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Clinical Study

Exercise-induced changes of cerebrospinal fluid vascular endothelial growth factor in adult chronic hydrocephalus patients

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a growth factor demonstrated to be a key factor in cerebral angiogenesis and neurogenesis. It has been considered a critical component in hippocampus neurogenesis and memory formation and has been observed to increase in the rat hippocampus after exercise. We previously found increased VEGF levels in experimental chronic hydrocephalus in several brain areas and cerebrospinal fluid (CSF), suggesting a role in the adaption to chronic hypoxia. Here we investigate the ability of moderate exercise to increase CSF-VEGF levels in adult chronic hydrocephalus patients. Lumbar CSF samples were collected from 17 normal pressure hydrocephalus patients. During CSF collection, 11 patients (exercise group) underwent a standard in-room occupational therapy session; six patients (no-exercise group) did not undergo a physical therapy session. CSF-VEGF levels were evaluated for increase related to exercise and the clinical response to CSF drainage. CSF-VEGF levels in the exercise group demonstrated significant increases 1–3 hours post-exercise compared with the levels 1–2 hours pre-exercise ($p = 0.04$), and also showed significantly higher levels than the no-exercise groups ($p = 0.03$). The post-exercise CSF-VEGF level in the group that did not clinically improve was significantly higher than both their own pre-exercise level ($p = 0.02$) and that seen in the clinically improving group ($p = 0.05$) after exercise. We conclude that CSF-VEGF levels can increase after moderate exercise even in elderly hydrocephalus patients. This suggests that a potential benefit of exercise, especially in CSF drainage non-improved patients, may exist via a central VEGF mechanism.

1. Introduction

Vascular endothelial growth factor (VEGF) plays a key role in many physiological and pathological processes including angiogenesis, neurogenesis, mitosis and vascular permeability [1]. It has also been considered a critical component of hippocampus neurogenesis and memory formation [2]. VEGF levels are up-regulated in various diseases, especially hypoxia and ischemia conditions such as in hydrocephalus [3]. In a previous study, we found an increase in VEGF receptor and/or ligand associated with chronic experimental hydrocephalus. Up-regulation of VEGF and its receptor occurred in multiple brain areas and cerebrospinal fluid (CSF), suggesting a role of VEGF in adaption to chronic hypoxia [3]. Recent studies further demonstrated that exercise could increase VEGF levels in the hippocampus, stimulating hippocampal neurogenesis and cognitive improvement [2,4].

Hydrocephalus, characterized by increased CSF volume and pressure with consequent ventriculomegaly and decreased cerebral blood flow (CBF) and brain tissue oxygen delivery, is often associated with impaired cognitive function [5]. Although shunting is a choice for hydrocephalus manipulation, the treatment of cognitive impairment still remains a major challenge. Recent research suggests that engaging in physical activity may reverse some aspects of age-related cognitive decline [6]. Regular exercise was found to be effective in increasing blood VEGF and blood flow rate, which could explain the observation of increased cognitive function after exercise [7,8]. However the mechanisms involved in these processes in central nervous system still remain unclear, especially the modulation of CSF-VEGF levels in response to exercise. In this study, we investigate the ability of moderate exercise to increase CSF-VEGF levels in adult hydrocephalus patients and analyze the possible correlation with clinical condition.

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2. Materials and methods

2.1. Patients and sample collection

This study was approved by the Institutional Review Board of the Cleveland Clinic (IRB #09-118), and written consent was obtained from all subjects. CSF was obtained from patients who were admitted to the hospital for evaluation of normal pressure hydrocephalus (NPH) as previously described [9]. In brief, patients with suspected NPH were admitted to the Cleveland Clinic for a therapeutic trial of lumbar CSF drainage. Ten milliliters of CSF were drawn every hour for 36 hours. This study took place on the second day of CSF drawing between 12 pm and 5 pm. CSF samples were obtained from 17 patients who were allocated to one of two groups: exercise group (N = 11) and no-exercise group (control, N = 6). In the exercise group, the patients were conducted with intervention of a standard occupational therapy regimen, while in the no-exercise control group the patients did not undergo exercise intervention.

The collected CSF samples were centrifuged at 1,000 relative centrifugal force for 10 minutes to remove possible contaminated blood cells [10], and aliquots were stored at −80 °C for VEGF measurement.

