Effects of Angiotensin-Converting Enzyme Inhibition and Statin Treatment on Inflammatory Markers and Endothelial Functions in Patients with Longterm Rheumatoid Arthritis

CANAN TIKIZ, OZAN UTUK, TIMUR PIRILDAR, OZGUR BAYTURAN, PETEK BAYINDIR, FATMA TANELI, HAKAN TIKIZ, and CIGDEM TUZUN

ABSTRACT. Objective. To investigate the effects of angiotensin-converting enzyme (ACE) inhibitors and statins (hydroxy-methyl-glutaryl-CoA reductase inhibitors) on inflammatory markers and endothelial functions in patients with rheumatoid arthritis (RA).

Methods. A total of 45 patients with longterm RA were randomized into 3 groups to receive 8 weeks of treatment with placebo (n = 15), simvastatin (20 mg/day, n = 15), or quinapril (10 mg/day, n = 15) as an adjunct to existing antirheumatic drug treatment. Factors with a role in the development of endothelial dysfunction, such as C-reactive protein (CRP), fibrinogen, nitric oxide (NO), and serum cytokine concentrations including interleukin 1ß (IL-1ß), IL-6, and tumor necrosis factor-α (TNF-α) were measured at baseline and in the posttreatment period. Brachial artery vasodilator responses were assessed by high resolution ultrasound to evaluate endothelial functions.

Results. Simvastatin treatment significantly decreased serum CRP and TNF-α [from 14 ± 6 to 7 ± 3 mg/l (p = 0.025) and 30 ± 5 to 16 ± 4 pg/ml (p = 0.012), respectively], while quinapril had no significant changes in these 2 measures. IL-1ß and IL-6 showed insignificant changes in patients in the 2 drug groups. Endothelium-dependent vasodilatation was improved significantly in the simvastatin group [from 5.3 ± 1.1% to 8.9 ± 1.4% (p = 0.025)], while there was no difference in endothelium-independent vasodilatation [9.0 ± 1.8% to 11.2 ± 2.5% (p = 0.17)]. The quinapril group showed no significant changes in both types of vasodilation although there was a tendency to an increase in endothelium-dependent vasodilatation [from 6.1 ± 0.8% to 7.8 ± 0.7% (p = 0.06)]. Treatment with the 2 drugs had no significant effects on resting arterial diameter.

Conclusion. We show that simvastatin 20 mg daily improves endothelial function in patients with RA. Its beneficial effect may be attributed to lowering CRP and TNF-α concentrations. ACE inhibition with daily 10 mg quinapril was found to have no significant effects on inflammatory markers and endothelial vasodilator response. (J Rheumatol 2005;32:2095–101)

Key Indexing Terms:
RHEUMATOID ARTHRITIS     ENDOTHELIAL FUNCTION     INFLAMMATORY MARKERS
STATIN                                                  ANGIOTENSIN-CONVERTING ENZYME INHIBITOR

Endothelial dysfunction is widely accepted as a key process in atherosclerosis and independently predicts cardiovascular mortality. It denotes impairment of endothelium-dependent vasodilatation and is associated with advanced age, hypercholesterolemia, cigarette smoking, hypertension, diabetes mellitus, high homocysteine concentrations, and inactivity. While the precise mechanism remains to be elucidated, endothelial dysfunction appears to develop after a short or transient period of an inflammatory process.

It is well documented that the number of cardiovascular events in patients with rheumatoid arthritis (RA) was 4 times higher than in individuals of the same age and sex without RA. The increased prevalence of cardiovascular complications in patients with RA is closely related to the presence of atherosclerosis, and accelerated atherogenesis is shown to be the major cause of morbidity and mortality in this group. In the rheumatic diseases, endothelial dysfunction has been described in states of necrotizing vasculitis, where the vessel wall is the primary target of the pathological process. RA, however, primarily affects the diarthrodial joints and not the vasculature. Nonetheless, many recent
studies describe endothelial dysfunction in patients with RA that could be reversed with anti-tumor necrosis factor-α (TNF-α) treatment.

In addition to anti-TNF-α treatment, angiotensin-converting enzyme (ACE) inhibitors and hydroxy-methyl-glutaryl-CoA reductase (HMG CoA) inhibitors were shown to improve endothelial dysfunction in patients with high cardiovascular risk. However, to date there are limited data on the effects of statins and ACE inhibitors on endothelial functions in patients with RA.

