Whole Blood Multiple Electrode Aggregometry Is a Reliable Point-of-Care Test of Aspirin-Induced Platelet Dysfunction

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BACKGROUND: Aspirin is one of the most commonly ingested over-the-counter drugs. In addition to its analgesic and antiinflammatory actions, it also potently inhibits platelet aggregation. Evaluation of aspirin-induced platelet dysfunction is relevant in various clinical situations, including during complex surgeries with high bleeding risk in individuals who have ingested aspirin. In this study, we examined the suitability of multiple electrode aggregometry (MEA) for time course assessment of the antiplatelet effects of a single oral dose of 500 mg aspirin. We also determined the applicability of this method in the point-of-care (POC) setting by comparing the results of the test after different time intervals after blood sampling.

METHOD: Twenty-four adult volunteers were enrolled in the study. After blood drawing at baseline, 500 mg aspirin was administered to all volunteers. Blood samples were taken at 4, 24, 56, 80, and 124 h after aspirin ingestion. At each time point, measurements were performed immediately and 30 and 60 min after drawing blood. Whole blood MEA was performed after stimulation with thrombin receptor activating peptide (TRAPtest, 32 μM) and arachidonic acid (ASPItest, 0.5 mM). Repeated measurement analysis of variance with a Bonferroni correction for multiple comparisons was performed to detect differences between time points. Assay imprecision was determined by calculating the coefficient of variation. The level of statistical significance was set to \( P < 0.05 \).

RESULTS: Platelet aggregation by ASPItest was markedly decreased 4 h after aspirin intake. From the second day after aspirin intake, ASPItest values recovered with high interindividual variability, and 5 days after aspirin intake, ASPItest values did not differ significantly from baseline. TRAP-induced platelet aggregation (TRAPtest) showed no systematic changes during the study period. The resting time of the sample did not affect TRAPtest or ASPItest values. The coefficients of variation were 10% for the ASPItest and 7% for the TRAPtest.

CONCLUSIONS: MEA reliably detected the effects of aspirin. Notably, 500 mg aspirin caused complete inhibition of arachidonic acid-induced platelet aggregation for 2 days in all volunteers. Aggregation returned to baseline values with a wide interindividual variation in time course by day 5. No resting time for the blood sample was required for ASPItest or TRAPtest. These assays can be implemented as real POC tests. The reproducibility of the assays studied here is within the range of modern POC analyzers.

increased risk of perioperative bleeding. Preoperative aspirin administration increases blood loss during bleeding-sensitive operations. Thus, the American College of Chest Physicians suggests that patients having noncardiac surgery who are at low risk for cardiac disease stop aspirin intake 7–10 days before surgery.

Quantitative measurement of the antiplatelet effect of aspirin has clinical relevance, as platelet inhibition differs among individuals. Although laboratory aspirin resistance, a condition of inadequate inhibition of platelet function by aspirin, depends considerably upon the assessment method, it is associated with an increased incidence of major thromboembolic events. The detection of residual aspirin-related platelet dysfunction could result in postponement of complex bleeding-sensitive surgical interventions. However, the exclusion of residual aspirin effects in individuals with suspected aspirin intake or in aspirin-treated patients with unknown compliance could prevent unnecessary postponement of bleeding-sensitive surgical interventions or transfusion of platelet concentrates. Therefore, convenient methods for measuring the degree of platelet inhibition by aspirin or other platelet inhibitors are highly desirable.

Multiple electrode aggregometry (MEA) (Multiplate®, Dynabyte, Munich, Germany) is a newly developed technique to test platelet function in whole blood, based on classical whole blood impedance aggregometry. MEA has recently been used to study the effects of aspirin, non-opioid analgesics, clopidogrel, anticoagulants, antifibrinolytics, and temperature on platelet aggregation. MEA has five MEA test cells for parallel testing, and the attachment of platelets to the electrodes was continuously measured for each sensor unit over a period of 6 min. Data were transformed to arbitrary AU and plotted as two separate aggregation curves versus time. Aggregation measured by MEA was quantified by arbitrary AU and time. Aggregation measured by MEA was quantified as the area under the aggregation curve (AUC, [AU*min]). Alternatively, the software of the analyzer allows for the expression of AUC values in [U], where 10 [AU*min] correspond to 1 [U].

