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Effects of gliclazide on platelet aggregation and the plasminogen activator inhibitor type 1 level in patients with type 2 diabetes mellitus

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Abstract

Vascular complications are a common factor determining morbidity and mortality of diabetic patients. In vitro studies have revealed that gliclazide has antiplatelet activities. To clinically assess this action, we measured the effects of gliclazide on platelet activities and abnormal fibrinolysis in patients with type 2 diabetes mellitus. We studied 14 patients aged 38 to 72 years (9 men and 5 women) with type 2 diabetes mellitus who have been treated with glibenclamide in our hospital for more than 6 months. We switched from glibenclamide to gliclazide using the average ratio of the respective doses, 2.5 vs 40 mg. We titrated the dose of gliclazide to keep the glycemic control at the same level as the previous (glibenclamide) treatment. We measured 10 μmol/L serotonin–induced or 0.5 μmol/L adenosine diphosphate (ADP)–induced platelet aggregate formation by particle counting using light scattering at baseline and up to 6 months after the switch. After switching to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced ($P < .05$, compared with the levels observed after glibenclamide treatment). The body mass index, fasting plasma glucose, immunoreactive insulin, homeostasis model assessment of insulin resistance, hemoglobin A_{1c} (HbA_{1c}), total cholesterol, triglycerides, high-density lipoprotein cholesterol, prothrombin time, activated partial thromboplastin time, fibrinogen, thrombin-antithrombin III complex, plasmin-α2-plasmin inhibitor complex, and plasma plasminogen activator inhibitor type 1 (PAI-1) were not changed. In the group with improved HbA_{1c} (n = 5), ADP-induced platelet aggregate formation and plasma PAI-1 level were significantly reduced ($P < .05$, compared with the group with aggravated HbA_{1c}, n = 9). Multiple regression analysis showed that percentage change of ADP-induced platelet aggregate formation (standardized $\beta = 0.540$, $P < .05$) was independently associated with percentage change of plasma PAI-1 level in addition to percentage change of HbA_{1c} (standardized $\beta = 0.657$, $P < .05$) ($R = 0.939$, $P < .05$) after switching to gliclazide. The other independent variants, like the final dose of gliclazide, homeostasis model assessment of insulin resistance, percentage change of prothrombin time, activated partial thromboplastin time, and total cholesterol, were not significantly associated with the percentage change of plasma PAI-1 level. These results indicate that gliclazide inhibits platelet aggregation via the serotonin pathway, independently of the metabolic control per se. Furthermore, in the patients with improved glycemic control, gliclazide could inhibit ADP-induced platelet aggregation and reduce PAI-I level. Taken together, the results show that gliclazide may be more useful for the prevention of diabetic vascular complications than glibenclamide.

1. Introduction

Atherosclerotic complications play a crucial role in the prognosis of type 2 diabetes mellitus (DM). It is fully recognized that long-term macrovascular complications are common factors determining morbidity and mortality in the diabetic population. The Diabetes Control and Complications Trial and UK Prospective Diabetes Study indicate a consistent relationship between hyperglycemia and the incidence of chronic vascular complications in type 1 and type 2 DM, respectively [1,2]. Platelet function in DM patients is enhanced and is correlated with both agonist-induced and spontaneous aggregation [3]. It is thought that long-term exposure to high glucose levels may enhance

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Table 1
Characteristics of enrolled patients

Age (y)	61.5 ± 2.6
Sex (M/F)	9/5
Height (cm)	165.3 ± 2.9
Weight (kg)	62.0 ± 3.1
BMI (kg/m^2)	22.5 ± 0.8
Duration of DM (y)	12.0 ± 1.8
HbA_{1c} (%)	7.4 ± 0.2
FPG (mg/dL)	165.0 ± 7.0
IRI (μU/mL)	7.8 ± 1.6
HOMA-R	3.0 ± 0.6

Data are expressed as mean ± SEM.

platelet function in DM patients. Moreover, rapid alterations of platelet aggregability in acute hyperglycemia have also been reported [4]. Intraplatelet serotonin (5-hydroxytryptamine; 5-HT) content is diminished and plasma levels of 5-HT are increased in DM patients [5].

