Journal of Steroid Biochemistry & Molecular Biology 152 (2015) 124–132

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Steroid hormones in prediction of normal pressure hydrocephalus

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ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 13 April 2015
Accepted 7 May 2015
Available online 11 May 2015

Keywords:
Normal pressure hydrocephalus
Cerebrospinal fluid
Neuroactive steroids
Aldosterone
Prediction

ABSTRACT

Normal pressure hydrocephalus (NPH) is a treatable neurological disorder affecting elderly people with the prevalence increasing with age. NPH is caused by abnormal cerebrospinal fluid (CSF) reabsorption and manifested as a balance impairment, urinary incontinence and dementia development. These symptoms are potentially reversible if recognized early. Diagnosis of NPH is difficult and can be easily mistaken for other neurodegenerative disorders, which makes NPH one of the major misdiagnosed diseases worldwide. The aim of the study was to find out the appropriate combination of indicators, based on CSF steroids, which would contribute to a clearer NPH diagnosis. The levels of CSF cortisol, cortisone, dehydroepiandrosterone (DHEA), 7α-OH-DHEA, 7β-OH-DHEA, 7-oxo-DHEA, 16α-OH-DHEA and aldosterone (all LC-MS/MS) were determined in our patients ($n = 30$; NPH, 65–80 years) and controls (n = 10; 65–80 years). The model of orthogonal projections to latent structures (OPLS) was constructed to predict NPH. Cortisone, 7α-OH-DHEA, 7β-OH-DHEA, 7-oxo-DHEA, aldosterone, 7α-OH-DHEA /DHEA, 7-oxo-DHEA/7α-OH-DHEA, 7β-OH-DHEA/7-oxo-DHEA and 16α-OH-DHEA/DHEA in the CSF were identified as the key predictors and the model discriminated patients from controls with 100% sensitivity and 100% specificity. The suggested model would contribute to early and accurate NPH diagnosis, enabling promptly treatment of the disease.

1. Introduction

Normal pressure hydrocephalus (NPH) is a treatable neurological disorder of unknown pathophysiology predominantly occurring in elderly people. It was first described in 1960s by Adams and Hakim [1] who characterized the disease by the triad consisting of gait disturbance, urinary incontinence and dementia development with enlarged brain ventricles and the absence of raised intracranial pressure [1]. A ventricle enlargement increases pressure on the brain, which may impair brain tissues and cause several brain malfunctions. These degenerative changes may be reversible if recognized early and adequately treated. On the other hand, left untreated normal pressure hydrocephalus can cause mental deterioration, lethargy, immobility and incontinence.

NPH is one of the most misdiagnosed worldwide diseases. It can occur with varying combinations or degrees of each of the clinic triad elements [2,3], which makes its diagnosis challenging. Generally, gait disturbance along with one of the additional features appear [4]. NPH diagnosis is generally based on clinical history, brain imaging (CT and MRI), physical ascertainment, physiological measurements and liquor dynamic tests [5]. Until recently, no distinct laboratory biomarker was discovered [6]. The diagnosis frequently overlaps with other neurological disorders and may be easily mistaken for Alzheimer (AD) and Parkinson (PD) disease, vascular dementia (VD), infectious diseases (borreliosis, HIV, syphilis) or urological diseases (urinary tract infections, benign prostate hyperplasia, prostate and urinary bladder cancer) [7]. For whatever reason the patient goes undiagnosed, the delay leads to further progression of the symptom and diminished recovery after a delayed surgery. The neuropathological changes in untreated NPH comprise the white matter damage and secondary neuronal disconnection as the result of the cerebral ventricle expansion [8,9]. The only accurate tool is liquor dynamic test. It is based on the lumbar drainage requiring the introduction of a catheter into the lower back for several days. Excessive CSF is drained to a connected collection system, enabling the controlled

