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Platelets in migraine

Abstract Modification in platelet activation and aggregation has been demonstrated in migraine patients both during and between attacks. A different pattern in the secretion of platelet products has also been observed in patients affected by migraine compared with controls. The most important variation concerns a hyosecretion of dense body products. Platelets share structural and functional analogies with monoaminergic neurons, in particular serotonergic neurons, and have been used as a peripheral model to study monoaminergic function in migraine patients. A reduced turnover of serotonin has been shown in migraine patients in the interictal period, whereas a reduced content of this monoamine was detected in patients with chronic daily headache (CDH), particularly in those with analgesic abuse. Nitric oxide metabolism was also studied in platelets of migraine patients between and during attacks. An increased activity of nitric oxide synthase (NOS),

which was more accentuated during attacks, was found in these patients compared to healthy individuals. The increase in platelet NOS activity was also confirmed in CDH patients in association with a reduction of serotonin content and increased calcium levels. Variations in aggregation to different agents and secretion may not, at the moment, be indicative of similar changes in cerebral circulation of migraine patients. Its occurrence should be confirmed in samples of jugular venous blood during attacks to establish a definite link with migraine pathogenesis. Moreover changes in serotonin and NO metabolism observed in platelets of migraine patients may only indirectly suggest similar modifications in the neuronal pathways involved in inducing and maintaining head pain in migraineurs.

Key words Platelets • Aggregation • Secretion • Serotonin metabolism • NO metabolism • Platelet enzymes • Migraine

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Introduction

Platelets are anuclear cellular elements of the blood which are primarily involved in hemostasis. Platelet functions include adhesion, secretion of factors implicated in aggrega-

tion and vascular tone modifications, thrombus formation and, finally, uptake of serotonin and aminoacids from plasma. The principal products secreted from platelets are adenosine nucleotide, serotonin (stored in the dense bodies), β -thromboglobulin and platelet factor 4 (contained in the α -granules). These products, together with thromboxane A₂,

synthesized from arachidonic acid, induce the secondary, irreversible aggregation of platelets which underlies thrombus formation. These events occur when the vessel wall is damaged, also in the cerebral circulation. One of the first pathogenetic hypotheses formulated for migraine stated that an alteration of platelet function contributed to vascular changes observed during attacks. These changes can also be investigated in the peripheral blood and involve some aspects of platelet function, such as reactivity and aggregability to stimulating agents, secretion from dense bodies and α -granules, as well as microaggregate formation.

Platelet activation and aggregation in migraine

Although certain alterations in platelet function and secretion have been found in migraine patients, their role in the pathogenetic events of a migraine attack remains to be established. The extreme discrepancies among data in this regard should be mentioned. It is mandatory, more than in other cellular models, to define the variability of the tests used in the experimental setting. Some of the main findings on platelet activation and aggregation between attacks are reported in Table 1 [1–7].

Platelet function is influenced by several factors, such as age, sex and stress; stress in particular is believed to be a precipitating factor of migraine attacks. Biochemical modifications found during stressful conditions, such as the increase in catecholamines and free fatty acids in blood, may in fact trigger the activation of platelets.

Taking these considerations into account, the most constant finding in migraine which is more evident during attacks is the increased aggregability of platelets, both spon-

taneously and subsequent to stimulation by aggregating agents, such as epinephrine, adenosine diphosphate and thrombin. Accordingly, drugs which reduce platelet aggregability, in particular non-steroidal anti-inflammatory drugs, 5HT₂ antagonists and beta-blockers, have been proposed for migraine prophylaxis.

However, a recent study by Joseph [8] using impedence-tometry on whole blood, which more faithfully explores platelet function in vivo, did not show significant variations in migraine patients outside attacks. The same author used the combined results of different tests exploring platelet function (e.g. adhesion, aggregation, secretion, plasma thromboxane levels and circulating microaggregates) to investigate the role of platelets during attacks. He found an increase in the index of platelet function only at the end of attacks, and challenged the hypothesis of platelet intervention as a *primum movens* of migraine attack [8].

Monamine metabolism in migraine: focus on serotonin

Platelets are structurally and functionally similar to monoaminergic neurons, in particular serotonergic neurons. The two cell types have a common ectodermic origin. They use Ca²⁺, intracellular phosphoinositol and prostaglandin pathways for secretion. They bind 5-hydroxytryptamine (5HT₂) and imipramine, and metabolize serotonin in a similar manner.

