Effects of blood pressure and the renin-angiotensin system on platelet activation in type 2 diabetes

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ABSTRACT

Aims/Introduction: Platelet-derived microparticles (PDMP) are released from the platelets either after activation or in response to physical stimulation *in vivo*. The present study examined the association between blood pressure and PDMP, and the effects of high-dose angiotensin receptor blockers (ARB) on PDMP in patients with type 2 diabetes.

Materials and Methods: The study subjects consisted of 28 type 2 diabetes patients with blood pressure ≥130/80 mmHg who were treated with valsartan (80 mg daily). The patients were randomly assigned to take either 80 mg of telmisartan (Tel group) or 160 mg of valsartan (Val group) and then were followed up for 24 weeks. Thereafter, the patients were switched to combination therapy (5 mg of amlodipine with 40 mg of telmisartan [Tel group] or 80 mg of valsartan [Val group]) for 12 weeks.

Results: Although the ambulatory blood pressure did not change, the PDMP levels were significantly decreased from baseline to week 24 (high dose ARB). In contrast, combination therapy reduced both blood pressure and PDMP levels compared with the baseline. Although the PDMP level was significantly correlated with the morning BP elevation at baseline and week 36 (combination therapy), this same relationship was not found at week 24. There were no significant differences in the blood pressure and PDMP levels between the two groups.

Conclusions: Patients with morning hypertension might be at risk for cardiovascular diseases. High-dose renin-angiotensin system inhibition and blood pressure control are both considered to reduce cardiovascular events in patients with type 2 diabetes.
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KEY WORDS: Platelet activation, Renin-angiotensin system, Morning blood pressure

INTRODUCTION

Diabetes mellitus is associated with atherosclerotic cardiovascular disease[1]. In addition, diabetic subjects have a greater burden of other atherogenic risk factors than non-diabetic subjects, including hypertension, obesity, chronic kidney diseases and dyslipidemia[2–4]. The abnormal metabolic state that accompanies diabetes also enhances platelet function[5]. Platelet activation might therefore contribute to the overall cardiovascular risk in diabetic patients, because platelets play a pivotal role in the pathogenesis of cardiovascular diseases[6,7]. Although there is strong evidence showing that platelet function is enhanced in diabetic patients, many earlier studies have assessed the platelet function by measuring the formation of platelet aggregates in response to exogenous stimulation with various agonists[8–10]. These conventional methods do not measure platelet micro-aggregate formation, which occurs during the initial process of platelet activation. Platelet-derived microparticles (PDMP) are released from the platelets either after activation or in response to physical stimulation under various conditions *in vivo*[11,12], and PDMP are responsible for the procoagulant activity of platelets[13].

As many as 40% of patients with type 2 diabetes are hypertensive at the time of diagnosis and, in approximately 50% of these patients, the elevation in blood pressure (BP) occurs before the onset of microalbuminuria[2]. The treatment of hypertension is particularly important in diabetic patients, both to prevent cardiovascular disease and to minimize the progression of renal disease and diabetic retinopathy[14]. There is also accumulating evidence that antihypertensive regimens that inhibit the renin-angiotensin system (RAS) might provide incremental diabetic end-organ protection[15–18]. In addition, higher doses of RAS blocking agents have also been shown to have more beneficial tissue protective effects than the usual doses of angiotensin II receptor blockers (ARB) [19]. In contrast, an abnormal circadian BP rhythm (nocturnal hypertension and/or morning hypertension) is a risk factor for cardiovascular disease[20], and it is also associated with diabetes[21], particularly in patients treated with an ARB[22]. Morning hypertension might contribute to increased prothrombotic activity, which might increase the risk

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for cardiovascular events[23,24]. It was also reported that the use of ARB inhibited platelet aggregation[25,26], although the anti-platelet effects were reported to differ among ARB[27]. Telmisartan, an ARB with one of the longest plasma half-lives among ARB, elicits a greater effect than valsartan on BP during the early morning period[28]. These findings suggested that the circadian BP rhythm might contribute to these differences because the half-life of ARB varies widely.

The objectives of the present study were to determine the association between the circadian rhythms of blood pressure and PDMP levels, and to examine whether a higher dose of RAS blocking agents might have a more beneficial effect on platelet activation in type 2 diabetic patients. Therefore, the 24-h ambulatory blood pressure and PDMP levels were measured in patients receiving the regular dose of ARB, a relatively high-dose ARB and combination therapy with regular dose ARB and amlodipine, a calcium channel blocker. In addition, the study compared the effects of telmisartan and valsartan on the PDMP levels.

