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Jean-Marie Launay
François Tabuteau
André Pradalier

Desialylated transferrin in plasma of patients with migraine without aura

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J.M. Launay • F. Tabuteau
CR Claude-Bernard,
Biochemistry Service,
Hôpital Lariboisière AP-HP, Paris, France

A. Pradalier (✉)
Internal Medicine Service,
Migraine and Headache Centre,
Hôpital Louis Mourier,
178 Rue des Renouillers, 92701
Colombes, France
E-mail: pradalier@ginkgo.fr
Tel.: +33-1-4760-6705
Fax: +33-1-4760-6072

Abstract Patients with migraine without aura (MO), either during or between attacks, present elevated histamine levels in platelet-poor plasma but normal whole blood histamine levels, compared with controls. This finding is usually interpreted as an increased histamine release from basophils due to unidentified histamine-releasing factors. Compared with 10 control plasma samples, each sample from 12 MO patients (5 during and 7 between in attacks) contained normal amounts of iron and immunologically reactive transferrin but decreased transferrin iron-binding capacity. As transferrin inhibits histamine release in vitro, such a functional abnormality, probably due to modifications of the transferrin glycan moiety (desialylated transferrin), may well account for the increased histamine release observed in MO patients. We suggest that glycan-modified transferrin may be related to migraine histamine-releasing factors.

Key words Histamine • Migraine without aura • Release • Sialic acid • Transferrin

Introduction

In migraine without aura (MO), many platelet abnormalities have been described [1–5]. However, these changes seem to be largely secondary phenomena, rather than primary causative factors [6–8]. More than twenty years ago, several groups [9–11] noted the presence of a serotonin-releasing factor in plasma drawn during a migraine attack. More recently, our own group [12, 13] detected two amine-releasing factors in the plasma of MO patients: a catecholamine-serotonin-releasing factor, detectable only during attacks, and a histamine-releasing factor (HRF) present both during and between attacks. The latter might account for the increased plasma histamine level observed in some migraine patients [14–16].

Numerous HRF and histamine-release inhibitory factors (HRIF) such as interleukin (IL)-3, IL-8, GM-CSF, reactive oxidants, CTAP III and NAP2 have already been described [17,18].

Transferrin (TF) not only transports iron in all extracellular fluids but also exerts many additional properties, including cell growth stimulation, bacteriostatic action and inhibition of histamine release from mast cells and basophils [19–23]. TF is a 75 kDa glycoprotein containing 679 amino acids and two glycan chains. For each, the last residue is a sialic (*N*-acetyl neuraminic) acid which can be hydrolyzed by neuraminidase. Circulating TF mainly originates from hepatocytes, its intracellular precursor being asialoapotransferrin. Transferrin receptors, which are present throughout the body, are abundant (10^6/cell) on the basophil plasma membrane [24]. They are also involved in the development of the nervous system [25].

Because of the known relationships between TF and histamine release, we decided to investigate the histamine-releasing ability of this glycoprotein in the plasma of MO patients.

Materials and methods

Blood samples were collected with ACD-A as anticoagulant from 12 women (aged 23–48 years) with MO but free from other pathological conditions (particularly diabetes and alcoholism). Informed consent to participate in the study was obtained in each. In 5 patients blood was drawn 1–2 h after the onset of an attack and from the other 7, during an attack-free period.

Migraine without aura was diagnosed according to the IHS classification [26]. Patients with tension-type headache (TTH) or migraine and TTH were excluded. Illness duration was 2–25 years, with a frequency of 2–6 attacks per month. Prophylactic antimigraine treatment was stopped for at least 10 days, and drugs used to treat individual migraine attacks were withheld for 48 hours before blood sampling. Ten healthy female volunteers, without history of migraine (aged 20–45 years, Blood Bank, Hôpital Saint-Louis, Paris) and with ABO, Rh and HLA groups similar to those of MO patients, served as controls.

Platelet-poor plasma (PPP) was obtained between 8 and 10 a.m. (to avoid any variation(s) due to nyctohemeral rhythms) by the procedure of Lorenz and Doenicke [27] and stored at –80° C until use. For release experiments, one volume of PPP was added to one volume of whole blood. This mixture was then incubated for 30 min at 37° C in a shaking water bath and centrifuged for 10 min at 4° C and 2500 g. The histamine contents of whole blood, PPP and supernatants from release experiments were measured radioenzymatically [28].

