Effects of febuxostat on platelet-derived microparticles and adiponectin in patients with hyperuricema

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Platelet-derived microparticles (PDMPs) and adiponectin play an important role in the development of atherothrombosis. We investigated the effect of febuxostat on circulating PDMP levels and adiponectin in hyperuricemic patients. Levels of PDMP and biomarkers were measured using an ELISA at baseline and after 2 and 6 months of treatment. Plasma levels of PDMPs and biomarkers were higher, while those of adiponectin were lower in hyperuricemic patients than in normouricemic controls. Uric acid and interleukin (IL)-6 levels decreased significantly in hyperuricemic patients after 2 months of febuxostat treatment. However, PDMP and biomarkers decreased significantly in hyperuricemic patients after only 6 months of febuxostat treatment and adiponectin increased significantly. These results suggest that the effects of febuxostat for PDMPs seen may be the effect on xanthine oxidase but not the decrease of uric acid, and febuxostat may be beneficial for primary prevention of atherothrombosis in hyperuricemic patients. *Blood Coagul Fibrinolysis* 26:887–892 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

A high level of serum uric acid is a risk factor for coronary artery atherosclerosis and hyperuricemia promotes the development of chronic kidney disease [1,2]. Increased serum uric acid is also closely associated with systemic inflammation [3]. Inflammation is characterized by elevated levels of acute phase proteins, such as fibrinogen and C-reactive protein, and elevated levels of cytokines such as interleukin (IL)-6 and tumour necrosis factor-α. All these biomarkers, which are cardiovascular risk factors simultaneously, are markedly elevated in patients with metabolic syndrome and diabetes mellitus [4].

Platelet-derived microparticles (PDMPs) play roles in normal haemostatic responses to vascular injury because they possess prothrombotic activity [5,6]. PDMPs are also released from platelets following physical stimulation under various conditions [5–8] and it is considered that PDMPs contribute to thrombin generation and thrombus formation by generating tissue factors [6,8]. Therefore, PDMPs ultimately cause vascular complications with the participation of the blood coagulation system.

Adiponectin, the most abundant adipose tissue-specific protein, is exclusively expressed in and secreted by adipose tissue [9]. Plasma adiponectin concentrations have been shown to be decreased in obese individuals with type 2 diabetes and to be closely related to whole-body insulin sensitivity [10,11]. The protein occurs in abundance in the circulation and stimulates nitric oxide production in vascular endothelial cells, which ameliorates endothelial cell function [12–14]. These observations suggest that the antiatherogenic properties of adiponectin may involve its nitric oxide-dependent antiplatelet effects.

Febuxostat was developed in Japan as a treatment for hyperuricemia because it was shown to decrease serum uric acid to therapeutic target levels and maintain these levels over the long term [15]. Unlike allopurinol, this drug does not inhibit nucleic acid metabolizing enzymes other than xanthine oxidase [16–18]. Xanthine oxidase is one of the major enzymatic sources of reactive oxygen species (ROS) and it catalyzes the oxidation of purine substrates, producing uric acid and ROS [16]. Xanthine oxidase has been reported to be upregulated by various inflammatory stimuli [17,18] and augmented xanthine oxidase eventually causes excess ROS formation leading to tissue damage. Furthermore, clinical studies, comparing allopurinol with febuxostat, have shown that febuxostat has a more potent uric acid lowering effect [19,20] and can potentially prevent xanthine oxidase-dependent tissue dysfunction. However, the effects of febuxostat on PDMPs and adiponectin are poorly understood. In this study, we have investigated the effects of febuxostat treatment on plasma levels of PDMPs, soluble (s)P-selectin and adiponectin in hyperuricemic patients, to determine whether febuxostat affects development of platelet-associated atherothrombosis.

