therapy) in the lymphocytes of children with NS receiving steroid therapy; c.G3435C, c.G2677T and c.G2677T polymorphisms of the MDR1 gene in steroid-sensitive and steroid-resistant patients with NS; and pre- and post-therapy MDR1 mRNA expression in peripheral blood lymphocytes in steroid-sensitive children have been evaluated in previous studies[1,2,16-18]. In this study, we examined renal P glycoprotein (rP-gp) expression in the renal tissue in patients with NS and investigated its relationship with response to steroid therapy.

Material and Methods

Patients

The study was performed with three groups of patients. Group1 consisted of patients with NS, Group 2 of patients with immunoglobulin (Ig) A nephropathy and Group 3 of healthy subjects who donated their kidney. The diagnosis of NS in Group 1 had been established by the presence of hypoalbuminemia (≤2.5 g/dl) in addition to massive proteinuria (≥40 mg/m^2/h) in otherwise healthy subjects[19]. In Group 1, patients who did not enter remission following a one-month prednisolone therapy at 60 mg/m^2/day were defined as steroid-resistant[20], who relapsed during the tapering off or within two weeks of the discontinuation of prednisolone therapy as steroid-dependent, and who experienced four or more relapses of NS episodes within 12 months as NS cases with frequent relapses[21]. Patients with family history of NS and syndromes were excluded from the study. Cumulative steroid dosage obtained prior to biopsy and frequency of relapses following the biopsy were calculated in Group 1. Mesangial proliferation and presence of immune deposits were evaluated using light microscopy and immunofluorescent microscopy, respectively, in Groups 1 and 2.

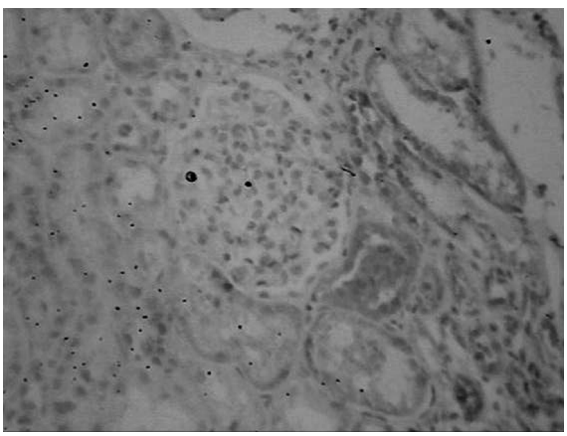

Fig. 1. In the donor group, immunostaining for P-gp was faint in the glomeruli and intense in the tubuli (hematoxylin & eosin [H&E], x100).

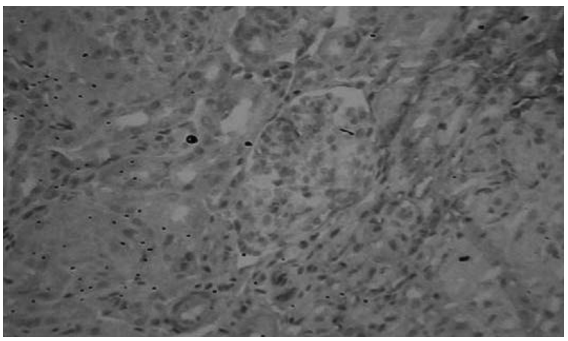

Fig. 2. In the nephrotic syndrome group, intense immunostaining for P-gp in the glomeruli and tubuli is shown (H&E, x40).

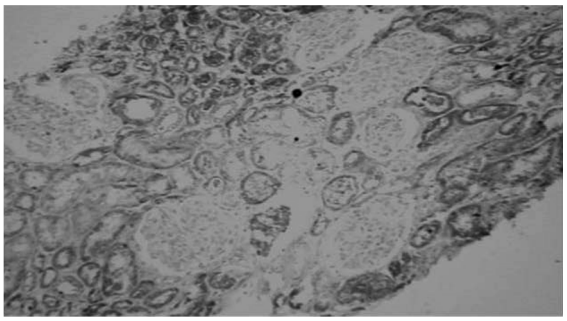

Fig. 3. In the IgA nephropathy group, immunostaining for P-gp was negative in the glomeruli and intense in the tubuli (H&E, x100).

