Responsiveness to aspirin in patients with unstable angina pectoris by whole blood aggregometry

J. B. Li, H. M. Dong, Z. Jian, X. J. Wu, X. H. Zhao, S. Y. Yu, L. Huang

SUMMARY

Aims: To evaluate aspirin responsiveness in patients with unstable angina pectoris (UAP) by whole blood aggregometry. Another goal was to differentiate aspirin-resistant patients into pharmacokinetic or pharmacodynamic type. Methods: We measured platelet aggregation by determining impedance values in 70 normal volunteers and 104 UAP patients on aspirin (100 mg/day ≥ 7 days) in four inducing conditions [1 µg/ml collagen, 2 µg/ml collagen, 5 µmol/l adenosine diphosphate (ADP) and 10 µmol/l ADP]. We calculated a cut-off value based on data from normal volunteers to define aspirin responsiveness in cases. Then, the correlation and agreement between the results in the four conditions was analysed to choose a preferred inducing condition for identification of aspirin resistance. All results from all samples were incubated with 0.1 mmol/l aspirin and measured again for aspirin-resistant classification. Results: Aspirin resistance was observed in 38 patients (36.5%), 51 patients (49.0%), 67 patients (64.4%) and 67 patients (64.4%), respectively, for 1 µg/ml collagen, 2 µg/ml collagen, 5 µmol/l ADP and 10 µmol/l ADP among 104 patients. Collagen at low concentration was suggested as a preferred agent for detecting aspirin inhibitory effect according to the coefficient of sensitivity. After incubation, only three among 38 aspirin-resistant patients showed normal platelet aggregation and were classified into pharmacokinetic type. Conclusions: In the presence of collagen at low concentration (1 µg/ml), the prevalence of aspirin resistance is about 36.5% in UAP patients, and according to a classification specific for resistant patients, most of the aspirin ‘resistance’ is just because of pharmacokinetic issues.

Introduction

For decades, aspirin has been the most commonly prescribed antiplatelet drug used worldwide for the prevention of thrombo-embolic vascular events (1). It acts promptly to inhibit cyclooxygenase-1 irreversibly within platelets, which prevents the formation of thromboxane A2, diminishing the platelet aggregation promoted by this pathway (2). The efficacy of aspirin has been documented in the recent meta analysis performed by the Antithrombotic Trialists’ Collaboration (3), and its use can reduce the risk of myocardial infarction, stroke or death by approximately 25% in patients with cardiovascular disease.

Despite its high efficacy, safety and low cost, aspirin may not benefit all patients equally, as a substantial proportion of patients receiving aspirin still suffer thrombo-embolic vascular events (4). Thus the concepts of ‘aspirin resistance’, 'aspirin failure’ or ‘aspirin non-response’ have evolved as an explanation of the fact that aspirin-treated patients exhibit normal platelet function (5,6). Underlying mechanisms of aspirin resistance include inadequate dose, drug interactions, genetic polymorphisms involved in thromboxane biosynthesis, up-regulation of non-platelet sources of thromboxane biosynthesis and increased platelet turnover (7). Previous studies addressing the prevalence of aspirin resistance in various populations with manifest cardiovascular diseases have shown that 5.5–45% of patients are aspirin resistant (1). Various data sources from randomised trials showed a significant association between laboratory measurements of aspirin resistance and the risk of clinical cardiovascular events during long-term treatment with aspirin (8–10).

Unstable angina pectoris (UAP) is usually, but not always, caused by atherosclerotic coronary artery
disease and is associated with an increased risk of subsequent myocardial infarction and cardiac death (11). It is recommended that aspirin be initiated as soon as the diagnosis of acute coronary syndrome (ACS) is made or suspected unless contraindicated. However, there are limited data documenting aspirin responsiveness in UAP patients. This study was designed to evaluate, by whole blood aggregometry (WBA), the response to aspirin among UAP patients. Another goal was to differentiate aspirin-resistant patients into pharmacokinetic or pharmacodynamic types.

