Cerebrospinal Fluid Ouabain-Like Compounds in Idiopathic Intracranial Hypertension

The Ouabain-IIH Study Group :

Neuro-Ophthalmology Service, Ibn Sina Hospital, Kuwait City, Kuwait: Raed Behbehani, MD, Asser Mabrook, MD

Emory Eye Center, Atlanta, Georgia: Beau B. Bruce, M.D.

Neuro-Ophthalmology Unit, Rabin Medical Center, Petah Tikva, Israel: Ehud Reich, M.D.; Hadas Stiebel-Kalish, M.D.

Department of Neurology, Northwestern University, Chicago, Illinois: Katherine Carroll, M.D.; Lyla Kamsheh, M.D.

University of Ottawa Heart Institute,: Frans Leenen, M.D.; Roselyn White, MLT.

Northern Neurosciences Inc.: Mark K. Borsody, M.D., Ph.D. *; Tamara N. Heaton, M.S.; Chisa Yamada, M.D.

* author for correspondence:

Northern Neurosciences, Inc.

88 Tarry Lane

Orinda, CA 94563

mborsody@northern-neurosciences.com

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Abbreviations: OLC, ouabain-like compounds; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbant assay; IIH, idiopathic intracranial hypertension; SEM, standard error of the mean

Abstract

Background: Ouabain-like compounds (OLC), present in serum and cerebrospinal fluid (CSF), are endogenous inhibitors of the sodium-potassium ATPase. Since the sodium-potassium ATPase is the rate-limiting step in CSF production, we hypothesized that idiopathic intracranial hypertension (IIH) patients will have reduced levels of CSF OLC.

Methods: In this multicenter study we assessed 31 patients with a new or established diagnosis of IIH. CSF was obtained via lumbar puncture with measurement of the opening pressure. CSF OLC were determined by ELISA. CSF OLC levels for the 31 IIH patients were compared to those of 31 historical controls using a Wilcoxon rank test.

Results: The mean CSF OLC for IIH patients was significantly lower than the mean CSF OLC for controls. This proved true when 14 samples with CSF OLC levels below threshold were included with values set at detection threshold (0.053 ng/mL ng/mL vs. 0.115 ng/mL, $p < 0.05$) or when the 14 samples were excluded (0.067 ng/mL vs. 0.115 ng/mL, $p < 0.001$).

Conclusions: Analysis of the combined dataset suggests that IIH patients have lower CSF OLC levels than controls. A definitive test of the hypothesis, and assessment of a causative role of low CSF OLC in IIH, will require a larger patient population subject to CSF OLC measures pre- and post-symptomatic treatment.

Introduction

Idiopathic intracranial hypertension (IIH) is a syndrome characterized by signs and symptoms of raised intracranial pressure in the absence of an identifiable cause [1]. In a previous pilot study, we hypothesized that ouabain-like compounds (OLC) levels in the cerebrospinal fluid (CSF) are reduced in IIH patients [4]. The hypothesis was based on the known action of OLC to inhibit the sodium-potassium ATPase [2, 5, 6], the rate-limiting factor in CSF production [2]. Indeed, OLC have been linked to several disorders of fluid volume outside of the brain [3]. If reduced levels of OLC allow for increased production of CSF that in turn would elevate intracranial pressure, we would expect to find lower CSF OLC in IIH patients than in control subjects. .

In a previous pilot study [4], we were unable to demonstrate a difference in CSF OLC levels between IIH patients and controls with a study population of 7 IIH patients and 31 control subjects. In this study, we collected CSF samples on 31 IIH patients and compared them to 31 historical controls from the previous study.

Methods

This protocol was approved by the Institutional Review Boards of Ibn Sina Hospital, Northwestern Memorial Hospital, Emory University, and the Rabin Medical Center. All enrolled patients were required to provide informed consent. New (n=28) or established (n=3) IIH patients were recruited. Data from control subjects (n=31) with various other conditions or suspected neurological disease from our previous study [4] were used in the analysis (Table 1). The only exclusion criterion for this study was

digoxin use, which can potentially interfere with the OLC assay, although no such exclusion was necessary.

CSF was withdrawn by routine lumbar puncture with the patient in the lateral decubitus position. Opening pressure was measured and recorded in all patients. Routine laboratory tests (i.e., cell counts, protein, and glucose levels) were performed on all CSF samples and additional tests were performed at the discretion of the treating physician in a manner appropriate to the patient's condition. Management of the patient's medical condition was not affected by enrollment in this study.

IIH was diagnosed according to the following criteria of Friedman and Jacobsen [7]: (i) neurological symptoms or signs consistent with elevated intracranial pressure, (ii) opening pressure > 25 cm H_2O in the relaxed, lateral decubitus position, and (iii) normal CSF studies allowing for a traumatic lumbar puncture.

