

Possible new role for angiotensin-converting enzyme inhibitors in treating glomerulonephritis

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دور إنجابي جديد محتمل لمثبطات الإنزيم المحوّل للأنجيوتنسين في معالجة التهاب كبيبات الكلى

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الخلاصة: أجري تعيين مقدار إنتاج عامل النمو المحوّل بيتا-1 في المصل لدى عشرة مرضى مصابين بارتفاع ضغط الدم الأساسي، ولدى 12 مريضاً مصاباً بالتهاب كبيبات الكلى (5 منهم مع ارتفاع ضغط الدم و7 منهم بدون ارتفاع ضغط الدم) ولدى عشرة من الشواهد الأصحاء. وقد أعطيت المصابون بالتهاب كبيبات الكلى الكابتوبريل المثبط للإنزيم المحوّل للأنجيوتنسين بمقدار 25-75 ميلي غرام يوميا لمدة أربعة أسابيع قبل إجراء قياس لليوريا في الدم وللكرياتينين في المصل وللبروتين في البول المجموع في فترة 24 ساعة ولعامل النمو المحوّل بيتا-1 في المصل. ولوحظ أن كلا من اليوريا والكرياتينين مرتفعتان ارتفاعاً يُعتدُّ به إحصائياً لدى المجموعتين المصابتين بارتفاع ضغط الدم والتهاب كبيبات الكلى أكثر مما لدى الشواهد، كما أنهما مرتفعتان لدى المصابين بالتهاب كبيبات الكلى أكثر مما لدى المصابين بارتفاع ضغط الدم. وكان عامل النمو المحوّل بيتا-1 مرتفعاً بشكل يُعتدُّ به إحصائياً لدى جميع المصابين بالتهاب كبيبات الكلى، بالمقارنة مع مجموعتي الشواهد أو المصابين بارتفاع ضغط الدم لوحده. لقد كان كل من التحول المصلي لإنتاج عامل النمو بيتا-1 وبروتين البول المجموع في فترة 24 ساعة منخفضاً بشكل واضح لدى مجموعة المصابين بالتهاب كبيبات الكلى.

ABSTRACT Serum transforming growth factor-beta (TGF-β1) production was estimated for 10 patients with essential hypertension, 12 patients with glomerulonephritis (5 hypertensive and 7 normotensive) and 10 healthy controls. The glomerulonephritis group received angiotensin-converting enzyme inhibitor captopril 25-75 mg/day for 4 weeks. Blood urea, serum creatinine, 24-hour urinary protein and serum TGF-β1 were then re-estimated. Urea and creatinine were significantly higher in the hypertension and glomerulonephritis groups than in the controls and also higher in the glomerulonephritis group than the hypertension group. TGF-β1 was significantly higher in the glomerulonephritis groups than in the control and hypertension groups. TGF-β1 and 24-hour urinary protein were significantly reduced in the glomerulonephritis group.

Nouveau rôle possible pour les inhibiteurs de l'enzyme de conversion de l'angiotensine dans le traitement de la glomérulonéphrite

RESUME La production du facteur de croissance transformant-bêta sérique (TGF-β1) a été évaluée chez 10 patients atteints d'hypertension artérielle essentielle, 12 patients atteints de glomérulonéphrite (5 hypertendus et 7 normotendus) et 10 témoins en bonne santé. Le groupe atteint de glomérulonéphrite a reçu l'inhibiteur de l'enzyme de conversion de l'angiotensine captopril 25-75 mg/jour pendant quatre semaines. L'urée sanguine, la créatininémie, la protéinurie 24 heures et le TGF-β1 sérique ont été alors ré-évalués. Les taux d'urée et de créatinine dans le groupe de patients atteints d'hypertension et de glomérulonéphrite étaient significativement plus élevés que ceux du groupe témoin et ils étaient également plus élevés dans le groupe atteint de glomérulonéphrite que ceux du groupe souffrant d'hypertension. Les taux de facteur de croissance TGF-β1 étaient significativement plus élevés dans le groupe de patients atteints de glomérulonéphrite que ceux du groupe témoin et du groupe souffrant d'hypertension. Les taux de TGF-β1 et la protéinurie 24 heures étaient significativement réduits dans le groupe de patients atteints de glomérulonéphrite.

