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Tocilizumab Increases Serum Adiponectin and Reduces Serum Fatty Acid Binding Protein 4 in Patients With Rheumatoid Arthritis

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Abstract

Background: Recently tocilizumab, a humanized anti-interleukin-6 receptor antibody (IL-6R Ab), was clinically demonstrated to ameliorate metabolic syndrome. However, it is unknown whether blocking the IL-6R with tocilizumab directly impacts the adiponectin and fatty acid-binding protein 4 (FABP4) levels.

Methods: In this study, we measured the serum adiponectin and FABP4 levels in 18 patients with rheumatoid arthritis (RA) 3 months after treatment with tocilizumab.

Results: Our study revealed that treatment with tocilizumab decreased serum FABP4 levels and increased serum adiponectin levels in patients with RA. We also assessed the production of adipocytokines stimulated by IL-6R Ab using adipocyte precursors obtained from human fat tissue. Tocilizumab did not increase local adiponectin levels; however, the suppression of adiponectin secretion by IL-6 was completely abolished. Tocilizumab also directly suppressed FABP4 in human adipocytes.

Conclusion: This suggests that treatment with tocilizumab may be a novel approach to coordinately regulate adiponectin and FABP4 levels.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 1% of the general population and is associated with increased mortality, predominantly as a result of increased risk of cardiovascular disease (CVD) [1, 2]. Although the awareness of increased cardiovascular risk in patients with inflammatory diseases is increasing, the traditional risk factors for CVD in some patients remain suboptimally managed [3]. Recently, it was suggested that adipose tissue plays a role in chronic inflammatory diseases. It synthesizes and releases highly bioactive substances, including classical adipokines (such as leptin and adiponectin), and various pro-inflammatory cytokines (including tumor necrosis factor-α and interleukin-6 (IL-6)), which are collectively termed adipo(cyto)kines [4]. However, its ability to synthesize pro-inflammatory cytokines is not well understood.

Fatty acid-binding protein 4 (FABP4, also designated aP2 or adipocyte FABP) is expressed in adipocytes and other tissues and integrates inflammatory and metabolic responses [5, 6]. The expression of both FABP4 and adiponectin is regulated by peroxisome proliferator-activated receptor (PPAR)-γ [7]. However, the two proteins are differentially regulated because higher serum FABP4 levels [8, 9] and lower serum adiponectin levels [10, 11] have recently been found to be associated with metabolic syndrome (MetS) and CVD. Therefore, accumulating evidence suggests that the adipokine levels may act as biomarkers to dictate drug or dietary treatment strategies.

Tocilizumab, a humanized anti-IL-6 receptor antibody that blocks IL-6 signaling, is a novel therapeutic strategy for various autoimmune and inflammatory diseases, such as RA, Castleman's disease, and juvenile idiopathic arthritis [12]. Although tocilizumab increased plasma adiponectin levels in patients with RA [13], the relationship between IL-6 and FABP4 is still controversial [14, 15], and the effects of tocilizumab on FABP4 are unknown.

The aim of the present study was to evaluate whether treatment with tocilizumab leads to changes in serum adipokine levels in patients with RA.

