Pediatr Nephrol (2001) 16:719–722

CLINICAL NEPHROLOGY / ORIGINAL ARTICLE

Alessandra Vitorino Naghettini
Paulo Cesar Koch Nogueira · Maria Juliano
Allain Amador Bueno · Dulce Elena Casarini
João Tomás de Abreu Carvalhaes

Tubular urinary enzymes in acute post-infectious glomerulonephritis

Received: 31 May 2000 / Revised: 25 April 2001 / Accepted: 27 April 2001

Abstract Tubular function of 17 pediatric patients with a mild form of acute post-infectious glomerulonephritis was prospectively evaluated by assessment of the urinary activity of proximal and distal tubule enzymes. Neutral-like endopeptidase (NEP-like) and angiotensin-converting enzyme (ACE) were the proximal tubule enzymes assessed, while prolyl-endopeptidase (PE) and serine-endopeptidase H1 and H2 were the distal tubule enzymes analyzed. Urine was collected at diagnosis (T0) and after 2 (T2) and 6 (T6) months of follow-up. NEP-like enzyme activity (nmol/mg creatinine; median±quartile range) was increased at diagnosis, and this remained stable during the first 6 months (T0 18.30±83.26, T2 17.32±49.56, T6 23.38±107.18). Urinary activity of the other enzymes was as follows: ACE (mU/ml per mg creatinine) T0 0.08±0.16, T2 0.06±0.10, T6 0.18±0.29; PE (nmol/mg creatinine) T0 6.70±84.87, T2 9.55±69.00, T6 13.67±28.70; serine-endopeptidase H1 (nmol/mg creatinine) T0 7.86±26.95, T2 17.17±59.37, T6 18.19± 79.14; and serine-thiol-endopeptidase H2 (nmol/mg creatinine) T0 3.06±21.97, T2 12.06±32.42, T6 16.22± 44.06. Thirty other healthy children matched for age and gender were considered as a control group. This group was assessed once and the results were: NEP-like activity 6.05±10.54, ACE 0.11±0.22, PE 7.10±13.36, H1 5.00±17.30, and H2 6.00±20.16. In conclusion, we observed that NEP-like and H1 enzymes exhibited significant increased urinary activity 6 months after the diagnosis. This increase occurred in spite of the disappearance of clinical symptoms, which occurred 2 months after the diagnosis. We believe that the increase in urinary enzymatic activity could be a manifestation of a silent tubular dysfunction following an episode of acute post-infectious glomerulonephritis.

Keywords Glomerulonephritis · Acute disease · Endopeptidases · Peptide hydrolases · Renal tubular function

A.V. Naghettini (✉)
Department of Pediatrics, Faculdade de Medicina, UFG, Goiânia, Goiás, Brazil
e-mail: naghetti@ terra.com.br
Fax: +55-62-2558401

A.V. Naghettini · P.C.K. Nogueira · J.T. de Abreu Carvalhaes
Pediatric Nephrology Unit, Department of Pediatrics, Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil

M. Juliano
Department of Medical Biophysics, Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil

A.A. Bueno · D.E. Casarini
Kidney and Hormone Laboratory, Nephrology Discipline, Department of Internal Medicine, Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil

P.C.K. Nogueira
Department of Pediatrics, Faculdade de Ciências Médicas de Santos, Brazil

Introduction

Acute post-infectious glomerulonephritis (AGN) is a well-known disease, usually associated with a favorable prognosis, and most frequently seen in children and adolescents. Clinical and laboratory aspects of AGN and its glomerular lesions have been extensively investigated in several studies [1, 2, 3]. In the majority of cases the disease is quite silent and mild severity is to be expected; only rarely are severe complications, such as acute renal failure or hypertensive encephalopathy, present. The course of the disease is also benign and the main symptoms usually disappear after 1–2 months of follow-up. The only symptom that persists is microscopic hematuria, which can be present up to 1 year following the diagnosis [4]. The integrity of tubular function has rarely been assessed in AGN, since the disease is believed to have a benign nature, and only a few studies to date have reported long-term glomerulotubular involvement [5, 6, 7, 8].

Urinary enzyme activity has been used as a method of assessment of tubular functional integrity. Some evidence suggests that there is a correlation between morphological lesions and cellular functional changes [9, 10,

11, 12], indicating that it might be possible to detect and follow renal tubular involvement by periodical assessments of urinary enzyme activity.

The present pilot study aims to evaluate urinary enzyme activity in a group of patients with a mild form of AGN (a frequent disease in our country), both in the acute phase and during a medium-term follow-up period, in order to assess the tubular function of this selected group of patients for whom usual clinical and laboratory data are expected to be uneventful.

