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ORIGINAL ARTICLE

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Effects of low-dosage simvastatin on rheumatoid arthritis through reduction of Th1/Th2 and CD4/CD8 ratios

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Abstract The objective of this study was to assess both the anti-inflammatory and immunomodulatory effects of low-dosage simvastatin on rheumatoid arthritis (RA). In each patient, simvastatin at 10 mg/day was administered for 12 weeks. The other treatments were unchanged at least 3 months before simvastatin administration to the end of the study. Patients were assessed for the improvement in clinical, laboratory, and immunological parameters of RA and for adverse events. Twenty-four patients with RA were enrolled. Clinical symptoms, including patient's assessment of pain and disease activity on visual analog scale (VAS), the swollen joint and tender joint counts, and handgrip strength significantly improved. Physician's assessment of disease activity on VAS, a period of morning stiffness and modified health assessment questionnaire showed a tendency of improving after administration of low-dosage of simvastatin. Of special interest was that the median levels of erythrocytes sedimentation rate, C-reactive protein, and rheumatoid factor were significantly decreased from 54.0 mm/h to 45.5 mm/h, from 1.50 mg/dl to 0.85 mg/dl, and from 57.0 IU/ml to 28.0 IU/ml, respectively, after administration of simvastatin. ACR20 and ACR50 responses were achieved in 62% and 38%, respectively, of simvastatin-treated patients for 12 weeks. Immunological assessment in peripheral blood revealed that the Th1/Th2 and CD4/CD8 ratios were significantly reduced by simvastatin. No adverse events were reported during simvastatin treatment. Immunomodulation through the alteration of Th1/Th2 and CD4/CD8 ratios may be a pharmacological mechanism in the anti-rheumatic effect of low-dosage simvastatin. Although it is necessary to evaluate the long-term effects of statins, low-dosage statins appear to be good as additional therapeutic agents.

Key words CD4/CD8 · Inflammatory · Rheumatoid arthritis · Simvastatin · Statin · Th1/Th2

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Introduction

Statins, which inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, are widely used as cholesterol-lowering medicines. Statins have been highlighted as anti-inflammatory drugs since the first clinical observation of pravastatin decreasing the incidence of severe acute rejections and, thereby, significantly improving the 1-year survival in heart transplant recipients.[1] The pleiotropic effect of these drugs has been supplemented by in vitro studies in which statins may affect the functions of the immune and inflammatory cells, including natural killer cells, monocytes, macrophages, and T cells.[2] It has been also known that statins inhibit the expression of adhesion molecules, monocytes chemotaxis, and matrix metalloproteinases activity.[2] Moreover, statins attenuate the secretion of pro-inflammatory cytokines interleukin (IL)-6 and IL-8 but not tumor necrosis factor-α (TNF-α) from activated macrophages.[2] Notably, from a recent report, it has been demonstrated that a certain type of statin inhibits the molecular association between leukocyte function antigen-1 (LFA-1) and intercellular adhesion molecule-1 by competitive binding to the L-site of LFA-1.[3] Another recent report has demonstrated that statins suppress T cell activation through the inhibition of interferon-γ (IFN-γ)-inducible major histocompatibility complex class II (MHC-II) expression.[4] These findings suggest that statins might inhibit antigen presentation to pro-inflammatory Th1

cells. Therefore, we addressed the question whether statins ameliorated rheumatoid arthritis (RA), a Th1 predominant disease.[5] In our previous report, RA patients with hypercholesterolemia were treated with low-dosage simvastatin at 10 mg per day for 12 weeks. A part of the clinical parameters significantly improved and the level of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) tended to decrease after simvastatin treatment.[5] These data suggested that simvastatin could suppress inflammatory variables as well as clinical symptoms of RA. However, we have not shown the pharmacological mechanism of simvastatin on the treatment of RA. We, therefore, evaluated immunological, inflammatory, and clinical parameters in RA patients regardless of the presence of hypercholesterolemia after low-dosage simvastatin treatment.

