Platelet models and their possible usefulness in the study of migraine pathogenesis

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Platelets may be linked to migraine. On the one hand they are activated during the migraine attack and thus may participate in the pathogenesis of the disorder (the nature of this activation is still unknown). In order to understand this platelet anomaly, we discuss the data available in the literature. In particular, we review recent in vitro studies of a-granules and dense bodies secretion, and aggregation induced by collagen and PAF. On the other hand, platelets share many metabolic characteristics with serotonergic neurons and endothelial cells. Accordingly, platelets have been used to investigate the possible role of serotonin turnover and nitric oxide function in migraine. In both cases, the data obtained have shown peculiar abnormalities that may explain pathogenetic and clinical aspects of primary headache. • *Nitric oxide, platelet activation, platemodels, serotonin*

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The increased urinary excretion of 5-hydroxyindo-lacetic acid (5HIAA) during headache attacks, reported by Sicuteri in 1961 (1), together with a decrease in platelet serotonin (5HT) levels during the prodromal phase, reported some years later by Anthony et al. (2), linked the platelet to the pathophysiology of migraine. As a consequence, in the last 20 years, many aspects of the biochemistry and behaviour have been tested in order to describe specific migraine platelet-related dysfunction(s). Some studies suggest that platelets of migraineurs have specific peculiarities (3-5), but others seem to indicate that platelet changes are secondary phenomena (6-8). Table 1 summarizes the main lines of platelet research in this topic (9-33).

The platelet function more extensively investigated is aggregation in platelet-rich plasma (PRP) with optical principle. With this technique, increased spontaneous aggregation (4), or following the administration of 5HT (4, 13, 34, 35) ADP (9, 14, 35, 37), epinephrine (9, 35, 38) or platelet-activating factor (PAF) (37, 39) has been observed during migraine attacks and/or in headache-free periods. These results, however, were not replicated in other studies (40-43). Moreover, when platelet aggregation was studied with collagen, the extent of aggregation was either similar to control subjects (11, 12) or significantly reduced (15) (Table 2).

Potential explanations for these conflicting results include: (i) the different diagnostic criteria used before the appearance of the IHS classification (44); (ii) a small number of patients was recruited in many studies and the results were obscured by the great intrinsic intra-individual variability of platelet activity; (iii) different methodologies, such as different concentrations of inducers, centrifugation time and pharmaceutical substances produced by different manufacturers, different timing of blood sampling (time of day, menstrual cycle, time passed since the last attack, fasting/non-fasting), blood sample procedures (venopuncture-induced platelet activation, body position, blood sample device: open system/ vacutainer, operators' experience, time of stasis),

Table 1. **Platelet "in vitro" studies in migraine.**

Agonist-induced receptor-mediated responses
Adhesion (9)
Mobilization of intracellular Ca^{2+}(10)
Secretion from dense and a-granules (11, 12)
Aggregation in platelet-rich plasma (4, 13, 14)
Aggregation in whole blood (11, 15)
Receptor studies
Serotonin uptake kinetics (16-18)
Fibrinogen (19)

Enzyme activity
MAO (20, 21)

Membranes
Fluidity (22, 23)
Fatty acid composition (24)

Morphological
No. of mitochondria (25)
No. of dense and a-granules (26, 27)
Platelet generation
Megakaryocyte regeneration time (28)
Whole blood platelet count (29)

Metabolism
Fosphoinositol cycle (30)
Serotonin catabolism (27)
Amino acids (31, 32)
Nucleotide content (33)

Table 2. Platelet aggregation studies in migraine.