Patients and methods

The study group comprised 17 children (13 boys) with a mean age of 6.7±2.3 years (range 2–10 years) and a clinical diagnosis of mild acute post-infectious glomerulonephritis syndrome. The syndrome was characterized by an appropriate history and clinical evidence of at least two of the following: hypertension, fluid retention, and/or hematuria (after an episode of infectious disease). All patients were assessed during the acute phase (T0 from onset of symptoms up to a maximum of 15 days later), and again at 2 (T2) and 6 (T6) months of follow-up.

A group of 30 healthy children was recruited in local schools as controls (22 boys, mean age 6.4±2.1 years, range 2.4–9.9 years). They were not taking medications nor presented with any renal or systemic disease. Evaluation of urinary enzyme activity was performed only once for this group, and they were compared with the patients only at T6, because this is a period usually characterized by remission of clinical symptoms.

Samples of 5 ml of urine were collected from patients and controls by spontaneous voiding in the morning, after discarding the first flush. This strategy was adopted in order to avoid confounding variables, such as urinary flow rate, physical exercise, and circadian rhythm. The urine samples were then stored at –20°C until biochemical analyses were performed. Collected urine was centrifuged at 3,000 rpm at 4°C and then 4 times concentrated in an Amicon concentrator. Samples were then incubated with 5 µg/ml substrate, in 50 nM TRIS-HCl buffer, pH 7.0, at 37°C [13].

Activity of the enzymes neutral-like endopeptidase (NEP-like), prolyl-endopeptidase (PE), serine-endopeptidase (H1), and serine-thiol-endopeptidase (H2) was determined by a spectrofluorimeter (F2000, Hitachi) with specific fluorogenic substrates derived from the substrate Abz-RPPGFSPFRQ-EDDnp (Abz-BKQ-EDDnp) (Abz=orthoaminobenzoic acid, EDDnp=2,4-dinitrophenyl ethylenediamine, BK=bradykinin, and Q=glutamine). Glutamine was used to adapt the solid-phase methodology. Results are expressed as nanomoles per milligram creatinine [13, 14, 15].

Angiotensin converting enzyme (ACE) activity was determined fluorimetrically by the quantity of L-His-Leu released and was expressed as milliunits per milliliter per milligram urinary creatinine. This is based on the conversion of the substrate analog, hippuryl-L-histidyl-L-leucine, to hippurate and L-histidyl-L-leucine, which is quantified spectrofluorimetrically [16, 17].

Samples of urine from the patients were also checked for pH, urine density, glycosuria, and hematuria. Hematuria was detected on centrifuged urine by light microscopy. Concentration of urinary protein was determined by the method of Bradford. Determination of urinary creatinine was performed according to the Jaffé reaction, an alkaline picrate method automated on a Cobas Mira Plus (Roche). The reaction was read by the absorbance at 520 nm and the quantity of creatinine was determined from a standard curve expressed in milligrams per milliliter of urine.

Blood samples were drawn only from patients at T0. Serum creatinine was determined according to the Jaffé reaction, while serum C3 was assessed by immunoenzyme reaction.

Since the numerical data had a skewed distribution, non-parametric tests were used for the statistical analysis of the results. Friedmann's post test [18] was used for analysis of variance. Analysis was complemented by the multiple comparisons test when a significant difference was found by Friedmann's analysis of variance [19]. Mann-Whitney U test for two independent samples [18] was used to compare controls with AGN patients at T6. Sperman's correlation coefficient was used to correlate enzyme activity with other parameters.

The protocol was reviewed and approved by our hospital's ethics committee. All patients and their guardians were informed about the study at the beginning of the survey and the children were included only after signing the informed consent form.

Results

All 17 patients fulfilled the clinical criteria for AGN. The most frequent sites of associated infections were the skin [11/17 (64.7%)] and the upper respiratory tract [6/17 (35.3%)]. One-third of the patients received antibiotics (benzathine penicillin) because of active infection at the time of the study. Only 6 children were admitted to hospital and all were discharged in good health. All patients presented with hematuria, while 14 children exhibited oliguria and edema; hypertension was noted in 10 patients at T0. Three patients with acute renal failure at the time of diagnosis were excluded from the study due to severe clinical presentation.