METHODS

Participants

Japanese type 2 diabetic patients who visited the outpatient clinic of the Department of Medicine, Shiga University of Medical Science Hospital were recruited into the present study. Type 2 diabetes mellitus was diagnosed in accordance with the World Health Organization criteria. The other inclusion criteria were a baseline BP ≥130/80 and <180/110 mmHg, and having used 80 mg valsartan daily for at least 3 months. The exclusion criteria were type 1 diabetes, treatment with antihypertensive agents other than ARB, treatment with antiplatelet agents, baseline estimated GFR <30 mL/min/1.73 m^2, baseline serum potassium >5.6 mmol/L, kidney or renal tract disease other than diabetic nephropathy, cardiovascular events (unstable angina, myocardial infarction, cerebral infarction, cerebral hemorrhage or transient ischemic attack) within the preceding 6 months, severe peripheral vascular disease and congestive heart failure. The study was approved by the Ethics Committee of Shiga University of Medical Science and was undertaken in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Study Design

Circadian BP Rhythm and PDMP

The 24-h ambulatory BP was measured every 30 min non-invasively with an automatic device (model TM-2431; A&D, Tokyo, Japan). The mean arterial pressure (MAP) was calculated as the diastolic BP (DBP) plus one-third of the pulse BP. Nocturnal BP was calculated as the average BP from the time the patient went to bed until the time the patient woke up. Daytime BP was calculated as the average of the remaining readings. Morning BP was defined as the average BP during the first 2 h after waking (four BP values), as described in a previous report[22]. The morning BP elevation was calculated as the morning BP minus the nocturnal systolic BP (SBP)[29]. Blood samples for PDMP measurement were collected from a peripheral vein into a Vacutainer containing EDTA-ACD (Nipro Co. Ltd, Osaka, Japan) with a 21-gauge needle to minimize platelet activation. Platelet-rich plasma (PRP) was then prepared by centrifugation at 150 *g* for 10 min at room temperature, added to a half volume of 0.1% EDTA/saline (final concentration 60% PRP) and centrifuged at 1500 *g* for 20 min. The above procedures were completed within 30 min of blood being drawn. ELISA samples were stored at −80°C until the assay. The circulating PDMP levels were then assayed by ELISA[30].

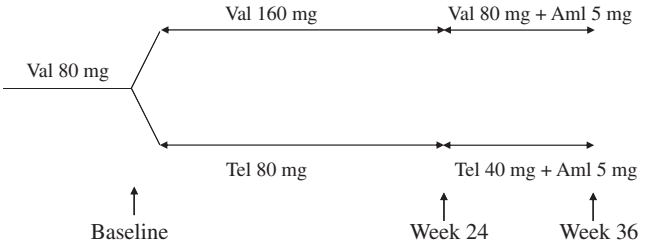

Figure 1 | A schematic illustration of the study protocol. The doses of valsartan were given once daily. Neither group was given non-angiotensin receptor blockers (ARB) antihypertensive treatment at baseline. Aml, amlodipine; Tel, telmisartan; Val, valsartan.

Comparison of Therapeutic Regimens

The design of the study is shown in Figure 1. After the baseline examinations, the patients were randomly assigned to receive either telmisartan (Tel group) or valsartan (Val group). All patients were switched from regular-dose ARB therapy (80 mg of valsartan) to relatively high doses of ARB therapy (160 mg of valsartan or 80 mg of telmisartan). Randomization was carried out using sealed envelopes containing one of the therapies prepared from a list of random numbers generated by a computer. After 24 weeks of relatively high-dose ARB therapy, the regimen was changed to combination therapy with a regular dose of the ARB (Val group, 80 mg of valsartan; Tel group, 80 mg of telmisartan) and amlodipine (5 mg daily). All antihypertensive drugs were given once daily in the morning. The patients were followed up every 4–8 weeks for 24 weeks with a relatively high dose ARB and for 12 weeks with a regular dose ARB and amlodiopine. Conventional BP was measured using a mercury sphygmomanometer with the patients in the sitting position after at least 5 min of rest. Blood samples were obtained and the 24-h ambulatory BP was measured at baseline and at the end of the study. Urinary albumin excretion was measured using the first morning urine samples. Patients received standard diabetes care throughout the study. PDMP were measured at a central laboratory (BML, Tokyo, Japan). All adverse events, medications and patient compliance data were recorded.

