

Plasma matrix metalloproteinase-9 and ACE-inhibitor-induced improvement of urinary albumin excretion in non-diabetic, microalbuminuric subjects

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Key words: angiotensin-converting enzyme, ACE-inhibitors, fosinopril matrix metalloproteinase-9, urinary albumin excretion

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Journal of the Renin-Angiotensin-Aldosterone System

(Including other Peptidergic Systems)

December 2007
Volume 8
Number 4

Abstract

Introduction. Elevated plasma matrix metalloproteinase-9 (MMP-9) levels have been suggested to precede the development of microalbuminuria. As angiotensin-converting enzyme (ACE) inhibitors effectively reduce urinary albumin excretion (UAE), in the present study we have investigated the potential association of plasma MMP-9 levels with UAE and treatment effects of ACE-inhibition.

Material and methods. In a placebo-controlled randomised trial we determined plasma MMP-9 levels at baseline and after three months of randomisation to either placebo (n=202) or fosinopril (20 mg/day, n=204) treatment.

Results. Baseline plasma MMP-9 levels were not related to baseline UAE ($r=-0.008$ ($p=0.871$)). Three months of fosinopril treatment effectively reduced UAE compared to placebo treatment (-10.4 ± 2.4 vs. 1.8 ± 1.3 mg/24 hours, $p<0.001$, respectively). However, fosinopril treatment failed to significantly change plasma MMP-9 levels compared to placebo (-0.47 ± 7.68 vs. 0.06 ± 9.20 , $p=0.646$, respectively). In addition, the change in UAE was not related with change in MMP-9 levels.

Conclusion. The effective reduction of UAE with fosinopril was not related to plasma MMP-9 levels.

Introduction

Microalbuminuria is an important marker of widespread endothelial damage.¹ It is strongly related to the development of cardiovascular morbidity and mortality in patients with hypertension or diabetes, as well as in the general population.²⁻¹⁰ The Prevention of Renal and Vascular ENdstage Disease Intervention Trial (PREVEND-IT) demonstrated that the angiotensin-converting enzyme (ACE) inhibitor fosinopril effectively reduces urinary albumin excretion (UAE) in microalbuminuric subjects.¹¹ The favourable effects of ACE inhibitors on UAE have been described previously by others as well.¹²⁻¹⁸ Surprisingly little is known about the mechanisms causing the reduced UAE by ACE-inhibitors, beyond blood pressure (BP) reduction.^{19,20} Knowledge of the mechanisms is important for the design of future drugs and therapies aimed at improving cardiovascular prognosis. Recently, it has been suggested that

the increased synthesis of collagen IV and thickening of the glomerular capillary basement membrane in particular, plays a pivotal role in the development of microalbuminuria.²¹ Matrix metalloproteinases (MMPs) are a large family of zinc-dependent matrix-degrading enzymes. In particular, MMP-9 (gelatinase B; 92 kDa type IV collagenase) is involved in the breakdown of native type IV, V and VII collagen, as well as gelatine and can damage the glomerular basement membrane. Increased plasma MMP-9 levels have been reported to precede microalbuminuria in diabetic subjects.²² These observations suggested a potential additional mechanism of action for ACE-inhibitors. Therefore, we examined the association of plasma MMP-9 levels with UAE and treatment effects of ACE-inhibition.

Material and methods

We included 376 non-diabetic, microalbuminuric patients from PREVEND-IT, an investigator initiated, randomised trial that used a two-by-two factorial design to compare the effect of statin therapy (pravastatin 40 mg) versus placebo and of ACE-inhibitor therapy (fosinopril 20 mg) versus placebo on cardiovascular events in microalbuminuric patients. A detailed description of the study's inclusion and exclusion criteria have been reported previously.^{11,23} For this analysis, we defined change in microalbuminuria the change obtained at the three-month follow-up visit. UAE was determined by nephelometry (Dade Behring Diagnostics, Marburg, Germany). Plasma MMP-9 was measured in duplicate with the use of a 2-site sandwich ELISA assay (Amersham Pharmacia Biotech, Buckinghamshire, UK), which measures MMP-9, ProMMP-9, and the ProMMP-9/TIMP-1 complex, with an assay range of four to 128 ng/mL.²⁴ The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the ethics committee of the University Medical Center Groningen. All subjects gave written informed consent.