Abbreviations: 7α-OH-DHEA, 7α-hydroxy-dehydroepiandrosterone; 7β-OH-DHEA, 7β-hydroxy-dehydroepiandrosterone; 7-oxo-DHEA, 7-oxo-dehydroepiandrosterone; HSD11B1, 11β-hydroxysteroid dehydrogenase type 1; HSD11B2, 11β-hydroxysteroid dehydrogenase type 2; 16α-OH-DHEA, 16α-hydroxy-dehydroepiandrosterone; AD, alzheimer's disease; BBB, blood-brain-barrier; CSF, cerebrospinal fluid; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; MR, mineralocorticoid receptors; NPH, normal pressure hydrocephalus; OPLS, the method of orthogonal projections to latent structures; PD, Parkinson disease; UPLC, ultra performance liquid chromatography; VD, vascular dementia.

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http://dx.doi.org/10.1016/j.jsbmb.2015.05.004
0960-0760/

simulation of the effects of a shunt on the brain (see shunt treatment below) [10]. The clinical response to a lumbar drainage test still remains the most accurate method for NPH diagnosis [10,11], though its sensitivity is limited [11].

There have only been a few epidemiological reports concerning NPH prevalence. The diversity of symptoms in patients with NPH leads to difficulty in determining inclusion criteria for research [12]. Recent published data showed the prevalence in community-dwelling elderly people of approximately 1% [13–15]. The most actual data show the prevalence of probable NPH 0.2% in patients aged 70–79 years and 5.9% in those aged 80 years and older [16]. Unfortunately, most patients with NPH are either underdiagnosed or misdiagnosed in clinical practice. The number of persons with NPH is probably much higher than the number of persons currently treated [16]. Data from 2009 showed that only 20% of subjects suffering from NPH are detected and adequately treated [17].

The most frequent therapeutic approach of NPH is the surgical introduction of ventriculoperitoneal shunt, draining the excessive CSF into the abdominal cavity. The selection of NPH patients profiting from shunt implantation is still problematic [5]. The financial demands of the shunt surgery and the necessity of lifelong monitoring in specialized centers of patients after surgery require a high positive predictive value of the preoperative tests. Diagnosis in the early stage gives the patients high probability to the disappearance of all the symptoms after shunt surgery [18,19]. Simple selection on the basis of imaging method is insufficient [20]. The predictive value ranges between 27 and 58%. To increase the predictive value of the shunt surgery, supplementary liquor dynamic tests have been introduced into clinical practice [2].

In the abovementioned diagnostic process, CSF is easily approachable biological material encouraging the biochemical analysis. Above many biochemical analytes, steroid hormones may serve as indicators of neurodegenerative disorders. The combination of steroids was reported as a discriminatory tool for AD and VD and for predicting schizophrenia [21,22]. Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), showing anti-inflammatory, antiglucocorticoid, immunoprotective and neuroprotective effects, are ones of the most abundant steroids in humans. Out of the adrenal synthesis, an important portion of DHEA is also known to be synthesized in the brain and therefore is together with its metabolites assigned among neurosteroids [23–25].

The best known neuroactive DHEA metabolites synthetized in the brain are 7-oxygenated derivatives such as 7α-OH-DHEA, 7β-OH-DHEA and their intermediate 7-oxo-DHEA [26–28]. These metabolites exhibit immunoprotective, antiglucocorticoid, antioxidant, antiapoptotic and neuroprotective activities commonly attributed to DHEA and may stay behind its protective activities [24,29–34]. 7α-OH-DHEA is a substrate for the type 1 11β-hydroxysteroid dehydrogenase (HSD11B1), which converts 7α-OH-DHEA into 7-oxo-DHEA and 7β-OH-DHEA and vice versa [35–37]. It should be pointed out, however, that the HSD11B1 is primarily responsible for the NADP(H)-dependent reduction of inactive cortisone to bioactive cortisol [35,37–39] in glucocorticoid responsive tissues including the brain. The hydroxylation of DHEA at carbon 16 resulting in 16α-OH-DHEA [29] is a concurrent metabolic reaction to 7-hydroxylation, as reported for the liver and adrenal cortex [40]. So far, there are no reports of 16α-OH-DHEA in the brain, but it has been identified in the CSF [41]. Increased levels of 16α-OH-DHEA have been linked to autoimmune diseases [42].

