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TXA_2 synthesis and COX1-independent platelet reactivity in aspirin-treated patients soon after acute cerebral stroke or transient ischaemic attack

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ABSTRACT

Introduction: The pharmacological target of aspirin is the inhibition of cyclooxygenase-1 (COX1) and thromboxane-A_2 (TX) synthesis. Very few data are available on TX assessment in patients with stroke. We studied platelet TX synthesis, COX1-independent platelet reactivity, the influence of platelet–erythrocyte interactions and the potential association between platelet responses and the severity of stroke, evaluated with a clinical score (NIHSS).

Material and Methods: We examined 157 aspirin-treated patients with acute stroke or TIA, 128 aspirin-free and 15 aspirin-treated healthy subjects (HS). Collagen-induced TX, platelet recruitment in whole blood and platelets ± erythrocytes (haematocrit 40%) were assessed in patients on daily-aspirin within three days from onset. Arachidonic-acid-, ADP-, thrombin-receptor activating peptide TRAP-, and collagen-induced aggregation were also evaluated.

Results: Partial TX inhibition (<95% inhibition *vs* aspirin-free controls) was observed in 13% of patients. This was associated with marked increases in COX1-dependent responses (arachidonic-acid- and collagen-induced aggregation and platelet recruitment; $P < 0.0001$) but not with differences in ADP- or TRAP-induced aggregation. Partial TX inhibition was independently associated with severe stroke (NIHSS ≥ 12) at both admission ($P < 0.05$) and discharge ($P < 0.05$). Among patients with fully blocked TX, those with elevated COX1-independent platelet reactivity (mean + 2SD of aspirin-treated HS) were most likely to suffer severe stroke ($P < 0.05$). Platelet–erythrocyte interactions enhanced platelet reactivity in these patients by COX1-dependent and -independent mechanisms ($P < 0.0001$).

Conclusions: TX inhibition by aspirin varied across patients. Partial TX inhibition and COX1-independent platelet hyperfunction were associated with more-severe stroke.

Introduction

Aspirin is the mainstay antiplatelet treatment for patients with acute stroke or transient ischaemic attack (TIA). The best-characterized effect of aspirin on platelets is the irreversible inhibition of both cyclooxygenase 1 (COX1) activity and the subsequent formation of thromboxane A_2 (TX), a potent platelet agonist and vasoconstrictor [1]. The consistent reduction in the risk of recurrence afforded by aspirin in patients with vascular diseases [2] highlights the importance of TX as a platelet-mediated factor underlying the growth and stabilization of thrombi [1]. However, there is growing *ex vivo* evidence that aspirin treatment has an individual effect on the inhibition of platelet function. The down-regulation of platelet function is less than expected in some patients, a phenomenon called "aspirin resistance," which has been predominantly studied in patients with ischaemic heart disease, whereas fewer data are available on patients with ischaemic stroke [3]. Moreover, most studies have evaluated the effects of aspirin on platelets using biological tests of platelet function rather than by assessing platelet TX synthesis, the pharmacological target of aspirin in platelets. We have found only two previous reports of the effects of aspirin on platelet TX inhibition, in 37 and 50 patients with acute stroke [4,5]. The urinary excretion of 11-dehydro-TXB_2 is reportedly elevated in aspirin-free and aspirin-treated patients with acute stroke [6,7], although this may derive from extra-platelet sources [8]. Importantly, the assessment of platelet TX production allows the accurate evaluation of the COX1-independent platelet responses in patients with fully blocked TX synthesis, which has not been studied in patients with acute stroke/TIA. Furthermore, although the prothrombotic effect of erythrocytes on platelet reactivity is one mechanism involved in the variable effects of aspirin [9–12], no data are available for patients with acute stroke.

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The aims of the present study were: a) to study the effects of aspirin on platelet TX synthesis and the effects of the level of TX inhibition on other markers of platelet reactivity in patients with acute stroke or TIA; b) to evaluate the possible association between platelet TX and the severity of stroke, as evaluated on clinical scales; and c) to assess the COX1-independent platelet response and the effects of platelet–erythrocyte interactions in patients with acute stroke.

Materials and Methods

Ethics Statement

The study conformed to the ethical guidelines for human research defined by the Declaration of Helsinki and was approved by the Institutional Review Board of the University Hospital La Fe. All patients and HS gave their written informed consent as approved by the Institutional Review Board.

Patients and Controls

Between March 2007 and March 2010, 240 consecutive patients with acute stroke or TIA hospitalized at the Stroke Unit of the University Hospital La Fe, were enrolled in platelet function studies during the acute phase of brain ischemia. Patients were admitted to the Stroke Unit if they had suffered a stroke less than 24 h before hospital admission or presented with recurring TIA. Of them, 157 patients were treated with aspirin as the only antiplatelet drug (male 90; 70 ± 12 years of age) (Fig. 1). Diagnoses were made with computed tomography (CT), Doppler sonography, and where appropriate, magnetic resonance imaging, cerebral angiography magnetic resonance, and cardiological studies (Holter and cardiac echography). Stroke was defined as a focal neurological deficit that lasted more than 24 hours and TIA as an episode of amaurosis fugax or focal cerebral dysfunction of ischemic origin with complete recovery within 24 h. Neurological severity was evaluated using the National Institutes of Health Stroke Scale (NIHSS) [13], and the modified Rankin scale [14] was used for functional evaluations. Patients with contraindications for aspirin, those treated with other antiplatelet drugs, nonsteroidal anti-inflammatory drugs (NSAID), or anticoagulants were excluded from the study. The choice of antiplatelet treatment and the dose of aspirin (100–500 mg) were at the criteria of the treating physician. The 500 mg dose was administered intravenously (lysine acetylsalicylate; Grunenthal Pharma, Madrid, Spain) to patients with swallowing difficulties.