Statistical Analysis

The results are expressed as the means ± standard deviation. The urinary albumin-to-creatinine ratio (ACR) was

logarithmically transformed and is presented as the geometric mean with 95% confidence intervals. Comparisons between the groups were carried out using either the χ^2-test or Fisher's exact test for categorical variables and Student's *t*-test for continuous variables. Changes in the parameters were evaluated by a repeated measures analysis of variance (ANOVA) with subsequent Fisher's test for continuous variables. Correlations between the BP values and PDMP levels were determined by the least-squares method. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

The present study evaluated 28 diabetic patients (male/female: 21/7) who had been treated with valsartan (80 mg daily) and had uncontrolled BP. The mean age of the patients was 58 ± 9 years. Although there were no significant relationships between the BP values and PDMP (24-h SBP: $r = 0.11$, $P = 0.57$; daytime SBP: $r = 0.09$, $P = 0.65$; nocturnal SBP: $r = 0.13$, $P = 0.53$; or morning SBP: $r = 0.22$, $P = 0.27$), the morning BP elevation was significantly correlated with the PDMP level ($r = 0.40$, $P = 0.03$; Figure 2a).

All 28 patients were randomly assigned to either the Tel or Val groups. The baseline clinical characteristics of the patients are summarized in Table 1. The mean age, sex distribution, serum lipid, glycated hemoglobin, renal function and urinary ACR were equivalent in the two groups. All therapeutic regimens were well tolerated, with no dose-related increases in adverse events, including hypotension and hyperkalemia. Significant reductions in the SBP of the ambulatory BP values were found from baseline to week 36 (combination therapy period), but not from baseline to week 24 (high dose ARB period; Table 2). The HbA_{1c} level did not change during the study periods (baseline 6.9 ± 1.2%, week 24 6.8 ± 0.9%, week 36 6.9 ± 0.8%). The ACR tended to decrease from baseline to week 24, but not to week 36 (baseline 40: [11–145] mg/gCr, week 24: 30 [8–105] mg/gCr, week 36: 36 [14–135] mg/gCr; mean [95% CI]). Table 3 lists the values of the PDMP during the study periods. The PDMP level significantly decreased from baseline to week 24 and 36 in both groups. The changes in ACR and PDMP were not substantially different between the Val group and the Tel group.

Morning BP elevation The correlation between the PDMP level and the morning BP elevation at week 24 and 36 was examined because the PDMP level significantly correlated with the morning BP elevation at baseline. Although the PDMP level significantly correlated with the morning BP elevation at week 36, this same relationship was not found at week 24 (Figure 2b,c). In both groups, morning BP elevation was not changed during the study periods. However, in nine patients with high morning BP elevation (>30 mmHg), the values of morning BP elevation were reduced by combination therapy, but not by high-dose ARB therapy (baseline 37.8 ± 4.5 mmHg, high-dose ARB 35.9 ± 7.1 mmHg, combination therapy 35.9 ± 7.1 mmHg). In contrast, PDMP levels tended to reduce by both treatment regimen (baseline 17.3 ± 8 U/mL, high dose ARB 11.6 ± 3.8 U/mL, combination therapy 11.0 ± 4.0 U/mL).