Determination of Renal P-Glycoprotein Expression

Tissue samples were obtained to determine rP-gp expression prior to non-steroid immunosuppressive therapy (cyclosporine) in Group 1, prior to any therapies in Group 2, and during the donation of kidney in Group 3. Kidney needle biopsies in 10% neutral buffered-formalin, prior to routine processing through to embedded blocking sections, were cut, and immunohistochemical reactions were performed

Table I. Age, Sex and Ratio of P-gp-Positive Glomeruli and Tubuli in the Groups

	Group 1 (n: 11)	Group2 (n: 9)	Group 3 (n: 7)	P
Age (year)*	5.8 ± 5.1 (1.0 – 17.0)	12.0 ± 5.1 (3.0 ± 18.0)	25.2 ± 9.1 (19.0-45.0)[1]	0.000
Sex (M/F) †	6/5	3/6	5/2	0.356
P-gp (+) glomeruli (%)*	33.8 ± 27.1 (0.0-100.0) [2]	0.0 ± 0.0	4.7 ± 4.6 (0.0-11.0)	0.000
P-gp (+) tubuli (%)*	67.7 ± 16.7 (40.0-100.0)	70.0 ± 19.8 (30.0-90.0)	80.7 ± 15.9 (50.0-95.0)	0.310

* One-way ANOVA
† Chi-square test
[1]Group 3 vs Group 1 and Group 2, p<0.05
[2]Group 1 vs Group 3 and Group 2, p<0.05

on paraffin-embedded tissue by an avidin-biotin peroxidase complex method. The antibodies used in the present study were monoclonal MDR antibodies (1:50, Neomarkers, Fremont, CA, USA). A renal pathologist independently analyzed the immunostained sections by light microscopy, and the final interpretation was based on agreeing assessments. P-gp expression was graded semi-quantitatively as negative (−) or positive (+) according to the color intensity[22,23].

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) 11.0 for Windows software was used to perform statistical analyses. A P value less than 0.05 was considered statistically significant. Chi-square test was used in the inter-group comparison of categorical data, one-way ANOVA was used in the comparison of more than two independent samples' mean, and correlation analysis was used to determine the relationship between two numerical variables.

Results

A total of 11 children (6 males) were included in Group 1. These patients consisted of 7 subjects with frequently relapsing NS, 3 subjects with steroid-resistant NS and 1 steroid-dependent NS, and their mean age was 5.8±5.1 years. There were 9 patients (3 males) in Group 2 with a mean age of 12.0±5.1 years. Group 3 consisted of 7 subjects (5 males) with a mean age of 25.2±9.1 years. Mean ages were significantly different in the three groups (p=0.000) (Table I).

The ratio of glomeruli with P-gp expression over total glomeruli was significantly higher in Group 1 compared to Group 3, whereas no P-gp expression was determined in the glomeruli of subjects in Group 2 (p=0.000). Tubular P-gp expression was similar in the three groups (Table I, Figs. 1-3).

There was no significant relation of the pre-biopsy cumulative steroid dose and total relapse rate to glomerular (r=0.455, p=0.160 and r=0.335, p=0.345, respectively) and tubular (r=-0.086, p=0.802 and r=-0.213, p=0.555, respectively) P-gp expression rate (Table II).

Table II. Relationships of Pre-Biopsy Cumulative Steroid Dose and Post-Biopsy Relapse Rate to the Ratio of P-gp (+) Glomeruli and Tubuli in the Study Group

	Pre-biopsy cumulative steroid dose (mg/m^2)		Post-biopsy relapse rate (per year)	
	r	p	r	p
P-gp (+) glomeruli (%)	0.455	0.160	0.335	0.345
P-gp (+) tubuli (%)	-0.086	0.802	-0.213	0.555

Mesangial proliferation was noted in 90.9% of subjects in Group 1 and 88.8% of subjects in Group 2. Mesangial IgM deposits were determined in 9 subjects of Group 1 (82%) and 4 subjects of Group 2 (44.4%).

