Reduced proteinuria using ramipril in diabetic CKD stage 1 decreases circulating cell death receptor activators concurrently with ADMA. A novel pathophysiological pathway?

Mahmut Ilker Yılmaz¹, Alper Sönmez², Mutlu Sağlam³, Halil Yaman⁴, Tuncer Çavuş⁴, Selim Kılıç⁵, Tayfun Eyileten¹, Kayser Caglar¹, Yusuf Öğüz¹, Abdulgaffar Vural¹, Mujdat Yenicesu¹ and Jonas Axelsson⁶

¹Department of Nephrology, ²Department of Endocrinology, ³Department of Radiology, ⁴Department of Biochemistry, ⁵Department of Epidemiology, Gülhane School of Medicine, ⁶Division of Renal Medicine, Department of Clinical Science, Intervention and Technology, Karolinska Institute, Stockholm, Sweden

Correspondence and offprint requests to: Mahmut Ilker Yılmaz; E-mail: mahmutiyilmaz@yahoo.com

Abstract

Background. Renin–angiotensin system (RAS) blockade improves proteinuria and the endothelial functions in diabetic nephropathy. Plasma asymmetric dimethylarginine (ADMA), abundant in the cell than in the plasma, is also improved by RAS blockade. We hypothesized that RAS blockade may reduce ADMA by reducing injurious cell death.

Methods. In a hypothesis-generating study, we assessed circulating levels of apoptotic signalling peptides in incident chronic kidney disease (CKD) stage 1 patients (aged >18 years with diabetes mellitus type 2 as the only cause of nephropathy) not previously prescribed statins or RAS blockade. Ninety-three (29 M, 47 ± 5 years) patients with CKD 1 diabetic nephropathy and 38 healthy subjects (20 M, 47 ± 5 years) were enrolled. Ramipril was given (5 mg daily for 12 weeks), and circulating ADMA, soluble Fas (sFas), myostatin and endothelial function [flow-mediated vasodilation (FMD); ultrasound)] were measured.

Results. After the study, ADMA, sFas, myostatin, insulin resistance, high-sensitive C-reactive protein (hsCRP), estimated glomerular filtration rate (eGFR), blood pressure and proteinuria levels were decreased, and FMD and serum albumin levels increased (P < 0.05 for all). ADMA and sFas levels were independently related to FMD levels both before (rho = −0.33; P < 0.005 and rho = −0.26; P < 0.02, respectively) and after (rho = −0.39; P < 0.001 and rho = −0.28; P < 0.002, respectively) ramipril treatment. Changes in sFas and ADMA were related to the change in FMD (−0.32; P > 0.004 and −0.31; P < 0.004, respectively).

Conclusion. A reduction of proteinuria in CKD 1 diabetic kidney disease is accompanied by lower circulating sFas, myostatin and ADMA, suggesting that increased cell death may contribute to ADMA formation and endothelial dysfunction in diabetic CKD.

Keywords: ADMA; angiotensin-converting enzyme inhibitor; chronic kidney disease; protein catabolism; proteinuria

Introduction

While proteinuria in early diabetic chronic kidney disease (CKD) is an established risk factor for cardiovascular morbidity and mortality [1,2], as well as for the risk of progression of renal disease [3], the mechanisms mediating this effect remain unclear. Indeed, interventional studies have failed to show an effect of renin–angiotensin system (RAS) blockade on survival in the absence of proteinuria [4]. Meanwhile, a series of epidemiologic studies have related a high plasma asymmetric dimethylarginine (ADMA) to established risk factors for CKD [5,6], cardiovascular disease (CVD) [6–8] and proteinuria [7,8] in patients with diabetes mellitus type 2 (DM). In congruence with studies in non-uraemic subjects [9], we have previously shown that increased circulating ADMA is also associated with endothelial dysfunction (ED) in CKD [10,11], thus likely constituting one risk factor for CVD. In vitro, apoptosis of endothelial vascular smooth muscle cells leads to significantly elevated ADMA [12], and persistent proteinuria has been shown to increase apoptosis in peritubular cells [13]. While the mechanisms for this remain unclear, increased oxidative stress has been linked to both apoptosis [14,15] and proteinuria [14,15] in diabetic animals. As intracellular levels of ADMA can be 5–20-fold those in plasma [16], we hypothesized that proteinuria may somehow be linked to increased necrosis and/or defective apoptosis, leading to increased circulating ADMA mediating CVD risk.

