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Neuron specific enolase in retinal detachment*

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Abstract

Purpose. Neuron Specific Enolase (NSE) is released following central nervous system (CNS) distress. As retina is part of the CNS, NSE levels were measured in the subretinal fluid (SRF), aqueous, and serum of patients with primary rhegmatogenous retinal detachment (RD).

Methods. Radioimmunoassay was used to determine NSE levels in the SRF, aqueous, and serum of 13 patients (28–92 years old, mean = 71 years) with RD. As controls, NSE was measured in the aqueous of 6 patients undergoing cataract surgery and in serum of 18 patients without ophthalmological or neurological diseases.

Results. SRF levels of NSE ranged from 50–200 µg/l (mean ± s.d. = 150 ± 57). NSE levels in aqueous from patients with RD were 2–140 µg/l (mean ± s.d. = 39 ± 42), significantly higher than in controls (0–6 µg/l; mean ± s.d. = 1.58 ± 2.24; $p = 0.04$). Serum NSE levels in RD patients ranged from 6.5–80 µg/l (mean ± s.d. = 26 ± 21) and was significantly higher than in controls (5.3 ± 1.66 µg/l; $p = 0.005$).

Conclusions. Retinal neuron injury in retinal detachment (RD) releases sufficient Neuron Specific Enolase (NSE) to be detected in subretinal fluid, aqueous, and even in serum. Thus, NSE could index disease severity in RD and provide a means by which to assess the response to neuroprotection in RD.

Keywords: retinal detachment; ischemia; neuron specific enolase; neuroprotection; vision recovery

Introduction

Enolase is an enzyme of the glycolytic pathway which is found in numerous isomeric forms. Alpha-gamma and gamma-gamma enolases constitute the so-called neuron-specific enolases (NSE). The gamma-enolase has been shown to be located in cells of neuroectodermal origin and constitutes approximately 1.5% of the total soluble protein in the brain. Gamma-enolase NSE is known to be present in neuronal cell cytoplasm and dendrites in the human central nervous system.[1–3] Previous studies[4,5] have identified NSE in human retinal neurons.

NSE is released from injured nerve cells and, as such, is a specific marker of neuronal distress. Cerebral ischemia in rats and humans induces NSE release to the extracellular matrix[1,2] accompanied by a loss of NSE staining in the damaged neurons.[1,3,6] The appearance of NSE in cerebrospinal fluid and peripheral blood reflects a washout phenomenon[1,3,6] where the serum levels of NSE are believed to correlate with the extent of neuronal damage.[7]

As rhegmatogeneous retinal detachment (RD) causes ischemic injury to retinal nerve cells, we hypothesize that patients with RD will have a release of NSE from the retina. This study was undertaken to determine whether NSE can be detected in the subretinal fluid, aqueous, and serum of patients with RD.

Methods

IRB-approved informed consent was obtained form thirteen patients, aged 28–92 years (mean 71 years) who presented with rhegmatogeneous retinal detachment (RD). Twelve scleral buckle procedures were performed under local anesthesia and one with general anesthesia. During surgery, exter-

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nal drainage of SRF was performed prior to cryotherapy, with a radial sclerotomy and perforation of the exposed choroid. No cases that had bleeding at the drainage site were included in the study. Aqueous was aspirated by paracentesis with a 30 gauge needle. Aqueous sampling by paracentesis was also performed during cataract surgery in 6 controls. Blood was drawn during surgery from a peripheral vein and immediately centrifuged at 5000 rpm for 10 minutes with the supernatant decanted for analysis. Additionally, peripheral blood was drawn from 18 normal controls with no history or evidence of neurologic or ophthalmic disease. All specimens were frozen and stored at −70°C.

Analytic methods

NSE concentrations were determined using a commercial (Pharmacia AB, Uppsala, Sweden) double antibody radioimmunoassay. Previous studies[8] have established the sensitivity and specificity of this assay. Furthermore, during every assay run calibration is performed using standards of purified NSE. Each sample assay determination is performed in duplicate for both standards and unknowns and a standard curve is prepared for each assay run. The test sample results are derived from the curve that was established using known quantities of NSE standards. Student's *t*-test was used for statistical analysis.

