# Diabetes Mellitus and Retinal Vein Occlusion as Risk Factors for Open Angle Glaucoma and Neuroprotective Therapies for Retinal Ganglion Cell Neuropathy

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## Abstract

Glaucoma, diabetic retinopathy (DR), and retinal vein occlusion (RVO) are major diseases that can lead to blindness and affect mainly the elderly population worldwide. The results of recent investigations have demonstrated that the death of retinal ganglion cells (RGCs) and their axons is the common pathological change in these three disease processes. The exact mechanism that is responsible for the onset and progression of RGC death and axonal degeneration in patients with glaucoma, DR and RVO has not been definitively determined. Thus, identifying the risk factors for the onset and the progression of RGC neuropathy can help in deciding not only the specific treatments but also whether the treatments should be initiated, withheld, or augmented in individuals with glaucoma, DR, and RVO. This review describes the major risk factors for the onset of glaucoma, and the factors associated with the progression of glaucoma that have been obtained from large population-based prevalence and incidence studies. In addition, the potential risk factors for glaucoma, diabetes mellitus, and RVO are discussed in terms of the results obtained by both clinical and laboratory studies. This review introduces potential neuroprotective therapies for damaged RGC in eyes with RGC neuropathy, and the factors that should be considered for a complete therapy for the RGC neuropathy involved in glaucoma, DR and RVO. Neuroprotective therapies combined with a reduction of the IOP should be considered for the complete management of RGC neuropathy involved in glaucoma, DR and RVO.

**Keywords:** Diabetes mellitus; Glaucoma; Retinal vein occlusion; Neuroprotective therapy

## Introduction

Diabetic retinopathy (DR), retinal vein occlusion (RVO), and glaucoma are the leading causes of blindness in the elderly population worldwide. They are associated with progressive optic nerve neuropathy with retinal ganglion cell (RGC) death and axonal degeneration. Although an improvement in the management of the risk factors and advances in the treatments for DR have contributed to reducing the risk of blindness [1-3], the incidence of type 2 diabetes has been increasing in the Asian populations [4,5]. The report from the International Diabetes Federation estimated that people with diabetes in the Asian Pacific region will increase from 137 million in 2010 to 214 million by 2030 [6]. According to the study performed by Kawasaki et al. as part of the Japan Diabetic Complications Study, the incidence of DR is 38.3/1,000 person-years and the progression rate is 21.1/1,000 person-years [7].

RVO is the second most common cause of vision decrease from retinal vascular diseases following DR [8]. In a cohort of adults in the United States of America (USA), the prevalence of RVO was 0.6 percent while the prevalence of central RVO was 0.1 percent with no significant difference between men and women. The highest prevalence of RVO was in patients of age 80 years and older (4.6 percent) [9].

An epidemiological study performed by the Japanese Ministry of Welfare in 2005 showed that glaucoma is the most common eye disease that causes blindness. According to the results of this study, over 3000 patients with glaucoma lose their vision each year in Japan. Thus, the current management and therapies for glaucoma are not sufficient to prevent the progression to blindness in patients with glaucoma. A recent population–based epidemiological study in Japan demonstrated that 5% of people over 40-years-of-age had glaucoma, and the prevalence of primary open angle glaucoma (POAG) is 3.9% [10]. Surprisingly, 90% of the eyes of Japanese with OAG are the normal tension type of glaucoma (NTG), and the prevalence of NTG is 3.6% in patients >40-years-of-age in Japan [10]. The average intraocular pressure (IOP) in patients with POAG is $15.4 \pm 2.8$ mmHg, which is significantly higher than that of normal subjects ($14.5 \pm 2.5$ mmHg) [1-10] even though the average difference in the IOP is only 0.9 mmHg. Thus, a reduction of the IOP the standard therapy for glaucoma may not be sufficient for the complete management of the patients with glaucoma in Japan. Other therapeutic modalities, such as neuroprotection, should be considered for a complete therapeutic regimen for glaucoma patients.

RGC death and axonal degeneration are most likely involved in the progression of DR, RVO, and glaucoma. Many neuroprotective therapies that prevent RGC death and axonal degeneration have been and are being investigated, and some of these neuroprotective factors may be used clinically in the near future. To establish neuroprotective therapies for DR, RVO and glaucoma, a determination of the exact mechanism that leads to a progression of RGC neuropathy to death and axonal degeneration is required [11]. However, the pathogenesis of the onset and progression of RGC neuropathy has still not been definitively determined. Identification of the risk factors for the onset and the progression of disease would help in determining the

pathogenesis of RGC neuropathy and in deciding not only the specific treatment but also whether the treatment should be initiated, withheld, or augmented in individuals with glaucoma, DR, and RVO.

