Circulation

American Heart Association®

Learn and Live℠

Angiotensin-Converting Enzyme Inhibition Improves Vascular Function in Rheumatoid Arthritis

Andreas J. Flammer, Isabella Sudano, Frank Hermann, Steffen Gay, Adrian Forster,
Michel Neidhart, Peter Künzler, Frank Enseleit, Daniel Périat, Matthias Hermann,
Juerg Nussberger, Thomas F. Luscher, Roberto Corti, Georg Noll and Frank
Ruschitzka

The online version of this article, along with updated information and services, is
located on the World Wide Web at:

http://circ.ahajournals.org/cgi/content/full/117/17/2262

Vascular Medicine

Angiotensin-Converting Enzyme Inhibition Improves Vascular Function in Rheumatoid Arthritis

Andreas J. Flammer, MD*; Isabella Sudano, MD, PhD*; Frank Hermann, MD; Steffen Gay, MD; Adrian Forster, MD; Michel Neidhart, PhD; Peter Künzler, MSc; Frank Enseleit, MD; Daniel Périat, MD; Matthias Hermann, MD; Juerg Nussberger, MD; Thomas F. Luscher, MD; Roberto Corti, MD; Georg Noll, MD; Frank Ruschitzka, MD

Background—The excess in cardiovascular risk in patients with rheumatoid arthritis provides a strong rationale for early therapeutical interventions. In view of the similarities between atherosclerosis and rheumatoid arthritis and the proven benefit of angiotensin-converting enzyme inhibitors in atherosclerotic vascular disease, it was the aim of the present study to delineate the impact of ramipril on endothelial function as well as on markers of inflammation and oxidative stress in patients with rheumatoid arthritis.

Methods and Results—Eleven patients with rheumatoid arthritis were included in this randomized, double-blind, crossover study to receive ramipril in an uptitration design (2.5 to 10 mg) for 8 weeks followed by placebo, or vice versa, on top of standard antiinflammatory therapy. Endothelial function assessed by flow-mediated dilation of the brachial artery, markers of inflammation and oxidative stress, and disease activity were investigated at baseline and after each treatment period. Endothelial function assessed by flow-mediated dilation increased from $2.85\pm1.49\%$ to $4.00\pm1.81\%$ ($P=0.017$) after 8 weeks of therapy with ramipril but did not change with placebo (from $2.85\pm1.49\%$ to $2.84\pm2.47\%$; $P=0.88$). Although systolic blood pressure and heart rate remained unaltered, diastolic blood pressure decreased slightly from 78 ± 7 to 74 ± 6 mm Hg ($P=0.03$). Tumor necrosis factor-α showed a significant inverse correlation with flow-mediated dilation ($r=-0.408$, $P=0.02$), and CD40 significantly decreased after ramipril therapy ($P=0.049$).

Conclusions—Angiotensin-converting enzyme inhibition with 10 mg/d ramipril for 8 weeks on top of current antiinflammatory treatment markedly improved endothelial function in patients with rheumatoid arthritis. This finding suggests that angiotensin-converting enzyme inhibition may provide a novel strategy to prevent cardiovascular events in these patients. (***Circulation***. 2008;117:2262-2269.)

Key Words: angiotensin-converting enzyme inhibitors ■ arthritis, rheumatoid ■ endothelium ■ inflammation

The striking similarities between atherosclerotic vascular disease and states of autoimmune inflammation such as rheumatoid arthritis (RA)[1] have prompted the hypothesis that inflammatory mechanisms responsible for synovial lesions in patients with RA also might be involved in endothelial cells and may thus facilitate the development of atherosclerotic lesions. This may explain the excess cardiovascular disease and the shortened life expectancy in patients with RA[2] and may provide a rationale for therapeutical interventions at an early stage of the disease process before overt cardiovascular disease has developed.

Clinical Perspective p 2269

Particularly, angiotensin-converting enzyme (ACE) inhibitors (ACEIs) have the potential to beneficially affect cardiovascular disease progression in patients with RA; the Heart Outcomes Prevention Evaluation (HOPE) trial and the European Trial on Reduction of Cardiac Events With Perindopril in Stable Coronary Artery Disease (EUROPA) showed the effectiveness of ACEIs such as ramipril and perindopril in reducing mortality and morbidity from cardiovascular events among patients with established disease or at high cardiovascular risk.[3,4] Intriguingly, the benefit observed in HOPE and

EUROPA was beyond what would have been expected from the blood pressure–lowering effects of the drug. Indeed, by inhibiting angiotensin II or by increasing the concentration of bradykinin, ACE inhibition is associated with a decrease in vascular NADPH activity and reactive oxygen species[5]; reduced activation of important signaling pathways, including nuclear factor-κB activation[6]; and an improvement in fibrinolysis[7] and endothelial function,[8] all of which may reduce the progression of atherosclerosis.

Recently, we provided the first evidence of endothelial dysfunction in RA patients in whom other traditional cardiovascular risk factors were absent.[9,10] Endothelial dysfunction has been implicated in the pathogenesis and clinical course of all known cardiovascular diseases and is associated with future risk of adverse cardiovascular events.[11–14] As such, the detection of vascular dysfunction at an early stage of the disease process long before symptoms of cardiovascular disease become evident provides a strong rationale for an intervention with a well-established strategy known to beneficially affect outcome in patients at increased cardiovascular risk. Hence, the present study investigated the effect of the ACEI ramipril on vascular function, inflammation, and oxidative stress in RA patients.