Values from aspirin-treated patients were significantly lower than those from healthy blood donors. However, thus far, no information is available regarding MEA determination of the time course of platelet inhibition after ingestion of a single high dose of aspirin.

MEA does not require a specialized coagulation laboratory and may be useful for point-of-care (POC) analysis. As for most platelet function tests, a resting time of 30 min after blood sampling before testing is recommended from the manufacturer for MEA analysis. This resting time may impede immediate detection of platelet dysfunction intraoperatively.

The degree of aspirin-induced platelet inhibition is of particular interest in the perioperative setting. We hypothesized that MEA could reliably detect the effect of aspirin at the POC. Therefore, we selected MEA for our study i) to assess the time course of the antiplatelet effect before and up to 5 days after the ingestion of 500 mg aspirin, and ii) to reappraise the need of a 30-min resting interval before measurement.

**METHODS**

**Study Population**

After IRB approval, 24 healthy adult volunteers gave written informed consent and were included in the study. All participants denied the intake of any medication during the previous 10 days and had no bleeding history.

**Blood Sampling and Intervention**

Blood was drawn from the antecubital vein by puncture without stasis using a 21G butterfly needle. The first 2 mL of blood was discarded. Blood was then collected into 4.5 mL tubes containing 25 µg/mL hirudin (Dynabyte, Munich, Germany) as an anticoagulant, according to the recommendations of the manufacturer. After blood drawing at baseline, all study participants took one tablet of 500 mg acetylsalicylic acid (Aspirin-Hexal®, Hexal AG, Holzkirchen, Germany) in the presence of a study advisor. Blood samples were then taken 4, 24, 56, 80, and 124 h after aspirin intake.

**Platelet Function Assays**

Platelet function analysis was performed using the Multiplate analyzer, a novel whole blood impedance aggregometer (Dynabyte, Munich, Germany). The device has five MEA test cells for parallel testing, and each test cell incorporates two independent sensor units. One unit consists of two silver-coated, highly conductive copper wires. Analysis is based on platelet adhesion upon activation, a property that results in aggregation onto the metal sensor wires in the test cell, thus increasing the electrical impedance between the wires. For measurement, 300 µL of preheated saline (37°C) and 300 µL hirudin-anticoagulated whole blood were placed into the test cell, and the sample was stirred using a teflon-coated electromagnetic stirrer (800 rpm) over a 3-min incubation period.

Platelet aggregation was initiated using arachidonic acid (AA) (ASPItest, 0.5 mM) or thrombin receptor-activating peptide (TRAP-6, TRAPtest, 32 µM) using reagents supplied by the manufacturer (Dynabyte, Munich, Germany). Increased impedance due to attachment of platelets to the electrodes was continuously measured for each sensor unit over a period of 6 min. Data were transformed to arbitrary AU and plotted as two separate aggregation curves versus time. Aggregation measured by MEA was quantified as the area under the aggregation curve (AUC, [AU*min]). Alternatively, the software of the analyzer allows for the expression of AUC values in [U], where 10 [AU*min] correspond to 1 [U]. The duplicate sensors served as an internal control to reduce the occurrence of systematic errors. Pearson’s correlation coefficients of the individual data points of the curves.
as assessed by the two electrode pairs, and the differences between the AUC values detected by each sensor unit and the mean AUC were calculated. When the values were outside the acceptable range (correlation coefficient <0.98, difference from the mean curve >20%), the results were flagged and the measurement was repeated. Measurements were performed at the POC immediately before and at 4, 24, 56, 80, and 124 h after aspirin ingestion. To study the effect of the resting, both assays were also performed immediately after blood drawing (T0) and after resting intervals of 30 (T30) or 60 (T60) min.