Both PPP iron content and total iron-binding capacity (TIBC), the usual functional measurements of TF, were routinely determined by colorimetry with the multiparameter analyzer Dimension (Dupont). PPP transferrin content was also determined by immunochemical titration, using both radial immunodiffusion (Boehringer) and immunoprecipitation in solution (BNA analyzer). TF sialylation was investigated in PPP through a lectin-enzyme immunoassay (LEIA) and desialylation was performed using a *Vibrio comma* neuraminidase [29].

The two-tailed non-parametric Kolmogorov-Smirnov (KS) test was used for comparisons. The chosen significance level was $p < 0.05$.

Results

Whole blood histamine levels of MO patients fell within the normal range, while PPP histamine levels were always increased ($p < 0.05$, KS test), as compared to controls, both during and between attacks (Table 1).

Mixing PPP of MO patients, obtained either during or between attacks, with control blood resulted in high ($p < 0.05$, KS test) supernatant histamine values as compared with control conditions (i.e. control PPP + control blood). In contrast, mixing control PPP with MO blood reduced ($p < 0.05$, KS test) supernatant histamine values, compared with MO conditions (i.e. MO PPP + MO blood). This finding no longer held true when a neuraminidase-treated control PPP was used (Table 2). Therefore, the PPP of MO patients is likely either to contain HRFs or to be deficient in histamine-release inhibitory factors (HRIF).

Control PPP treated with neuraminidase (which abolishes sialylation) increased ($p < 0.05$, KS test) histamine release, leading to histamine levels similar to those present in MO PPP. This finding led us to suspect the presence of desialylated TF in the plasma of MO patients.

Accordingly, despite unchanged plasma iron and immunochemically measured TF levels (Table 3), TIBC and LEIA-TF levels were significantly decreased ($p < 0.05$, KS test) in MO patients, compared with controls. Thus, both the functional capacity and the sialylation of TF were decreased in MO patients.

Table 1 Histamine levels in whole blood and in platelet-poor plasma (PPP) of controls and patients with migraine without aura (MO) during and between attacks. Values are mean ± SEM (range)

Histamine	Controls (n = 10)	MO patients	
		Attack-free (n = 7)	Attack (n = 5)
Whole blood (μM)	0.39 ± 0.04 (0.21–0.66)	0.50 ± 0.04 (0.32–0.64)	0.48 ± 0.05 (0.34–0.60)
Platelet-poor plasma (nM)	2.5 ± 0.5 (0.5–5.0)	9.9 ± 0.4* (8.4–11.8)	16.3 ± 0.3*§ (15.4–16.9)

* $p < 0.05$ vs. controls, KS test

§ $p < 0.05$ vs. attack-free MO patients, KS test

Table 2 Histamine levels in supernatant mixtures of whole blood and platelet-poor plasma (PPP) (1:1, v/v) from either controls or migraine without aura (MO) patients during and between attacks. Values are mean ± SEM (range)

	Control PPP	Control PPP + neuraminidase	MO PPP	
			Attack-free (n = 7)	Attack (n = 5)
Whole blood				
Control	2.1 ± 0.8 (0.2–8.3)[a]	–	7.9 ± 0.6* (5.7–10.4)	10.1 ± 0.5* (8.9–11.7)
MO attack-free, n = 7	4.1 ± 0.4[§] (2.2–5.6)	12.8 ± 0.7* (10.7–15.8)	14.3 ± 0.6* (11.9–16.8)	–
MO attack, n = 5	4.9 ± 0.6[§] (2.6–6.3)	13.5 ± 0.7* (10.9–14.9)	–	15.2 ± 0.4* (14.3–16.4))

[a] n = 10
* $p < 0.05$ vs. controls, KS test
[§] $p < 0.05$ vs. corresponding MO situation, KS test

Table 3 Plasma iron and transferrin (TF) data for controls and MO patients during and between attacks. Values are mean ± SEM (range)

	Controls (n = 10)	MO patients	
		Attack-free (n = 7)	Attack (n = 5)
Iron (μM)	18.0 ± 1.6 (11.6–25.6)	18.7 ± 1.8 (11.6–26.8)	17.6 ± 2.3 (12.2–24.4)
TIBC (μM)	50.2 ± 1.6 (41.4–59.4)	43.3 ± 0.9* (40.4–46.6)	44.8 ± 0.4* (44.0–46.0)
IC-TF (g/l)	3.64 ± 0.09 (3.24–3.98)	3.61 ± 0.09 (3.25–3.96)	3.43 ± 0.06 (3.30–3.59)
LEIA-TF (g/l)	3.53 ± 0.07 (3.25–3.97)	2.67 ± 0.04* (2.50–2.80)	2.69 ± 0.06* (2.50–2.82)

