Effect of a Treatment Strategy Consisting of Pravastatin, Vitamin E, and Homocysteine Lowering on Carotid Intima-Media Thickness, Endothelial Function, and Renal Function in Patients With Mild to Moderate Chronic Kidney Disease

Results From the Anti-Oxidant Therapy in Chronic Renal Insufficiency (ATIC) Study

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Background: Patients with chronic kidney disease have an increased risk of cardiovascular disease. Oxidative stress has been proposed to play a role in the development of cardiovascular disease among these patients.

Methods: We conducted a randomized, double-blind trial in 93 patients (Cockcroft-Gault equation: creatinine clearance, 38±15 [mean±SD] mL/min per 1.73 m² [0.63±0.25 mL/s per m²]) to investigate the effect of a treatment strategy designed primarily to achieve stepwise oxidative stress reduction on common carotid intima-media thickness (CC-IMT), brachial artery flow-mediated dilatation (BA-FMD), albuminuria, and renal function. The treatment group received a regimen of pravastatin to which vitamin E supplementation was added after 6 months and homocysteine-lowering therapy after another 6 months. Blood pressure in both groups was managed according to a standard protocol. The placebo group received matching placebos. Measurement of CC-IMT and BA-FMD was performed at randomization after 6, 12, and 18 months. Patients were followed up for 2 years. Generalized estimating equations were used for analysis.

Results: Compared with placebo, active treatment was associated with a decrease in CC-IMT (after 18 months: from 0.68 to 0.63 mm in the treatment group and from 0.65 to 0.71 mm in the placebo group; \( P < .001 \)), an increase in BA-FMD (after 18 months: from 4.66% to 7.56% in the treatment group and from 6.21% to 4.73% in the placebo group; \( P < .001 \)), and an attenuated increase in urinary albumin excretion over time (\( P = .04 \) for between-group difference after 24 months), but no effect was observed on renal function.

Conclusion: In patients with mild to moderate chronic kidney disease, 18 months of a treatment strategy along with well-controlled blood pressure reduced CC-IMT and urinary albumin excretion and increased BA-FMD.

Trial Registration: clinicaltrials.gov Identifier: NCT00384618

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Patients with mild to moderate chronic kidney disease (CKD) have an increased risk of cardiovascular disease, which cannot fully be explained by the presence of known cardiovascular risk factors such as hypertension, diabetes, smoking, and dyslipidemia. Therefore, other atherothrombotic mechanisms play a role. In the last few years, compelling evidence has emerged pointing to the contributing role of oxidative stress in the pathogenesis of cardiovascular complications in CKD. Oxidative stress in patients with CKD has been attributed to the effects of uremic toxins, angiotensin II, proinflammatory cytokines, and hyperhomocysteinemia.

Statins have been shown to reduce oxidative stress in hypercholesterolemic patients. Vitamin E supplementation and homocysteine-lowering therapy have also been shown to reduce oxidative stress in several patient populations. However, in dialysis patients, studies aimed at reducing cardiovascular events with statins and homocysteine-lowering therapy have...
have not shown positive results. A possible explanation for these disappointing findings is that patients at the start of dialysis often have advanced cardiovascular disease,\(^2\) which may be difficult to reverse in this phase. However, only a few cardiovascular intervention studies have been performed on patients with mild to moderate CKD,\(^20\) and most of the large intervention trials with statins have excluded patients with moderate renal failure.

In view of these considerations, we designed the Anti-Oxidant Therapy in Chronic Renal Insufficiency (ATIC) Study to examine the effect of a treatment strategy primarily designed to achieve a stepwise reduction of oxidative stress in a population of patients with mild to moderate CKD\(^21\) and well-controlled blood pressure. The treatment strategy consisted of pravastatin, vitamin E, and homocysteine-lowering therapy on common carotid intima-media thickness (CC-IMT) (a strong surrogate marker of cardiovascular risk in the general\(^22\) and the dialysis\(^23\) populations), brachial artery flow-mediated dilatation (BA-FMD) (a marker of endothelial function that can be impaired by increased oxidative stress\(^24,25\)), estimated glomerular filtration rate (eGFR), and urinary albumin excretion. Plasma-oxidized low-density lipoprotein (oxLDL)\(^26\) and malondialdehyde\(^27\) were measured as oxidative stress parameters. Interventions were added to the regimen every 6 months to investigate both the effects of individual interventions and the effects of the entire strategy on the end points.