Materials and methods

Patients and controls

In a hypothesis-generating study, we prospectively studied a selection of patients referred to the Department of Nephrology, Gülhane School of Medicine outpatient clinics during the period 1 January 2007–1 May 2009. Patients older than 18 years with stage 1 CKD were enrolled.
The inclusion criteria were 24-h protein excretion <500 mg/day, systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, and having diabetes mellitus type 2 as the only cause of nephropathy (renal biopsy and medical history). Among the 341 patients who fulfilled the above criteria, 252 patients were excluded because of the following reasons: being previously treated by angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARBs, medical history) or statins, having obesity [body mass index (BMI) >30 kg/m²], dyslipidemia (total cholesterol >200 mg/dl, fasting triglycerides >150 mg/dl), nephritic syndrome, history of CVD [medical history, abnormal electrocardiogram (ECG, see below)] or smoking. Thus, the final cohort comprised 89 patients (29 M, 47 ± 5 years). The duration of proteinuria and diabetic nephropathy in these patients before and after initial diagnosis were not known.

We also recruited 38 healthy subjects (20 M, 47 ± 5 years) to serve as controls. These individuals had no known diseases and were not currently taking any drugs. The control subjects were subject to the same inclusion and exclusion criteria as the patients. Informed consent to participate in the study was obtained from both patients and controls. The drug ethical committee of Gülhane School of Medicine approved the study, which was also registered at clinicaltrials.gov (NCT00893425).

Baseline characterization

Recruited patients were evaluated by standard physical examination, chest X-ray, baseline ECG, two-dimensional echocardiography and routine clinical laboratory tests, including liver and kidney function tests and 24-h urinary protein measurements. Arterial blood pressure was measured in the right arm by mercury sphygmomanometer three times in a resting condition in the morning, and mean values were calculated for diastolic and systolic pressures. Mean arterial pressure (MAP) was calculated as DBP + (SBP – DBP) / 3.

Intervention and follow-up measurements

In an open-label trial, patients were given an ACE inhibitor (ramipril, 5 mg once per day) for 12 weeks immediately following baseline measurements. During the study period, serum creatinine and potassium concentrations were measured every 2 weeks, and the dose of ramipril was titrated to achieve a maximum reduction of proteinuria without significant hypotensive episodes and/or a serum potassium concentration ranging between 99% and 103 % with an overall recovery of 101%. We also recruited 38 healthy subjects (20 M, 47 ± 5 years) to serve as controls. These individuals had no known diseases and were not currently taking any drugs. The control subjects were subject to the same inclusion and exclusion criteria as the patients. Informed consent to participate in the study was obtained from both patients and controls. The drug ethical committee of Gülhane School of Medicine approved the study, which was also registered at clinicaltrials.gov (NCT00893425).

Glomerular filtration rate (GFR) assessment

GFR was calculated according to the simplified version of the Modification of Diet in Renal Disease (MDRD) Study prediction equation formula [GFR = 186 × Pcr × 1.154 × age −0.203 × 1.212 (if black) × 0.742 (if female)] as defined by Levey [17].

Blood chemistry

Morning blood samples were collected from patients and control subjects after 12 h of fasting. Subjects were asked to refrain from physical activity for at least 30 min prior to the blood draw. In addition to routine clinical laboratory tests, serum myostatin, myoglobin, ADMA, soluble Fas (sFas) (CD95) concentrations and basal insulin levels were analysed (as described below) from all patients (Figure 1). After the intervention period, we obtained blood samples for the measurement of serum myostatin, myoglobin, sFas (CD95), high-sensitive C-reactive protein (hsCRP) and insulin levels. The measurements of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol and fasting plasma glucose (FPG) were performed by enzymatic colorimetric method using an Olympus AU 600 auto-analysers and reagents from Olympus Diagnostics GmbH (Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald’s formula [18]. The serum basal insulin value was determined by the coated tube method (DPC-USA). In particular, insulin resistance index homeostasis model assessment–insulin resistance (HOMA–IR) was computed with the formula: HOMA–IR = FPG (mg/dl) × [immunoreactive insulin (IRI) (μU/ml)] / 405 [19]. All samples were run in triplicates.