Statistical Analysis

Statistical analysis was performed using Sigma Stat software (Version 3.1, Jandel, San Rafael, CA). For sample size analysis, we defined differences in platelet aggregation between analyses at T0, T30, and T60 exceeding 15% as unacceptably high. A difference larger than 15% would suggest an impact of the sample resting time on the measurement results which would discard a real POC suitability of these assays. Thus, the minimum detectable differences in the means were 165 and 135 AU·min for TRAPtest and ASPITest, respectively (15% of the expected baseline values). To detect significant differences in platelet aggregation at T0, T30, and T60, sample size analyses (expected standard deviation 170 and 150 AU·min in TRAPtest and ASPITest, respectively, desired power 0.8, \( P < 0.05 \)) resulted in sample sizes of 22 and 25 for TRAPtest and ASPITest, respectively. As the expected differences in platelet aggregation in the ASPITest before and after aspirin treatment were much higher than 15%, no sample size analysis for this objective was performed.

One-way repeated measures analysis of variance was used to detect differences between the time points. If the normality test (Kolmogorov-Smirnov) failed, Friedman repeated measures analysis of variance on ranks was used. In case of significant differences in the means (or medians), a Bonferroni (or Dunn) post hoc multiple comparison procedure versus control was applied according to the distribution of the data.

Variability of measurements was quantified using the mean of the standard deviations of the three consecutive measurements (T0, T30, and T60) at each measuring point and in each subject, and are expressed as a percentage of the mean values (coefficient of variation, CV, %).

Results are expressed as the mean ± sd or as the median. The level of statistical significance was set to \( P < 0.05 \).

RESULTS

Of the 24 volunteers studied, 11 (46%) were men. The mean age was 31.1 ± 3.7 yr and the mean Body Mass Index was 23.2 ± 2.8 kg/m². Bleeding and drug history were negative for all study participants.

The baseline range of MEA values in the different assays was determined as the range between the minimum and maximum values obtained from the baseline measurement 30 min after blood drawing, including the data from all volunteers. Baseline values were in the range from 596 to 1197 AU·min for ASPITest and 897-1469 AU·min for TRAPtest (Fig. 1). Four hours after aspirin ingestion each volunteer had MEA values below the cutoff of 300 AU·min for therapeutic aspirin effect in ASPITest (Fig. 1). Nonresponders were not observed.

Platelet aggregation after stimulation with AA and TRAP (mean and positive sd) is shown in Figure 2.

Figure 1. Distribution of baseline values and values 4 h after aspirin ingestion in ASPITest and TRAPtest, obtained 30 min after drawing blood. The straight horizontal line shows the upper cutoff value for the full aspirin effect as determined by von Pape et al.\(^9\)

Figure 2. Platelet aggregation after activation with arachidonic acid (AA) in ASPITest or with TRAP-6 in TRAPtest at T0, T30, and T60. The bars show the means, and error bars indicate the positive sd. *\( P < 0.001 \) as compared with baseline at T0, #\( P < 0.001 \) compared with baseline at T30, and §\( P < 0.001 \) as compared with baseline at T60.
The mean AA-induced platelet aggregation in ASPItest was significantly decreased 4 h after aspirin intake, with stepwise normalization of aggregation by 124 h after drug ingestion (Table 1).

Aspirin-induced platelet inhibition was homogenous for up to 24 h after aspirin intake, as all study participants showed a therapeutic aspirin effect (AUC < 300 AU*min) at 4 and 24 h (Fig. 3). On day 3, at 56 h, platelet aggregation as measured by ASPItest was <300 AU*min in 63% (15 of 24) of volunteers and on day 4 at 80 h in 8% (2 of 24) of the volunteers. None of the subjects showed a therapeutic antiplatelet effect of aspirin on day 6 (124 h) after drug administration.

