Platelet–leukocyte interaction and platelet activation in migraine: a link to ischemic stroke?

J A Zeller, K Frahm, R Baron, R Stingele, G Deuschl

Objectives: Migraine has been identified as an independent risk factor for ischemic stroke. Both neurogenic inflammation and platelet activation have been linked to the pathophysiology of migraine. Increased platelet activation results in up-regulation of specific binding to leukocytes which promotes pro-inflammatory leukocyte secretion and their tethering to endothelium, a mechanism that has been demonstrated in stroke and which could provide a link to migraine. We aimed to determine whether platelet–leukocyte aggregation is increased in migraine patients outside an acute attack.

Methods: Seventy two patients with migraine according to IHS criteria were compared to a control group (n=72). Whole blood flow cytometry was used to quantify the activation dependent P selectin on the platelet, and to assess the fraction of platelets bound to the different leukocyte subsets.

Results: Migraine patients showed significantly more platelet–leukocyte aggregates compared to the control subjects (p=0.003). This effect was driven by an increased polymorphonuclear cell–platelet aggregation (p=0.003) whereas platelet aggregation with monocytes and lymphocytes was not. Platelet activation was also increased (p=0.001).

Conclusions: In migraine pro-inflammatory platelet adhesion to leukocytes occurs during the headache free interval similar to that seen in acute coronary and cerebrovascular syndromes. This may suggest a link between migraine and stroke on a cellular level.
history of cardiac or cerebrovascular disease, diabetes, and acute infection. Both patients and controls were free from acetylsalicylic acid or other non steroidal antiinflammatory drugs for at least 2 weeks, and nobody in the control group was taking tricyclic antidepressants.

**Methods**

Venous blood was drawn from an antecubital vein between 10 a.m. and 1 p.m., anticoagulated with 3.8% sodium citrate and processed after 10 min of resting time without further manipulation. Blood samples were kept at body temperature at all times. For the flow cytometric assays, we used direct fluorescent markers (all commercially available; Coulter Immunotech, Krefeld, Germany). Whole blood was diluted 1:10 with warmed HEPES buffer and two aliquots of 50 μl were incubated with CD 61-PE (an activation independent subunit of the GP IIb/IIIa complex) to immunologically identify all platelets. Simultaneously, in a one step procedure, the sample for measuring platelet activation was stained with anti CD 62-P. The other sample was double stained with the panleukocytic marker CD 45 to identify leukocytes.

After incubation for 5 min, the process was stopped using cold buffer, immediately followed by flow cytometry. Platelets were identified by their size and granularity properties using forward/sideward scatter and staining with the panthrombocytic marker CD 61-PE. By double gating of CD 45 positivity, forward and sideward scatter properties we discriminated between leukocyte subsets. Then the double fluorescent particles positive for both the leukocytic CD 45 and platelet CD 61 epitope were counted as platelet–leukocyte aggregates and platelet–leukocyte subset aggregates, respectively. Since red cells are not specifically stained and unspecific fluorescence is far below detection threshold, it is not necessary in this assay to perform red cell lysis, thus avoiding further artefacts. Measurements were performed with an Epics XL cytometer (Coulter Immunotech, Krefeld, Germany). This assay is an established procedure in various academic institutions in our country and has been in routine use in our laboratory for the 3 years prior to this investigation.

**Statistical analysis**

All values are expressed as means (SD). The Mann–Whitney U test was used to compare groups, and p<0.05 was considered significant in two tailed tests.

**RESULTS**

A total of 72 migraine patients (59 women and 13 men) were included in this study. Two thirds of the patients suffered from migraine without aura and one third from migraine with aura, a ratio commonly seen in the distribution of migraine subtypes in specialised clinics. Within the patient group there were no significant differences regarding age or sex distribution. Since we deliberately examined migraine patients outside an acute attack, none of the subjects had experienced headaches in the 24 h prior to blood sampling. Nineteen of the 72 patients (26%) reported a migraine attack 1–3 days prior to the investigation, and 17 had used an oral or subcutaneous triptane. Recent migraine attack and triptane intake did not differ statistically between both migraine groups. In the control group (as in control groups in previous studies), there was no gender difference regarding platelet–leukocyte adhesion or platelet activation. Thus we regarded the gender mismatch between controls and migraine patients as insignificant.

