Study of Platelet Activation in Migraine: Control by Low Doses of Aspirin

GIOVANNI D'ANDREA, M.D., MORENO TOLDO, M.D., ANTONIO CANANZI, M.D., AND FRANCESCO FERRO-MILONE, M.D., PH.D.

SUMMARY Although platelet activation is known to occur during migraine attacks, the cause-effect relationship remains to be determined. This problem was approached by studying the possible occurrence of platelet activation in vivo in headache-free periods of subjects affected by common or classic migraine, and, subsequently, by verifying the possibility of its pharmacological control through administration of a classic anti-aggregation drug such as aspirin (ASA). The plasma levels of beta-thromboglobulin (β-TG) and platelet factor 4 (PF4), indices of the occurrence of platelet activation in vivo, were therefore first assayed in both groups of migraine sufferers in the absence of therapy and then during the administration of aspirin (50 mg/daily).

In the group of 15 patients affected by classic migraine, basal plasma levels of β-TG and PF4 were significantly higher than control subjects. On the other hand, only β-TG plasma levels were significantly higher in the group of 18 patients affected by common migraine. Patients suffering from classic migraine showed a high incidence of platelet activation (>90%) in comparison with common migraine patients (~33%). This suggests that platelet activation occurs in vivo in migraine patients also during headache-free periods.

Administration of aspirin to the patients affected by common and classic migraine caused a decrease in plasma β-TG and PF4 concentration. Consequently, pharmacological treatment with aspirin in adequate dose may prove to be helpful in diminishing the vascular side-effects known to occur in migraine sufferers.

The evidence now available suggests an involvement of platelets in migraine pathogenesis.1-5 In comparison with normal control subjects, migraine sufferers have shown higher levels of platelet circulating micro-aggregates during attacks and in prodromal periods.6 Lower disaggregability assayed by Davis method has also been reported.7 In vitro platelet sensitivity to substances such as 5-HT and ADP is higher in migraine patients than in controls.8,9 During both headache-free periods and attacks, platelet enzyme defects (MAO, phenolsulphontransferase P)6,10 and anomalous serotonin uptake have been reported.6,11 Nevertheless a specific role of platelets in migraine pathogenesis is still a debated question.

If the stimulus is sufficiently intense, platelets are activated. The process of platelet activation occurs in two successive steps: platelets first undergo a change in shape and release into the plasma substances contained in their cytoplasmatic granules (release reaction).12 Dense bodies release serotonin, nucleotides, calcium etc. (release I); alpha-granules release, together with other substances, beta-thromboglobulin (β-TG), platelet factor 4 (PF4) and platelet-derived growth factor (PDGF) (release II). The presence of β-TG and PF4 in plasma is considered an index of platelet activation in vivo.13

Elevated β-TG plasma concentrations have been reported during migraine attacks in 6 out of the 12 migraine sufferers examined (Gawel et al, 1979).14 D’Andrea et al have recently shown the occurrence of high PF4 as well as β-TG plasma concentrations during migraine attacks in 9 subjects suffering from common migraine. Moreover, high β-TG and PF4 plasma levels were also observed during headache-free periods in 6 out of the 12 patients studied. On the contrary, whilst levels decreased during attacks, platelet serotonin remained unmodified in headache-free periods.15

A more extensive study of the possible occurrence of platelet activation in vivo in headache-free periods in subjects suffering from either common or classic migraine may perhaps yield some further information about: a) the incidence of platelet activation in common and/or classic migraine, b) the relevance of this to migraine pathogenesis or to accompanying side-effects.16 Possible predisposition of migraine sufferers to cerebrovascular and cardiac diseases is known.17,18 Indeed, the “aura” symptomatology, which usually accompanies classic migraine, is considered an expression of cerebral ischemic attacks.17,18

The aim of this work was to study the occurrence of platelet activation in headache-free periods of both subjects affected by common or classic migraine and, at the same time, to verify the possibility of its pharmacological control by administration of a classic anti-aggregation drug such as aspirin (ASA). β-TG and PF4 plasma levels were therefore first assayed in both groups of migraine sufferers in complete absence of any therapy and subsequently during ASA administration (50 mg/daily).