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Introduction

There is experimental and human evidence that inhibition of the renin-angiotensin system by angiotensin-converting enzyme inhibitors (ACE-I) consistently reduces urinary protein excretion rate and retards the development of renal injury [1]. Thus, in rats with subtotal nephrectomy, in diabetic rats and in male Munich Wistar Fromter/Ztm (MWF/Ztm) rats that spontaneously develop renal failure with age, ACE-I are effective in reducing blood pressure, proteinuria and glomerulosclerosis [2]. Also they give comparable protection in immunologic models of progressive renal disease (passive Heymann nephritis in the rat) that closely resemble human membranous nephropathy [3]. Together these data can be taken to suggest that reducing angiotensin II (Ang II) activity protects from the development of proteinuria and renal injury [4]. Besides lowering intraglomerular pressure [5], improving glomerular size selectivity [6] and limiting mesangial cell proliferation and matrix synthesis by direct action of Ang II [7], autocrine activation of growth factors is another possible mechanism [8]. Thus Ang II potentially induces in the kidney the expression of transforming growth factor-beta (TGF- β) [8], a cytokine involved in cell proliferation, monocyte migration and matrix synthesis [9].

TGF- β is important in wound healing and tissue repair. Overproduction of TGF- β can result in excessive deposition of scar tissue and fibrosis [10]. TGF- β stimulates the deposition of extracellular matrix by different mechanisms: TGF- β stimulates directly the synthesis of extracellular matrix molecules including fibronectin, collagen and proteoglycan and blocks the degradation of matrix by inhibiting the secretion of proteases and stimulating the

production of protease inhibitors. TGF- β also modulates the expression of integrin matrix receptors on cells in a manner that facilitates cell matrix adhesion and matrix deposition. It also autoinduces its own secretion [11].

This study determined the serum level of TGF- β 1 in patients with essential hypertension and in patients with glomerulonephritis and demonstrated the effect of ACE-I on TGF- β 1 levels, degree of proteinuria and kidney function in patients with glomerulonephritis.

Methods

Patients who were scheduled for routine follow-up in the general medicine and nephrology outpatient clinics of Kasr al-Aini Hospital, Cairo, Egypt, were included in this study. All patients were subjected to full history taking, full clinical examination, urine analysis, blood urea [12], serum creatinine [13], fasting and two-hour postprandial blood glucose [14] and urine examination for microalbuminuria [15]. Patients who were positive for microalbuminuria had their urine examined for 24-hour urinary protein. Patients with serum creatinine more than 3 mg/dL or pyuria and those receiving ACE-I were excluded.

The patients were divided into two groups. Group I comprised 10 patients with essential hypertension without microalbuminuria (5 males and 5 females). Their mean age was 52.4 ± 10.5 years. Clinical examination revealed left ventricular hypertrophy in 3 patients and accentuated second heart sound A2 in 5 patients. Fundus examination was normal for all.

Group II comprised 12 patients with glomerulonephritis (5 males and 7 females). Of these, 7 patients had lupus

nephritis, 3 patients had focal segmental glomerulonephritis and 2 had membranous nephritis; diagnosis was made according to renal biopsy. Their mean age was 39.8 ± 8.4 years. Clinical examination revealed hypertension in 5 and normal blood pressure in 7. Nine patients had lower limb oedema. Fundus examination was normal for all patients.

Ten healthy persons (6 males and 4 females) of mean age 48 ± 6.9 years served as the control group.

Serum TGF- β 1 was estimated for all subjects. The glomerulonephritis group received the ACE-I captopril 25–75 mg/day for four weeks to keep systolic pressure between 110–140 mmHg and diastolic pressure between 70–90 mmHg. Blood urea, serum creatinine, 24-hour urinary protein excretion and serum TGF- β 1 were then re-estimated.

The TGF- β enzyme-amplified sensitivity immunoassay (EASIA) (MedGenix, Brussels) was used. This is a solid phase enzyme-immunoassay performed on microtitre plates, in which a fixed amount of TGF- β labelled with horseradish peroxidase (HRP) competes with unlabelled TGF- β 1 present in standard or extracted samples for a limited number of binding sites on a specific coated antibody. After 2 hours incubation at room temperature with continuous shaking, the microtitre plate was washed to stop the competition reaction. The chromogenic solution tetramethylbenzidine (TMB) was added and incubated for 30 minutes. The reaction was stopped with the addition of stop solution and the microtitre plate was read at the appropriate wavelength. The amount of the substrate was determined calorimetrically by measuring the absorbance that was inversely proportional to TGF- β concentration. A standard curve was plotted and

the TGF- β 1 concentration in the samples was determined by interpolation from the standard curve [16].

Data were analysed using SPSS, version 8. Quantitative data were analysed using Student t-test for comparison means of two groups. Paired t-test was used to compare between means before and after intervention. Values less than 0.05 were significant. The Pearson correlation coefficient was used to express the relationship between TGF- β 1 and variables [17].

Results

The laboratory data of all groups was collected (Table 1). There were significantly higher blood urea ($P < 0.03$) and creatinine ($P < 0.02$) levels in the essential hypertension group than in the controls. There were significantly higher urea ($P < 0.01$) and creatinine ($P < 0.01$) levels in the glomerulonephritis group than in the controls. Furthermore, there were significantly higher urea ($P < 0.04$) and creatinine ($P < 0.03$) in the glomerulonephritis group than the hypertension group.