Results

Preoperative clinical findings are shown in Table 1. The extent of RD ranged from 3 clock hours to total RD and the macula was involved in each case. The onset of symptoms ranged from 2 days to 6 weeks prior to surgery.

Quantitative analysis of samples

Table 1 contains the results of the NSE assays. In RD patients, NSE levels in SRF ranged from 50 to 200 µg/l with a mean = 150 ± 57, and aqueous NSE levels were 2–140 µg/l (mean = 39 ± 42). In contrast, aqueous NSE levels in control patients undergoing cataract surgery ranged from 0–6 µg/l (1.58 ± 2.24), significantly lower than in patients with RD ($p = 0.04$). Although SRF levels of NSE showed a tendency to be higher in patients with extensive retinal detachment, there was no statistical significance for this finding. There was also no correlation between NSE levels and the duration of RD.

In the serum of RD patients, NSE levels were 6.5–80 µg/l (mean = 26 ± 21) (Table 1). The serum levels of NSE in 18 controls without neurological or eye diseases was 5.3 ± 1.66 µg/l, significantly lower than serum levels in patients with RD ($p = 0.005$).

Discussion

Neuron Specific Enolase, a marker of CNS injury, is released from retinal neurons in patients with RD. In these patients, as compared to controls, NSE is found in relatively high levels in SRF, aqueous, and serum.

Subretinal fluid only exists in patients with RD. However, as the subretinal space is embryologically in continuity with the subarachnoid space, NSE levels in normal cerebrospinal fluid (CSF) could serve as a basis of comparison for subretinal fluid NSE levels. As reported by Yanfeng *et al.*,[9] NSE levels (determined using the same assay employed in this study) in the CSF of adults without neurological disease were 1.92 ± 1.92 µg/l. In the SRF of patients with RD, NSE levels were

Table 1. Patient data and NSE assay results.

Patient	age	sex	Duration of RD in days (*)	Extent of RD (§)	Capsule intact (+) or open (−)	SRF (µg/ml)	Aqueous (µg/ml)	Serum (µg/ml)
1	63	m	42	0.4	+	120	24	57
2	74	f	3	0.3	−	200	33	80
3	82	f	6	0.6	+	200	36	32
4	74	m	14	0.33	+	50	24	20
5	74	m	28	0.5	+	93	15	18
6	68	m	21	0.4	+	70	18	24
7	28	m	3	0.6	+	98	17	20
8	71	f	3	0.25	+	145	13	7
9	67	m	3	0.66	−	200	2	29
10	74	f	6	0.42	+	200	40	10
11	76	m	4	0.5	+	200	120	6.5
12	92	f	42	1	+	170	140	15.5
13	73	m	14	1	+	200	25	14

m = male; f = female; RD = retinal detachment; SRF = subretinal fluid.
* as best could be determined from the patient history.
§ expressed as ratio of clock-hours of RD/12.

significantly higher, ranging from 50 to 200 µg/l with a mean of 150 ± 57 µg/l. These levels were comparable to those in the CSF of asphyxiated full term infants with stage 3 hypoxic ischemic encephalopathy (149 ± 48 µg/l).[10] These findings suggest that RD causes significant release of NSE into the surrounding milieu, as well as systemically. The finding that NSE levels in aqueous of patients with RD are significantly elevated above those levels in patients undergoing cataract surgery suggests that once released from detached retina, NSE readily diffuses forward into the anterior chamber. That aqueous NSE levels do not correllate with serum NSE levels (Fig. 1) suggests that aqueous NSE does not derive from serum. Rather, it is more likely that aqueous NSE derives directly from detached retina by diffusion through vitreous to the anterior segment and that serum NSE derives in part from aqueous outflow, and in part from uptake posteriorly, across the damaged blood-retinal barrier present in RD.