Authors	Year	PRP		WB	Agonist
Hilton & Cummings	1972	↑	⇑		5HT
Deshmukh & Meyer	1972	↑	⇑		ADP epinephrine
Kalendovsky & Austin	1975	↑	⇑		epinephrine
Couch & Hassanein	1977		⇑		ADP
Hanington et al	1981		⇑		5HT
Jones et al	1982		⇑		5HT
Manotti et al	1983		⇑		ADP
Kruglak et al.	1984	↑	⇔		ADP
		↔	⇔		epinephrine
Wuther et al.	1985		⇔		ADP
Lechner et al.	1985	↑	⇑		ADP epinephrine 5HT
Hanon et al.	1985	↔	⇔		ADP norepinephrine 5HT collagen thrombin
Kozubski & Stanczyk	1985	↓	⇑		
Joseph et al.	1986			⇔	collagen
Kofacs et al.	1991	↑	⇑		ADP
		↔	⇓		collagen
		↔	⇑		PAF
D'Andrea et al.	1993		⇔		collagen
			⇔		PAF
D'Andrea et al.	1993			⇓	collagen

↔ ↑ ↓ = during migraine attack; ⇔ ⇑ ⇓ = during headache-free period; PRP = platelet rich plasma; WB = Whole blood.

medication and diet and (iv) the use of one test as an index of global platelet activity.

In our opinion, the finding that seems more consistent is the platelet release reaction during mi-graine attack (45, 46), the origin of which is unknown. The knowledge of why platelets are activated and the pathophysiological and clinical consequences of this event may be important in understanding the role of platelets in this disorder. In this article, we therefore focus on the nature of platelet activation in migraine, the possible inducers and their specific pathways. In addition, we examine the usefulness of the platelet as readily available blood elements which would be used as a model of CNS serotonergic metabolism of nitric oxide (NO) in migraine.

The nature of platelet activation in migraine

Several studies indicate that platelets are activated during a migraine attack, as reflected by increased plasma concentrations of ß-TG and PF_4 paralleled by a decrease of serotonin platelet levels (46-48). Despite this evidence of in vivo platelet hypersecretion during the attack, there have been few "in vitro" studies of platelet-dense body (11) and none of a-granule secretion. Accordingly, we determined interictal 5HT and PF_4 secretion (markers of dense body and a-granules release) and aggregation in PRP induced by increasing doses of collagen (0.5 and 2.0 µg/ml) and PAF (0.1 and 1.0 µM) in 62 migraine with aura patients (MA), 41 migraine without aura patients (MwA), and in 26 healthy controls.

There were no significant abnormalities of platelet aggregation compared to healthy controls. PF_4 secretion, induced by PAF, was significantly increased in MwA and there was a similar trend also in those suffering MA. In contrast, PF_4-induced by collagen was reduced in MA. The MA group showed elevated intraplatelet levels of 5HT and enhanced 5HT secretion induced by both inducers (Table 3).

Platelet as a possible model for studying serotonin CNS metabolism

Platelets share with serotonergic neurons many morphologic, biochemical and pharmacological characteristics: uptake, storage and calcium-dependent release of serotonin from dense organelles and cytosolic enzymes (e.g. MAO) that catabolize 5HT to 5HIAA (49, 50). Hence, the platelet can be a suitable model to study CNS serotonergic metabolism and may be used to investigate its possible role in migraine pathogenesis.

During migraine attack, serotonin levels decrease in platelets, whereas they are normal in headache-free periods (47). However, recent studies have shown high serotonin platelet levels in MA sufferers (27, 51) and increased number of dense bodies (26, 27) in both migraine types. In contrast to the traditional theory of migraine as a low serotonin syndrome (52, 53), these findings suggest that the metabolism and transport capacity of platelet serotonin may be increased in migraine.

Table 3. **Basal platelet levels of serotonin, platelet secretion of serotonin and platelet factor 4 after stimulation with collagen and PAF in patients suffering from migraine with and without aura and in healthy controls.**

