RESEARCH ARTICLE

Acetyl salicylic acid resistance and inhibition to platelet aggregation in normal healthy subjects

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Abstract:

Background. Aspirin (ASA), widely used as an antiplatelet agent to reduce high risk of arterial thrombosis. ASA resistance is of unknown etiology describing decreased platelet activation. The adverse side-effects are mainly gastro-intestinal bleeding with larger ASA dose but low doses are less effective in inhibiting platelet aggregation. The objective is to determine if 50 mg ASA is as effective as 100 mg use in normal healthy subjects.

Materials and Methods. Eighteen subjects (females n=15, males n=3) with mean age 41.7 ± 7.4 years completed the study protocol. The inhibition rate was analysed in 15 subjects as three subjects were non-responders to 50 mg ASA and two to 100 mg ASA. Platelet aggregation was performed with agonist arachidonic acid. Transmission max % was use for analysis.

Results and Discussion. The 18 normal subjects had normal platelet count of mean 301 ± 72.3 x109/L. Significant (P=<0.001) platelet inhibition was seen in 15 subjects as three subjects were non-responders to 50 mg ASA and two to 100 mg ASA. Platelet aggregation was performed with agonist arachidonic acid. Transmission max % was use for analysis.

Conclusion. The study showed that 100 mg ASA dose is better suited for antiplatelet therapy compared to 50 mg. Significantly greater platelet inhibition was seen with 100 mg ASA with no gastro-intestinal bleeding or other side-effects reported. ASA resistance was seen in 16.7% of subjects ingesting 50 mg ASA and 11.1% in 100 mg dose.

Keywords: ASA, resistance, inhibition
Introduction

Platelets play an important role in the pathogenesis of arterial-thrombosis, activated platelet initiate thrombus formation and antiplatelet therapy modifies these properties involving aspirin (acetyl salicylic acid, ASA) (1). ASA is widely used as an antiplatelet agent to reduce the risk of non-fatal stroke, non-fatal myocardial infarction and vascular death in patients at high risk of arterial thrombosis. It was reported to reduce mortality in acute coronary syndrome (ACS) and improve the condition (2). However, ASA failed to inhibit platelet aggregation in acute myocardial infarction (AMI) (3) and their use in AMI is of little or no use (4). ASA resistance is of unknown etiological phenomenon describing decreased platelet activation by ASA. The existence of ASA resistance affects more than 50% with cardiovascular disease (1) and 25.8% in severe symptomatic peripheral artery disease (PAD) (5). In stable coronary artery disease (CAD) 36% of Asian Indian patients were non-responders to ASA have been reported (6). Abnormal platelet aggregation studies due to aspirin-like defect was seen in up to 9% of platelet donors (7). The adverse effects of ASA are mainly gastro-intestinal bleeding and this effect are greater with daily 300 mg ASA use (8). Very low doses of ASA spare prostacyclin formation and reduce gastro-intestinal side effects but are less effective in inhibiting platelet aggregation. Doses of 100 and 162.5 mg ASA had shown greater inhibition than lower doses (9). Doses higher than 162 mg ASA, only 6% did not show any response in another study (10). Aspirin resistance is associated with an increased risk of severe stroke and large infarct volume in patients taking aspirin before stroke onset (11) and between 5% to 65% was seen in patients with ischemic stroke (12).

Platelet aggregation is performed to identify and quantitate platelet response and monitor platelet inhibition by drug therapy. It is based on the addition of platelet agonist to a blood sample (usually platelet rich plasma). It may be assessed using various agonists such as adenosine di-phosphate (ADP), collagen and others. Arachidonic acid (AA), a precursor of thromboxane A2 and hydroxyl fatty acids liberated from human platelets on activation converts the enzyme cyclooxygenase-1 (COX-1) into a potent inducer of platelet aggregation. Ingestion of ASA inhibits COX-1 thus inhibits platelet aggregation. The aggregation of platelets is an essential physiologic life-saving process of blood coagulation. The role of platelets in haemostasis involves adherence to sites of injury, activation of internal signalling pathways, aggregation to form plugs and the acceleration of the coagulation reactions to form thrombin. Platelet aggregation particularly at site of plaque rupture results in thrombus formation blocking normal blood circulation in the heart musculature in acute coronary syndrome (13). Platelet function may be impaired if any of the pathways mediated by activation process by agonists are defective.