Patients and methods

Patients

This study was approved by the ethics committees of two participating hospitals (Tokyo University Hospital and Saitama Medical University Hospital), and signed consents were obtained from all patients before they were enrolled in the study. Recruitment of subjects was done from March 2001 to March 2004. Patients who fulfilled the American College of Rheumatology (ACR) criteria for RA were treated in each hospital. RA patients regardless of the presence of hypercholesterolemia were enrolled in this study. We excluded patients who had already taken statins or fibrates and developed any adverse reactions to statins earlier. In addition, patients who had severe renal or liver dysfunction were also excluded.

Study design

All patients received simvastatin at 10 mg per day for 12 weeks. Treatments for RA, including corticosteroids, methotrexate, sulfasalazine, bucillamine, auranofin, and mizoribine were not changed at least 3 months before simvastatin administration to the end of the study. Other medications also were unchanged during this study without tapering corticosteroids. Clinical and immunological parameters were evaluated before simvastatin therapy and at the end of the administration of simvastatin. Physical examinations included tender joint count (0–68), swollen joint count (0–66), patient's self-assessment of pain [visual analog scale (VAS) 0–10], patient's self-assessment of disease activity on VAS (0–10), Stanford modified health assessment questionnaire (mHAQ) score,[6] and physician's global assessment of disease activity on VAS (0–10). Total clinical response to simvastatin was evaluated according to the ACR 20% improvement (ACR20) and the ACR 50% improvement (ACR50) response criteria.[7] Inflammatory parameters, which were examined before the treatment and at 4, 8, and 12 weeks after starting simvastatin, included ESR (normal value <10 mm/h), CRP (normal value <0.3 mg/dl), and rheumatoid factor (RF) level (normal value <20 IU/ml). Immunological parameters including the numbers of T cells, B cells, and NK cells, the Th1/Th2 ratio, the CD4/CD8 ratio, and the expression of human leukocyte antigen D-related (HLA-DR) on CD3, CD19, and CD14 positive cells, were measured by flow cytometry. The populations of lymphocytes were evaluated by multiparameter flow cytometry with two- or three-color analyses. The using fluorescein isothiocyanate (FITC)-labeled specific monoclonal antibodies including CD3, CD4, CD8, CD14, CD19, CD56, and HLA-DR were purchased from Becton Dickinson (San Jose, CA, USA). Flow cytometric analysis was performed with a fluorescence activated cell sorting (FACS) Calibur cell sorter (Becton Dickinson Biosciences, San Jose, CA, USA) and analyzed with CellQuest software. The proportion of lymphocytes stained with each monoclonal antibody was converted to the absolute per microliter by multiplying the absolute number of lymphocytes per microliter derived from complete blood count. The Th1 cells produce mainly IFN-γ, whereas the Th2 cells secrete mainly IL-4. The Th1/Th2 ratio was assessed as the ratio of intracytoplasmic expression of IFN-γ and IL-4 in CD4 positive T cells by flow cytometry. Peripheral blood mononuclear cells, which were obtained from whole blood by centrifugation over Ficoll (Amersham Pharmacia Biotech, Amersham, UK), were activated by phorbol myristate acetate (PMA) (Sigma–Aldrich, St. Louis, MO, USA) and ionomycin (Sigma–Aldrich) and brefeldin-A (Sigma–Aldrich) were added to inhibit cytokine secretion leading to intracellular accumulation. Subsequently, the cells were incubated with phycoerythrin-cyanine (PC)-5-anti-CD4 antibody. Intracellular cytokine staining was performed with using antibodies: FITC-anti-IFN-γ (Becton Dickinson) and phycoerythrin (PE)-anti-IL-4 (Becton Dickinson) in CD4 positive T cells by flow cytometry.

Statistical analysis

Outcome measures before and at the end of simvastatin treatment were compared using paired *t* test or Wilcoxon's signed rank test. A *P* value of less than 0.05 was considered to be significant.

