Urol Res (2005) 33: 465–469
DOI 10.1007/s00240-005-0509-z

ORIGINAL PAPER

Hakan Vuruskan · Zulkuf Caliskan · Yakup Kordan
Cuneyt Ozakin · Ismet Yavascaoglu · Bulent Oktay

Elevated plasma concentrations of transforming growth factor-beta 1 in patients with unilateral ureteral obstruction

Received: 28 February 2005 / Accepted: 3 May 2005 / Published online: 29 November 2005

Abstract We measured plasma concentrations of TGF-beta 1 in patients with obstructive ureteral calculi and compared them with the plasma concentrations of healthy volunteers. The present study was a prospective study containing a homogenous group of patients with unilateral ureteral obstruction (UUO). The study consisted of patients with ureteral stones less than 7 mm in diameter that caused mild to moderate obstruction. All patients were referred by the emergency department of our hospital and examined between April 2003 and April 2004. The presence and characteristics of both stone and obstruction were determined by plain abdominal x-ray and gray-scale ultrasonography (US). Blood samples were collected from both patients and control individuals on admission and 1 week after conservative follow-up. The plasma TGF-beta 1 concentration was determined using a quantitative sandwich enzyme immunoassay specific for TGF-beta 1. There were 35 patients with 20 women and 15 men (average age 26.8 ± 5.9 years), and 15 volunteers in the control group, with nine women and six men (average age 24.2 ± 4.5 years). Average stone size was 5.6 mm ± 1.2 mm (range 3.5–7) for the patient group. US showed the presence of mild hydronephrosis in 24 and moderate hydronephrosis in 11 patients. Plasma concentrations of TGF-beta 1 in patients with ureteral obstruction (1,117 ± 5.8 ng/ml, range 36–2,442 ng/ml) were significantly higher than those in the healthy control group (32 ± 4 ng/ml) on admission ($P < 0.001$). There was a significant increase in TGF-beta 1 plasma concentrations in the patient group (33,525 ± 6.8 ng/ml, range 1,107–73,288 ng/ml) after 1 week follow-up ($P < 0.001$). Ureteral obstruction increases plasma TGF-beta 1 concentrations in patients with ureteral stones as in UUO models in animal studies. A concomitant treatment with an anti-fibrotic agent may reduce the incidence of renal injury during obstruction.

Keywords TGF-beta 1 · Obstructive nephropathy · Ureteral calculus · Interstitial fibrosis

H. Vuruskan (✉) · Z. Caliskan · Y. Kordan · I. Yavascaoglu
B. Oktay
Department of Urology, Medical Faculty, Uludag University, Bursa, Turkey
E-mail: vuruskan@uludag.edu.tr
Tel.: +90-224-4428146
Fax: +90-224-4428688

C. Ozakin
Department of Microbiology, Medical Faculty, Uludag University, Bursa, Turkey

Introduction

Ureteral obstruction is a common clinical finding in urology practice. Ureteral calculus is the most frequent cause of unilateral ureteral obstruction (UUO). A conservative approach can be used in a large number of patients having a ureteral stone with a diameter of less than 7 mm. In 98% of patients, such small ureteral stones are passed spontaneously [1, 2]. In clinical practice, due to certain variables, damage to renal function is unpredictable with temporary obstruction. Most of our knowledge on UUO is derived from studies on experimental animal models. Increased expression of transforming growth factor-beta 1 (TGF-beta 1) in intrinsic renal cells or macrophages invading the kidney is due to elevated concentrations of angiotensin II. TGF-beta 1 is a key mediator of renal fibrosis in obstructive nephropathy. It increases the synthesis of matrix proteins while decreasing that of proteases which could degrade matrix [3, 4]. Recovery after relief of obstruction is dependent on several factors, including the duration of the obstruction, its location, whether it is partial or complete, and the presence of intercurrent infection [5, 6]. At a very early stage of obstruction, a number of pharmacologic interventions, such as ACE inhibitors and antagonists, arginine, estrogenic protein-1, pirferidon, and atorvastatin, may ameliorate the increased expansion of the interstitial volume, decrease the expression of TGF-beta 1, and downregulate the

production of extracellular matrix and the infiltration of the interstitium by macrophages [3, 7, 8]. Increased concentrations of TGF-beta 1 have been described in animal models of UUO, particularly those associated with scarring [9–11]. Since TGF-beta 1 plays an essential role in the progression of obstructive uropathy, we measured its plasma concentrations in patients with obstructive ureteral calculi and compared these to the plasma concentrations of healthy volunteers. The present study was a prospective study containing a homogenous group of patients with UUO.