This increase in plasma 5-HT may reflect enhanced release of platelet 5-HT by hyperactive platelets that may contribute to the pathogenesis of atherosclerosis. The measurement of 5-HT–induced platelet aggregation is therefore a useful method to evaluate the risk of diabetic complications in DM patients [5].

A technique for studying platelet aggregation by particle counting using light scattering may detect subtle changes in platelet activation [6]. Hypercoagulability and decreased fibrinolysis, including increased plasma plasminogen activator inhibitor type 1 (PAI-1) level, are often found and are considered to be risk factors of cardiovascular diseases and glucose intolerance, especially in patients with non–insulin-dependent DM [7]. Gliclazide is a second-generation sulfonylurea with the potency of free radical scavenger activity. Some studies have shown that gliclazide has beneficial effects on the hemorrheologic abnormalities seen in diabetic vascular disease [8-12].

To assess this clinically, we measured platelet activities and fibrinolysis in patients with type 2 DM treated with gliclazide; and we compared the results with those obtained in patients treated with glibenclamide.

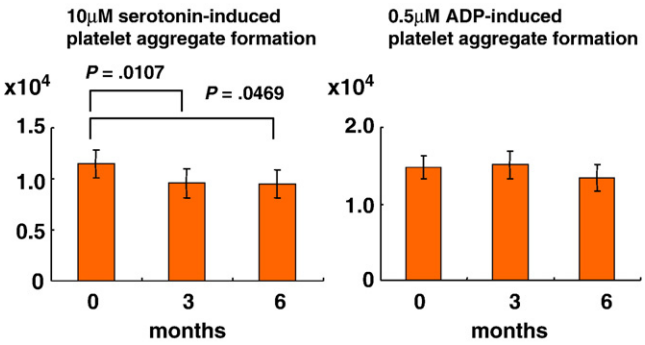

Fig. 1. Effects of gliclazide on platelet aggregation. Data are expressed as mean ± SEM.

2. Subjects and methods

2.1. Subjects

Fourteen patients with type 2 DM (9 men and 5 women; age [mean ± SEM], 61.5 ± 2.6) were randomly chosen as subjects. They were admitted to our metabolic ward between the years 2001 and 2002. Diagnosis of diabetes was based on World Health Organization 1998 criteria. All patients were treated with diet and glibenclamide. All the procedures in the study and the protection of the patients' private information were approved by the ethical committee of Hyogo College of Medicine. Informed consent was obtained from each patient before enrollment in the study.

2.2. Experimental protocol

We switched from glibenclamide to gliclazide using the average ratio of the respective doses, 2.5 vs 40 mg (1.25-20 mg in 2 patients, 2.5-40 mg in 6 patients, 5.0-80 mg in 2 patients, and 7.5-120 mg in 3 patients). We titrated the dose of gliclazide to keep the glycemic control at the same level as in the glibenclamide control. Patients' blood was assayed 3 times: before switching from glibenclamide to gliclazide and then 3 and 6 months after switching.

2.3. Blood sample preparation

Blood was collected in fasting condition on the respective mornings. Venous blood was drawn into 3.8% sodium citrate (1:9 vol/vol). Platelet-rich plasma (PRP) and platelet-poor plasma were obtained by centrifugation of the citrated blood at room temperature for 10 minutes at 150*g* and for 15 minutes at 3000*g*, respectively. The platelet count in PRP was adjusted to 2×10^{11}/L with platelet-poor plasma.