	Serotonin (ng/10^9 plts)			PF_4 (UI/ml)		
	Controls	Migraine with aura	Migraine without aura	Controls	Migraine with aura	Migraine without aura
Basal value		855.3	$1396.7^{****/DDDD}$	833.9		
	(449.9)	(1168.3)	(534.0)			
Collagen						
0.5 µg/ml	432.7	$703.3^{****/DDDD}$	345.3	4196.2	$3460.2^{**/DD}$	3901.5
	(373.5)	(667.2)	(255.0)	(2300.4)	(2093.9)	(2355.0)
2.0 µg/ml	543.0	$960.5^{****/DDDD}$	516.3	5745.9	$5049.2^{*/DDDD}$	5990.5
	(396.3)	(890.5)	(360.3)	(2506.5)	(2453.5)	(3353.1)
PAF						
0.1 µM	272.5	$449.0^{***/DD}$	243.9	1318.7	1786.3	2007.0^{*}
	(390.8)	(566.6)	(283.9)	(1355.8)	(1586.2)	(2270.0)
1.0 µM	405.8	$653.7^{***/DD}$	395.0	3109.8	4346.3^{**}	$5325.0^{****/DDD}$
	(403.9)	(633.7)	(323.9)	(1875.1)	(2664.3)	(3617.8)

Mean values and (SD) are shown.

* Indicates a statistically significant difference between a migraine group and the control group.

^{D} Indicates a statistically significant difference between the two migraine groups.

$^{*/D}$ $p < 0.05$; $^{**/D}$ $p < 0.01$; $^{***/D}$ $p < 0.001$; $^{****/D}$ $p < 0.0001$.

Table 4. Platelet levels of 5HT and 5HIAA in migraine patients and controls.

	Healthy controls (C)	MA vs C	Migraine with aura (MA)	MwA vs C	Migraine without aura (MwA)	MwA vs MA
Serotonin (µg/10^9 platelets)						
All subjects	0.84 *(0.59)*	$p < 0.04$	1.36 *(1.13)*		0.84 *(0.55)*	*p < 0.01*
Males	0.63 *(0.24)*	$p < 0.05$	1.27 *(1.18)*		0.71 *(0.32)*	$p < 0.04$
Females	1.14 *(0.82)*		1.53 *(1.02)*		1.01 *(0.75)*	
Foll	1.51 *(1.25)*		1.17 *(0.86)*		0.98 *(0.91)*	
Ovu	1.05 *(0.58)*	$p < 0.04$	2.24 *(1.71)*		1.12 *(0.79)*	$p < 0.01$
Lut	1.02 *(1.33)*		1.47 *(1.47)*		*0.90 (0.75)*	
5-HIAA (ng/10^9 platelets)						
All subjects	6.8 *(4.8)*	*p < 0.05*	11.1 *(9.9)*		9.5 *(6.6)*	
Males	6.2 *(5.6)*		10.9 *(11.4)*		9.9 *(7.9)*	
Females	7.3 *(3.3)*		11.7 *(6.7)*	$p < 0.04$	9.1 *(4.4)*	
Foil	*5.7 (5.6)*		10.7 *(10.3)*		10.3 *(5.5)*	
Ovu	10.0 *(7.9)*		12.4 *(8.5)*		8.4 *(6.1)*	
Lut	6.8 *(4.7)*	$p < 0.04$	12.9 *(10.1)*		8.2 *(6.2)*	*p < 0.05*

Against this background we studied 5HT and 5HIAA levels in platelet and plasma of 62 MA, 41 MwA patients, during headache-free, and in 26 healthy control subjects. Blood samples were drawn on one occasion in men, in early follicular, mid-luteal and late luteal phases in females. The mean value of these three samples was used for the statistical evaluation. Platelets and plasma 5HT and 5HIAA were significantly increased in MA but not in MwA (Tables 4, 5). In female MA and MwA patients, the highest peak of 5HT and 5HIAA levels occurred in the ovulatory phase, whereas in controls in the follicular phase.