All newly-diagnosed cases of IIH had cerebral venous thrombosis excluded by appropriate neuroimaging. All patients in this study were examined with lumbar puncture as part of the standard diagnostic and therapeutic requirements for their condition; no effort was made to enroll patients for the sole purpose of sample collection. Patients were enrolled in this study in a consecutive manner.

OLC were measured in CSF samples by means of ELISA [8]. Extracted CSF samples or ouabain standards and rabbit anti-ouabain antiserum were added to each well in an ovalbumin/ouabain-coated enzyme immunoassay plate. Plates were then incubated at room temperature for 2 hours and rinsed. Anti-ouabain antibodies remaining bound to the ovalbumin-ouabain were reacted with goat anti-rabbit IgG-peroxidase conjugate (Jackson Immunolabs) for 1 hour at room temperature. After being washed, the amount

of peroxidase enzyme remaining in each well was determined by the addition of 3,3'5,5-tetramethylbenzidine base substrate solution. The absorbance in each well was measured at 450 nm with the use of a microplate recorder. The concentration of OLC in each sample was calculated from the reduction in absorbance according to the ouabain standard curve. The threshold for detection of OLC was dependent upon the sample volume available (typically 1-3mL), e.g., with 0.038 ng/mL (0.065nM) being the detection threshold for OLC in a 3 ml sample. This is comparable to the sensitivity for OLC measured in body fluids by ELISA or radioimmunoassay, as reviewed by Semra et al [9]. The concentration of OLC was not normalized to CSF protein concentration.

The CSF OLC levels for IIH patients were compared to that of 31 historical controls using a Wilcoxan rank test. All data are displayed as the mean ± SEM. Unpaired t-tests were used to assess potential subgroup differences within the IIH patient population. $P < 0.05$ was defined as significant. Linear regression analysis was performed in the whole subject population (combined IIH and control groups) to determine correlation between CSF OLC and opening pressure.

Results

The demographic and clinical characteristics of the IIH patients and control subjects are shown in Table 1. In the 31 IIH patients recruited in this study, the average opening pressure was 39.4 ± 1.6 cm H_2O (range: 27-60 cm H_2O). Three patients had a previous diagnosis of IIH: their opening pressures were 42, 36, and 28 cm H_2O, with the latter two measures obtained in patients taking acetazolamide without symptomatic relief.

Seven more patients had been started on acetazolamide at the time of lumbar puncture for treatment of presumptive IIH; their opening pressures averaged 41.3 ± 4.2 cm H_2O (range: 28-56 cm H_2O). The average opening pressure of all 9 IIH patients taking acetazolamide was not significantly different from that of IIH patients who were not taking acetazolamide (39.2 ± 1.9 cm H_2O vs. 39.2 ± 3.5 cm H_2O; $p = 0.99$).

Fourteen CSF samples belonging to IIH patients were found to be below the detection threshold for OLC. There was no difference between opening pressures in those patients with OLC levels below the detection threshold (36.8 ± 2.0 cm H_2O) and those above it (37.7 ± 1.9 cm H_2O; $p = 0.27$). In order to account for OLC levels below the detection threshold, we compared the means of OLC levels between the IIH patients and control subjects with and without the 14 IIH patients who had CSF OLC levels below the detection threshold. When the 14 patients were included in the analysis, their CSF OLC levels were arbitrarily set at the best possible detection threshold value based on the individual sample size.

Linear regression of the combined IIH patient and control groups demonstrated a significant negative correlation when the entire population of IIH and control subjects was included ($r = -0.463$, $p < 0.0001$; Figure 1) or when the 14 patients who had CSF OLC levels below detection threshold were excluded ($r = -0.387$, $p < 0.0001$).

Comparing the two groups, the mean CSF OLC in IIH patients was significantly lower than controls (0.053 ng/mL vs. 0.115 ng/mL, $p < 0.05$) including all IIH patients. When we excluded the 14 CSF OLC measurements below the detection threshold, the mean CSF OLC for IIH patients was still significantly lower than that of controls (0.067 ng/mL vs. 0.115 ng/mL, $p < 0.001$).

Conclusion

The underlying cause of IIH is unknown [7,10,11]. One possible mechanism is the overproduction of CSF [12]. OLC are endogenous substances with steroid and non-steroid components [18,19] that are found in the CSF of mammals. As one might suspect based on observations with ouabain itself, the OLCs potentially inhibit CSF production and therefore may play role in the regulation of intracranial pressure. The mechanism-of-action for this would likely be inhibition of the sodium-potassium ATPase [2].

Our regression analysis involving both IIH patients and control subjects demonstrated an inverse relationship between CSF OLC levels and opening pressure consistent with the potential regulatory role for OLCs over intracranial pressure. It is important to note that, despite a common binding site on the sodium-potassium ATPase, CSF OLC are not structurally identical with ouabain [16], and that their physiological role in setting intracranial pressure is not yet established.

