

The Effect of Mild Hyperuricemia on Urinary Transforming Growth Factor Beta and the Progression of Chronic Kidney Disease

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Key Words

Uric acid · Hypertension · Chronic kidney disease · Angiotensin

Abstract

Although mild hyperuricemia is common in patients with renal disease, it has usually been considered a marker of reduced nephron mass rather than a risk factor for progression of kidney disease. On the other hand, experiments in a rat model demonstrated important deleterious effects of mild hyperuricemia on several aspects of renal structure and function. In the present investigation, the impact of the discontinuation of allopurinol therapy on the control of hypertension and the rate of progression of chronic kidney disease was considered. The present work involved 50 patients, suffering from stage 3 and 4 chronic kidney disease. All of them were on chronic allopurinol therapy for the treatment of mild hyperuricemia. Their blood pressure, serum creatinine and uric acid levels were followed for 12 months following allopurinol withdrawal. Urinary transforming growth factor beta-1 (TGF- β_1) was assayed by a solid-phase enzyme-linked immunosorbent assay. After allopurinol withdrawal, significant worsening of hypertension, significant acceleration of the rate of loss of kidney function and a significant increase in the urinary excretion of TGF- β_1 were observed in the group of patients who were not receiving pharmacological blockers of the renin-angiotensin system. In conclusion,

asymptomatic hyperuricemia has a deleterious effect on the progression of chronic kidney disease and the control of hypertension. This effect was blocked by treatment with renin-angiotensin system blockers. Copyright © 2007 S. Karger AG, Basel

Introduction

The association between hyperuricemia, hypertension and renal disease has been known since 1879 [1]. Since that time, many authorities have attributed this association to a simple clustering of hyperuricemia with well-established cardiovascular and renal risk factors, and an elevated serum uric acid level by itself has generally been regarded as insignificant or incidental [2, 3]. However, two studies have found that hyperuricemia is an independent risk factor for progression of renal disease in patients with immunoglobulin A (IgA) nephropathy [4, 5]. In a recent study [6] involving 6,400 subjects with normal kidney function, a serum uric acid >8 mg/dl was associated with a 10-fold increased risk for the development of renal insufficiency within 2 years in women and a 2.9-fold increased risk in men. This increased risk was independent of age, blood pressure, total cholesterol, body mass index, blood glucose, smoking, proteinuria and hematuria. In the same study, elevated uric acid was more predictive of renal insufficiency than proteinuria.

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Table 1. Age, gender and kidney function at the time of allopurinol withdrawal

	Group 1 (n = 15)	Group 2 (n = 12)	Group 3 (n = 23)
Age, years	47.8 ± 4.6	43.8 ± 5.5	44.5 ± 4.8
Male/female	10/5	7/5	14/9
Serum creatinine, mg/dl	3.5 ± 0.9	3.41 ± 1	3.35 ± 1.1
Creatinine clearance, ml/min	28.7 ± 7.7	30 ± 8.1	32.3 ± 10
Δ Serum creatinine, mg/year ^a	0.5 ± 0.1	0.52 ± 0.3	0.4 ± 0.2
Δ Creatinine clearance, ml/min/year ^a	-3.9 ± 2	-4.1 ± 2.2	-3.6 ± 2

No significant differences were found between the studied groups. To convert serum creatinine in mg/dl to μmol/l, multiply by 88.4.

^a Average rate of change during the year preceding allopurinol withdrawal.

Experiments on an animal model of mild hyperuricemia demonstrated clearly that mild hyperuricemia induces salt-sensitive hypertension [7], a hypertension-independent renal arteriopathy [8], glomerular hypertension [9, 10], activation of the rennin-angiotensin system and progressive renal failure [7]. The older animal models of hyperuricemia were associated with severe hyperuricemia and the development of crystal-induced nephropathy with tubular obstruction and interstitial inflammation, findings that resemble human acute gouty nephropathy. In contrast, the oxonic acid-fed rat model develops mild hyperuricemia and a non-crystal deposition nephropathy that better mimics human asymptomatic hyperuricemia [7].

