ACE Inhibitors Improve Diabetic Nephropathy Through Suppression of Renal MCP-1

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OBJECTIVE — Chemokines play an important role in the pathogenesis of diabetic nephropathy. Angiotensin II induces several fibrogenic chemokines, namely monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor-β. The progression of diabetic nephropathy can be retarded by ACE inhibitors (ACEIs) in patients with type 1 and type 2 diabetes. We examined if blockade of the renin-angiotensin system lowered urinary levels of the chemokine MCP-1 and correlated urinary MCP-1 (uMCP-1) with parameters of renal function and glucose and lipid metabolism before and after 1 year of treatment with an ACE inhibitor.

RESEARCH DESIGN AND METHODS — In 22 patients with type 2 diabetes and diabetic nephropathy in stages 3–5, treatment with the ACEI lisinopril was initiated. Before treatment and after 12 months of continuous therapy, proteinuria, creatinine clearance, uMCP-1 levels, BMI, HbA1c, and serum cholesterol were assessed.

RESULTS — Lisinopril treatment improved renal function. Proteinuria decreased from 410 ± 662 mg per 24 h to 270 ± 389 mg per 24 h. Creatinine clearance rose from 61 ± 26 to 77 ± 41 ml/min. Urinary MCP-1 levels decreased from 0.366 ± 0.22 ng/mg creatinine to 0.08 ± 0.096 ng/mg creatinine. The change in uMCP-1 correlated significantly (r = 0.61, P < 0.001) with the change in proteinuria. No other parameter correlated with the improvement in renal function.

CONCLUSIONS — Blockade of the renin-angiotensin system in type 2 diabetic patients with diabetic nephropathy reduces uMCP-1 levels and improves renal function. Because MCP-1 induces monocyte immigration and differentiation to macrophages, which augment extracellular matrix production and tubulointerstitial fibrosis, pharmacological reduction of angiotensin II may also exert its beneficial effects in diabetic nephropathy by downregulation of renal MCP-1.

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Diabetic nephropathy due to type 2 diabetes is becoming the single most important reason for end-stage renal disease in the western world (1). Besides glomerular damage and glomerulosclerosis, diabetic nephropathy is characterized by aseptic tubulitis and tubulointerstitial fibrosis (2). Macrophages and macrophage products play an important pathogenic role in tubulointerstitial inflammatory and noninflammatory conditions and have been implicated as effector cells of tubulointerstitial damage in diabetic nephropathy (3,4). Increased numbers of glomerular and interstitial macrophages have been observed in rat models of experimental diabetes and in biopsies of patients with diabetic nephropathy (5). Monocytes are attracted to the place of organ damage by monocyte-specific chemokines. Monocytes follow an endothelial-bound gradient of chemokine molecules and transmigrate from the vascular bed into the tissue at the point of the highest chemokine concentration (6). Therefore, systemic levels of chemokines do not reflect their local generation. A meaningful determination of chemokine expression can only be done in the anatomical compartment where chemokines are produced or in the near vicinity thereof. In renal diseases, this is either the kidney itself or urine.

Monocyte chemoattractant protein-1 (MCP-1) is the strongest known chemotactic factor for monocytes and is upregulated in many renal diseases (7), including diabetic nephropathy (8). Renal MCP-1 expression is induced by elevated glucose levels, tubular reabsorbed protein, and probably advanced glycosylated end products (9). In diabetes, the tissue renin-angiotensin system (RAS) of the kidneys is activated. Studies in type 1 (10) and type 2 (11) diabetes showed that abrogation of angiotensin (AT)-II activity either with ACE inhibitors (ACEIs) or AT-II type 1a receptor antagonists slowed the loss of renal function more effectively than treatment with other antihypertensive drugs even in the absence of systemic hypotension. Additional evidence from experiments with specific AT-1a receptor antagonists showed that the nephroprotective effect of ACEIs is indeed due to inhibition of the RAS and not due to other ACE-mediated processes (12,13).