Measurement of ADMA

Measurements of ADMA were accomplished by HPLC, using the method described by Chen et al. [20]. In brief, to 1 ml serum, 20 mg of 5-sulfosalisilic acid (5-SSA) was added, and the mixture was left in an ice-bath for 10 min. The precipitated protein was removed by centrifugation at 2000 g for 10 min. Ten microlitres of the supernatant which was filtered through a 0.2-μm filter was mixed with 100 μl of derivatization reagent [prepared by dissolving 10 mg o-phthalaldehyde in 0.5 ml of methanol, 2 ml of 0.4 M borate buffer (pH 10.0) and 30 μl of 2-mercaptoethanol] and then injected into the chromatographic system. Separation of ADMA was achieved with a 150 × 4-mm I.D. Nova-pak C18 column with a particle size of 5 μm (Waters Inc., Milford, MA, USA) using 50 mN sodium acetate (pH 6.8), methanol and tetrahydrofuran as mobile phase (A, 82:17:1; B, 22:77:1) at a flow rate of 1.0 ml/min. The area of peak detected by fluorescent detector (excitation: 338 nm; emission: 425 nm) was used for quantification of ADMA levels in serum. The variability of the method was <7%, and the detection limit of the assay was 0.01 μM. The mean inter-assay coefficients of variation (CV) of amounts for ADMA derivatives were 2.55%, and intra-assay coefficients of variation were 2.91%.

Serum sFas measurement

We used enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA) to determine serum sFas levels. The calculated overall intra-assay coefficient of variation was 3.7%, and the calculated overall inter-assay coefficient of variation was 4.5%. After spiking of human sFas into normal human serum, average spike recoveries ranged from 91% to 110% and, overall mean recovery of 97% was found. On serial, 2-fold dilution recovery ranged between 99% and 103% with an overall recovery of 101%. We measured all samples in duplicates and used the mean values.

Serum myostatin measurement

Again, ELISA kits were used according to the manufacturer’s instructions (Uscnlife Science & Technology Co., Ltd, Wuhan, China) to determine myostatin levels. The calculated overall intra-assay coefficient of variation was 3.4%, and the calculated overall inter-assay coefficient of variation was 6.7%. We measured all samples in duplicates and used the mean values.
**Paired samples**

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**Serum myoglobin measurement**

Serum myoglobin levels were quantified by the Elecsys 1010 (Roche Diagnostics, Indianapolis, IN, USA) using a commercial specific kit (Roche Diagnostics).

**hsCRP assessment**

Serum samples were diluted with a ratio of 1/101 with the diluent solution. Calibrators, kit controls and serum samples were all added on each microwell with an incubation period of 30 min. After three washing intervals, 100 μl enzyme conjugate (peroxidase-labelled anti-CRP) was added on each microwell with an incubation period of 30 min. After three washing intervals, 100 μl enzyme conjugate (peroxidase-labelled anti-CRP) was added onto each well for additional 15-min incubation in room temperature in the dark. The reaction was stopped with a stop solution, and photometric measurement was performed at the 450-nm wavelength. The amount of serum samples was calculated as milligram per litre with a graphic that was made by noting the absorbance levels of the calibrators.

**Endothelial function tests**

Endothelial dysfunction was assessed according to the method described by Celemajer et al. [21]. Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA, USA) with a 12-Mhz probe. All vasoactive medications were withheld for 24 h before the procedure. The subjects remained at rest in the supine position for at least 15 min before the examination. Each subject's right arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single 2D frames. All ultrasound images were recorded on Super Video Home System (S-VHS) videotape for subsequent blinded analysis. The maximum flow-mediated vasodilation (FMD) diameters were calculated as the average of the three consecutive maximum diameter measurements after hyperaemia and nitroglycerin, respectively. The FMD levels were then calculated as the percent change in diameter compared with baseline resting diameters.

**Statistical analysis**

All the statistical analyses were performed by using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) statistical package. Non-normally distributed variables were expressed as median (range), and normally distributed variables were as mean ± SD as appropriate. A P-value <0.05 was considered to be statistically significant. One-sample Kolmogorov–Smirnov test was used for analysis distribution of data. One-way ANOVA, Student’s t-test and paired sample t-test were used to compare numeric data. Spearman’s rank correlation was used to determine correlations with continuous variables. Stepwise multivariate regression analysis was used for independent variables.