Materials and methods
Patients

The study group included 51 normouricemic controls and 69 hyperuricemic patients. However, seven patients

Table 1 Clinical characteristics and various factors of the hyperuricemic patients and normouricemic controls

	Normouricemic controls	Hyperuricemic patients	P
N	51	62	
Men/women (no.)	29/22	38/24	
Age (years)	56 ± 13	73 ± 10	<0.01
BMI (kg/m^2)	24.1 ± 3.6	25.9 ± 3.9	N.S.
HbA1c (%)	6.1 ± 2.4	7.6 ± 2.8	<0.05
UA (mg/dl)	4.23 ± 0.83	8.29 ± 1.21	<0.001
DM (%)	46.8	68.7	<0.05
HT (%)	55.2	66.9	N.S.
HC (%)	23.2	35.5	N.S.
Smoking (%)	33.4	41.8	N.S.
P-CAD (%)	6.5	17.8	<0.05
PDMP (U/ml)	12.7 ± 7.3	23.9 ± 8.1	<0.001
IL-6 (pg/ml)	3.1 ± 1.4	4.7 ± 2.2	<0.05
sP-selectin (ng/ml)	198 ± 52	251 ± 72	<0.05
sE-selectin (ng/ml)	75.9 ± 11.3	92.6 ± 14.1	<0.01
sVCAM-1 (ng/ml)	587 ± 138	902 ± 216	<0.001
MCP-1 (ng/ml)	262 ± 74	421 ± 101	<0.01
Adiponectin (μg/ml)	5.17 ± 1.61	3.78 ± 1.53	<0.01

Data are shown as mean ± SD. DM, diabetes mellitus; HbA1c, haemoglobin A1c; HC, hypercholesterolemia; HT, hypertension; MCP-1, monocyte chemoattractant protein-1; P-CAD, previous history of coronary artery disease; PDMP, platelet-derived microparticle; interleukin-6; sE-selectin, soluble E-selectin; sP-selectin, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule; UA, uricemic acid. P value, hyperuricemic patients vs. normouricemic controls.

dropped out because of disease aggravation or patient removal. Therefore, 62 patients were analysed in this study (Table 1 baseline data). From September 2011 to June 2014, normouricemic controls and hyperuricemic patients were selected from patients admitted to our hospitals. The protocol of this study was approved by the Institutional Review Board (IRB) of the medical institution, and written informed consent was obtained from each individual prior to the start of the trial in accordance with the guideline for good clinical practice (GCP).

The participation criteria included the absence of a history of inflammatory, coronary artery or cerebrovascular disease for 3 months prior to enrolment, as well as the absence of clinically detectable renal (serum creatinine ≥2.0 mg/dl), hepatic (elevated serum transaminase), infectious (fever or elevated white blood cell count) or malignant disease (as determined by ultrasonography or computed tomography). Other uric acid lowering agents were withheld, owing to their potential influence on data interpretation. These medications were stopped at least 2 weeks prior to the initiation of febuxostat therapy. Of the 62 hyperuricemic patients, 43 had type 2 diabetes (Table 1); of these, 14 were being treated with sulfonylureas, 10 with α-glucosidase inhibitors and eight with insulin injections. The age range of eligible patients was 20–90 years.

Study design

The 62 study participants had a serum uric acid more than 8 mg/dl and were not on antihyperuricemic therapy. The primary endpoint was serum uric acid level, PDMPs and adipinectin after treatment. Secondary endpoints were as

follows: IL-6, sP-selectin, sE-selectin, soluble vascular cell adhesion molecules (VCAM)-1 and monocyte chemotactic peptide 1 (MCP)-1.

The target serum uric acid level was less than 6.0 mg/dl and the dose of the test drug, febuxostat, was increased up to a maximum of 60 mg/day. No other changes to the pharmacologic regimens of the patients were made during the course of the trial. In addition, patient food habits, such as diet, were not altered during the study. Clinical and biochemical data determined before and after 6 months of therapy with febuxostat were analysed.