We investigated and compared the effects of ACE inhibition with quinapril and hydroxy-methyl-glutaryl-CoA reductase (HMG CoA) inhibition with simvastatin on endothelial functions determined by flow mediated and glycerol trinitrate (GTN) induced vasodilatation using high-sensitivity brachial ultrasonography in patients with longterm RA. We also evaluated the inflammatory markers that may have an important role in development of endothelial dysfunction, including fibrinogen, C-reactive protein (CRP), nitric oxide (NO), and cytokines such as interleukin 1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α).

MATERIALS AND METHODS

Patient population. In this randomized, placebo controlled study, 45 patients with longterm RA (41 women, 4 men, mean age 48 yrs, mean disease duration 8.7 yrs) recruited from Celal Bayar University between September 2003 and July 2004 were included. Patients fulfilled the 1987 American Rheumatism Association criteria for RA and were receiving disease modifying antirheumatic drugs (DMARD) with and without steroids and nonsteroidal antiinflammatory drugs (NSAID). Baseline variables included demographic characteristics and disease duration; the Disease Activity Score (DAS28) was obtained from 28-joint counts as described. We also recorded patients’ DMARD use from their charts.

Patients were excluded for the following reasons: history of coronary bypass grafting, myocardial infarction within 28 days, left ventricular dysfunction (ejection fraction < 40%), total cholesterol > 240 mg/dl, uncontrolled hypertension (> 160/95 mm Hg), significant valvular heart disease, type I diabetes mellitus, or smoking within 6 months before screening. Patients were also excluded if they had received any kind of ACE inhibitor or angiotensin II receptor blockers within 6 months of randomization; or patients with type II diabetes mellitus, or smoking within 6 months before screening.

RESULTS

Assessment of endothelial function. Patients were studied in the morning, between 9:00 and 11:00 AM, in a quiet room with a temperature of 20–24°C, after an overnight fast. Each patient avoided alcohol, caffeine, and cigarette smoking the night before, and all additional drug treatment (simvastatin, quinapril, and placebo) was withheld for at least 24 h before the second test. The subject lay at rest for at least 10 min before a first resting screen was recorded. Flow-mediated dilatation and GTN-induced vasodilatation (0.4 mg sublingual Nitrolingual spray; Pohl-Boskamp, Hohenlockstedt, Germany) of the brachial artery was determined by high-resolution ultrasound with a 7.5 MHz linear array transducer (Siemens Sonoline Elegra) according to a validated method by the same physician (PB), who was blinded to patient data. Briefly, the arterial diameter was measured at a fixed distance from an anatomical marker such as a bifurcation and calculated as the mean value of 2 measurements at the end of the diastole, concurrent with the onset of the QRS complex. The average diameter of the artery then was calculated over 3 cardiac cycles. After taking the baseline measurements, increased flow was induced by inflation of a brachial cuff placed on the proximal limb and inflated to a pressure of 50 mm Hg above systolic pressure for 5 min. After release, the arterial diameter was recorded every 15 s for 3 min. Post-test arterial diameter measurements were made 60 s after cuff deflation. Fifteen minutes was allowed for vessel recovery before GTN application, and the same procedure was repeated after GTN application. To evaluate the reproducibility of imaging and to assess intraobserver variability, a second assessment was done for the same patients on consecutive days in 15 randomly selected patients, and the stored images were then analyzed offline blinded to patients’ details. The correlation coefficients were r = 0.92 for baseline, r = 0.94 for reactive hyperemia, and r = 0.90 for GTN induced vasodilatation.

Statistical analysis. SPSS v. 11.0 was used for statistical analysis. Data are expressed as mean ± standard error (SEM). Baseline characteristics were compared using the Mann-Whitney U test or chi-square test, as appropriate. Differences and percentage changes in baseline characteristics and biochemical markers between the 3 groups in the pre- and posttreatment periods were investigated by Kruskal-Wallis test, and post-hoc analyses were performed using Mann-Whitney U tests. Wilcoxon rank test was used for paired comparisons. Percentage changes in vasodilatory responses were calculated as [(posttreatment value – pretreatment value)/pretreatment value × 100]. P values < 0.05 were considered to be significant.

