CHAPTER V

DISCUSSION

5.1 Effect of Aspirin on Platelet Aggregation and Serum Thromboxane B_2 in Type 2 Diabetic Patients

Aspirin acts by inhibit COX, and there by reduces thromboxane A_2 which is an activator of platelet aggregation. The clinical pharmacology of aspirin has been characterized by measuring arachidonic acid induced platelet aggregation, which reflects thromboxane dependent platelet function; and by measuring serum thromboxane, which reflects the inhibitory effect of aspirin on thromboxane production. In this study, subjects with type 2 diabetic were evaluated using in vitro platelet aggregation studies and determination of the serum thromboxane metabolite. Compared to diabetic patients in control group, most of aspirin-treated diabetic patients had lower platelet function and lower thromboxane B_2 level. 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 (median 0.19 [0.04-0.37] vs 5.53 [3.00-8.32] ng/ml, $p<0.001$). Platelet aggregation induced by 1 mmol/l arachidonic acid and 10 umol/l ADP in diabetic with aspirin had significantly lower compared with type 2 diabetic patients without aspirin ($17.71 \pm 17.99\%$ vs $79.34 \pm 7.34\%$, $p=0.000$ and $61.76 \pm 9.165\%$ vs $72.09 \pm 8.26\%$, $p=0.000$, respectively). 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/d aspirin was significantly lower than in diabetic patients who treated with 300 mg/d aspirin.

Previous study on the relationship between aspirin dosage and serum thromboxane B_2 levels in patients with vascular disease reported similar results[68]. Compared with an aspirin dosage of 325 mg/d, changing the dosage to 81 or 1300 mg/d resulted in statistically significant increases in serum thromboxane B_2. Previous studies of low-dose aspirin in healthy subjects[69, 70] 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. Thromboxane inhibition of approximately 95% is required to limit platelet aggregation [71]. In this study, serum thromboxane concentrations in all doses of aspirin group, 60 mg/d of aspirin group and 300 mg/d of aspirin group were 3.43%, 4.95% and 0.90% of the serum thromboxane B_2 concentration in the control group, respectively. Serum thromboxane was more than 95% inhibitied when taking aspirin which met the requirement for limitation of the platelet aggregation in most patients. Since taking 60 mg/d of aspirin (the lowest dose) was enough to inhibit approximately 95% of serum thromboxane which is the requirement for limitation of platelet aggregation, further increased in the dosage of aspirin was not required, therefore, taking 60 mg/d or 300 mg/d of aspirin did not show statistically significantly different of platelet aggregation. This result was consistent with the result reported by Tohgi et al.[54]. They compared platelet aggregation induced by 1 uM ADP, 5 uM ADP and 10 uM ADP in poststroke patients before and after aspirin medication. With the aggregation induced by 1 uM ADP and 5 uM ADP, the rate of aggregation increased remarkably after 40 mg/d aspirin and increased further after higher aspirin doses. For 10 uM ADP induced aggregation, the mean aggregation was 66.4% after 40 mg/d aspirin and did not change substantially after higher aspirin doses. This can explain that 10 uM ADP is a strong agonist. The ability of aspirin to inhibit platelet aggregation induced by strong stimuli appears to reach its maximum with low-dose aspirin. On the other hand, the aggregation of platelet rich plasma induced by weaker stimuli (1 and 5 uM ADP) could not be inhibited or was less inhibited by 40 mg/d aspirin compared with higher doses. These apparently paradoxical findings may be explained that 1 uM ADP induces mainly the primary aggregation and that aspirin essentially inhibits the second aggregation. With this fact, 10 uM ADP induced aggregation of platelet rich plasma is recommended for monitoring the effect of aspirin.

5.2 Prevalence of Aspirin Resistance in Type 2 Diabetic Patients

Although the benefits of aspirin are widely accepted, many individuals treated with aspirin do not achieve the inhibitory response anticipated on the basis of laboratory measurements of platelet activation and aggregation, a phenomenon termed "aspirin resistance". Antiplatelet drugs that are effective and safe in one individual may not effective in another.

Antiplatelet drugs that are effective and safe in one individual may not effective in another. Aspirin is a weak platelet inhibitor, it may not provide sufficient antithrombotic therapy in some clinical or experimental circumstances. The primary prevention project (PPP) trial failed to show a clear benefit of aspirin therapy in diabetic patients with a non-significant reduction (10%) of cardiovascular events compared to a significant risk reduction of 41% in non-diabetic subjects[14]. Due to these results Sacco et al.[14] reported a lower efficacy of low dose aspirin therapy for primary prevention of CVD in diabetic patients.