Methods

Patients and normal volunteers
A total of 104 consecutive patients (57 men and 47 women, aged 64.8 ± 9.4 years) admitted to the Institute of Cardiovascular Science, Xinqiao Hospital of Third Military Medical University, China with UAP were eligible for inclusion. All patients had received aspirin 100 mg daily for secondary prevention for ≥ 1 week prior to admission. Compliance with aspirin dose and treatment was ascertained by a personal interview at the time of inclusion. Exclusion criteria were use of other doses of aspirin; concurrent ingestion of clopidogrel, ticlopidine, dipyridamole or non-steroidal anti-inflammatory drugs; the presence of a history of bleeding disorders; platelet count within 1 month of enrolment; family or personal myeloproliferative disease; major surgical procedure 1 week prior to admission. Compliance with aspirin during the measurements has been verified by colorimetric detection of aspirin hydrolysis under assay conditions. Incubation with 0.1 mmol/l aspirin at room temperature for 20–30 min. Platelet aggregation was then stimulated in the four inducing conditions (1 and 2 μg/ml collagen, 5 and 10 μmol/l ADP) using a whole blood aggregometer described below. The stability of aspirin during the measurements has been verified by colorimetric detection of aspirin hydrolysis under assay conditions. Incubation with 0.1 mmol/l aspirin can nearly inhibit thromboxane formation completely (12), and most research workers chose such a concentration to assess the response to aspirin in vitro (13–15).

Blood sampling
Blood samples from UAP patients were drawn 1–24 h after administration of the last dose of aspirin by antecubital venipuncture, using a 21-gauge butterfly needle. One tube of 2.7 ml venous blood anticoagulated with ethylenediaminetetraacetic acid (BD Vacutainer®; Belliver Industrial Estate, Plymouth, UK) was collected for measurements of leucocyte, haemoglobin, erythrocyte, hematocrit and platelet count (Sysmex SE-9000, Sysmex, Japan). Additional venous blood was drawn into three 2.7 ml vacutainer tubes containing 3.8% sodium citrate for platelet aggregation trials and measurement of fibrinogen. As for the normal group, two sample tubes of whole blood anticoagulated with 3.8% sodium citrate for assessment of platelet aggregation were obtained. The blood samples taken for studying platelet function were kept supine at room temperature before analysis. All blood samples were processed within 3 h of collection. To avoid possible observer bias, blood samples were coded and analysed with no observer knowledge of experimental group. Sampling procedures and platelet studies were performed by laboratory assistants not informed of the experimental protocol.

Incubation in vitro with aspirin
To maximise inhibition of cyclooxygenase-1–mediated platelet aggregation, aliquots of citrated whole blood from all samples were also incubated with 0.1 mmol/l aspirin at room temperature for 20–30 min. Platelet aggregation was then stimulated in the four inducing conditions (1 and 2 μg/ml collagen, 5 and 10 μmol/l ADP) using a whole blood aggregometer described below.

Definition of UAP
Following the ACC/AHA 2007 Guidelines: the Management of Patients With Unstable Angina/Non ST-Elevation Myocardial Infarction (11), the diagnosis of UAP was based on the clinical presentation of patients manifesting ≥ 1 of the following histories: (i) rest angina (angina occurring at rest and prolonged, usually > 20 min), (ii) new-onset (< 2 months) severe angina and (iii) increasing angina (previously diagnosed angina that has become distinctly more frequent, longer in duration or lower in threshold). In addition, all patients had transient ST-segment depression ≥ 0.05 mV or T-wave inversion ≥ 0.2 mV during a symptomatic episode at rest and without any increase of biomarkers for myocardial infarction (creatinine kinase, creatine kinase-myocardial band and troponin I).
Platelet aggregation test

Electrical aggregometry (Model 560CA, Chrono-Log, Haverton, PA, USA) measures the impedance between a pair of electrodes immersed in diluted whole blood. The increase in impedance is associated with the amount of platelet aggregates deposited on the electrodes after the addition of a platelet agonist. Briefly, measurements were performed at 37 °C and a stirring speed of 1000 rpm. According to the recommendations of the manufacturer, citrate blood (500 μl) pipetted into a cuvette was diluted 1-to-1 with 0.9% sodium chloride and prewarmed for 5 min at 37 °C. After a pair of platinum electrodes was placed into the cuvette, the agonist’s collagen (final concentration 1 and 2 μg/ml) and ADP (final concentration 5 and 10 μmol/l) were added to the samples for in vitro activation of the platelets. The impedance value reflecting the extent of platelet aggregation was read as ohms (Ω) of aggregation within 6 min. In addition, a graphical printout (i.e. chart tracing) of each electrical impedance aggregometry tracing is provided by the aggrolink software. A more detailed description of the method has been reported elsewhere (16).

