



Regular Article

# Effect of increasing doses of aspirin on platelet function as measured by PFA-100 in patients with diabetes

Adnan Abaci<sup>a,\*</sup>, Yucel Yilmaz<sup>b</sup>, Mustafa Caliskan<sup>b</sup>, Fahri Bayram<sup>c</sup>,  
Mustafa Cetin<sup>d</sup>, Ali Unal<sup>d</sup>, Servet Cetin<sup>b</sup>

<sup>a</sup>Department of Cardiology, Gazi University School of Medicine, Ankara 06550, Turkey

<sup>b</sup>Department of Cardiology, Erciyes University School of Medicine, Kayseri, Turkey

<sup>c</sup>Department of Endocrinology, Erciyes University School of Medicine, Kayseri, Turkey

<sup>d</sup>Department of Hematology, Erciyes University School of Medicine, Kayseri, Turkey

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## KEYWORDS

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## Abstract

**Introduction:** Platelets of diabetic patients have been reported to be less sensitive to aspirin. The aim of this study is to compare a medium (300 mg) and low (100 mg) dose of aspirin on platelet function in diabetic patients.

**Methods:** We have included one hundred and two patients with type 2 diabetes mellitus. Platelet function was measured as closure time (CT) with the Platelet Function Analyzer (PFA)-100™ before the administration of aspirin. Initially the patients were given 100 mg aspirin once daily for seven days, and then the measurements were repeated. If the CT exceeded the upper limit of 300 s, the study was terminated. If not, the patients continued the aspirin therapy with a dose of 300 mg daily for another seven days, and the CTs were measured again.

**Results:** After taking 100 mg aspirin, the CT significantly increased from  $126 \pm 29$  s to  $256 \pm 66$  s ( $p < 0.001$ ). In 68 of 102 (67%) patients, the CT increased to 300 s. In the remaining 34 patients, the baseline CT was  $113 \pm 29$ , and increased to  $170 \pm 45$  s after 100 mg aspirin ( $p < 0.001$ ). In these patients, there was a further increase in the CT from  $170 \pm 45$  to  $229 \pm 75$  s following 300 mg aspirin ( $p < 0.001$ ). On average, the CT was increased by 60% and 39% following ingestion of 100 and 300 mg aspirin, respectively. CT > 300 s were obtained in 15 (44%) of 34 patients after 300 mg aspirin.

\* Corresponding author. Gazi Universitesi Tip Fakultesi, Kardiyoloji Anabilim Dalı, Beşevler/Ankara 06550, Turkey. Tel.: +90 312 318 6447; fax: +90 312 212 9012.

E-mail address: abaci@gazi.edu.tr (A. Abaci).

*Conclusions:* Although, a daily dose of 100 mg aspirin effectively inhibited platelet function in a majority of diabetics, a considerable proportion of patients showed a greater platelet inhibition with the use of 300 mg aspirin. The PFA-100™ closure time may be used to separate those patients who require a higher dose of aspirin to achieve desired antiplatelet effect.

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Aspirin reduces the risk of cardiovascular events in all patients with coronary artery disease [1]. However, the optimal dosage of aspirin is subject to great debate. Meta-analysis of the randomized trials failed to reveal greater benefit from doses  $\geq 350$  mg compared with lower doses [1]. The ACC/AHA guidelines for the treatment of acute myocardial infarction recommend an initial dose of 162–325 mg to achieve full antiplatelet effect rapidly [2]. Although, comparison of the two lower doses of aspirin with one another has not been performed, 75–325 mg of aspirin daily is recommended in patients with chronic stable angina for long-term therapy [3].

Diabetes is associated with an increased risk of cardiovascular events, even in the absence of diagnosed cardiovascular disease [1]. The American Diabetes Association recommended low-dose (75–150 mg daily) aspirin as a primary prevention strategy not only in high-risk diabetic patients but for anyone with diabetes who is  $>30$  years of age and has no known contraindications [4]. Platelets of patients with diabetes exist in a relatively activated state, and synthesize significantly higher amounts of thromboxane than those from nondiabetic controls [5–7]. Consequently, diabetics have increased platelet aggregation and thrombus formation [8,9]. Aspirin-induced suppression of platelet thromboxane synthesis is lower in diabetic patients compared to nondiabetic individuals [10] and a higher incidence of aspirin resistance in diabetics has been reported [11]. Therefore, low-dose aspirin might be inadequate for inhibition of platelet function, and relatively higher doses of aspirin may be needed in diabetic patients. The aim of the study was to compare the medium (300 mg) and low (100 mg) dose of aspirin on platelet function in diabetic patients.