Serum NSE levels are generally low in subjects without CNS disease or tumors containing neural elements.[2,3] This was confirmed in our control group, where serum NSE levels were 5.3 ± 1.66 µg/l. Yet, the average NSE level in the serum of patients with RD was 26 ± 21 µg/l, which is significantly higher than in individuals without CNS disease[6,9] and also higher than the levels found in the control subjects in this study ($p = 0.005$). Indeed, the serum concentrations of NSE in patients with RD (6.5–80 µg/l) were as high as serum NSE levels in patients with cerebral hypoxia or intracerebral hemorrhage,[3] as well as other acute neurological diseases.[6,11,12] Considering that RD is associated with a breakdown in the blood-retinal barrier,[13] the access of NSE into the systemic circulation is perhaps predictable, although the high levels of serum NSE detected in this study are somewhat surprising.

The time course of NSE release from detached retina is difficult to evaluate solely on the basis of this study. It is known[12] that within 4 hours after a single session of electroconvulsive therapy there are increased serum levels of NSE with a peak of 65 µg/l after 12 hours. These serum NSE levels return to normal (below 15 µg/l) after 50 hours. Histologic studies[14,15] of retinal cell changes in RD found damage to the photoreceptors early in the course of RD, with the other layers of the retina being affected later in the course. Measurements of retinal S-antigen in human subretinal fluid found increasing levels during the first 2 weeks of RD that attained a steady-state thereafter.[16] In our study of NSE in patients with RD there was no correlation of NSE levels in any compartment (SRF, aqueous, serum) with the duration of RD. This is probably due to our relatively small study population and the likelihood that defining the duration of RD solely on the patient's subjective experience is unreliable.

In summary, this study demonstrates that NSE is released from retinal neurons into the subretinal space, aqueous, and serum of patients with rhegmatogeneous retinal detachment. Since NSE correlates with neuronal distress and possibly survival as well,[17] the findings in subretinal fluid suggest that the study of NSE in RD may provide better understanding of the pathophysiologic effects of RD on vision. Measuring NSE levels in SRF and/or aqueous may also prove useful as an outcome measure of the response to therapy with agents intended to mitigate against ischemia-reperfusion injury and

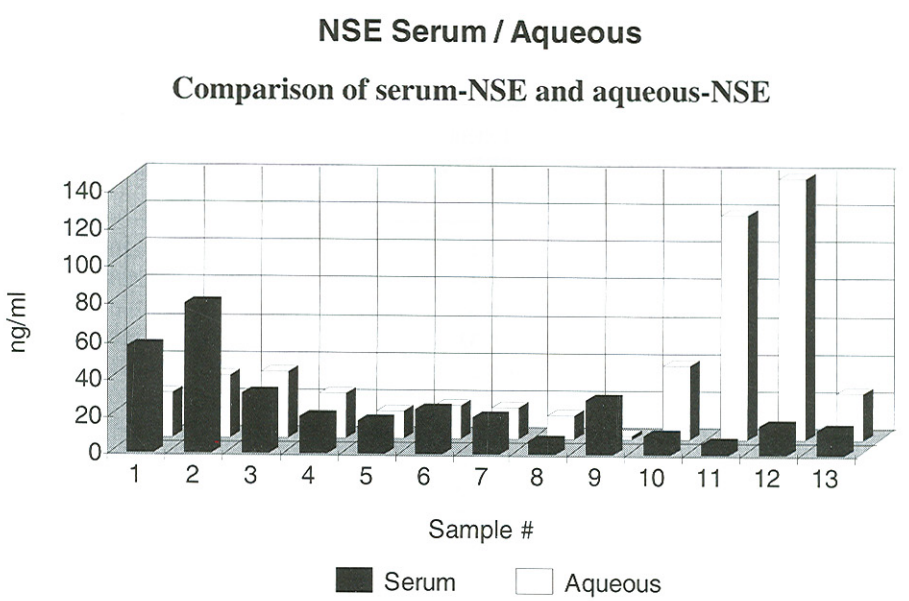

Figure 1. The bar graph of NSE values in serum (black columns) are paired with NSE values in aqueous (white columns) by patient. The results show that NSE levels in the serum are not correlated with NSE levels in aqueous, suggesting that the anterior chamber is not the primary route by which NSE gains access to the systemic circulation. (Sample numbers 1 to 13 on the abscissa correspond to patient numbers 1 to 13 in Table 1.)

to increase neuronal survival. This will likely improve post-operative vision recovery in longstanding RD and cases involving the macula. Lastly, the findings in serum suggest that this assay could provide a systemic marker of retinal detachment, perhaps useful for screening or other population-based studies.