Platelet as a tool for studying nitric oxide in migraine

Nitric oxide (NO), an endogenous gas molecule, is a potent antiaggregant and myorelaxant agent. Also, NO is considered an intracellular messenger via

***Table 5.* Plasmatic levels of 5HT and 5HIAA in migraine patients and healthy controls.**

	Healthy controls (C)	MA vs C	Migraine with aura (MA)	MwA vs C	Migraine without aura (MwA)	MwA vs MA
Serotonin (ng/ml)						
All subjects	0.34 *(0.52)*	$p < 0.05$	1.93 *(3.00)*		0.92 *(1.22)*	
Males	0.15 *(0.22)*		1.50 *(2.44)*		0.56 *(0.97)*	
Females	0.62 *(0.73)*		2.79 *(3.82)*		1.45 *(1.40)*	
Foll	0.24 *(0.40)*		2.26 *(6.52)*		1.24 *(2.10)*	
Ovu	0.77 *(1.79)*		2.36 *(5.25)*		4.42 *(10.5)*	
Lut	0.88 *(1.76)*		4.31 *(7.98)*		0.85 *(1.68)*	
5-HIAA (ng/ml)						
All subjects	0.60 *(0.65)*	$p < 0.05$	1.40 *(2.04)*		0.54 *(0.53)*	$p < 0.02$
Males	0.58 *(0.71)*		0.91 *(1.05)*		0.64 *(0.58)*	
Females	0.64 *(0.59)*		2.38 *(3.04)*		0.37 *(0.40)*	$p < 0.03$
Foll	0.75 *(1.39)*		1.02 *(1.52)*		0.41 *(1.04)*	
Ovu	0.47 *(0.70)*		3.99 *(8.05)*		0.62 *(1.38)*	
Lut	0.36 *(0.44)*		1.64 *(2.36)*		0.98 *(1.29)*	

balance that regulates vascular tone *(55,* 56). Plate-lets, endothelium and neurons possess the NO biosynthetic pathway (54). The human platelet colla-gen-induced aggregation is accompanied by a con-centration-dependent increase of cGMP. On the other hand, the extent of platelet aggregation by collagen, but not by other inducers, is increased and cGMP levels are reduced when platelets are incubated with N-monomethyl-L-arginine (L-NMMA), a selective inhibitor of NOS and NO synthesis (57). Therefore, platelets can be used as a model for studying the L-arginine-nitric oxide metabolism in migraine (58). We measured interictal plasma and platelets levels of L-arg in 32 MA, 26 MwA patients and in 19 healthy controls. Also, we studied whole blood platelet aggregation, induced by collagen and PAF, in 62 MA, 41 MwA patients between attacks and 26 healthy controls. MA and MwA patients showed a reduced aggregation to collagen compared with healthy subjects, whereas PAF-induced aggregation was similar in the three groups (Fig. 1). Platelet L-arg levels were significantly increased in MA, whereas the plasma levels were in the same range in migraineurs and healthy controls (Fig. 2).

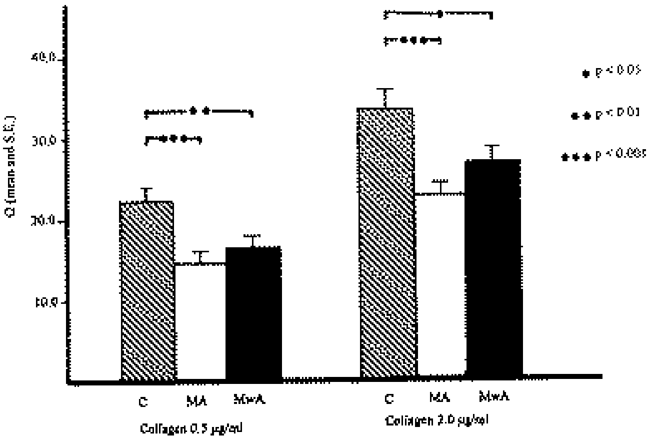

Fig. 1 Platelet aggregation induced by collagen in migraine with aura (MA), migraine without aura (MwA) and healthy controls (C).