Criteria for aspirin resistance

To define aspirin responsiveness, we calculated a cut-off by subtracting 2SD from the mean 6-min impedance measured in normal subjects with every stimulating agent. Thus, when a patient had an agonist-induced impedance value greater than the cut-off value, this patient was categorised as meeting the criteria for aspirin resistance. Among aspirin-resistant patients, one having impedance more than the cut-off value in spite of incubation would be considered as manifesting a pharmacodynamic type of aspirin resistance. Conversely, one with impedance less than the cut-off value after incubation was classified as a pharmacokinetic type.

Statistics

Continuous variables were presented as mean ± standard deviation (M ± SD) and categorical variables were presented as frequencies and percentages. The significance of any difference between cases and normal subjects was tested by means of Independent-Samples t-test for age and χ² test for gender. Correlations between results obtained in the various inducing conditions, irrespective of aspirin resistance or sensitivity, were established using Kendall’s W-test and Pearson correlation analysis. The agreement between the aspirin resistance status assessed in the different inducing conditions was evaluated with the use of the kappa statistic. A box-plot was used to illustrate the distribution of impedance values, and a scatter plot showed the correlation between impedances with the same agonists but different concentrations. Test probabilities were two-sided, and p-values < 0.05 were considered to be statistically significant. The statistical analyses were performed with spss package for Windows version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Impedance results

The impedance value reflecting the extent of platelet aggregation with addition of agonists was read after an additional 6 min. Impedance values are directly proportional to the extent of aggregation. To evaluate the imprecision of the assay (coefficient of variation, CV), samples were measured from 10 subjects five times, and the mean CVs were 5.1%, 6.0%, 5.4% and 5.1% for 1 μg/ml collagen, 2 μg/ml collagen, 5 μmol/l ADP and 10 μmol/l ADP, respectively. The mean of results in normal subjects were 14.6 ± 2.1 (Ω) (1 μg/ml collagen), 16.7 ± 2.9 (Ω) (2 μg/ml collagen), 12.5 ± 2.2 (Ω) (5 μmol/l ADP) and 12.6 ± 2.1 (Ω) (10 μmol/l ADP) (Table 1), and the reference intervals (M ± 2SD) were 10.4–18.8 (Ω), 10.9–22.5 (Ω), 8.2–16.9 (Ω) and 8.4–16.8 (Ω), respectively. As a result, among 104 patients, aspirin resistance was observed in 38 patients (36.5%), 51 patients (49.0%), 67 patients (64.4%) and 67 patients (64.4%), respectively, based on four cut-off values in the different inducing conditions (Table 1). As we can see, the prevalence assessed by collagen was less

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Normal group (Ω)</th>
<th>Cut-offs (Ω)</th>
<th>Cases (Ω)</th>
<th>Prevalence of AR n (%)</th>
<th>CS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 μg/ml collagen</td>
<td>14.6 ± 2.1</td>
<td>10.3</td>
<td>8.3 ± 4.5</td>
<td>38 (36.5%)</td>
<td>42.9</td>
</tr>
<tr>
<td>2 μg/ml collagen</td>
<td>16.7 ± 2.9</td>
<td>10.9</td>
<td>11.1 ± 4.8</td>
<td>51 (49.0%)</td>
<td>33.5</td>
</tr>
<tr>
<td>5 μmol/l ADP</td>
<td>12.5 ± 2.2</td>
<td>8.2</td>
<td>9.9 ± 4.7</td>
<td>67 (64.4%)</td>
<td>20.5</td>
</tr>
<tr>
<td>10 μmol/l ADP</td>
<td>12.6 ± 2.1</td>
<td>8.5</td>
<td>10.6 ± 5.3</td>
<td>67 (64.4%)</td>
<td>15.9</td>
</tr>
</tbody>
</table>

AR, aspirin resistance; ADP, adenosine diphosphate; CS, coefficient of sensitivity: (MEANnormal – MEANcase)/MEANnormal.
than that assessed by ADP, especially for collagen at a low concentration.

