ORIGINAL

Effect of Janus kinase inhibition by tofacitinib on body composition and glucose metabolism

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Abstract : Tofacitinib is the first Janus Kinase (JAK) inhibitor to treat moderately to severely active RA. In this study, we investigated whether the effect of tofacitinib have any effects on body composition in mice and female patients with RA. Female C57BL/6 mice fed with a high-fat diet were treated with 30 mg/kg/day tofacitinib or vehicle for 70 days. Following treatment, trunk muscle, subcutaneous fat, and visceral fats were measured using X-ray computed tomography CT scan. Glucose tolerance and insulin sensitivity were assessed. In female RA patients treated with biological disease modified anti-rheumatic-drugs (biological DMARDs) or tofacitinib (n=4 per group), we also evaluated the body composition after 3 months from the start of treatment initiation using bioelectrical impedance analysis. Treatment with tofacitinib did not affect the body weight, and body composition in C57BL/6 mice. It also did not affect glucose, and insulin tolerance in mice. In patients with RA, treatment with biological DMARDs did not affect the body composition whereas the muscle mass was unchanged after receiving tofacitinib and the fat mass was significantly increased. J. Med. Invest. 65 : 166-170, August, 2018

Keywords **:** *Rheumatoid arthritis, Tofacitinib, Body composition*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, joint tenderness, and destruction of synovial joints, affecting more number of women than men. Systemic inflammation in RA causes muscle wasting and compensatory increase in the body fat, without a significant change in the body weight (1-2). Several studies have indicated that patients with RA have an abnormal body composition (3-4). In addition, our previous study reported that sarcopenia was identified in 48.3% and 54.0% of women and men with RA, respectively, which is more frequent than the incidence in the Japanese elderly people (unpublished). Sarcopenia is a condition that is characterized by progressive and generalized loss of skeletal muscle mass and strength, which causes a risk of adverse health outcomes, including physical disability, poor quality of life (QOL), and death (5). Therefore, early identification and treatment of sarcopenia in patients with RA are important.

Owing to advances in the pharmacologic treatment of RA over the past decade, the prognosis of patients with RA has improved dramatically (6). Currently, patients with RA are treated with traditional anti-rheumatic drugs, nonsteroidal anti-inflammatory drugs, steroids, biologic disease modified anti-rheumatic-drugs (biological DMARDs), or Janus kinase (JAK) inhibitors.

Tofacitinib is the first JAK inhibitor approved for use to treat patients with moderately to severely active RA who show inadequate response to or cannot tolerate methotrexate (MTX) (7). The JAK enzymes (JAK1 and JAK3) have essential roles in the intracellular signaling transduction of cytokines, leading to joint inflammation and damage (8). By inhibition of cytokine signaling and decreased inflammation, tofacitinib would improve the RA pathology and patients' condition.

Biological DMARDs are administered by subcutaneous or intravenous injections, which are sometimes limited or inconvenient, whereas tofacitinib is administered orally, which relieves patients from the burden of injections. It is recognized that tofacitinib will be used more frequently or without biological DMARDs in the future if sufficient experiences are accumulated through clinical data.

Most therapeutic RA agents have been recently reported to affect body composition, particularly decrease muscle and increase body fat, and sarcopenic obesity has been identified in many patients. In contrast, treatment with anti-tumor necrosis factor-α (TNF-α) agent is known to maintain body composition (9-11). Because sarcopenia or decreased muscle mass is associated with bad prognosis, considering the influence on body composition in selecting RA therapeutic agents is important. JAK inhibition has been reported to decrease lipolysis and increase body fat in mice (12). In this study, we investigated whether tofacitinib has any effects on body composition in mice and patients with RA.

MATERIALS AND METHODS

Animals : High fat fed C57BL/6 mice with Tofacitinib treatment

All animal experiments were approved by the institutional animal care and use committee at the University of Tokushima Graduate School (Tokushima, Japan).