Result

Patients' characteristics

There were 3 men and 21 women with a median age of 61 years (range 46–81) enrolled in the study. The median duration after the diagnosis of RA was 7 years (range 0.7–28). The numbers of patients in Stages I, II, III, and IV by Steinbrocker classification were 3, 9, 5, and 7, respectively. The numbers of patients in Class I, II, III, and IV by RA classification were 8, 6, 8, and 2, respectively. All patients have received various kinds of therapy, including corticosteroids ($n = 14$), methotrexate ($n = 11$), sulfasalazine ($n = 10$), bucillamine ($n = 6$), auranofin ($n = 5$), and mizoribine ($n = 2$).

The median duration of morning stiffness was 30 min (range 0–420). The median right and left handgrip strengths were 144 mmHg (range 45–300) and 128 mmHg (range 20–300), respectively. The median counts of tender joint and swollen joint were 8 (range 2–29) and 4 (range 0–19), respectively. The median VAS values in the self-assessment of pain and disease activity by patients were 3.5 (range 0.5–10) and 4.5 (range 0.6–8.2), respectively. The median mHAQ score was 8. The median VAS value in physician's global assessment of disease activity was 3.4 (range 0.5–6.3). The median values of CRP and ESR were 1.50 mg/dl (range 0.1–6.9) and 54.0 mm/h (range 9–140), respectively. The median value of RF was 57.0 IU (range 10–2160). The median total cholesterol level was slightly higher than normal.

Simvastatin efficacy

The outcome of the parameters measured after treatment was evaluated at the end of simvastatin treatment. Simvastatin was discontinued in two patients after 4 weeks and in one patient after 8 weeks, because of their refusal to continue with the treatment. The remaining 21 patients completed the treatment for 12 weeks.

The changes in the clinical, laboratory, and immunological parameters before and at the end of simvastatin treatment are shown in Table 1. Most clinical findings tended to improve after simvastatin administration. In particular, the VAS values of patient's assessment of pain and disease activity and handgrip strength were significantly improved. In addition, significant reductions in the tender joint and the swollen joint counts were recorded at the end of simvastatin therapy. Notably, the values of ESR, CRP, and RF significantly decreased from 54.0 to 45.5 mm/h ($P = 0.029$), 1.50 to 0.85 mg/dl ($P = 0.044$), and 57.0 to 28.0 ($P = 0.027$), respectively. The serial changes in ESR and CRP levels after simvastatin therapy are shown in Fig. 1a, b. The significant reductions in ESR and CRP were already observed in the first 4 weeks after the simvastatin treatment. The reductions in ESR and CRP at the initial 4 weeks were greater than those at 12 weeks. These findings showed that the duration of the anti-inflammatory effect of simvastatin appears short. Figure 1c shows the ACR20 and ACR50 responses at 4 weeks and 12 weeks, respectively. The ACR20 response was 14/24 (58.3%) at 4 weeks, and 13/21 (61.9%) at 12 weeks. The ACR50 response was 2/24 (8.3%) at 4 weeks, and 8/21 (38.1%) at 12 weeks.

For the immunological evaluations, the Th1/Th2 ratio was significantly reduced from 5.9 (range 3.0–73) to 5.7 (range 3.0–30), ($P = 0.0018$; Fig. 2a). In addition, the CD4/CD8 ratio was significantly decreased from 2.2 (range 0.50–6.5) to 1.8 (range 0.51–5.7), ($P = 0.028$; Fig. 2b). However, the numbers of HLA-DR positive T cells, B cells, and monocytes did not change. These results may suggest that the improvement in the clinical and laboratory parameters in RA by low-dosage simvastatin could be due to its anti-inflammatory effects through the suppression of the Th1/Th2 and CD4/CD8 ratios.

Adverse effects

No adverse effects were noted during simvastatin treatment.