There are no universally accepted clinical recommendations for the management of mild and moderate asymptomatic hyperuricemia in patients with chronic kidney disease apart from dietary protein and purine restriction [11]. The lack of an animal model, concerns about the side effects of allopurinol [12], the nonspecific pathological findings associated with mild hyperuricemia [13] and confusion with lead nephropathy [14] have contributed to the current clinical uncertainty.

Transforming growth factor-β₁ (TGF-β₁) is a prosclerotic cytokine involved in the synthesis of extracellular matrix, decreasing its degradation and stimulating the synthesis of integrin matrix receptors [15]. Progressive nephropathies are characterized by the enhanced accumulation of extracellular matrix in the kidney, and overproduction of TGF-β₁ can result in pathological tissue fibrosis through the accumulation of extracellular matrix proteins [16]. Many studies have shown that TGF-β₁ expression is increased in human glomerulopathies, including diabetic nephropathy, lupus nephritis, human immunodeficiency virus nephropathy and IgA nephropathy [17–19].

In the present investigation, chronic allopurinol therapy was withdrawn from a group of patients with chronic kidney insufficiency and asymptomatic hyperuricemia. The reason for allopurinol withdrawal was concern about possible toxicity of a non-evidence-based use of the drug. The impact of the discontinuation of allopurinol therapy on the control of hypertension and the rate of progression of chronic kidney disease was considered. The possible role of angiotensin II in mediating uric acid-induced renal injury was investigated. Urinary TGF-β₁ was measured to detect a possible state of intrarenal inflammation in asymptomatic hyperuricemia.

Methods

The current investigation involved 50 patients (19 females, table 1). They all gave informed consent to participate in the study. They were suffering from stage 3 (n = 36) and stage 4 (n = 14) non-diabetic chronic kidney disease. Hypertension was the cause of renal disease in all of them. All of them had echogenic kidneys on abdominal ultrasound examination with variable degrees of renal atrophy. None of them had a nephrotic range proteinuria, biopsy-proven glomerulonephritis, or metabolic, obstructive or autosomal dominant polycystic kidney disease. All of them were on chronic allopurinol therapy for the treatment of mild hyperuricemia for at least 1 year before its withdrawal. Their mean serum uric acid level before starting allopurinol therapy was 9.6 ± 1.4 mg/dl (571 ± 83 μmol/l), range 7.8–10.4 mg/dl (464–619 μmol/l). The mean dose of allopurinol was 250 ± 50 mg/day, range from 100 to 400 mg/day. The mean duration of allopurinol therapy was 17 ± 3.4 months, range 13–20 months. Group 1 included 15 patients whose antihypertensive regimen included fosinopril, 20 mg/day while group 2 consisted of 12 patients whose antihypertensive regimen included losartan, 50 mg twice daily. Group 3 consisted of 23 patients on other antihypertensive therapies not including angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). Their blood pressure, kidney function and uric acid levels were measured monthly during the year before al-

Table 2. Uric acid level in the studied groups

	-Group 1	Group 2	-Group 3
Uric acid before starting allopurinol, mg/dl	9.8 ± 1.6	9.7 ± 1.8	9.5 ± 2
Allopurinol effect compared to pretreatment level, %	-21.5 ± 8	-23 ± 9	-22.5 ± 6
At 2 weeks after stopping allopurinol, %	+17 ± 7	+18 ± 6	+21 ± 5.8
At 12 months after stopping allopurinol, %	+25 ± 6	+26 ± 7	+33 ± 8

No significant differences were found between the groups. In group 3, 17 patients only were eligible for consideration at 12 months after allopurinol withdrawal. To convert serum uric acid in mg/dl to $\mu\text{mol/l}$, multiply by 59.48.

lopurinol withdrawal, 2 weeks after stopping allopurinol and monthly for 12 months following allopurinol withdrawal. Monthly revision of the antihypertensive therapy and dose titration and/or addition of a new class of antihypertensive drugs were done. However, the de novo addition of an ARB or ACEI was a reason for exclusion from further analysis. The serum creatinine and uric acid were determined by an autoanalyzer. Creatinine clearance was estimated using the modification of diet in renal disease (MDRD) formula [20]. Twenty-four-hour urinary protein excretion rate was measured by the turbidimetric assay [21].