Materials and Methods

1) Patients and Controls

Thirty-three patients were examined, 18 with common migraine and 15 with classic migraine, each type differentially diagnosed according to the criteria defined by “Ad Hoc Committee on Classification of Headache.”19 All subjects were female, non-smokers,
aged 20–50 years (table 1). The case history of each patient indicated occurrence of migraine attacks for at least one year, with a frequency of 1–10 attacks per month (table 1). The patients were hospitalized as long as diagnostic examination was carried out and then invited to come to hospital for blood collection in headache-free periods. None of the patients had metabolic diseases and arterial hypertension. The neurologic examination and cranial, sinus, cervical and thoracic radiographs were all negative. The common routine laboratory hematochemical tests were normal. No patient had received drugs known to be active on platelets for at least ten days prior to the execution of the tests described below.

In all the patients, values of BTG and PF4 plasma levels were obtained from the average of the determinations carried out daily for 10 days.

The control group consisted of 19 normal subjects, aged 35–45 years, most of them laboratory and hospital personnel, all non-smokers. The values of control BTG and PF4 plasma levels were the average result of six consecutive daily determinations.

Informed consent was obtained after the nature of the procedure had been fully explained to the subjects.

2) Modality of Aspirin Treatment

The 33 migraine patients were invited to take ASA orally (50 mg/daily). After ten days without interruption of treatment, and then for at least another ten days, blood samples were collected every day for plasma BTG and PF4 determinations. Only 18 patients (7 affected by common migraine and 11 by classic migraine) followed the instructions completely for ASA treatment.

3) BTG and PF4 Assay

Blood (2.7 ml) was drawn by the same operator without stasis and immediately placed into plastic tubes containing "Edinburgh mixture" (0.3 ml) consisting of 0.1 ml 10% EDTA Na2, 0.1 ml theophylline (5.4 mg/ml) and 0.1 ml prostaglandin E1 (1 mg/ml). The tubes were inverted three times, kept in ice and spun within one hour at 2,300 g for 30 minutes at 0–4°C to obtain platelet-poor-plasma (PPP). The PPP samples were frozen at −20°C and tested within two weeks. BTG and PF4 were assayed on PPP samples using AMERSHAM and ABBOTT radioimmunoassay kits respectively, as previously described.20

Results

a) Subjects Affected By Common Migraine

As reported in table 2, the mean of the relative values of BTG plasma levels, assayed in 18 patients affected by common migraine during headache-free periods, is significantly more elevated (p < 0.02 Welch two-tail test) than the mean observed in healthy control subjects. On the other hand, although the mean relative values of PF4 plasma levels are higher than controls, the difference is not statistically significant. Nevertheless, careful analysis of the distribution of mean values of each patient in comparison with each control clearly indicates that 4 out of the 18 patients not only have higher BTG levels but also higher PF4 levels (fig. 1).

b) Subjects Affected By Classic Migraine

In the patients affected by classic migraine elevated BTG plasma levels were also found to occur. BTG values were more elevated than those assayed in common migraine sufferers and, when compared with control subjects, the difference was highly significant (p < 0.001 Welch two-tail test).

The same was found about PF4 values, the difference between classic migraine sufferers and control subjects being also highly significant (p < 0.001 Welch two-tail test) (table 3). The analysis of the distribution of the mean values of BTG and PF4 levels per single patient clearly demonstrated that almost all the patients suffering from classic migraine have BTG and PF4 levels higher than the values ± standard deviation of the control subjects (fig. 1).

c) Effects Of Aspirin Administration

Administration of aspirin (50 mg/daily) to both the patients affected by common migraine (table 4) and classic migraine (table 5) led to a decrease in plasma BTG and PF4 concentrations. In comparison with pre-treatment levels, aspirin therapy reduced the BTG and PF4 plasma levels in 7 common migraine sufferers,

