Original Paper

Role of Metabolites of Nitric Oxide and Arginase in the Pathogenesis of Glomerulonephritis

ANGELA CIUNTU[1]

[1]*Pediatric Department, State University of Medicine and Pharmacy „Nicolae Testemitanu", Chișinău*
Paediatrics National Institute of Health Care for Mother and Child, Nephrology Unit, R.Moldova

ABSTRACT: Purpose: The aim of the study is to assess the level of nitric oxide metabolites and arginase in the urine of children with glomerulonephritis depending on clinical evolutional stages of the disease. Materials and methods: The prospective study included 65 children with primary glomerulonephritis, 25 children with steroid-sensitive nephrotic syndrome (SSNS) and 20 children with steroid-resistant nephrotic syndrome (SRNS), 20 children with mixed form of chronic glomerulonephritis(CGN). Results: Thus in the SRNS group, during relapse period the concentration of NO metabolites in urine was increased by 4,2 times, while in SSNS by 3,0 times in comparison with the control group. The concentration of NO metabolites in the urine increased by 4,8 times during relapse CGN mixed form in comparison to the control values. During remission, the levels of NO metabolites in the urine remain increased in both groups. In relapse of SSNS arginase levels in the urine increased by 4,5 times in comparation to SRNS, thus the concentration of arginase was reduced. During remission period arginase levels in the urine were practically reduced to the levels of the control group. In the mixed form of CGN, relapse period arginase levels in the urine were increased by 2,9 times and during remission were decreased by almost 1,9 times in comparision to the control group. Conclusions: Assesment of NO metabolites and arginase in urine can be used as a diagnostic method in order to monitor renal disease process, evolution and effectiveness of the applied treatment.

KEYWORDS: *metabolites of nitric oxide, arginase, glomerulonephritis, children*

Introduction

Nitric oxide (NO) is one of the most important paracrine modulators and mediators in the control of renal functions such as global and regional renal blood flow, renal autoregulation, glomerular filtration, renin secretion and excretion of salts. NO plays an important role in the pathogenesis of several kidney diseases such as diabetic nephropathy, inflammatory glomerular disease, acute renal failure, septic shock, chronic renal failure and nephrotoxicity of drugs, transmitting both beneficial effects through its hemodynamic functions and adverse effects through its cytotoxicity when it is produced in large amounts by inducible nitric oxide synthase (iNOS) [3,6].

There are three distinct isoforms of NOS; neuronal NOS (NOS-1 or nNOS), endothelial NOS (eNOS or NOS-3) and inducible NOS (iNOS or NOS-2). These isoforms show different tissue distribution, intracellular localization, molecular regulation, enzyme kinetics and calcium dependence [6].

The three isoforms of NOS have been found to be expressed in kidneys. In kidneys endothelial nitric oxide synthase (eNOS) is considered to be important in maintaining of the glomerular filtration rate, regional vascular tone and renal blood flow [6]. Neuronal nitric oxide synthase (nNOS) is expressed mainly in macula densa and is involved in the control of glomerular hemodynamics throught feedback and renin release. NO is synthesized by Nitric Oxide Synthase (NOS) using L-arginine as a substrate for NO synthesis.

L-arginine is also the substrate for arginase, a group of enzymes that are involved in tissue repair processes and which metabolise L-arginine to L-ornithine and ureea, and thus compete with the oxid- nitric synthase (NOS) for the common substrate of L-arginine.

Mammalian arginase has two isoenzymes (arginase – 1 and arginase - 2), which are encoded by different genes in the tissue distribution, subcellular localization and immunological reactivity and are regulated independently[14]. Arginase - 2 is the predominant expressed isoform in kidneys.

Both arginase isoforms are induced by various pathologic stimuli, they contribute to vascular cell dysfunction and remodeling of vessel wall remodeling in several diseases[21]. Strong evidences show that increased arginase expression and / or the activity in endothelial cells limit NO bioavailability receiving eNOS coupling this leading to oxidative stress and vascular inflammatory responses[13,22].Role of arginase in the reduction of endothelial NO production is well known due to the studies of Yang and Ming [22].