2.2. Exercise regimen

Members from the Cleveland Clinic Occupational Therapy team spent approximately 20 minutes working with each patient at around 1–2 pm on the day of study participation. The participants (exercise patients) were worked at a mild-moderate level according to the Borg Scale of Perceived Exertion (approximately 11–14 rating) [11]. The patients were asked to spend approximately 5 minutes working on an upper body bike (Marcy Cardio Mini Cycle, model #NS912), followed by bicep curls, shoulder and arm presses with 1–3 pound weights, depending on physical ability. Patients were then asked to do external shoulder rotation exercises with a resistance band. The goal for each exercise was to do three sets of 10 repetitions. While the exercises and order of exercises remained the same, some variations in the exercise were present due to the varied conditions of each patient. Regardless of physical condition, careful consideration was taken in order to ensure that exertion levels remained consistent for all patients. Patients were given the option to take short (2–5 minute) rest breaks when needed in order to maintain a safe heart rate throughout the session. All exercises were conducted through the upper extremities in order to avoid the lumbar catheter disturbance.

2.3. Evaluation of hydrocephalus and Evans' ratio measurement

We evaluated the severity of ventriculomegaly with Evans' ratio. Measurement of Evans' ratio is a unidimensional measurement of ventricular width on CT scan or MRI, which is standard clinical practice to determine ventricle size. This value is defined as the ratio of the maximum width of the anterior ventricular horns at the level of the foramen of Monro to the maximum width of the calvarium at the same level. A ratio greater than or equal to 0.3 has been accepted clinically as ventriculomegaly.

2.4. Lumbar CSF drainage trial for NPH and evaluation of response to CSF drainage

The protocol of lumbar CSF drainage has been used in the Cleveland Clinic for a therapeutic trial as part of the diagnostic work-up and evaluation of possible shunt placement for NPH. The protocol involves evaluating the patient through functional improvement before and after a 36 hour (10 ml/hour) CSF removal via a lumbar catheter. Cognitive and gait behavioral testing before and after trial CSF removal, as well as reports from the patient and family, are used to evaluate clinical improvement or response. The test calculates the scores for cognitive function, gait, and patient and family opinions about improvement. The total improvement score calculation is based on following criteria: "0" indicates no change; "1" slight change; and "2" significant change. The test result gives a single improvement score from 0–6 yielded from a sum of three considerations: gait testing, neuropsychological testing, and patient/family reporting. Overall, a total improvement score of >3 indicated clinical improvement or "CSF drainage responder" status and a shunt placement is recommended.

2.5. Immunoassay for VEGF

VEGF levels were determined using a quantitative sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's instruction (Quantikine Human VEGF Immunoassay, DVE00, R&D Systems, Minneapolis, MN, USA). The sensitivity of the assay was 5 pg/ml VEGF. Two hundred microliters of each sample or standards was used per evaluation. All samples and standards were assayed in duplicate and analyzed in 96-well microtiter plates. Optical densities were determined by using an Epoch Microplate Spectrophotometer (Revision: G, BioTek Instruments, Winooski, VT, USA) at 450 nm with a correction at 570 nm. The blank was subtracted from the duplicate readings for each standard and sample. Standard curve and concentrations of the samples were calculated using Gene5 Microplate Data Collection & Analysis Software (BioTek Instruments).

2.6. Statistical analysis

Data of VEGF levels, age, and Evans' ratio were presented as mean ± standard deviation and analyzed by one way analysis of variance followed by subsequent two-tailed unpaired Student's t-tests. CSF drainage testing scores were presented as median (range) and analyzed by Mann–Whitney Rank Sum Test. $p < 0.05$ was considered as the level of significance.

3. Results

3.1. Patient demographics, exercise regimen, and CSF drainage response

The demographic characteristics of the subjects are shown in Table 1. Lumbar CSF samples were obtained from 17 patients. The mean age was 74 years (range 60–85) and 8/17 (47%) were male. No patients reported symptoms suggesting infection at the time of lumbar drainage, which was confirmed with routine CSF laboratory analysis.

Of the 17 NPH patients tested, 11 patients underwent a standard occupational therapy regimen while six patients did not

Table 1
Demographic characteristics of all patients

	Exercise	No-exercise	*p* value
Patients, n	11	6	
Age, years, mean ± SD	72.8 ± 3.2	75.4 ± 4.9	0.52
Male/female	5/6	3/3	
Evans' ratio, mean ± SD	0.38 ± 0.03	0.35 ± 0.02	0.35
Total CSF drainage testing score, median (range)	3 (0–4)	2 (1–3)	0.21

SD = standard deviation.