For the measurement of PAI-1, blood was centrifuged for 15 minutes at 3000*g*; and the supernatant was kept at −80 °C until assayed. Fasting plasma glucose (FPG), immunoreactive insulin (IRI), hemoglobin A_{1c} (HbA_{1c}), fasting serum concentrations of total cholesterol (T-Chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-Chol), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), thrombin-antithrombin III complex (TAT), and plasmin-α2-plasmin inhibitor complex (PIC) were also measured. Total cholesterol, TG, and HDL-

Table 2
Effects of gliclazide on metabolic factors

	Before	3 mo	6 mo
BMI (kg/m^2)	22.5 ± 0.8	22.3 ± 0.8	21.9 ± 0.8
HbA_{1c} (%)	7.4 ± 0.2	8.0 ± 0.3	7.7 ± 0.3
FPG (mg/dL)	165.0 ± 7.0	166.0 ± 9.9	170.0 ± 8.0
IRI (μU/mL)	7.8 ± 1.6	7.5 ± 1.5	7.4 ± 1.1
HOMA-R	3.0 ± 0.6	3.0 ± 0.8	3.0 ± 0.4
T-Chol (mg/dL)	205.0 ± 8.3	205.0 ± 8.7	201.0 ± 9.4
TG (mg/dL)	121.0 ± 22.1	102.0 ± 10.6	135.0 ± 19.3
HDL-Chol (mg/dL)	50.0 ± 2.6	50.0 ± 2.3	48.0 ± 1.8

Table 3
Effects of gliclazide on coagulation test and PAI-1

	Before	3 mo	6 mo
PT-INR	0.93 ± 0.01	0.92 ± 0.01	0.92 ± 0.02
APTT (s)	25.8 ± 0.7	25.8 ± 0.6	26.6 ± 0.4
Fbg (mg/dL)	300.0 ± 20.7	301.0 ± 9.5	340.0 ± 11.8
TAT (ng/mL)	50.2 ± 22.2	15.3 ± 5.4	25.8 ± 7.8
PIC (μg/mL)	0.8 ± 0.1	0.8 ± 0.1	1.3 ± 0.3
PAI-1 (ng/mL)	42.0 ± 5.6	35.4 ± 4.9	36.4 ± 5.3

Data are expressed as mean ± SEM. INR indicates international normalized ratio.

Chol were assayed using an autoanalyzer (JCA-BM 2250; Nihon Denshi, Akishima, Tokyo, Japan), while HbA_{1c} was measured by high-performance liquid chromatography (HLC-723G7 system; Tosoh, Tokyo, Japan). The subjects were then divided into 2 groups depending on whether their HbA_{1c} levels were improved or aggravated 6 months after switching from glibenclamide to gliclazide.

2.4. Platelet aggregation

Platelet aggregation was monitored with an AG10 aggregometer (Kowa, Tokyo, Japan) that determines the size and number of platelet aggregates based on particle counting using light scattering [6,13]. A laser beam (675 nm) is passed through a platelet suspension, and the intensity of light scattering provides information on the number and size of aggregates. Data were recorded as a 2-dimensional graph showing the change over time of total light intensity expressed as cumulative summation. The total light intensities of small aggregates were determined. Particles with an intensity of 25 to 400 mV represent small aggregates consisting of less than 100 platelets. Platelet-rich plasma (180 μL) was placed in a cuvette and incubated for 3 minutes at 37°C while rotating at 1000 rpm. Subsequently, 20 μL of 5-HT (final concentration, 10 μmol/L) or adenosine diphosphate (ADP) (0.5 μmol/L) was added; and the formation of platelet aggregates was monitored for 5 minutes. For this experiment, we determined the peak level of aggregate formation.

2.5. Statistical analysis

Values are presented as means ± SEM. Correlations were assessed using Spearman rank correlation test. Multiple regression analysis was performed to assess the combined influence of variables on percentage change of plasma PAI-1 levels. The Wilcoxon signed rank test or the Mann-Whitney *U* test were used for comparison. Differences were considered significant at $P < .05$. All the statistical analyses were performed using StatView J-5.0 software (SAS Institute, Berkeley, CA).

3. Results

3.1. Clinical characteristics of the patients

The clinical characteristics of the enrolled patients in the study are summarized in Table 1. The mean FPG was 165.0 ± 7.0 mg/dL (reference range, 70-110 mg/dL), and HbA_{1c} was 7.4% ± 0.2% (reference range, 4.0%-5.4%).