### METHODS

#### PATIENTS

Between May 2001 and December 2002, patients with a creatinine clearance of 15 to 70 mL/min per 1.73 m\(^2\) (0.25-1.17 mL/s per m\(^2\)) (according to the Cockcroft-Gault equation) from 7 outpatient clinics in Amsterdam, the Netherlands, were screened for eligibility for participation in the ATIC Study, a randomized, double-blind, placebo-controlled trial investigating the effects of oxidative stress–lowering treatment on vascular structure and function in nondiabetic patients with chronic renal failure who had no manifest arterial occlusive disease.

#### DESIGN

Participants were randomized after stratification for prior use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, creatinine clearance (between 15-39 and 40-70 mL/min per 1.73 m\(^2\) [between 0.25-0.65 and 0.66-1.17 mL/s per m\(^2\)]), and age (between 20-49 and 50-80 years). Randomization was carried out centrally by means of a computer-generated sequence involving randomized blocks of 4, and sealed envelopes were kept by 1 hospital pharmacist. Unblinding was performed after the data analysis. After randomization, participants in the treatment group were treated with pravastatin (40 mg/d), vitamin E (\(\alpha\)-tocopherol acetate) (300 mg/d) was added to the regimen 6 months later, and homocysteine-lowering therapy (folinic acid [5 mg/d], pyridoxine hydrochloride [100 mg/d], and cyanocobalamin [1 mg/d] in 1 tablet) was added 6 months after that. Patients continued this triple therapy for another 12 months (Figure 1). Patients in the placebo group received matching placebos at the onset and 6 and 12 months later. Adherence to therapy was assessed by counting leftover pills. Subjects who were not using angiotensin-converting enzyme inhibitors or angiotensin receptor blockers at inclusion received an angiotensin-converting enzyme inhibitor (fosinopril [10 mg/d]) for at least 2 weeks before the baseline measurements and randomization. Those who were taking angiotensin receptor blockers continued taking them. During the following visits, blood pressure was controlled according to a standard protocol in which hydrochlorothiazide (a loop diuretic) was administered if the eGFR was <30 mL/min, metoprolol succinate, amiodipine mesilate, or doxazosin were added in that order to achieve a blood pressure of less than 140/90 mm Hg. Measurement of the CC-IMT and BA-FMD and laboratory tests were performed in all cases at randomization and at 6, 12, and 18 months after randomization. Laboratory tests were also performed after 24 months. We excluded individuals with diabetes mellitus (American Diabetes Association criteria), active vasculitis, nephrotic syndrome, renal transplantation, a fasting total cholesterol level higher than 270 mg/dL (7.00 mmol/L), cholesterol-lowering therapy within 3 months prior to inclusion, or ischemic coronary, cerebrovascular, or peripheral arterial disease. Ninety-three patients (out of 118 eligible patients) took part in the study (Figure 1). Written informed consent was obtained from all participants, and the study was approved by the ethical committees at each center.

#### PROCEDURES

Patients were examined in fasting state in a temperature-controlled (25°C) room. Data were collected with regard to age, medications, and smoking status (having smoked in the past year), and a history was obtained to exclude peripheral, cerebral, and coronary vascular disease. After 30 minutes of rest,
CAROTID ARTERY ULTRASONOGRAPHY

The CC-IMT measurements were performed using a medical scanner (Scanner 330, Pie Medical, Maastricht, the Netherlands) with a linear array transducer of 7.5 MHz attached to a data registration and processing unit (Wall Track System II; Pie Medical) as described in detail elsewhere.28,29

BRACHIAL ARTERY ULTRASONOGRAPHY

The measurement protocol for BA-FMD has also been described in detail elsewhere.30,31 Briefly, baseline diameter (mean of 3 measurements) and peak flow velocity (mean of 2 measurements) were determined. A pressure cuff, placed on the forearm, was then inflated and kept constant at suprasystolic pressure. After 5 minutes, the cuff was released to increase blood flow. After cuff release, maximum peak flow velocity was measured within 15 seconds and diameter was measured at 45, 90, 120, 150, and 300 seconds. The BA-FMD was calculated as the percentage of change in the maximum postocclusion diameter of the brachial artery relative to the mean baseline diameter.