Methods

Patients

Thirteen patients with RA (4 of 7 American Rheumatism Association criteria fulfilled) who had normal blood pressure, were not treated for hypertension, and were on a stable background medication for at least 3 months were enrolled. Eleven patients were included in the analysis (4 men, 7 women; all white; mean age, 54.9 ± 11.2 years); 2 patients withdrew consent for personal reasons. Patients were screened at the University Hospital Zurich (Switzerland). Patients with previous myocardial infarction, coronary interventions, or coronary surgery and patients taking statins or ACEIs within the last 6 months were excluded. Further exclusion criteria were low-density lipoprotein >4.9 mmol/L, obesity (body mass index >35 kg/m^2), anemia, kidney disease (creatinine >150 μmol/L), insulin-dependent diabetes mellitus, chronic heart failure (New York Heart Association class >I), pregnancy, malignancy, chronic inflammatory disease other than RA, predisposition to angioedema, and smoking. Patients did not take study drugs or receive standard treatment on the day of examination to allow us to obtain results at trough levels. The local ethics committee of the University Hospital Zurich approved the study protocol. All procedures were in accordance with institutional guidelines, and all research was carried out in compliance with the Helsinki Declaration.

Study Protocol

The effect of an 8-week treatment with either ramipril or placebo was studied using a randomized, double-blind, crossover protocol. After written informed consent was given, baseline characteristics, including physical examination, ECG, blood sample, and a noninvasive assessment of endothelial function, were obtained. Arterial blood pressure was measured by sphygmomanometer in the sitting position according to guidelines.[15] The patients were then randomly assigned to receive either ramipril 10 mg (uptitration scheme; starting with 2.5 mg/d in the first week, then 5 mg/d in the second week, followed by 2×5 mg/d [10 mg] for the final 6 weeks) followed by placebo or vice versa. The uptitration scheme was chosen to avoid clinical manifest hypotension in these normotensive patients. The target dose (10 mg/d) was according to the maximum dose allowed in Switzerland, which is based primarily on the results of the HOPE study.[4] The individual antirheumatic drug therapy was continued unchanged throughout the study. The above-mentioned examinations were repeated after 8 weeks of treatment with the first and 8 weeks of treatment with the second study drug. At weeks 1 and 2 in each treatment period, a safety visit was scheduled.

Assessment of Endothelial Function

Flow-mediated dilation (FMD) was the predefined primary end point of the study.

FMD- and glycerol trinitrate (GTN) (0.4 mg sublingual, Nitrolingual Spray, Pohl-Boskamp, Hohenlockstedt, Germany)–induced vasodilation of the brachial artery was assessed at baseline and after each treatment period by a high-resolution ultrasound vessel wall tracking device with a 10-MHz linear-array transducer (WTS-2, Pie Medical, Bilthoven, the Netherlands) according to guidelines.[16,17] Reactive hyperemia reflects endogenous nitric oxide formation, resulting in endothelium-dependent vasodilation, whereas GTN acts as an exogenous nitric oxide donor directly on vascular smooth muscle cells, inducing endothelium-independent vasodilation. FMD of the brachial artery was induced by release of a wrist cuff inflated to 220 mm Hg for 5 minutes. After release, we recorded the arterial diameter every 15 seconds for 2 minutes. After GTN application, we recorded the diameter every 30 seconds for 6 minutes. Arterial flow velocity was obtained by pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) in the center of the artery.

Assessment of Inflammatory and Oxidative Stress Markers

Markers of vascular inflammation and oxidative stress represented the predefined secondary end point of the study. Isoprostane was measured from plasma with an 8-isoprostane enzyme immunoassay (Cayman Chemicals, Ann Arbor, Mich). Interleukin (IL) -1β, IL-6, and tumor necrosis-α (TNF-α) were measured by ELISA using the commercially available Quantikine HS kits (R&D Systems, Abingdon, UK). For bradykinin and sCD40, enzyme immunoassay kits (Bachem, Bubendorf, Switzerland, and Bender MedSystems, Vienna, Austria, respectively) were used. Myeloperoxidase was determined by ELISA using the myeloperoxidase ELISA kit (Immunodiagnostic AG, Bersheim, Germany).

Assessment of Plasma Renin Activity and Aldosterone

Plasma renin activity was measured by trapping generated angiotensin I by high-affinity antibodies and subsequent radioimmunoassay.[18] Aldosterone was measured by a direct radioimmunoassay using high-affinity antibodies produced in a New Zealand White rabbit.[19]

Cell Culture

Human umbilical vein endothelial cells were cultivated in EGM-2 growth medium (both from Lonza, Basel, Switzerland) to a confluence of 80% to 90% under standard conditions (37°C, 5% CO$_2$). Human umbilical vein endothelial cells were prestimulated with the inflammatory cytokines IL-1β (1 ng/mL) and TNF-α (10 ng/mL) (R&D Systems) for 1 hour before the addition of ramipril (100 nmol/L). Stimulated but untreated cultures served as controls. After 24 hours, total RNA was extracted and used for differential gene expression analysis by Affymetrix GeneChip technology (Affymetrix, Santa Clara, Calif).

Affymetrix GeneChip

Total RNA was isolated with the RNeasy MiniPrep Kit (Qiagen, Basel, Switzerland), including treatment with RNase-free DNase. Quality and concentration of the RNA were checked first with the Nanodrop ND-1000 Spectrophotometer (Witec AG, Littau, Switzerland) and a second time with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Calif). Double-stranded cDNA was synthesized from 5 μg total RNA with the GeneChip One-Cycle cDNA Synthesis Kit, including labeling with the One-Cycle Target Labeling Assay (both from Invitrogen, Basel, Switzerland). The labeled cRNA was hybridized with the probe sets present on the Human

Table 1. Baseline Characteristics of the Study Population

Characteristics	
Age, y	54.9±11.2
Gender, F/M	7/4
CV risk factors, n	
Dyslipidemia	4/11
Hypertension	0/11
Previous nicotine consumption	2/11
Diabetes mellitus	0/11
Positive family history for CV disease	1/11
Medication, n	
NSAIDs	6/11
COX-2 inhibitors	3/11
Prednisone	6/11
Methotrexate	6/11
TNF-α inhibitors	3/11
Aspirin	3/11
Folic acid	4/11

CV indicates cardiovascular; NSAIDs, nonsteroidal antiinflammatory drugs; and COX-2, cyclooxygenase-2. Data are shown as mean±SD or as absolute numbers.

Genome U133 Plus 2.0 Gene Expression Array (Affymetrix) using the Fluidics Station 450 according to standard protocols. The hybridization picture was scanned with a GeneChip Scanner 3000 and further analyzed with the GCOS software.