RESULTS

The baseline demographics and clinical characteristics of the patients are summarized in Table 1. The groups were matched in terms of age, sex, body mass index, and cardiovascular risk factors such as hypertension and diabetes mellitus. Disease Activity Scores (DAS28) and the RA treatment protocols were also similar to each other. Table 2 shows basal clinical measures such as mean arterial press-
sure, fibrinogen, CRP, NO, lipid measurements, and cytokine levels. As shown, all measures were similar among the 3 groups. Table 3 summarizes percentage changes between pre- and posttreatment values. The quinapril group showed decreased mean arterial pressures compared to the other groups, as expected. Total cholesterol and LDL cholesterol were significantly decreased in the simvastatin group compared to others. Changes in Lp(a), apo-A, apo-B, and NO were similar in the simvastatin and quinapril groups and showed no statistical differences (p > 0.05). Simvastatin treatment significantly decreased serum CRP and TNF-α levels: from 14 ± 6 to 7 ± 3 mg/l (p = 0.025) and 30 ± 5 to 16 ± 4 pg/ml (p = 0.012), respectively; while no significant changes were observed in the quinapril group. IL-1β and IL-6 showed insignificant changes in both the simvastatin and quinapril groups. Table 4 illustrates the percentage changes in endothelium-dependent and independent vasodilatation in placebo, simvastatin, and quinapril groups. Endothelium-dependent vasodilatation was improved significantly in the simvastatin group (from 5.3% ± 1.1% to 8.9% ± 1.4%; p = 0.025), while there was no difference in endothelium-independent vasodilatation (9.0% ± 1.8% to 11.2% ± 2.5%;

Table 1. Demographic variables and baseline characteristics of patients. Data are given as mean ± standard error of mean (SEM).

<table>
<thead>
<tr>
<th></th>
<th>Placebo, n = 15</th>
<th>Simvastatin, 20 mg/day, n = 14</th>
<th>Quinapril, 10 mg/day, n = 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration, yrs</td>
<td>100</td>
<td>10 (3)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>Rheumatoid factor positive, %</td>
<td>100</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>DAS28</td>
<td>4.57 (1.5)</td>
<td>4.60 (1.65)</td>
<td>4.59 (1.16)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>48 (10)</td>
<td>46 (8)</td>
<td>50 (10)</td>
</tr>
<tr>
<td>Male/female</td>
<td>1/9</td>
<td>2/9</td>
<td>1/10</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27 (1.2)</td>
<td>25 (1)</td>
<td>26 (2)</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.74 (0.08)</td>
<td>0.82 (0.13)</td>
<td>0.69 (0.11)</td>
</tr>
<tr>
<td>Hypertension, n (%)*</td>
<td>2 (13)</td>
<td>3 (21)</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)*</td>
<td>2 (13)</td>
<td>3 (21)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Treatment, (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NSAID</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>16</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>66</td>
<td>71</td>
<td>57</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>33</td>
<td>35</td>
<td>28</td>
</tr>
</tbody>
</table>

No significant differences were noted between baseline characteristics, risk factors, and medications between groups. BMI: body mass index, DAS: Disease Activity Score, NSAID: nonsteroidal antiinflammatory drug.

Table 2. Basal values of mean arterial pressure (MAP) and biochemical markers of patients. Data are mean ± standard error of mean (SEM).