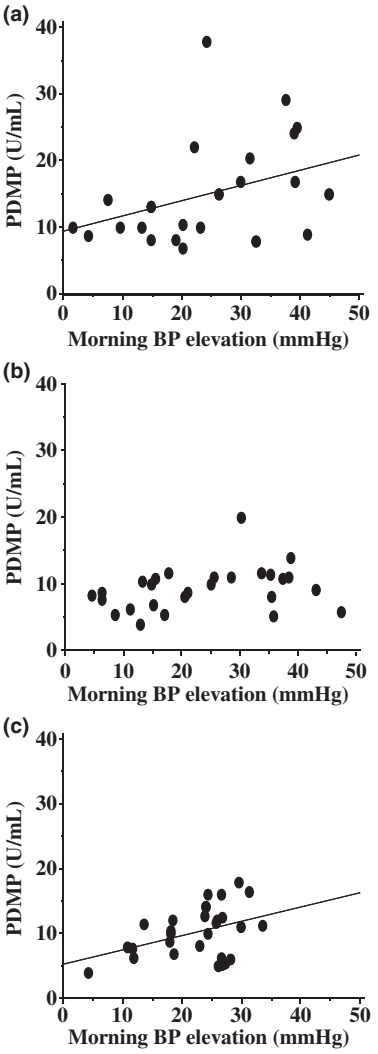

Figure 2 | Correlation between PDMP and the morning BP elevation at baseline (a), week 24 (b) or week 36 (c). The levels of platelet-derived microparticles (PDMP) and the morning blood pressure (BP) elevation were significantly correlated at baseline ($r = 0.40$, $P = 0.035$) and week 36 ($r = 0.41$, $P = 0.03$). However, no significant correlation between the levels of PDMP and morning BP elevation was found at week 24 ($r = 0.25$, $P = 0.19$).

DISCUSSION

The present study showed the morning BP elevation to be associated with platelet activation and that ARB could reduce the platelet activity in patients with type 2 diabetes in a BP independent manner. In addition, the ACR, a risk factor for renal and cardiovascular disease, also decreased after the relatively high dose of ARB therapy. These results suggest that higher doses of ARB are therefore required to optimize tissue protection in diabetes.

The morning elevation in BP is associated with an increased risk of cardiovascular events, such as myocardial infarction and stroke, particularly in patients with diabetes[23]. A variety of

Table 1 | Clinical characteristics of studied patients

Parameters	Total (n = 28)	Tel group (n = 14)	Val group (n = 14)
Age (years)	58 ± 9	57 ± 7	60 ± 10
BMI (kg/m^2)	26.7 ± 3.3	26.9 ± 3.7	26.6 ± 3.0
T cholesterol (mg/dL)	203 ± 35	216 ± 27	190 ± 37
TG (mg/dL)	155 ± 112	132 ± 110	178 ± 112
HDL Chol (mg/dL)	46 ± 14	46 ± 15	46 ± 14
HbA1c (%)	6.9 ± 1.2	6.9 ± 1.3	6.9 ± 1.2
Scr (mg/dL)	0.81 ± 0.12	0.81 ± 0.13	0.82 ± 0.12
eGFR (mL/min/1.73 m^2)	73 ± 12	71 ± 9	69 ± 6
PDMP (U/mL)	14.0 ± 7.8	15.5 ± 9.1	12.7 ± 6.2
ACR (mg/g Cr)	40 (11–145)	52 (11–237)	30 (111–84)

Data are expressed as the mean ± SD or the number of patients. The ACR was logarithmically transformed and is presented as the geometric means with their 95% confidence intervals. Tel; Telmisartan; Val; valsartan; BMI, body mass index; T, total; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; ACR, albumin/creatinine excretion ratio; PDMP, platelet-derived microparticles. *P < 0.05 *vs* baseline.

factors contribute to the early morning prothrombotic state, including increased endovascular shear stress, increased coagulation, platelet aggregation, blood viscosity and reduced fibrinolysis[23]. In addition, the activity of the RAS is the highest in the morning. Therefore, in addition to strict BP control, antihypertensive therapy targeting the morning BP elevation using RAS inhibitors could potentially have a beneficial effect in terms of protecting against renal and cardiovascular diseases.

Platelet aggregation might increase in the morning, as do the frequencies of the onset of myocardial infarction and sudden cardiac death[31]. An increase in the RAS activity is thought to play an important role in the enhancement of platelet aggregation because angiotensin II potentiates epinephrine-induced platelet aggregation[32,33]. The platelet function is also enhanced in diabetes[8–10]. However, many of the earlier studies assessed the platelet function by measuring the formation of platelet aggregates in response to exogenous stimulation with various agonists [3,9,10,18]. These methods do not assess platelet activation, which is the initial process of platelet aggregation. The present study assessed the PDMP level, which is observed as the vesicles released from platelets after adhesion to vessel walls[34,35]. Therefore, circulating PDMP are a marker for platelet activity *in vivo*. In fact, circulating PDMP have been reported to be associated with acute and chronic atherothrombotic events[30,36–38]. The circulating level of PDMP is higher in type 2 diabetic patients that in patients without diabetes[39]. However, the effect of the morning BP elevation on the PDMP level in diabetic patients has not been reported. In the present study, the PDMP level significantly correlated with the morning BP elevation at baseline. Furthermore, in patients with high morning BP elevation, morning BP elevations were reduced and PDMP levels tended to reduce by treatment with low dose ARB and amlodipine. Taken together with the findings from previous studies, the current results show that platelet activation occurs in the systemic circulation of patients with type 2 diabetes, particularly in those with morning BP elevations, which might be associated with cardiovascular events.