Data were normalized (measurements <0.01 were set to 0.01, and normalization per chip was set to the 50th percentile) and analyzed with GeneSpring Microarray Analysis Software (Silicon Genetics, Redwood City, Calif). Filters were set on 2-fold regulation; expres-

sion levels had to be >2.0; and flags had to be present or marginal in at least 1 of 2 compared samples.

Statistical Analysis

Results are presented as mean±SD. Measurements of FMD- and GTN-induced vasodilation represent the maximal increase in brachial diastolic arterial diameter and are expressed as percentage increase from baseline. For differences in parameters in the ramipril and placebo groups, we used 2-tailed paired Student t test. For correlation between FMD and TNF-α, we used Pearson correlation analysis. A value of $P \leq 0.05$ was considered statistically significant.

The power calculation was based on the hypothesis of the superiority of ramipril, assuming results similar to those in our previous study of RA[9]: endothelial function improved from 3.2% to 4.1% (SD, 1%). No change was expected in the placebo group. With a significance level of $\alpha=0.05$ and power of 0.8, we calculated a needed sample size of 12 patients to provide a crossover design. Statistical analyses were performed with SPSS 11.0.4 for Mac OS X (SPSS Inc, Chicago, Ill).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Study Cohort

A total of 13 patients were enrolled; however, 2 patients withdrew their informed consent shortly after inclusion. Therefore, 11 patients were included in the analysis; their baseline demographic, laboratory, and clinical characteristics are presented in Tables 1 and 2.

Primary End Point

Effect of Ramipril on Vascular Function

ACE inhibition improved endothelium-dependent vasodilatation after 8 weeks of treatment with ramipril (from

Table 2. Laboratory and Clinical Response to Placebo and Ramipril

	Baseline	Placebo	Ramipril	*P*, BL/Pl	*P*, BL/Ra	*P*, Pl/Ra
Laboratory						
Hb (n=7)	12.8±1.14	13.0±0.93	13.1±0.71	0.28	0.96	0.86
Tc (n=7)	204±84	263±65	260±107	0.27	0.23	0.54
Lc (n=7)	6.04±1.59	6.60±1.99	7.07±2.34	0.38	0.58	0.57
Sodium, mmol/L	140.0±1.7	140.0±3.5	139.5±1.4	1	0.65	0.83
Potassium, mmol/L	3.9±0.2	3.9±0.3	4.0±0.3	1	0.29	0.16
Urea, mmol/L	5.5±2.1	5.6±2.7	5.3±2.0	0.31	0.29	0.31
Creatinine, μmol/L	80.0±9.0	82.7±11.0	83.1±14.1	0.06	0.24	0.84
Protein, g/L	74.3±6.2	75.3±5.5	76.2±6.5	0.51	0.38	0.49
Cholesterol, mmol/L	5.2±1.5	5.2±1.7	5.2±1.7	0.61	0.69	0.45
HDL, mmol/L	1.4±0.4	1.4±0.4	1.4±0.4	0.76	0.22	0.54
LDL, mmol/L	3.0±1.3	3.1±1.4	3.0±1.2	0.52	0.43	0.76
TG, mmol/L	2.0±1.4	1.8±1.1	2.2±1.4	0.52	0.18	0.01
Clinical						
SBP	122.5±7.7	122.0±8.2	119±10.8	0.75	0.13	0.31
DBP	77.9±6.7	75.6±7.0	73.6±5.6	0.13	0.028	0.21
Heart rate	71±11	70±12	71±8	0.66	1	0.71
DAS28	3.7±1.4	3.4±1.2	3.7±1.1	0.36	0.41	0.99

BL indicates baseline; Pl, placebo; Ra, ramipril; Hb, hemoglobin; Tc, thrombocytes; Lc, leucocytes; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure; and DAS28, disease activity score in 28 joints. Data are shown as mean±SD.
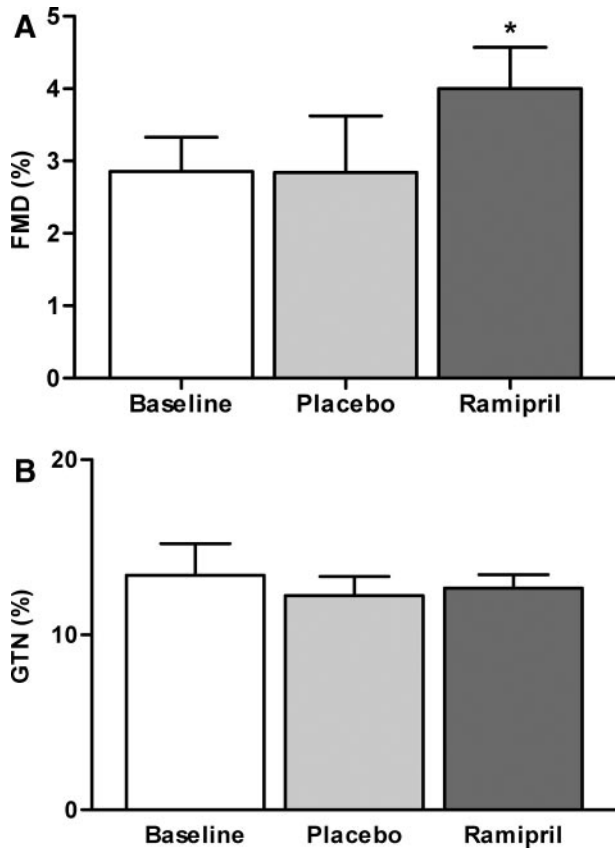
Figure 1. Vascular function. ACE inhibition improved endothelium-dependent vasodilatation after 8 weeks of treatment with ramipril (from 2.85±1.49% to 4.00±1.81%; P=0.017), whereas placebo had no effect (from 2.85±1.49% to 2.84±2.47%; P=0.88). Endothelium-independent vasodilatation assessed by GTN remained unaltered (from 13.40±5.68% to 12.67±2.44% with ramipril [P=0.67] and to 12.25±3.4% with placebo [P=0.5], respectively).