## Methods

We included the patients with type 2 diabetes mellitus who did not use aspirin or other drugs known to modify platelet function for at least two weeks prior to beginning of the study. Patients with an active duodenal or gastric ulcer, a history of

previous upper gastrointestinal hemorrhage, a platelet count less than  $150 \times 10^9/L$ , hemoglobin  $<12$  g/dL, or known intolerance to aspirin were not included in the study. Diabetes was defined if the patients were taking insulin or oral hypoglycemic drugs on the basis of elevated ( $>7.0$  mmol/L) levels of fasting blood glucose on at least two separate assessments. The study was approved by the local ethics committee of our institution, and written informed consent was obtained in all patients before the study.

## Blood sampling

Blood samples for the determination of platelet function was obtained from the antecubital vein using a 19-gauge needle and collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) anticoagulated with 3.8% sodium citrate. Throughout the study, same type of Vacutainer tubes were used. One additional tube of blood anticoagulated with ethylenediaminetetraacetic acid was collected for the hematocrit and platelet count analysis. The blood was drawn 2 h after the last dose of aspirin. Daily variation of platelet aggregation has been reported in healthy subjects [12]. Therefore, platelet function studies were performed at the same time of day in all patients in this study.

## Platelet function

Platelet function was measured by the Platelet Function Analyzer (PFA)-100™ assay which is a simple and easy to use system that can provide a quantitative measure of platelet function in vitro using anticoagulated whole blood [13,14]. The test cartridge simulates an injured blood vessel and measures the time required to form a platelet plug (defined as CT) that occludes a microscopic aperture cut into a collagen/epinephrine or collagen/ADP coated membrane under high shear flow condition [13,14]. The collagen/epinephrine cartridge is the primary cartridge for detection of aspirin effect on platelet function. Previous studies have shown that effect of aspirin treatment on platelet function can be measured with the PFA-100

**Table 1** The baseline characteristics of the study patients

	Total (n=102)
Duration of diabetes (years)	6.4 ± 6.1
Age (years)	50 ± 11
Women	61 (59.8)
Serum cholesterol (mmol/L)	5.14 ± 1.37
Hypertension	48 (47.1)
Smoking	22 (21.6)
Medication use	
Insulin	46 (45.1)
β-blocker	7 (6.9)
ACEI	41 (40.2)
ARB	4 (3.9)
CCB	13 (12.7)
Statin	12 (11.8)
HbA1c (%)	8.1 ± 2.4
Baseline CT (s)	126 ± 29 (71–182)
Hematocrit	
Before the aspirin	0.41 ± 0.05
After the aspirin	0.41 ± 0.05
Platelet count (×10 <sup>9</sup> /L)	
Before the aspirin	275 ± 76
After the aspirin	275 ± 70

Values are given as mean ± SD (range) or number (%). CT=closure time, ACEI=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker, CCB=calcium canal blocker, NS=non-significant.

system [14,15]. CT measurements were performed using collagen/epinephrine cartridges according to the manufacturer's instruction not earlier than 30 min after and within 2 h of blood sampling. The maximal CT for collagen/epinephrine cartridges is 300 s and values greater than 300 s are reported as non-closure. Platelet function was determined before the administration of aspirin. Initially the patients were given 100 mg aspirin once daily for seven days, the measurements were then

repeated. If the CT exceeded the upper limit of 300 s, the study was terminated. If not, the patients continued the aspirin therapy at a dose of 300 mg daily for seven days, and the CT was again measured.

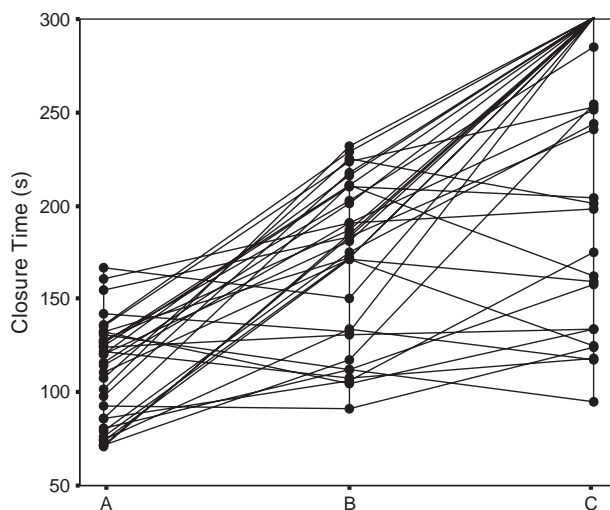
## Statistical analysis

Continuous variables were given as mean values ± SD, and categorical variables as percentages. A chi-square test was used for comparison of categorical variables. Comparison of continuous variables were performed by means of Student's *t* test. The relations between the variables and the baseline CT were evaluated using the Spearman's correlation coefficient or the Pearson's correlation coefficient. Statistical analysis was performed by use of the SPSS statistical software package (version 10.0). A *p* value of <0.05 was considered statistically significant.