TGF- β 1 levels in all groups are given in Table 2. TGF- β 1 was significantly higher in the hypertension and glomerulonephritis groups and in the glomerulonephritis subgroups (hypertensive and normotensive) than in the controls ($P < 0.001$). There was significant higher TGF- β 1 in the total glomerulonephritis group and in the subgroups (with and without hypertension) than in the essential hypertension group ($P < 0.001$). There was no significant difference in TGF- β 1 between the hypertensive and normotensive glomerulonephritis patients.

The effects of ACE-I captopril in glomerulonephritis patients are given in Table

Table 1 Blood urea, serum creatinine and blood glucose levels of controls and study groups of patients

Test	Controls	Essential hyper-tension group	Glomerulo-nephritis group
Blood urea (mg/dL)	24.50 ± 5.00	43.10 ± 14.60 ^a	58.00 ± 32.60 ^{a,b}
Serum creatinine (mg/dL)	0.90 ± 0.16	1.07 ± 0.10 ^a	1.50 ± 0.60 ^{a,b}
Fasting blood glucose (mg/dL)	82.60 ± 8.50	89.50 ± 12.57	89.00 ± 11.60
Two-hour postprandial blood glucose (mg/dL)	96.90 ± 10.13	107.00 ± 27.30	104.80 ± 13.50

Values are mean levels ± standard deviation.

^aP < 0.05, diseased groups versus controls.

^bP < 0.05, essential hypertension group versus glomerulonephritis group.

Table 2 Transforming growth factor-beta (TGF-β1) levels for controls and study groups of patients

Group	No. of patients	TGF-β1 levels (ng/dL)
		Mean ± s
Controls	10	36.3 ± 12.9
Essential hypertension group	10	80.4 ± 18.2 ^a
Glomerulonephritis group	12	386.7 ± 88.8 ^{a,b}
Hypertensive	5	393.3 ± 92.2 ^{a,b}
Normotensive	7	379.1 ± 83.5 ^{a,b}

^aP < 0.05, diseased groups versus controls.

^bP < 0.05, essential hypertension group versus glomerulonephritis group.

s = standard deviation.

3. There were significant reductions in 24-hour urinary protein and TGF-β1 after giving captopril to these patients ($P < 0.005$ and $P < 0.001$, respectively). There were no significant changes in urea and creatinine.

There was no significant correlation between TGF-β1 and 24-hour urinary protein in patients with glomerulonephritis.

Discussion

In the present study we found significantly higher TGF-β1 in patients with essential hypertension than in the control group. Our results agree with Li et al. [18] and Suthanthiran et al. [19]. Suthanthiran et al. found higher plasma TGF-β1 and mRNA in hypertensive patients compared with normotensive subjects. They concluded that TGF-β1 hyperexpression is a risk factor for hypertension and hypertension complications. Moreover, Li et al. found a significant positive correlation of TGF-β1 circulating levels and blood pressure. Their observation supported the idea that genetically determined TGF-β1 concentrations may play a role in blood pressure regulation in humans via stimulation of endothelin I and/or renin secretion [18].

In the present study, patients with glomerulonephritis (total and subgroups) had significantly higher serum TGF-β1 than normal healthy controls. Kanai et al. [20] found increased urinary excretion of TGF-β in patients with focal segmental glomerulonephritis and with lupus nephritis but not in IgA nephropathy and memb-

Table 3 Urea, creatinine, 24-hour urinary protein and serum transforming growth factor-beta (TGF- β 1) levels for the glomerulonephritis group, before and after captopril treatment

Test	Before captopril treatment	After captopril treatment	P-value
Blood urea (mg/dL)	58.0 \pm 32.7	60.5 \pm 38.4	NS
Serum creatinine (mg/dL)	1.5 \pm 0.6	1.6 \pm 0.0	NS
24-hour urinary protein (g/day)	4.4 \pm 3.1	2.6 \pm 1.6	< 0.005
Serum TGF- β 1 (ng/dL)	386.7 \pm 88.8	306.4 \pm 60.0	< 0.001

Values are mean levels \pm standard deviation.
NS = not significant.

ranous glomerulonephritis. Honkanen et al. [21] found significantly increased urinary excretion of TGF- β in patients with membranous nephritis compared with healthy controls. Experimental and human studies have demonstrated that renal injury is associated with increased expression of TGF- β 1 by glomerular mesangial cells and that increased production of TGF- β plays a key role in renal fibrosis [22]. An increased glomerular TGF- β staining was observed in human renal biopsies of 10 patients with post-streptococcus glomerulonephritis [23]. It has been reported that increased renal expression of TGF- β after renal injury occurs in response to many factors such as Ang II, hypoxia, autoantibodies and the immune complex [24].