3.2. Change of platelet aggregation and metabolic factors after switching to gliclazide

After switching from glibenclamide to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced ($P = .0107$, compared with glibenclamide treatment) after 3 months and ($P = .0469$, compared with glibenclamide treatment) after 6 months, although the ADP-induced platelet aggregate formation was not changed at all (Fig. 1). The switch from glibenclamide to gliclazide did not modify body mass index (BMI), FPG, IRI, homeostasis model assessment of insulin resistance (HOMA-R), HbA_{1c}, T-Chol, TG, and HDL-Chol (Table 2).

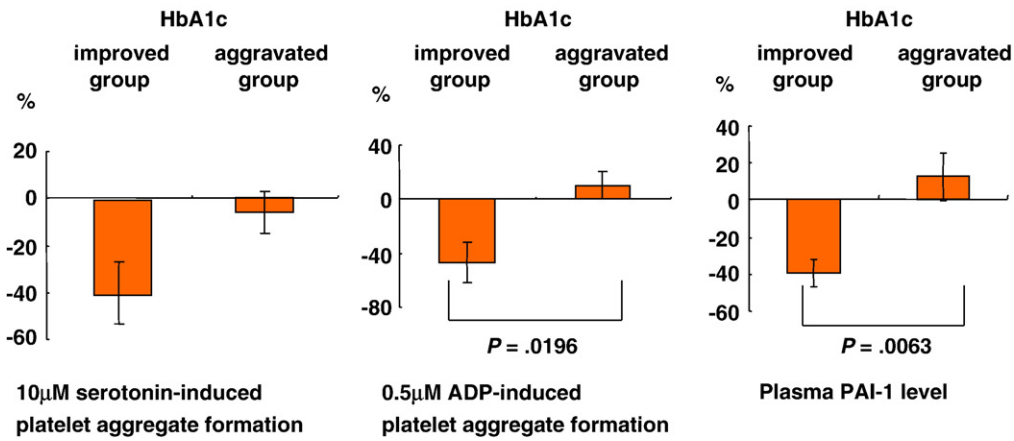

Fig. 2. Percentage change of platelet aggregate formations by 10 μmol/L serotonin or 0.5 μmol/L ADP and plasma PAI-1 level depend on the change of glycemic control after switching to gliclazide. Data are expressed as mean ± SEM.