<table>
<thead>
<tr>
<th></th>
<th>Placebo, n = 15</th>
<th>Simvastatin, 20 mg/day, n = 14</th>
<th>Quinapril, 10 mg/day, n = 14</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm/Hg</td>
<td>89 (1.9)</td>
<td>91 (2.4)</td>
<td>90 (2.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Fibrinogen, mg/l</td>
<td>375 (31)</td>
<td>426 (54)</td>
<td>361 (48)</td>
<td>0.45</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>12 (3)</td>
<td>14 (6)</td>
<td>10 (2)</td>
<td>0.72</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>210 (6)</td>
<td>234 (12)</td>
<td>216 (8)</td>
<td>0.21</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>122 (8)</td>
<td>130 (12)</td>
<td>126 (11)</td>
<td>0.10</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>138 (13)</td>
<td>149 (32)</td>
<td>148 (29)</td>
<td>0.90</td>
</tr>
<tr>
<td>Lp(a), mg/dl</td>
<td>35 (10)</td>
<td>37 (8)</td>
<td>24 (6)</td>
<td>0.65</td>
</tr>
<tr>
<td>NO, µmol/l</td>
<td>41 (2)</td>
<td>42 (3)</td>
<td>44 (5)</td>
<td>0.61</td>
</tr>
<tr>
<td>Apo-A, g/l</td>
<td>1.5 (0.04)</td>
<td>1.6 (0.11)</td>
<td>1.5 (0.06)</td>
<td>0.18</td>
</tr>
<tr>
<td>Apo-B, g/l</td>
<td>0.58 (0.05)</td>
<td>0.76 (0.06)</td>
<td>0.71 (0.07)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cytokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1ß, pg/ml</td>
<td>4.8 (0.7)</td>
<td>6.3 (1.7)</td>
<td>4.0 (0.8)</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>17 (8)</td>
<td>18 (8)</td>
<td>14 (4)</td>
<td>0.40</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>27 (4)</td>
<td>30 (5)</td>
<td>27 (4)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

p = 0.17) (Figures 1 and 2). The quinapril group showed no significant changes in these responses, although there was a tendency to an increase in endothelium-dependent vasodilation (from 6.1% ± 0.8% to 7.8% ± 0.7%; p = 0.06). Treatment with each drug had no significant effects on resting arterial diameter.

**DISCUSSION**

The novel finding of our study was that treatment with simvastatin 20 mg daily for 2 months significantly improved endothelial-dependent vasodilatation in patients with longterm RA under conventional antirheumatic drug treatment, while no significant change was observed with quinapril 10 mg daily. Simvastatin was also shown to decrease the inflammatory markers including CRP and TNF-α significantly, while the placebo and quinapril groups showed no significant changes in any measured inflammatory marker.

Increased prevalence of cardiovascular morbidity and mortality due to premature atherosclerosis has been observed in patients with autoimmune diseases such as RA and systemic lupus erythematosus. The mechanisms that cause acceleration in the atherosclerotic process in this patient group are still under investigation. Recent reports describe that endothelial dysfunction is one of the main factors involved in this pathological process. Inflammatory mechanisms implicated in the development of synovial lesions in RA have been suggested to involve the vessel wall and to initiate the development of atherosclerosis, and many recent studies describe endothelial dysfunction in patients with RA.

Statins and ACE inhibitors are 2 classes of drugs whose effects on endothelial functions have been widely investigated. Statins, which are mostly used for lipid modulation, have been shown to improve endothelial vasodilator response in patients with atherosclerosis. This beneficial effect was attributed to their antiinflammatory and immunomodulatory properties, which were independent of...
their cholesterol-lowering function. Ex vivo studies revealed that statins suppress adhesion molecules, leukocyte cytokine release, and MHC-II expression. Statins also improve the stability of the mRNA for endothelial nitric oxide synthase (eNOS), and by so doing enhance the generation of NO from the endothelium, which has a vasodilatory effect. ACE inhibitors were also shown to improve endothelial vasodilator responses through decreased levels of angiotensin II and increased levels of bradykinin and NO. Not only longterm treatment but also acute administration of ACE inhibitors has been shown to augment endothelial-dependent vasodilatation in coronary circulation. However, to date there are no data regarding the effects of statins and ACE inhibitors on endothelial functions in patients having chronic inflammation as in RA.

In our comparative study in patients with RA, we tested 2 groups of agents previously shown to improve endothelial functions in patients with coronary atherosclerosis. Our results showed that only simvastatin was associated with a significant improvement in endothelial-dependent vasodilatation. To our knowledge this is the first demonstration of such an improvement. Recently, Van Doornum, et al published their results on the effects of atorvastatin 20 mg daily in patients with longterm RA. They showed a significant reduction in arterial stiffness measured by pulse-wave analysis after 12 weeks of atorvastatin treatment. Our findings confirm their results, although we observed a significant improvement with a different method.