**Platelet–leukocyte aggregation**

Migraine patients showed a significantly increased number of platelet–leukocyte aggregates (5.70% vs 3.89% in controls, p = 0.003). This was due to the increased proportion of platelets aggregating with polymorphonuclear cells (7.26% vs 4.98%, p = 0.002; table 1). Aggregation of platelets with monocytes and lymphocytes was, although raised, not significantly increased in this sample size. Analysis of age dependency (two groups above and below the 50th percentile) and of the influence of prophylactic medication (47 with, 24 without) revealed no statistical difference.

**Platelet activation**

During the headache free interval investigated here, migraine patients expressed more of the activation dependent platelet epitope P selectin compared to the control subjects (1.41 (0.22) arbitrary units, p = 0.001; table 1). This was mainly influenced by the highly significant (1.42 (0.21), p<0.001) difference in patients suffering from migraine without aura; in those affected by migraine with aura the difference failed to reach significance in our sample (1.39 (0.23), p = 0.082). As seen with aggregation, values were not age or drug dependent.

**DISCUSSION**

This study represents the first investigation of intercellular communication between platelets and leukocytes in migraine patients. The hypothesised significant increase in intercellular platelet–leukocyte interaction does exist in migraine patients similar to that seen in many patients with acute or early postacute cerebral ischaemia. In addition, those without aura have a higher baseline of platelet activation compared to controls.

Platelet activation has been investigated before by measuring plasma levels of the platelet secretion products or urinary platelet metabolites. Using a photometric technique, whole blood flow cytometry, the externalisation of activation dependent platelet epitopes can be quantified on a cell to cell level in an automated procedure. In this way, dilution effects in the whole body plasma compartment and possible artefacts by metabolic processes can be avoided. We use P selectin antibodies which quantify the surface expression of the CD 62P epitope (P selectin) on the platelet. This alpha granule protein is externalised on to the platelet surface during platelet activation. The epitope has been shown to be a reliable activation marker in cardiac and neurovascular patients. Also, direct detection of both leukocyte and platelet specific cell properties allows immediate ex vivo identification of aggregates without further artefact prone immunostaining and microscopy.

Cytometric measurement of activation dependent platelet epitopes aims directly at the platelets’ cellular surface with very little ex vivo manipulation, and is less prone to artefacts.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Platelet–leukocyte aggregates (percent of platelet population (SD)) and platelet activation (mean fluorescence in arbitrary units (SD))</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Controls, n = 72</td>
</tr>
<tr>
<td>Leukocyte–platelet aggregation</td>
<td>3.89 (2.19)</td>
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<tr>
<td>Polymorph–platelet aggregation</td>
<td>4.98 (3.10)</td>
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<tr>
<td>Monocyte–platelet aggregation</td>
<td>5.57 (4.21)</td>
</tr>
<tr>
<td>Lymphocyte–platelet aggregation</td>
<td>0.58 (0.67)</td>
</tr>
<tr>
<td>Mean platelet activation</td>
<td>1.29 (0.18)</td>
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</tbody>
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The observation of augmented platelet activation in migraine patients confirms previous reports that used indirect methods. We suggest that enhanced platelet activation in migraine patients is a marker of the inflammatory process in the trigeminovascular system and cellular interaction rather than a precipitating factor in migraine pathogenesis.

As regards isolated lymphocyte properties, altered white cell function in migraine has been reported: both a significant rise in CD3+ (T lymphocyte) CD16/56+ (natural killer lymphocytes) classified lymphocytes in migraine without aura patients outside an attack were seen as well as raised B lymphocyte counts and decreased CD8 (T suppressor) positive lymphocytes in migraine patients when compared to controls. Empi et al described an increased CD4 (T helper) count in patients with migraine with or without aura compared to controls. In addition they found significantly higher proportions of integrin high expressing T helper cells in subjects with migraine without aura compared to migraine with aura.

