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Headache Medicine in Brazil: Research Submission

Glutamate Levels in Cerebrospinal Fluid and Triptans Overuse in Chronic Migraine

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Objective.—Chronic migraine (CM) is a common disorder, affecting 2% to 3% of the general population. Glutamate is implicated in cortical spreading depression, trigeminovascular activation, central sensitization, and may be linked to migraine chronification. Triptans brought a novel option for the acute migraine treatment. As the development of central sensitization impacts upon the effectiveness of triptan therapy, we hypothesized that glutamate might be related to triptan response mechanisms.

Methods.—We studied 19 patients diagnosed with CM according to the International Headache Society (2004) criteria. Patients were divided in those overusing analgesics (NSAIDs); those without overuse, and those overusing triptans.

Results.—Cerebrospinal fluid (CSF) glutamate levels were similar in patients overusing acute medications (0.335 ± 0.225 μmol) compared to those without overuse (0.354 ± 0.141 μmol), P = NS). In contrast, patients overusing triptans had CSF glutamate levels significantly lower than that observed in nonoverusers (0.175 ± 0.057 vs 0.354 ± 0.141 μmol, $P = 0.015$), and significantly higher than controls (0.175 ± 0.057 vs 0.109 ± 0.066 μmol, $P = 0.039$). In triptan overusers, CSF glutamate levels, although lower, were not significantly different from patients overusing other types of analgesics.

Conclusions.—Our study showed lower glutamate levels in CSF of CM patients overusing triptans. Glutamate may be implicated in triptan response mechanisms, triptans may work in part by reducing extracellular glutamate levels in the brain.

Key words: migraine, glutamate, triptans, overuse

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Chronic migraine (CM) is a common disorder, affecting 2% to 3% of the general population, being one of the most debilitating and difficult-to-treat headache disorders.[1] The vascular hypothesis of migraine has now been superseded by a more integrated theory involving vascular and neuronal components.[2] Neurotransmitters and intracellular mediators have been scarcely investigated in pathophysiology of CM.

Glutamate, the major excitatory neurotransmitter in the central nervous system, has been shown

to be involved in migraine mechanisms. Glutamate is implicated in cortical spreading depression, trigeminovascular activation, central sensitization, and may be linked to migraine chronification since altered glutamate levels have been reported in migraine patients.[3] For instance, glutamate levels in cerebrospinal fluid (CSF) and in plasma of patients with episodic migraine have been reported to be higher than those observed in controls,[4,5] and glutamate CSF has been shown to be elevated in CM patients[6] as well as in CM patients with fibromyalgia and pressure allodynia.[7]

Triptans brought a novel option for the acute migraine treatment, acting at several steps during the progression of the migraine attack. As the development of central sensitization impacts upon the effectiveness of triptan therapy occurs,[8] early intervention, before sensitization onset, has been shown better efficacy. Since glutamate is linked to central sensitization,[9] we hypothesized whether it might be related to triptan response mechanisms. Accordingly, here we investigated glutamate levels in CSF in CM patients treated with different acute medications, such as analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), and triptans in order to test this hypothesis.

PATIENTS AND METHODS

We studied 20 patients diagnosed with CM according to the International Headache Society criteria.[10] All patients underwent a lumbar puncture in order to rule out idiopathic intracranial hypertension (IIH), which is present in 5% to 14% of chronic daily headache series.[11,12] In our sample, IIH was ruled out in all patients. All patients overused analgesics or NSAIDs according to the revised criteria for medication overuse,[13] except for item (c). Headache does not have developed or markedly worsened during medication overuse.

Nineteen patients were included in this study (13 women 6 men; mean age 42.89 years, SD 11.64 years), mean frequency of headache 28.94 days per month, SD 2.43 days; mean baseline pain intensity 0 to 10 5.1, SD 1.3; mean exacerbations pain intensity 0 to 10 9.2, SD 0.8. One patient was excluded because she overused triptans and analgesics.

Patients were divided in 3 groups: Group 1 comprised 8 patients overusing analgesics such as NSAIDs; Group 2 comprised 5 CM patients without overuse of medications; and Group 3 comprised 6 patients overusing triptans (Table). The glutamate levels from CSF were measured by high-performance liquid chromatography as previously reported.[9]

Table.—Patients' Profile of Triptans Overuse in Group 3

Patient No.	Acute Medication	No. of Days/Month
1	Rizatriptan 10 mg	12
2	Rizatriptan 10 mg + Orphenadrine 70 mg, dipyrone 600 mg, caffeine 100 mg	12
3	Naratriptan 2.5 mg	30
4	Rizatriptan 10 mg + Orphenadrine 70 mg, dipyrone 600 mg, caffeine 100 mg	12
5	Naratriptan 2.5 mg	30
6	Rizatriptan 10 mg	30

Control CSF specimens were also obtained from 19 age- and sex-matched subjects who underwent lumbar puncture for other diagnostic purposes. In all these subjects, CSF and blood examinations and, when needed, instrumental investigations, including neuroimaging, were performed in order to exclude related central nervous system (CNS) (multiple sclerosis, vasculitis, and other autoimmune diseases affecting the CNS) or systemic diseases (diabetes, renal or hepatic dysfunction, inflammatory diseases). Neurodegenerative diseases were also excluded in these subjects. The CSF samples were on ice to prevent conversion of glutamate to glutamine.