Besides the systemic and renal hemodynamic effects of AT-II, it has been shown recently that AT-II directly induces the expression of MCP-1 in vascular smooth muscle cells (14). Furthermore, in experimental nephritis, AT-II activates the transcription factor nuclear factor-κB and initiates MCP-1 synthesis in renal cells with subsequent interstitial recruitment of monocytes and interstitial fibrosis; this effect could be blocked by ACE inhibition (15). The main renal source of MCP-1 is the tubular epithelial

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Abbreviations: ACEI, ACE inhibitor; AT, angiotensin; MCP-1, monocyte chemoattractant protein-1; RAS, renin-angiotensin system; uMCP-1, urinary MCP-1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
Nephropathy and MCP-1

cells (16). Therefore, blockade of the RAS might reduce the renal production of MCP-1, and the resulting decrease in monocyte immigration and monocyte activity would subsequently ameliorate interstitial fibrosis and, consequently, stabilize or improve renal function. To test this hypothesis, we investigated the relationship between urinary MCP-1 (uMCP-1) and renal function in patients with type 2 diabetes and diabetic nephropathy. In the absence of urinary tract infection, uMCP-1 reflects the renal production of this chemokine. Therefore, we measured uMCP-1 before and after 1 year of treatment with the ACEI lisinopril.

**RESEARCH DESIGN AND METHODS**

**Patients**

Twenty-two patients with type 2 diabetes and diabetic nephropathy in stages 3–5 according to the definition of Mogersen et al. (17) (microalbuminuria of \( \geq 30 \) mg/l) who had not been previously treated with an ACEI or an AT-II receptor antagonist were included. All patients received either conventional or intensified insulin therapy; no oral antidiabetics drug were given. The characteristics of the patients at baseline are shown in Table 1.

Patients with hypertension were included if blood pressure with previous antihypertensive medication was <150/90 mmHg. Antihypertensive medication was continued unchanged, and lisinopril was given as an add-on medication. All patients were given lisinopril (Acerbon; Astra, Wesel, Germany) in a dose ranging from 5 mg to maximally 20 mg per day for 12 months once daily. The mean ± SD daily dose was 9.2 ± 2.1 mg lisinopril. Compliance was checked by telephone interviews with patients and general practitioners after 6 months. Patients were reexamined after 12 months. At entry and after 12 months of continuous administration of lisinopril, the following parameters were determined: serum creatinine, HbA1c, serum total cholesterol, C-reactive protein, 24-h microalbuminuria/proteinuria, 24-h creatinine clearance, and uMCP-1 levels. Urine and blood samples were taken when no clinical and laboratory signs of systemic infection were present. Urinary tract infection at the time of sampling was excluded by normal urine sediment. Urinary albumin was measured by nephelometry (Beckman Instruments, Fullerton, CA); normal values are <20 mg per 24 h. Creatinine clearance and total creatinine excretion were measured in a 24-h urine collection. HbA1c was determined with high-pressure liquid chromatography (Menarini HA 8140). Fasting total serum cholesterol levels were assayed by standard enzymatic methods.

**MCP-1 assay**

MCP-1 urine levels were measured with a solid-phase enzyme linked immunosorbent assay (Quantikine MCP-1 ELISA; R&D Systems, Minneapolis, MN). All assays were done in duplicate. The mean variation in the samples of one patient was <5%. The minimum detectable MCP-1 level with this kit is 5 pg/ml. There is no cross-reactivity with the closely related chemokines MIP-1α, MIP-1β, and MCP-2, -3, and -4.

**Statistical methods**

For statistical analysis, the SPSS software package (SPSS, Chicago, IL) was used. All results are given as means ± SD. The Mann-Whitney test for paired samples was used. Correlations were calculated with the Pearson product moment correlation coefficient. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Treatment effects**

After 1 year of treatment with lisinopril, there were no changes in the physical characteristics of the patients (Table 2). Urinary concentrations of MCP-1 at baseline correlated significantly with microalbuminuria/proteinuria. Under lisinopril treatment, drastically lowered MCP-1 concentrations in urine (\( P = 0.001 \), Fig. 1) were found. Creatinine clearance improved and microalbuminuria/proteinuria decreased (\( P < 0.002 \), Fig. 2). The reduction of uMCP-1 (\( \Delta \) uMCP-1) correlated highly significant (\( r = 0.61, \ P < 0.001 \)) with the change in microalbuminuria/proteinuria (\( \Delta \) urinary protein, Fig. 3). Serum creatinine remained stable during treatment. Addition of lisinopril did not significantly lower blood pressure and had no effects on BMI. Neither serum total cholesterol levels nor glycated hemoglobin levels correlated with uMCP-1 or proteinuria.

**CONCLUSIONS** — In this study, we describe a drastic reduction of urinary levels of the chemokine MCP-1 in patients with diabetic nephropathy who were treated with the ACEI lisinopril. Furthermore, the observed improvement

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<tr>
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Data are means ± SD.