REPRODUCIBILITY

All ultrasound measurements at each visit were performed by a single observer who was blinded to the treatment allocation. Reproducibility was assessed in 10 healthy subjects (43 ± 13 [mean ± SD] years) who were examined by the same observer twice, 3 weeks apart. The intraobserver coefficient of variation (CV) (SD of the mean difference/2 × pooled mean.) was 10% for the CC-IMT measurement and 15% for the BA-FMD measurement.

LABORATORY ANALYSES

Serum creatinine concentration was assessed by a kinetic Jaffe method. Plasma total (free plus protein-bound) homocysteine was measured with an automated fluorosecence polarization immunoassay analyzer (IMx; Abbott Laboratories, Abbott Park, Ill), with an interassay CV of less than 4%.32 Renal function was estimated by the Modification of Diet in Renal Disease (MDRD) study equation (eGFR in milliliters per minute per 1.73 m², per Levey equation)7 and by the Cockcroft-Gault and Dubois formulas (creatinine clearance in milliliters per minute per 1.73 m²).33,34 Urinary albumin was measured in a 24-hour urine collection at each visit and analyzed using a microalbumin assay (Beckman Array 360 Analyzer; Beckman Instruments Inc, Fullerton, Calif). All analyses were performed according to the intention-to-treat principle. Outcome variables were analyzed with generalized estimating equations, an established longitudinal data analysis technique.36 In the primary generalized estimating equations model, the outcome variable studied (eg, CC-IMT or BA-FMD) was analyzed as a dependent variable using treatment strategy (1, intervention group; 0, placebo group) as a key independent variable adjusted for time and, if appropriate, for previous observations using extra independent variables. Also, to evaluate effect modification, the product term of group and time (group × time) was added as an independent variable. In case of skewed data, analyses were performed after log transformation.

Data are presented in graphs indicating means with standard errors. All these variables except urinary albumin were normally distributed. P < .05 was considered statistically significant.

Table 1 shows the baseline characteristics of the participants. Of 93 patients who were included in the study, 6 withdrew after undergoing the baseline measurement and 87 underwent the second measurement and were included in the final analysis (Figure 1). After 2 years, 72 patients in the treatment group and 77 patients in the placebo group were still taking the drugs. Compliance at each follow-up visit was defined as consumption of at least 80% of the scheduled tablets since the previous visit.

Four patients in the treatment group and 2 patients in the placebo group consumed more than 60% but less than 80% of the allocated tablets during the study period; all other participants took at least 80% of their scheduled tablets.

COMMON CAROTID INTIMA-MEDIA THICKNESS

After 18 months, the mean CC-IMT had decreased from 0.68 to 0.63 mm in the treatment group, whereas it had increased from 0.65 to 0.71 mm in the placebo group (P < .001 for between-group difference) (Figure 2). After adjustment for baseline values of CC-IMT, the treatment strategy was associated with a CC-IMT lowering of 0.13 mm (95% confidence interval [CI], 0.10-0.16 mm) at 18 months. The largest change in CC-IMT (from 0.68 to 0.65 mm in the treatment group; P < .001 for between-group difference after 6 months) was seen in the first 6 months of therapy. After adjustment for baseline values of CC-IMT, the treatment strategy was associated with a CC-IMT lowering of 0.07 mm (95% CI, 0.05-0.09 mm) after 6 months.
After 18 months, the BA-FMD had increased from 4.66% to 7.56% in the treatment group, whereas it had decreased from 6.21% to 4.73% in the placebo group (P < .001 for between-group difference after 18 months) (Figure 3). After adjustment for baseline values of BA-FMD and time, the treatment strategy was associated with a BA-FMD increase of 3.18% (95% CI, 1.23%-5.13%) after 18 months. After 6 months, the BA-FMD had increased from 4.66% to 6.73% in the treatment group and from 6.21% to 6.43% in the placebo group (P = .11 for between-group difference after 6 months) (Figure 3).