It has been postulated that enhanced platelet activity is a major factor in atherogenesis and thrombogenesis associated with cardiovascular events in diabetic conditions. Not only high blood pressure, but also poor glycemic control has been reported to increase in the PDMP levels[39]. However, in the present study, glycated hemoglobin levels were not changed during the study periods. Therefore, glycemic control level did not play a major role in reducing PDMP levels in the present study.

The activation of platelet angiotensin receptors might contribute to the further progression of thrombotic events[32]. Previous *in vitro* studies have suggested that valsartan elicits significant inhibition of human platelets[40]. Furthermore, valsartan significantly inhibits the platelet aggregation and the antiplatelet properties of valsartan are more profound in patients with diabetes when compared with the nondiabetic patients[26]. Other ARB, including irbesartan[41] and losartan[42], were reported to have

Table 2 | Effects of treatment regimens on the blood pressures

	Total (n = 28)			Tel group (n = 14)			Val group (n = 14)		
	Baseline	Week 24	Week 36	Baseline	Week 24	Week 36	Baseline	Week 24	Week 36
24 h mean									
SBP	136 ± 12	131 ± 9	129 ± 8*	138 ± 11	133 ± 8	128 ± 7*	134 ± 12	131 ± 10	129 ± 9*
DBP	82 ± 8	79 ± 9	77 ± 7*	83 ± 7	81 ± 10	80 ± 7	80 ± 8	78 ± 8	75 ± 6
Daytime									
SBP	140 ± 12	136 ± 10	133 ± 9*	141 ± 11	137 ± 8	134 ± 8*	139 ± 13	136 ± 12	132 ± 8*
DBP	84 ± 8	81 ± 9	79 ± 8*	85 ± 8	82 ± 10	81 ± 8	83 ± 9	80 ± 9	77 ± 7
Night-time									
SBP	127 ± 13	122 ± 12	118 ± 13*	130 ± 14	123 ± 14	114 ± 13*	123 ± 12	120 ± 11	121 ± 13
DBP	76 ± 8	74 ± 8	73 ± 6	78 ± 8	76 ± 8	76 ± 6	73 ± 8	72 ± 7	71 ± 6

Data are expressed as the mean ± SD (mmHg). Tel, Telmisartan; Val, valsartan; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure. *P < 0.05 *vs* baseline.

Table 3 | Effects of treatment regimens on platelet-derived microparticles

	Baseline	Week 24	Week 63
Total (n = 28)	14.2 ± 7.6	9.4 ± 3.2*	10.1 ± 3.8*
Val group (n = 14)	15.5 ± 9.1	9.6 ± 3.8*	10.9 ± 3.9*
Tel group (n = 14)	12.9 ± 5.8	9.2 ± 2.7*	9.3 ± 3.7*

Data are expressed as the mean ± SD (U/mL). Tel, telmisartan; Val, valsartan; ACR, albumin/creatinine excretion ratio; PDMP, platelet-derived microparticles. *P < 0.05 *vs* baseline.

similar effects on platelets, whereas candesartan[27] and telmisartan[42] failed to reduce platelet activation. However, these data were based on *in vitro* studies, and the mechanism responsible for the difference remains unclear. The present study found that higher doses of ARB, telmisartan or valsartan could reduce platelet activity *in vivo*. In addition, the PDMP levels were significantly decreased in a BP-independent manner by higher doses of ARB. These results show that the inhibition of RAS might therefore play a major role in the inhibition of platelet aggregation by an ARB. However, the present study could not address whether the incidence of future renal and/or cardiovascular disease is higher in patients with elevated PDMP levels. Further prospective follow-up studies are therefore needed to answer this question.

The reason why the positive relationship between PDMP level and morning BP was not observed at week 24 is not known. High dose ARB seems to reduce the PDMP level in a blood pressure independent manner and the effect of morning blood pressure was masked in our small number of patients examined. To determine the effects of high dose ARB on platelet activation, larger prospective randomized studies are needed.

In conclusion, the morning elevation in BP is therefore considered to be associated with an enhanced platelet activity. High-dose ARB therapy reduced the platelet activity in a BP-independent manner. Therefore, high-dose RAS inhibition and/or strict BP control for 24 h were considered to reduce cardiovascular events in patients with type 2 diabetes.

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