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Mean Values BTG and PF4 Plasma Levels in Headache-free Periods (18 Patients with Common Migraine)</th>
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<tbody>
<tr>
<td>Patients</td>
<td>Welch two-tail test</td>
</tr>
<tr>
<td>BTG*</td>
<td>52.2 ± 34.7</td>
</tr>
<tr>
<td>PF4*</td>
<td>12.2 ± 13.6</td>
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</tbody>
</table>

*The values are expressed as ng/ml.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Mean Values BTG and PF4 Plasma Levels in Headache-free Periods (18 Patients with Common Migraine)</th>
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</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Welch two-tail test</td>
</tr>
<tr>
<td>BTG*</td>
<td>95.6 ± 13.6</td>
</tr>
<tr>
<td>PF4*</td>
<td>33.4 ± 29.7</td>
</tr>
</tbody>
</table>

*The values are expressed as ng/ml.
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although the difference was not statistically significant (table 4).

On the other hand, ASA treatment significantly reduced the elevated β-TG (p < 0.005 t-paired test) and PF4 (p < 0.01 t-paired test) plasma levels found in 11 subjects affected by classic migraine (table 5), and in 5 out of them it brought the β-TG and PF4 values within normal control limits (fig. 2).

Discussion

The assay of the plasma concentrations of β-TG and PF4 levels reported indicates that platelet activation occurs in vivo in migraine patients during headache-free periods. Patients suffering from classic migraine show an extremely high incidence of platelet activation (> 90% of cases studied), when compared with common migraine sufferers (~ 33% of patients). Consequently, as platelet behaviour is qualitatively, even if not quantitatively, similar in both types of migraine, the above data suggest that the focal symptoms which characterize the classic migraine attack might not be related to platelet release.

Platelet activation is known to occur also during cerebral21-28 and cardiac26-28 ischemic attacks. Common and classic migraine sufferers have been reported to be affected by cerebral ischemic attacks,17,18 the incidence of which seems to be higher in the latter.17 Computerized axial tomography (CT-scan) has shown the occurrence of focal hypodense areas of infarctual nature during headache.31,32 In certain cases the neurological deficits which accompany migraine are permanent.33 Cortical atrophy and ventricular enlargement have been documented by CT-scan30 in many chronic migrainous subjects. Although the cause-effect relationship between platelet activation and migraine pathogenesis is still uncertain, it seems therefore probable that platelet activation plays a not-negligible role in the cerebral ischemic accidents reported to occur in migraine subjects.

Is it possible to manipulate such anomalous platelet function thereby improving the patient’s condition? As previously mentioned, aspirin was used to investigate its effects on platelet activation in migraine patients. ASA is known to be a potent anti-platelet aggregation drug.31-34 Such activity has been shown in vitro to be primarily due to an irreversible block of cyclo-oxygenase activity,35,36 the enzyme which catalyzes the transformation of arachidonic acid into the cyclic endoperoxides PGG2 and PGH2, intermediate products, in the production of the "aggregatory" thromboxane A2.37 The irreversibility of the aspirin effect is consequent on the anuclear platelet incapability of enzyme re-synthesis.38 On the other hand, blockade by aspirin of endothelial cyclo-oxygenase activity results in lower production of prostacyclin, an "anti-aggregatory" agent.39 This "aspirin therapy dilemma" may be bypassed by using very low doses of aspirin.40-43 Low doses of aspirin (50 mg/daily) have been reported to inhibit in vitro only platelet cyclo-oxygenase activity and to result in reduced formation of salicylate, which is known to disturb aspirin antiaggregatory effects.44 It is to be stressed that data emphasize lack of aspirin efficacy in women following administration of high doses of aspirin.45

As reported in the results, aspirin administration at the doses of 50 mg/daily reduces in migraine sufferers

