Brain-derived Neurotrophic Factor in the Aqueous Humor of Glaucoma Patients

Tsutomu Igarashi¹, Kenji Nakamoto¹, Maika Kobayashi¹, Hisaharu Suzuki¹, Takeshi Arima¹, Yutaro Tobita¹, Kazuhiro Takao¹, Toru Igarashi², Takahisa Okuda¹, Takashi Okada⁴ and Hiroshi Takahashi¹

¹Department of Ophthalmology, Nippon Medical School, Tokyo, Japan  
²Department of Pediatrics, Nippon Medical School, Tokyo, Japan  
³Division of Legal Medicine, Department of Social Medicine, Nihon University School of Medicine, Tokyo, Japan  
⁴Division of Molecular and Medical Genetics, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Background: Brain-derived neurotrophic factor (BDNF) may be involved in the pathogenesis of glaucoma. BDNF concentrations reported in previous studies have varied widely, and the concentration of BDNF in aqueous humor is unknown. In this study, BDNF concentrations in the aqueous humor of glaucoma patients and control patients were measured with ELISA kits.

Methods: This prospective, observational study examined BDNF levels in aqueous humor in 62 eyes of 43 patients who underwent cataract surgery or trabeculectomy (11 glaucoma patients and 32 non-glaucoma cataract patients as controls). BDNF concentrations were examined by 4 different enzyme-linked immunosorbent assay (ELISA) techniques.

Results: The mean ± SD patient age was 72.0 ± 10.1 (range 35 to 87) years. Two of the techniques detected no BDNF in aqueous humor in any samples (n=3 and n=9, respectively); the average value was less than zero. An ultrasensitive ELISA kit did not yield reliable measurements. Finally, in an even more sensitive ELISA (Simoa-HD1), performed by an outside contractor, 25 (54.3%) eyes were below the detection limit, including 20 (55.6%) control and 5 (50%) glaucoma cases. For eyes with detectable BDNF, the overall BDNF concentration was 0.158 pg/mL (n=21): 0.196 pg/mL (n=16) in controls and 0.034 pg/mL (n=5) in glaucoma cases.

Conclusions: BDNF level in aqueous humor varies widely. (J Nippon Med Sch 2021; 88: 128–132)

Key words: brain-derived neurotrophic factor, BDNF, glaucoma, aqueous humor, anterior chamber

Introduction

Glaucoma is a group of eye diseases that result in damage to the optic nerve, with progressive degeneration of retinal ganglion cells (RGCs)¹. Risk factors for glaucoma include high intraocular pressure (IOP), and standard treatment focuses on reduction of IOP by medication or surgery. However, a subset of glaucoma, known as normotensive glaucoma (NTG), does not respond to such treatment². Therefore, factors other than IOP may be involved in the pathogenesis of the disease³, and depletion of brain-derived neurotrophic factor (BDNF) is a candidate factor.

BDNF is a member of the neurotrophin family of growth factors, which are a critical component for building and preserving neurons⁴. BDNF expression was investigated in glaucoma models⁵⁷. BDNF is transported retrogradely from the superior colliculus to the RGCs in the optic nerve, and this flow is inhibited by acute IOP elevation¹. Blockade of axonal transport may cause deficits in BDNF and thus RGC death in glaucoma⁵⁷. BDNF is vital in maintaining RGCs and had a potent protective effect in various models of experimental glaucoma⁸¹².

Correspondence to Tsutomu Igarashi, MD, PhD, Department of Ophthalmology, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan  
E-mail: tutomu@nms.ac.jp  
https://doi.org/10.1272/jnms.JNMS.2021_88-305  
Journal Website (https://www.nms.ac.jp/sh/jnms/)
Clinically, serum BDNF concentrations were lower in patients with primary open angle glaucoma and NTG than in normal patients\(^{13,18}\).

Three studies of BDNF in aqueous humor used enzyme-linked immunosorbent assays (ELISAs). Zhang et al. reported BDNF levels of 0.84 pg/mL in glaucoma patients and 0.89 pg/mL in controls (p=0.11)\(^9\), and Shpak et al. reported levels of 35.2 pg/mL in glaucoma patients and 54.6 pg/mL in controls (p<0.001)\(^{17}\). However, Uzel reported levels of 9.36 ng/mL in glaucoma patients and 12.05 ng/mL in controls (p=0.011)\(^{18}\), approximately 10,000 times higher than those reported by Zhang et al. and Shpak et al. Measured values vary widely, and the concentration of BDNF in aqueous humor is unknown. In this study, BDNF concentrations in the aqueous humor of glaucoma patients and control patients were measured with various ELISA kits.