The individual course of the antiplatelet effect of aspirin was determined by calculating the individual aggregation responses at each measuring point relative to the baseline value (AUC<sub>4h</sub>/AUC<sub>baseline*100</sub>, AUC<sub>24h</sub>/AUC<sub>baseline*100</sub>, etc.), using the values obtained for ASPItest at T30. Aggregation response at 4 h was in the range of 7%–24% (mean 18%). At 24 h, uniform suppression of AA-induced platelet aggregation was still found, with an aggregation response in the range of 7%–35% (mean 18%). In contrast, marked interindividual variability was observed at 56 and 80 h after aspirin intake (Fig. 3). At 56 h, the individual aggregation response ranged from 9% to 61% (mean 28%), and at 80 h it ranged from 10% to 91% (mean 61%). On day 6 (124 h) after aspirin intake, the aggregation response reached in mean 89% of the baseline value (range, 47%–117%).

Platelet aggregation returned to the baseline range (596–1197 AU*min) in none of the volunteers by 56 h, in 33% by 80 h and in 88% by 124 h.

Platelet aggregation in TRAPtest showed no significant changes during the 5-day study period after aspirin intake (Table 1, Fig. 2).

There were no significant differences in AUC values when samples were measured immediately after drawing blood or after a 30 or 60 min rest (Fig. 2). Therefore, we treated the measurements at the three resting time points as triplicates to calculate the imprecision of the assays. We quantified this by determining the CV at the measuring points with mean values within the baseline ranges (baseline and 124 h)

### Table 1. Mean ± SD of Arachidonic Acid- and TRAP-Induced Platelet Aggregation in ASPItest and TRAPtest at all Measuring Points (Baseline, 4, 24, 56, 80, and 124 h) and Resting Intervals (T0, T30, T60), n = 24

<table>
<thead>
<tr>
<th>Measuring point</th>
<th>ASPItest (AUC, [AU*min])</th>
<th>TRAPtest (AUC, [AU*min])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0 Mean ± SD</td>
<td>T30 Mean ± SD</td>
</tr>
<tr>
<td>Baseline</td>
<td>839 ± 164</td>
<td>889 ± 157</td>
</tr>
<tr>
<td>4 h</td>
<td>137 ± 44</td>
<td>155 ± 44</td>
</tr>
<tr>
<td>24 h</td>
<td>151 ± 71</td>
<td>160 ± 63</td>
</tr>
<tr>
<td>56 h</td>
<td>207 ± 144</td>
<td>245 ± 126</td>
</tr>
<tr>
<td>80 h</td>
<td>485 ± 167</td>
<td>527 ± 172</td>
</tr>
<tr>
<td>124 h</td>
<td>711 ± 138</td>
<td>798 ± 156</td>
</tr>
<tr>
<td>T0</td>
<td>1173 ± 170</td>
<td>1155 ± 147</td>
</tr>
<tr>
<td>T30</td>
<td>1210 ± 209</td>
<td>1204 ± 144</td>
</tr>
<tr>
<td>T60</td>
<td>1163 ± 148</td>
<td>1168 ± 168</td>
</tr>
<tr>
<td>T0</td>
<td>1209 ± 143</td>
<td>1186 ± 104</td>
</tr>
<tr>
<td>T30</td>
<td>1192 ± 132</td>
<td>1233 ± 148</td>
</tr>
<tr>
<td>T60</td>
<td>1160 ± 127</td>
<td>1167 ± 146</td>
</tr>
</tbody>
</table>

T0 = immediate measurement after blood drawing; T30 = 30; T60 = 60 min after blood drawing; AUC = platelet aggregation [AU*min]; sd = standard deviation.

### Figure 3. Individual platelet aggregation after stimulation with arachidonic acid (AA) (ASPItest). Each plot depicts the time course of the aspirin effect as determined by multiple electrode aggregometry (MEA) in one volunteer after a 30 min rest of the sample.