Measurement of platelet-derived microparticle, soluble molecules and adiponectin

Fasting blood samples from patient and control individual peripheral veins were collected into vacutainers containing EDTA-ACD (NIPRO Co. Ltd., Osaka, Japan) using 21-gauge needles to minimize platelet activation. Samples were gently mixed by inverting the tubes once or twice and were then kept at room temperature for a maximum period of 2–3 h. Immediately after centrifugation at 8000g for 5 min, 200 μl of the upper layer supernatant from the 2 ml samples was collected to avoid contamination with platelets. The collected samples were stored at −40°C until analysis.

PDMP levels were measured twice and mean values were recorded. Furthermore, some basic studies were carried out prior to this assessment using clinical specimens. An ELISA kit used for PDMP measurements was obtained from JIMRO Co. Ltd. (Tokyo, Japan) [10,21]. Plasma sP-selectin, sE-selectin, sVCAM-1, MCP-1 and IL-6 were measured using an mAb-based ELISA kit purchased from Invitrogen International Inc. (Camarillo, California, USA), while plasma adiponectin was measured with an Adiponectin ELISA kit purchased from Otsuka Pharmaceuticals Co. Ltd (Tokyo, Japan). Recombinant products and standard solutions provided with the commercial kits were used as positive controls in each assay. All kits were used in accordance with the manufacturer's instructions.

Effect of uric acid for platelet-derived microparticles in normal platelet-rich plasma

Platelet-rich plasma of healthy persons ($n = 3$) were treated with purified uric acid (Wako Pure Chemical Industries, Ltd, Osaka, Japan) of various concentrations (1–32 mg/dl) for 30 min. After treatment, PDMPs were collected by the above-mentioned method. PDMP levels were measured five times by the ELISA method, and finally mean volumes were recorded.

Statistics

Data were expressed as the mean ± SD and analysed using multiregression analysis, as appropriate. Between-group comparison of values was made with the Newman–Keuls test and Scheffe's test. The correlation between uric acid

Table 2 Multiregression analysis on uric acid in hyperuricemic patients

Analysis	Univariate		Univariate	
	β	P	β	P
Age (years)	0.6216	<0.00001*	0.5049	0.00487*
Sex (men)	−0.0359	0.25231		
BMI (kg/m²)	0.2241	0.06773		
HbA1c (%)	0.3175	0.04315*	0.2415	0.10234
DM (%)	0.3971	0.01397*	0.2851	0.08951
HT (%)	0.1346	0.08791		
HC (%)	−0.0657	0.23169		
P-CAD (%)	−0.0392	0.32861		
PDMP (U/ml)	0.6506	<0.00001*	0.4156	0.00562*
IL-6 (pg/ml)	0.1766	0.21778		
sP-selectin (ng/ml)	0.4586	0.00141*	0.3378	0.02316*
sE-selectin (ng/ml)	0.3917	0.00749*	0.2791	0.08846
sVCAM-1 (ng/ml)	0.4325	0.00164*	0.3171	0.06625
MCP-1 (pg/ml)	0.5784	0.00008*	0.4019	0.00892*
Adiponectin (μg/ml)	−0.6393	<0.00001*	−0.5175	0.00212*

β, standardized regression coefficients; DM, diabetes mellitus; HbA1c, haemoglobin A1c; HC, hypercholesterolemia; HT, hypertension; MCP-1, monocyte chemoattractant protein-1; P-CAD, previous history of coronary artery disease; PDMP, platelet-derived microparticle; interleukin-6; sE-selectin, soluble E-selectin; sP-selectin, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule; UA, uricemic acid. *Statistically significant.

and after continuous-response variables was assessed using multivariate linear regression analysis. *P* values less than 0.05 were considered statistically significant. Analysis was performed using the StatFlex program (ver. 6).

Results

Plasma levels of PDMPs, IL-6, sP-selectin, sE-selectin, sVCAM-1 and MCP-1 were higher, while those of adiponectin were lower in hyperuricemic patients than in normouricemic controls (Table 1). We investigated 15 variables for hyperuricemic patients using multiregression analysis (Table 2). Univariate analysis showed that age, HbA1c, diabetes mellitus, PDMP, sP-selectin, sE-selectin, sVCAM-1, MCP-1 and adiponectin were significantly associated with uric acid (Table 2). In addition, age, PDMP, sP-selectin, MCP-1 and adiponectin were significant factors in the multivariate model with uric acid (Table 2).

