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Research Article

PLATELET AGGREGATION AND SERUM THROMBOXANE B_2 LEVEL AFTER TAKING 60 MG/DAY OF ASPIRIN IN TYPE 2 DIABETIC THAI PATIENTS

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ABSTRACT

This study is objected to determine whether 60 mg/day dose of aspirin inhibit platelet aggregation capacity adequately to be clinically effective in diabetic patients. Total 97 diabetic patients who were taking low doses of aspirin participated in the study; among these, 75 patients were taking 60 mg/day of aspirin. Besides, 32 diabetes patients who were not taking aspirin were recruited as the control group. Platelet function was assessed by optical platelet aggregation technique using arachidonic acid and ADP as agonists and serum thromboxane B_2 level was determined by enzyme immunoassay (EIA) technique. Platelet functions as assessed by optical platelet aggregation after taking 60 mg/day of aspirin did not differ from those obtained after taking 300 mg/day of aspirin. The frequency of aspirin resistance after 60 mg/day of aspirin was similar the results previously reported for higher doses of aspirin. The serum thromboxane was more than 95% inhibited after taking 60 mg/day of aspirin. Aspirin 60 mg/day may be sufficient to be used in average type 2 diabetic patients for prevention of adverse cardiovascular events.

Keywords: Aspirin, Serum thromboxane level, Platelet aggregation, Diabetes, Aspirin resistance

INTRODUCTION

Aspirin therapy for platelet inhibition is recommended for diabetic patients as a mean to reduce risk for atherothrombotic vascular events[1]. Aspirin irreversibly inhibits cyclooxygenase-1 (COX-1), thereby blocking the production of thromboxane A_2, a powerful activator of platelet aggregation [2]. There is a general agreement that the inhibition in terms of platelet thromboxane forming capacity, as assessed from determination of serum thromboxane level should be more than 95%, to be clinically effective [3].

A proportion of patients experience recurrent atherothrombotic events despite antiplatelet therapy which lead to the concept of "resistance" to antiplatelet agent [4, 5]. Aspirin resistance involves inadequate inhibition of the COX-1-mediated thromboxane A_2 pathway [6]. The prevalence of aspirin resistance reported by investigators varies extensively, 5-60% of adult patients [7-10]. The results were varying due to differences in the definition of resistance, methods of platelet investigation or type of assay used, dose of aspirin, and patient population under consideration[10]. Two principally different methods of laboratory control for platelet sensitivity to aspirin are available: measurement of platelet function or measurement of inhibition of thromboxane formation.

The American Diabetes Association (ADA) and the American Heart Association (AHA) recommends the use of low-dose aspirin (75-162 mg/day) in diabetic patients as a primary and secondary prevention of cardiovascular events [11]. Recently, clinical data were not support the use of aspirin doses greater than 75 to 81 mg/d. Besides, higher dosages were associated with increased risk of bleeding complications [12]. Recent review panel endorsed by the American Diabetes Association, the American Heart Association, and the American College of Cardiology Foundation published a revised recommendation in the journal Diabetes Care. The experts found the risks of aspirin-related side effects, such as stomach bleeding and the much lower chance of bleeding strokes, must be carefully weighed against the potential benefits of using aspirin especially in diabetic men younger than 50 and diabetic women younger than 60 who have no other risk factors [13].

Few years ago, diabetic patients in Thailand were commonly prescribed with 60 mg/day dose of aspirin as a prevention of cardiovascular events. This present study was therefore aimed to determine whether 60 mg/day dose of aspirin could be an initial dose which was as effective as the higher recommended low dose of aspirin, which are more frequently prescribed worldwide. Platelet function and serum thromboxane B_2 of patients who received 60 mg/day dose of aspirin and those who received higher low doses of aspirin would be measured and compared. Prevalence of aspirin in type 2 diabetic patients treated with 60 mg/day of aspirin would be determined via optical platelet aggregation technique using arachidonic acid and adenosine diphosphate (ADP) as agonists. The percentage of aspirin resistance obtained would be compared to those previously reported for those higher low doses of aspirin. Serum thromboxane B_2 would also be determined and compared between type 2 diabetic patients who received and did not receive 60 mg/day of aspirin.