Table 1 Clinical and laboratory values (mean±standard deviation) in acute post-infectious glomerulonephritis patients at the time of diagnosis (T0), and after 2 (T2) and 6 (T6) months. For normal children (control group) the analysis was confined to one period (*BP* blood pressure)

	T0 (*n*=17)	T2 (*n*=17)	T6 (*n*=17)	Control (*n*=30)
Systolic BP (mmHg)	128±25	88±9	91±9	92±10
Diastolic BP (mmHg)	88±20	58±7	56±8	51±11
Hematuria (rbc/ml urine)	378,000±38,522	12,176±17,278	8,058±18,403	
Serum complement C3 (mg/dl)	42±28	118±36	133±42	
Serum creatinine (mg/dl)	0.8±0.6	0.7±0.2	0.6±0.2	
Serum albumin (g/dl)	4.2±0.7	4.7±0.4	4.8±0.4	
Urine protein (mg/24 h)	455±776	58±29	106±231	
Urine pH	5.5±0.6	5.9±0.7	5.7±0.6	
Urine density	1023±6	1020±5	1018±7	

Table 2 Urinary enzymatic activity (median±quartile range) in acute post-infectious glomerulonephritis patients at the time of diagnosis (T0), and after 2 (T2) and 6 (T6) months. For normal children (control group) the results are confined to one period (*NEP* neutral-like endopeptidase, *ACE* angiotensin converting enzyme, *PE* prolyl-endopeptidase)[a]

Enzymes	T0 (*n*=17)	T2 (*n*=17)	T6 (*n*=17)	Control (*n*=30)	*P*
NEP-like (nmol/mg creatinine)	18±83	17±50	23±107	6±11	<0.05*
ACE (mU/ml per mg creatinine)	0.1±0.2	0.1±0.1	0.2±0.3	0.1±0.2	NS
PE (nmol/mg creatinine)	7±85	10±69	14±29	7±13	NS
H1 (nmol/mg creatinine)	8±27	17±59	18±79	5±17	<0.05*
H2 (nmol/mg creatinine)	3±22	12±32	16±44	6±20	NS

* Control less than patients at T6, according to Mann-Whitney U test
[a] T0=T2=T6 by Friedmann Post test

All patients showed a benign clinical and laboratory course and had no clinical involvement in the 2nd month of follow-up, as shown in Table 1. Enzymatic activity in patients with AGN at three different stages of follow-up, as well as the differences between this group and controls at T6, are shown in Table 2. NEP-like enzyme activity was increased at T0, and this was maintained during the first 6 months; this was significantly different from controls. However, there were minimal variations in ACE during the entire follow-up period and results for this enzyme did not attain statistical significance. We also observed a significant rise of enzymuria for enzymes with primary activity in the distal tubule (PE, H1, and H2) up to 6 months of follow-up. H1 activity in patients was significantly different from controls at T6 (Table 2). We found no meaningful correlation of urinary enzymatic activity and clinical and laboratory data.

Discussion

Although investigating the epidemiology of the disease was not our goal, the triggering infection is likely to have a streptococcal origin, such as impetigo and tonsillitis, due to the great prevalence of these conditions. Pyoderma represented the main cause, probably related to the climate of the region (hot and dry), the presence of insects, and the low socioeconomic level of the population, factors which have been described in previous studies [20, 21, 22, 23].

Increased amounts of urinary enzymes have been used as initial markers of renal injury. Their quantitative assessment and monitoring represent a promising non-invasive diagnostic technique for evaluation of acute and chronic nephron damage, since excretion of these enzymes usually rises before other markers, such as serum creatinine, urinary glucose, phosphate, amino acids, and/or proteins [11, 12].

Casarini et al. [24] and Di Marco et al. [13] demonstrated that endopeptidases and exopeptidases are excreted by the kidney. The initial portion of the proximal tubule secretes ACE and NEP-like, while the final segment of the distal tubule secretes PE, serine endopeptidase, carboxypeptidase, and NEP. The excretion of urinary enzymes may vary according to age, sex, and physical exercise. Different urinary flow rates, physical exercise, and circadian rhythm are typical short-term physiological variables that influence the pattern of excretion [11, 25].

The urinary activity of NEP-like was high in the initial phase of glomerulonephritis and at 6 months, with a significant difference between patients and controls. NEP-like is amply distributed throughout the human body (kidneys, central nervous system, lungs, male genital tract, plasma membrane of neutrophils). Its molecular weight is high (94 kilodaltons), its serum concentration is very low, and, under normal conditions, NEP is not filtered by the glomeruli [26]. The presence of neutrophils in the mesangium during the acute phase of the disease might account for the increased amounts of this enzyme at the onset of symptoms (T0) [27]. However, the persistence of increased excretion at T6 might reflect a lesion of the tubular surface, since NEP is an enzyme located in the brush border of the proximal tubule cells [26, 28].