**Materials and Methods**

**Study Population**

This prospective, observational study adhered to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the Nippon Medical School Hospital (approval number: 227026). This study was registered in the Japanese UMIN Clinical Trials Registry (clinical trial identifier: UMIN000021304) before patient enrollment. Written informed consent was obtained from all participants before any clinical evaluations were performed.

From September 2016 to December 2019, BDNF levels in aqueous humor were examined in 62 eyes of 43 patients who underwent cataract surgery or trabeculectomy (11 glaucoma patients and 32 non-glaucoma cataract patients as controls) in the Department of Ophthalmology of Nippon Medical School in Tokyo. Eight patients were excluded in accordance with the exclusion criteria. The mean ± SD patient age was 72.0 ± 10.1 years (range 35 to 87 years). Glaucoma was diagnosed by evaluating IOP, gonioscopy, optic nerve head change, and presence of a visual field defect (Humphrey Field Analyzer, Zeiss, Oberkochen, Germany).

Exclusion criteria included severe ophthalmic disease, such as corneal dystrophy, degenerative retinal disease, and uveitis, and any ophthalmic surgery within 3 months. Moreover, patients with diseases that could affect BDNF concentration, such as depression, epilepsy, and Alzheimer disease, were excluded.

**Preparation of BDNF from the Anterior Chamber**

Anterior aqueous humor (100 μL or more) was aspirated from the anterior chamber. Samples were frozen on dry ice within 1 minute and stored at −80°C until use in experiments 1 and 2. In experiments 3 and 4, after 100 μL of the sample was dispensed, and 33.3 μL of the protein-stabilizing cocktail solution (#89806, Thermo Fisher Scientific K.K., Tokyo, Japan) was added, samples were immediately frozen on dry ice and stored at −80°C.

**Experiment 1**

BDNF levels were measured with a human BDNF Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), in accordance with the manufacturer’s protocol (n=3). The standard range is 62.5 to 4,000 pg/mL, and sensitivity is 20 pg/mL. If the sample was sufficient, two replicates were used.

**Experiment 2**

BDNF levels were measured with an ultrasensitive human BDNF ELISA kit (Aviscera Bioscience, Inc., Santa Clara, CA, USA) in accordance with the manufacturer’s protocol (n=9). The standard range is 11 to 750 pg/mL, and sensitivity is 3 pg/mL. If the sample was sufficient, two replicates were used.

**Experiment 3**

After the protein-stabilizing cocktail solution was added and stored within 1 minute, BDNF levels were measured with an ultrasensitive human BDNF ELISA kit (Aviscera Bioscience) in accordance with the manufacturer’s protocol (n=5). If the sample was sufficient, two replicates were used.

**Experiment 4**

After the protein-stabilizing cocktail solution was added and stored within 1 minute, the samples were sent to SCRUM Inc (Tokyo, Japan), a company specializing in life sciences. The company measured BDNF levels with an in-house digital ELISA on the Simoa-HD1 Platform (Quanterix, Lexington, MA, USA), which measures low concentrations with precision\(^{20}\). The lower limit of detection (LLoD) is 0.008 pg/mL, and the lower limit of quantitation is 0.027 pg/mL. If the sample was sufficient, two replicates were used (n=41).

**Statistical Analysis**

The mean values of measurements were calculated for each group, and comparisons between groups were made with the unpaired t-test (Excel; Microsoft, Tokyo, Japan). A p value of <0.05 was considered significant.

**Results**

**Experiment 1**

BDNF could not be detected in aqueous humor in any of the samples (n=3). The average value was less than...
Figure 1 BDNF concentrations in aqueous humor as determined with an ultrasensitive human BDNF ELISA kit (Aviscera Bioscience) and protein-stabilizing cocktail solution. (A) BDNF in control was 1.13 pg/mL (n=3) and BDNF in glaucoma was 0.69 pg/mL. The standard range of this ultrasensitive human BDNF ELISA kit (Aviscera Bioscience) is 11 to 750 pg/mL, and sensitivity is 3 pg/mL. (B) Each value was determined after drawing a linear approximation curve.