**Results**

**Baseline characteristics**

Baseline clinical and laboratory characteristic as well as vascular measurements for the study population are shown in Table 1. There were no differences between diabetic patients and control groups with respect to age, sex, eGFR and BMI. As expected, serum ADMA, sFas, 24-h proteinuria, creatinine kinase, myostatin, myoglobin, HbA1c, serum albumin, HOMA, SBP, DBP and hsCRP levels were higher in diabetic patients. FMD levels were lower in diabetic patients than those of the controls (Table 1). At baseline, FMD was negatively correlated with both serum ADMA, sFas, 24-h proteinuria, myostatin and hsCRP le-
levels, and positively correlated with eGFR levels before the treatment period.

The effect of ACEI treatment

No significant changes in baseline parameters occurred in the untreated controls. Following 12 weeks of RAS blockade, ADMA, sFas, myostatin, myoglobin, HOMA index, hsCRP, eGFR, SBP, DBP, HbA1c and proteinuria levels were all significantly decreased, while FMD and serum albumin levels were significantly increased. The lipid parameters and BMIs of the patients did not change significantly during the study period. The negative correlation between FMD levels and ADMA, sFas, 24-h proteinuria, myostatin and hsCRP was present also after the 12-week treatment period as well (Table 2). Furthermore, sFas levels were positively correlated with ADMA, myoglobin, myostatin, LDH, HOMA, 24-h proteinuria and hsCRP, and negatively correlated with FMD and eGFR levels before the treatment period (Figure 2).

Correlates of changes in sFas, myostatin and ADMA

The correlations between changes in cell death markers and ADMA with other parameters are shown in Table 3.

Multivariate regression analysis

We next investigated the independence of the observed correlations with FMD using a multiple regression model incorporating variables expected to influence FMD (sex, age, 24-h proteinuria, eGFR and hsCRP), as well as ADMA, sFas and myostatin (Table 2). Briefly, ADMA and sFas levels were independently related to FMD both before (beta = −0.33, P = 0.005 and beta = −0.26, P = 0.02, respectively) and after (beta = −0.39, P < 0.001 and beta = 0.28, P = 0.003, respectively) ACEI treatment (Table 2). Additionally, in a third model, we investigated the independent predictors of the change in proteinuria (Table 4A) and FMD (Table 4B) following ACEI therapy. Briefly, changes in proteinuria were related to myoglobin (<0.001) and creatinine kinase (0.04), while changes in FMD were independently related only to changes in sFas (P = 0.03) and ADMA (P = 0.04).

Discussion

In a hypothesis-generating study of 93 patients with stage 1 CKD due to type 2 DM, studied before and after 12 weeks of RAS blockade (using ramipril), we found circulating levels of myostatin and sFas, two cell death mediators, to be independently related to the degree of proteinuria, as well as to endothelial dysfunction (ED), and circulating ADMA, a potent inhibitor of nitric oxide (NO) synthesis in endothelial cells [16]. Furthermore, the degree of reduction in proteinuria achieved also predicted changes in sFas, myostatin, ADMA and ED independently of changes in inflammation, serum albumin and blood pressure. In 38 untreated controls, these levels did not change during the same time period.

It has long been known that the advent of proteinuria is a poor prognostic factor in patients with type 2 diabetes [1,2], but the mechanisms behind have remained unclear. Multiple studies demonstrate that a reduction in blood pressure and proteinuria using RAS blockers improves endothelial function and diminishes the risk for early death from CVD proportionally to the reduction in proteinuria [4,22]. Furthermore, initiation of RAS blockade lowers circulating levels of the NO synthesis inhibitor ADMA [11] in diabetic CKD, while hypertension [9,23] and CKD [5] are both associated with elevated ADMA that has also been found to correlate with the degree of ED in diabetic kidney disease [8,11].

As intracellular ADMA is 5–20-fold higher than those in plasma [16], we hypothesized that leakage from the intracellular space caused by necrosis and/or faulty apoptosis during proteinuria could contribute to high ADMA levels in these patients. Further supporting a link between proteinuria and cell death, in patients with immunoglob-
ulin A (IgA) nephropathy, administration of the RAS blocker lisinopril has been reported to decrease circulating levels of the intracellular cell death protein bcl-2 [24], while animal data suggest that blocking the pro-apoptotic MAPK pathway reduces proteinuria and CVD in diabetic rats [25].

