ELSEVIER

Clinical Significance of Serum Antibody Against Neuron-Specific Enolase in Glaucoma Patients

Yoko Ikeda,* Ikuyo Maruyama,[†,‡]
Mitsuru Nakazawa[†] and Hiroshi Ohguro[†]

**Department of Ophthalmology, Kyoto Prefectural University School of Medicine, Kyoto, Japan; [†]Department of Ophthalmology, Hirosaki University School of Medicine, Hirosaki, Japan; [‡]Department of Ophthalmology, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan*

Purpose: In a recent study, we found the presence of serum autoantibody against neuron-specific enolase (NSE) in glaucoma patients. The purpose of the present paper is to investigate further the clinical significance of the presence of the serum antibody against NSE in glaucoma patients.

Methods: Serum autoantibody against NSE was examined by Western blot analysis in 143 patients with glaucoma (normal tension glaucoma [NTG], 45 cases; primary open angle glaucoma [POAG] 98 cases). Clinical characteristics including visual acuity, visual field, intraocular pressure (IOP), and optic disc features were compared between the serum autoantibody-positive and the serum autoantibody-negative patients.

Results: Maximum IOP in the serum anti-NSE antibody-positive patients was significantly lower than that in the negative patients ($P < .05$). However, no statistical differences were observed in visual field loss, disc cupping, or other clinical factors. During the clinical course, rates of the presence of anti-NSE antibody were significantly higher in the early stages of POAG ($P < .0001$) with visual field deterioration than without it. Although it was not statistically significant, the positive rates of serum anti-NSE antibody were relatively higher in the later stages of POAG and NTG with visual field deterioration than without it.

Conclusion: The present observations suggest that the presence of serum autoantibody against NSE may be clinically useful for predicting the progression of visual field loss in POAG patients. **Jpn J Ophthalmol 2002;46:13–17** © 2002 Japanese Ophthalmological Society

Key Words: Apoptosis, autoimmune response, ganglion cell, glaucoma.

Introduction

Glaucomatous optic neuropathy is characterized by loss of retinal ganglion cells and their axons, excavated appearance of the optic nerve head, and progressive loss of visual field sensitivities.[1] In terms of its pathology, apoptotic cell death of retinal ganglion cells is known to be involved, based upon postmortem studies of human eyes with primary open-angle glaucoma (POAG)[2] or neovascular glaucoma,[3] and experimental glaucoma models with elevated intraocular pressure (IOP).[4,5] As the molecular mechanism triggering the apoptosis, deprivation of neurotrophic factors,[4] ischemia,[6] chronic elevation of glutamate,[7] and disorganized nitric oxide (NO) metabolism[8] have been implicated. In addition it was reported that autoimmune responses toward rhodopsin,[9] 60-kDa heat shock protein (hsp 60),[10] 27-kDa heat shock protein (hsp 27), and α-crystallin[11] may be related to the apoptotic cell death process in some glaucoma patients, particularly in those patients with normal tension glaucoma (NTG). Recently, we have found that approximately 20% of POAG patients possessed se-

Received: October 10, 2000
Correspondence and reprint requests to: Hiroshi OHGURO, MD, Department of Ophthalmology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan

Published by Elsevier Science Inc.
0021-5155/02/$–see front matter
PII S0021-5155(01)00455-5

Jpn J Ophthalmol
Vol 46: 13–17, 2002

rum antibody against neuron-specific enolase (NSE), and that maximum IOP levels in POAG patients with anti-NSE antibody were statistically lower than in POAG patients without the antibody. However, other clinical factors, including visual field defects, medication, and optic nerve cupping, were comparable between the antibody-positive and -negative POAG patients.[12] In addition, injection of the patient's serum into the vitreous cavity of a Lewis rat caused reduction in the b-wave in electroretinography (ERG) and TdT-dUTP terminal nick-end labeling (TUNEL)-positive staining within the retinal ganglion cells, and these effects were comparable to those caused by the excitotoxicity induced by N-methyl-D-aspartate (NMDA). Therefore, based upon these findings we suggested that serum anti-NSE antibody may be one of the clinical factors responsible for deteriorating glaucomatous optic neuropathy. A rat model intravitreously injected with the anti-NSE antibody has proved to be useful for understanding the molecular pathology of glaucoma and the evaluation of several anti-glaucoma drugs.