The majority of blood serotonin is localized in platelet dense bodies and is released from platelets after activation. There is a reduced turnover of this monoamine between attacks while it is released during migraine crises. The latter finding was supported by early studies which demonstrated

Table 1 Aggregation and activation of platelets from migraine patients, during headache-free periods

Reference	Year	Patients	Results
Hedman et al. [1]	1988	MA	Decreased beta-adrenoreceptor response to stimulation with isoprenaline
Herman et al. [2]	1989	MA and MWA	Decreased sensitivity of platelets to PAF
Takeshima et al. [3]	1989	Migraine, type not specified	Increased activation during cold pressure test
Kovacs et al. [4]	1990	MA	Increased activation to ADP and PAF
D'Andrea et al. [5]	1994	MA and MWA	Decreased collagen-induced aggregation
Kitano et al [6]	1994	MA and MWA	Continuous activation measured as increased values of 11-dehydrothromboxane B2
Tozzi et al. [7]	1996	MA and MWA	Perturbed platelet viscosity; Decreased stimulation-induced influx of external calcium through the platelet membrane; Hyperaggregation to adenosine diphosphate and collagen

MA, migraine with aura; MWA, migraine without aura; PAF, platelet-activating factor

a reduced platelet content of serotonin that was attributed to an unidentified plasma releasing factor. The principal data on serotonin metabolism in migraine outside the attacks are summarized in Table 2 [9–17].

Differences in platelet serotonin content have been observed between migraine with aura and migraine without aura patients assessed between attacks [15]. Changes in serotonin content were also noticed in women with menstrual migraine, in relation to the cyclic hormonal variations. In these women, platelet serotonin levels peaked in the follicular period and decreased in the luteal phase, when 5HIAA values were particularly increased [16, 17]. These data suggest an aberration in menstrual cycle-related changes of serotonin in women migraineurs, possibly due to increased catabolism or reduced synthesis which predisposes these patients to migraine attacks.

The decreased secretion from platelet dense bodies in migraine was suggested by the finding of higher norepinephrine content [18]. This observation, together with adenosine triphosphate (ATP) hyposecretion, is evidence of sympathetic hypofunction in migraine.

The defective turnover of platelet serotonin between attacks may reflect a hyposerotonergic status at the central level. This hyposerotonergic status is more accentuated in chronic daily headache, in particular when associated with analgesic abuse, as demonstrated by several recent studies [19–21].

Alterations in serotonin metabolism and turnover are reflected by the different patterns of expression of 5HT₂ receptors. In migraine patients, particularly in those with aura, a down-regulation of these receptors was observed even in headache-free period, whereas in transformed migraine the most relevant finding was an up-regulation of the same receptors. These observations support the hypothesis of a derangement of the serotonin system related to the chronicization of headache in these patients [22, 23].

Other neurotransmitters in platelets of migraine patients

Variations in the levels of other neurotransmitters in platelets of migraine patients have also been observed. The most suggestive finding is the higher values of glutamate in platelets of migraine patients compared with controls, implicating an analogous alteration in the central nervous system. Increased platelet glutamate may underlie the NMDA-mediated events hypothesized to be involved in the aura pathogenesis [24, 25].

Elevated values of substance P were found in platelets of migraine patients during attacks, as were reduced levels of 5-HT; a negative correlation between the two neurotransmitters was noted. In migraine patients an uptake of substance P, released by trigeminal nerves, may explain

Table 2 Serotonin metabolism in platelets from migraine subjects, during headache-free periods

Authors	Year	Patients	Results
D'Andrea et al. [9]	1987	MA and MwA	Decreased 5HT turnover
D'Andrea et al. [10]	1989	MA	Increase in the content of serotonin, not evident in MA Decrease in 5-HIAA (also in MwA)
D'Andrea et al. [11]	1989	MA and MwA	High NE levels and low 5-HT/NE ratio in platelets of patients with MwA; High 5-HT levels and high 5HT/NE ratio in platelet of patients with MA
Joseph et al. [12],	1989	Migraine patients (migraine form is not specified)	Increased number of dense bodies; Altered coupling of 5HT secretion from dense bodies and ionised calcium; Decreased serotonin secretion
Riddle et al. [13]	1989	MA and MwA	Increase in the number of dense bodies
Joseph et al. [14]	1989	MA and MwA	Abnormal sensitivity to PAF
D'Andrea et al. [15]	1994	MA	Increased basal platelet 5HT and increased 5HT secretion induced by both collagen and PAF (not evident in MwA)
D'Andrea et al. [16]	1995	MM, TTH and controls	Plasma and platelet 5HT peak in MM in ovulatory phase; 5HT peak evident in follicular phase in TTH and controls
Fioroni et al. [17]	1996	MM	Reduced 5HT and increased 5HIAA in luteal phase, suggesting a greater susceptibility to attacks in this period

MA, migraine with aura; MwA, migraine without aura; 5HT, serotonin; NE, norepinephrine; PAF, platelet-activating factor; MM, menstrual migraine; TTH, tension-type headache; 5HIAA, 5-hydroxyindolacetic acid

the increase of this neuropeptide in platelets. Substance P may contribute to trigger attacks by releasing 5-HT by platelets [26].

Another suggestive finding in migraine was the increased metenkephalin secretion from platelets during attacks, which has been interpreted as a compensatory mechanism antagonizing the serotonin release from platelets occurring ictally [27].

Platelets as a model for the study of NO metabolism

Platelets possess a constitutive form of nitric oxide synthase (NOS) which intervenes in the formation of NO from L-arginine [28]. The most specific agent able to activate NOS in platelets is collagen [29].