The objective of this small cohort study is to determine whether acetyl salicylic acid of 50 mg dose is as effective as 100 mg dose in normal healthy subjects.
Material and Methods

The study received ethical approval from the Health Research Ethical Committee of North Sumatera (No:357/KOMET/FK USU2013), Medical School, University of Sumatera Utara, Medan, Indonesia. It was conducted at the Department of Clinical Pathology, Faculty of Medicine, University of North Sumatera/Haj Adam Malik Hospital. The inclusion criteria for the study: age above 18 years old, not on any medication for the past 10 days, and agreed to give informed consent. The exclusion criteria: body mass index (BMI) >30, known medical disorders.

Subjects. Initially 46 subjects gave their informed consent but 12 failed to turn up for the study. Of the remaining 34 subjects, only 18 subjects completed the protocol study whilst the remainder failed to turn up for the 100mg ASA protocol and therefore was excluded from the analysis. Data from the 18 subjects (female n= 15, male n= 3) who completed the 50 mg and 100 mg ASA protocol were therefore analysed. Their mean age was 41.7 ±7.4 years ranging between 31 years and 50 years old. The study subjects were healthy individuals recruited from within the institution, they had no known medical history and have given written informed consent to participate in the study.

Blood sampling. After having fasted overnight, a clean venepuncture was performed and blood collected into EDTA anticoagulant (2 mL, BD Vacutainer) for platelets and 3.6 mL blood into 0.4 mL 3.8% sodium citrate for preparation of platelet rich plasma (PRP). Blood sampling was performed pre-ASA ingestion and 3 days later after daily ingestion of 50 mg ASA. A wash-out period of 7 days was instituted before another blood sampling performed after daily ingestion of 100 mg ASA for 3 days. Citrated blood was centrifuged at room temperature at 150g for 10 mins for PRP and again centrifuged at 2000g for 15 minutes to obtain platelet poor plasma (PPP). Platelet aggregation was performed within two to three hours from blood sampling.

Laboratory analysis. Platelets (EDTA blood) and PRP were determined in the automatic cell counter Sysmex XT 4000 (Kobe, Japan). The ex-vivo platelet aggregation studies was performed using as agonist arachidonic acid (AA, PT Helena Laboratories (Australia) Pte Ltd, product 5364) at final concentration of 500 µg/mL to the PRP in the platelet aggregometer, AggRAM (Helena Biosciences, Gateshead, UK)). PPP was calibrated as maximum transmission at 100%.

Statistical analysis. The Statistical Package for Social Sciences (SPSS 22 IBM Corp. USA) was used to perform statistical analysis. Paired samples t-test was performed to compare differences in platelet aggregation inhibition with 50 mg and 100mg. Three subjects were considered non-responders to 50 mg ASA and remaining two to both 50 mg and 100 mg ASA. They were excluded from the final analysis. A P value of less than 0.05 was considered statistically significant.
**Results**

*Platelets and Platelet Rich Plasma*

Platelet numbers of the 18 normal subjects was mean 301 ± 72.3 x109/L and ranged between 204 and 448 x109/L. The mean platelet numbers in PRP was 240 ± 56.3 x 109/L and ranged between 185 and 360 x 109/L. For platelet aggregation studies platelet numbers in the PRP sample should be in the region of between 150 to 400 x 109/L.

Platelet aggregation (T max%) and inhibition to 50 mg and 100 mg acetyl salicylic acid (ASA) in normal healthy subjects compared with pre-ASA (excluding three non-responders to ASA).

Significant inhibition (P= <0.001) of ex-vivo platelet aggregation to 50 mg ASA (mean 50.3%, ranged between 10.6% to 85.5%) and 100 mg dose (mean 75.7%, ranged between 11.8% to 93.6%) compared with pre-ASA state in the 15 normal subjects was seen. Further enhanced inhibition (P= <0.001) with 100 mg dose compared to 50 mg dose was also seen (Table 1).

