Patient Population

From January 2016 to June 2016, a total of 196 patients on chronic aspirin treatment were screened. All patients were on aspirin 100 mg/die. Patients were on aspirin therapy for primary or secondary prevention as per judgement of treating physician. Patients were excluded if any of the following criteria were present: use of any drug, other than aspirin, known to interfere with platelet function (nonsteroidal anti-inflammatory agent, cilostazol, P2Y12 receptor inhibitors) in the previous 10 days; use of oral or intravenous anticoagulant therapy in the previous 2 days; the presence of acute or chronic inflammatory diseases; aspirin use less than 2 months.

Patient compliance to antiplatelet treatment was assessed by interview and by arachidonic acid–induced (0.75 mM) platelet aggregation (if < 10% indicative of compliance to treatment). Nine patients were excluded due to arachidonic acid–induced platelet aggregation higher than 10% after 4 minutes, suggesting lack of patient compliance to aspirin treatment; 11 patients were excluded due to aspirin treatment less than 2 months. Platelets from patients who had collagen-induced (4 μg/mL) platelet aggregation higher than 40% were in vitro aspirinated (100 μM, 20′, 37°C) and subsequently collagen-induced (4 μg/mL) platelet aggregation was performed. From our population, 54 patients were excluded due to collagen-induced (4 μg/mL) platelet aggregation less than 40% after 4 minutes of stimulation. Therefore, a total of 122 patients were eligible for the study. Patients with less than 40% aggregation were considered as sensitive to aspirin treatment (n = 67), while patients with higher than 40% aggregation were considered as having HARPR (n = 55).

Patients were considered hypertensive if blood pressure was higher than 140/90 in three different measurements when patients were in a supine position for at least 10 minutes, or if they were treated with antihypertensive drugs; hypercholesterolemic if serum cholesterol was greater than 220 mg/dL or if they were treated with a lipid lowering agent; smokers if they smoked more than five cigarettes per day.

Aspirin and Salicylic Acid Intraplatelet Concentration

To extract aspirin and salicylic acid from platelets, PFP (200 mL) was treated with 15 mL H3PO4 (7 M), 80 mg NaCl, 40 mL H2O/CH3OH: 1/1, and 50 mL internal standard solution. Hexane (8 mL) was then added and centrifuged at 2,500 × g for 15 minutes at 4°C. The organic (upper) layer was separated and evaporated to dryness under nitrogen stream.

The residue was dissolved in mobile phase (acetonitrile/water 30/70), and injected into HPLC (Binary LC pump 250; Perkin Elmer, Waltham, Massachusetts, United States). Aspirin and salicylic acid were analysed using RP8 (Pinnacle C8; Restek Corporation, Bellefonte, Pennsylvania, United States) column and eluted with acetonitrile/water (30/70) mobile phase. UV detector (UV/VIS 200, Perkin Elmer) set at 229 nm was used to identify and quantify the analytes (26).