For measuring urinary TGF- β_1 excretion, a 2.0-ml urine sample was centrifuged for 5 min to remove cells and particulate matter, and the supernatant was stored at -80°C . Before assay, specimens were thawed, placed in a filter unit (Centricon-10 filter; Amicon, Danver, Mass., USA), concentrated 25-fold by centrifugation for 60 min at 6,500 rpm, acid activated, and then neutralized as previously described [22]. Urinary TGF- β_1 was assayed by solid-phase enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Abingdon, UK) according to the manufacturer's instructions. Urinary creatinine concentration was determined simultaneously and TGF- β_1 was expressed in nanograms per gram creatinine. For each patient, the 12 monthly observations of serum creatinine and creatinine clearance during the year before stopping allopurinol and the 12 observations during the year following its withdrawal were used to calculate an average rate of change of the corresponding parameter during the corresponding year. The computed slope of the best-fit straight line representing the relation between time and either creatinine clearance or serum creatinine was considered the average rate of change of the corresponding parameter during the year. The unpaired 2-tailed Student t test was employed to compare the means of the groups, χ^2 to compare the incidence of nonparametric events between groups, and the paired t test to compare between TGF- β_1 before and 1 month after stopping allopurinol in group 3. Significance was set at $p < 0.05$. Statistical analysis was done on a personal computer using commercially available software.

Results

There were no statistically significant differences in serum creatinine, creatinine clearance and the rate of deterioration of kidney function between the three groups of the study before allopurinol withdrawal (table 1).

Table 3. Mean number of antihypertensive drugs across the studied groups

	Group 1	Group 2	Group 3
Before allopurinol withdrawal	2.17	2.13	2.35
2 weeks after withdrawal	2.17	2.13	2.69
1 month after withdrawal	2.17	2.2	2.78
6 months after withdrawal	2.25	2.2	3.06 ^a
12 months after withdrawal	2.33	2.67	3.12 ^a

^a Seventeen patients only.

Table 2 shows the serum uric acid levels of the studied subjects. Allopurinol therapy was equally effective in reducing uric acid levels in the three groups of the study. Similarly, differences in the antihypertensive regimens were not associated with significant differences in the degree of rise in uric acid levels after stopping allopurinol. Two weeks after allopurinol withdrawal, a significant increase in both systolic 155.6 ± 18.4 versus 124.5 ± 7.4 mm Hg ($p < 0.05$) and diastolic blood pressures 93.5 ± 5.3 versus 80.5 ± 3.2 mm Hg ($p < 0.05$) was noted in group 3, while no significant increase in blood pressure was noted in group 1 or group 2 (table 2). During the 12 months following allopurinol withdrawal, worsening of hypertension necessitating the addition of a new class of (or more) antihypertension medication occurred in 2/15 patients in group 1, 2/12 of patients in group 2, and 10/23 in group 3 who were significantly more likely to have worsening hypertension compared to patients in group 1 ($p < 0.05$) and group 2 ($p < 0.05$). Table 3 shows the mean number of antihypertensive medications in the studied groups.

Six patients in group 3 needed the de novo addition of either an ACEI or ARB for the control of worsening hypertension. After excluding them, 44 patients were eligible for further analysis. Table 4 shows serum creatinine

Table 4. Blood pressure before and after stopping allopurinol

	Group 1	Group 2	Group 3
<i>Systolic pressure, mm Hg</i>			
Upon allopurinol withdrawal	123.6 ± 6	120.4 ± 5.2	124.5 ± 7.4
2 weeks after withdrawal	125.1 ± 6*	123 ± 5.2*	155.6 ± 18.4*
12 months after withdrawal	124 ± 6.3	124 ± 5.7	129 ± 7.8
<i>Diastolic pressure, mm Hg</i>			
Upon allopurinol withdrawal	82.4 ± 3	79.6 ± 2.8	80.5 ± 3.2
2 weeks after withdrawal	80.5 ± 3.2*	80.2 ± 2.5*	93.5 ± 5.3*
12 months after withdrawal	82.6 ± 3	77.9 ± 3.3	83.5 ± 3.4

In group 3, 17 patients only were eligible for consideration at 12 months after allopurinol withdrawal. * $p \leq 0.01$.