**DISCUSSION**

The main findings of this study were that i) MEA reliably detected the antiplatelet effect of aspirin and AA-induced platelet aggregation returned to baseline values with a wide interindividual variation in time course by day 5, and ii) no resting time for the blood samples was required before determination of ASPItest and TRAPtest.

Notably, 500 mg aspirin caused complete inhibition of AA-induced platelet aggregation in all volunteers (Fig. 1). A dose of 500 mg was selected for this study as it corresponds to the typical dose of aspirin ingested as an analgesic in Europe and is between 1 and 2 tablets of the over-the-counter United States dose (325 mg). Nonresponsiveness to aspirin was not observed in our study, probably due to the high dosage administered. The available prevalence data for aspirin resistance are inconsistent and vary from 5% to 80%, mainly depending on the diagnostic method used. In two recent studies, several assays were compared for...
assessment of the effects of aspirin on platelet function,7,19 but only a moderate correlation was found between the different methods. Thus, definition of laboratory aspirin resistance demands clarification. Although 5%–80% is a very wide range, laboratory aspirin resistance is nonetheless associated with impaired outcome in patients with cardiovascular, cerebrovascular, or peripheral arterial diseases.6 Therefore, the clinical impact of aspirin resistance is significant, and good standardized and validated bedside tests are needed to reliably quantify the antiplatelet effect of aspirin.

Some studies have linked MEA data to bleeding4,10,20 or thrombotic outcomes.21 A very recent investigation of 100 patients undergoing cardiac surgery suggested that preoperative ASPITest in MEA may be a more sensitive predictor of platelet transfusion than patient self-reporting on aspirin intake.10 The cutoff for an abnormal aggregation response of AUC <510 AU*min determined in that study, however, is not applicable to our data because the authors used heparinized blood.10 For MEA analysis hirudin is the best investigated and standardized anticoagulant; however, some centers use heparinized blood and report reproducible results.10,22 Additionally, the manufacturer of the analyzer recommends the use of hirudin-anticoagulated blood collection tubes. Citrated samples are not recommended for MEA analysis, as this calcium-chelating anticoagulant does not preserve the physiological concentrations of ionized calcium and magnesium, which are essential for platelet aggregation.8 In most studies using MEA, hirudin blood has been used9,11,12,14–16,21,23; therefore, we also selected hirudin as the anticoagulant for this study.

For the first 2 days after aspirin intake, we observed a more than 80% suppression of AA-induced platelet aggregation in all volunteers. This suppression was comparable with results reported for MEA during long-term aspirin treatment.9 We could also confirm the cutoff value for a therapeutic aspirin effect used in our study, as 300 AU*min clearly separated the baseline values from those obtained 4 h after aspirin ingestion (Fig. 1). Platelet function normalized gradually between the third and fifth days after drug ingestion. The time course of the antiplatelet effect of aspirin as assessed by MEA in our study was in accordance with results obtained via other monitoring techniques such as thromboxane B2 production24 or PFA-100,25 despite the fact that the administered doses in these studies were lower.

Although various studies have confirmed a correlation of the results obtained by MEA and PFA-100 under aspirin treatment,9,13,26 assessment of platelet function by MEA might be superior in the perioperative setting. PFA-100 is not specific to the aspirin-sensitive cyclooxygenase-1 (COX-1) pathway and has been reported to have limited predictivity for perioperative bleeding and transfusion requirements.27,28 Furthermore, PFA-100 closure-time has been shown not to be reliable in cases of low platelet count (PC) or hematocrit,29 thus limiting its intraoperative usefulness in patients with blood loss.