Uric acid and IL-6 levels decreased significantly in hyperuricemic patients after 2 and 6 months of febuxostat treatment (Fig. 1). However, PDMP, sP-selectin, MCP-1, sE-selectin and sVCAM-1 decreased significantly in hyperuricemic patients after only 6 months of febuxostat treatment and adiponectin increased significantly (Fig. 1 and 2). On the contrary the enhancing effect of uric acid on PDMPs was not observed in an in-vitro experiment using platelet-rich plasma of healthy persons (Fig. 3).

Discussion

Uric acid has been shown to be a predictor and an independent risk factor for atherothrombotic diseases,

Fig. 1
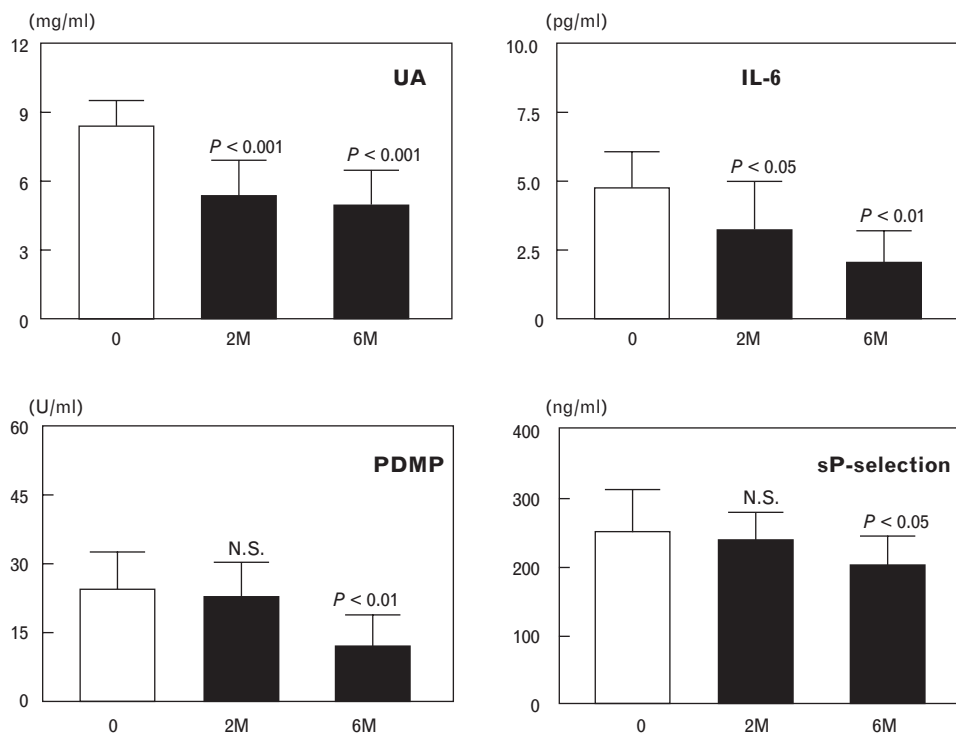
Changes in the plasma levels of uric acid (UA), interleukin-6 (IL-6), platelet-derived microparticle (PDMP) and sP-selectin before and after febuxostat treatment in hyperuricemic patients Data are shown as mean ± SD. *P* value, 0 vs. 2 or 6 months. N.S., not significant.
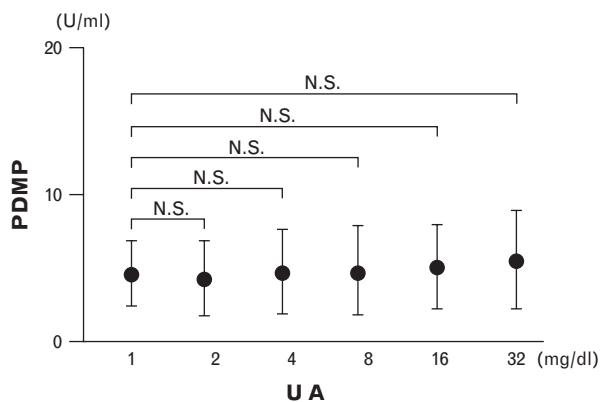
Fig. 2
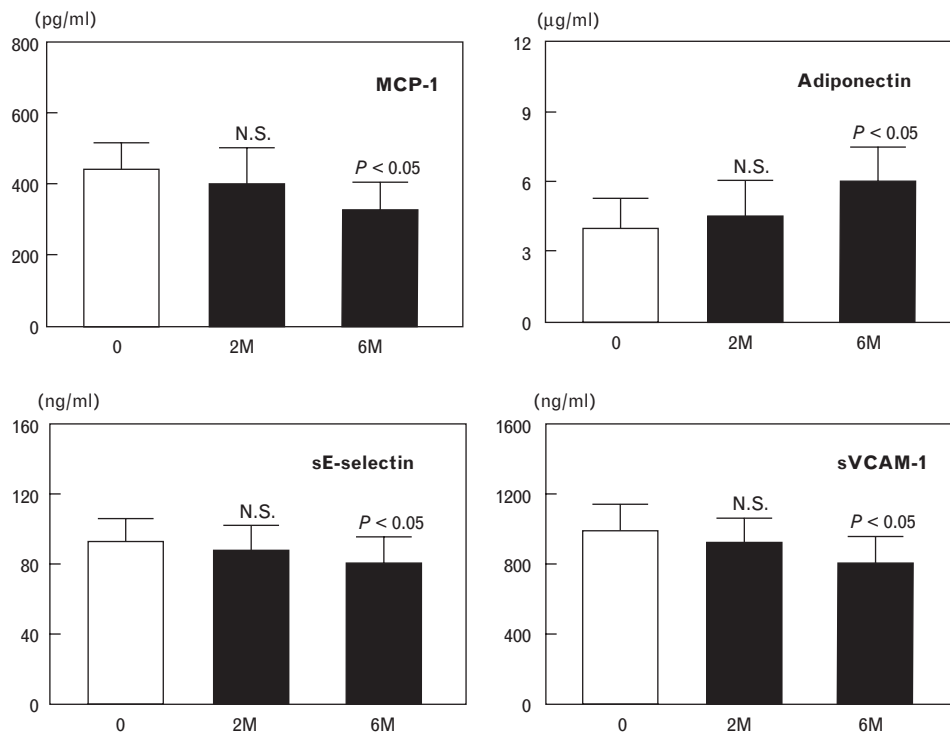
Changes in the plasma levels of monocyte chemoattractant protein-1 (MCP-1), adiponectin, sE-selectin and soluble vascular cell adhesion molecule-1 (sVCAM-1) before and after febuxostat treatment in hyperuricemic patients. Data are shown as mean ± SD. *P* value, 0 vs. 2 or 6 months. N.S., not significant.

such as diabetes mellitus, cerebrovascular disease and acute coronary syndrome [22,23]. In addition, a close association between elevated uric acid and numerous markers of inflammation has been noticed [24]. These findings suggest that uric acid is one of the determinants for a vascular event in atherosclerosis. As usual, allopurinol has long been regarded as a first-line drug for the

Fig. 3

Effect of uric acid (UA) for platelet-derived microparticles (PDMP) in normal platelet-rich plasma. Data are shown as mean ± SD. N.S., not significant.

treatment of hyperuricemia. However, this drug has been reported to cause some adverse reactions such as renal dysfunction and hypersensitivity vasculitis [1,15]. Unlike allopurinol, febuxostat is more reliable and it has been reported to have a stronger effect on hyperuricemia than allopurinol [25]. In the present study, uric acid levels were also significantly lower in patients after treatment with febuxostat.