**Correlation and agreement between the various conditions**

Kendall’s Coefficient of Concordance and Pearson correlation analysis were calculated to assess correlations between the different inducing conditions. In Kendall’s W-test, the mean ranks were 1.97, 3.05, 2.38 and 2.60, respectively, for 1 µg/ml collagen, 2 µg/ml collagen, 5 µmol/l ADP and 10 µmol/l ADP. We concluded that the impedance values measured by WBA demonstrated a consistency among the applied conditions (α = 0.134, p < 0.001). Also, the correlation between these was significant by Pearson correlation analysis (r = 0.476–0.750, p < 0.01), especially between 5 and 10 µmol/l ADP. According to cut-off points, agreement between different conditions was evaluated by Kappa statistics, and the degree of agreement in relation to aspirin resistant status was presented as κ value (κ = 0.304–0.706, p < 0.01) (Table 2), which supports this statistical significance. Overall, a correlation between these four inducing conditions in the study, irrespective of different agonists or different concentrations of the same agonist was verified (Figure 1).

In spite of the fact that the prevalence of aspirin resistance indicated by the use of ADP was higher than that indicated by collagen, some studies have demonstrated that the use of ADP was far less sensitive than the use of collagen in detecting the inhibitory potency of aspirin. That is to say, the aggregation induced by ADP may not reflect the platelet inhibitory effect of aspirin accurately.

Aspirin resistance can be defined as a failure of aspirin to inhibit blood aggregation, and our group was set up to determine the profiles of aspirin resistance in patients with UAP. To compare and verify the sensitivity between ADP and collagen in assessing aspirin resistance in this study, (MEANnormal – MEANcase)/MEANnormal in this study was defined as coefficient of sensitivity (CS) for measuring the inhibitory effect of aspirin. As we can see from (Table 1) and (Figure 2), the CS of collagen is distinctly greater

<table>
<thead>
<tr>
<th>Agents</th>
<th>1 µg/ml collagen</th>
<th>2 µg/ml collagen</th>
<th>5 µmol/l ADP</th>
<th>10 µmol/l ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 µg/ml collagen</td>
<td>1*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2 µg/ml collagen</td>
<td>0.732*</td>
<td>1*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5 µmol/l ADP</td>
<td>0.476*</td>
<td>0.613*</td>
<td>1*</td>
<td>–</td>
</tr>
<tr>
<td>10 µmol/l ADP</td>
<td>0.503*</td>
<td>0.536*</td>
<td>0.750*</td>
<td>1*</td>
</tr>
</tbody>
</table>

*p < 0.01. ADP, adenosine diphosphate.

**Figure 1** Results of 1 and 2 µg/ml collagen (A), 5 and 10 µmol/l adenosine diphosphate (ADP) (B) in UAP patients on aspirin. Results of aggregometry correlated linearly between two different concentrations of the same agonist.
than the CS of ADP, particularly a 42.9% for 1 μg/ml collagen.

Preferred agent and the corresponding prevalence of aspirin resistance

These data suggested that collagen would be a preferred agent for identifying aspirin resistance in our study, with more accurate results and fewer false negative results. The mean value was 14.6 ± 2.1 (Ω) with 1 μg/ml collagen and 10.3 Ω was calculated as the cut-off value. Based on these values, cases could be divided into two groups: aspirin-resistant and aspirin-sensitive. Among 104 patients, 38 patients (36.5%) with impedance more than 10 Ω were considered to be aspirin resistant. We also compared the characteristics of aspirin-resistant patients (n = 38) to those of aspirin-sensitive patients (n = 66) (Table 3). Aspirin-resistant patients had more leucocytes [(6.32 ± 1.55) · 10^9/l vs. (5.77 ± 1.19) · 10^9/l, p = 0.04] and higher platelet counts [(165 ± 65) · 10^9/l vs. (134 ± 37) · 10^9/l, p = 0.01]. There were no differences in other characteristics between these two groups.