Patients and methods

The population in our study consisted of patients with ureteral stones less than 7 mm in diameter causing mild to moderate obstruction. We excluded patients with diabetes, autoimmune disease, malignancy, hypertension and recurrent stone disease to avoid other causes of TGF-beta 1 concentration elevation. All patients were referred by the emergency department of our hospital and examined in the Department of Urology between April 2003 and April 2004. The patient group consisted of 35 individuals, including 20 women and 15 men (average age 26.8 ± 5.9 years). There were 15 volunteers in the control group, including nine women and six men (average age 24.2 ± 4.5 years). All patients and volunteers were Caucasians. The presence and characteristics of the stones and obstruction were determined by plain abdominal x-ray and gray-scale ultrasonography (US). All patients underwent these imaging studies at admission and 1 week later. The degree of hydronephrosis was classified as minimal, moderate and severe (Malave et al. [12]). All patients were allowed to use symptomatic therapy with injections of 75 mg diclofenac if required. After 1 week follow-up, patients who failed to expel the stone underwent ureteroscopy. Blood samples were collected from the patients and control group at admission and 1 week after conservative follow-up.

Determination of plasma TGF-beta 1 concentrations

Venous blood (5 ml) was immediately placed on ice after being collected in precooled EDTA-containing tubes. The samples were centrifuged at 2,500 rpm for 30 min to remove platelets; the top 0.6 ml of the platelet-poor plasma was preserved at −80°C until assayed. The plasma TGF-beta 1 concentration was determined using a specific quantitative sandwich enzyme immunoassay (R and D Systems, Minneapolis, USA) according to the manufacturer's instructions. To activate the latent TGF-beta 1 into the immunoreactive form, the samples were activated by acid and then neutralized [9]. A total of 0.5 ml sample was briefly acidified with 0.5 ml 1.25 mol/l acetic acid/10 mol/l urea plus 50 mg of phenylmethyl-sulfonyl fluoride; five samples were neutralized with 0.5 ml of 2.7 mol/l NaOH/0.1 mol/l HEPES free acid. Serially diluted standards and samples with calibrator diluents in the immunoassay kit were incubated in a 96-well plate coated with TGF-beta 1 receptor type II. After washing away any unbound protein, a polyclonal antibody specific for TGF-beta 1 conjugated to horseradish peroxidase was added to the wells to sandwich the TGF-beta, which was immobilized during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and the color development at 450 nm was measured. The TGF-beta 1 content was determined by extrapolation from a standard curve.

Plasma concentrations of TGF-beta 1 from the patient and control groups on admission and 1 week later were compared. The Wilcoxon test was used for statistical analysis. TGF-beta 1 concentrations were also compared by Kruskal-Wallis test according to the severity of obstruction. Significance was defined as $P < 0.05$.

This study was performed after approval by the Ethics Committee of Uludag University, Bursa, Turkey.

Results

The male to female ratio was 1:1.75 for the patient group and 1:1.5 for the control group. Average stone size was 5.6 mm ± 1.2 mm (range 3.5–7) for the patients. In 28 of 35 patients (80%) stone presence was determined by x-ray. The presence of stone was diagnosed in all patients with US, and US showed the presence of mild hydronephrosis in 24 and moderate hydronephrosis in 11 patients. Stone expulsion was observed in 30 patients (85.7%) after 1 week of follow-up. No patient was hospitalized for recurrent colic and no urinary tract infection was recorded.

Plasma concentrations of TGF-beta 1 in patients with ureteral obstruction (1,117 ± 5.8 ng/ml, range 36–2,442 ng/ml) were significantly higher than those in the healthy control group (32 ± 4 ng/ml) at admission ($P < 0.001$). There was a significant elevation in

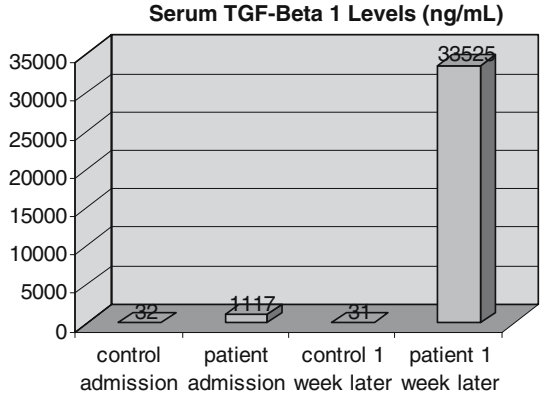

Fig. 1 Plasma concentrations of TGF-beta 1 for patients and control groups at admission and 1 week later