Female C57BL/6 mice were obtained from Japan SLC (Shizuoka, Japan) and used at 8 weeks of age for experiments. Mice were

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housed in temperature (23±3°C) and humidity-controlled conditions with a 12-h light/12-h dark cycle. Mice were given free access to water and a high-fat diet (60% calories from fat ; Oriental Yeast Ltd, Tokyo, Japan). They were divided into two groups : control or treatment with tofacitinib. Body weight and food intake of each mouse were measured once a week.

Treatment with tofacitinib in C57BL/6 mice

Tofacitinib (LC Laboratories, Woburn, MA) was dissolved in a sterile solution of 50% dimethyl sulfoxide, 40% water, and 10% polyethylene glycol 300. Tofacitinib was administered 30 mg/kg/day for 70 days via subcutaneous injection. The control group was administered a corresponding volume of sterile solution without tofacitinib.

X-ray computed tomography scan

Trunk muscle and subcutaneous fat, visceral fat and % of body fat were measured in mice under isoflurane anesthesia, using LaTheta X-ray computed tomography (CT) scanner LCT-200 (HITACHI, Tokyo, Japan). Data were analyzed using LaTheta software (HITACHI, Tokyo, Japan).

Oral glucose tolerance tests and insulin tolerance tests

Blood glucose levels were measured in tail vein blood samples using a glucometer (Arklay, Kyoto, Japan). For oral glucose tolerance test (OGTT), blood glucose level was measured at 0, 15, 30, 60, and 120 min after an oral glucose load (1.5g/kg), following an overnight fast. For insulin tolerance test (ITT), blood glucose levels were measured at 0, 15, 30, 60, and 120 min after an intraperitoneal injection of insulin (0.75 U/kg), following a 6-h fast.

Subjects : female RA patients assessed by BIA

This study was approved by the ethical committee of Tokushima University Hospital (Tokushima, Japan). Written informed consent was obtained from each patient before enrollment. Pregnant women and patients with pacemakers were excluded.

We included eight female patients aged 20 years and older, who fulfilled the American College of Rheumatology/European League Against Rheumatism classification criteria (13). The patients were enrolled from June 2014 to December 2014 at Tokushima University Hospital. The enrolled patients were divided into two groups : treatment with tofacitinib or with other biological DMARDs (TNF-α or IL-6 inhibitor). All patients were outpatients without other severe disease such as diabetes and hypertension and asked about disease duration and previous medication use including steroids.

RA disease activity score

Disease activity 28 (DAS28) was used to assess RA disease activity. The DAS28 is a composite score derived from four measures, including the number of swollen and tender joints (out of the 28), pain score on the visual analog scale (VAS), and serum C-reactive-protein (CRP) levels(14).

Body composition measurements by bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) is a noninvasive and widely used method for measuring body composition. In this study, body composition was measured using InBody720 (InBody, Tokyo, Japan) during the morning > 2 h after breakfast. The following measurements were obtained : body weight, skeletal muscle mass, and fat mass.

Statistical analysis

Data were expressed as means± standard deviation (SD). Values were analyzed using unpaired Student *t* test or paired *t*-test. A p value of < 0.05 was considered statistically significant. All analyses were performed using Statcel Ver.3.0 (OMS Publishing, Saitama, Japan).

RESULTS

Effect of tofacitinib on body composition in mice

We first examined body weight and body composition by using X-ray CT scan to study the effect of tofacitinib on body composition in C57BL/6 mice treated with tofacitinib. Treatment with tofacitinib did not affect body weight and body composition (data not shown). Based on this result, tofacitinib administration to normal mice might not affect body composition. We subsequently used C57BL/6 mice fed with a high-fat diet for 4 weeks to cause weak inflammation. Figure 1A shows that treatment with tofacitinib did not affect body weight without affecting food intake. In addition,