**Table 3**: Comparison of characteristics between AR and AS cases

<table>
<thead>
<tr>
<th></th>
<th>Aspirin resistant (n = 38)</th>
<th>Aspirin sensitive (n = 66)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.6 ± 10.8</td>
<td>65.5 ± 8.5</td>
<td>0.343</td>
</tr>
<tr>
<td>Male (%)</td>
<td>21 (55.3%)</td>
<td>36 (54.5%)</td>
<td>0.944</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.1 ± 2.5</td>
<td>23.0 ± 4.1</td>
<td>0.888</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>7 (18.4%)</td>
<td>15 (22.7%)</td>
<td>0.605</td>
</tr>
<tr>
<td>Atrial fibrillation (%)</td>
<td>6 (15.8%)</td>
<td>14 (21.2%)</td>
<td>0.499</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>22 (57.9%)</td>
<td>41 (62.1%)</td>
<td>0.671</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5 (13.2%)</td>
<td>9 (13.6%)</td>
<td>0.945</td>
</tr>
<tr>
<td>Hyperlipidaemia (%)</td>
<td>4 (10.5%)</td>
<td>8 (12.1%)</td>
<td>0.806</td>
</tr>
<tr>
<td>Cerebrovascular disease (%)</td>
<td>9 (23.7%)</td>
<td>9 (13.6%)</td>
<td>0.192</td>
</tr>
<tr>
<td><strong>Medication at</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin 100 mg (%)</td>
<td>38 (100%)</td>
<td>66 (100%)</td>
<td>–</td>
</tr>
<tr>
<td>Long-acting nitrates (%)</td>
<td>28 (73.7%)</td>
<td>55 (83.3%)</td>
<td>0.238</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>19 (50.0%)</td>
<td>37 (56.1%)</td>
<td>0.551</td>
</tr>
<tr>
<td>β-blockers (%)</td>
<td>12 (31.6%)</td>
<td>20 (30.3%)</td>
<td>0.892</td>
</tr>
<tr>
<td>ACEIs (%)</td>
<td>20 (52.6%)</td>
<td>30 (45.5%)</td>
<td>0.481</td>
</tr>
<tr>
<td>ARBs (%)</td>
<td>11 (28.9%)</td>
<td>16 (24.2%)</td>
<td>0.598</td>
</tr>
<tr>
<td>Calcium antagonists (%)</td>
<td>12 (31.6%)</td>
<td>19 (28.8%)</td>
<td>0.764</td>
</tr>
<tr>
<td>Diuretics (%)</td>
<td>13 (34.2%)</td>
<td>22 (33.3%)</td>
<td>0.927</td>
</tr>
<tr>
<td><strong>Laboratory parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocyte (10^9/l)</td>
<td>6.32 ± 1.55</td>
<td>5.77 ± 1.19</td>
<td>0.044</td>
</tr>
<tr>
<td>Haemoglobin (g/l)</td>
<td>126.97 ± 12.22</td>
<td>126.18 ± 12.77</td>
<td>0.758</td>
</tr>
<tr>
<td>Erythrocyte (10^12/l)</td>
<td>4.16 ± 0.40</td>
<td>4.18 ± 0.48</td>
<td>0.854</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.0 ± 9.3</td>
<td>38.8 ± 3.9</td>
<td>0.343</td>
</tr>
<tr>
<td>Platelet count (10^12/l)</td>
<td>165 ± 65</td>
<td>134 ± 37</td>
<td>0.01</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>265.0 ± 42.8</td>
<td>259.7 ± 42.7</td>
<td>0.550</td>
</tr>
</tbody>
</table>

ACEIs, angiotensin converting enzyme inhibitors; ARBs, adrenergic receptor blockers; AR, aspirin resistance; AS, aspirin sensitivity.
Incubation of aspirin in vitro