2.85±1.49% to 4.00±1.81%; P=0.017), whereas placebo did not change endothelial function (from 2.85±1.49% to 2.84±2.47%; P=0.88; Figure 1). Endothelium-independent vasodilatation assessed by GTN remained unaltered (from 13.40±5.68% to 12.67±2.44% with ramipril [P=0.67] and to 12.25±3.4% with placebo [P=0.5], respectively). Percentage changes in flow velocity (from resting condition to reactive hyperemia) were similar between groups (baseline visit, 197±58%; after placebo, 230±94%; and after ramipril, 187±68%; P between groups=0.41)

Secondary End Points

Markers of Oxidative Stress, Inflammation, and the Renin-Angiotensin-Aldosterone System
A significant reduction in CD40 levels occurred after ramipril: CD40 decreased from 84.3±85.6 to 63.1±83.8 pg/mL (P=0.049) after ramipril therapy, whereas the change was not significant after placebo (P=0.29) (Figure 2A). TNF-α tended to decrease from 1.74±1.82 to 1.21±1.28 pg/mL (P=0.22) in the ramipril group. Interestingly, a significant inverse correlation of TNF-α with FMD was found (r=−0.408, P=0.02; Figure 2B and 2C).

Other inflammation parameters like high-sensitivity C-reactive protein, IL-1, IL-6, myeloperoxidase, and blood

sedimentation rate, as well as the oxidative stress parameter 8-isoprostane, were not influenced by an 8-week treatment of ramipril (Table 3).

Renin activity significantly increased after ramipril therapy but remained unchanged after placebo (from 1.00±0.86 to 3.37±3.6 ng · mL^{-1} · h^{-1} after ramipril [P=0.022] and to 0.94±0.5 ng · mL^{-1} · h^{-1} after placebo [P=0.73]), indicating adherence to therapy throughout the study (Figure 2D). The ratio of aldosterone to renin changed from 16.82±10.23 to 52.53±67.26 (P=0.13) and remained unchanged after placebo (23.02±30.62; P=0.44).

Effect of Ramipril on Clinical and Laboratory Parameters
Eight weeks of treatment with ramipril tended to decrease systolic blood pressure and to significantly decrease diastolic blood pressure compared with baseline (from 122.5±7.7 to 119±10.8 mm Hg [P=0.13] and from 77.9±6.7 to 73.6±5.6 mm Hg [P=0.028], respectively; Figure 3). Disease activity score in 28 joints, heart rate, creatinine, potassium, and cholesterol remained unaltered after treatment with ramipril or placebo (Tables 1 and 2).

GeneChip Analysis of Human Umbilical Vein Endothelial Cells
The differential gene expression analysis in stimulated and nonstimulated cells revealed plenty of off-target effects of ramipril. For example, leukotriene B4 12-hydroxydehydrogenase was upregulated >2-fold. In addition, ACE inhibition downregulates proinflammatory arachidonate lipoxygenase 3 by >50%. Moreover, the dual-specificity phosphatase 6 is also downregulated, indicating modulation of phosphorylation of the mitogen-activated protein kinase pathway. Finally, in nonstimulated endothelial cells, ramipril induces an 8-fold and a 2.5-fold upregulation of carbonic anhydrase VB-like protein and angiomotin, respectively. However, the data warrant further validation and investigation.

Discussion
In this study, we provide evidence that long-term ACE inhibition with 10 mg ramipril improves endothelial function in the brachial artery of patients with RA.

Endothelial dysfunction plays a pivotal role in the pathogenesis and clinical course of cardiovascular diseases and predicts future risk of adverse cardiovascular events.[11–14] Importantly both ACEIs and angiotensin receptor blockers improve endothelial function in patients with atherosclerotic vascular disease.[20–24]

Similar to patients with classic cardiovascular risk factors, RA patients are characterized by impaired endothelial function compared with normal subjects.[9,10,25–27] Because RA patients suffer from premature atherosclerosis and a life expectancy that is reduced up to 10 years because of cardiovascular comorbidities compared with age-related controls,[2] any additional gain in maintaining endothelial integrity, as shown in our study, may indicate the potential of a therapeutic intervention such as ACEI in patients with RA.