Figure 2 BDNF concentrations in aqueous humor as determined with an in-house digital ELISA on the Simoa-HD1 Platform and protein-stabilizing cocktail solution. (A) The number of eyes below the detection limit was 25 (54.3%): 20 (55.6%) in the controls and 5 (50%) in glaucoma patients. (B) In examined eyes with detectable BDNF, mean BDNF was 0.158 pg/mL overall, 0.196 pg/mL in controls, and 0.034 pg/mL in glaucoma patients.

Experiment 2

BDNF could not be detected in aqueous humor in any of the samples (n=9). The average value was less than zero.

Experiment 3

The average BDNF concentration in aqueous humor was 0.95 pg/mL (n=5). BDNF concentration was 1.13 pg/mL (n=3) in controls and 0.69 pg/mL (n=2; Fig. 1A) in glaucoma cases. The standard range of this ultrasensitive human BDNF ELISA kit (Aviscera Bioscience) is 11 to 750 pg/mL, and sensitivity is 3 pg/mL. Each value was determined by drawing a linear approximation curve (Fig. 1B). Because ultrapure water that contained no BDNF showed a BDNF concentration of 1.1 pg/mL, and the concentrations of four samples were less than 1.1 pg/mL, these results were deemed unreliable.

Experiment 4

A total of 46 eyes were examined: 36 control eyes and 10 glaucoma eyes. In experiment 4, BDNF was measured with an in-house digital ELISA on the Simoa-HD1 Platform. Because the LLoD was 0.0081 pg/mL, the detection limit was less than 0.008 pg/mL. Twenty-five (54.3%, Fig. 2A) eyes were below the detection limit, including 20
(55.6%) control eyes and 5 (50%) glaucoma cases. After examining eyes with detectable BDNF, the overall BDNF concentration was 0.158 pg/mL (n=21): 0.196 pg/mL (n=16) in control cases and 0.034 pg/mL (n=5) Fig. 2B in glaucoma cases.

### Discussion

BDNF is transported anteriorly and retrogradely through optic nerve fibers and the BDNF receptor TrkB is expressed in RGCs, which degenerate in glaucoma patients. BDNF dysfunction is a putative cause of RGC death in glaucoma. Experimental studies have shown that reduced axonal transport induced by elevated IOP results in reduced BDNF and subsequent RGC death.

BDNF in aqueous humor is considered an important biomarker for glaucoma. Therefore, in this experiment, BDNF concentrations were also measured in the aqueous humor of glaucoma patients. The most commonly used ELISA kit (human BDNF Quantikine ELISA kit; R&D Systems), the most sensitive commercially available ELISA kit (ultrasensitive human BDNF ELISA kit; Aviscera Bioscience), and Simoa, an ultrasensitive digital ELISA contract analysis service, were used. However, BDNF concentrations were still below the measurement limit. Conventional ELISA measurements are limited to pg/mL levels of detection. Simoa (Single-Molecule Array) is a single-molecule digital detection technology with a femtomolar (fg/mL) detection sensitivity, ie, 1,000 times higher than that of conventional ELISA. Even with this technique, the detection rate was below 50%, and BDNF concentration in measurable samples was 0.2 pg/mL or less. The protein-stabilizing cocktail is a versatile stabilizing solution that increases the shelf-life of proteins during storage. However, the protein-stabilizing cocktail did not result in dramatic improvement. A potential reason why we were unable to measure BDNF in aqueous humor is that it is present in very small amounts and degraded before measurement. Sakane et al. reported that the therapeutic protein of BDNF has a short half-life in vivo (<2 minutes). Vitreous BDNF has not been measured because of its short half-life but might be more abundant than in the aqueous humor, as the vitreous contacts the retina. In the future, measurement of BDNF in the vitreous may be useful.

Zhang et al. used multiplex bead-based immunoassays (Luminex, Austin, TX, USA) with Human Neurodegenerative Disease Panel 3, which includes BDNF with a detection limit of 2.4 pg/mL. Their average measured BDNF concentration was 0.89 pg/mL, which was below the detection limit. As was the case for our experiment 3, these data may be unreliable. Shpak et al. used the BDNF Emax ImmunoAssay System kit (Promega Corporation, Madison, WI, USA), which has a detection limit of 7.8 pg/mL. Their average measured BDNF concentration in glaucoma patients was 35.2 pg/mL, which is within the measurement range. Uzel et al. used the RayBio human BDNF ELISA kit (Raybiotech Inc, Peachtree Corners, GA, USA), which has a detection limit of 66.6 pg/mL. The average BDNF concentration in glaucoma patients was 9.36 ng/mL. This value is extremely high and thus interesting.