Table 5. Kidney function, urinary protein excretion rate and TGF- β_1 after stopping allopurinol

	Group 1	Group 2	Group 3
Serum creatinine (12 months after stopping allopurinol), mg/dl	4 ± 0.5*	4 ± 1*	5.8 ± 1.1*
Creatinine clearance (12 months after stopping allopurinol), ml/min	24.2 ± 7.7*	28 ± 8.1*	18.2 ± 8.8*
Δ Serum creatinine, mg/year ^a	0.5 ± 0.1*	0.6 ± 0.3*	1.7 ± 0.6*
Δ Creatinine clearance, ml/min/year ^a	-3.9 ± 2*	-4.1 ± 2.2*	-14.1 ± 5.3*
Protein excretion upon withdrawal, g/dl	0.68 ± 0.2	0.73 ± 0.3	0.61 ± 0.2
2 weeks after withdrawal, g/dl	0.7 ± 0.3	0.77 ± 0.4	0.7 ± 0.3
12 months after withdrawal, g/dl	0.66 ± 0.4	0.75 ± 0.4	0.73 ± 0.4
TGF- β_1 upon withdrawal, ng/g	21.3 ± 5.8	19.5 ± 6.3	20.1 ± 7.9 ^b
TGF- β_1 1 month after withdrawal, ng/g	22.2 ± 6.3	21.6 ± 7	34 ± 8.4 ^b

In group 3, serum creatinine, creatinine clearance, Δ serum creatinine, Δ creatinine clearance and proteinuria at 12 months after withdrawal were considered in 17 patients only. To convert serum creatinine in mg/dl to μ mol/l, multiply by 88.4. * $p \leq 0.01$.

^a Average rate of change during the year after allopurinol withdrawal.

^b $p < 0.05$ when tested by the paired t test.

and creatinine clearance 12 months after allopurinol withdrawal as well as the rate of change of the same variables after allopurinol withdrawal. There was a significant acceleration of the rate of loss of kidney function during the 12 months following allopurinol withdrawal in group 3 compared to group 1 and group 2. Urinary protein excretion rate was not significantly affected by allopurinol withdrawal (table 5). No significant differences in urinary TGF- β_1 were detected before allopurinol withdrawal between the three groups of the study (table 5). A significant increase in urinary TGF- β_1 was detected 1 month after allopurinol withdrawal in group 3 34 ± 8.4 versus 20.1 ± 7.9 ng/g creatinine while no significant change was detected in group 1 or group 2.

Discussion

In the present investigation, the clinical course of a group of patients with chronic renal failure, hypertension and asymptomatic hyperuricemia was considered. In the absence of pharmacological blockage of the renin-angiotensin system as little as 20% increase in serum uric acid was associated with a significant worsening of hypertension detectable as early as 2 weeks after stopping allopurinol therapy while no similar deterioration of hypertension was detected in the patients receiving either ACEIs or ARBs. This trend towards worsening hypertension persisted throughout the study as reflected by the need to escalate the antihypertension

therapy in the group of patients not receiving either ACEIs or ARBs.

The association between hyperuricemia and hypertension is strong. Hyperuricemia is observed in 25% of untreated hypertensive subjects, 40% of those on treatment, and 75% of those with malignant hypertension or renal dysfunction [23]. However, the current clinical ideology considers asymptomatic hyperuricemia to be a result of simple clustering of cardiovascular risk factors rather than a noxious substance of its own [2, 3, 11, 23].

Waring et al. [24] reported that uric acid infusion in healthy humans resulted in impaired acetylcholine-induced vasodilatation in the forearm, thereby documenting impaired endothelial nitric oxide release. Serum uric acid and serum nitric oxide levels also vary during the day in a reciprocal pattern [25]. Two human studies reported the association between hyperuricemia and activation of the renin-angiotensin system: an old one [26] reported a positive correlation between the angiotensin II level and serum uric acid concentration and a recent study [27] reported the same finding in childhood hypertension.

