Background - Objectives

All relevant guidelines recommend low-dose aspirin since daily secondary prevention of myocardial infarction, stroke, and cardiovascular death (1-3). However, patients who develop aspirin resistance are increasingly becoming an issue of clinical concern, since they could be sub-optimally protected from cardiovascular disease (4-7). Aspirin resistance can be evaluated by biological testing and the rate of occurrence of outcome events (4-7). However, the proportion of aspirin-resistant patients varies greatly from one report to another ranging from 0% to 6% from cardiovascular disease patient series (6,7). The reasons for such variability are partly explained through several studies that have evaluated the effect of the aspirin dose as a parameter to explain aspirin resistance (8-10). The mechanism of action of aspirin and its antiplatelet effect has been long studied, mainly in healthy volunteers. The peak plasma levels of endogenous thromboxane A2 (TXA2) following 80 mg aspirin is 40-50% lower than aspirin (11). Thromboxane A2 is synthesized by cyclooxygenase 2 (COX-2), which is induced by aspirin, and plays its role on the platelet surface, first by generating various COX-2 products such as prostaglandin D2 (PGE2), prostaglandin E2 (PGE2), prostaglandin F2 alpha (PGF2a), thromboxane A2 (TXA2), and leukotrienes C4 (LTC4), and then by inhibiting platelet aggregation (12). However, there is no definitive consensus on the role of aspirin resistance and its influence on platelet function in patients with coronary artery disease. In the present study, we aimed to evaluate the time-dependent antiplatelet effect of low-dose aspirin in patients undergoing elective surgery, and to assess the reproducibility of on-treatment aspirin resistance.

Methods

Patients

The present study was conducted according to the Declaration of Helsinki. Patients were healthy volunteers (n=47) who were taking low-dose aspirin (100 mg/day) for at least 1 week before undergoing elective surgery. The study was approved by the ethics committee of the hospital (IRB number: 2017/0012). All patients provided informed consent before participating in the study. We excluded patients who had a history of cardiovascular disease, diabetes, or chronic kidney disease. In addition, the aspirin dose was stopped 72 hours before surgery. Blood samples were drawn before surgery and 24 hours after aspirin intake.

Blood sampling

Blood samples were collected from a peripheral vein in 10 mL vacutainer tubes containing citrate anticoagulant (final concentration 1:9). The citrate tubes were centrifuged at 1000 ×g for 10 minutes at room temperature. The supernatant plasma was immediately frozen and stored at -80°C until platelet aggregation and thromboxane B2 synthesis were measured. Samples were stored at -80°C until use. The samples were run in a triplicate manner on the same day.

Light Transmission Aggregometry (LTA)

Aggregation was performed by aggregating the platelets with 1 µmol/L ADP in a Ristocetin C assay to assess the aggregation response of platelets to ADP. The aggregation was measured using a LTA-100 aggregometry (Prometheus Medical, Munich, Germany). Each sample was run in three replicates and the mean of the three values was used for analysis. The aggregation response was calculated as a percentage of the aggregation response of a positive control (active) sample, defined as 100%.

Whole blood TXB2 assay

Whole blood was prepared from citrated tubes for the determination of TXB2 as described previously (13) and performed on an immunometric assay system (Pentra® 129 Platelet Function System, Biocare Medical, Walnut Creek, CA). The platelet sodiated TXB2 (P-129-010) calibrators were prepared with TXA2 from rat brain tissue. The TXB2 calibrators were constructed with TXA2 to allow for the measurement of TXB2 in the presence of citrate. The TXB2 assay was performed as recommended by the manufacturer on the Pentra platform. TXB2 concentrations were calculated as the mean of the three replicates and the mean of the three values was used for analysis. The TXB2 assay was used for the calculation of the percentage of TXB2 synthesized in whole blood samples.

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Results

150 patients were included, all receiving standard recommended treatment for CAD. Aspirin dose was in the lower range (75–100 mg once daily) for 60% of the patients (39 with 75 mg/day and 21 with 100 mg/day) and in the higher range (160–250 mg once daily) for 40% (28 with 160 mg/day and 12 with 250 mg/day).

As shown in Figure 1 (black squares), seven patients in the total patient population (4.7%) demonstrated significant AA induced platelet aggregation 2 hours after aspirin intake (peak effect) as compared to 37 patients (24.7%) who demonstrated significant aggregation 24 hours after aspirin ingestion (trend effect – p=0.0001).

Influence of patient characteristics on 24-hour aspirin efficacy

The results of the present study were further analyzed by the endogenous TXB2 synthesis on a per-patient basis. As shown in Table 1, the platelet reactivity to ADP was significantly lower in patients with a high endogenous TXB2 synthesis compared to patients with a low endogenous TXB2 synthesis. This finding indicates that the platelet reactivity to aspirin may vary among patients. Additionally, the platelet reactivity to ADP was significantly lower in male patients compared to female patients. These findings suggest that the platelet reactivity to aspirin may vary among patients based on their sex.

Conclusions

In conclusion, our study demonstrates that, in patients chronically treated with recommended daily low-dose aspirin, aspirin “resistance” increases progressively to reach nearly 25% of patients by 24 hours after last aspirin intake. Such a “resistance” is also observed in patients treated with high doses of aspirin (>100 mg and ≥250mg daily). Any biological assessment of aspirin efficacy should take time since last aspirin intake into consideration. Several studies are needed to assess whether these results are clinically relevant, and if this type of aspirin “resistance” could be avoided by repeated low-dose aspirin use daily instead of increasing the dose of a single daily intake without increasing the side effects such as gastrointestinal toxicity.