ACE has been detected in many tissues (lung, kidney, blood plasma). In the kidney, it is located on the surface of the brush border [29]. In our study, ACE showed low urinary activity during the whole follow-up period. In other studies, a low concentration of this enzyme was observed compared with NEP [30, 31]. Our data do not allow us to conclude whether this low ACE activity occurred independently of high NEP-like activity, or if partial degradation of the enzyme occurred during the purification method [30].

Serine proteinases are not filtered by the glomeruli and it is known that PE, H1, and H2 have activity in the distal portion of the kidney, but the specific cellular site has not been defined yet [24, 32, 33]. A progressive increase in urinary activity of H1 and H2 was observed in our patients at T2 and T6. This is in agreement with the hypothesis that tubular function might be altered during a period characterized by the remission of clinical symptoms of AGN. In accordance with this hypothesis, previ-

ous studies have established by morphometric methods that the distal tubule is mostly affected by interstitial damage, whatever the type of glomerulonephritis. Moreover, due to its proximity to other nephron segments, it can be affected even when it is not the primary site of damage [34]. However, our data showed a wide range of variation and should be interpreted with caution.

In conclusion, we observed that some enzymes exhibited increased urinary activity 6 months after the diagnosis of AGN. This is in contrast to the observed clinical picture, which was characterized by the disappearance of clinical symptoms with no apparent sequelae. This suggests that there is a silent tubular dysfunction in AGN, in spite of the favorable short-term clinical course of the disease. We do not know the implications of this phenomenon in the long-term evolution of these patients. However, it would be interesting to investigate whether the observed enzymuria persists for a longer period of follow-up in AGN patients, and whether it occurs in other forms of glomerulonephritis.