Although there have been many clinical studies on the risk factors for the progression of ocular hypertension (OHT) to glaucoma, we believe that these risk factors are not appropriate for Japanese patients with glaucoma because 90% of the patients with OAG have NTG in Japan. Thus, we have carefully reviewed the risk factors for OAG obtained from clinical and laboratory studies. In addition, the potential risk factors for glaucoma, including diabetes mellitus and RVO, are discussed in terms of the results obtained by both clinical and laboratory studies. We have also reviewed the results of recent studies that have suggested that neuroprotective therapies should be part of the complete therapy for RGC neuropathy in glaucoma, DR, and RVO patients.

## Risk Factors for Open Angle Glaucoma

The risk factors that have good evidence for being significantly associated with OAG are listed in Table 1.

### Intraocular pressure (IOP)

Both the average IOP [12] and the diurnal fluctuations in the IOP [13,14] are strong risk factors for OAG. Although 90% of the OAG in Japan is NTG, a higher mean IOP is still a significant risk factor for OAG [12]. A multivariate analysis of the data of the Tajimi Study showed that the odds ratio (OR) for higher IOP was 1.13 [12]. Other studies also support the idea that even when the IOP was within the normal range, a higher mean IOP was still a risk factor for OAG [15,16]. Thus, a reduction of the IOP should be an important target of the therapy for eyes with OAG. In fact, the Ocular Hypertension Treatment Study (OHTS) reported that when the mean IOP is increased by 1 mmHg, the risk of progression from OHT to glaucoma is increased by 10% [17,18]. The results of the Collaborative Normal-Tension Glaucoma Study (CNTGS), a multi-center based study in USA and Canada, suggested that a reduction of the IOP by 30% from baseline is effective and decreases the risk of a progression of the glaucomatous neuropathy significantly [19,20].

### Age

An older age has been consistently identified as a significant risk factor for an increased prevalence, incidence, and progression of OAG [12,21-25]. This effect of age is probably enhanced by the normal decrease in the number of RGCs and their axons that occurs with increasing age. Several studies have reported that the RNFL thickness decreases from 0.05 μm/year [26] to 0.44 μm/year [27] in healthy volunteers aged from 6 to 79 years. Thus, older people have an increased risk of developing OAG.

### Larger cup-to-disk ratio

Many studies have shown that a larger cup-to-disk ratio is a strong and consistent risk factor for OAG [17,18,28-30]. In fact, a large cup-to-disk ratio is a clinical sign of glaucomatous optic neuropathy and an essential parameter for making a diagnosis of OAG especially when the IOP is within the normal range. However, patients with large cup-to-disk ratios may already have structural optic disc damages and visual field defects that can be detected by frequency doubling technology or short-wavelength automated perimetry.

### Myopia

Myopia is a significant risk factor for OAG especially those eyes with low average IOPs [12,31], and the odds ratio is 2.60 for moderate to high myopia in Japanese individuals [12]. Population-based studies also found that myopia is associated with OAG [32-34]. Several studies reported that the RNFL thickness is influenced by the axial length of the eye [35-36]. Kita et al. investigated the RNFL thickness in glaucomatous eyes and found that when normal eyes were compared to glaucomatous eyes, there were a greater number of myopic eyes in the glaucoma group [36].

### Central corneal thickness

In Japanese individuals, the central corneal thickness is not a significant risk factor for OAG [12], which is not consistent with the results of other studies such as that of the OHTS which had 90% Caucasians [17,18]. When eyes with thicker corneas are measured by Goldmann applanation tonometry, the IOP is recorded to be higher than the true IOP. On the other hand, when patients with thin corneas are measured by Goldmann applanation tonometry, their IOPs are recorded to be lower than the true IOP. Thus, thin corneas are reported to be a significant risk factor for the progression from OHT to POAG [17,18]. However, thin corneas do not appear to be associated with NTG [12].

### Exfoliation syndrome

There is strong relationship between the exfoliation syndrome and glaucoma [24,37]. In the Early Manifest Glaucoma Trial (EMGT) [24] in patients with exfoliation syndrome, the exfoliation syndrome was found to be a major risk factor for the progression of OAG, and this risk was independent of the IOP in these patients. The Blue Mountain Eye Study [37] in the USA found glaucoma in 14.2% of eyes with the exfoliation syndrome, which was significantly more than eyes without the exfoliation syndrome (1.7%) after adjustments were made for other glaucoma risk factors including the IOP.

### Ethnicity

Although ethnicity is not an independent risk factor, black individuals are more likely to have OAG than any other ethnicities [17,18]. In the clinic however, it does not seem to be so important whether this risk is independent because black individuals have many risk factors such as larger cup-to-disc ratios, thinner corneas, higher IOPs, and lower access to clinical examinations [38-40]. Thus, clinicians should carefully observe black individuals who generally have a higher risk of OAG.