Table 1. Changes in clinical, laboratory, and immunological parameters before versus at the end of low-dosage simvastatin treatment

	Before	End	*P*
ESR (mm/h)	54.0	45.5	0.029*
CRP (mg/dl)	1.50	0.85	0.044*
RF (IU/ml)	57.0	28.0	0.027*
Total cholesterol (mg/dl)	244	188	<0.0001*
Duration of morning stiffness (min)	30	20	0.17
Grip strength (Rt), mmHg	144	190	0.027*
Grip strength (Lt), mmHg	128	186	0.031*
Patient's assessment of pain (VAS)	3.5	2.3	0.036*
Patient's assessment of disease activity (VAS)	4.5	2.6	0.049*
mHAQ	8.0	2.5	0.51
Physician's assessment of disease activity (VAS)	3.4	2.1	0.17
Tender joint count	8.0	2.5	0.0001*
Swollen joint count	4.0	0.5	0.012*
Th1/Th2	5.9	5.7	0.0018*
CD3 (μl^{-1})	1070	957	0.046*
CD19 (μl^{-1})	112	91	0.027*
CD56 (μl^{-1})	168	167	0.79
HLA-DR+ CD3+/CD3+ (%)	13.2	14.0	0.17
HLA-DR+ CD19+/CD19+ (%)	97.5	98.0	0.50
HLA-DR+ CD14+/CD14+ (%)	99.4	99.2	0.48
CD4/CD8	2.2	1.8	0.028*
MCP-1 (pg/ml)	177	233	0.31

Indicated values are the median; *$P < 0.05$

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; HLA-DR, human leukocyte antigen D-related; VAS, Visual Analog Scale; mHAQ, modified health assessment questionnaire; MCP-1, monocyte chemoattractant protein-1

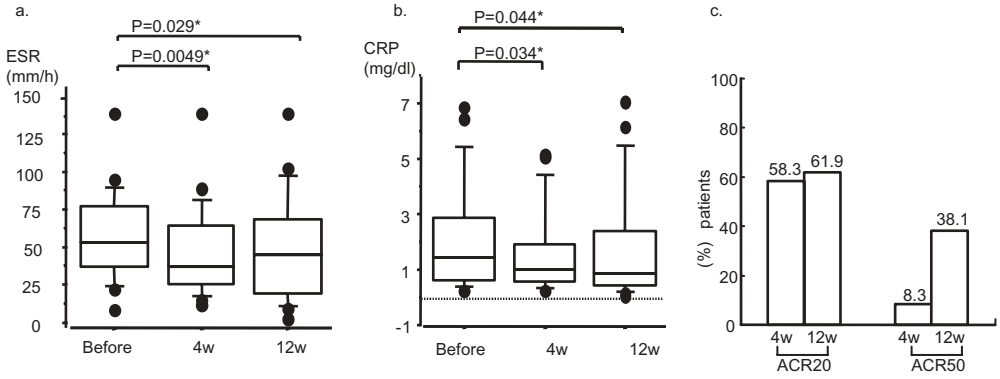

Fig. 1. Effects of simvastatin on rheumatoid arthritis. **a, b** Changes in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels after simvastatin treatment. *Y*-axis shows inflammatory parameters: **a** ESR values, **b** CRP values. In the *X*-axis, *Before*, *4w*, and *12w* represent the durations after the simvastatin treatment. The *box* and *whisker plot* show 10, 25, 50, 75, and 90 percentile values. Outliers are indicated by *dots*. **c** ACR20 and ACR50 responses at 4 weeks and 12 weeks after simvastatin treatment