The protocol was approved by the local ethics committee and all patients gave written consent to these study. The subjects' consent was obtained according to the Declaration of Helsinki.

CSF Analysis.— *High Performance Liquid Chromatography (HPLC).*—The HPLC was performed as previously described by our group.[9] In summary the Waters chromatography system consisted of the Model 600E gradient pump, a 717 plus auto sampler coupled with a 2475 fluorescence detector (excitation 330 nm, emission 450 nm). The CSF samples were deproteinized with 10% trichloroacetic acid (1:0.2 v/v). The resulting mixture was kept at 0°C for 15 minutes and then centrifuged at 3000 g for 10 minutes at 4°C. Supernatant (100 μL) was added to 400 μL of 0.2 M sodium bicarbonate. Amino acid determination

was performed using a precolumn derivatization procedure. The reagent OPA/BME (Serva, Heidelberg, Germany) was prepared 24 hours prior to use and the derivatization was performed by the 717 auto sampler, just before injection. OPA (150 μL) was transferred to the sample vial. After mixing, 20 μL was injected onto a reversed phase Nova-Pak C18 (3.9 $\times$ 150, 4 mm) column (Waters) equilibrated at 25°C with mobile phase A (0.05 M sodium phosphate buffer pH 5.5/15% methanol) at a flow rate of 1 mL/min. The mobile phase B consisted of sodium phosphate pH 5.5/80% methanol. Glutamate was eluted after the establishment of a linear gradient as previously described by Cavalheiro et al.[14] The elution program consisted of 20 minutes of elution with only the mobile phase A and a gradual change to mobile phase B during a period of 40 minutes, followed by 20 minutes with pure mobile phase B.. After this the column was re-equilibrated with the mobile phase A during 10 minutes, before the next injection. Standard solutions containing glutamate were employed to establish calibration curves (correlation coefficients of 0.99 or better in all cases).

Statistical Analysis.—The values in μmol/L were expressed as mean $\pm$ SD. One-way within-subjects ANOVA was carried out to compare glutamate levels from CSF. A Student *t*-test was used to compare all groups. Five percent for 2-sided tests was chosen as a minimum level of statistical significance.

RESULTS

All CSF samples presented normal levels of protein, glucose, lactate as well as cell count. CM patients from all groups showed higher CSF glutamate levels when compared with values found in control subjects ($P < .01$). CSF glutamate levels were similar in patients overusing acute medications (0.335 μmol $\pm$ 0.225) and in those without the overuse of drugs (0.354 μmol $\pm$ 0.141, $P =$ NS). In contrast, patients overusing triptans had CSF glutamate levels lower than the observed in nonoveruser CM patients (0.175 $\pm$ 0.057 vs 0.354 $\pm$ 0.141 μmol, $P = .015$), but significantly higher than controls (0.175 $\pm$ 0.057 vs 0.109 $\pm$ 0.066 μmol, $P = .039$. In patients overusing triptans, CSF glutamate levels, although lower, were not significantly different from patients overusing other types of analgesics (group 1) (0.175 $\pm$ 0.057 vs 0.335 $\pm$ 0.225 μmol, $P = .116$) (Figure). The demographic and clinical variables (age, gender, headache frequency, duration, and severity) did not correlate with glutamate levels in our sample population.

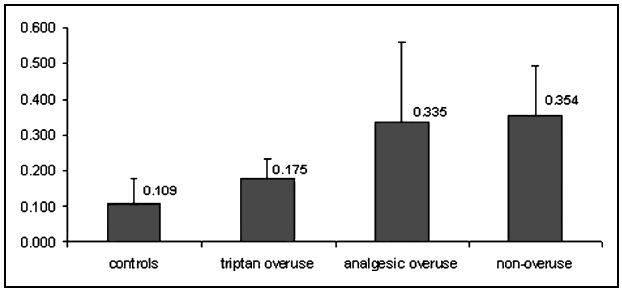

Figure.—CSF glutamate levels (μmol/L) in chronic migraine patients overusing triptans, analgesics, nonoverusers, and controls.

COMMENTS

The present work showed low CSF glutamate levels in CM patients overusing triptans when compared with nonoveruser CM patients. This difference was not observed when we compared patients overusing other drugs than triptans with nonoverusers.