BDNF has great potential as a neuroprotective factor. In recent years, ocular gene therapy has been performed frequently. Use of an adenovirus-associated virus vector for gene transfer into the inner retina is a new strategy for BDNF replacement. An effective gene transfer method of intravitreal injection has also been developed for non-human primates. The possibility of ocular gene therapy with BDNF for glaucoma requires monitoring of BDNF in the retina. Because it is impossible to measure BDNF in the retina, measurement of BDNF in aqueous humor is important. Past and present evidence indicates that BDNF levels in aqueous humor vary widely. The present experiments used Simoa, an ultrasensitive ELISA, but 50% or more of the samples were below the detection limit. In the future, this will be important basic data, because of the role of BDNF gene expression in ocular gene therapy.

### Funding

This work was supported in part by a Grant-in-Aid for Scientific Research (c) (16K11276 and 19K10001) from the Ministry of Education, Science and Culture of Japan and MEXT (Ministry of Education, Culture, Sports, Science and Technology).

### Conflict of Interest

The authors declare no conflict of interest.

### References

1. Quigley HA. Neuronal death in glaucoma. Prog Retin Eye Res. 1999 Jan;18(1):39–57.
2. Anderson DR, Graham S, Pillunat L. Normal-tension glaucoma. J Glaucoma. 2005 Apr;12(2):164–6.
3. Miglior S, Bertuzzi F. Relationship between intraocular pressure and glaucoma onset and progression. Curr Opin Pharmacol. 2013 Feb;13(1):32–5.
4. Lambert WS, Clark AF, Wordinger RJ. Neurotrophin and Trk expression by cells of the human lamina cribrosa following oxygen-glucose deprivation. BMC Neurosci. 2004 Dec 3;5:51.
5. Gupta V, You Y, Li J, et al. BDNF impairment is associated with age-related changes in the inner retina and ex-
accerbates experimental glaucoma. Biochim Biophys Acta. 2014 Sep;1842(9):1567–78.
6. Ko ML, Hu DN, Ritch R, Sharma SC, Chen CF. Patterns of retinal ganglion cell survival after brain-derived neurotrophic factor administration in hypertensive eyes of rats. Neurosci Lett. 2001 Jun 8;305(2):139–42.
7. Pease ME, McKinnon SJ, Quigley HA, Kerrigan-Baumrind LA, Zack DJ. Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. Invest Ophthalmo vis Sci. 2000 Mar;41(3):764–74.
8. Chen H, Weber AJ. BDNF enhances retinal ganglion cell survival in cats with optic nerve damage. Invest Ophthalmo vis Sci. 2001 Apr;42(5):966–74.
9. Klocker N, Kerner P, Weishaupt JH, Labes M, Ankerhold R, Bahr M. Brain-derived neurotrophic factor-mediated neuroprotection of adult rat retinal ganglion cells in vivo does not exclusively depend on phosphatidyl-inositol-3'-kinase/protein kinase B signaling. J Neurosci. 2000 Sep 15;20(18):6962–7.
10. Ma YT, Hsieh T, Forbes ME, Johnson JE, Frost DO. BDNF injected into the superior colliculus reduces development- retinal ganglion cell death. J Neurosci. 1998 Mar 15;18 (6):2097–107.
11. Mey J, Thanos S. Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res. 1993 Feb 5;602 (2):304–17.
12. Peinado-Ramon P, Salvador M, Villegas-Perez MP, Vidal-Sanz M. Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study. Invest Ophthalmo vis Sci. 1996 Mar;37(4):489–500.
13. Ghaffariyeh A, Honarpisheh N, Heidari MH, Puyan S, Abasov F. Brain-derived neurotrophic factor as a biomarker in primary open-angle glaucoma. Optom Vis Sci. 2011 Jan;88(1):80–5.
14. Ghaffariyeh A, Honarpisheh N, Shakes Y, et al. Brain-derived neurotrophic factor in patients with normal-tension glaucoma. Optometry. 2009 Nov;80(11):635–8.
15. Igarashi T, Nakamoto K, Kobayashi M, et al. Serum brain-derived neurotrophic factor in glaucoma patients in Japan: An observational study. J Nippon Med Sch. 2020; 87(6):339–45.