Here, to study further the clinical significance of the presence of the anti-NSE antibody in glaucoma patients, we have performed a clinical characterization of a greater number of glaucoma patients.

Materials and Methods

The studies were performed in accordance with the guidelines set by Hirosaki University and the Declaration of Helsinki on Biomedical Research Involving Human Subjects. The protocols were approved by the institution's Committee for the Protection of Human Subjects.

Patients

One hundred and forty-three patients with glaucoma (NTG, 45 cases; POAG, 98 cases) from Sapporo Medical University were included in the present study after obtaining fully informed consent. The diagnostic criteria for NTG were as follows: (1) the presence of normal open iridocorneal angles, (2) no evidence of IOP higher than 21 mm Hg, (3) glaucomatous changes in visual field and optic nerve cupping, and (4) the absence of alternative causes of optic neuropathy. For the diagnosis of POAG, the criteria were identical to those of NTG, except that IOP had to be higher than 21 mm Hg. The IOP was measured using a Goldmann applanation tonometer. The visual field was examined at least twice a year by a Goldmann perimeter and/or a Humphrey visual field analyzer (Humphrey-Zeiss, San Francisco, CA, USA) using the central 30-2 program. Peripheral venous blood samples were immediately subjected to serum separation and stored at −80°C before use. The follow-up period was 63.5 ± 51.3 weeks for POAG and 51.0 ± 44.8 weeks for NTG.

Criteria for Progression of Glaucomatous Visual Field Loss

To study the relationship between presence of the serum anti-NSE antibody and glaucoma progression, clinical data of the right eyes were used. Glaucomatous visual field loss was considered to have progressed if one or more of the following occurred; (1) widening by 5° or more of nasal step or other peripheral defect found by kinetic perimetry; (2) the appearance of a new scotomatous defect; and (3) a change in a scotoma from relative to absolute.[13–15] To evaluate the presence of progression of glaucomatous visual field loss, all patients who met the following criteria were included: (1) visual fields show well-documented progression of defects at least two times; (2) maintenance of adequate visual acuity for reliable plotting of the field; (3) no other ocular abnormality exists that would affect the visual field; and (4) at least 12 months of visual field follow-up.

Western Blot Analysis

Western blot analysis was carried out as described previously.[16] For isolation of bovine retinal soluble protein fraction, 10 frozen bovine retinas were homogenized in 10 mM hepes buffer (pH 7.5) containing 100 mM NaCl, 1 mM benzamidine, and 0.1 mM leupeptine and centrifuged at 50,000 *g* for 1 hour. The supernatant containing approximately 0.1 mg protein was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis using a 12.5% polyacrylamide gel. Separated proteins in a gel were electrotransferred to polyvinylidene difluoride membranes in 10 mM bis-tris propane buffer (pH 8.4) and 10% methanol solution. After blocking nonspecific binding by 2% skim milk in phosphate buffered saline, the membrane was probed successively with diluted patient serum and horseradish peroxidase-labeled anti-human IgG (Funakoshi, Tokyo). Specific antigen/antibody binding was visualized by a chemiluminescence system (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Statistical Analysis

The clinical data including age, maximum and mean IOPs, and disc cupping, and the experimental data of ERG amplitudes are shown as mean ± SD.

Significant differences between groups were found using the Mann-Whitney test with a significance level of less than $P < .05$. Positive rates of progression of visual field losses between anti-NSE antibody-positive and -negative groups were statistically analyzed by the chi-square test with a significance level of less than $P < .05$.

Results

As shown in Table 1, serum antibody against NSE was recognized in 20% of the glaucoma patients. As we reported previously[12] using a small number of patients, (1) in POAG, maximum and mean IOP levels were statistically and relatively lower in the antibody-positive patients than in the -negative patients; and (2) other clinical factors including, visual field defects, medication, and optic nerve cupping, were almost identical between the two groups of POAG and NTG patients. These findings were once again observed in the present study using a large number of patients.