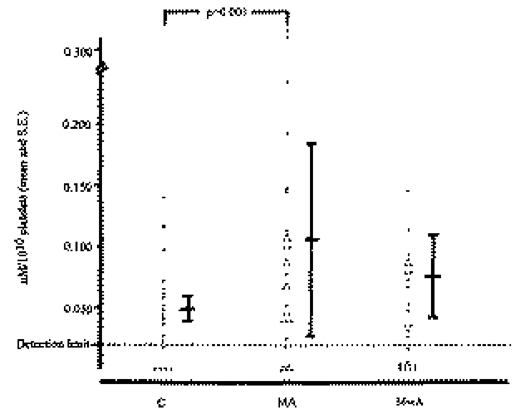

Fig. 2: Platelet L-arginine levels in migraine with aura (MA), migraine without aura (MwA) and healthy controls (C)

Our study indicates that the platelet hyporesponsiveness to collagen stimulation may be linked to an increased availability of the amino acid precursor and an increased NO synthesis.

Discussion

The first aim of these studies was to investigate in vitro aggregation and secretion in PRP, to clarify the origin of platelet activation in migraine. Platelet aggregation in both migraine types was not different from that of control subjects. In contrast, secretion from dense and a-granules showed specific peculiarities. PF_4 release, induced by PAF, was significantly enhanced in both migraine types, suggesting an up-regulation of platelet receptors. The PAF platelet hypersensitivity, detected when patients were headache-free, may indicate a contact between platelets and PAF during previous attacks. PAF plasma levels were never measured, but it is probable that substance P (SP) and calcitonin gene-related peptide (CGRP), released from activated trigeminal-vascular endings during migraine attacks (59) may induce PAF synthesis via endothelial and whole blood cell activation (60). Platelets are very sensitive to this inducer in concentrations as low as 1-10 nM (61) and, hence, this agent could be the mysterious plasmatic factor that causes platelet release in migraine (62-24).

PF_4 release induced by collagen was reduced in MA patients. This may be related to the specific platelet activation pathway induced by collagen, which implies a production of NO that may be enhanced in this pathological condition.

The increased 5HT release with both inducers may be considered a platelet peculiarity of MA patients, probably related to the high platelet basal levels of the indole. Increased availability and release of platelet 5HT may explain short and mild attacks often reported in MA (65).

The second goal was to study 5HT metabolism using platelets as a model. Levels of 5HT and 5HIAA were elevated in platelets and plasma of MA, but normal in MwA patients. The elevated amount of 5HT together with an increased carried capacity as shown by own previous morphological studies may be responsible for the low frequency of attacks in MA compared to that of MwA (65). In addition, 5HT levels showed different menstrual cycle-related fluctuations in female patients and controls: the highest peak appeared at mid-cycle in migraineurs and in the early follicular phase in healthy women. It is possible that the altered peripheral serotonin metabolism and menstrual cycle periodicity reflect similar changes in the CNS. There are several indications that peripheral and central turnover is modulated by a common pacemaker (66-69). On the other hand, the phase-shifted rhythmicity of platelet serotonin levels in female migraineurs may be related to impaired regulation of biological serotonergic variation. Probably dysregulated serotonergic rhythmicity may render migraineurs particularly vulnerable to trigger factors at certain time points of biological rhythms, perhaps explaining the circadian, seasonal and menstrual cycle-related variations in the frequency of migraine.

Finally, the platelet seems to be a promising tool for studying the role of NO in migraine. Decreased collagen-induced aggregation in whole blood, together with increased platelet arginine levels indicate the possibility of an elevated NO synthesis. This anomalous behaviour is not evident when platelets are stimulated in PRP, because blood centrifugation inevitably causes a red cell lysis with haemoglobin liberation. It is well known that haemoglobin inhibits the action of NO on platelet activation (57). Therefore, when experiments were carried out in PRP, secretion is a more sensitive index than aggregation in revealing the platelet hypofunction to collagen stimulation. Platelet abnormalities in migraine may reflect similar changes in endothelium and neurons (70, 71). The generalized altered NO synthesis may be involved in various migraine-related features such as dense and a-granule hyposecretion to collagen (15), occurrence of spreading depression (72, 73) induced by neuronal release of glutamate (31, 70, 74) and regional cerebral blood flow changes (75, 76).

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