Although some previous data proposed that diabetic patients are less responsive to aspirin therapy than other high-risk patients, this study investigated the frequency of aspirin resistance in Thai patient with type 2 diabetes taking 60-300 mg/d aspirin. The method used was optical platelet aggregation with 1 mmol/l arachidonic acid and 10 umol/l ADP. Aspirin resistance in this study was defined as a maximal aggregation $\geq$ 20% with 1 mmol/l arachidonic acid and maximal aggregation $\geq$ 70% with 10 umol/l ADP. Semi-responder was defined as meet one but not both of the above criteria. Aspirin sensitive was defined as a maximal aggregation < 20% with 1 mmol/l arachidonic acid and maximal aggregation > 70% with 10 umol/l ADP. Frequency of aspirin resistance in this study was 6.19%, aspirin semi-responder was 25.77% and aspirin sensitive was 68.04%. The frequency of aspirin resistance in this study was lower as compared to other studies in diabetic patients that used other method to assess aspirin resistant, but is concordant with earlier studies in non-diabetic group assessed by optical platelet aggregation.

Previous studies have reported prevalence of aspirin resistance range from 0.4%-60% depended on the population studied and the laboratory test used to diagnose aspirin resistance. Gum et al.[15, 17] conducted a prospective trial in 326 patients with stable coronary artery disease who were treated with 325 mg of aspirin for $\geq$ 7 days. Optical platelet aggregometry, was used to test for aspirin resistance. Aggregation is induced by ADP and arachidonic acid. Aspirin resistance was defined as a mean aggregation $\geq$ 70% with 10 umol/l ADP and a mean aggregation $\geq$ 20% with 0.5 mg/ml arachidonic acid. The investigators found that 5.5% of them were aspirin resistant and 23.8% were aspirin semi-responders. They also analyzed aspirin resistance by Platelet Function Analyzer (PFA 100), a device that simulates platelet function in vitro at high shear. Aspirin resistant defined by PFA-100 were 9.5%. After

two-year follow up, the investigators found that aspirin resistance according to the optical platelet aggregation was significantly associated with an increased risk of death, myocardial infarction or cerebrovascular accident compared with aspirin sensitive patients (24% vs 10%, p=0.03). However, there was no relation between aspirin resistance measured by PFA-100 and subsequent adverse cardiovascular events (15.1% aspirin resistant vs 12.9% aspirin sensitive, p=0.4).

Fateh-Moghadam and co-workers[72] evaluated the prevalence of aspirin resistance in 172 patients with type 2 diabetes using 100 mg aspirin daily.. Resistance to aspirin was assessed by PFA-100. Aspirin resistance defined as a normal collagen/epinephrine induced closure time (82-165 seconds). Aspirin responders were defined when closure time was $\geq$ 300 seconds. They found that 21.5% were aspirin resistant, 16.9% were semi-responders and 61.6% were responders. Another study investigated the prevalence of type 2 diabetes mellitus reported 16.2 % of aspirin resistance with Ultegra Rapid Platelet Function Assay[73]

There are several methods to measure aspirin resistance include optical platelet aggregometry, whole-blood aggregometry, PFA-100, Rapid platelet function assay (RPFA, VerifyNow®) and thromboxane metabolites. Different methods have reported a wide range of estimates of aspirin resistance in selected populations, with poor concordance among those methods. Definitions of aspirin resistance or nonresponse vary according to the method of platelet function testing used. Also, different techniques define levels of platelet aggregation in units that cannot be directly compared. In addition, definitions of aspirin resistance within a particular technique are also subject to variation. Finally, the setting of criteria defining aspirin resistance is often arbitrary, and there is no standardized protocol in administering tests.

Recent systematic review found that optical aggregation shows a prevalence significantly lower than the prevalence from the point of care devices, for instance PFA-100 and rapid platelet function assay (RPFA) [74]. However, there is poor concordance between optical aggregation and PFA-100 method [74, 75]. In addition, aspirin resistant patients defined by optical aggregation had been shown to have threefold increase in the risk of cardiovascular events[17]. But this association has not been found with PFA-100[75]. It might be due to the fact that the results of the PFA-100 assay are sensitive to many variables (other than thromboxane A_2 production) including platelet count, red blood cells, and plasma von Willebrand factor

characterized by high shear, plasma von Willebrand factor is a major determinant of closure time. As a result, the effect of aspirin on closure time of the collagen-epinephrine cartridge can be outweighed by other variables, such as von Willerbrand factor, that are not affected by aspirin. This could be one explanation for the high proportion of patients with short closure time, despite being treated with aspirin, and the poor agreement between the PFA100 and optical aggregation (K=0.1, 95% CI 0.04-0.25). This discrepancy suggests that optical aggregatory using arachidonic acid as agonist reflect biochemical action of aspirin most directly whereas PFA-100 or RPFA measures different aspect of platelet function [74].

5.3 Factors Influencing Platelet Response to Aspirin in Diabetic Patients

5.3.1 Blood glucose

This study found that HbA1c correlated with platelet aggregation induced by arachidonic acid. Although earlier studies of aspirin resistant in diabetic group have not found this relationship, in vitro study of Watala et al.[76] found that platelet protein glycation, HbA1c, is associated with the aspirin insensitivity in diabetic patients. Since aspirin is capable of acetylating in a nonselective manner all susceptible amino and hydroxyl groups in a variety of platelet and plasma proteins. Thus, glucose and aspirin compete each other for the free amino groups in platelet proteins. An impaired platelets response to clinical doses of aspirin in diabetic patients, associated with the extent of protein glycation, may result from diminished platelets' susceptibility to becoming acetylated by aspirin [76].