To study the relationship between presence of the serum anti-NSE antibody and glaucoma stages, the antibody-positive rates of patients were compared with their stages of glaucomatous visual field loss. As shown in Table 2, in POAG, the positive rates of patients varied among their Aulhorn-Greve stages; in NTG, the positive rates decreased with advancing stage; and in neither the POAG nor the NTG patients was a systematic pattern of increase observed with advancing stage.

To elucidate the relationship between glaucoma progression and the presence of the antibody, rates of visual field deterioration were compared between the antibody-positive and -negative groups. In terms of the presence of visual field deterioration, the previously described criteria (1)–(3) in the Materials and Methods section were used, and 78 of 98 patients with POAG and 37 of 45 patients with NTG met those criteria. Twenty-five patients (POAG, 15 cases and NTG, 10 cases) were judged by static perimetry and the others by both static and kinetic pe-

Table 1. Clinical Characteristics of Anti-NSE Antibody-Positive and -Negative Glaucoma Patients*

	POAG (n = 98, 182 eyes)		NTG (n = 45, 90 eyes)	
	Anti-NSE (+) (n = 22, 40 eyes)	Anti-NSE (−) (n = 77, 142 eyes)	Anti-NSE (+) (n = 10, 20 eyes)	Anti-NSE (−) (n = 35, 70 eyes)
Sex				
Male	10	42	2	17
Female	12	35	8	18
Age				
Mean ± SD	64.6 ± 9.8	62.3 ± 13.0	66.7 ± 12.0	62.3 ± 11.8
Maximum IOP (mm Hg)				
Mean ± SD	22.5 ±2.3†	24.9 ± 4.8†	17.7 ± 1.59	17.5 ± 1.8
Mean IOP (mm Hg)				
Mean ± SD	17.2 ± 4.1	18.4 ± 3.3	14.4 ± 1.0	14.5 ± 1.5
Visual field loss				
Nasal step	14 (35.0%)	42 (29.6%)	10 (50.0%)	47 (67.1%)
Paracentral scotoma	6 (15.0%)	36 (25.4%)	10 (50.0%)	27 (38.6%)
Arcuate scotoma	9 (22.5%)	27 (19.0%)	3 (15.0%)	20 (28.6%)
Central island	1 (2.5%)	5 (3.5%)	0	1 (1.4%)
Temporal island	0	3 (2.1%)	0	1 (1.4%)
Eyedrops used				
β-blocker	20 (50.0%)	100 (70.4%)	9 (45.0%)	29 (41.1%)
Myopic	9 (22.5%)	32 (22.5%)	0	3 (4.3%)
PG derivative	13 (32.5%)	81 (57.0%)	9 (45.0%)	41 (58.6%)
Adrenergic	8 (20.0%)	20 (14.1%)	1 (5.0%)	6 (8.6%)
CA inhibitor	1 (2.5%)	3 (2.1%)	0	0
Disc cupping				
Mean ± SD	0.70 ± 0.22	0.75 ± 0.19	0.74 ± 0.17	0.78 ± 0.18
Operation				
Trabeculectomy	7 (17.5%)	24 (16.9%)	1 (5.0%)	2 (2.9%)

*NSE: neuron-specific enolase, POAG: primary open-angle glaucoma, NTG: normal tension glaucoma, IOP: intraocular pressure, PG: prostaglandin, CA: carbonic anhydrase. Male and female numbers represent persons, but numbers of visual field loss, eye drops and operation represent numbers of eyes. POAG patients with no episode of IOP higher than 21 mm Hg were excluded from present study.

†$P < .05$ (Mann-Whitney test).