References

1. Tejani A, Ingulli E (1990) Post-streptococcal glomerulonephritis. Nephron 55:1–5
2. Cole BR, Salinas-Madrigal L (1994) Acute proliferative glomerulonephritis and crescentic glomerulonephritis. In: Holliday MA, Barratt MT, Avner ED (eds) Pediatric nephrology, 3rd edn. Williams and Wilkins, Baltimore, p 697
3. Brouhard BH, Travis LB (1992) Acute postinfectious glomerulonephritis. In: Edelmann CM (ed) Pediatric kidney disease, 2nd edn. Little Brown, Boston, p 1199
4. Habib R, Gonzales-Burchard G (1983) Glomérulonéphrite aiguë post-infectieuse. In: Royer P, Habib R, Mathieu H, Broyer M (eds) Néphrologie pédiatrique, 3rd edn. Flammarion Médicine-Sciences, Paris, p 294
5. Bohle A, Mackensen-Haen S, Von Gise H, Grund K-E, Wehrmann M, Batz C, Bogenschütz O, Schmitt H, Nagy J, Müller CA, Müller GA (1990) The consequences of tubulointerstitial changes for renal function glomerulopathies. A morphometric and cytological analysis. Pathol Res Pract 186: 135–144
6. Bohle A, Wehrmann M, Batz C, Bogenschütz O, Batz C, Vogl W, Schmitt H, Müller CA, Müller GA (1992) The long-term prognosis of the primary glomerulonephritides. A morphological and clinical analysis of 1747 cases. Pathol Res Pract 188:908–924
7. Parra G, Romero M, Henriquez LA, Roche C, Pineda R, Rodriguez-Iturbe B (1994) Expression of adhesion molecules in poststreptococcal glomerulonephritis. Nephrol Dial Transplant 9:1412–1417
8. Cleper R, Davidovitz M, Halevi R, Eisentein B (1997) Renal functional reserve after acute poststreptococcal glomerulonephritis. Pediatr Nephrol 11:473–477
9. Rebel W, Bertsch T, Bode G, Bleuel H (1992) Enzymuria as an indicator of renal pathomorphology. In: Jung K, Mattenheimer J, Burchardt U (eds) Urinary enzymes in clinical and experimental medicine, 2nd edn. Springer, Berlin Heidelberg New York, p 43
10. Jung K (1992) Urinary enzymes in renal-associated and urological disorders. In: Jung K, Mattenheimer H, Burchardt U (eds) Urinary enzymes in clinical and experimental medicine, 2d edn. Springer, Heidelberg Berlin New York, p 169
11. Jung K (1994) Urinary enzymes and low molecular weight proteins as markers of tubular dysfunction. Kidney Int 46:29–33
12. Scherberich JE (1990) Urinary proteins of tubular origin: basic immunochemical and clinical aspects. Am J Nephrol 10:43–51
13. Di Marco GS, Quinto BMR, Juliano M, Carmona AK, Stella RCR, Plavnik FL, Casarini DE (1998) Purification and characterization of neutral endopeptidase-like enzyme from human urine. J Hypertens 16:1971–1978
14. Chagas JR, Juliano L, Prado ES (1991) Intramolecular quenched fluorogenic tetrapeptide substrate for tissue and plasma kallikreins. Anal Biochem 192:419–425
15. Oliveira MC, Hirata IY, Chagas JR, Boschove P, Gomes R A, Figueiredo AF, Juliano L (1992) Intramolecular quenched fluorogenic peptide substrates for human renin. Anal Biochem 203:39–46
16. Friedland J, Silverstein E (1976) A sensitive fluorimetric assay for serum angiotensin-converting enzyme. Am J Clin Pathol 66:416–424
17. Casarini DE, Boim MA, Stella RCR, Krieger-Azzolini MH, Krieger JE, Schor N (1997) Angiotensin I-converting enzyme activity in tubular fluid along the rat nephron. Am J Physiol 272:F405–F409
18. Siegel S, Castellan NJ (1988) Non-parametric statistics, 2nd edn. McGraw-Hill, New York, p 399
19. Hollander M, Wolfe DA (1973) Non-parametric statistical methods. Wiley, New York, p 503
20. Tewodros W, Muhe L, Daniel E, Scahlén C, Kronvall GA (1992) One-year study of streptococcal infections and their complications among Ethiopian children. Epidemiol Infect 109:211–225
21. Zoric D, Kelmendi M, Shehu B, Krdzic J (1997) Acute poststreptococcal glomerulonephritis in children. Adv Exp Med Biol 418:125–127
22. Keller CK, Andrassy R, Ritz E (1994) Postinfectious glomerulonephritis – is there a link to alcoholism? Q J Med 87:97–102
23. Van Buynder PG, Gaggin JA, Martin D, Pusgley D, Mathews JD (1992) Streptococcal infection and renal disease markers in Australian aboriginal children. Med J Aust 156:537–540
24. Casarini DE, Boim MA, Stella RCR, Schor N (1999) Endopeptidases (kininases) are able to hydrolyze kinins in tubular fluid along the rat nephron. Am J Physiol 277:F66–F74
25. Feldmann D, Flandrois C, Jardel A, Phan T-M, Aymard P (1989) Circadian variations and reference intervals for some enzymes in urine of healthy children. Clin Chem 35:864–867
26. Nortier J, Abramowicz D, Najdovski T, Kinnaert P, Vanherweghem J-L, Goldman M, Deschodt-Lanckman M (1993) Urinary endopeptidase 24.11 as a new marker of proximal tubular injury – preliminary study in kidney transplant recipients. Contrib Nephrol 101:169–176
27. Nortier JL, Deschodt-Lanckman MM, Simon S, Thielemans NO, Prez EG, Depierreux MF, Tielemans RC, Lauwerys RR, Bernard AM, Vanherweghiem J-L (1997) Proximal tubular injury – Chinese herbs nephropathy: monitoring by neutral endopeptidase enzymuria. Kidney Int 51:288–293
28. Nortier J, Bernard A, Roels H, Deschodt-lankman M, Gueuning C, Lauwerys R (1997) Urinary neutral endopeptidase in workers exposed to cadmium: interaction with cigarette smoking. Occup Envirom Med 54:432–436
29. Erdös EG, Skidgel RA (1987) The angiotensin I-converting enzyme. Lab Invest 56:345–348
30. Skidgel RA, Davis RM, Erdös EG (1984) Purification of a human urinary carboxipeptidase (kininase) distinct from carboxipeptidase A,B, or N. Anal Biochem 140:520–531
31. Skidgel RA, Schulz WW, Tam L-T, Erdös EG (1987) Human renal angiotensin I converting enzyme and neutral endopeptidase. Kidney Int 31:45–48
32. Davin J-C, Davies M, Foidart J-M, Foidart JB, Dechene CA, Mahieu PR (1987) Urinary excretion of neutral proteinase in nephrotic rats with a glomerular disease. Kidney Int 31:32–40
33. Casarini DE, Stella RCR, Araújo MS, Sampaio CAM (1993) Purification and characterization of endopeptidase H2, a kinin inactivating serine proteinase (kininase) from human urine. Braz J Med Biol Res 26:15–29
34. Khan TN, Sinniah R (1993) Morphometric study showing the importance of distal tubular damage in impaired creatinine clearance. Am J Nephrol 13:178–183