## Results

One hundred and two patients with type 2 diabetes mellitus were included in the study. The baseline characteristics of the study subjects are given in Table 1. Of the patients, 46 (45.1%) were insulin-treated and 56 (54.9%) were treated by oral agents. The duration of diabetes ranged from 1 month to 25 years, averaging 6.4 ± 6.1 years. The mean level of HbA1c was 8.1 ± 2.4%. There were no hemorrhagic complications due to aspirin.

Before aspirin administration, the CT ranged 71–182 s, (mean 126 ± 29, median 129.5 s). Patients receiving insulin had a similar baseline CT as



**Figure 1** Closure times before (A), after 100 mg (B) and 300 mg (C) doses of aspirin in 34 patients in which the closure times did not exceeded the upper limit of 300 s. \**p* < 0.05 (100 mg vs. before treatment and 300 mg vs. 100 mg).

compared to patients receiving oral hypoglycemic agents ( $124 \pm 27$  vs  $128 \pm 31$  s, respectively). There is no relation between the baseline CT and age, gender, hypertension, smoking, serum cholesterol level, hematocrit, platelet count, and HbA1c.

At the end of one week of 100 mg per day aspirin administration, the CT significantly increased to  $256 \pm 66$  s (range 91–300) ( $p < 0.001$ ). The CT was the same in both genders after the administration of aspirin. In 68 of 102 (67%) patients, the CT increased to 300 s, which is the upper limit for the collagen/epinephrine cartridges. In these patients the baseline CT was  $132 \pm 28$  s.

In the remaining 34 patients, the baseline CT was  $113 \pm 29$  s (range 71–167), and increased to  $170 \pm 45$  s (range 91–232) after 100 mg aspirin daily ( $p < 0.001$ ) (Fig. 1). With these patients, there was a further increase in CT from  $170 \pm 45$  s to  $229 \pm 75$  s (range 95–300) following 300 mg aspirin daily ( $p < 0.001$ ). On average, the CT was increased by 60% and 39% following ingestion of 100, and 300 mg aspirin, respectively. In fifteen (44%) of the 34 patients, the CT increased to the upper limit of 300 s after 300 mg aspirin daily.

## Discussion

Our results showed that aspirin at 100 mg daily was effective in the inhibition of platelet function as assessed by PFA-100 in majority of patients. However, there was a further increase in platelet inhibition with higher aspirin doses in patients who have not achieved the desired level of antiplatelet effects from 100 mg aspirin daily. The discussion on the correct dosage of aspirin in platelet inhibition seems to be never ending. Meta-analysis of randomized trials of antiplatelet therapy failed to reveal greater benefit from doses  $\geq 350$  mg compared with lower doses [1]. Therefore, it is now accepted that high doses of 500–1500 mg aspirin daily are no more effective than medium doses of 160–325 mg/day or low doses of 75–150 mg/day [1]. However, there is no large scale, randomized clinical study comparing the medium (160–325) and low dose (75–150) aspirin regimens.

Preference of a low dose of aspirin is due to fewer gastrointestinal side effects and undesired cyclooxygenase inhibition of the vascular wall [16,17]. Good clinical practice should be recommended in the use of the lowest dose of aspirin shown effective in the prevention of events. Although the results of the previous studies demonstrated preventive effects of low or medium

doses of aspirin, there may be no “ideal” dose of aspirin for all patients. If optimal dose of aspirin for a particular patient can be known, a greater proportion of patients may benefit from aspirin because the sensitivity of platelets to aspirin differs between patients. However, there is no study to detect the difference between individualized aspirin dosage and the fix low dose aspirin. Whether individualized aspirin dosage is superior remains an open question. Platelet function tests may be used to differentiate those patients most likely to benefit from a higher aspirin dosage. The PFA-100™ appears to be a simple test for the assessment of antiplatelet effect of aspirin that could be easily employed as a routine test in the clinical practice. However, studies relating to failure of prolonged CT with aspirin and the clinical outcome is lacking.