Table 1 Mean value of plasma concentrations of TGF-beta 1 in patients with ureteral stone according to severity of hydronephrosis

Severity of hydronephrosis	No. patients	TGF-beta 1(ng/ml) at admission	*P*	TGF-beta1(ng/ml) 1 week later	*P*
Mild	24	624	< 0.01	28,427	< 0.01
Moderate	11	2,192.5		44,648	

TGF-beta 1 plasma concentration (33,525 ± 6.8 ng/ml, range 1,107–73,288 ng/ml) in patients after 1 week follow-up ($P < 0.001$). In the control group there was no significant change (31 ± 3 ng/ml). A summary of the plasma TGF-beta 1 concentrations of patients and controls is given in Fig. 1. Plasma concentrations were normal at admission in two patients (8.5%) with mild hydronephrosis. After 1 week follow-up elevated plasma concentrations were also found in these patients.

The TGF-beta 1 concentrations at admission were higher in patients with moderate hydronephrosis compared to patients with mild hydronephrosis. The increase in TGF-beta 1 concentrations during 1 week follow-up was also higher in the former group (Table 1) ($P < 0.01$).

Discussion

Several animal studies have investigated the effects of UUO on renal injury and function. UUO is a well characterized model for experimental hydronephrosis which results in the tubulointerstitial fibrosis of obstructed kidney [13–15]. The in vivo effects of urinary tract obstruction have been less well studied. In this study, we aimed to provide a model of UUO in a homogenous group of patients. Our main finding is that TGF-beta 1 concentrations increase in the plasma of patients with ureteral obstruction, as seen in UUO animal model studies.

The renin-angiotensin system (RAS) is activated after the onset of ureteral obstruction, and angiotensin II may contribute to the initiation of the subsequent deterioration in the obstructed kidney [9]. Angiotensin II directly induces TGF-beta 1. Expression of TGF-beta mRNA increases in obstructed kidney. TGF-beta 1 is a cytokine, which stimulates extracellular matrix synthesis and inhibits its degradation [10, 16]. Interstitial fibrosis is thought to develop as a result of an imbalance between extracellular matrix synthesis, deposition and degradation [3]. Infiltrating macrophages may also play a role in propagating the initial glomerular injury to the development of interstitial fibrosis with TGF-beta 1 stimulating matrix accumulation [17]. The degree of interstitial fibrosis is a most helpful measure of the degree of renal injury, correlating with the impairment in renal function [18, 19]. Renal biopsy is the way to determine the degree of interstitial fibrosis due to obstructive uropathy, but it is not possible in in vivo studies. Urinary concentrations of TGF-beta 1 and urinary markers such as beta 2-microglobulin or N-acetyl-glucosaminidase have been used in numerous studies to monitor renal injury induced by obstruction [20–22]. In the present study, we found a remarkable elevation in plasma TGF-beta 1 concentration in patients with ureteral calculi both at administration and 1 week later. The increase in TGF-beta 1 mRNA levels was demonstrated as a characteristic finding for the progression of interstitial fibrosis during and after relief of ureteral obstruction in animal models [23, 24].

Beyond nephrolithiasis, renal cell injury caused by UUO and high urinary supersaturation might be preconditions for nephrolithiasis. In patients with renal stone disease, hyperoxaluria and calcium oxalate crystal deposition induce renin synthesis, activating the RAS system. UUO-induced renal fibrosis involves many molecular species, immune regulators and cellular signaling pathways, some of which are involved in the pathogeneses of nephrolithiasis. Angiotensin II upregulates the synthesis and production of osteopontin (OPN). OPN has a number of functions involving the regulation of osteoclast function during bone formation, renal stone formation, tumorigenesis and the transformation and accumulation of macrophages. OPN coats the luminal surfaces of renal tubuler epithelium causing crystal adherence. An intranephronal environment which is created by UUO could help stone formation by starting initial crystallization on basement of renal tubules [25]. A dramatic increase in renal OPN and mRNA levels was reported in renal tubules in angiotensin II induced tubulointerstitial nephritis [26]. Umekawa et al. demonstrated that the interruption of RAS by either angiotensin receptor blocking or inhibition of ACE might reduce crystal deposition within the kidneys and prevent further stone formation [27].