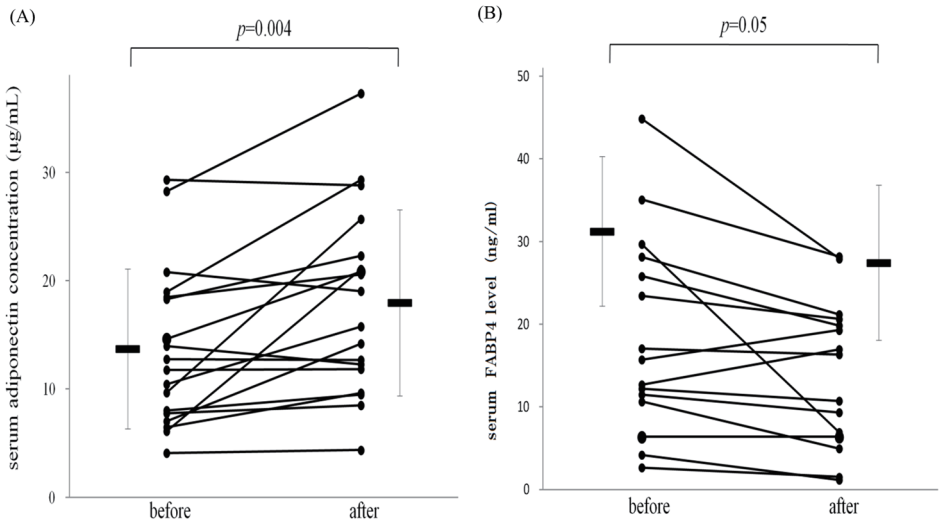

Figure 1. Treatment with tocilizumab increased serum adiponectin (A) and decreased fatty acid-binding protein 4 levels (B) in patients with RA. Bars represent mean ± SEM.

Materials and Methods

Patients

From July through December 2008, 16 patients (two males and 14 females) with active RA starting tocilizumab treatment were enrolled in this study between July and December 2008 at Osaka University Hospital. All patients had no history of medication use associated with PPAR-γ agonist (e.g., hypertension and diabetes) before enrolling in this study. They received a fixed dose of tocilizumab (8 mg/kg) in a single 1-h infusion every 4 weeks. Sixteen patients (89%) were also treated with oral prednisolone. Patient symptoms were assessed by disease activity score (DAS)-28, based on 28-joint counts for swelling and tenderness, tender joint count, and patient assessment. Serum FABP4 levels were measured using a human adipocyte FABP ELISA (BioVendor, Modrice, Czech Republic), and adiponectin levels were measured using an ELISA kit (Otsuka Pharmaceutical, Tokushima, Japan).

This study conformed to the Clinical Research Guideline of Osaka University Hospital and was approved by the institutional ethics committee. We obtained written informed consent to participate in this study from all patients.

Adipocyte culture and effects of tocilizumab on adipocytokine production in differentiated adipocytes

Preadipocytes were isolated from patients undergoing elective surgery who gave informed consent, as previously described [16]. Cells were differentiated into adipocytes by incubation in adipocyte differentiation medium (DM-2, Zen-bio®) at 37 °C with 5% CO_2. After 8 days, cells were plated in fresh adipocyte medium (AM-1, Zen-bio®) and treated with drug-containing medium every 48 h. The effects of tocilizumab and 10 μmol/L pioglitazone hydrochloride on adiponectin and FABP4 secretion were analyzed on day 12 after 48 - 96 h of treatment.

Immunoblotting of FABP4

Human FABP4 expression was assessed by western blotting. In brief, lysates from human adipocytes were collected and separated on 16% SDS-PAGE gels, and transferred to PVDF membranes. Membranes were blocked for 1 h at room temperature in blocking buffer (5% skim milk in 10 mmol/L Tris, 100 mmol/L NaCl, 0.1% Tween 20, pH 7.5), and then incubated with anti-FABP4 antibody (A-FABP C-15, Santa Cruz) at a dilution of 1:1,000 for 1 h at room temperature. After washing (3 × 5 min in 1 × TBS-0.05% Tween), membranes were incubated with secondary anti-mouse (Dako) or anti-goat (Wako) antibodies conjugated to horseradish peroxidase. The immune complexes were detected using ECL Advanced Western Blot Detection System (GE Healthcare, Buckinghamshire, UK).

Statistical analysis

All results are presented as mean ± SEM. The differences in CRP, DAS-28, and serum adipokine levels before and after tocilizumab treatment were analyzed using paired *t*-test. Student's *t*-test was used to compare the differences between the control and treatment groups. Values of $P < 0.05$ were considered to be statistically significant.