Bedside testing of aspirin by the PFA-100™ may allow us to measure the antiplatelet effect of aspirin and the ideal dose of aspirin for a particular patient may be determined. In our study, although 100 mg aspirin was effective for the majority of diabetic patients, some patients showed a greater platelet inhibition with the use of 300 mg aspirin. In accordance with the results of our study, Watala et al. have recently shown that the inhibitory effect of 150 mg aspirin a day on platelet function is less profound in diabetic patients compared to nondiabetic individuals and have suggested that at least some patients with diabetes might require higher aspirin doses [11]. Indeed, the results of the primary prevention project trial [18] suggested that low-dose aspirin might be less effective in primary prevention of cardiovascular disease in diabetic patients as compared to nondiabetics.

There are small-sized studies investigating the effect of different doses of aspirin on platelet function in various diseases or health subjects [19–27]. A majority of these studies showed that aspirin inhibited platelet function in a dose dependent manner [21–27]. A dose-related prolongation of bleeding time has been demonstrated in healthy subjects [21]. Helgason et al. [22] showed that inhibition of platelet function is dose dependent in patients taking aspirin for stroke prevention and increase of aspirin dose resulted in complete inhibition of platelet function in more patients. Tohgi et al. [23] showed that with higher doses, platelet aggregability and thromboxane A2 production were inhibited more conspicuously and in a greater proportion of stroke patients. Two recent studies have also shown that aspirin inhibited platelet function in a dose dependent manner in patients with stable coronary artery disease [24] or stroke [25]. To our knowledge, there is no study

comparing two different doses of aspirin on platelet function in diabetic patients.

Aspirin resistance has been reported in 5.5% and 60% of patients using different techniques and different definitions [28]. Patients with diabetes have been reported to have a higher rate of aspirin resistance in comparison to nondiabetic control subjects [11]. Our results also indicated that the prevalence of aspirin resistance may be related to aspirin dose and aspirin resistance can be overcome in some patients by increasing the aspirin dose.

## Limitations

We did not measure the von Willebrand factor (vWF) levels, which is a limitation of our study. As platelet plug formation under shear stress depends on vWF levels, the measured CT negatively correlates with vWF levels [13–15,29]. High levels of vWF may provide an explanation for the poor aspirin response to aspirin in some individuals. Although we did not measure serum salicylate levels, compliance was ascertained by pill count at the time of blood sampling. Since we only included diabetic patients, our results cannot be generalized to all patients in which aspirin therapy is indicated. Although there was a further inhibition of platelet function with higher aspirin doses in patients who have not achieved the desired level of antiplatelet effects from 100 mg aspirin daily, we did not follow the patients for the clinical consequences of dose escalation. Clinical trials are needed to evaluate the efficacy of different doses of aspirin with regard to the incidences of cardiovascular complications of type 2 diabetes. Although the results of our study cannot be used as a basis for recommending individualized aspirin dosing, it may stimulate such a study in this field.

In conclusion, although a daily dose of 100 mg aspirin effectively inhibited platelet function in majority of diabetics, a considerable proportion of patients showed a greater platelet inhibition with the use of higher doses. The PFA-100 closure time may be used to differentiate between those patients who require a higher doses of aspirin to get a desired antiplatelet effect.