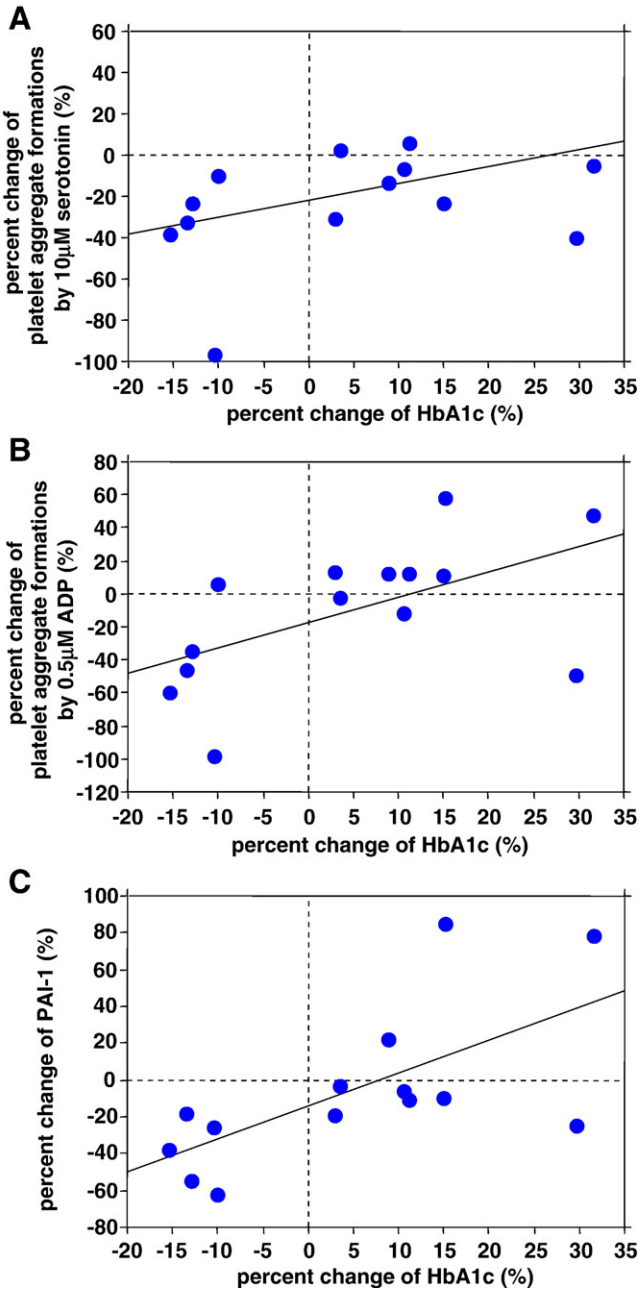

Fig. 3. A, Correlation between percentage change of HbA_{1c} and platelet aggregate formations by 10 μmol/L serotonin after switching to gliclazide (r = 0.39, P = .1712). B, Correlation between percentage change of HbA_{1c} and platelet aggregate formations by 0.5 μmol/L ADP after switching to gliclazide (r = 0.56, P = .0370). C, Correlation between percentage change of HbA_{1c} and PAI-1 after switching to gliclazide (r = 0.65, P = .0115).

3.3. Change of the levels of coagulation factors and PAI-1 after switching to gliclazide

After switching from glibenclamide to gliclazide, PT, APTT, Fbg, TAT, PIC, and PAI-1 were not changed significantly, although PAI-1 would tend to decrease (Table 3).

3.4. Relationship between platelet aggregate formation, plasma PAI-1, blood pressure, and glycemic control

At the end of the 6-month gliclazide treatment and compared with the group of patients with aggravated levels of HbA_{1c} (n = 9), patients with improved HbA_{1c} levels (n = 5) had significantly reduced ADP-induced platelet aggregate formation (P = .0196) and plasma PAI-1 levels (P = .0063) (Fig. 2).

Linear regression analysis showed that percentage change of HbA_{1c} correlated positively with both percentage change of platelet aggregate formation by 0.5μmol/L ADP (r = 0.56, P = .0370) (Fig. 3B) and percentage change of PAI-1 (r = 0.65, P = .0115) (Fig. 3C) after switching to gliclazide.

The mean systolic blood pressure (135.9 ± 3.9 mm Hg) in the group of patients with aggravated levels of HbA_{1c} was not significantly different from the mean systolic blood pressure (124.0 ± 9.2 mm Hg) in the group of patients with improved HbA_{1c} levels. The mean diastolic blood pressure (76.7 ± 2.4 mm Hg) in the former group was not significantly different from the mean diastolic blood pressure (70.2 ± 6.2 mm Hg) in the latter group. Percentage change of mean systolic blood pressure (−5.6% ± 4.3%) in the former group was not significantly different from percentage change of the mean systolic blood pressure (2.1% ± 2.5%) in the latter group. Percentage change of mean diastolic blood pressure (−2.0% ± 3.1%) in the former group was not significantly different from percentage change of the mean diastolic blood pressure (6.8% ± 2.7%) in the latter group.

3.5. Relationship between plasma PAI-1 and various factors

Multiple regression analysis showed that, after switching to gliclazide, the percentage change of ADP-induced platelet aggregate formation (r = 0.540, P = .0401) was independently associated with the percentage change of plasma PAI-1 level in addition to the percentage change of HbA_{1c} (r = 0.657, P = .0310) (R = 0.939, P = .0188) (Table 4). The other independent variants including the final dose of gliclazide, HOMA-R, percentage change of PT–international normalized ratio, APTT, and T-Chol were not significantly associated with percentage change of PAI-1.