Our group[10] and Tikiz et al[28] demonstrated a beneficial effect of simvastatin on endothelial function and inflamma-
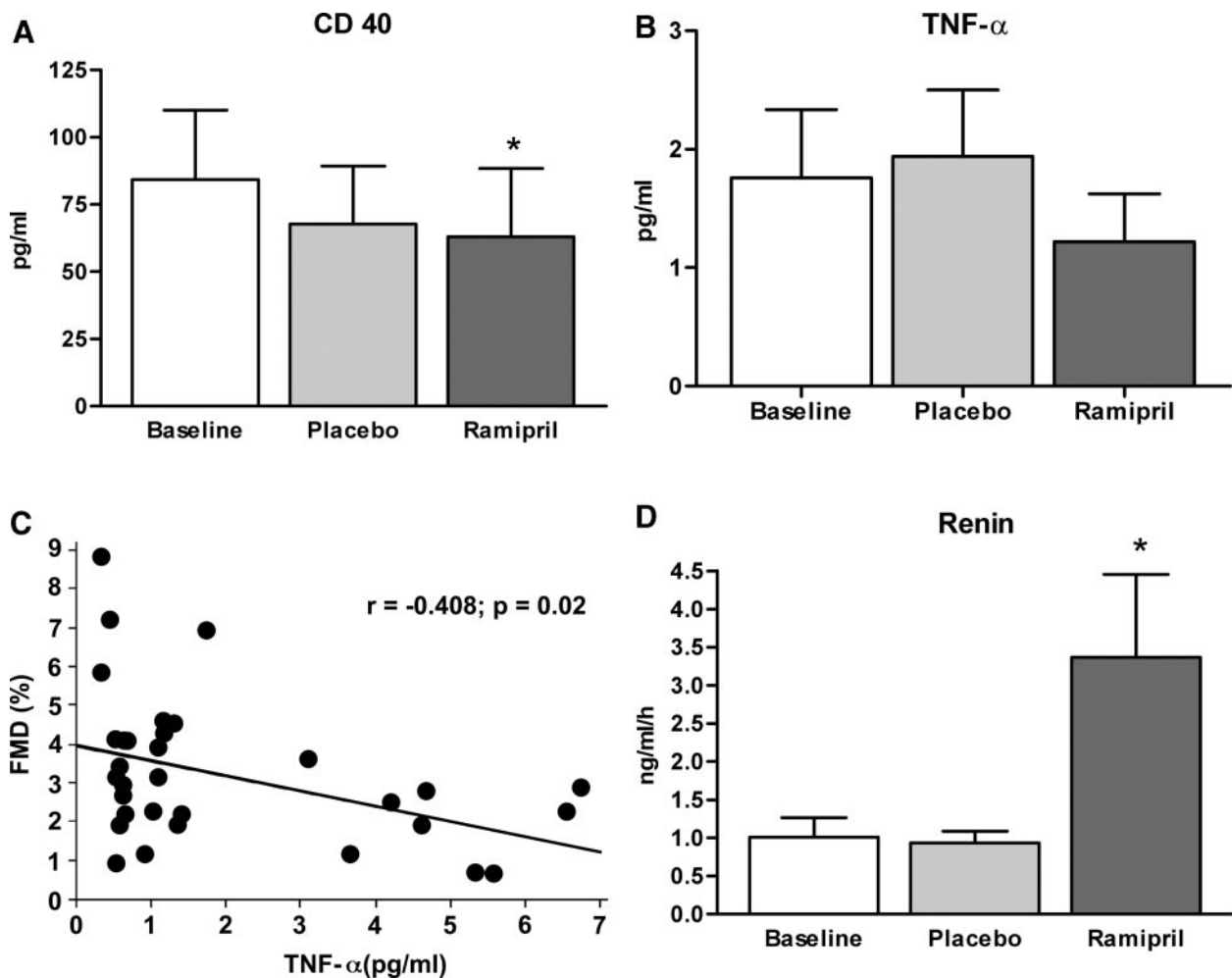
Figure 2. Inflammatory markers and renin activity. A significant change in CD40 levels occurred after ramipril: CD40 decreased from 84.3±85.6 to 63.1±83.8 pg/mL ($P=0.049$) after ramipril therapy, whereas the change was not significant after placebo ($P=0.29$). TNF-α tended to improve after ramipril (from 1.74±1.82 to 1.21±1.28 pg/mL; $P=0.22$), whereas it remained stable after placebo (1.94±1.78 pg/mL; $P=0.75$). TNF-α significantly inversely correlated with FMD ($r=-0.408$, $P=0.02$). Renin activity increased significantly from 1.00±0.86 to 3.37±3.6 ng · mL^{-1} · h^{-1} after ramipril ($P=0.02$) and remained unchanged after placebo (0.94±0.50 ng · mL^{-1} · h^{-1}; $P=0.73$).

tory parameters in RA patients. In contrast, ACE inhibition with quinapril failed to ameliorate endothelial function in the latter study.[28] This discrepancy compared with the present study may be related to the rather low dosage of quinapril used by Tikiz et al (10 mg), which may not provide potent ACE inhibition. The dose of ramipril used in the present study, on the other hand, markedly suppresses ACE, as reported previously.[29] In addition, it was recently shown in hypertensive patients that ramipril 10 mg, the dose also used in the HOPE trial,[4] induced a greater improvement in nitric oxide–dependent vasodilation compared with a lower dose (5 mg).[30] Similarly, the Study to Evaluate Carotid Ultrasound Changes in Patients Treated With Ramipril and Vitamin E (SECURE) trial showed a more pronounced reduction in intimal-medial thickness progression of the carotid artery with 10 versus 5 mg.[31]

Whereas ACE inhibition is associated with reduced atherosclerotic vascular events, mainly because it lowers blood pressure, the clinical relevance of its potential pleiotropic effects on oxidative stress and inflammation is still a matter

of debate.[32] It is of note that angiotensin II upregulates inflammatory TNF-α and IL-6 gene expression in different vascular beds, as well as macrophages and cardiac fibroblasts.[33] In addition, in animal models of atherosclerosis and vascular injury, ACE inhibition is associated with reduced lesion formation.[34,35]

In RA patients, increased ACE activity, along with upregulation of proinflammatory cytokines, particularly TNF-α, IL-1, and IL-6, has been demonstrated in synovial fluid and tissue and in blood monocytes.[36–38] Because these cytokines appear to affect expression of cellular adhesion molecules on endothelial cells, TNF-α in particular has been implicated in the initiation of vascular inflammation.[39] Indeed, in the present study, plasma levels of CD40, another important modulator in the inflammation pathway[40] and a member of the TNF-α superfamily, were reduced and the circulating levels of proinflammatory TNF-α tended to decrease after ramipril therapy. Interestingly, we also found a significant inverse correlation between TNF-α and FMD, thus indicating a key role of TNF-α in vascular inflammation and endothelial

Table 3. Parameters of Inflammation, Oxidative Stress, and the Renin-Angiotensin-Aldosterone System