Box-plots (Figure 3) illustrated the distribution of impedance values with the preferred agent (1 μg/ml collagen) before and after incubation of 0.1 mmol/l aspirin in vitro. Apparently, aspirin could inhibit platelet aggregation completely. The linear chart (Figure 4) showed that among aspirin-resistant patients, the values of only three cases were still in the normal range in spite of incubation, and being 11, 12 and 12 Ω, respectively. Based on the typological approaches used by Weber (12) and Ivandic (14), this study demonstrated that aspirin resistance can be classified into two distinct types – a pharmacokinetic or a pharmacodynamic type. The three patients with impedance > 10 Ω were considered as manifesting a pharmacodynamic type of aspirin resistance. Conversely, aspirin resistant patients with impedance ≤ 10 Ω were classified as a pharmacokinetic type. Among 38 aspirin-resistant patients with UAP, the prevalence of the pharmacodynamic type was only 7.9%.

Discussion

This is the first study to detect the prevalence of aspirin resistance in patients with UAP. Prior studies (meta analyses and systematic reviews) have demonstrated a significant association between aspirin resistance and the risk of major adverse cardiovascular events (9,17). UAP is associated with an increased risk of subsequent myocardial infarct and cardiac death (11), and trials of aspirin in UAP have consistently documented a benefit of its use compared with placebo (18). Thus identification of the prevalence of aspirin resistance in UAP patients is of great clinical importance.

Platelet aggregation studies play an important role in the assessment of aspirin effect, although there is no standardised method at present for detecting aspirin resistance. Currently, there are various platelet function tests widely used in patients with ACS, such as optical aggregometry, an aggregometer with laser light scattering, measurement of 11-dehydrothromboxane B₂, PFA-100, the VerifyNow System.
Aggregation was defined by arachidonic acid-induced maximal responsive to clopidogrel. And aspirin non-response to clopidogrel treatment was defined as a patient non-responsive to ADP-induced maximal platelet aggregation after clopidogrel treatment was defined as a patient non-responsive to clopidogrel. And aspirin non-response was defined by arachidonic acid-induced maximal aggregation \( \geq 60\% \). A new aggregometer with laser light scattering was used to study platelet aggregation in patients with ACS (21), and this device could be sensitive enough to identify small aggregates consisting of only two or three platelets, which allowed a precise assessment of aggregation processes in the setting of ACS. As we have noted, it has been suggested that aspirin resistance can be defined as the failure of suppressing thromboxane biosynthesis in aspirin-treated patients. Thus, Cipollone et al. (22) identified aspirin-insensitive patients with UAP by measuring 11-dehydro-thromboxane B\(_2\) in urine samples. In addition, Fuchs et al. (23) elucidated that shortened closure time values reflected biologically relevant platelet hyperfunction in patients with ACS, because they predicted recurrent ACS by a use of PFA-100. Moreover, it has been reported (24) that 19% (\( n = 20 \)) of 105 patients with ACS was aspirin resistant by PFA-100, and in the follow up, aspirin-resistant patients were at higher risk for major adverse cardiovascular events compared with patients having aspirin-sensitive platelet aggregation. Currently, Atiemo et al. (25) assessed platelet function by using PFA-100, flow cytometry and light transmittance aggregometry in patients presenting with chest pain, and found that patients with abnormal PFA-100 closure times had increased platelet aggregation and activation. However, this aspirin resistance identified by PFA-100 was not associated with clinical events in the index hospitalisation. The VerifyNow System, one of point-of-care assays, was also used to evaluate the relationship between aspirin responsiveness/resistance and diabetic outcomes in patients with ACS (26). The results showed that aspirin resistance (aspirin reaction units > 550) was associated with an increase in major adverse cardiovascular events overall.