Using this method, our results do not support lymphocytic reaction with memory cells, which represents a longer standing process of inflammation indicative of immunologically active inflammation in migraine patients, but acute phase leukocyte subsets (as seen by the increased neutrophil fraction attached to platelets). This suggests that even during the quiescent phase increased inflammatory signalling occurs. This may involve kinins like interferons and tumour necrosis factor and soluble adhesion molecules which lead in a partly self-propagating process to direct intercellular interaction among different subsets of leukocytes and between leukocytes and platelets.

Increased platelet–leukocyte adhesion has also been described in patients at risk of reocclusion after coronary stenting and in patients with unstable angina; this could be reduced by blockage of the activation dependent receptor GP IIb/IIIa receptor.

This observation of increased intercellular communication in both cardiovascular disease and migraine may provide a direct link to ischemic stroke for which migraine has been proven to be an independent risk factor, particularly in premenopausal women. In a recent review of migraine, haemostasis, and ischemic stroke in young women, the authors found it impossible to conclude from previous trials how migraine and cerebral ischemia are connected because of contradicting results and conflicting techniques. Likewise, studies on the incidence of antiphospholipid antibodies or prothrombotic genetic risk factors (mainly factor V Leiden and prothrombin mutation) in migraine patients were not promising, particularly since the latter are known to mainly affect venous system thrombosis, hence not a platelet initiated mechanism. However, it seems reasonable to study risk factors for increased platelet aggregability in the light of previous reports of patients with essential thrombocytopenia and migraine whose symptoms were reduced by antiplatelet therapy. Additional data suggest a preventive effect of aspirin alone or in combination with dipyridamole on migraine recurrence. We strongly support this approach: patients with acute ischemic stroke of large vessel but not of cardiac origin show increased platelet activation and, particularly following infections, increased leukocyte–platelet aggregates. The role of this intercellular communication is still subject to research: possible explanations include a causative model where infection via platelet and leukocyte activation triggers thrombus formation as well as post-thrombotic processes where platelet stimulated leukocyte activation adds to excitotoxic substance release. Increased platelet activation in essential thrombocythaemia leading to stroke has also been reported. These coincidences of altered platelet function in migraine patients, as reported here, and those suffering from stroke, myocardial infarction, and thrombocytopenia are promising links that should be further pursued. Although primary platelet dysfunction as an initiator for migraine is unlikely, the increased activation and leukocyte, namely neutrophil, aggregation constitute a prothrombotic risk that eventually may lead to ischemic stroke. It must also be considered that inflammation and infection itself have been proven to be independent risk factors of ischemic stroke.

In migraine pro-inflammatory platelet adhesion to leukocytes occurs during the headache free interval similar to that seen in acute coronary and cerebrovascular syndromes. This may suggest a link between migraine and stroke on a cellular level. Studies with correlation of this haemostasiological parameter to other indicators of altered coagulation biochemistry in migraine patients and comparisons between migraine patients with and without stroke, and subgroup analysis of data from stroke prevention trials on the efficacy of antiplatelet therapy in migraine patients will aid the understanding of underlying mechanisms and the deduction of possible prophylactic concepts.

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Competing interests: none declared

REFERENCES


HISTORICAL NOTE

Migraine treated by Shakespeare’s son-in-law, Dr John Hall

The name of Dr John Hall is familiar to students of Shakespeare, but less known by medical biographers. He was born c. 1575 and died in 1635,1 an eminent Stratford physician and herbalist. Early descriptions of migraine in Greco Roman times are well known.2 In De Captitis Passione, Caelius Aurelianus, born in AD 400 in Algeria, described hemicrania, although the term hemiconvulsion was not used. Willis’s De Heads and Headaches of 1644, as Select observations on English bodies, or cures both empirical and historicall performed upon very eminent persons in desperate diseases1: 2004 (in press).

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