Results

The body weights of the patients were unchanged during the

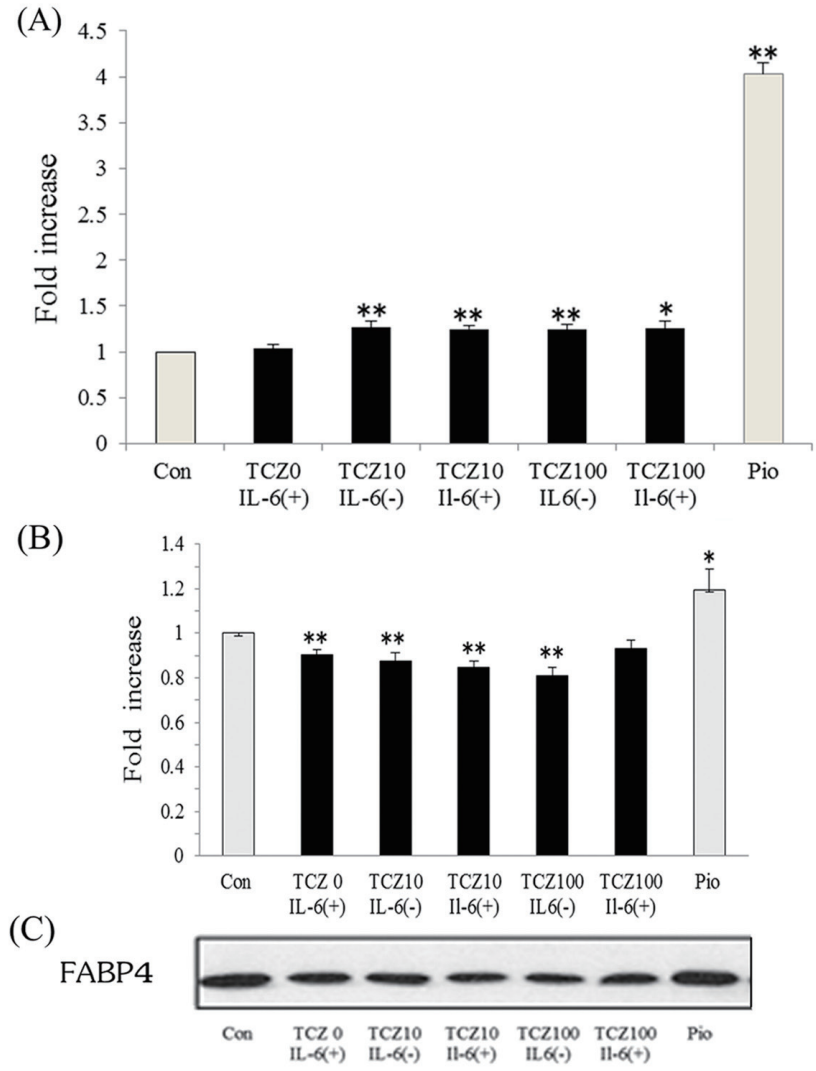

Figure 2. (A) Effects of interleukin-6 (IL-6) and tocilizumab on adiponectin production in human adipocytes. Cells were differentiated for 12 days, and then treated without (control) or with 10 μg/mL IL-6 antibody, and/or 100 μg/mL, or 10 mg/mL tocilizumab. Data are presented as means ± SEM of at least four independent experiments. *P < 0.05; **P < 0.01 vs. control. Pio: pioglitazone. (B) Effects of interleukin-6 (IL-6) and tocilizumab on fatty acid-binding protein 4 levels in human adipocytes. Cells were differentiated for 12 days and treated without (control) or with 10 μg/mL IL-6 antibody, and/or 100 μg/mL, 10 mg/ml or tocilizumab. Data are expressed as means ± SEM of at least four independent experiments.*P < 0.05; **P < 0.01 vs. control. Pio: pioglitazone. (C) A representative western blot of fatty acid-binding protein 4 expression in human adipocytes treated without (control) or with 10 μg/mL interleukin-6 antibody, and/or 100 μg/mL, or 10 mg/mL tocilizumab. Pio: pioglitazone.

study. Treatment with tocilizumab for 3 months significantly suppressed the inflammatory process, demonstrated by a decrease in mean serum CRP levels from 1.8 ± 0.4 to 0.2 ± 0.1 mg/dL (P < 0.01). Levels of DAS-28 also improved significantly, from 3.9 ± 0.3 to 1.8 ± 0.4 (P < 0.01). The baseline serum adiponectin concentrations were 13.7 ± 1.7 μg/mL, which increased significantly to 18.0 ± 2.1 μg/mL (P < 0.01, Fig. 1A) after treatment with tocilizumab for 3 months. In contrast, baseline FABP4 concentrations were 31.3 ± 9.0 ng/mL, which decreased to 27.4 ± 9.4 ng/mL after treatment (P = 0.05, Fig. 1B). Although previous reports suggested that treatment with steroids significantly decreased serum adiponectin levels [17], oral prednisolone did not affect serum FABP4 or adiponectin levels in our study (data not shown).