**RENAL FUNCTION**

After 24 months, the mean eGFR (MDRD formula) had decreased from 35 to 33 mL/min per 1.73 m² (from 0.58 to 0.53 mL/s per m²) in the placebo group and increased from 32 to 35 mL/min per 1.73 m² (from 0.53 to 0.58 mL/s per m²) in the treatment group (P = .89 for between-group difference after 18 months). After 6 months, the BA-FMD had increased from 4.66% to 6.73% in the treatment group and from 6.21% to 6.43% in the placebo group (P = .11 for between-group difference after 6 months) (Figure 3).
group difference) (Figure 4). After adjustment for baseline values and time, the treatment strategy was associated with a 0.10 mL/min per 1.73 m² (0.002 mL/s per m²) (95% CI, −2.11 to 1.92 mL/min per 1.73 m² [95% CI, −0.042 to 0.044 mL/s per m²]) MDRD increase at 24 months and a 0.06 mL/min per 1.73 m² (0.001 mL/s per m²) (95% CI, −0.035 to 0.032 mL/s per m²) MDRD decrease at 18 months.

FIGURE 4. Change in mean (SE) estimated glomerular filtration rate (eGFR) based on the Modification of Diet in Renal Disease formula (milliliters per minute per 173 m², per Levey equation 7), with the P values for between-group differences. For 0 to 6 months, P=.79; for 0 to 12 months, P=.60; for 12 to 18 months, P=.21; for 18 to 24 months, P=.39; and for 0 to 24 months, P=.33. Error bars indicate SE.

The main finding of this study is that, in patients with mild to moderate nondiabetic CKD who had no manifest arterial occlusive disease and had well-controlled blood pressure, 18 months of treatment with an oxidative stress–lowering strategy consisting of pravastatin, vitamin E, and homocysteine-lowering therapy resulted in a statistically significant improvement in BA-FMD (Table 2). There was no significant reduction of plasma malondialdehyde (P=.13) during the study period (Table 2). There were 19 dropouts (11 from the treatment group and 8 from the placebo group) and 6 cardiovascular events during the study (Table 3).

COMMENT

The main finding of this study is that, in patients with mild to moderate nondiabetic CKD who had no manifest arterial occlusive disease and had well-controlled blood pressure, 18 months of treatment with an oxidative stress–lowering strategy consisting of pravastatin, vitamin E, and homocysteine-lowering therapy resulted in a statistically significant reduction in CC-IMT (P<.001) and a statistically significant improvement in BA-FMD (P=.001). There was no statistically significant effect on eGFR (P=.89). However, treatment was associated with an attenuated increase in urinary albumin excretion over time.
Cardiovascular morbidity and mortality are extremely high in patients with end-stage renal disease, and the results of intervention studies aimed at the reduction of cardiovascular events with statins and homocysteine-lowering therapy in these patients have been disappointing. These results may suggest that the extent and nature of vascular disease in patients with end-stage renal disease makes such treatment options less effective than in other patient groups. Therefore, we evaluated whether intervention at an earlier stage of CKD (Kidney Disease Outcomes Quality Initiative [K/DOQI] stages 2 through 4) would have beneficial effects on strong surrogate estimates of cardiovascular outcome, ie, CC-IMT, BA-FMD, and urinary albumin excretion. Furthermore, and in contrast with most other lipid trials, the design of our study included formal control of blood pressure (<140/90 mm Hg) using a strict protocol. Blood pressure control is extremely important, as hypertension is very frequent in patients with CKD, and adequate blood pressure control in patients with mild to moderate renal disease slows the decline of the eGFR and decreases cardiovascular morbidity and mortality. Renin-angiotensin system blockade, in particular, has been shown to reduce proteinuria and to retard the progression of CKD, in part independent of blood pressure lowering. Therefore, the results of the present study should be interpreted as the effect of the treatment strategy in conjunction with well-controlled blood pressure.

Very few data are available on the effects of statins on cardiovascular outcomes in patients with mild to moderate CKD (K/DOQI stages 2 through 4) and adequately controlled blood pressure, because patients with moderate CKD (stages 3 and 4) were usually excluded from the large cardiovascular outcome trials with statins, and/or blood pressure control was not included in the design of those trials. On the other hand, subgroup analyses of a limited number of lipid trials (Anglo-Scandinavian Cardiac Outcomes Trial and the Pravastatin Pooling Project) do suggest that statin treatment may reduce cardiovascular events in patients with stages 1 through 3 CKD. These data provide an indirect indication of the beneficial effects of lipid lowering in stages 1 to 3 CKD. It is important to realize, however, that patients with stage 4 CKD (eGFR, 15-29 mL/min per 1.73 m²) were absent or the numbers were too small for analysis in these trials. In a recent study, Isbel et al showed that, when compared with usual care, a multiple risk factor intervention program in a population of patients with stages 4 and 5 CKD was not associated with reduction in CC-IMT or with improvement in endothelial function. However, only 25% of the patients in Isbel and colleagues’ study had stage 4 CKD, and those patients were not analyzed separately.