Dramatic changes in the metabolism of arginine may lead to vascular dysfunctions [18] due to the increase of arginase activity inclusively vascular dysfunction in diabetes and other diseases [4,16]. Therefore the study of the

level of NO metabolites and arginase in children with glomerulonephritis and nephrotic syndrome is important because it could suggest milestones for diagnosis and monitoring of the effectiveness of the treatment applied in these diseases.

The study purpose is to assess the level of nitric oxide metabolites (NO) and arginase in the urine of children with glomerulonephritis on different clinical evolutional stages of the disease.

Material and method

This study has a prospective character and was performed in State University of Medicine and Pharmacy" Nicolae Testemitanu"(SUMPh), Paediatrics National Institute of Health Care for Mother and Child, Department of Nephrology and in the Laboratory of Biochemistry SUMPh "NicolaeTestemitanu". It is based on biological samples collected according to the principles of contemporary research, approved by the Ethics Committee of Research of SUMPh "Nicolae Testemitanu" (favorable reviewal 13.05.2015, official record No.55). Diagnostic algorithm of nephrotic syndrome (NS) was based on the guide KDIGO 2012 and the essential criteria for inclusion were children with NS with preserved renal function, whose parents have consented their participation in this study. Children with congenital or secondary NS and those with chronic kidney diseases (CKD) stage II-V chronic kidney disease were excluded from the study.

The comparison group was constituted by 20 practically healthy children of the same age without any acute or chronic diseases or other antecedents at the enrollment.

The study included 65 children with primary glomerulonephritis, 25 children with steroid-sensitive nephrotic syndrome (SSNS) and 20 children with steroid-resistant nephrotic syndrome (SRNS), 20 children with mixed form of chronic glomerulonephritis(CGN),which includes NS in association with hypertension and hematuria. Children were divided into two groups according to the response to the treatment with glucocorticoids: SSNS and SRNS. Patients with SSNS and SRNS were divided into 2 subgroups according to the disease activity (SSNS relapse, SSNS remission, SRNS relapse, SRNS remission).

Nephrotic syndrome (NS) was diagnosed by the presence of edema, massive proteinuria (> 40 mg/m²/h or the ratio of protein/ urinary creatinine> 2.0 mg/mg) and hypoalbuminemia (<2.5 mg / dL) [9] .

Steroid-sensitive nephrotic syndrome (SSNS) was dominated by the normalization of urinalysis within 4 weeks rarely 8 weeks after administration of glucocorticoids and installation of complete remission [9].Steroid-resistant nephrotic syndrome (SRNS) was dominated in the case of maintaining of the level of proteinuria to <3 g / dL over the 8 weeks of treatment with prednisolone at a dose of 2 mg/kg/ 24 h and subsequently carrying out pulse therapy with prednisolone 20-30 mg/kg /24h N 3 (not more than 1 g during a course) [9].

Complete remission has been found in the case of resolution of edema, normalization of serum albumin up to 3,5 g / dl and reduction of proteinuria up to <4 mg/m^2/ h (100 mg/m^2/24h) in three consecutive urinalysis [9].

Relapse (recurrence) was defined as a recurrence of massive proteinuria (>40 mg/m^2/h or the ratio of protein / urinary creatinine> 2.0 mg/mg or albuminuria ≥ 2 + within 3 consecutive days, usually with recurrence edema [9].

Determination concentration of nitric oxide (NO) metabolites was performed according to the method of Metelskaya V.A.,Gumanova N.G.[12] and arginase activity in urine by the method Erisir M. et.al., 2005[5].

To assess the significant difference of performed indices were used statistical methods which appreciated arithmetic average size [X], average squared deviation, error of median arithmetic average size [± m]. It has also been used nonparametric statistical test "Mann-Whitney U" and the selected level of statistically significance was $p < 0.05$ (StatsDirect statistical software, version 1.9.5, 2001).