The increase in plasma TGF-beta 1 concentration was correlated with the severity of hydronephrosis in patients. Two patients with normal TGF-beta 1 concentrations on admission may be explained by their seeking treatment at the onset of obstruction. We used US with plain radiography for the assessment of ureteral obstruction and stones. US is highly accurate in detecting mild to severe hydronephrosis, however, dilatation of the collecting system does not imply the presence of ureteral obstruction or determine its severity [28, 29]. The determination of obstruction lacked standardization; however, the contribution of clinical findings during obstruction enhanced the reliability of the results of imaging studies.

In acute as well as chronic glomerular diseases, the upregulation of TGF-beta 1 and the accumulation of extracellular matrix are concomitantly observed [30]. Hankonen et al. reported a correlation between urinary TGF-beta 1 excretion and interstitial inflammation in

patients with membranous glomerulonephritis [31]. Hellmich et al. found significantly higher serum concentrations of TGF-beta 1 in patients with diabetic nephropathy compared with patients without renal involvement [32]. In renal transplant recipients, Boratynska demonstrated that urine secretion of TGF-beta1 was increased in chronic renal graft rejection and urine secretion of TGF-beta 1 was associated with arterial hypertension, degree of interstitial tissue fibrosis, and progression of graft insufficiency [33]. On the other hand, Haramaki et al. [34] and De Muro et al. [35] found enhancement of urine TGF-beta 1 concentrations without interstitial inflammation in patients with membranous glomerulonephritis.

Although the clinical significance of renal injury secondary to obstruction by ureteral calculi remains questionable in healthy patients with two normal kidneys, those with a solitary kidney or compensated chronic renal insufficiency and recurrent stone disease may experience some degree of renal failure. The results of the present study have demonstrated that the increase of TGF-beta 1 concentration due to ureteral obstruction is similar in humans to that found in animal studies, and may cause interstitial fibrosis. This suggests that concomitant treatment with an anti-fibrotic agent may reduce the incidence of renal injury during obstruction. Further trials are needed to investigate the time-course of TGF-beta 1 levels after recovery from obstruction.

Acknowledgment This study was performed with financial support from the Research Foundation of Uludag University from Bursa, Turkey.