![Table 1—Characteristics of the patients at baseline](image)
in proteinuria correlated well with the reduction of MCP-1 levels. There was no correlation of uMCP-1 with glycemic control, serum lipid levels, or blood pressure. These results are consistent with the findings in an animal model of diabetic nephropathy. In rats with streptozotocin-induced diabetes, treatment with ACEI specifically decreased renal MCP-1 mRNA and improved renal function without affecting the levels of other fibrogenic chemokines (18). In this animal model, improvement of proteinuria correlated closely with the reduction in renal MCP-1 mRNA. Glomerular and tubulointerstitial monocyte/macrophage infiltration was significantly reduced in the animals treated with ACEI or AT-II type 1a receptor antagonists. The extent of tubulointerstitial lesions is a prognostic factor for the progression of diabetic nephropathy (19).

The results of our study indicate that increased intrarenal production of MCP-1 may be a pathogenic pathway also in human diabetic nephropathy. Although higher urinary levels of MCP-1 have been detected in patients with diabetic nephropathy in the macro- than in the microalbuminuric range (20,21), the connection between AT-II and MCP-1 in diabetic nephropathy is not completely understood. Because systemic blood pressure remained virtually unchanged in the study subjects, we think that predominantly nonhemodynamic actions of AT-II are responsible for the observed decrease in uMCP-1 and the accompanying improvement of renal function. Furthermore, blockade of the AT-II type 1a receptor or treatment with ACE-I is effective in reducing proteinuria in chronic renal diseases without systemic hypertension (22).

The importance of nonhemodynamic mechanisms of AT-II in diabetic nephropathy has been stressed recently (23). AT-II is a direct stimulus of MCP-1 in vascular smooth muscle and in the mesangial cells of the kidney (24). AT-II itself has monocyte chemoattractant activity, and activated macrophages themselves—besides being potent sources of MCP-1—can generate AT-II via an intrinsic ACE pathway (25,26). Moreover, the infusion of AT-II into rats leads to an influx of macrophages into the kidney, which can be attenuated by the administration of an AT1a-receptor antagonist (27). In experiments with AT-1a-receptor−deficient mice, diminished expression of MCP-1 led to markedly less interstitial fibrosis and preserved renal function in a mouse

**Figure 1**—Change of uMCP-1 levels in individual patients before and after 1 year of ACEI therapy.
model of anti-Gbm nephritis (28). Interstitial monocyte infiltration, extracellular matrix accumulation, and fibrosis of the kidney interstitium are also characteristic histological features of diabetic nephropathy in man (29). Monocytes move along a concentration gradient to the place with highest expression of MCP-1, which in the kidneys are the proximal tubular cells (30). Here, activated monocytes/macrophages induce proliferation and transformation of dormant fibrocytes to myofibroblasts, and interstitial scarring and tubular atrophy ensues (31).

Also, increased glomerular capillary pressure and subsequently increased protein filtration can lead to protein overload of proximal tubular epithelial cells. In response to protein overload, tubular cells can secrete MCP-1 into the tubulus and in the adjacent interstitium (32,33). Amelioration of glomerular capillary hypertension via blockade of the RAS could theoretically diminish the amount of protein in the tubular fluid, and MCP-1 secretion would subsequently decrease. Because of the impressive reduction of uMCP-1 levels in all patients and not only in those with frank proteinuria, we think that this mechanism is probably only functional in patients with a daily protein excretion in the nephrotic range.

In our study, treatment with statins for hyperlipidemia was initiated concomitantly with ACEI therapy in eight patients, and because lovastatin (34) and atorvastatin (35) have been shown to reduce MCP-1 expression in glomerular cells and vascular smooth muscle cells, respectively, we correlated the reduction in cholesterol levels in statin-treated patients with uMCP-1; however, changes in cholesterol levels did not influence uMCP-1 levels.

Therefore, we conclude that in patients with type 2 diabetes and diabetic nephropathy, blockade of AT-II with ACEI may exert its beneficial effect on renal function also via suppression of the AT-II–induced tubular production of the chemokine MCP-1. This possibly results in a decrease in the number and the activity of interstitial monocytes. Subsequently, the progression of tubulointerstitial fibrosis and tubular atrophy might be slowed. This explanation for the findings in this study is consistent with the theory that AT-II/MCP-1–induced renal fibrosis is an important part of diabetic nephropathy as well as of other noninflammatory renal diseases. Long-term studies concerning the role of MCP-1 and of its specific pharmacological suppression in diabetic nephropathy are needed to further clarify this issue.

References

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