Differences between treatment groups were evaluated by Student *t*-test, χ^2 , or Wilcoxon test, when appropriate. We initially used Pearson correlation coefficients followed by standard

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Accepted for publication 3rd September 2007

JRAAS 2007;8:177–80

Table 1

Baseline characteristics of all patients, and of patients allocated to placebo and fosinopril treatment.

	Total group (n=376)	Fosinopril (n=186)	Placebo (n=190)
Age	51.1±11.6	50.9±11.8	51.3±11.4
Male gender	248 (66.0%)	125 (67.2%)	123 (64.7%)
Caucasian	362 (96.3%)	174 (93.5%)	188 (98.9%)
Current smokers	119 (31.2%)	61 (32.8%)	58 (30.5%)
Blood pressure			
Systolic	131.1±18.5	130.3±19.1	132.4±18.5
Diastolic	76.2±9.7	76.1±9.5	76.2±10.0
sMDRD (ml/min/1.73m ²)	82.2±13.2	81.7±13.8	82.7±12.7
Lipids			
Total cholesterol	5.86±1.04	5.96±1.05	5.75±1.01
LDL cholesterol	4.14±0.94	4.23±0.98	4.05±0.90
HDL cholesterol	1.03±0.31	1.01±0.32	1.05±0.29
Triglycerides	1.55±1.06	1.60±1.02	1.49±1.10*
BMI	26.4±4.2	26.4±4.1	26.3±4.1
Beta blocking agent (%)	1.6	0.5	2.6
Pravastatin	50.0	50.0	50.0
Nitrate (%)	0.5	0.5	0.5
Diuretic (%)	0.5	1.1	0
Digoxin (%)	0.5	0.5	0.5
Anti-platelet agent (%)	1.9	1.1	2.6

Key: * = p<0.05. sMDRD = simplified Modification of Diet in Renal Disease equation; BMI = body mass index.

linear regression analysis to evaluate the relationship between variables (after transformation to natural logarithm for skewed variables) at baseline. All analyses were performed using SPSS version 11.5 software (SPSS, Chicago, IL, USA).

Results

Detailed baseline characteristics are presented in table 1. At baseline, MMP-9 plasma concentration (interquartile range) in the placebo and fosinopril group were 6.97 (5.20–10.24) and 7.35 (5.59–11.05) (p=0.312), respectively. The MMP-9 concentrations were not correlated with baseline UAE (r=-0.008, p=0.871) or renal function (r=-0.039, p=0.460). MMP-9 levels were also not significantly correlated with any other biochemical baseline variable.

Mean daily dose of fosinopril after three months of treatment was 16.8 mg. Three months of placebo treatment resulted in a non-significant increase of UAE (1.8±1.3 mg/24 hour, p=0.151 *vs.* baseline). In contrast, fosinopril treatment resulted in a significant decrease in UAE (-10.4±2.4 mg/24 hour, p<0.001 *vs.* baseline, and p<0.001 *vs.* placebo group). The median MMP-9 concentration did not change significantly in either the placebo or fosinopril group (figure 1).

Table 2 summarises the association between the changes in the levels of MMP-9 and the change in UAE in both groups. Multivariate linear regression analysis, corrected for age, gender, MAP, BMI and

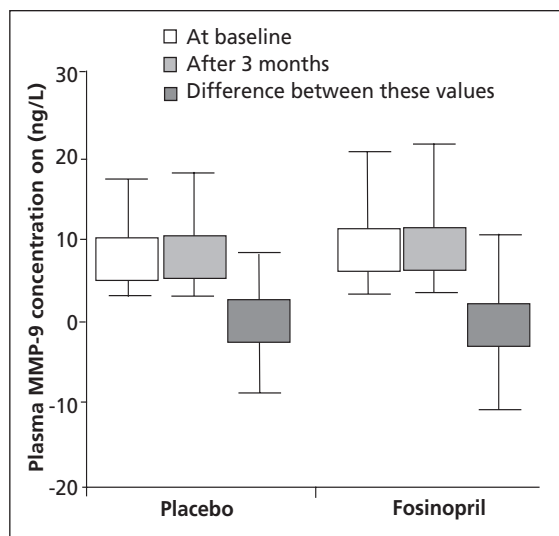


Figure 1 Box and whiskers plot of plasma MMP-9 concentrations in both placebo and fosinopril treated patients at baseline, after three months, and the absolute difference between these values. None of the differences between the groups reached statistical significance.