Non-responders or resistance to 50 mg ASA (16.7%) was seen in 3 normal subjects. One subject was resistant to 50 mg ASA only but respond to 100 mg dose (77.4% inhibition). With 100 mg ASA the resistance rate is 11.1% (2/18) in this small cohort study. Their results (T max%) are shown in Table 2.

**Table 1.** Ex-vivo platelet aggregation (Tmax%) and inhibition to 50 mg and 100 mg acetylsalicylic acid (ASA) in normal healthy subjects compared with pre-ASA (excluding three non-responders to ASA).

<table>
<thead>
<tr>
<th>Platelet aggregation: n=15</th>
<th>Tmax (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- ASA Mean (SD)</td>
<td>55.8 (17.2)</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>32.2 – 86.1</td>
<td></td>
</tr>
<tr>
<td>50 mg ASA (mean SD)</td>
<td>26.9 (13.1)</td>
<td>50.3 (21.9)</td>
</tr>
<tr>
<td>Range</td>
<td>9.3 - 47.4</td>
<td>16 – 85.5</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100 mg ASA mean (SD)</td>
<td>13.0 (10.3)</td>
<td>75.7 (19.3)</td>
</tr>
<tr>
<td>Range</td>
<td>4.3 – 45.0</td>
<td>11.8 – 93.6</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Comparison between 50 mg and 100 mg ASA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 2.** Acetylsalicylic acid non-responders to ex-vivo platelet aggregation in three healthy individuals with one subject not-responding to only 50 mg ASA.

<table>
<thead>
<tr>
<th>Platelet aggregation T max (%)</th>
<th>pre-ASA</th>
<th>50 mg ASA</th>
<th>100 mg ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>47.2</td>
<td>42.2</td>
<td>71.1</td>
</tr>
<tr>
<td>2.</td>
<td>42.2</td>
<td>60.9</td>
<td>56.5</td>
</tr>
<tr>
<td>3.</td>
<td>44.3</td>
<td>45.2</td>
<td>10.0 (77.4% inhibition)</td>
</tr>
</tbody>
</table>

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Discussion and conclusion

Aspirin (acetyl salicylic acid) is widely used as an antiplatelet agent to reduce the risk of non-fatal stroke, non-fatal myocardial infarction and vascular death in patients at high risk of arterial thrombosis. It was reported to reduce mortality in acute coronary syndrome (ACS) and improve the condition (2). However, ASA failed to inhibit platelet aggregation in acute myocardial infarction (AMI) (3) and their use in AMI is of little or no use (4). ASA resistance has been shown to be associated with an increased risk of severe stroke and large infarct volume in patients taking aspirin before stroke onset (11). High platelet activity contributes to poor prognosis but the mechanism of ASA resistance describing decreased platelet activation remains unclear (14). Ingestion of ASA inhibits COX-1 activity thus inhibits platelet aggregation. Abnormal platelet aggregation studies due to aspirin-like defect was seen in up to 9% of platelet donors (8). The adverse effects of ASA are mainly gastro-intestinal bleeding and this effect are greater with daily 300 mg ASA use (8). In our small study the subjects had normal platelet counts and had no known medical history to report but only 88.9% respond to 100 mg ASA and 83.3% to 50 mg ASA. Greater inhibition to platelet aggregation was seen in 100 mg compared to 50 mg ASA suggesting the effects of 100 mg ASA is better suited for therapy as an antiplatelet agent. We report here that 11.1% of normal subjects are ASA resistance to 100 mg ASA and 16.7% to 50 mg ASA dose which is much higher than reported for platelet donors of up to 9% (7) and 6% in another study with 162 mg ASA (10). In stable coronary artery disease of Asian Indian patients there were 36% of non-responders to ASA therapy have been reported (6). There were no reports on gastro-intestinal bleeding or other side-effects in the study subjects probably it may be due to the short duration of ASA use.

In conclusion, the study showed that 100 mg ASA dose is better suited for antiplatelet therapy compared to 50 mg dose. Significantly greater platelet inhibition was seen with 100 mg ASA use with no gastro-intestinal bleeding or other side-effects reported. ASA resistance was seen in 16.7% of subjects ingesting 50 mg ASA and 11.1% in 100 mg ASA in this short duration study.

Acknowledgments

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References

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