A whole blood aggregometer which we used in this study was introduced by Cardinal and Flower in 1980 (27). Some studies have suggested that the latter assay appears to have its advantages. First, WBA offers a more physiologic assessment of platelet response to antiplatelet drugs with the presence of prostacyclin and thromboxane A\(_2\) (30), as well as injury to platelets and loss of large and hyperactive ones. Fourthly, WBA has higher sensitivity in detecting drug-related platelet dysfunction verified in some aspirin-treated cases (31). However, every coin has two sides. Clearly, this methodology – WBA – has its own disadvantages. Platelet aggregation studies must be performed within 3 h after blood collection, and a delay may influence the results significantly (32). Improper venipuncture and sample collection may cause platelet activation. Additionally, erythrocytes and leucocytes in whole blood have been shown to regulate actively the availability of ADP in blood, and when used as an agonist, ADP is believed to be necessary to elicit platelet aggregation in whole blood (31,33). And even some studies have shown that the results of platelet aggregation in whole blood and platelet rich plasma were not closely correlated (34,35). As for thrombocytopenic samples, the conclusive interpretation of WBA results is limited, because the reference range for normal samples cannot be used. Furthermore, there are many drugs which can affect platelet function (31), including non-steroidal anti-inflammatory drugs, some antibiotics, diuretics, etc. Thus, proper interpretation of WBA results requires taking into account the patient’s drug history. Generally, WBA is a rapid, simple and accurate method for monitoring antiplatelet therapies, and nowadays has been a useful tool for physicians to assess platelet function, especially platelet aggregation in clinical practice. Mueller et al. (36) reported that among 100 patients with intermittent claudication who underwent elective percutaneous balloon angioplasty, there was considerable variation in the response to aspirin and that incomplete platelet inhibition by aspirin was associated with an increased risk of reocclusion following peripheral angioplasty. Willoughby et al. (37) evaluated platelet aggregation of 51 patients with ACS within 24 h of coronary care unit admission using WBA, as well for identification of platelet nitric oxide (NO) responsiveness, and in this study a
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sodium nitroprusside (NO donor) response of < 32% inhibition of aggregation was categorised as an ‘impaired’ platelet NO response. They finally demonstrated that impaired platelet NO responsiveness is a novel and independent predictor of increased mortality and cardiovascular morbidity in patients with high-risk ACS during a median of 7 years of follow up. Thus, WBA was also chosen by us for identifying aspirin-resistant patients in this study.

We measured the responsiveness to aspirin of UAP patients with two kinds of agonists – collagen (1 and 2 μg/ml) and ADP (5 and 10 μmol/l). It has been shown that aspirin could not inhibit collagen- or ADP-induced platelet aggregation completely. According to the corresponding cut-offs, the results of assessment show statistical associations between the four inducing conditions, but the prevalence of aspirin resistance detected by collagen was less than that detected by ADP, especially collagen at low concentration (1 μg/ml). However, ADP is not specific to the cyclooxygenase pathway, and its use may translate into a higher rate of falsely aspirin-resistant patients (38). In our study, after coefficients of sensitivity were calculated and compared with one another, the CS of 1 μg/ml collagen (42.9%) was the highest of all. It could be concluded that collagen at low concentration is considerably more sensitive than ADP to the inhibitory effect by aspirin, which was in conformity with prior results. Sathiropas (39) studied the sensitivity of WBA for the detection of minimal aspirin effect on platelet aggregation, and found that it depended on the agonist type and concentration. Finally, he concluded that in the presence of low concentration of collagen (1 μg/ml), aggregation is sensitive to inhibition by aspirin; this agreed with the same suggestion by Weber (12). Vargaftig et al. (40) who demonstrated that, with collagen at high concentrations, no inhibition was seen irrespective of the aspirin dose. On these bases, we suggest that aspirin resistance should be assessed with collagen at a low concentration (1 μg/ml). In whole blood, collagen can stimulate thromboxane A2 synthesis from blood cell membrane-derived arachidonic acid by an action upon known collagen receptors, and perhaps integrates all relevant sources of thromboxane A2 formation and thromboxane-independent platelet aggregation as potential causes of aspirin non-responsiveness.