Jpn J Ophthalmol
Vol 46: 13–17, 2002

Table 2. Positive Rates of Serum Anti-NSE Antibody in Glaucoma Patients at Different Aulhorn-Greve Stages of Visual Field Defects

	Aulhorn-Greve Stages						
POAG (n = 98, 182 eyes)	0	1	2	3	4	5	6
Positive rates of anti-NSE antibody (%)	0/6 (0.0)	13/72 (18.1)	15/37 (40.5)	3/21 (14.3)	1/13 (7.7)	6/23 (26.1)	2/10 (20.0)
NTG (n = 45, 90 eyes)							
Positive rates of anti-NSE antibody (%)	2/6 (33.3)	8/30 (26.7)	6/26 (23.1)	2/10 (20.0)	1/7 (14.3)	1/8 (12.5)	0/3 (0.0)

NSE: neuron-specific enolase, POAG: primary open-angle glaucoma.

rimetry. As shown in Table 3, rates of the presence of anti-NSE antibody were significantly higher in the early stages of POAG (Aulhorn-Greve stages 0–2) with visual field deterioration than without it. It was not statistically significant, but in the later stages of POAG (Aulhorn-Greves stages 3–6) and NTG the rates of the presence of anti-NSE antibody were higher with visual field deterioration than without it.

Discussion

Elevation of IOP is known to be a major causative factor in the progression of glaucomatous visual field losses.[17] However, several clinical investigations have revealed that approximately 20–30% of POAG patients showed progressive deterioration of visual field defects even though the IOP levels were controlled at the lower levels.[18] Therefore, this observation suggests that in addition to elevated IOP, some unknown mechanisms must be present causing glaucoma progression. In our previous study, we found that serum autoantibody toward NSE was recognized in approximately 20% of glaucoma patients, and maximum and mean IOP levels were significantly and relatively lower, respectively, in the anti-NSE antibody-positive POAG patients than in those without the antibody.[12] In addition, we also found that intravitreal injection of purified anti-NSE antibody induced experimental retinal ganglion cell apoptosis, which was similar to that caused by NMDA administration.[12] Therefore, we suggested that circulating anti-NSE antibody within the blood flow reached the retina and possibly caused retinal ganglion cell damage. If our speculation is correct, autoimmune reaction toward NSE may be one of the factors causing glaucoma progression besides elevated IOP. In fact, in our present study, we found that in the antibody-positive patients the rates of visual field deterioration were significantly higher in the early stages of POAG than in the antibody-negative patients, and were relatively higher in the late stages of POAG and NTG. Therefore, we suggest that the anti-NSE antibody may be one of the useful factors for predicting glaucoma progression.

Table 3. Relationship Between Presence of Anti-NSE Antibody and Visual Field Deterioration in Glaucoma Patients*

	Positive Rates of Anti-NSE Antibody (%)	
POAG (n = 78, 78 eyes)	Aulhorn-Greve stages 0–2	Aulhorn-Greve stages 3–6
VF deterioration (+)	10/16 (62.5%)†	3/20 (15.0%)
VF deterioration (−)	1/31 (3.2%)†	1/11 (9.1%)
NTG (n = 37, 37 eyes)		
VF deterioration (+)	4/9 (44.4%)	1/5 (20.0%)
VF deterioration (−)	3/15 (20.0%)	0/8 (0%)

*Criteria for visual field deterioration are described in Materials and Methods section. All numbers represent numbers of persons. NSE: neuron-specific enolase, POAG: primary open-angle glaucoma, VF: visual field, NTG: normal tension glaucoma.

†$P < .0001$ (chi-square test)

Several reports have been published on the autoimmune factors in glaucoma. Cartwright et al[19] reported the coexistence of immune-related diseases in NTG cases. Wax et al[20] reported an increased incidence of paraproteinemia and antibodies to extractable nuclear antigens and several components of retina in NTG patients. Clinically, a prevalence of NTG and POAG in patients with collagen diseases has been reported.[21] Among the retinal autoantigens recognized by serum from glaucoma patients, several antigens, including rhodopsin,[9] heat shock proteins,[10,11] and NSE[12] have been found so far. We do not know why more than one antigen is present as autoantigen in glaucoma patients. As a possibility, it was considered that such autoimmune reactions may be secondarily associated with the destruction of retinal ganglion cells. In the present study, although such a possibility was not completely ruled out, we could not find any correlation between the positivity of the anti-NSE antibody and the glaucoma stage by the Aulhorn-Greve classification. However, even though the anti-NSE antibody is produced second-

ary to the retinal damage in glaucoma, our present observation that the positivity of the serum anti-NSE antibody correlated with the POAG progression suggests that the presence of serum anti-NSE antibody may be useful in monitoring the disease progression of POAG.