Next, the effect of IL-6 and tocilizumab on adiponectin and FABP4 was assessed in human adipocytes. After treatment of differentiated adipocytes with 100 μg/mL tocilizumab, adiponectin secretion was increased approximately 1.25 ± 0.04-fold (P < 0.01) compared with control. Tocilizumab treatment enhances adiponectin secretion by suppressing IL-6 (Fig. 2A). In contrast, FABP4 expression was decreased approximately 0.85 ± 0.03-fold (P < 0.01) in cells treated with tocilizumab compared with control. However, pioglitazone substantially increased FABP4 expression approximately 1.19 ± 0.10-fold

(Fig. 2B, C).

Discussion

In this study, we found for the first time that treatment with tocilizumab, an IL-6R Ab, for 3 months reduced serum FABP4 levels in patients with RA. Both adiponectin and FABP4 are downstream targets of PPAR-γ in adipocytes. PPAR agonists such as telmisartan and thiazolidines induce the secretion of adiponectin [16] and simultaneously increase serum FABP4 levels [18, 19]. A previous study demonstrated olmesartan, which has little PPAR-γ activity, decreased serum FABP4 in patients with hypertension [20], while little is known about the effect of medication on FABP4 levels.

FABP4 promotes atherosclerotic diseases by acting on macrophages [21]. FABP4-deficient macrophages display defects in cholesterol accumulation and decreased pro-inflammatory cytokines TNFα, IL-6, and MCP-1 levels by reducing IκB kinase and NF-κB activity [22]. Although FABP4 is a circulating protein, the mechanism by which it enters the circulation is unknown. In both cross-sectional and prospective studies, serum FABP4 levels were positively correlated with lipid profiles, hyperglycemia, and non-alcoholic fatty liver diseases [23]. In addition, a 12-year community-based cohort study in a Chinese population indicated that plasma FABP4 levels were a strong predictor of CVD [24]. Because elevated FABP4 levels are a risk factor for CVD in patients with end-stage renal disease [8] and are correlated with numerous metabolic syndrome symptoms [9], the up-regulation of FABP4 may induce unfavorable side effects in patients treated with PPAR agonists.

Tocilizumab, a monoclonal antibody that blocks both membrane-bound and circulating IL-6R has anti-inflammatory actions that extend beyond reducing the concentrations of C-reactive protein and fibrinogen [25]. Patients with chronic inflammatory diseases, such as RA, are at increased risk of developing CVD [3], which is in part caused by increased IL-6 levels. Although further studies are required to confirm that these effects are also mediated by factors including macrophages and IL-6 *in vivo*, the present results suggest that changes in adiponectin and FABP4 levels reflect metabolic defects in adipose tissue and thus may be a useful biomarker of CVD in patients with RA.

Tocilizumab was identified as an agent that may help prevent coronary heart disease [26]. Because the suppression of adiponectin gene expression by IL-6 is mediated in part by p44/42 MAP kinase [27], the inhibition of this signaling pathway by tocilizumab may induce adiponectin secretion. The concurrent decrease in FABP4 levels and increase in adiponectin levels induced by tocilizumab may help prevent CVD and metabolic syndrome. However, additional studies are required to define the mechanism behind the differential effects of tocilizumab on FABP4 and adiponectin expression. For example, tocilizumab may act as a selective PPAR gamma modulator (SPPARM) [28] to reduce oxidative stress [29] or improve hypoxia [30]. However, SPPARM exhibited limited effects on FABP4 gene expression in mature 3T3-L1 adipocytes [28] and inhibited the differentiation of human preadipocytes compared with PPAR-γ agonists. SPPARM also displayed a diminished ability to induce FABP4 mRNA expression compared with rosiglitazone [29], whereas in human trophoblasts cultured under hypoxic conditions, the expression of FABP4 was enhanced [30].

In conclusion, tocilizumab treatment decreases FABP4 levels in patients with RA and could provide a novel therapeutic approach to prevent CVD.

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