Our group found an increase in platelet NOS activity, both basal and collagen-stimulated, in migraine patients assessed between attacks [30]. This was accompanied by a significant rise in the intracellular messenger cyclic guanosine monophosphate (cGMP) [30]. This increase may account for some alterations in platelet function observed in migraineurs, such as the increase in platelet content of L-arginine and the reduced responsiveness to collagen; the latter finding was first shown by D'Andrea et al. [5] and later confirmed by our group [4, 30]. A recent study in migraine patients confirmed the activation of NOS in platelets, especially during attacks, by demonstrating increased NO end-products compared with headache-free period [31].

The significance of the previously described changes to migraine pathogenesis remains to be established. The increase in NO formation may represent a mechanism to compensate for the aggregation induced by unknown factors, and therefore may be a short-acting feedback mechanism in peripheral and also cerebral circulation. On the other hand, the increase in NO synthase activity of platelets, which is present also in headache-free periods, could reflect a more generalized increase in NO synthesis, as suggested by Olesen et al. [32], who considered this messenger the "key molecule" of migraine.

Our group also found an increased activity in the L-arginine/NO pathway in the late luteal phase in women with menstrual migraine. This increase, together with neurotransmitter modifications and hormonal changes, may account for the greater susceptibility to attacks in the perimenstrual period [33].

The significant increase in NOS activity in patients with chronic daily headache, associated with reduced serotonin levels, suggests the failure of this pathway to antagonize the increase in cytosolic calcium [21]. This leads to the depletion of serotonin in the platelet dense bodies.

Considering platelets as a peripheral model of serotonergic neurons, an analogous depletion at the central level may contribute to maintain attacks and lead to the chronicization of head pain [34]. As stated before, serotonergic hypofunction could be accentuated by analgesic abuse.

Platelet enzymes in migraine

The first research carried out in platelets concerned the enzymes monoamine oxidase (MAO), present in platelets mainly in the B isoform which uses tyramine and tryptamine as substrates, and the enzyme phenol sulphatase, whose substrate is unknown. These studies found an altered activity of both enzymes, particularly in patients who reported certain foods as trigger factors [35–39].

The alteration of MAO-B activity in platelets was reported particularly during migraine attacks, and was more accentuated in women with migraine related to menstrual cycle; this change is not specific for migraine as it was also found in patients with tension-type headache [16].

An analogous alteration in the activity of phenolsulphotransferase has been hypothesized to promote the absorption of toxic substances by intestinal tract and to trigger migraine attacks, at least in a subgroup of migraine patients [37, 38].

A defect of superoxide dismutase has also been observed in migraine [40] and suggests that patients with migraine (with or without aura) are vulnerable to oxidative stress. The role of the impairment of this radical-scavenging enzyme remains to be established [40].

Secretion products from platelets in migraine

Studies on the secretion products of platelets from migraine patients have given contrasting results. One showed an increased platelet adenosine content compared with control subjects, in agreement with the finding of a reduced adenosine release from dense bodies accompanied by the activation of A₂ receptors [41]. The inhibited adenosine release may participate in the rapid elimination of serotonin in migraine sufferers [41]. An increase in β -thromboglobulin, a 35 000 dalton protein, released from α -granules as a consequence of platelet activation has also been observed during migraine [15]. This increased secretion was accompanied by a similar increase in thromboxan and prostacyclin, evidence of a more generalized platelet activation [42].

A different behavior of platelet factor 4 (PF4) secretion induced by collagen was observed in migraine with and without aura patients. Platelets of the former patients exhibited a reduced secretion, not observed in the latter. The

reduced PF4 secretion was coupled with an increased basal intraplatelet serotonin concentration and secretion induced by collagen, suggestive of a different serotonin turnover in this migraine subtype. However a similar trend in PF4 secretion induced by PAF was observed in the two migraine forms, but in migraine with aura patients the increased secretion observed was greater than that found in migraine without aura patients, reflecting an abnormal α -granule secretion [15].

Conclusions

Although data on platelets in migraine are contrasting and inconclusive, some important concepts can be derived from studies carried out in this regard. The findings of platelet dysfunctioning, as well as alterations in neurotransmitter content and secretion, all refer to the peripheral circulation.

The alterations detected, in particular those concerning serotonin, are nonetheless suggestive of analogous functional abnormalities in central pathways involved in head pain in migraine, given the analogies between platelets and monoaminergic neurons.

Variations in platelet aggregation and secretion to different agents cannot, at the moment, be assumed as indicative of similar changes in cerebral circulation in migraine. Experimental evidence from the neurogenic inflammation model suggests an activation of platelets, but this finding should be confirmed in samples of jugular blood of migraine patients assessed during attacks.

The role of PAF, considered by some authors to be pivotal in the pathogenetic events involved in migraine attack, should be confirmed, especially because of the extreme difficulty in quantifying it. Even for PAF, conclusive information should be derived from samples of jugular blood of migraine patients taken during the crises, when a reliable test for detecting this factor becomes available.

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