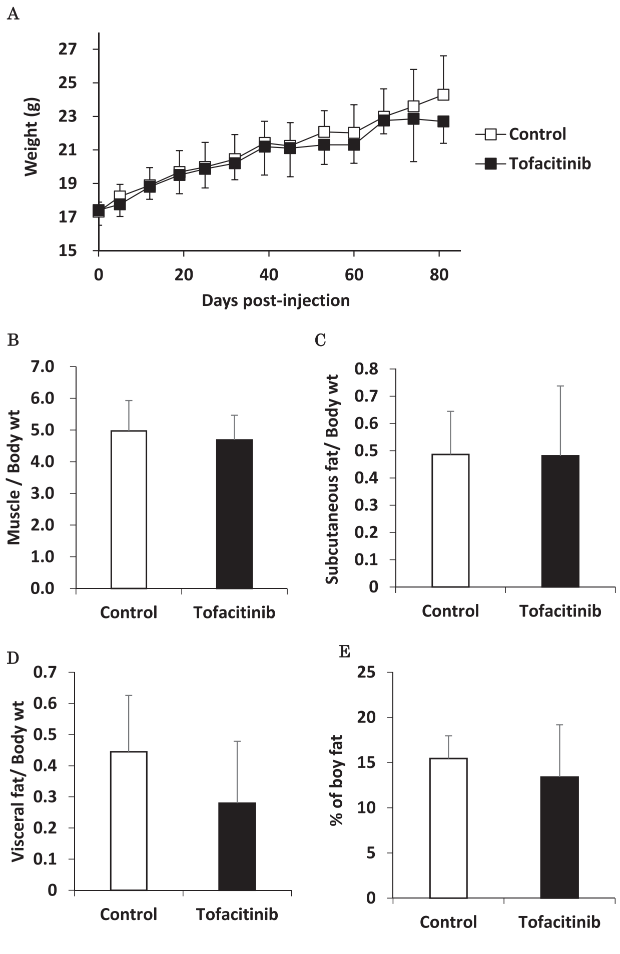

Figure 1 Treatment with tofacitinib did not affect body weight and body composition.
Mice were fed with a high-fat diet and were treated with 30 mg/kg/day tofacitinib or vehicle for 70 days (tofacitinib, n=9 ; vehicle, n=7). Body weight was measured weekly (A). Body composition was assessed using computed tomography, with the trunk muscle content (g/body weight ; B), subcutaneous fat content (g/body weight ; C), visceral fat content (g/body weight ; D), and the relative body fat content (E) quantified. Values are presented as mean± standard deviation (A-E). Control : vehicle-treated group (open squares in glaph A) ; tofacitinib : tofacitinib-treated group (closed squares). Control : vehicle-treated group (open columns in glaph B-E) ; tofacitinib : tofacitinib-treated group (closed columns).

no differences of %volume were found in the trunk muscle and subcutaneous fat or visceral fat between the two groups (Fig 1B-E). We also examined OGTT and ITT. Treatment with tofacitinib did not affect glucose and insulin tolerance (Figure 2A and 2B).

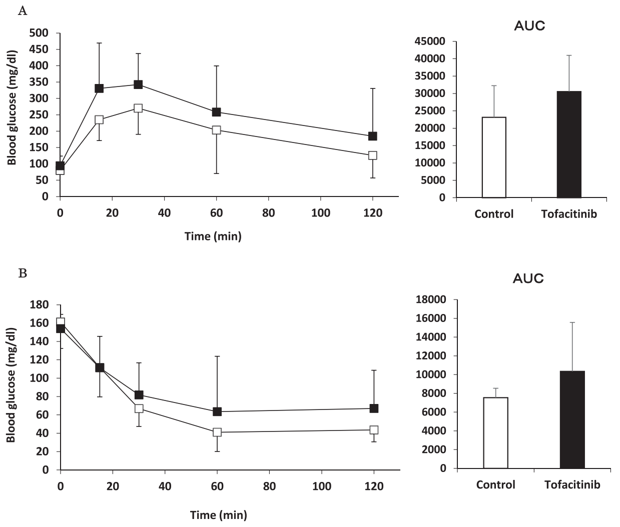

Figure 2 Treatment with tofacitinib did not affect glucose and insulin tolerance.
Glucose (A) and insulin (B) tolerance tests were performed in mice fed with high-fat diet after an overnight (16 h) (A) or 6-h (B) fast. Mice were administered an oral dose of 1.5 g/kg glucose (A) or 0.75 U/kg insulin by intraperitoneal injection (B). Blood glucose was measured at the indicated times. Glucose utilization and insulin sensitivity were determined from the area under the curve (AUC ; inset). Open squares : vehicle-treated group ; closed squares : tofacitinib-treated groups. Values are presented as means± standard deviation.