Table 2

Relationship between urinary albumin excretion and plasma MMP-9 (both at three months and delta) stratified according to randomisation to placebo or fosinopril.

Variables	Placebo		Fosinopril	
	Std beta*	p-value	Std beta*	p-value
3 Month urinary albumin excretion				
MMP-9	-0.008	0.879	0.050	0.501
Delta urinary albumin excretion				
Delta MMP-9	0.033	0.663	-0.067	0.376

Key: Presented are standardised-beta and p-values from standard linear regression analysis for the relationship between the dependent variable urinary albumin and the independent variable MMP-9, adjusted for age, sex, body mass index and mean arterial pressure (at three months or delta respectively) * = Adjusted for MAP at three months, and delta MAP, respectively; BMI = Body Mass Index; MAP = mean arterial pressure; MMP-9 = matrix metalloproteinase 9; Std beta = standardised beta.

baseline UAE demonstrated that change in plasma MMP-9 was not associated with the change in UAE from baseline in patients treated with fosinopril.

Discussion

Microalbuminuria is an important marker of widespread endothelial damage and predicts future cardiovascular events.¹⁻¹⁰ However, determinants of microalbuminuria and efficacy of ACE-inhibitors in reducing microalbuminuria, beyond BP reduction, are not well understood. In the present study we demonstrated that in patients with microalbuminuria, but without overt cardiovascular disease, three months of fosinopril treatment indeed significantly reduces microalbuminuria, but not plasma MMP-9 levels. Besides beneficial renal haemodynamic effects it has been suggested that ACE-inhibitors affect glomerular albumin loss through additional mechanisms. An important role has been attributed to the interplay between the renin-angiotensin system activity and MMP-9 in determining UAE.^{22,25,26} Recently, it has been suggested that in non-insulin dependent diabetes mellitus patients the development of microalbuminuria was preceded by a significant increase in plasma MMP-9 concentration.²² Intriguingly, in the microalbuminuric subset, therapy with an ACE-inhibitor for six months, abolished the increments in MMP-9 and decreased, in parallel, urinary protein excretion. Another group observed that type 2 diabetic patients with macroalbuminuria had significantly increased urinary MMP-9 levels compared to healthy adults. Furthermore, they found that urinary levels of MMP-9 were related to the clinical severity of the disease and concluded that measurement of urinary MMP-9 might be useful

for evaluating the degree of renal injury.²⁶ However, these previous studies involved less than 50 patients each and should be considered severely underpowered. Although we studied a non-diabetic population we hypothesised similar relationships between MMP-9 and UAE and anticipated modification by ACE-inhibitor treatment. This was not the case. Considering our sample size and our randomised, placebo-controlled study design we provide strong evidence for the absence of a relationship between plasma MMP-9 levels and UAE and potential modification by ACE-inhibitors. A limitation of the current study is that urinary MMP-9 levels were not determined. Future studies should move beyond plasma MMP-9 measurements to establish a mechanism of UAE and efficacy of ACE-inhibitors.

Conclusions

In this large randomised placebo-controlled clinical trial we could not confirm findings of previously performed small scale studies suggesting an important role for MMP-9 plasma levels explaining the efficacy of ACE-inhibitors in reducing UAE.

Acknowledgements

This research was supported by a grant from Boehringer Ingelheim BV (the Netherlands). P van der Harst is supported by the Innovational Research Incentives Scheme program of the Netherlands Organisation for Scientific Research (NWO VENI, grant 916.76.170). P van der Harst is a research fellow of the Netherlands Heart Foundation (grant 2006T003) and the Interuniversitair Cardiologisch Instituut Nederland (ICIN).

References

1. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 1989;**32**:219-26.
2. Yuyun MF, Khaw KT, Luben R *et al*. A prospective study of microalbuminuria and incident coronary heart disease and its prognostic significance in a British population: the EPIC-Norfolk study. *Am J Epidemiol* 2004;**159**:284-93.
3. Hillege HL, Fidler V, Diercks GF *et al*. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;**106**:1777-82.
4. Gerstein HC, Mann JF, Yi Q *et al*. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA* 2001;**286**:421-6.
5. Bigazzi R, Bianchi S, Baldari D, Campese VM. Microalbuminuria predicts cardiovascular events and renal insufficiency in patients with essential hypertension. *J Hypertens* 1998;**16**:1325-33.
6. Jensen JS, Feldt-Rasmussen B, Borch-Johnsen K, Clausen P, Appleyard M, Jensen G. Microalbuminuria and its relation to cardiovascular disease and risk factors. A population-based study of 1254 hypertensive individuals. *J Hum Hypertens* 1997;**11**:727-32.