In this context, we studied two other—upstream—cell death mediators, sFas and myostatin. sFas is the soluble form of the membrane-bound Fas receptor, the most studied cell death receptor. It is involved in mediating glomerular and mesangial cell injury [26], apoptosis of myocytes following cardiac ischaemia [27], as well as an important regulator of immunity [28]. Myostatin is a transforming growth factor-beta superfamily member and is known as an inhibitor of skel muscle cell proliferation and differentiation. Exposure to myostatin induces G1 phase cell cycle arrest through downregulation of Cdk4 activity via promotion of cyclin D1 degradation [29]. These two proteins thus represent diverse pathway both leading to apoptosis. We found the circulating levels of both markers to decrease with decreasing proteinuria and concomitantly with an improvement of ED, as well as with a decrease in circulating creatinine kinase and myoglobin—both markers of myocyte damage. Meanwhile, circulating ADMA and hsCRP, both related to CVD risk and ED in CKD [11,30,31], decreased. It should however be noted that our study does not identify the cause and effect of the observed relationships. Indeed, while increased sFas and myostatin with uncontrolled proteinuria may represent increased apoptotic signalling, they—like ADMA—may also simply reflect intracellular leakage due to uncontrolled cell death. Alternate pathways mediating the observed effects may also be proposed. Thus, angiotensin II has been reported to mediate skeletal muscle oxidative stress and inflammation, leading to insulin resistance [32,33], which in turn is known to influence endothelial reactivity [30].

### Table 3. Univariate correlates of changes in sFas (A), myostatin (B) and ADMA (C)

<table>
<thead>
<tr>
<th></th>
<th>(A) Change in sFas (pg/ml) (rho, P)</th>
<th>(B) Change in myostatin (ng/ml) (rho, P)</th>
<th>(C) Change in ADMA (μmol/l) (rho, P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in sFas (pg/ml)</td>
<td></td>
<td>0.33; P &lt; 0.01</td>
<td>0.36; P &lt; 0.001</td>
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<tr>
<td>Change in ADMA (μmol/l)</td>
<td>0.36; P &lt; 0.001</td>
<td>NS</td>
<td></td>
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<tr>
<td>Change in FMD (%)</td>
<td>−0.32; P &lt; 0.01</td>
<td>−0.32; P &lt; 0.005</td>
<td></td>
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<tr>
<td>Change in myostatin (ng/ml)</td>
<td>−0.33; P &lt; 0.01</td>
<td>−</td>
<td>NS</td>
</tr>
<tr>
<td>Change in proteinuria (mg/day)</td>
<td>0.31; P &lt; 0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Change in myoglobin (ng/ml)</td>
<td>0.34; P &lt; 0.01</td>
<td>0.23; P &lt; 0.05</td>
<td></td>
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<tr>
<td>Change in creatinine kinase (ng/ml)</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Change in lactate dehydrogenase (mg/dl)</td>
<td>0.25; P &lt; 0.05</td>
<td>NS</td>
<td></td>
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<tr>
<td>Change in hsCRP (mg/l)</td>
<td>NS</td>
<td>−0.26; P &lt; 0.05</td>
<td></td>
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<tr>
<td>Change in MAP (mmHg)</td>
<td>NS</td>
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### Table 4. Independent predictors of the change in proteinuria (A) and FMD (B) following ACEI therapy