References

1. Hubner WA, Irby P, Stoller ML (1993) Natural history and current concepts for the treatment of small ureteral calculi. Eur Urol 24: 172
2. Ueno A, Kawamura T, Ogawa A, Takayasu H (1977) Relation of spontaneous passage of ureteral calculi to size. Urology 10: 544
3. Klahr S (2001) Urinary tract obstruction. Semin Nephrol 21: 133
4. Sharma K, Ziyadeh FN (1994) The emerging role of transforming growth factor-beta in kidney diseases. Am J Physiol 266: 829
5. Ito K, Chen J, El Chaar M et al. (2004) Renal damage progresses despite improvement of renal function after relief of UUO in adult rats. AJP Renal Physiol 287: 1283
6. Shokeir AA, Provoost AP, Nijmaan RJM (1999) Recoverability of renal function after relief of chronic partial upper urinary tract obstruction. BJU Int 83: 11
7. Manucha W, Oliveros L, Carrizo L, Seltzer A, Valles P (2004) Losartan modulation on isoforms and COX-2 expression in early renal fibro genesis in unilateral obstruction. Kidney Int 65: 2097
8. Mizuguchi Y, Miyajima A, Kosaka T et al. (2004) Atorvastatin ameliorates renal tissue damage in unilateral ureteral obstruction. J Urol 172: 2456
9. Ishidoya S, Morrisey J, McCracken R, Reyes A, Klahr S (1995) Angiotensin II receptor antagonist ameliorates renal tubulointerstitial fibrosis caused by unilateral ureteral obstruction. Kidney Int 47: 1285
10. Kaneto H, Morrisey J, Klahr S (1993) Increased expression of TGF-beta 1 mRNA in the obstructed kidney of rats with unilateral ureteral ligation. Kidney Int 44: 313
11. Oka A, Toshino N, Miyauchi A, Yokoyama M (1999) Expression of growth factors after release of ureteral obstruction in the rat kidney. Int J Urol 6: 607
12. Malave SR, Neiman HL, Spies SM et al. (1980) Diagnosis of hydronephrosis: comparison of radionuclide scanning and sonography. Am J Roentgenol 135: 1179
13. Ulm AH, Miller F (1962) An operation to produce experimental reversible hydronephrosis in dogs. J Urol 8: 261
14. Klahr S (1991) New insights into the consequences and mechanisms of renal impairment in obstructive nephropathy. Am J Kidney Dis 18: 689
15. Nagle RB, Bulger RE, Cutler RE, Jervis HR, Benditt EP (1973) Unilateral obstructive nephropathy in the rabbit. I. Early morphologic, physiologic and histochemical changes. Lab Invest 28: 456
16. Roberts AB, McCune BK, Sporn MB (1992) TGF-Beta regulation of extracelluler matrix. Kidney Int 41: 557
17. Diamond JR, Kees-Folts D, Ding G, Frye JE, Restrepo NC (1994) Macrophages, monocyte chemoattractant peptide-1 and TGF-beta 1 in experimental hydronephrosis. Am J Physiol 266: 926
18. Bole A, Mackensen-Haen S, Von Gisse H (1987) Significance of tubulointerstitial changes in the renal cortex for the excretory function and concentration ability of the kidney: a morphometric contribution. Am J Nephrol 17: 421
19. Wen JG, Frokier J, Jorgensen TM, Djurhuus JC (1999) Obstructive nephropathy: an update of the experimental research. Urol Res 27: 29
20. Carr MC, Peters CA, Retik AB, Mandell J (1994) Urinary levels of the renal tubular enzyme N-acetyl-glucosaminidase in unilateral obstructive uropathy. J Urol 151: 442
21. Huland H, Gonnermann D, Werner B, Possin U (1998) A new test to predict reversibility of hydronephrotic atrophy after stable partial unilateral ureteral obstruction. J Urol 140: 1591
22. Assimos DG, Boyce WH, Furr EG et al. (1987) Urinary enzyme levels after extracorporeal shock wave lithotripsy (ESWL). J Urol [Suppl] 137: 143A
23. Ogata Y, Ishidoya S, Fukuzaki A et al. (2002) Upregulated expression of transforming growth factor-beta, type IV collagen and plasminogen activator inhibitor-1 mRNA are decreased after release of unilateral ureteral obstruction. Tohoku J Exp Med 197: 159
24. Fukuda K, Yoshitomi K, Yanagida T, Tokumoto M, Hirakata H (2001) Quantification of TGF-Beta 1 mRNA along rat nephron in obstructive nephropathy. Am J Physiol Renal Physiol 281: 513
25. Xie Y, Sakatsume M, Nishi S et al. (2001) Expression, roles, receptors and regulation of osteopontin in the kidney. Kidney Int 60: 1645
26. Giachelli CM, Pichler R, Lombardi D et al. (1994) Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis. Kidney Int 45: 515
27. Umekawa T, Hatanaka Y, Kurita T, Khan SR (2004) Effect of angiotensin II receptor blockage on osteopontin expression and calcium oxalate crystal deposition in rat kidneys. J Am Soc Nephrol 15: 635
28. Ellenbogen PH, Scheible FW, Talner LB et al. (1978) Sensitivity of gray scale ultrasound in detecting urinary tract obstruction. Am J Roentgenol 130: 731
29. Baumgartner BR, Steinberg HV, Ambrosse SS et al. (1987) Sonographic evaluation of renal stones treated by extracorporeal shock-wave lithotripsy. Am J Roentgenol 149: 131
30. Border WA, Noble NA (1993) Cytokines in kidney disease: the role of transforming growth factor-beta. Am J Kidney Dis 22: 105
31. Hankonen E, Teppo AM, Tornroth H, Groop PA, Gronhagen-Riska C (1997) Urinary transforming growth factor-beta 1 in membranous glomerulonephritis. Nephrol Dial Transplant 12: 2562

32. Hellmich B, Scheller M, Schatz H, Pfeiffer A (2000) Activation of transforming growth factor-beta 1 in diabetic kidney disease. Metabolism 49: 353
33. Boratynska M (1999) Urine excretion of transforming growth factor-beta 1 in chronic allograft nephropathy. Ann Transplant 2: 23
34. Haramaki R, Tamaki K, Fujisawa M et al. (2001) Steroid therapy and urinary transforming growth factor-beta 1 in Ig-A nephropathy. Am J Kidney Dis 38: 1191
35. De Muro P, Faedda R, Freso P et al. (2004) Urinary transforming growth factor-beta 1 in various types of nephropathy. Pharmacol Res 49: 293