	Baseline	Placebo	Ramipril	P, BL/Pl	P, BL/Ra	P, Pl/Ra
Renin activity, ng · mL^{-1} · h^{-1}	1.00±0.86	0.94±0.50	3.37±3.60	0.73	0.02	0.037
Aldosterone, pg/mL	122.5±49.24	126.1±64.29	134.3±56.90	0.83	0.45	0.69
Aldosterone/renin, ng/dL:ng · mL^{-1} · h^{-1}	16.82±10.23	23.02±30.62	52.53±67.26	0.44	0.13	0.24
hsCRP, mg/L	19.0±43.2	14.5±28.9	19.3±46.4	0.44	0.25	0.42
BSR, mm/h	20.1±23.2	19.6±20.9	19.3±23.9	0.86	0.65	0.93
TNF-α, pg/mL	1.74±1.82	1.94±1.78	1.21±1.28	0.75	0.22	0.24
IL-1, ng/mL	0.75±1.85	1.07±2.31	0.85±1.82	0.2	0.53	0.42
IL-6, pg/mL	11.2±12.6	14.2±12.7	10.2±10.3	0.35	0.81	0.38
CD40, pg/mL	84.3±85.6	67.7±71.6	63.1±83.8	0.29	0.049	0.69
MPO, pg/mL	83.01±43.48	44.29±22.42	67.5±43.59	0.03	0.41	0.19
Apolipoprotein A1, g/L	1.62±0.28	1.64±0.34	1.6±0.30	0.58	0.32	0.33
lso-8, pg/mL	0.42±0.30	0.26±0.09	0.27±0.14	0.15	0.13	0.85

BL indicates baseline; Pl, placebo; Ra, ramipril; hsCRP, high-sensitivity C-reactive protein; BSR, blood sedimentation rate; MPO, myeloperoxidase; and 8-Iso, 8-isoprostane. Data are shown as mean±SD.

dysfunction in patients with RA. This is further supported by recent findings that ACE inhibition with quinapril reduces articular TNF-α production[41] and by beneficial effects of anti–TNF-α treatment on endothelial function in RA.[9,42,43]

It is of note that all patients in this study presented with moderate disease activity; they had to remain clinically stable to allow comparable conditions during the repetitive assessment of the end points at different time points throughout the study. Circulating levels of these cytokines may, however, only partially reflect local vascular changes.

Because high-dose ACE inhibition markedly improved endothelial function with a modest reduction in markers of
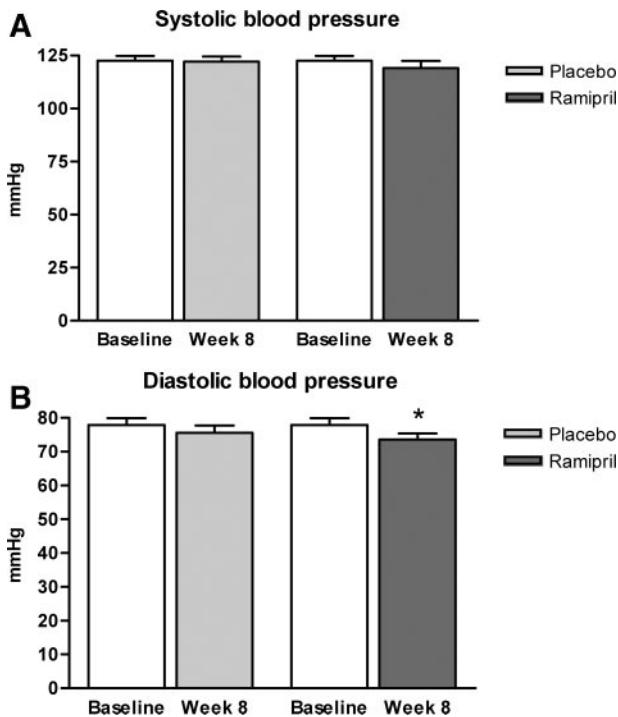

Figure 3. Blood pressure. Eight weeks of treatment with ramipril tended to decrease systolic blood pressure (A) and to significantly decrease diastolic blood pressure (B) vs baseline (from 122.5±7.7 to 119±10.8 mm Hg [P=0.13] and from 77.9±6.7 to 73.6±5.6 mm Hg [P=0.028], respectively).

vascular inflammation or oxidative stress, the benefit provided by ramipril also could be related to the small blood pressure reduction and/or an upregulation of endothelial nitric oxide expression and activity as demonstrated in experimental studies.[44]

However, because the results of the present study indicate a role of the inflammatory cytokines, particularly TNF-α, in vascular dysfunction in patients with RA, the differential gene expression analysis in stimulated and nonstimulated cells revealed plenty of off-target effects of ramipril, some of which conceptually could have contributed to the beneficial effects of the ACE inhibition on vascular function. In particular, leukotriene B4 12-hydroxydehydrogenase was upregulated. Importantly, leukotriene B4 12-hydroxydehydrogenase constitutes an important inactivation pathway for these key lipid mediators involved in inflammation.[45] In addition, differential gene expression analysis unexpectedly revealed that ACE inhibition downregulates proinflammatory arachidonate lipoxygenase 3. Moreover, the dual-specificity phosphatase 6 also is downregulated, indicating modulation of phosphorylation of the mitogen-activated protein kinase pathway. In nonstimulated endothelial cells, ramipril induces an upregulation of carbonic anhydrase VB-like protein and angiomotin. Angiomotin, a newly discovered molecule that regulates the migration and tubule formation of endothelial cells, has thus been implicated in the control of angiogenesis.[46]

Taken together, differential gene expression analysis results demonstrate novel off-target effects of ramipril that, in view of the multiple background therapies of the present study population, are beyond the scope of the present study but warrant further investigation, particularly in RA patients.

Our study has several limitations. Although the study was powered for and successful in its primary end point, it was not powered to detect significant differences in the secondary end points, markers of vascular inflammation and oxidative stress. Although we could demonstrate a significant reduction in CD40 concentration and an inverse correlation between TNF-α and FMD, definitive statements based on the results of the secondary end points should be interpreted with

caution. The results of our study could be influenced by background treatment such as methotrexate, corticosteroids, and nonsteroid anti-inflammatory drugs. However, only patients with moderate disease activity were included, so all background medications, including nonsteroid anti-inflammatory drugs, were kept stable throughout the entire course of this crossover study.

Triglycerides are slightly but significantly elevated after ramipril therapy compared with placebo therapy. Because triglycerides are known to impair endothelial function,[47] the significant beneficial impact of ACE inhibition on endothelial function may even be underestimated in the present study.

Conclusions

Eight weeks of therapy with ramipril on top of standard antiinflammatory treatment significantly improves endothelial function in RA patients. Because cardiovascular disease is considered the leading cause of mortality in RA and is responsible for approximately half the deaths observed in RA, the results of the present study underscore the need for large-scale prospective randomized clinical trials with ACE inhibition in these patients at high cardiovascular risk.