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References

1. Horn M, Seger F, Schlote W. Neuron-specific enolase in gerbil brain and serum after transient cerebral ischemia. *Stroke*. 1995;26:290–297.
2. Barone FC, Clark RK, Price WJ, White RF, Feuerstein G, Storer BL, Ohlstein EH. Neuron-specific enolase increases in cerebral and systemic circulation following focal ischemia. *Brain Res*. 1993;623:77–82.
3. Schaarschmidt H, Prange HW, Reiber H. Neuron-specific enolase concentrations in blood as a prognostic parameter in cerebrovascular disease. *Stroke*. 1994;25:558–565.
4. Chevez P, Font RL. Practical applications of some antibodies labelling the human retina. *Histol Histopathol*. 1993;8:437–442.
5. Dunker S, Sebag J, Sadun AA, DeGiorgio CM. Neuron-specific enolase in the human retina. *Invest Ophthalmol Vis Sci*. 1997;38:S319.
6. Cunningham RT, Morrow Jl, Johnston CF, Buchanan KD. Serum neurone-specific enolase concentrations in patients with neurological disorders. *Clinica Chimica Acta*. 1994; 230:117–124.
7. Rabinowicz AL, Correale J, Boutros RB, Couldwell WT, Henderson CW, DeGiorgio CM. Neuron-specific enolase is increased after single seizures during inpatient video/EEG monitoring. *Epilepsia*. 1996;37:122–125.
8. Pahlman S, Esscher T, Bergvall P, Odelstad L. Purification and characterization of human neuron-specific enolase: Radioimmunoassay development. *Tumour Biol*. 1984;5(2): 127–139.
9. Yanfeng L, Xinde W, Zhenhua Y. Neuron-specific enolase in patients with acute ischemic stroke and related dementia. *Chin Med J* 1995;108:221–223.
10. Garcia-Alix A, Cabanas F, Pellicer A, Hernanz A, Stiris TA, Quero J. Neuron-specific enolase and myelin basic protein: Relationship of cerebrospinal fluid concentrations to the neurologic condition of asphyxiated full-term children. *Pediatrics*. 1994;93:234–240.
11. Cunningham MW, Winder J, McKinstry S, Lawson JT, Johnston CF, Hawkins SA, Buchanan KD. Serum neuron-specific enolase as an indicator of stroke volume. *Eur J Clin Invest*. 1996;26:298–303.
12. Greffe J, Lemoine P, Lacroix C, Brunon A-M, Terra J-L, Dalery J. Increased serum levels of neuron-specific enolase in epileptic patients and after electroconvulsive therapy – A preliminary report. *Clinica Chimica Acta*. 1996;244: 199–208.
13. Cantrill HL, Pederson JE. Experimental retinal detachment. VI. The permeability of the blood-retinal barrier. *Arch Ophthalmol*. 1984;102(5):747–751.
14. Erickson PA, Fisher SK, Anderson DH, Stern WH, Borgula DA. Retinal detachment in the cat: The outer nuclear and outer plexiform layers. *Invest Ophthalmol Vis Sci*. 1983;24:927–942.
15. Fisher SK, Anderson DH. Cellular effects of detachment of the neural retina and the retinal pigment epithelium. In: Ryan, SJ, ed. *Retina*, Vol. 3 (ed: Glaser BM): 1994;2035–2062.
16. Sebag J, Tuyen W, Faure JP, Chauvaud D, Pouliquent Y. Retinal S-Antigen in human subretinal fluid. *Invest Ophthalmol Vis Sci*. 1987;28:2038–2041.
17. Li A, Lane WS, Johnson LV, Chader GJ, Tombran-Tink J. Neuron-specific enolase: A neuronal survival factor in the retinal extracellular matrix? *J Neurosci*. 1995;15:385–393.