Effect of tofacitinib on body composition in patients with RA

We subsequently compared the body composition in female patients with RA treated with tofacitinib and those treated with other biological DMARDs. The characteristics of patients are listed in Tables 1 and 2.

We evaluated the body composition before and 3 months after treatment initiation. Treatment with biological DMARDs did not affect body weight, skeletal muscle mass, and body fat mass (Figure 3A-C). By contrast, the body weight of patients treated with tofacitinib tended to increase ($p=0.06$). Although treatment with tofacitinib did not affect muscle mass, body fat mass was significantly increased ($p< 0.05$). The DAS-28 scores improved in both groups (Fig 3D).

DISCUSSION

In this study, we investigated the effect of tofacitinib on body composition in mice fed with a high-fat diet and patients with RA. Our results show that tofacitinib did not affect body weight and body composition in mice treated with tofacitinib. Previous studies have suggested that JAK-STAT signaling pathways play important roles in adipose tissue function (15-16). Shi *et al*. reported that adipocyte-specific deficiency of JAK2 in mice impairs lipolysis and increases body weight (12). However, our results in mice treated with tofacitinib did not show weight gain and increased fat mass.

Table. 1
Characteristics of participants and disease

	Biological DMARDs (n=4)	Tofacitinib (n=4)	P value
Age (years)	57.0± 14.7	55.3± 19.5	0.891
Sex : Male : Female	0 : 4	0 : 4	
Disease duration (years)	5.8± 10.8	8.5± 6.6	0.679
Steroids dairy dose (mg)	1.1± 1.4	3.3± 5.3	0.485
DAS28 CRP	5.0± 0.7	5.1± 0.8	0.742
Energy intake (kcal/kgBW/day)	30.7± 7.9	35.9± 6.1	0.337

Data is presented as mean± standard deviation (SD). The two groups compared using unpaired Student's t test.

Table. 2
Difference of body composition data between biological DMARDs and Tofacitinib groups

	Biological DMARDs (n=4)	Tofacitinib (n=4)	P value
Body mass index (kg/m^2)	24.4± 5.1	24.0± 4.9	0.924
Body fat mass (%)	32.9± 8.0	32.3± 4.8	0.906
Skeletal muscle mass(kg)	22.3± 3.3	20.4± 4.0	0.490
Skeletal muscle index (kg/m^2)	5.0± 0.7	6.4± 1.1	0.918
Body cell mass (kg)	26.7± 3.6	24.6± 4.4	0.500
Edema (%, n)	0 (0)	25 (1)	0.500

Data is presented as mean ± standard deviation (SD) or proportions (%). The two groups compared using unpaired Student's t test.