5.3.2 Dosage of aspirin

Another factor to consider in aspirin non-response is the dosage used. Most studies examined aspirin resistance in dosages between 80 and 325 mg/d [15, 48, 72, 74, 77, 78]. This study is the first report of aspirin resistance at 60 mg/d aspirin. Interestingly, frequency of aspirin resistance in 60 mg/d did not differ from higher dose of aspirin.

Although low-dose aspirin is demonstrated to inhibit COX-1 completely[42], some patients may require higher doses to achieve the desired antiplatelet effect. This idea was explored in several cerebrovascular studies in the early 1990s that showed that resistance could be overcome by increasing the dose of aspirin[79, 80]. Conversely, other trials failed to confirm a dose response. Weksler et al. demonstrated that 3-7 days of treatment with aspirin 40 mg/day prevented platelet aggregation and thromboxane A_2 formation as effectively as higher doses in patients with previous cerebral ischemia. A comparative trial evaluated outcomes in patients who underwent carotid endarterectomy and were taking low-dose (81-325 mg/d) or high-dose (650-1,300 mg/d) aspirin[81]. The combined rate of stroke, MI, or death was less in the low-dose group than in the high-dose group at 30 days (5.4% vs 7.0%, p=0.07) and 3 months (6.2% vs 8.4%, p = 0.03). A meta-analysis conducted by the Antiplatelet Trialists' Collaboration found similar efficacy for vascular events with aspirin in daily doses of 75-325 mg compared with 500-1,500 mg.

Although no optimal aspirin dose for the prevention of vascular events has been recommended, higher aspirin doses may not be practical in many patients because of dose-dependent side effects, such as gastrointestinal bleeding.

5.3.3 Insulin resistance and platelet response to aspirin

In this study we have found that in individuals with type 2 diabetes, insulin resistance is not associated with platelet non responsiveness to aspirin. While insulin sensitivity as measured by HOMA-IR increases the risk for Type 2 diabetes[82, 83], and may be associated with cardiovascular disease in those without diabetes[83, 84], it does not appear to contribute to the increase in the risk for cardiovascular disease after onset of Type 2 diabetes[85]. There is a possibility that insulin resistance is associated with cardiovascular risk prior, but not after, the diagnosis of diabetes when insulin resistance may stabilize[86]. In a meta-analysis of mostly non-diabetic populations, insulin resistance as measured by insulin concentration was shown to be weakly associated with cardiovascular disease[87].

5.3.4. Patient characteristics associated with aspirin resistance

Some studies reported correlation between patient conditions with aspirin resistance. These included female gender and older age[15]. However, no study could make a definite statement about clinical predictors of aspirin resistance. This study did not find any association between patient characteristics and aspirin resistance. It may be due to small sample size in this study, thus the correlation was not detected.

5.4 Management of Aspirin Resistance

The first step in evaluating a potentially aspirin resistant is to examine dosage, compliance and possible drug interactions (pseudo-aspirin resistance). If non exists, then a true cause of aspirin resistance or a failure of aspirin to inhibit platelet function may be assumed. Increasing the aspirin dose may be considered; however little evidence supports reversal of resistance by such means. The alternative is substitution for, or adjunctive antiplatelet therapy with, an agent having a different mechanism of action, such as clopidogrel or dipyridamole. Their introduction can only be based on clinical judgment and benefit can only be presumed. Some study support the superiority of combination antiplatelet therapy over aspirin alone in selected patient group [88], benefit that could speculatively be due to aspirin resistant patients. However, several studies have suggested that the addition of clopidogrel does not overcome aspirin resistance[89-91].

5.5 Study Limitations

There are some limitations to this study. Aspirin use was based on answers to questionnaires only. Salicylate levels or pill counts were not performed. Therefore, it is possible that some of the subjects had not taken aspirin regularly. Salicylate levels or pill counts were not performed. Therefore, it is possible that some of the subjects had not taken aspirin regularly. Aggregation studies and thromboxane level were performed only once. No baseline had performed before taken aspirin. Another limitation is limited number of subjects. This may cause statistical errors. Some differences among population may not have been detected. In addition,

there were many confounders that might affect the results but we did not evaluate. For instance, patient stress and some food such as garlic, ginger, onion, and chillis have been reported that can induce platelet activation. The generalizability of the findings of this study may not extend to age glucose control and severe or acute condition that not included in this study.

Although this study showed that 60 mg/d and 300 mg/d aspirin was equally inhibited platelet aggregation in T2DM and aspirin resistance was not common in T2DM, further studies are needed to determine long term clinical outcome of these patients.