MATERIALS AND METHODS

The present study was approved by the ethics committees of Ramathibodi Hospital, Bangkok, Thailand. Written informed consent was obtained from all participants. Patients with type 2 diabetes mellitus, who had taken 60 mg/day up to 325 mg/day of aspirin for more than 14 days, were eligible for enrollment. Exclusion criteria included: injection of insulin, taking other antiplatelet medications such as clopidogrel or ticlopidine, use of other drugs containing aspirin or nonsteroidal anti-inflammatory agents or cyclooxygenase-2 inhibitors, administration of warfarin or heparin.

Medical records were reviewed for age, gender, history of cardiovascular disease and concomitant medications. Blood samples were obtained 24 ± 2 hours after the administration of the last dose of aspirin. Platelet function was assessed by optical platelet aggregation using a Chronolog Lumi-Aggregometer (model 560 Ca, Chronolog, Inc.). Platelets in platelet rich plasma (PRP) were stimulated with 1 mmol/l of arachidonic acid and 10 μM of ADP. Aggregation was expressed as the maximal percent change in light transmittance from baseline, using platelet-poor plasma as a reference. Each sample was analyzed in duplicate. All platelet aggregation tests were performed within two hours after blood collection. Aspirin resistance was defined as a mean aggregation of ≥ 20% with 1 mM arachidonic acid and a mean aggregation of ≥ 70% with 10 μM of ADP. Aspirin semiresponders were defined as meeting one, but not both of the above criteria [7]. Serum thromboxane B_2 level was determined by enzyme immunoassay (EIA) technique (Thromboxane B_2 EIA Kit Cayman chemical Co., cat no. 519031.1). Fasting plasma glucose, HbA1c, lipid profile, and complete blood count were sent to analyze at the central lab of Ramathibodi Hospital.

Patient characteristics and laboratory data were presented as mean ± S.D. Gender was summarized as frequency (percentage). Percent platelet aggregation and serum thromboxane B_2 level were summarized as means, medians, and interquartile (IQ) ranges. Student's *t* test was used to compare percent platelet aggregation

between different dosages of aspirin. Comparisons of serum thromboxane B_2 level between different doses of aspirin were performed by Mann-Whitney *U* test. Analysis of variance (ANOVA) technique and Turkey post hoc comparisons were used to compare serum thromboxane B_2 level and clinical data among aspirin resistance, aspirin semiresponders, and aspirin sensitive patients. A two tailed *p* value of < 0.05 was considered statistically significant. Data was analyzed using computer programs SPSS for windows (Statistical Package for Social Science for windows) version 11.5.

RESULTS

The total 97 diabetic patients who were taking low doses of aspirin participated in this study; among these, 75 patients were taking 60 mg/day of aspirin. Besides, 32 diabetic patients who were not taking aspirin were participated as the control group.

Effect of dosage of aspirin on platelet aggregation induced by arachidonic acid

In patients who were taking either dose of aspirin, the mean platelet aggregations induced by arachidonic acid were significantly lower than those who did not received aspirin (p<0.001) (Table 1). However, 300 mg/day aspirin did not show significantly lower platelet aggregation value than 60 mg/day aspirin. Most patients in 60 mg/day group had platelet aggregation less than 25%; however, there were 2 patients who had platelet aggregation between 35 and 45, and 8 patients who had platelet aggregation between 65 and 85. Of the 16 patients who received 300 mg/day aspirin, 15 had platelet aggregation ≤ 20% while platelet aggregation of 1 patient was 45%.

Effect of dosage of aspirin on platelet aggregation induced by ADP

In patients who were taking 60 mg/day and 300 mg/day doses of aspirin, the mean platelet aggregations induced by ADP were significantly lower than those who did not receive aspirin (p<0.001) (Table 2). However, no significant differences in the means of ADP induced platelet aggregation were found among patients who were taking different doses of aspirin.

Effect of dosage of aspirin on serum thromboxane B_2 level

Table 3 illustrated serum thromboxane B_2 levels after taking different dosages of aspirin. Serum thromboxane B_2 level of patients after taking 300 mg/day of aspirin was significantly lower than those after 60 mg/day (median = 0.046 ng/ml vs 0.274 ng/ml, p<0.001). The median reduction of serum thromboxane B_2 in 60 mg/day aspirin group and 300 mg/day aspirin group as compared to that in non-aspirin group were 95.04% and 99.17%, respectively.