TIBC, transferrin iron binding capacity; *IC-TF*, immunochemically measured transferrin; *LEIA-TF*, transferrin measured by a lectin-enzyme immunoassay
* $p < 0.05$ vs. controls, KS test

Discussion

According to previous studies [12, 14, 16], higher PPP histamine levels were present in MO patients either during or between attacks, compared with controls, despite unchanged whole blood histamine levels. This finding is usually considered as representing an increased histamine release from basophils, with their numerous TF receptors [25], due to unidentified histamine-releasing factors [7, 12–14]. The first approach on this subject dates back more than twenty years [30] and other groups reported a histamine release model from basophils [31].

The present study corroborates these findings. Mixing MO PPP with control whole blood increased the histamine content of supernatants, as compared to those obtained after mixing control PPP and MO whole blood. In MO plasma, we also found normal iron and immunochemically measured TF concentrations but decreased TIBC-measured TF (reflecting TF functionality) and LEIA-measured TF (reflecting sialylation), indicating a defect in TF post-translational events, i.e. a qualitative defect in TF. Moreover, we observed that treatment of control PPP with neuraminidase (which abolishes sialylation) significantly increased histamine release.

Taken as a whole, these findings are in agreement with the previously reported [19, 20] inhibition of histamine release by normal TF and suggest that, in MO patients, a defect in TF sialylation (occurring during or after its synthesis) reduces TF's ability to inhibit histamine release. This property may be connected (at least in part) to the previously described histamine-releasing factors (HRFs) detected in the plasma of migraine patients. This is indirectly reasserted by the absence of any IgE-dependent histamine release [32] in the investigated patients and by the normal plasma levels of the main HRFs, i.e. IL-3, IL-5, GM-CSF, and MCP1-4 (unpublished results).

Some TF variations have already been reported: poor plasma storage conditions artefactually increase TF levels, whereas both TIBC and immunochemically measured TF levels are lowered in hepatitis, cancer or haemochromatosis [21, 22, 24]. In the present study, a significant TIBC decrease was associated with a normal immunochemically measured TF level in the plasma of MO patients. Both sampling procedures and assay methods [25, 27] were carefully selected as were the patients, who were drug-free and healthy, apart from their migraine.

Qualititative TF alterations, largely related to its sialic acid content, have been reported in alcoholism, cirrhosis, cancer and pregnancy [21] but this is the first time, to our knowledge, that a TF desialylation has been reported in migraine patients. The number of studied patients is quite small. Obviously, the present preliminary findings need to be asserted in a larger number of patients. If confirmed, future issues concerning a defect in TF sialylation in MO patients might be: (i) the determination of glycan structures and molecular mass of TF glycovariants, (ii) the investigation of sialylation of other glycoproteins, including iron-binding proteins such as lactoferrin, and (iii) the effect of sialylated and desialylated TF on histamine release.

The relation of the present finding to some pathogenic mechanisms of migraine should also be addressed. As expected from the model of histamine-induced migraine, PPP histamine is increased especially during attacks ([7], Table 1, and unpublished data obtained from MO patients assessed both between and during attacks). This increase of histamine should be better clarified in the context of migraine pathogenesis as well as its source (basophils, as suspected from the present in vitro experiments, but also mast cells, or possibly both).

TF has been identified as one of the iron-binding proteins responsible for an inhibitory effect on histamine release from basophils and mast cells [19, 20, 33, 34]. The dose-response curves in these studies revealed that inhibition of histamine release is dependent on the degree of TF iron saturation. Accordingly, in the present study the plasma TIBC of MO patients was significantly lower than that of controls. Liver endothelium, which is involved in TF desialylation (a process selective for its triantennary chain) also functions in the transport and removal of TF from the circulation [35]. Is this true only for liver endothelium or does it also occur in the endothelium of cranial vessels? This may be important for some pathogenic events underlying migraine attacks, such as neurogenic inflammation in which mast cells and brain endothelium seem to be involved. If a systemic TF defect is demonstrated in migraine, this can have a great impact on brain vessels, contributing to exacerbate plasma protein extravasation and other events involving histamine via H1 receptors and other neurotransmitters including NO.

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