In the present study TGF- β 1 was significantly higher in patients with glomerulonephritis than in patients with essential hypertension without renal involvement. TGF- β 1 was also elevated in our patients with glomerulonephritis both with and without hypertension. Therefore, a factor or factors other than hypertension causes elevation of TGF- β 1 in glomerulonephritis. A possible factor may be related to Ang II because of the reduction of TGF- β 1 levels in serum in our study after treatment with

ACE-I captopril. It has been reported that Ang II is a potent inducer of TGF- β 1. The induction of TGF- β by Ang II is a protein kinase C-dependent effect, similar to TGF- β 1 induction by glucose [25]. Also Ang II may play a role in glomerular TGF- β 1 autoinduction; thus it may play an outstanding role in the switch of mechanisms that are involved in the healing process of renal lesions. Such action would promote lesion progression towards a chronic fibrotic disease (glomerulosclerosis) rather than remodelling to the original anatomical functional structure. Ang II stimulates renal tubular hypertrophy and mesangial cell and matrix overexpression via autocrine stimulation of TGF- β 1 in tissue culture [26].

TGF- β 1 plays a role in the pathogenesis of experimental models of glomerulonephritis. Ang II induces the expression of TGF- β 1 in the kidney, a cytokine involved in cell proliferation, monocyte migration and matrix synthesis [27]. It has been reported that rat mesangial cells in culture exposed to Ang II increased TGF- β 1 mRNA and protein, which in turn promoted the synthesis of extracellular matrix [28]. In antithymocyte serum-induced glomerulonephritis in rats (which resembles human IgA nephropathy and mesangial prolife-

rative forms of lupus nephritis) after a single injection of antithymocyte serum (ATS) there is mesangiolysis and monocyte/macrophage infiltration of glomeruli, followed by mesangial proliferation and matrix expansion, and TGF- β is overexpressed in the glomeruli [29]. Treatment with TGF- β 1 neutralizing antibodies limited the severity of the disease and prevented matrix deposition [30]. TGF- β 1 induces the synthesis and overexpression of many mitogenic and chemical mediators, such as platelet derived growth factor [31], endothelin I [32] and monocyte chemoattractant protein-1 [33] and may contribute to glomerular cell proliferation and monocyte/macrophage recruitment.

The effect of ACE-I on TGF- β 1 was studied in patients with glomerulonephritis. We found that serum levels of TGF- β 1 and 24-hour urinary protein decreased significantly with no significant difference in urea and creatinine after treatment with the ACE-I captopril. Our results agree with Peters et al. [22] and Campistol et al. [34]. Peters et al. studied the effect of treatment of experimentally-induced glomerulonephritis in rats with increased doses of the ACE-I enalapril or the Ang II antagonist losartan. Six days after disease induction, the therapeutic effect on the glomerular TGF- β 1 overexpression was evaluated. Both enalapril and losartan reduced TGF- β 1 overproduction in a dose-dependent manner. Campistol et al. [34] studied the effect of losartan on the plasma level of TGF- β 1 in renal transplant patients with chronic allograft nephropathy. They found that losartan significantly decreased the

plasma levels of TGF- β 1 and concluded that these results could play a decisive role in treatment and prevention of chronic nephropathy and not only graft nephropathy.

The mechanism by which ACE-I reduces the serum TGF- β 1 level is unknown. It is likely that inhibition of ACE levels leads to reduction of Ang II, which would lower the production of TGF- β 1 in a variety of cell types. In cell culture studies, Ang II stimulates mRNA and protein levels [35]. Zoja et al. [4] studied the effects of ACE-I lisinopril and Ang II receptor antagonist L-158,809 in rats with ATS-induced glomerulonephritis. Both were effective in preventing proteinuria and reduced glomerular cell proliferation and macrophage infiltration and prevented the formation of microaneurysms. These effects were associated with reduction in glomerular TGF- β 1 mRNA expression and with normalization of excessive urinary excretion of TGF- β 1 that likely reflects the renal synthesis of the protein. Thus, ATS-blocking Ang II synthesis or activity prevents proteinuria and, by reducing excessive renal TGF- β 1, possibly limits glomerular cell proliferation and inflammatory cell infiltration.

It can be concluded that TGF- β 1 is elevated in patients with essential hypertension and chronic glomerulonephritis. Use of ACE-I may reduce proteinuria and TGF- β 1. Reduction of TGF- β 1 is a possible mechanism of reduction of proteinuria in patients with glomerulonephritis; however, the effects of other mechanisms cannot be ruled out.

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