The current investigation proves that the renin-angiotensin system is the main mediator of the hypertension-inducing effect of mild hyperuricemia in the human. It was believed that at advanced stages of hypertension, the structural damage in the kidney becomes the main mediator of hypertension and lowering the serum uric acid would not affect the progression of hypertension and renal disease [3]. In the present investigation, the effect of hyperuricemia on the perpetuation of hypertension was detectable even in the presence of advanced renal disease while blocking the renin-angiotensin system achieved a clinically significant protection against hypertension after allopurinol withdrawal.

Withdrawal of allopurinol was also associated with acceleration of the rate of kidney function loss in the absence of pharmacological blockade of the renin-angiotensin system. Neither worsening of hypertension nor an increase in proteinuria may explain the observed clinical deterioration. Hypertension remained well controlled, albeit at the expense of escalating the antihypertensive drug therapy. Similarly, no increase in proteinuria was detected during the study. Hyperuricemia induces both functional and structural renal changes in the rat model of mild hyperuricemia. The functional changes include impairment of nitric oxide release [7], glomerular hypertension [9], an early hyperfiltration phase [10], activation of the renin-angiotensin system [7], both salt-resistant and salt-dependent hypertension [7] as well as a state of

intrarenal inflammation [28, 29]. The structural changes include an early increase of kidney weight, glomerular hypertrophy [10], angiotensin-dependent and independent arteriopathy [7], as well as inflammatory interstitial infiltrates and fibrosis. Uric acid is a potent stimulant of vascular formation of C-reactive protein and lipopolysaccharide-induced cytokine production [28, 29]. These changes culminate in a shrunken failing kidney [7]. Uric acid also stimulates rat vascular smooth muscle cell proliferation in vitro [30]. Vascular smooth muscle cells do not express a receptor for uric acid but rather have organic anion transporters that allow urate uptake [31]. Once inside the vascular smooth muscle cell, uric acid activates specific mitogen-activated protein kinases with the de novo induction of cyclooxygenase-2, local thromboxane formation with upregulation of platelet-derived growth factor and its receptor [31, 32]. Similarly, hyperuricemia induces an increase in monocyte chemoattractant protein-1, which is known to have a key role in stimulating macrophage infiltration in atherosclerotic vessels [32]. Hyperuricemia universally increases cytokine production from the vascular endothelium after lipopolysaccharide challenge [29]. Similarly, uric acid is a potent stimulus for endothelial C-reactive protein secretion [28]. Intrarenal inflammation is now conceived as the underlying mechanism of the maintenance as well as the progression of systemic hypertension, salt sensitivity, nephrosclerosis, interstitial inflammation and ultimately renal failure in human patients [33, 34].

Allopurinol withdrawal was associated with a significant increase in urinary TGF- β_1 in the absence of pharmacological blockade of the renin-angiotensin system denoting the relation between hyperuricemia and intrarenal inflammation and cytokine production. A finding that is very similar to the well-documented pathophysiology of mild hyperuricemia in the oxonic acid-fed rat model [29, 30, 32].

Both losartan and fosinopril were capable of blocking the deterioration of kidney function following allopurinol withdrawal while in the absence of pharmacological blockade of the renin-angiotensin system an equivalent rise of serum uric acid led to a significant acceleration of kidney function loss. These findings are consistent with a physiopathological model in which uric acid, angiotensin II and TGF- β_1 work in succession along a single pathological pathway in which uric acid amplifies the noxious effects of angiotensin II and TGF- β_1 acts as a final prosclerotic cytokine.

The findings in this study have striking similarities to the animal model of mild hyperuricemia. The well-docu-

mented protective effect of allopurinol in the animal model of mild hyperuricemia may therefore prompt the clinical consideration of allopurinol trial in patients with chronic kidney disease and asymptomatic hyperurice-

mia. The observed protective effect of the blockers of the renin-angiotensin system against asymptomatic hyperuricemia may be particularly advantageous in the clinical management of patients with chronic kidney disease.

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