## References

- [1] Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ* 2002;**324**:71–86.
- [2] Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: executive summary: a report of the ACC/AHA Task Force on Practice Guidelines. *Circulation* 2004;**110**:588–636.
- [3] Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, et al. ACC/AHA 2002 guidelines update for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2003;**107**:149–58.
- [4] American Diabetes Association. Aspirin therapy in diabetes. *Diabetes Care* 2000;**23**(Suppl. 1):S61–2.
- [5] Mustard JF, Packham MA. Platelets and diabetes mellitus. *N Engl J Med* 1984;**311**:665–7.
- [6] Di Minno G, Silver MJ, Cerbone AM, Riccardi G, Rivellese A, Mancini M, et al. Increased binding of fibrinogen to platelets in diabetes: the role of prostaglandins and thromboxane. *Blood* 1985;**65**:156–62.
- [7] Davi G, Rini GB, Aversa M, Novo S, Di Fede G, Pinto A, et al. Thromboxane B2 formation and platelet sensitivity to prostacyclin in insulin-dependent and insulin-independent diabetics. *Thromb Res* 1982;**26**:359–70.
- [8] Aronson D, Rayfield EJ, Chesebro JH. Mechanisms determining course and outcome of diabetic patients who have had acute myocardial infarction. *Ann Intern Med* 1997;**126**:296–306.
- [9] Bell DSH. Diabetes mellitus and coronary disease. *Coron Artery Dis* 1996;**7**:715–22.
- [10] Mori TA, Vandongen R, Douglas AJ, McCulloch RK, Burke V. Differential effect of aspirin on platelet aggregation in IDDM. *Diabetes* 1992;**41**:261–6.
- [11] Watala C, Golanski J, Pluta J, Boncler M, Rozalski M, Luzak B, et al. Reduced sensitivity of platelets from type 2 diabetic patients to acetylsalicylic acid (aspirin)—its relation to metabolic control. *Thromb Res* 2004;**113**:101–13.
- [12] Fujimura A, Ohashi K, Ebihara A. Daily variations in platelet aggregation and adhesion in healthy subjects. *Life Sci* 1992;**50**:1043–7.
- [13] Mammen EF, Comp PC, Gosselin R, Greenberg C, Hoots WK, Kessler CM, et al. PFA-100 system: a new method for assessment of platelet dysfunction. *Semin Thromb Hemost* 1998;**24**:195–202.
- [14] Kundu SK, Heilman EJ, Sio R, Garcia C, Ostgaard RA. Characterization of an in vitro platelet function analyzer, PFA-100™. *Clin Appl Thromb/Hemost* 1996;**2**:241–9.
- [15] Homoncik M, Jilma B, Hergovich N, Stohlawetz P, Panzer S, Spelser W. Monitoring of aspirin (ASA) pharmacodynamics with the platelet function analyzer PFA-100. *Thromb Haemost* 2000;**83**:316–21.
- [16] Weksler BB, Pett SB, Alonso D, Richter RC, Stelzer P, Subramanian V, et al. Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. *N Engl J Med* 1983;**308**:800–5.
- [17] Hampton KK, Cerletti C, Loizou LA, Bucchi F, Donati MB, Davies JA, et al. Coagulation, fibrinolytic, and platelet function in patients on long term therapy with aspirin 300 mg or 1200 mg daily compared with placebo. *Thromb Haemost* 1990;**64**:17–20.
- [18] Sacco M, et al, on behalf of the PPP Collaborative Group. Primary prevention of cardiovascular events with low-dose aspirin and vitamin E in type 2 diabetic patients. *Diabetes Care* 2003;**26**:3264–72.
- [19] Boss AH, Boysen G, Olseen JS. Effect of incremental doses of aspirin on bleeding time, platelet aggregation and thromboxane production in patients with cerebrovascular disease. *Eur J Clin Invest* 1985;**15**:412–4.
- [20] Dabaghi SF, Kamat SG, Payne J, Marks GF, Roberts R, Schafer AI, et al. Effects of low-dose aspirin on in vitro

- platelet aggregation in the early minutes after ingestion in normal subjects. *Am J Cardiol* 1994;**74**:720-3.
- [21] Buchanan MR, Brister SJ. Individual variation in the effects of ASA on platelet function: implications for the use of ASA clinically. *Can J Cardiol* 1995;**11**:221-7.
- [22] Helgason CM, Tortorice KL, Winkler ER, Penney DW, Schuler JJ, McClelland TJ, et al. Aspirin response and failure in cerebral infarction. *Stroke* 1993;**24**:345-50.
- [23] Tohgi H, Konno S, Tamura K, Kimura B, Kawano K. Effect of low-to-high doses of aspirin on platelet aggregability and metabolites of thromboxane A2 and prostacyclin. *Stroke* 1992;**24**:1400-3.
- [24] Malhotra S, Sharma YP, Grover A, Majumdar S, Hanif SM, Bhargava VK, et al. Effect of different aspirin doses on platelet aggregation in patients with stable coronary artery disease. *Intern Med J* 2003;**33**:350-4.
- [25] Gan R, Teleg RA, Florento L, Bitanga ES. Effect of increasing doses of aspirin on platelet aggregation among stroke patients. *Cerebrovasc Dis* 2002;**14**:252-5.
- [26] Feng D, McKenna C, Murillo J, Mittleman MA, Gebara OC, Lipinska I, et al. Effect of aspirin dosage and enteric coating on platelet reactivity. *Am J Cardiol* 1997;**80**:189-93.
- [27] Hart RG, Leonard AD, Talbert KL, Pearce LA, Cornell E, Bovill E, et al. Aspirin dosage and thromboxane synthesis in patients with vascular disease. *Pharmacotherapy* 2003;**23**:579-84.
- [28] Wong S, Appleberg M, Ward CM, Lewis DR. Aspirin resistance in cardiovascular disease: a review. *Eur J Vasc Endovasc Surg* 2004;**27**:456-65.
- [29] Chakroun T, Gerotziafas G, Robert F, Lecrubier C, Samama MM, Hatmi M, et al. In vitro aspirin resistance detected by PFA-100™ closure time: pivotal role of plasma von Willebrand factor. *Br J Haematol* 2004;**124**:80-5.