7. Allen KV, Walker JD. Microalbuminuria and mortality in long-duration type 1 diabetes. *Diabetes Care* 2003;**26**:2389-91.
8. Romundstad S, Holmen J, Kvenild K, Hallan H, Ellekjaer H. Microalbuminuria and all-cause mortality in 2,089 apparently healthy individuals: A 4.4-year follow-up study. The Nord-Trondelag Health Study (HUNT), Norway. *Am J Kidney Dis* 2003;**42**:466-73.
9. Borch-Johnsen K, Feldt-Rasmussen B, Strandgaard S, Schroll M, Jensen JS. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol* 1999;**19**:1992-7.
10. Kannel WB, Stampfer MJ, Castelli WP, Verter J. The prognostic significance of proteinuria: the Framingham study. *Am Heart J* 1984;**108**:1347-52.
11. Asselbergs FW, Diercks GF, Hillege HL *et al.* Effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria. *Circulation* 2004;**110**:2809-16.
12. Parving HH, Hommel E, Smidt UM. Protection of kidney function and decrease in albuminuria by captopril in insulin dependent diabetics with nephropathy. *BMJ* 1988;**297**:1086-91.
13. Ravid M, Savin H, Jutrin I, Bental T, Katz B, Lishner M. Long-term stabilizing effect of angiotensin-converting enzyme inhibition on plasma creatinine and on proteinuria in normotensive type II diabetic patients. *Ann Intern Med* 1993;**118**:577-81.
14. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators. *Lancet* 2000;**355**:253-9.
15. Ruggenti P, Fassi A, Ilieva AP *et al.* Preventing microalbuminuria in type 2 diabetes. *N Engl J Med* 2004;**351**:1941-51.
16. Viberti G, Mogensen CE, Group LG, Pauls JF. Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. European Microalbuminuria Captopril Study Group. *JAMA* 1994;**271**:275-9.
17. Jafar TH, Schmid CH, Landa M *et al.* Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease. A meta-analysis of patient-level data. *Ann Intern Med* 2001;**135**:73-87.
18. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). *Lancet* 1997;**349**:1857-63.
19. van de Wal RM, Gansevoort RT, van der Harst P *et al.* Predictors of Angiotensin-Converting Enzyme Inhibitor-Induced Reduction of Urinary Albumin Excretion in Nondiabetic Patients. *Hypertension* 2006;**48**:870-6.
20. van de Wal RM, Plokker HW, Lok DJ *et al.* Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition. *Int J Cardiol* 2006;**106**:367-72.
21. Tsilibary EC. Microvascular basement membranes in diabetes mellitus. *J Pathol* 2003;**200**:537-46.
22. Ebihara J, Nakamura T, Shimada N, Koide H. Increased plasma metalloproteinase-9 concentrations precede development of microalbuminuria in non-insulin-dependent diabetes mellitus. *Am J Kidney Dis* 1998;**32**:544-50.
23. Diercks GF, Jansen WM, van Boven AJ *et al.* Rationale, design, and baseline characteristics of a trial of prevention of cardiovascular and renal disease with fosinopril and pravastatin in nonhypertensive, nonhypercholesterolemic subjects with microalbuminuria (the Prevention of Renal and Vascular Endstage Disease Intervention Trial [PREVEND IT]). *Am J Cardiol* 2000;**86**:635-8.
24. Sundstrom J, Evans JC, Benjamin EJ *et al.* Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: the Framingham Heart Study. *Circulation* 2004;**109**:2850-6.
25. Boffa JJ, Lu Y, Placier S, Stefanski A, Dussaule JC, Chatziantoniou C. Regression of renal vascular and glomerular fibrosis: role of angiotensin II receptor antagonism and matrix metalloproteinases. *J Am Soc Nephrol* 2003;**14**:1132-44.
26. Tashiro K, Koyanagi I, Ohara I *et al.* Levels of urinary matrix metalloproteinase-9 (MMP-9) and renal injuries in patients with type 2 diabetic nephropathy. *J Clin Lab Anal* 2004;**18**:206-10.