Table 4
Multiple regression analysis with percentage change of plasma PAI-1 level

	Regression coefficient	SEM	Standardized regression coefficient	P
Percentage change of ADP-induced platelet aggregate formation	0.539	0.207	0.540	.0310
Percentage change of HbA_{1c}	1.809	0.645	0.657	.0401

4. Discussion

We found that platelet aggregate formation induced by 5-HT was significantly reduced after switching from glibenclamide treatment to gliclazide under the same conditions of metabolic control. Serum advanced glycation end products (AGEs) are significantly higher in DM subjects compared with healthy subjects [14], and our previous study indicated that enhancement of 5-HT–induced platelet aggregation in DM is dependent on the increased level of AGEs [15]. Gliclazide may therefore decrease the effect of AGEs on the enhancement of 5-HT–induced platelet aggregate formation in type 2 DM patients. When we switched from glibenclamide to gliclazide, BMI, FPG, IRI, HbA_{1c}, T-Chol, and TG were not changed at all. These results indicate that gliclazide inhibits platelet aggregation via the serotonin pathway, independently of the metabolic and/or glycemic control per se. Although gliclazide is a more potent ADP-induced platelet aggregation inhibitor than glibenclamide [16], ADP-induced platelet aggregate formation was not changed in our study when we switched from glibenclamide to gliclazide. We reported that ADP-induced platelet aggregation is increased by AGEs; but this increment is diminished by addition of sarpogrelate, a selective 5-HT receptor antagonist [15]. In the group with improved HbA_{1c}, ADP-induced platelet aggregate formation and plasma PAI-1 level were significantly reduced compared with the group with aggravated HbA_{1c}. Although a relationship between the level of blood pressure (particularly hypertensive levels) and platelet activation has been reported, there was no significant difference of hypertensive levels between the groups with aggravated and improved HbA_{1c} levels. The percentage change of ADP-induced platelet aggregate formation was independently associated with the percentage change of plasma PAI-1 level in addition to percentage change of HbA_{1c} after switching to gliclazide by multiple regression analysis. In some reports, an improved metabolic control of type 2 DM could significantly decrease the elevated concentrations of PAI-1. The decrement in PAI-1 is induced by drugs with dissimilar effects on insulin secretion (ie, glipizide gastrointestinal therapeutic system and metformin), emphasizing the important contribution that metabolic control has on this process [17].

Furthermore, in patients with improved glycemic control, gliclazide could inhibit ADP-induced platelet aggregation and PAI-1 level. Gliclazide rather than glibenclamide has been reported to attenuate the progression of carotid intima-media thickness in subjects with type 2 DM [18]. Furthermore, in a population-based case-control and follow-up study, the risk of myocardial infarction would appear to be higher among users of old sulfonylureas including glibenclamide (adjusted odds ratio, 2.07; 95% confidence interval, 1.81-2.37) than among users of new sulfonylureas including gliclazide (adjusted odds ratio, 1.36; confidence interval, 1.01-1.84) [19]. Recently, in the ADVANCE trial (Action in Diabetes and Vascular disease: preterAx and diamicroN modified release Controlled Evaluation), an intensive glucose-control strategy using gliclazide (modified release) and other drugs as required lowered the average HbA_{1c} value to 6.5% in a broad range of patients with type 2 DM and reduced the incidence of the combined primary outcome of major macrovascular or microvascular events [20]. We should have listed the limitations of the study in the interpretation of the results. All patients were switched from glibenclamide to gliclazide. Although we have claimed that the subjects were under the same conditions of metabolic control, at the end of the 6-month period, there was a group with aggravated levels of HbA_{1c} and a group with improved HbA_{1c} levels. To compare the 2 drugs, half the patients should have continued on glibenclamide; or, more practicable with the small number, a cross-over design could have been used. This would permit comparing the 2 drugs in the patients with matching HbA_{1c} levels.

In conclusion, the study results demonstrate that gliclazide inhibits serotonin-induced platelet aggregation independently of glycemic control, although being less effective on ADP-induced aggregation, and may have a better effect on the reduction of platelet aggregability than glibenclamide. Very importantly, this study supports previous results showing the reduction in platelet aggregability and reduction in PAI-1 level with the improvement in glycemic control. Therefore, gliclazide may be more useful for the prevention of diabetic vascular complications than glibenclamide via beneficial and pleiotropic effects on the hemorrheologic abnormalities.

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