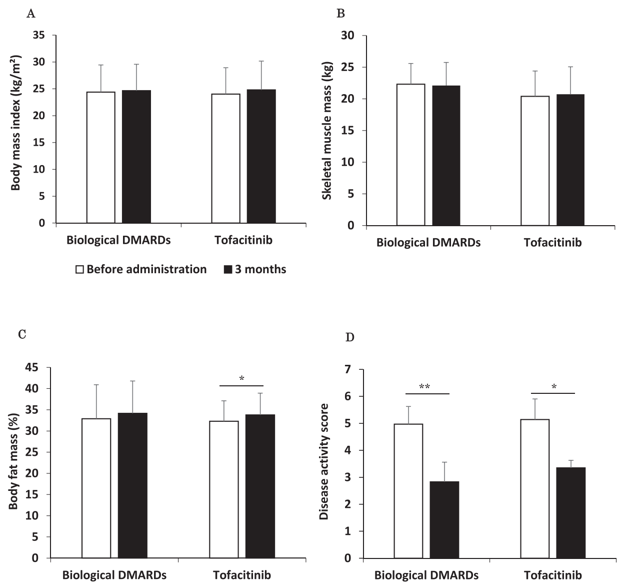

Figure 3 Treatment with biological DMARDs did not affect body composition, whereas treatment with tofacitinib significantly increased body fat mass.
We evaluated body composition 3 months after treatment initiation in patients treated with biological DMARDs or tofacitinib (n=4 per group). Body mass index (A), skeletal muscle mass (B), and body fat mass (C) were measured using bioelectrical impedance analysis (BIA). Disease activity score was also caluculated (D). Values are presented as means± standard deviation. Open bars : before treatment, closed bars : 3 months after treatment initiation. *$P< 0.05$, **$P< 0.01$ 3 months after treatment initiation vs. before treatment.

The JAK-STAT pathway might occur in most cells and mediate the action of numerous cytokines, growth factors, energy expenditure, and cellular differentiation (17). Tofacitinib inhibits JAK enzyme, JAK1, JAK2, and JAK3, and shuts down the signaling of inflammatory cytokines by binding to cytokine receptors on the surface of immune cells, which improves the pathology of RA. However, the amount of tofacitinib affecting the JAK signal of adipose tissues is still unclear. Because previous studies only used knockout mice that target JAK-STAT activators, the mechanism of JAK inhibition caused tofacitinib does not involve lipolysis and body fat accumulation i.e., treatment with tofacitinib may not act as strongly as in the knockout mice.

In the present study, patients with RA had a higher percentage of body fat, which was comparable among patients treated with biological DMARDs (32.9± 8.0%) and those with tofacitinib (32.3± 4.8%). A variety of factors are involved in the increase of fat, including inflammatory cytokines, glucocorticoid use, and decreased physical activity due to pain. Indeed, our patients with tofacitinib used about 3-fold steroids compared to patients with DMARDs. This might affect on body fat percent. Several reports have demonstrated that anti-TNF-α therapy for RA has not been effective in obese patients (5-6). Jhun *et al.* also demonstrated that obesity aggravates joint inflammation in a collagen-induced arthritis model (7). Thus, a higher percentage of body fat increases RA severity. The suppression of the appropriate disease activity may be the most important strategy to treat metabolic abnormalities.

In the patients treated with tofacitinib, the body weight tended to increase, and fat mass significantly increased. Various factors may have contributed to body weight gain and fat increase. Because tofacitinib can be taken orally, which is safer than subcutaneous injection, it is suitable for use by elderly people. Although no significant difference was found in age between the biological DMARDs and tofacitinib groups, only the tofacitinib group included elderly people over 75 years. Furthermore, patients with high use of steroids were also included in the tofacitinib group. Steroid use is very common factor to increase body fat. The large variation because of the small sample size is the limitation of this study. Further investigation is needed with a large sample size.

In addition, the DAS in both groups of patients improved after treatment initiation. Because of the improved symptoms, dietary intake and physical activity may have changed, possibly leading to weight change. The effect of biological DMARDs on body composition has also been reported. Treatment with anti-TNF has been reported to not change body composition in the short term (after 12 weeks of therapy) (8-9). Biological DMARDs have been reported to preserve fat-free mass. Our results were consistent with the previous study. We also showed that tofacitinib maintained fat-free mass similar to biological DMARDs. However, regarding fat mass, some report showed that anti-TNF therapy increases body fat mass in early RA (10). Our results were not consistent with this point. Examination of the fat of each part, such as trunk fat, is important.

In this study, different results were occurred in mice and patients. One of reasons might because of our mice model. We did not find any change of body fat when we use mele mice with normal chow, we have tried touse female with high fat diet to induce weak inflammation. It might be better to use some RA model mice such as SKG mice. Also, as we described above, there were some variations between patients, especially steroid use, we further need to see the effect of tofacitinib on body composition.

Taking together, there is still possibility that tofacitinib may improve body composition.

However, whether tofacitinib is superior to methotrexate in the long-term treatment of RA remains unknown. Additional larger studies are needed to explore this possibility.

CONFLICT OF INTEREST DISCLOSURE

There are no conflict of interest.

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