Therefore, to our knowledge, the ATIC Study is the first randomized, placebo-controlled trial examining the effect of an oxidative stress–lowering strategy in a population of patients with mild to moderate nondiabetic stages 3 and 4 CKD without manifest cardiovascular disease. Also, and in contrast to the above-mentioned studies, 45% (42/93) of our patient population had K/DOQI stage 4 CKD, equally divided between the treatment and the placebo groups (22 patients in the treatment group and 20 patients in the placebo group). Most of the remaining patients (48/93) had K/DOQI stage 3 CKD (25 patients in the treatment group and 23 patients in the placebo group). The treatment strategy had beneficial effects on the CC-IMT and BA-FMD in patients with stages 3 and 4 CKD (data not shown).

The systolic blood pressure did not differ statistically significantly (P = .14) between the 2 groups at any point, and adjustment for systolic, diastolic, or mean blood pressure difference did not alter the CC-IMT, BA-FMD, or urinary albumin excretion results. Therefore, according to our study findings, we conclude that the treatment strategy described herein in conjunction with adequately controlled blood pressure has beneficial effects for patients with stage 3 or 4 CKD who have no prior cardiovascular disease.

A few studies have demonstrated a renoprotective effect of statins. However, most of these studies were short term, with small patient populations, or subgroup analyses from large statin trials involving subjects at high risk for cardiovascular events but with mild CKD or normal renal function at baseline. After 2 years of treatment in our patient population, we could not demonstrate a statistically significant effect on the eGFR between the groups. Our study was not powered, and the follow-up period may have been too short to demonstrate any effect on the eGFR.
However, we were able to demonstrate a significant attenuation of the increase in urinary albumin excretion over time in the treatment group. Additional analyses suggested that these effects were limited to individuals with urinary albumin excretion of more than 30 mg/24 h, but the hazards of such analyses are well known, and, clearly, this result requires confirmation. Urinary albumin excretion may be a marker of endothelial dysfunction, as shown in our population (Figure 3), and thereby to the reduction of urinary albumin excretion.

We studied a selected population of patients with mild to moderate CKD. Our study had limited power and was too short to detect an effect on clinical cardiovascular endpoints. In other populations, large trials with vitamin E and homocysteine lowering have not shown any beneficial effects on cardiovascular events. However, during the design period of our study, the then-available information suggested that these vitamins could have beneficial effects in patients with renal failure because these patients were known to have increased oxidative stress. Also, small studies with vitamin E in dialysis patients at that time showed some promising results. We decided to use the treatment strategies concomitantly to reduce the number of patients needed to perform this study and to achieve a maximum oxidative stress reduction in the treatment group. Furthermore, we decided to add interventions sequentially and planned to evaluate the effects of individual treatments. We expected (in retrospect, wrongly) the maximum effect of each intervention to be achieved within 6 months after the given intervention and/or that the additional effect of the next step would be clearly distinguishable from the effects of the previous step. Decreases in CC-IMT and improvement in BA-FMD were observed during the whole study period (Figures 2 and 3). In retrospect, we are unable to draw any conclusions on the individual effects of these interventions. Also, the treatment modalities of the present study certainly have effects independent of oxidative stress lowering. We therefore cannot draw any conclusions as to whether the observed improvements were the results of the oxidative stress lowering or of other effects such as reduction of lipid levels.

In conclusion, in nondiabetic patients with mild to moderate CKD with adequately controlled blood pres-
sure and without clinical features of atherosclerosis, a treatment strategy consisting of pravastatin, vitamin E, and homocysteine-lowering therapy resulted in a significant reduction in CC-IMT and a significant improvement in endothelial function and urinary albumin excretion. No significant effect on eGFR was seen. These results suggest, but do not prove, that this treatment strategy might safely reduce the burden of cardiovascular events in this population. Thus, larger studies carried out over a long period with clinical end points will be required to confirm and validate these results.

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