Our previously published results indicated the connection between NPH and inflammatory changes in the brain [43]. Bearing in consideration the immunoprotective and anti-inflammatory properties of DHEA and its metabolites, their synthesis may be altered as a result of abovementioned changes in the NPH affected brain.

The immunoprotective properties of DHEA and its 7-oxygenated metabolites may be counter regulated by cortisol and its biologically inactive form - cortisone [44]. CSF cortisol serves as an important regulator of the inner milieu of brain ventricles. Taking into account its large excess over aldosterone, it competes for the mineralocorticoid receptors (MR) in the brain [45].

Aldosterone (controlling the sodium and water homeostasis) is primarily synthesized in the adrenals and binds to specific MR located in target epithelial cells [46,47]. Although the peripheral aldosterone can cross the blood-brain-barrier (BBB), much of it may be returned back by the multidrug-resistant P-glycoprotein [48], which limits aldosterone penetration into the CNS [49]. The BBB transport of aldosterone is substantially less than other steroid hormones, most likely due to its high polarity. However, there is evidence that aldosterone can be synthesized in extraadrenal tissues including the central nervous system [47,50–53]. Concerning this assumption we hypothesized, that the CSF aldosterone levels may be altered in NPH affected brain. The aforementioned data indicated that a combination of some steroids in the CSF may serve as a basis for the efficient classification of NPH. Therefore, in the present study we attempted to find out an efficient model for diagnosis of NPH, which is based on the optimum combination of steroid hormones which allows for the shunt surgery to be proposed in time.

2. Materials and methods

2.1. Chemicals and reagents

Cortisol, cortisone and dehydroepiandrosterone (DHEA) were purchased from Koch-Light Laboratories Ltd. (Colnbrook, Great Britain); 7α-OH-DHEA, 7β-OH-DHEA, 7-oxo-DHEA, 16α-OH-DHEA and D3-DHEA were from Steraloids (Newport, USA). D4-Cortisol was from CDN isotopes (Ponte-Claire, Canada). Aldosterone, D7-Cortisone, D7-Aldosterone, 2-hydrazinopyridine, ammonium formate, diethyl-ether and trifluoroacetic acid were from Sigma-Aldrich (St. Louis, USA). LC-MS grade methanol and water as well as diethyl ether were from Merck AG (Darmstadt, Germany). The physiological solution was from B-Braun (Melsungen AG, Germany).

2.2. Subjects

The patient group included 30 subjects (15 men, 15 women) 65–80 years old, BMI 22.3–32.9 (mean 27.21) with gait disturbance—30 patients (pts), memory impairment—21 pts and urinary incontinence—14 pts. As an onset of clinical symptoms is slow, the gradual duration of clinical presentation cannot be exactly determined. Non-obstructive idiopathic normal-pressure hydrocephalus was diagnosed on the basis of a combination of clinical presentation, NMR imaging and a lumbar drainage test [54]. All except four patients suffered of diseases common in their age group: arterial hypertension—17 pts; dyslipidemia—9 pts; diabetes mellitus—5 pts; benign prostatic hyperplasia—4 pts; hyperuricemia—2 pts; renal insufficiency— 2 pts; coronary heart disease—1 pt, steatosis—1pt. All patients were treated with adequate medication and none of the used drugs can be suspected of elevation of plasmatic steroid levels. CSF was collected on the beginning of lumbar drainage test. The ventriculoperitoneal shunt was introduced to all of the patients diagnosed with NPH after finishing the lumbar drainage test. The shunt implementation led to an improvement of the disease symptoms in all patients. The control group (10 subjects; 3 men, 7 women) consisted of age-matched subjects, without apparent symptoms of hydrocephalus. To ensure that control group consists of neurologically healthy patients, samples were obtained from patients with incidental