Discussion

Corticosteroid therapy is used to suppress the increased lymphocyte activity in the treatment of patients with NS characterized with T-cell activation. Several patients with NS have comparable biochemical parameters and clinical signs; however, the course of the disease is different. Several mechanisms have been proposed for non-responsiveness to steroid. These mechanisms include the disease severity, patient incompliance, abnormalities in glucocorticoid metabolism, poor absorption of the medication (particularly in severe hypoalbuminemia), and resistance due to glucocorticoid receptor or post-receptor abnormalities[17]. P-gp is an intracellular transport protein that contributes to the above mechanisms by excreting steroids from the cell. Therefore, the relationship between P-gp expression and response to steroid therapy in patients with idiopathic NS has been investigated in several studies[16,18,24]. In the study of Wasilewska et al.[16], P-gp expression was examined in CD3-positive lymphocytes. CD3/P-gp expression was found significantly higher in children with NS compared to the controls and in steroid-dependent and frequently relapsing children with NS compared to not frequently relapsing children with NS, and higher CD3/P-gp expression was observed in the steroid-dependent group. In addition, authors in another study investigated P-gp expression in CD3 lymphocytes during prednisolone therapy and determined that P-gp expression was higher in patients with NS during the treatment compared to the steroid-off period[16]. Therefore, it has been reported that P-gp expression is induced by steroid therapy. The hypothesis that P-gp expression is induced by steroid therapy is supported by the facts that 1) no relationships have been determined previously between P-gp expression and age of normal bone marrow cells[25], 2) greater glomerular P-gp expression was determined in Group 1 compared to Groups 2 and 3 in our study, and 3) the lack of glomerular P-gp expression despite mesangial proliferation in Group 2 similar to Group 1. We suggested that expression of rP-gp might be increased in mesangial proliferation considering the facts that the efficacy of isolated steroid therapy is not obvious and reliable in patients with IgA nephritis[26], and this is more prevalent in severe mesangial proliferation[27]; mesangial hypercellularity is a marker of prognosis in NS[28]; and P-gp is also expressed by mesangial cells. Therefore, we suggest that P-gp expression is induced by steroid therapy and this is independent of the mesangial proliferation.

Previous studies have demonstrated a positive correlation between total dosage of prednisolone and P-gp, and reported that P-gp expression was associated with the number of NS episodes. Authors have reported that the rate of P-gp expression did not differ significantly between patients with a single NS episode and controls, whereas the rate was significantly higher in patients with consecutive episodes (with at least 12 months of remission without steroid)[17]. In our study, there was a positive correlation between the rate of P-gp (+) glomeruli and total steroid dosage in subjects with NS; however, this correlation was not statistically significant. This insignificant correlation might also be explained by the low number of patients in our study.

P-glycoprotein is an intracellular transport protein encoded by the MDR1 gene, and several studies have investigated MDR1 expression and polymorphisms in patients with NS. In the study of Funaki et al.[18], MDR1 mRNA expression was demonstrated to be variable in peripheral blood nucleotide cells and was reduced following complete remission. In another study, MDR1 activity and mRNA expression were determined to be higher in subjects with NS resistant to steroid, cyclophosphamide or cyclosporine compared to subjects sensitive to these drugs[19].

There are studies on the relationship between genetic variations in MDR1 and development and progression of certain diseases with failure of cellular barrier functions in the blood-brain barrier (Parkinson's disease), small intestine (ulcerative colitis) or kidneys (non-clear cell renal carcinoma)[29-31]. These studies have reported that MDR1 3435 TT genotype and/or T allele had higher frequency in patients compared to controls. Increased disease risk has

been attributed to decreased P-gp expression and/or failure of the tissue barrier followed by inadequate defense of the body against environmental and metabolic toxins[32,33]. This hypothesis was supported also in our study with the statistically insignificant negative correlation between rP-gp expression and frequency of relapses. In the study of Choi et al.[34], however, no differences were determined in the same genotype, and the level of P-gp expression was not reported to be a significant pathogenetic factor. However, the same authors determined that MDR1 1236 CC or TC genotype and C allele were markedly increased in steroid-responding NS patients compared to non-responding patients and that TGC haplotype was decreased. Therefore, MDR1 1236 CC genotype and C alleles have been reported to be good predictors of the initial response to steroids. In addition, P-gp also plays a role in the transport of cytokines including interleukin (IL)-2 and interferon (IFN)-gamma in peripheral lymphocytes, suggesting a significant a role in the pathogenesis of NS35. However, this suggestion has not been proven yet.

Studies performed up to date have been on lymphocytes. Our study is the first to demonstrate P-gp expression in renal tissue of patients with NS. The results of this study have suggested that glomerular P-gp expression is increased in frequently relapsing, steroid-dependent or steroid- resistant childhood NS. However, to support this notion, further studies should be performed investigating the relationship between rP-gp expression and response to steroid in a greater number of pediatric patients with NS.

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