We also observed that simvastatin caused a significant decrease in CRP and TNF-α levels, which may explain its beneficial effects on endothelial functions. Our findings are in agreement with others who predicted a significant decrease in CRP levels after statin treatment in different patient populations. Moreover, in a small sample size preliminary study, Abud-Mendoza, et al indicated that atorvastatin 20 mg daily decreased CRP levels and improved clinical status in patients with RA. Additionally, in a recent study, McCarey, et al investigated the effects of atorvastatin 40 mg daily in 116 patients with RA and found significantly improved clinical status measured by DAS28 and decreased CRP and IL-6 levels at the end of 6 months. Our findings are similar to these 2 studies, although we were primarily interested in endothelial functions, not in clinical improvement. In light of these studies and the fact that elevated plasma CRP level is associated with endothelial dysfunction and high mortality, it seems reasonable that drugs that decrease CRP may improve endothelial function and decrease mortality in patients under high atherosclerotic risk. From this point of view, statins seem to be an important optional class of drugs in the treatment of patients with high cardiovascular risk if their CRP-lowering effect can be confirmed in studies with large patient groups.

Circulating measures of cytokines including IL-1, IL-6, and TNF-α were also shown to increase inflammatory processes and predict cardiovascular events. In one study, local administration of TNF-α and IL-1 was shown to impair endothelial vasodilatation induced by bradykinin. TNF-α also blocks the activation of eNOS, the main determinant of flow-dependent vasodilatation. Recent evidence suggests that TNF-α plays a pivotal role in the initiation and progression of inflammation in both RA and atherosclero-
sis34. Moreover, TNF-α was found to modulate hepatic synthesis of CRP45. This interaction between 2 inflammatory markers may help to explain the decrease of TNF-α and CRP levels in our study. The effects of statins on TNF-α have been investigated, with results similar to our findings. In 2 studies, simvastatin was found to decrease TNF-α levels in hypercholesterolemic patients46,47, and in another, pravastatin was observed to decrease TNF-α in human monocytes48. Interestingly, the rapid positive effect of a TNF-α blocker, infliximab, on endothelial functions highlights the importance of TNF-α in the mechanism of atherosclerosis mediated by endothelial dysfunction in RA13. Supporting this observation, Hurlimann, et al demonstrated an improvement in endothelial functions in patients with RA after 12 weeks of treatment with infliximab12.

Considered together, CRP and TNF-α seem to have important roles in the pathogenesis of inflammation in joints and vessel walls, and their decrease with any drug could improve inflammation in both joints and vascular endothelium. Potential dual effects in reducing inflammation and vascular risk may accrue with the use of statins in RA. As well, because endothelial reactivity of the brachial artery shows a close correlation with that seen in the coronary arteries49, our results support the idea that statins can decrease future cardiovascular events in patients with RA.

Interestingly, quinapril showed no significant change in either endothelium-dependent or independent vasodilatation. Quinapril was chosen as an ACE inhibitor because it has been shown to have high tissue specificity for ACE, and the association of the drug with the enzyme is markedly prolonged compared with other ACE inhibitors50. Moreover, clinical studies have shown that quinapril compared to other ACE inhibitors exerts beneficial effects in endothelial dysfunction15,16. In our study, although a tendency to improvement in endothelium-dependent vasodilatation was observed in the quinapril group, this increase did not reach statistical significance. Moreover, the lack of effect on CRP or TNF-α levels in the quinapril group could partially explain the lack of efficacy. It is also possible that the lack of efficacy of quinapril may be related to improper dosage or length of treatment. Because higher doses of quinapril (20–40 mg/day) usually cause severe hypotension in a normotensive population, higher doses with ACE inhibitors might cause significant problems during the treatment period. Nevertheless, studies with higher doses would be required to determine if these groups of drugs have no significant beneficial effect on endothelial functions.

This is the first study to show that simvastatin 20 mg daily improves endothelial function in patients with RA. Its beneficial effect may be attributed to its lowering levels of CRP and TNF-α. ACE inhibition with quinapril 10 mg daily was found to have no significant effect on inflammatory markers and endothelial vasodilator response. Our data confirm the antiinflammatory effects of statins and support their therapeutic potential for preventing cardiovascular events in patients with systemic inflammatory disease such as RA. However, because of the small number of patients studied, the results of our preliminary study also need to be supported by other studies with larger patient groups.

REFERENCES

17. Anderson TJ, Meredith IT, Yeung AJ, Frei B, Selwyn A, Gans P.