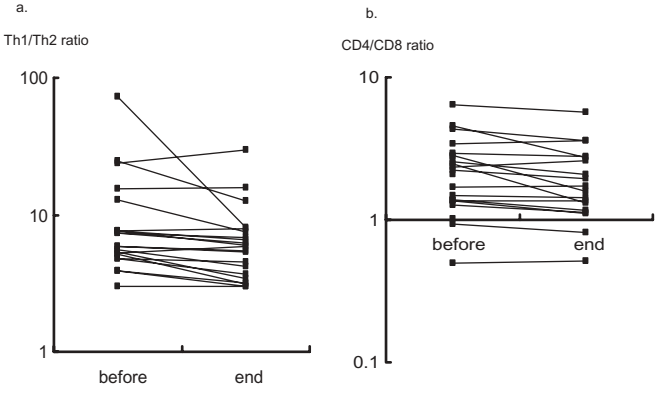

Fig. 2. Reduction of Th1/Th2 (**a**) and CD4/CD8 (**b**) ratios after simvastatin treatment

Discussion

We showed that the addition of low-dosage simvastatin to conventional immunosuppressive therapies improved the clinical, biological, and immunological parameters in RA patients. Of particular interest is the dramatic and rapid effect of statins on inflammatory markers such as CRP and ESR. Recently, two articles in which the authors evaluated the effects of statins on RA patients have been published.[8,9] Adud-Mendoza et al.[8] reported that either treatments of atorvastatin at 20 mg/day for 8 days or simvastatin at 40 mg/day for 4 weeks showed a reduction in the CRP level and a clinical improvement. In another report by McCarey et al.[9] TARA (trial of atorvastatin in RA) results showed that atorvastatin at 40 mg/day for 6 months significantly decreased the levels of CRP and ESR by 50% and 28%, respectively, in a placebo-controlled randomized trial. DAS 28 EULAR response was achieved more frequently in the atorvastatin group (31%) than in the placebo group (10%). However, the dosage of statins in these studies was higher than that approved for use in Japan. Therefore, we evaluated whether simvastatin at 10 mg/day can improve RA in this study. In spite of the low dosage of simvastatin at 10 mg/day, its efficacy was similar to those in the two other studies using higher dosages. In addition, the ACR20 response ratio at 62% was as excellent as that in patients who received tumor necrosis factor blocking agents, including infliximab, etanercept, and adalimumab.[10] However, we considered that this efficacy meant not only statin monotherapy but also low-dosage simvastatin plus disease modifying antirheumatic drugs (DMARDs) combination therapy. Therefore, we recommended low-dosage statin in addition to other DMARDs as a treatment for RA.

Although the mechanism of the anti-inflammatory effect of statins has been shown in several in vitro studies, little has been shown from in vivo studies. Thus, we analyzed several immunological parameters, including the numbers of T cells, B cells, and NK cells, the MHC-II expression on T cells, B cells, and monocytes, the Th1/Th2 ratio, and the CD4/CD8 ratio. We could not find significant changes in the MHC-II expression level on T and B cells, and monocytes. On the other hand, simvastatin significantly reduced the Th1/Th2 and CD4/CD8 ratios. Although we could not prove directly that the reduction of Th1/Th2 ratio caused by inhibition of MHC-II expression on T and B cells, and monocytes, decreasing the production of Th1 cytokines by low-dosage simvastatin might lead to inhibition of T cell activation and proliferation. In addition, it has been demonstrated that statins are beneficial for various animal models of Th1 diseases, including central nervous system demyelinating disease, a model of multiple sclerosis,[11] collagen-induced arthritis,[12] and allergic asthma.[13] The reduction of CD4/CD8 ratio also suggested the down-regulation of T cell-mediated immune responses. Therefore, our results suggest that low-dosage simvastatin reduces disease activity of RA through modulation of Th1 and CD4 predominant conditions in the disease in vivo.

In summary, this is the first clinical study to evaluate simultaneously the effects of low-dosage statin on both RA disease activity and immunological responses. The addition of low-dosage simvastatin to conventional immunosuppressive therapy not only reduced the CRP and ESR levels but also improved the clinical symptoms of RA patients in a short period. Moreover, the present study suggests that the

anti-inflammatory mechanisms of statins may have appeared through the suppression of Th1/Th2 and CD4/CD8 ratios. Considering the wide margin of safety of statins, a placebo-controlled study with larger number of subjects and longer treatment duration is warranted.

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