Results

The average age of the onset of NS was 6.4 ± 0.50 years; for the mixed form of chronic glomerulonephritis (CGN) 9,1 ± 0,99 years and its disease duration was 2,9 ± 0,47 years. The clinical manifestations of NS were represented by edema which constituted 92,5±2,9%, anasarca -60,0±5,5%, rare urination – 48,83± 5,6%, headache -22,5±4,7%, dyspnea-1,3±0,8% and algic syndrome -8,8±3,2%. Paraclinical examinations have determined a hypoproteinemia up to 52,9 ± 0,91g/l, seric albumins – 24,03± 2,82g/l, increase of lipid metabolism indices - total lipids increased up to 9,53± 0,98 mmo/l, cholesterol – 8,48 ± 0,35

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mmol/l, β-lipoproteins – 99,4 ± 2,75 arbitrary units, serum ureea – 6,2 ± 0,53 mmol/l, creatinine – 0,060 ± 0,04 mmol / l, proteinuria up to 5,5 ± 0,66 g/ l in urine within 24 hours.

Dynamics of nitric oxide metabolites and arginase in the urine of patients with glomerulonephritis is shown in Table 1.

Table 1. Level of nitric oxide metabolite (NO) and arginase activity in the urine of children with glomerulonephritis

	Group of patients	**NO M/ mM creatinine**		**Arginase activity, nkat/ mM creatinine**	
		relapse	remission	relapse	remission
1	Controls (n=25)	10,90±0,28		0,18±0,04	
2	SS nephrotic syndrom (n=25)	32,5±2,98**	14,0±1,11* p_1<0,01	0,82±0,23*	0,20±0,03 p_1<0,05
3	SR nephrotic syndrome (n = 20)	45,6±3,41*** p_2<0,01	24,5±1,48*** p_1<0,01	0,12±0,02 p_2<0,05	0,16±0,04 p_1>0,05
4	CGN mixed form (n = 20)	52,7±9,45***	28,0±1,89** p_1<0,05	0,53±0,09**	0,34±0,04* p_1>0,05

Note: statistically significant difference compared to control group values: * p <0.05; ** p <0.01; *** p <0.001. p_1-authenticity compared with the index registered under relapse stage. p_2-authenticity in comparison of SRNS with SSNS.

The data in Table 1 showed an increase level of NO metabolites in urine, in all groups of patients.

The level of NO metabolites in urine was increased in SRNS compared to SSNS. Thus, in the group SRNS relapse period the level NO metabolites in the urine increased 4,2 times (45,6 ± 3,41 M / mM creatinine), while in SSNS 3,0 times (32,5 ± 2,98 M /mM creatinine) compared to the control group (10,90 ± 0,28 M/mM creatinine).

The concentration of NO metabolites in the urine has increased 4,8 times (52,7 ± 9,45 nM/mM creatinine) in relapse of mixed form of CGN, compared to control values. During remission NO metabolites in the urine remained to be increased in both groups.

The arginase activity in the urine in various clinical variants of GN undergoes changes of different intensity and orietantion. The results show that in the group SSNS during relapse the arginase activity in urine increased by 4,5 times (0,82 ± 0,23 nkat/mM creatinine) compared to SRNS where arginase concentration decreased to 0,12 ± 0,02 nkat / mM creatinine.

During SN remission period arginase levels in the urine was practically reduced to the levels of the control group. In mixed form of CGN , during relapse arginase levels in urine increased by 2,9 times (0,53±0,09 nkat/mM creatinine) and during remission decreased by almost 1,9 times (0,34 ± 0,04 nkat/mM creatinine) compared with the control group.

Discussion

This study present increased values of nitric oxide metabolites in all clinical forms of GN, during the relapse period, thus exceeding by 3-5 times those of the control group. During remission was observed reduction of the level of nitric oxide metabolites compared to initial values that exceeded by 2 times the control group. This study remarked that arginase activity in urine in various clinical variants of GN, undergoes changes of different types and intensity. This indicates the persistence of the inflammatory process in kidneys, being related to the induction of iNOS.