We hypothesized that IIH patients would exhibit reduced concentrations of OLC in the CSF. This would lead to increased CSF production and the elevated intracranial pressure characteristic of the IIH. The hypothesis implies that CSF OLC levels would be lower in IIH patients in comparison with controls, which we were unable to demonstrate in our earlier study that was based on only 7 IIH patients. Here we have found that the OLC CSF levels in IIH patients are significantly lower than those of controls. If this relationship is specific for IIH, a useful corollary would be to show that CSF OLC levels are not reduced in other diseases with increased intracranial pressure. The essentially irreversible action of OLCs on the sodium-potassium ATPase [17] would require that one

evaluate a disease with a chronic elevation in intracranial pressure that nonetheless requires a lumbar puncture as a routine part of its medical care. To our knowledge, there are not many suitable diseases for such a study.

Our analytic methodology is limited by OLC assay sensitivity and the nature of the control group. Despite the similar protocols between this and our previous study [4], in the current study some CSF samples had OLC level below the detection threshold. However, even with the exclusion of those patients, CSF OLC levels were significantly lower in the IIH group than in controls. This problem should be readily surmountable in future studies by collection and analysis of larger volumes of CSF from IIH patients.

Since we did not concurrently enroll control subjects for this study, we had to use control subjects from our previous study [4]. That control group cannot be said to represent the normal population due to the variety of other medical diagnoses carried by the subjects in that group. Although this may be a source of bias, we did not believe it to be ethically acceptable to collect CSF on normal subjects solely for study purposes. More appropriate comparison groups would consist of patients being assessed for the possible diagnosis of IIH who on lumbar puncture fail to have elevated intracranial pressure, and the previously-mentioned lumbar puncture-requiring disease with chronically-elevated intracranial pressure.

Although we cannot conclude a causal relationship between reduced CSF OLC levels and elevated opening pressure in IIH based on the current analyses, our findings invites further consideration for this possible pathophysiologic mechanisms of IIH. Whether or not OLC play a causative role in IIH would best be demonstrated by administering OLCs and demonstrating a reduction in the elevated intracranial pressure

and alleviate the symptoms of IIH. While performing such a study would be operationally very difficult, it should be noted that the ouabain-like sodium-potassium ATPase inhibitor digoxin (itself a ouabain-like compound) has been historically used as a treatment of IIH [13,14].

In lieu of an OLC administration study, we would propose to assess CSF OLC levels for responsiveness to other treatments that decrease intracranial pressure in IIH. If CSF OLC levels are reduced in IIH patients as a reaction / epiphenomenon of the disease, then successful symptomatic treatment for the disease should allow CSF OLC levels to increase (i.e., normalize). If, however, the primary / causative defect in IIH is a reduction in CSF OLC levels, successful symptomatic treatment of the disease should have no effect on CSF OLC levels. Such a study might be conducted as part of a larger treatment trial for IIH.

Future studies should also consider, if possible, the nature of the sodium-potassium ATPase in IIH patients, for the enzyme may partly explain characteristics of the disease such as age and sex predilection. The target of the OLCs itself exhibits various isoforms that range in their affinity for ouabain more than 1000-fold [20], and sex steroids may regulate their activity [22]. Furthermore, a study of ouabain binding kinetics to lymphocyte sodium-potassium ATPase identified differences according to gender and, in women, a relation to subject age [21]. Larger studies of this type that incorporate IIH patients would be needed

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Table 1: Demographic and Clinical Characteristics of Study Patients

	IIH	Control
sex (# female / male)	28 / 3	19/12
average height (cm)	163.4 ± 1.5	169.3 ± 1.4
average weight (kg)	93.3 ± 4.1	68.7 ± 2.6
body mass index (kg/m^2*)*	35.1 ± 1.7	23.8 ± 0.7
pre-existing medical conditions	1 case with head trauma, shingles, major depressive disorder, hypertestosteronism, irritable bowel syndrome, gastric ulcers, diabetes, hypertension with glomerulonephritis, iron deficiency anemia, asthma, ADHD	3 cases with hypertension 2 cases with arthritis 2 cases with migraine 2 cases with coronary artery disease 1 case with sarcoid, histocytosis, lymphoma, seizure
Oral contraceptive use	2	3
Glucocortcoid use	4	5
Vitamin A use	0	0

Figure 1: Linear regression analysis between the cerebrospinal fluid (CSF) ouabain-like compounds (OLC) in control subjects (crosses) and IIH patients, and the CSF opening pressure (OP). IIH patients with detectable OLC levels are displayed as circles. IIH patients with OLC levels below detection threshold (triangles) have their measures set at half of the detection threshold as determined by the volume of CSF analyzed from that patient. Negative correlation was observed ($r = -0.463$, $p < 0.0001$).