In the present study, we observed a wide interindividual variability in the attenuation of the aspirin effect beginning at 56 h after administration (Fig. 3). Here, the possible effect of PC may be discussed. Within normal ranges (164–395/nL), no significant impact of PC on MEA results has been shown.9 As marked variability was observed only in ASPITest (intersubject variabilities of 58% and 33% at 56 and 80 h, respectively) but not in TRAPtest (intersubject variabilities of 10% and 11% at 56 and 80 h, respectively), differences in PC may not explain the observation of this phenomenon only in ASPITest. There are a number of further possible reasons for the variability of the aspirin effect, including those arising from patient compliance, individual platelet turnover after aspirin ingestion, the individual importance of the thromboxane A2-pathway, platelet hyperreactivity, molecular genetic variants of COX-1, and drug interactions with other COX-1 inhibitors.6,30 Considering that all study participants were young, healthy volunteers without any medications or comorbidity taking a witnessed dose of aspirin, it is likely that aspirin-treated patients would show even greater variability in the time course of aspirin activity.31,32

The observed interindividual variability at 56 and 80 h after aspirin intake may have clinical implications for the perioperative management of aspirin-treated patients. At 56 h, only 63% of volunteers exhibited therapeutic suppression of platelet activity (AUC < 300 AU*min) after taking 500 mg aspirin. In these volunteers, a moderate, nontherapeutic aspirin-induced platelet inhibition still has to be assumed, as platelet aggregation reached the normal range (596–1197 AU*min) in none of these subjects at 56 h. Although the clinical relevance of this moderate aspirin effect is not known, discontinuing aspirin for more than 2 days before surgery might result in near-normal platelet aggregation for a longer period than desirable. This could lead to unnecessary postponement of intervention or may increase the prothrombotic risk in patients with cardiovascular diseases. Recommendations for an optimal time for cessation of aspirin treatment before surgery remain contradictory.1,25,33 The observed interindividual variability of the aspirin effect in the present study and the consequent need for individual risk assessment supports the importance of preoperative assessment of platelet function when patients have reported aspirin intake before invasive procedures.

As expected, aspirin intake did not affect platelet aggregation in TRAPtest (Fig. 2). TRAP acts via the thrombin receptor, and the resulting massive thrombin stimulation of platelets bypasses the inhibition of COX-1 by aspirin. Thus, impairment of TRAPtest might be an indicator of global platelet function impairment. AA-induced platelet aggregation in
ASPItest can more specifically detect platelet inhibition by aspirin or other COX-1 inhibitors.

Another important finding of this study was that platelet aggregation in response to AA and TRAP-6 was not affected by the resting time of the sample before testing, i.e., when samples were examined immediately or 30 or 60 min after collection. For nearly all platelet function tests, including MEA, it is recommended to wait 30 min after blood sampling before performing measurement. For appraisal of POC suitability, however, comparison of the results obtained after immediate measurement and different resting periods is essential. Our findings indicate that the recommended resting time of 30 min is not necessary for monitoring platelet function using ASPItest or TRAPtest. These assays can be implemented as real POC tests.

The results obtained with MEA were consistent and reproducible, with assay imprecision (CV) values of 10% and 7% for ASPItest and TRAPtest, respectively; this is within the range of modern laboratory POC testing.

There were some limitations to our study. First, our study was not designed to evaluate the clinical predictivity of MEA results. We could not determine whether patients with low aggregation results in ASPItest actually showed increased bleeding, or whether patients with high aggregation results in ASPItest actually showed more prothrombembolic events. Second, as mentioned we did not measure the PC, as young and healthy volunteers not taking any medications are very unlikely to have abnormal PC. Third, we did not compare our results to an established platelet function test other than MEA. However, this study was not intended to provide a comparison among different techniques. Although MEA is a relatively new method for whole blood impedance aggregometry, various studies have confirmed the correlation between the results obtained by MEA and light transmission aggregometry, and MEA and flow cytometry.

Despite its limitations, our study has important clinical implications. We confirmed the duration of aspirin action as measured by MEA. MEA accurately detected the time-dependent antiplatelet effect of aspirin and provided reproducible platelet aggregation results. The time course of the attenuation of aspirin effects in this cohort of healthy volunteers was much more variable than the time of onset. Likewise, it is not necessary to allow blood samples to rest before analysis. Both ASPItest and TRAPtest can be determined as real POC assays immediately after blood drawing.

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REFERENCES