Acknowledgments

Sources of Funding

Disclosures

References

1. Pasceri V, Yeh ET. A tale of two diseases: atherosclerosis and rheumatoid arthritis. *Circulation*. 1999;100:2124–2126.

2. Solomon DH, Karlson EW, Rimm EB, Cannuscio CC, Mandl LA, Manson JE, Stampfer MJ, Curhan GC. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. *Circulation*. 2003;107:1303–1307.

3. Fox KM. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet*. 2003;362:782–788.

4. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med*. 2000;342:145–153.

5. Zhang H, Schmeisser A, Garlichs CD, Plotze K, Damme U, Mugge A, Daniel WG. Angiotensin II-induced superoxide anion generation in human vascular endothelial cells: role of membrane-bound NADH-/NADPH-oxidases. *Cardiovasc Res*. 1999;44:215–222.

6. Hernandez-Presa M, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M, Egido J. Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation*. 1997;95:1532–1541.

7. Hamdan AD, Quist WC, Gagne JB, Feener EP. Angiotensin-converting enzyme inhibition suppresses plasminogen activator inhibitor-1 expression in the neointima of balloon-injured rat aorta. *Circulation*. 1996;93:1073–1078.

8. Dohi Y, Criscione L, Pfeiffer K, Luscher TF. Angiotensin blockade or calcium antagonists improve endothelial dysfunction in hypertension: studies in perfused mesenteric resistance arteries. *J Cardiovasc Pharmacol*. 1994;24:372–379.

9. Hurlimann D, Forster A, Noll G, Enseleit F, Chenevard R, Distler O, Bechir M, Spieker LE, Neidhart M, Michel BA, Gay RE, Luscher TF, Gay S, Ruschitzka F. Anti–tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. *Circulation*. 2002;106:2184–2187.

10. Hermann F, Forster A, Chenevard R, Enseleit F, Hurlimann D, Corti R, Spieker LE, Frey D, Hermann M, Riesen W, Neidhart M, Michel BA, Hellermann JP, Gay RE, Luscher TF, Gay S, Noll G, Ruschitzka F. Simvastatin improves endothelial function in patients with rheumatoid arthritis. *J Am Coll Cardiol*. 2005;45:461–464.

11. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, Kiowski W, Luscher TF, Mancia G, Natali A, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Spieker LE, Taddei S, Webb DJ. Endothelial function and dysfunction, part II: association with cardiovascular risk factors and diseases: a statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens*. 2005;23:233–246.

12. Fischer D, Rossa S, Landmesser U, Spiekermann S, Engberding N, Hornig B, Drexler H. Endothelial dysfunction in patients with chronic heart failure is independently associated with increased incidence of hospitalization, cardiac transplantation, or death. *Eur Heart J*. 2005;26:65–69.

13. Katz SD, Hryniewicz K, Hriljac I, Balidemaj K, Dimayuga C, Hudaihed A, Yasskiy A. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. *Circulation*. 2005;111:310–314.

14. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000;101:948–954.

15. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Struijker Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Kjeldsen SE, Erdine S, Narkiewicz K, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Cifkova R, Dominiczak A, Fagard R, Heagerty AM, Laurent S, Lindholm LH, Mancia G, Manolis A, Nilsson PM, Redon J, Schmieder RE, Struijker-Boudier HA, Viigimaa M, Filippatos G, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Kiowski W, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Viigimaa M, Waeber B, Williams B, Zamorano JL. 2007 Guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J*. 2007;28:1462–1536.

16. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257–265.

17. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, Lerman A, Mancia G, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Schiffrin EL, Taddei S, Webb DJ. Endothelial function and dysfunction, part I: methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens*. 2005;23:7–17.

18. Nussberger J, Fasanella d'Amore T, Porchet M, Waeber B, Brunner DB, Brunner HR, Kler L, Brown AN, Francis RJ. Repeated adminis-

tration of the converting enzyme inhibitor cilazapril to normal volunteers. *J Cardiovasc Pharmacol*. 1987;9:39–44.

19. Nussberger J, Waeber B, Brunner HR, Burris JF, Vetter W. Highly sensitive microassay for aldosterone in unextracted plasma: comparison with two other methods. *J Lab Clin Med*. 1984;104:789–796.

20. Butler R, Morris AD, Struthers AD. Lisinopril improves endothelial function in chronic cigarette smokers. *Clin Sci (Lond)*. 2001;101:53–58.

21. Cheetham C, O'Driscoll G, Stanton K, Taylor R, Green D. Losartan, an angiotensin type I receptor antagonist, improves conduit vessel endothelial function in type II diabetes. *Clin Sci (Lond)*. 2001;100:13–17.

22. Ghiadoni L, Magagna A, Versari D, Kardasz I, Huang Y, Taddei S, Salvetti A. Different effect of antihypertensive drugs on conduit artery endothelial function. *Hypertension*. 2003;41:1281–1286.

23. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Luscher TF, Klibaner MI, Haber HE, Uprichard AC, Pepine CJ, Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND (Trial on Reversing Endothelial Dysfunction) Study. *Circulation*. 1996;94:258–265.

24. O'Driscoll G, Green D, Maiorana A, Stanton K, Colreavy F, Taylor R. Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol*. 1999;33:1506–1511.

25. Bergholm R, Leirisalo-Repo M, Vehkavaara S, Makimattila S, Taskinen MR, Yki-Jarvinen H. Impaired responsiveness to NO in newly diagnosed patients with rheumatoid arthritis. *Arterioscler Thromb Vasc Biol*. 2002; 22:1637–1641.

26. Gonzalez-Gay MA, Gonzalez-Juanatey C, Ollier WE. Endothelial dysfunction in rheumatoid arthritis: influence of HLA-DRB1 alleles. *Autoimmun Rev*. 2004;3:301–304.