Activated platelets and PDMPs may cause capillary microembolization secondary to the formation of microaggregates [26]. PDMPs play an important role in the clotting process, so an increase in PDMPs is likely to cause hypercoagulability [6,26]. We previously reported that PDMP levels were significantly increased in atherothrombotic patients [27]. Because PDMPs promote the expression of adhesion molecules by monocytes and endothelial cells [26,28], it seems possible that these microparticles may participate in the development or progression of atherosclerosis [28].

In the present study, febuxostat therapy significantly decreased PDMP levels. Although we did not show any direct changes in platelet function, febuxostat therapy also decreased expression of another platelet activation marker, sP-selectin, in our patients with hyperuricemia. However, we cannot conclude whether the same mechanism caused these changes or not. If

anything, different mechanisms are a possibility. Uric acid and IL-6 levels already decreased significantly after 2 months of febuxostat treatment. This result suggests that there is an association between a decrease in uric acid and inflammation, because IL-6 is a proinflammatory cytokine [29,30]. However, PDMP and sP-selectin decreased significantly after 6 months of febuxostat treatment. This result suggests that the improvement of PDMPs after febuxostat treatment was independent from the decrease of uric acid. Indeed, the uric acid of various concentrations did not affect PDMP levels was not observed in an in-vitro experiment using platelet-rich plasma of healthy persons. We accordingly guess that the effects of febuxostat for PDMPs seen may be the effect on xanthine oxidase, because it is previously reported that xanthine oxidase eventually causes excess ROS formation, leading to tissue damage [17,31]. Therefore, it is possible that febuxostat can prevent xanthine oxidase-dependent tissue dysfunction [19].

The plasma level of adiponectin is decreased in obese individuals [9,26] and is closely related to whole-body insulin sensitivity [12]. A significant decrease of plasma adiponectin has also been found in patients with type 2 diabetes [12]. Adiponectin has been reported to suppress the attachment of monocytes to endothelial cells [32] and plays a role in protection against vascular injury, so hypoadiponectinemia is associated with endothelial dysfunction [26]. Hypoadiponectinemia also seems to cause platelet activation. The level of nitric oxide, which regulates platelet activation, has been shown to be decreased by hypoadiponectinemia because adiponectin stimulates nitric oxide production by vascular endothelial cells [13,14,26]. Thus, platelet activation occurs because of low nitric oxide concentrations in individuals with hypoadiponectinemia. Therefore, the increase of adiponectin caused by febuxostat may have an antiplatelet effect via promotion of nitric oxide production.

We postulate that one possibility for the mechanism underlying adiponectin elevation by febuxostat treatment is the participation of ROS. Oxidative stress plays a pivotal role in the pathogenesis of various diseases [33]. In diabetes, oxidative stress impairs glucose uptake in muscle and fat and decreases insulin secretion from pancreatic ß-cells [34,35]. Inflammation is closely linked to the formation of ROS [33,36], and preadipocytes produce ROS [33,36,37]. Finally, ROS cause hypoadiponectinemia [33,37]. Febuxostat may have a possible effect on ROS, as suggested by previous reports demonstrating the anti-inflammatory effect of febuxostat that may relate to its ability to block the production and/or activity of ROS [31,38]. Therefore, the improvement of hyperuricemia by febuxostat could be due to alteration of the posttranslational modification of adiponectin. However, further studies are necessary to elucidate the effects of febuxostat itself on adiponectin production.

Conclusion

Febuxostat directly or indirectly increased circulating adiponectin levels in hyperuricemic patients. In addition, febuxostat treatment led to a decrease in PDMPs and sP-selectin related to platelet activation. Febuxostat may be beneficial for the primary prevention of atherothrombosis in hyperuricemic patients. However, validation of this hypothesis will require a large clinical trial.

Conflict of interest

There are no conflicts of interest.

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