<table>
<thead>
<tr>
<th>(A) Change in proteinuria (mg/day) (r² = 0.31)</th>
<th>Univariate rho (P)</th>
<th>Multivariate beta (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in sFas (pg/ml)</td>
<td>0.31 (0.006)</td>
<td>NS</td>
</tr>
<tr>
<td>Change in ADMA (μmol/l)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Change in FMD (%)</td>
<td>−0.24 (0.04)</td>
<td>NS</td>
</tr>
<tr>
<td>Change in myostatin (ng/ml)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Change in myoglobin (ng/ml)</td>
<td>0.52 (&lt;0.001)</td>
<td>0.44 (&lt;0.001)</td>
</tr>
<tr>
<td>Change in creatinine kinase (ng/ml)</td>
<td>0.38 (0.001)</td>
<td>0.21 (0.04)</td>
</tr>
<tr>
<td>Change in hsCRP (mg/l)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Change in MAP (mmHg)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Change in lactate dehydrogenase (mg/dl)</td>
<td>0.24 (0.04)</td>
<td>NS</td>
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<table>
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<tr>
<th>(B) Change in FMD (%) (r² = 0.15)</th>
<th>Univariate rho (P)</th>
<th>Multivariate beta (P)</th>
</tr>
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<tbody>
<tr>
<td>Change in sFas (pg/ml)</td>
<td>−0.32 (0.004)</td>
<td>−0.24 (0.03)</td>
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<tr>
<td>Change in ADMA (μmol/l)</td>
<td>−0.32 (0.005)</td>
<td>−0.23 (0.04)</td>
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<tr>
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<td>Change in MAP (mmHg)</td>
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<tr>
<td>Change in lactate dehydrogenase (mg/dl)</td>
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Should cell death signalling be shown to be increased in diabetic nephropathy, several likely mechanisms may be involved. Firstly, many studies have demonstrated an increased oxidative and inflammatory stress in CKD, and these factors have also been linked to increased apoptosis. Indeed, as expected, inflammation as measured by hsCRP correlated with changes of both ED and cell death receptors in our study. Furthermore, shear stress is known to be increased in patients with uraemic ED and is also associated with circulating levels of endothelial microparticles though to represent the remnants of endothelial cell lysis [34]. In the present study, while baseline MAP decreased significantly following ramipril therapy, the decrease was not significantly associated with either changes of IMT, sFas or myostatin. Finally, a novel pathway linking reduced endothelial dysfunction and cell death to RAS blockade appears to be the recently demonstrated ability of angiotensin II receptor type 1 knock-out mice to live longer and develop less cardiovascular damage due to increased expression of the cell survival protein visfatin [35]. Interestingly, we recently reported that the circulating visfatin correlates with the degree of ED in diabetic CKD [31], while an improved endothelial function following renal transplantation is concurrent with decreasing visfatin levels in the circulation [36]. Future studies may shed more light on the putative link between cell death, survival factors and their links to diabetic risk factors.

A number of weaknesses in this initial hypothesis-generating protocol should be kept in mind when interpreting our findings. Firstly, this was a biochemical analysis of patient blood samples and does not include direct histopathological proof of cell death. Furthermore, the sequential nature of the observations as well as the lack of a longitudinal control group limits the usefulness of our data. Nonetheless, the present study suggests, but does not conclusively prove, novel links between reduced cell death signalling following RAS therapy, reduced levels of circulating ADMA and amelioration of endothelial dysfunction in diabetic CKD.

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Conflict of interest statement. J.A. is the recipient of lecturing fees from Baxter Healthcare as well as research grant support from Sanofi–Aventis. The authors have no relationships or financial interests with companies related to the findings of this work.

References
Erythropoietin is reduced by combination of diuretic therapy and RAAS blockade in proteinuric renal patients with preserved renal function

Maartje C.J. Slagman¹, Steef J. Sinkeler¹, Marc H. Hemmelder², Femke Waanders¹, Liffert Vogt¹, Hanneke C. Kluin-Nelemans³, Gerjan Navis¹ and Gozewijn D. Laverman¹

¹Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, Groningen, The Netherlands, ²Division of Nephrology, Department of Internal Medicine, Medical Center Leeuwarden, Leeuwarden, The Netherlands and ³Department of Hematology, University Medical Center Groningen, Groningen, The Netherlands

Correspondence and offprint requests to: Gozewijn D. Laverman; E-mail: g.d.laverman@int.umcg.nl

Abstract

Background. Renin–angiotensin–aldosterone system (RAAS) blockade improves prognosis in renal patients, but usually requires diuretic co-treatment. RAAS blockade can decrease erythropoietin (EPO) and/or haemoglobin (Hb) levels. Diuretics decrease EPO in rodents, but their effect on EPO and Hb in humans is unknown.

Methods. Proteinuric renal patients with preserved renal function were treated during 6-week periods with placebo, losartan 100 mg/day (LOS) and LOS plus hydrochlorothiazide 25 mg/day (LOS/HCT), in random order.

Results. Hb was inversely related to proteinuria, and EPO levels were inappropriately low in relation to Hb. Hb was lowered by LOS with and without HCT. EPO was decreased by LOS/HCT, but not by LOS.

Conclusions. EPO and Hb are reduced by HCT added to LOS in proteinuric renal patients with preserved renal function. We hypothesize that EPO reduction by HCT is caused by a decrease in renal oxygen requirement, which is the main stimulus for EPO production, due to the inhibition of active tubular sodium reabsorption. Further studies should explore the exact mechanism of this phenomenon and its clinical impact.

Keywords: diuretics; erythropoietin; haemoglobin; proteinuria; renal disease; renin–angiotensin–aldosterone system blockade

Introduction

Blockade of the renin–angiotensin–aldosterone system (RAAS) with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) reduces