16. Oddone F, Roberti G, Micera A, et al. Exploring serum levels of brain derived neurotrophic factor and nerve growth factor across glaucoma stages. PloS One. 2017;12 (1):e0168565.
17. Chapak AA, Guekht AB, Druzhkova TA, Kozlova KI, Gulyaeva NV. Brain-derived neurotrophic factor in patients with primary open-angle glaucoma and age-related cataract. Curr Eye Res. 2018 Feb;43(2):224–31.
18. Uzel MM, Elgin U, Boral B, et al. The effect of trabeculectomy on serum brain-derived neurotrophic factor levels in primary open-angle glaucoma. Graefes Arch Clin Exp Ophthalmo. 2018 Jun;256(6):1173–8.
19. Zhang Y, Yang Q, Guo F, Chen X, Xie L. Link between neurodegeneration and trabecular meshwork injury in glaucomatous patients. BMC Ophthalmo. 2017 Nov 28;17 (1):223.
20. Wilson DH, Rissin DM, Kan CW, et al. The Simoa HD-1 Analyzer: A novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing, J Lab Autom. 2016 Aug;21(4):533–47.
21. Butowt R, von Bartheld CS. Anterograde axonal transport of BDNF and NT-3 by retinal ganglion cells: roles of neurotrophic receptors. Mol Cell Neurosci. 2005 May;29(1):11–25.
22. Caleo M, Menna E, Chierzi S, Cenni MC, Maffei L. Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system. Curr Biol. 2000 Oct 5;10 (19):1155–61.
23. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and re-growth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A. 1994 Mar 1;91(5):1632–6.
24. Cellerino A, Kohler K. Brain-derived neurotrophic factor/ neurotrophin-4 receptor TrkB is localized on ganglion cells and dopaminergic amacrine cells in the vertebrate retina. J Comp Neurol. 1997 Sep 15;386(1):149–60.
25. Perez MT, Caminio E. Expression of brain-derived neurotrophic factor and of its functional receptor in adult rat retina. Neurosci Lett. 1995 Jan 2;183(1-2):96–9.
26. Wahlin KJ, Adler R, Zack DJ, Campochiaro PA. Neurotrophic signaling in normal and degenerating rodent retinas. Exp Eye Res. 2001 Nov;73(5):693–701.
27. Quigley HA, McKinnon SJ, Zack DJ, et al. Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest Ophthalmo Vis Sci. 2000 Oct;41(11):3460–6.
28. Géral C, Angelova A, Lesieur S. From molecular to nanotechnology strategies for delivery of neurotrophins: emphasis on brain-derived neurotrophic factor (BDNF). Pharmaceutics. 2013 Feb 5;5(1):127–67.
29. Chiwa W, Bartlett CA, Petraus S, Fitzgerald M, Harvey AR. Intravitreal application of AAV-BDNF or mutant AAV-CRMP2 protects retinal ganglion cells and stabilizes axons and myelin after partial optic nerve injury. Exp Neurol. 2020 Apr;326:113167.
30. Igarashi T, Miyake K, Kobayashi M, et al. Tyrosine triple mutated AAV2-BDNF gene therapy in a rat model of transient IOP elevation. Mol Vis. 2016;22:816–26.
31. Osborne A, Khatib TZ, Songra L, et al. Neuroprotection of retinal ganglion cells by a novel gene therapy construct that achieves sustained enhancement of brain-derived neurotrophic factor/tropomyosin-related kinase receptor-B signaling, Cell Death Dis. 2018 Sep 26;9(10):1007.
32. Ren R, Li Y, Liu Z, Liu K, He S. Long-term rescue of retinal ganglion cells and visual function by AAV-mediated BDNF expression after acute elevation of intraocular pressure. Invest Ophthalmo Vis Sci. 2012 Feb;53 (2):1003–11.
33. Gamlin PD, Alexander JJ, Boye SL, Witherspoon CD, Boye SE. Sublum injection of AAV for gene delivery to the retina. Methods Mol Biol (Clifton, NJ). 2019;1950:249–62.
34. Takahashi K, Igarashi T, Miyake K, et al. Improved intravitreal AAV-mediated inner retinal gene transduction after surgical internal limiting membrane peeling in cynomolagus monkeys. Mol Ther. 2017 Jan 4;25(1):296–302.