According to the study [2] the production of nitric oxide (NO) from L-arginine O2 nitric oxid synthase (NOS) is reduced in kidney diseases. Chronic NOS inhibition produces hypertension and renal dysfunction in animals and probably NO deficiency contributes to the progression of renal disease in humans. Net NO deficiency can develop in a response to decreased substrate availability and the action of endogenous NOS inhibitors.

Relevant studies demonstrated that the inhibition of arginase help to reduce the mesangial expansion and glomerular hypercellularity in diabetes, indicating a possible contribution of arginase in the initiation and / or progression of diabetic renal fibrosis. Immunohistochemical studies have identified the expression of arginase-2 in proximal and collecting tubules[19].

The studies put in evidence the presence of vascular dysfunction in experimental models of diabetes [7,17,20] or in diabetic patients [1] is associated with increased arginase-1 [1,17] or arginase-2 [7], the vascular function is normalized by inhibiting the activity of arginase or genetic ablation of arginase -2 [7,20].

Since arginase -2 is colocalized in the mitochondria, it has been proposed that arginase-2 may be involved in the production of proline, while colocalization of arginase-1 and ornithine decarboxylase (ODC) would favor synthesis of polyamine [15].

According to the study [10] data related to arginine metabolism is disturbed in obstructive nephropathy. Induction of arginase-2 may lead to the activation of the pathway of proline formation instead of polyamine synthesis and finally result in synthesis/storage of collagen. In obstructive nephropathy it demonstrated the increase of expression of arginase-2 in the kidney. Analysis of mRNA expression reported the role of arginase with pro-fibrotic effect that is activated in obstructive nephropathy.

Recent studies indicate that arginase-2 plays a major role in induction of diabetic renal injury and blockage of arginase-2 activity or expression could be a new therapeutic approach in the treatment of diabetic nephropathy [14].

It has been found that pharmacological blockage or genetic deficiency of arginase-2 confer renal protection in experimental models of diabetes [8]. Inhibition of arginase mediates renal tissular protection of diabetic nephropathy through NOS dependent mechanisms which have NOS dependent effects on the recruitment of macrophage in the kidneys.

Experimental studies [11] suggest that arginase does not contribute to endothelial dysfunction in CKD; In conclusion, the transport affected by L-arginine may play an important role in the reduction of availability of substrate in production of nitric oxide, that leads to endothelial dysfunction.

Arginase inhibition effects have been compared with the treatment the angiotensin-converting enzyme inhibitor captopril, which was administered in early and advanced diabetic nephropathy. Early treatment with captopril reduced the level of albuminuria, histological changes and macrophage infiltration in the kidneys without affecting other parameters, while late treatment with captopril proved to be ineffective. These data demonstrate the importance of arginase inhibition as a potential new therapeutic target in early and late stages of diabetic renal injury[23].

Conclusions

1.The level of NO metabolites in the urine increased significantly in all clinical variants of GN. The most significant increase of NO was attested in CGN mixed form during relapse , thus level of NO in urine was increased by 4,8 times, and during remission was decreased by almost 2,6 times in comparison to the control group.

2.Arginase activity in urine was increased in all variants of clinical periods of GN. In SSNS group arginase activity in urine during the relapse period increased by 4,5 times compared to SRNS. During remission arginase activity in urine was reduced practically to the levels of the control group. In CGN mixed form arginase levels in the urine were increased by 2,9 times and during remission were decreased by almost 1,9 times compared to the control group.

3.Assesment of NO metabolites and arginase in urine can be used as a diagnostic method in order to monitor renal disease process, disease evolution and effectiveness of applied treatment.

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Corresponding Author: Angela Ciuntu, Assoc.Professor, Pediatric Department, State University of Medicine and Pharmacy „Nicolae Testemitanu", Paediatrics National Institute of Health Care for Mother and Child, Nephrology Unit, 165, Stefan cel Mare bd., MD-2004,Chișinău, Rep. of Moldova;
e-mail: angela.ciuntu@yahoo.com