27. Hansel S, Lassig G, Pistrosch F, Passauer J. Endothelial dysfunction in young patients with long-term rheumatoid arthritis and low disease activity. *Atherosclerosis*. 2003;170:177–180.

28. Tikiz C, Utuk O, Pirildar T, Bayturan O, Bayindir P, Taneli F, Tikiz H, Tuzun C. Effects of angiotensin-converting enzyme inhibition and statin treatment on inflammatory markers and endothelial functions in patients with longterm rheumatoid arthritis. *J Rheumatol*. 2005;32:2095–2101.

29. Brown NJ, Vaughan DE. Angiotensin-converting enzyme inhibitors. *Circulation*. 1998;97:1411–1420.

30. Ghiadoni L, Versari D, Magagna A, Kardasz I, Plantinga Y, Giannarelli C, Taddei S, Salvetti A. Ramipril dose-dependently increases nitric oxide availability in the radial artery of essential hypertension patients. *J Hypertens*. 2007;25:361–366.

31. Lonn E, Yusuf S, Dzavik V, Doris C, Yi Q, Smith S, Moore-Cox A, Bosch J, Riley W, Teo K. Effects of ramipril and vitamin E on atherosclerosis: the Study to Evaluate Carotid Ultrasound Changes in Patients Treated With Ramipril and Vitamin E (SECURE). *Circulation*. 2001;103: 919–925.

32. Pitt B. ACE inhibitors for patients with vascular disease without left ventricular dysfunction: may they rest in PEACE? *N Engl J Med*. 2004; 351:2115–2117.

33. Nakamura A, Johns EJ, Imaizumi A, Yanagawa Y, Kohsaka T. Effect of beta(2)-adrenoceptor activation and angiotensin II on tumour necrosis factor and interleukin 6 gene transcription in the rat renal resident macrophage cells. *Cytokine*. 1999;11:759–765.

34. Hernandez-Presa MA, Bustos C, Ortego M, Tunon J, Ortega L, Egido J. ACE inhibitor quinapril reduces the arterial expression of NF-kappaB-dependent proinflammatory factors but not of collagen I in a rabbit model of atherosclerosis. *Am J Pathol*. 1998;153:1825–1837.

35. Akishita M, Shirakami G, Iwai M, Wu L, Aoki M, Zhang L, Toba K, Horiuchi M. Angiotensin converting enzyme inhibitor restrains inflammation-induced vascular injury in mice. *J Hypertens*. 2001;19: 1083–1088.

36. Goto M, Fujisawa M, Yamada A, Okabe T, Takaku F, Sasano M, Nishioka K. Spontaneous release of angiotensin converting enzyme and interleukin 1 beta from peripheral blood monocytes from patients with rheumatoid arthritis under a serum free condition. *Ann Rheum Dis*. 1990; 49:172–176.

37. Veale D, Yanni G, Bresnihan B, FitzGerald O. Production of angiotensin converting enzyme by rheumatoid synovial membrane. *Ann Rheum Dis*. 1992;51:476–480.

38. Walsh DA, Suzuki T, Knock GA, Blake DR, Polak JM, Wharton J. AT1 receptor characteristics of angiotensin analogue binding in human synovium. *Br J Pharmacol*. 1994;112:435–442.

39. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation*. 2003;108:2957–2963.

40. Schonbeck U, Libby P. CD40 signaling and plaque instability. *Circ Res*. 2001;89:1092–1103.

41. Dalbeth N, Edwards J, Fairchild S, Callan M, Hall FC. The non-thiol angiotensin-converting enzyme inhibitor quinapril suppresses inflammatory arthritis. *Rheumatology (Oxford)*. 2005;44:24–31.

42. Bilsborough W, Keen H, Taylor A, O'Driscoll GJ, Arnolda L, Green DJ. Anti-tumour necrosis factor-alpha therapy over conventional therapy improves endothelial function in adults with rheumatoid arthritis. *Rheumatol Int*. 2006;26:1125–1131.

43. Cardillo C, Schinzari F, Mores N, Mettimano M, Melina D, Zoli A, Ferraccioli G. Intravascular tumor necrosis factor alpha blockade reverses endothelial dysfunction in rheumatoid arthritis. *Clin Pharmacol Ther*. 2006;80:275–281.

44. Quaschning T, d'Uscio LV, Shaw S, Luscher TF. Vasopeptidase inhibition exhibits endothelial protection in salt-induced hypertension. *Hypertension*. 2001;37:1108–1113.

45. Clish CB, Levy BD, Chiang N, Tai HH, Serhan CN. Oxidoreductases in lipoxin A4 metabolic inactivation: a novel role for 15-onoprostaglandin 13-reductase/leukotriene B4 12-hydroxydehydrogenase in inflammation. *J Biol Chem*. 2000;275:25372–25380.

46. Troyanovsky B, Levchenko T, Mansson G, Matvijenko O, Holmgren L. Angiomotin: an angiostatin binding protein that regulates endothelial cell migration and tube formation. *J Cell Biol*. 2001;152:1247–1254.

47. Sattar N, Petrie JR, Jaap AJ. The atherogenic lipoprotein phenotype and vascular endothelial dysfunction. *Atherosclerosis*. 1998;138:229–235.

CLINICAL PERSPECTIVE

Patients with rheumatoid arthritis demonstrate a >2-fold increase in cardiovascular morbidity and mortality, even though traditional cardiovascular risks often are absent. An increasing body of evidence suggests that the systemic inflammation associated with rheumatoid arthritis also contributes to accelerated atherosclerosis in rheumatoid arthritis patients. In view of the still-persisting uncertainty about how to handle and reduce the risk of future cardiovascular disease in patients with rheumatoid arthritis, aggressive control of traditional risk factors and vessel wall inflammation is needed. Whether and to what degree the intriguing effects of angiotensin-converting enzyme inhibition with ramipril in improving endothelial function, which although clinically well established remains a surrogate measure of outcome, may translate into clinical benefits for our patients with rheumatological diseases who are at particularly high cardiovascular risk need to be tested in large-scale clinical trials.

Go to http://cme.ahajournals.org to take the CME quiz for this article.