For a comparison, WBA has been widely used by other studies. Ivandic et al. (14) examined 66 healthy control individuals and 144 aspirin users with stable coronary artery disease by WBA, and a 6-min impedance > 8 Ω with collagen (mean – 2SD of the controls) was taken as evidence of non-responsive-ness. Finally, they found that collagen could identify 15% non-responsive aspirin users. Lordkipanidze et al. (38) reported that the prevalence of aspirin resistance in 201 patients with stable coronary artery disease receiving daily aspirin therapy (≥ 80 mg) was 18.0% for WBA. In the study with arachidonic acid as an agonist, a cut-off value of 3 Ω was chosen, based on previous results obtained in their laboratory from healthy volunteers. Moreover, with the accepted reference ranges of 14–22 Ω for collagen-induced aggregation and 5–17 Ω for aggregation in the presence of arachidonic acid (41,42), Watala et al. (43) considered values higher than 14 Ω for collagen and higher than 0 Ω for arachidonic acid as the hallmarks of non-responsiveness to aspirin. This study observed that among patients with UAP more than one-third (36.5%) appeared to be aspirin resistant, and the phenomenon was significantly more prevalent compared with patients with stable coronary artery disease in prior studies (44,45). The mechanism underlying aspirin resistance is unclear, although several theories have been proposed (46). As for this study, there may be several possible causes for the higher prevalence of aspirin resistance in UAP patients. First, we measured platelet function of defined UAP patients, and at the time of UAP, platelets from aspirin-resistant patients may be more sensitive and activable by agonists including ADP and collagen. This hypersensitivity could provide a possible explanation for the phenomenon (47,48). Secondly, increased platelet turnover with transient expression of inducible cyclooxygenase-2 may contribute to platelet aggregation insensitive to aspirin (49). Thirdly, the objects of the study were Chinese; perhaps genetic polymorphisms involved in thromboxane biosynthesis among different populations can affect the responsiveness of platelet to aspirin. Fourthly, previous studies on acute myocardial infarction have shown that it is possible to be resistant to aspirin during an acute event (50,51), and some platelet activating factors such as epinephrine, thrombin and ADP, which have been found to be increased at AMI, may account for some cases of aspirin resistance (52,53). Thus, we hypothesise that the high prevalence of aspirin resistance in UAP patients may also be associated with the elevated blood levels of activating factors, which needs to be verified in the future studies.

In vitro incubation with aspirin helped us classify aspirin resistance. To determine the maximal inhibition that can theoretically be achieved, we incubated samples from potentially non-responsive patients with 0.1 mmol/l aspirin. In the pharmacokinetic type of resistance, oral aspirin treatment is ineffective, but incubation with aspirin in vitro inhibits collagen
induced platelet aggregation, and it appears that an inadequate dose has an association with aspirin resistance of this type. In the pharmacodynamic type, neither oral aspirin treatment nor incubation of aspirin in vitro inhibits collagen-induced platelet aggregation. This typology may be helpful in clinical practice for deciding whether a patient with pharmacokinetic resistance should receive a higher dose of aspirin to prevent the increased risk of thrombo-embolic events, or if a patient with pharmacodynamic resistance should take an alternative antiplatelet drug. However, to elucidate the mechanism(s) of aspirin resistance and to optimise aspirin therapy in aspirin-resistant patients, there is need for large scale, prospective and randomised clinical investigations.

In conclusion, WBA can be used widely as a simple and rapid bedside test to detect responsiveness to aspirin in coronary artery disease, and collagen at a low concentration is a considerably more sensitive indicator than ADP of the alteration of platelets caused by aspirin. With 1 μg/ml collagen, the prevalence of aspirin resistance (36.5%) among UAP patients is higher than those with stable coronary artery disease in prior studies. Also, in vitro incubation with aspirin may help classify resistance in clinical practice for a pharmaceutical strategy of aspirin in cardiovascular disease.

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Author contributions

Jiabei Li and Lan Huang contributed to the concept of the paper and drafted the manuscript. Hongmei Dong and Zhao Jian were responsible for operational aspects of the study, including collecting the data. Xiaoqing Wu, Xiaohui Zhao and Shiyong Yu helped in data analysis. All authors were actively involved in data collection and critical discussion of the paper.

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