Table 1: Aspirin dosage and platelet aggregation induced by arachidonic acid

Dose (mg/day)	N	Mean %aggregation	SD	Median	P25	P75
0	32	79.34	7.34	81.00	74.25	84.00
60	75	18.38*	19.91	11.00	9.00	17.00
100	1	9.00	-	9.00	-	-
120	2	18.00	1.41	18.00	-	-
150	3	12.67	2.52	13.00	-	-
300	16	15.31*	8.79	13.00	9.50	17.25

* p<0.001 compared with non-aspirin group; P25 = 25th percentile, P75 = 75th percentile.

Table 2: Aspirin dosage and platelet aggregation induced by ADP

Dose (mg/day)	N	Mean %aggregation	SD	Median	P25	P75
0	32	72.09	8.25	72.50	66.00	78.00
60	75	62.54*	8.77	63.00	57.00	68.00
100	1	70.00	-	70.00	-	-
120	2	66.00	5.66	66.00	-	-
150	3	50.67	10.60	49.00	-	-
300	16	60.00*	10.54	56.50	50.25	72.25

* p<0.001 compared with non-aspirin group; P25 = 25th percentile, P75 = 75th percentile.

Table 3: Aspirin dosage and serum thromboxane B_2 levels

Dose		Serum thromboxane B_2 (ng/ml)				
(mg/day)	N	Mean	SD	Median	P25	P75
0	32	6.582	5.161	5.526	3.004	8.318
60	75	0.701	1.601	0.274^{A}	0.085	0.537
100	1	0.210	-	0.210	-	-
120	2	0.082	0.090	0.082	0.018	0.146
150	3	0.192	0.201	0.136	0.024	0.415
300	16	0.073	0.078	0.046^{B}	0.011	0.131

P25 = Percentiles 25 P75 = Percentiles 75; ^{A} p<0.001 compared to non-aspirin group.; ^{B} p<0.001 compared to non-aspirin group and 60 mg/day group.

Frequency of aspirin resistance in patients taking 60 mg/day of aspirin

The total number of patients who were taking 60 mg/day of aspirin and agreed to participate in this study was 75. Aspirin resistance and aspirin semiresponders were detected in 4 (5.3%) and 20 (26.7%) patients participated in the study respectively. Aspirin resistance was not related to age, gender, fasting plasma glucose, HbA1c, total cholesterol, LDL-cholesterol, HDL-cholesterol, and adiponectin (Table 4). Median serum thromboxane B_2 levels of aspirin resistance, aspirin semiresponders, and aspirin sensitive groups were 0.316, 0.338, and 0.182 ng/ml respectively (p=0.172).

Table 4: Demographic and clinical characteristics of patients

	Aspirin resistance (n = 4)	Aspirin semiresponders (n = 20)	Aspirin sensitive (n = 51)	*p*
Age (y)	61.5 (49-67)	60.2 (48-73)	58.1 (35-74)	0.540
Female (%)	25	75	56.8	
BMI	27.13 ± 3.34	26.43 ± 3.99	27.29 ± 4.17	0.462
SBP	139.33 ± 10.69	130.10 ± 10.80	132.0 ± 8.98	0.782
DBP	84.33 ± 5.5	79.05 ± 7.99	82.27 ± 7.07	0.184
HbA1c	6.95 ± 0.60	7.46 ± 1.46	7.24 ± 1.02	0.630
Total cholesterol	140.50 ± 23.87	179.65 ± 33.65	173.02 ± 41.45	0.991
Hb	14.15 ± 1.37	12.89 ± 1.31	12.92 ± 1.43	0.595
Hct	41.25 ± 4.48	38.48 ± 4.06	38.34 ± 4.14	0.554
Plt (x 10^3)	284.5 ± 37.12	284.40 ± 81.02	290.94 ± 72.94	0.721
Adiponectin	7.53 ± 2.23	8.61 ± 4.94	9.28 ± 4.36	0.440
Serum thromboxane B_2 (ng/ml)	0.315 (0.082-1.926)	0.338 (0.205-0.985)	0.182 (0.066-0.508)	0.172

DISCUSSION

Effect of dosage of aspirin on platelet aggregation and serum thromboxane B_2 level