![Figure 1. Mean plasma β-TG and PF4 concentrations in headache-free periods: 18 common migraine (left side) and 15 classic migraine patients (right side).](http://stroke.ahajournals.org/)

**Table 4.** Mean Values BTG and PF4 Plasma Levels in 7 Patients with Common Migraine Before and During ASA (50 mg/daily) Administration

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>During</th>
<th>t-paired test</th>
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<tbody>
<tr>
<td>BTG*</td>
<td>47.6 ± 38.4</td>
<td>38.4 ± 27.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>PF4*</td>
<td>8 ± 8.2</td>
<td>6.9 ± 8.4</td>
<td>n.s.</td>
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</tbody>
</table>

*The values are expressed as ng/ml.

**Table 5.** Mean Values BTG and PF4 Plasma Levels in 11 Patients with Classic Migraine Before and During ASA (50 mg/daily) Administration

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>During</th>
<th>t-paired test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTG*</td>
<td>89.6 ± 61</td>
<td>63.7 ± 50.6</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>PF4*</td>
<td>29.7 ± 34</td>
<td>17.6 ± 23.8</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

*The values are expressed as ng/ml.
the elevated plasma β-TG and PF4 levels, markers of platelet activation in vivo. No effect was observed in subjects with normal values. Nevertheless, we suspect that, if we had studied for aspirin therapy the common migraine patients with high β-TG and PF4 plasma levels, a significant aspirin-induced decrease would have occurred as well. Provided that high β-TG and PF4 plasma levels indicate a risk of cardiac and cerebrovascular accidents, such low dose aspirin therapy might therefore be useful for reducing the possible vascular side-effects in migraine sufferers.

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References
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Effects of Mild Hypercapnia on Somatosensory Evoked Potentials in Experimental Cerebral Ischemia

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SUMMARY In a previous report, the authors demonstrated the effectiveness of mild hypercapnia in enhancing decreased perfusion flow in ischemic, non-infarcted brain tissues. However, the previous work lacked in verification of improvement of suppressed brain function. Therefore, this report was attempted to evaluate the effect of hypercapnia on somatosensory evoked potential (SEP), using the similar ischemic model as previously. The results showed that mild hypercapnia of 43 to 55 mm Hg range was beneficial not only for enhancing decreased perfusion flow but also for restoring suppressed SEP. This report seems to be the first publication which verifies a presence of correlation between local cortical blood flow (LCBF) and SEP under mild hypercapnia in mildly to moderately ischemic brain tissues.

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IN THE PREVIOUS REPORT,1-6 the authors demonstrated the effectiveness of mild hypercapnia in enhancing decreased perfusion flow in ischemic, non-infarcted brain tissues, produced by occlusion of the canine middle cerebral artery. The beneficial range of PaCO2 for restoring the reduced flow was between 45 and 55 mm Hg. Moreover, the use of mannitol combined with hypercapnia did not produce any additional benefit in restoration of reduced blood flow. However it must be pointed out that the previous work lacked evidence of improvement of function, under mild hypercapnia which significantly improved the perfusion flow. This report was focused on evaluating restoration on somatosensory evoked potential (SEP), which seems to be one of the most reliable parameters for assessment of brain function, under the similar range of hypercapnia as the one in the previous.

Materials and Methods

General Procedure
Twenty-two mongrel dogs, unselcted as to age and sex, and weighing approximately 8 kg were initially anesthetized with intramuscular injection of ketamine hydrochloride 8 mg/kg and intravenous pentobartal 25 mg/kg. Additional pentobarbital was given through femoral cannula as necessary. Respiration was artificially controlled by animal respirator. The femoral artery was cannulated for continuous recording of blood pressure and sampling of blood gas analysis (Blood Microsystem ABL2, Copenhagen). Mean systemic arterial pressure (MSAP) was maintained between 110 and 120 mm Hg. The hypercapnic state was maintained by regulation of respiratory rate and volume with gas containing CO2 of 10% and O2 of 90%.

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