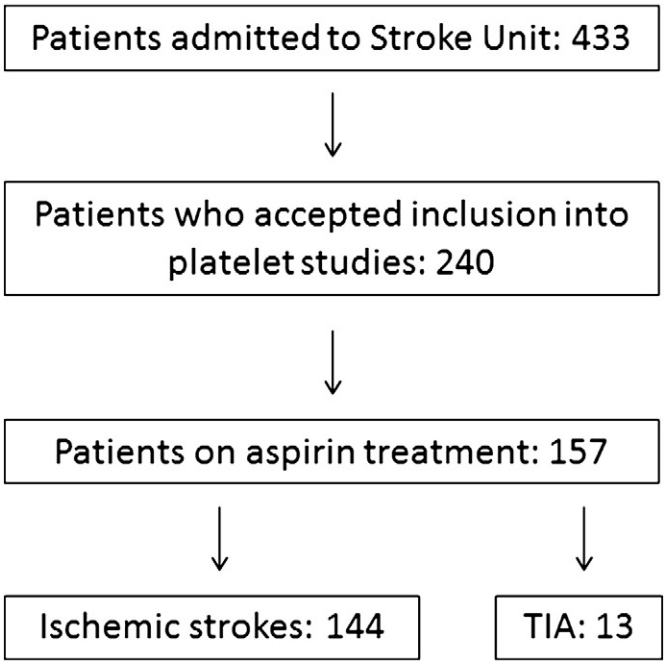

Fig. 1. Flow chart of patients included in the study.

Platelet function was evaluated in patients with Stroke (n = 144) or TIA (n = 13) within three days after the disease onset. All patients were receiving aspirin daily from the onset of the acute event. Blood collection took place within 4 and <24 h after the last dose of aspirin. In 43 patients, platelet function studies were performed after administration of the first dose of aspirin, while in 66 and 48 patients were performed after the second and third doses of aspirin, respectively.

Healthy subjects (HS; n = 128, male 51, aged 45.4 ± 12 years) from a working population with no clinical signs of vascular disease were evaluated as controls for platelet TX synthesis and recruitment. These parameters were also determined in 15 HS 2 h after they had ingested a single dose of 500 mg of aspirin, to assess the maximal effects of the drug on TX synthesis and platelet recruitment. HS had not taken any medication for ≥15 days before blood collection or before administration of the single dose of aspirin. The blood chemistry and haematological parameters of the HS were all within the normal ranges.

The platelet function assays were performed in a single specialized laboratory at the University Hospital La Fe.

Blood Cell Collection and Processing

For the study of platelet function, citrate-anticoagulated (3.2%) venous blood was collected from the patients and HS into siliconized glass tubes (Vacutainer; Becton Dickinson, Madrid, Spain). In the patients, blood collection was performed within 72 h after the onset of the acute event. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by differential centrifugation [12,15]. Following the removal of PRP, PPP, and buffy coats, 1 mL of erythrocytes was removed from the central area of the erythrocyte zone in the tube. The PRP samples were capped and stored at 22 °C under 5% CO_2–air for use in the platelet aggregation and recruitment studies within 4 h of venipuncture [12,15]. All platelet function tests in a patient were performed on the single day in which blood for platelet function studies was collected.

Platelet Aggregation

Platelet aggregation induced with arachidonic acid (AA, 1 mM), adenosine diphosphate (ADP, 3 μM), thrombin-receptor-activating peptide (TRAP, 15 μM) (all from Sigma-Aldrich, Madrid, Spain), or 1 μg/mL collagen (Nycomed Pharma GmbH, Munich, Germany) was determined by optical aggregometry (Chrono-Log 540, Havertown, PA, USA) as the change in light transmission. Aggregation was recorded for 5 min and the maximal intensity of aggregation was expressed as a percentage aggregation.

Measurement of Platelet Activation and Recruitment

Platelet activation and recruitment were evaluated independently using the activation–recruitment system, a two-stage *in vitro* procedure described previously [10–12,15]. PRP, platelets + erythrocytes (PRP + red blood cells [RBCs], haematocrit 40%), or whole blood were incubated for 10 min at 37 °C. Collagen (1 μg/mL) was added as the platelet agonist and the tube contents were mixed by inversion (10 s). A cell-free releasate was obtained by centrifugation (13,000 ×*g*, 1 min), the activation step. An aliquot of the releasate of collagen-(1 μg/mL)-stimulated whole blood was used to assess platelet TXB_2, the stable metabolite of TXA_2 [10–12,15]. In addition, 50 μl of the releasate of the collagen activation of PRP, PRP + RBCs or whole blood was used immediately as an inducer of platelet aggregation in the PRP assay system (to