Most of aspirin-treated diabetic patients had lower platelet function and lower thromboxane B_2 level as compared to diabetic patients in the control group. Serum level of thromboxane B_2 in diabetic patients who received aspirin was significantly lower than the level in diabetic patients who were not treated with aspirin. Platelet aggregation induced by 1 mmol/l arachidonic acid and 10 μmol/l ADP in diabetic with aspirin were significantly lower compared with type 2 diabetic patients without aspirin. Among aspirin-treated group, serum thromboxane B_2 was depended on dose of aspirin. Median [interquartile] serum thromboxane in diabetic patients who treated with 60 mg/day aspirin was significantly higher than in diabetic patients who were treated with 300 mg/day aspirin. However, the serum thromboxane concentrations in either dose (60-300 mg/day) of aspirin group, 60 mg/day of aspirin group and 300 mg/day of aspirin group were 3.43%, 4.95%, and 0.90% of the serum thromboxane B_2 concentration in the control group, respectively. These results indicated that serum thromboxane was more than 95% inhibited when taking aspirin which met the requirement for limitation of the platelet aggregation in most patients. Since taking 60 mg/day of aspirin (the lowest dose) was enough to inhibit approximately 95% of serum thromboxane which is the requirement for limitation of platelet aggregation, further increase in the dosage of aspirin might not be required. Taking 60 mg/day or 300 mg/day of aspirin did not show statistically significantly different of platelet aggregation. This result was consistent with the result reported by Tohgi et al.[3] They reported that when 10 μM ADP was used to induce aggregation, the mean aggregation was 66.4% after 40 mg/day of aspirin and did not change substantially after higher aspirin doses. They also indicated that 40 mg/day of aspirin was able to inhibit 85% serum thromboxane B_2. Previous studies of low-dose aspirin in healthy subjects have shown that the inhibition of thromboxane synthesis is dose-dependent, non-linear relationship. The dose-response effect reaches a plateau at approximately 80 mg.

Frequency of aspirin resistance in type 2 diabetic patients taking 60 mg/day of aspirin

Some previous data reported that diabetic patients were less responsive to aspirin therapy than other high-risk patients [14, 15]. This study investigated the frequency of aspirin resistance in Thai patient with type 2 diabetes taking 60 mg/day of aspirin. The method used was optical platelet aggregation with 1 mmol/l arachidonic acid and 10 μmol/l ADP as agonists. Aspirin resistance in this study was defined as a maximal aggregation ≥ 20% with 1 mmol/l arachidonic acid and maximal aggregation ≥ 70% with 10 μmol/l ADP. Semi-responder was defined as meet one but not both of the above criteria[7]. Frequency of aspirin resistance found in this study was 6.19%, aspirin semi-responder was 25.77%, and aspirin sensitive was 68.04%. The frequency of aspirin resistance found in this study was lower as compared to other study in diabetic patients where different methods of aspirin resistant assessment were used [8]; however, the result was in concordant with earlier studies in non-diabetic group where aspirin resistant was assessed by optical platelet aggregation[7, 10]. Most studies examined aspirin resistance in dosages between 81 and 325 mg/day[10, 16, 17]. This study is the first report of aspirin resistance at 60 mg/day aspirin. Interestingly, frequency of aspirin resistance while taking 60 mg/day did not differ from that reported for higher doses of aspirin. Some studies reported correlation between patient conditions with aspirin resistance. These included female gender and older age[7]. However, no study could make a definite statement about clinical predictors of aspirin resistance. This study did not find any association between characteristics and aspirin resistance which might be due to the small size included.

There were several limitations in this study. The number of subjects participated was small. Aspirin compliance was based on question and answer. Neither measurement of salicylate level nor pill count was performed. Aggregation function and thromboxane level were determined only once, no baseline data before aspirin were recorded. Several confounding factors which might influence platelet activation, such as stress and type of food consumed were not evaluated. Laboratory-defined values only were determined, long term clinical outcomes of these patients have not been observed.

CONCLUSION

The results obtained from this study demonstrated that 60 mg/day of aspirin might be sufficient to be used in average type 2 diabetic patients for primary and secondary prevention of adverse cardiovascular events. Since higher dose of aspirin involve higher risk of bleeding side effects, the lowest dosage which could provide high enough effect might be an appropriate dose to be recommended. However, more work is needed to clarify the clinical significance of this finding.

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