References

1. Shields MB, Ritch R, Krupin T. In: Ritch R, Shields MB, Krupin T, eds. The glaucomas. St. Louis: Mosby, 1996:717–25.
2. Kerrigan LA, Zack DJ, Quigley HA, Smith SD, Pease ME. TUNEL-positive ganglion cells in human primary open-angle glaucoma. Arch Ophthalmol 1997;115:1031–5.
3. Okisaka S, Murakami A, Mizukawa A, Ito J. Apoptosis in retinal ganglion cell decrease in human glaucomatous eyes. Jpn J Ophthalmol 1997;41:84–8.
4. Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest Ophthalmol Vis Sci 1995;36:774–86.
5. Garcia-Valenzuela E, Shareef S, Walsh J, Sharma SC. Programmed cell death of retinal ganglion cells during experimental glaucoma. Exp Eye Res 1995;61:33–44.
6. Levin LA, Louhab A. Apoptosis of retinal ganglion cells in anterior ischemic optic neuropathy. Arch Ophthalmol 1996;114:488–91.
7. Dreyer EB, Zurakowski D, Schumer RA, Podos SM, Lipton SA. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch Ophthalmol 1996;114:299–305.
8. Neufeld AH, Hernandes R, Gonzalez M. Nitric oxide synthase in the human glaucomatous optic nerve head. Arch Ophthalmol 1997;115:497–503.
9. Romano C, Barrett DA, Li Z, Pestronk A, Wax MB. Anti-rhodopsin antibodies in sera from patients with normal-pressure glaucoma. Invest Ophthalmol Vis Sci 1995;36:1968–75.
10. Wax MB, Tezel G, Saito I, et al. Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma. Am J Ophthalmol 1998;125:145–57.
11. Tezel G, Seigel GM, Wax MB. Autoantibodies to small heat shock proteins in glaucoma. Invest Ophthalmol Vis Sci 1998;39:2277–87.
12. Maruyama I, Ohguro H, Ikeda Y. Retinal ganglion cells recognized by serum autoantibody against γ-enolase found in glaucoma patients. Invest Ophthalmol Vis Sci 2000;41:1657–65.
13. Werner EB, Drance SM, Schulzer M. Trabeculectomy and the progression of glaucomatous visual field loss. Arch Ophthalmol 1977;95:1374–7.
14. Kidd MN, O'Connor M. Progression of field loss after trabeculectomy: a five-year follow-up. Br J Ophthalmol 1985; 69:827–31.
15. Shirakashi M, Iwata K, Sawaguchi S, Abe H, Nanba K. Intraocular pressure-dependent progression of visual field loss in advanced primary open-angle glaucoma: a 15-year follow-up. Ophthalmologica 1993;207:1–5.
16. Ohguro H, Ogawa K, Nakagawa T. Recoverin and hsc 70 are found as autoantigens in patients with cancer-associated retinopathy. Invest Ophthalmol Vis Sci 1999;40:82–9.
17. Quigley HA, Maumenee E. Long-term follow-up of treated open-angle glaucoma. Am J Ophthalmol 1979;87:519–25.
18. Katz J, Gilbert BA, Quigley HA, Sommer A. Estimating progression of visual field loss in glaucoma. Ophthalmology 1997;104:1017–25.
19. Cartwright MJ, Grajewski AL, Friedberg ML, Anderson DR, Richards DW. Immune related disease and normal-tension glaucoma. A case-control study. Arch Ophthalmol 1992; 110:500–42.
20. Wax MB, Barrett DA, Pestronk A. Increased incidence of paraproteinemia and autoantibodies in patients with normal-pressure glaucoma. Am J Ophthalmol 1994;117:561–8.
21. Yamamoto T, Maeda M, Sawada A, et al. Prevalence of normal-tension glaucoma and primary open-angle glaucoma in patients with collagen diseases. Jpn J Ophthalmol 1999;43:539–42.