Glutamate is one of the putative candidates in the development and maintenance of chronic headache via its action on ionotropic and metabotropic receptors.[15,16]

Several lines of investigation have shown that glutamate levels are altered in plasma, platelets, and CSF of patients with migraine when compared with control subjects what has been considered to indicate a systemic dysfunction of the glutamatergic system in this syndrome.[4-6,17-21] Ferrari et al[21] demonstrated that between attacks, patients with migraine had substantially high levels of serum glutamate and aspartate than controls or patients presenting tensional headache. In addition, Cananzi et al[18] evaluated the glutamate concentration in migraine patients with and without aura and found higher levels of this amino acid in plasma and platelet. Increased CSF glutamate level has also been reported in migraine patients with or without aura[5] and in patients with chronic daily headache.[6] Previous data from our group[7] have indicated that CSF glutamate levels in CM patients with and without fibromyalgia were increased in those CM patients

with widespread pain. Glutamate levels have also been correlated with pressure allodynia levels in these populations.[9]

The N-methyl-D-aspartate (NMDA) and non-NMDA receptor activation by glutamate released by central nociceptor terminals induces calcium entry into the dorsal horn neurons, in neurons of the trigeminal nucleus caudalis and also in supraspinal structures, structures that have been considered to participate in the processing of head pain.[22-25] Activation of NMDA receptors makes the spinal cord neurons more responsive to all nociceptive and nonnociceptive inputs, resulting in central sensitization. Central transmission and hyperexcitability are mediated by excitatory amino acids (aspartate and glutamate), and by neurokinins (in particular the substance P) and other sensory neuropeptides (CGRP) acting on glutamate and neurokinin receptors, respectively.[6,23,24] Central trigeminal neurons that receive convergent input from external stimuli may be sensitized and this mechanism of central sensitization has been implicated in migraine physiopathogenesis.

Berstein et al[26,27] have shown that during the migraine pain, cutaneous allodynia may progress within an attack in 80% of patients. Initial peripheral activation and sensitization affect intracranial blood vessels and the meninges. Later, peripheral pain fibers become hypersensitive mediating throbbing pain of migraine that worsens during coughing, bending over, and rapid head movements. If additional central sensitization of the trigeminal nucleus caudalis occurs, than cutaneous allodynia develops. Based on these aspects, the pathophysiological mechanisms underlying migraine support the early use of antimigraine drugs targeting peripheral nociceptors, before the development of central sensitization.[8,28,29] Theoretically, early treatment before the evolution of the pain to moderate or to severe intensity could avoid or reduce the sensitization and, consequently, improve the treatment response.

Cady et al[30] found that early intervention by the use of triptan administered when the pain is mild produced a greater pain-free response. However, when administered later, during the attack or when the pain had become moderate to severe, the clinical response is less significant. In general, more patients reached a pain-free status when treated with triptan during the mild pain phase than those receiving the drug when pain is graded from moderate to severe. This study also showed that in all headaches treated, the pain-free response at 2 hours was 50% with sumatriptan versus 0% for placebo, when the pain was mild, compared with 27% versus 6%, when pain was moderated or severe. Another study[31] with migraine patients treated with almotriptan 12.5 mg for headaches attacks of any severity showed similar results. At 2 hours, 84% of mild attacks versus 53% of moderate or severe attacks treated with almotriptan were pain free. In another clinical trial, 93% of patients with migraine but no allodynia treated with triptan were pain free at 2 hours compared with only 15% of patients with migraine experiencing allodynia.[32]

Accordingly, we could consider that the administration of triptans in early stages of migraine attack, ie, 30 to 60 minutes after pain arises, is able to block neuropeptides release from both the peripheral and central nerve endings, causing these neurons to become quiescent, thus decreasing the pain and throbbing and avoiding the development of allodynia. If allodynia and central sensitization are related to glutamate levels, and triptan response related to allodynia, one may speculate that glutamate levels may mediate this response.

One proposed site for triptan action is within the trigeminocervical complex. It has been assumed, based on observations of inhibition of plasma protein extravasations, and due to the localization of 5-HT_{1D} mRNA in the trigeminal ganglion, that triptans block trigeminal transmission by a prejunctional mechanism. It has been shown that some component of transmission across the trigeminal nucleus involves glutamatergic mechanisms.[33-36]

Our study showed lower glutamate levels in CSF of CM patients, who overused triptans, when compared to nonoveruse patients. Triptans may work in part by reducing extracellular glutamate, as reflected by the decrease in CSF levels. Since glutamate is involved in central sensitization, triptans may reduce this process in CM patients. We cannot suggest from our data that triptans are more indicated than other antimigraine drugs for CM treatment, but further clinical studies are necessary to clarify if triptans could prevent

migraine chronification, reduced headache frequency, or be used as a transitional treatment in CM. Triptans may affect glutamate neurotransmission by several hypothetical ways such as inhibiting the glutamate release, affecting the glutamate receptors binding site, increasing the glutamate uptake by glial cells and/or by neuronal transporters, or decreasing the neuronal firing in the trigeminal nucleus. Many variables could interfere with the data presented in our study, including clinical differences between groups, current pain status, triptan overuse, triptan efficacy in headache attacks, but our sample size was not enough to detect such differences. In addition, the group that did not overuse did not have significantly different glutamate levels, but again the sample size may have interfered with the results. The lower glutamate levels in these patients may therefore not represent a mode by which triptans work, but may have many other explanations. Further studies to investigate whether triptans work in part by lowering extracellular glutamate are required to explore this pathophysiology, as well as to test any current interpretation.

Conflict of Interest: None

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