### Optic disc crescent and disc hemorrhages

The incidence of optic disc crescents or peripapillary chorioretinal

| Risk Factors for Prevalence | Risk factors for Incidence | Risk Factors for Progression |
|---|---|---|
| IOP (higher, variations) | IOP (higher, variation) | IOP (higher, variation) |
| Age (older) | Age (older) | Age (older) |
| Cup-to-disk ratio (greater) | Cup-to-disk ratio (greater) | Cup-to-disk ratio (greater) |
| Myopia | Central corneal thickness (thinner) | Central corneal thickness (thinner) |
| Exfoliation syndrome | Exfoliation syndrome | Disk hemorrhage |
| Ethnicity (Black) | Ethnicity (Black) | Ethnicity (Black) |
| Disk crescent | | Disk crescent |

IOP=intraocular pressure. In all risk factors described in the table, there is strong evidence that they are associated with open angle glaucoma from evidence-based studies

**Table 1:** Risk factors associated with open-angle glaucoma.

atrophies is higher in patients with NTG than in normal subjects. In addition, the crescent is wider in patients with NTG. Disc hemorrhages are significantly associated with the size of the disc crescent and retinal nerve fiber layer defects in eyes with OAG [41] especially in eyes with NTG [42]. Disc hemorrhages are a strong risk factor for the progression of OAG [24] especially NTG [43,44]. The higher incidence of wide disc crescents and disc hemorrhages in patients with NTG suggests that the ischemic damage surrounding the optic disc may be associated with the pathogenesis of glaucomatous optic neuropathy.

### Visual field defects

The prognosis of the fellow eyes of NTG patients with unilateral field loss is significantly correlated with the severity of the visual field damage in the affected eye at presentation [45]. In addition, the presence of the visual field defects at the fixation point increases the risk of a progression of the visual field defect close to fixation leading to a decrease in the visual acuity in patients with NTG [46].

### Minor risk factors for OAG

There are other risk factors for OAG but the evidence for them is relatively weak. Further studies must be done to confirm that they are clinically relevant risk factors. Some minor factors for OAG are listed below.

### Family history

Although there are some studies suggesting a significant association of family history with OAG [15,47-49], other studies fail to confirm this relationship [12,17,18,43]. Unfortunately, self-reports of OAG or report of a family history of OAG are not trustworthy [48]. Thus, direct examination of relatives [49,50] should be done before conclusions are made on the presence of a family history of OAG. *Myocilin* and *optineurin* are reported to be the genes whose mutations are associated with OAG [51,52]. However, very few of the OAG patients have these gene mutations [51,52]. Although the genetic factors have low attributable risk for OAG, the results of the Rotterdam Study suggested that non-genetic factors such as environmental factors play a critical role in the development of OAG in family members [40]. Further studies with the exact classification of family history of OAG patients are needed to confirm whether a significant association of family history with OAG exists.

### Migraine

Although the Tajimi study in Japan did not report a significant association of migraine with OAG [12], there is some evidence that there is a significant association [43,53,54]. However, these findings should be accepted with some caution because the definition of migraine varied in these studies.

### Gender

In the CNTGS, the risk ratio for women and glaucoma was 1.85, and women are considered to be at significant higher risk factor for the development and progression of NTG [43]. However, the Tajimi study [12] and another investigation [15] did not confirm a gender associated risk factor for OAG.

Other factors including body mass index, and caffeine and alcohol use have not been found to be significantly associated with OAG. Cardiovascular diseases appear not to be associated with OAG, but uncontrolled systematic hypertension may be associated with OAG [55,56]. The Egna-Neumarkt Study reported that lower perfusion pressure is a major risk factor for OAG [56]. However, the association of hypertension with OAG may be due to the significant correlation of hypertension and age [12].

A recent meta-analysis found that individuals who were currently smoking have a significantly higher risk for developing OAG [57].

## Diabetes Mellitus As Risk Factor For Open Angle Glaucoma

DR is a major disease worldwide and is important because it can progress to a decrease of vision in a high percentage of the individuals. The results of recent studies have shown that not only vascular abnormalities but also neuronal abnormalities are associated with the pathogenesis of the early stage of DR [58,59]. The neuronal abnormalities, such as RGC death, affect the visual function of diabetic patients and this is especially important because the death of neurons is irreversible in adults. Knowledge of such early changes involved in the pathogenesis of DR is significant because it can lead to the development of new therapeutic strategies [60].

The evidence of an association of diabetes mellitus with OAG is not very strong (Table 2). A recent meta-analysis suggested that diabetes is a significant risk factor for having OAG [61], but the definition of OAG was not standardized in the different studies. Two of 12 studies included in the meta-analysis used only the IOP as a definition of OAG and did not consider optic nerve damage and visual field defects. Thus, these studies most likely included only eyes with OHT and did not include eyes with NTG. In addition, diabetic patients have a better chance of having their OAG detected because of their frequent fundus examinations. Thus, a detection bias may be included in these studies of diabetic patients.

It should also be noted that the diagnosis of diabetes mellitus may not have been made accurately in many of these studies. For example, the Tajimi Study [12], used self-presentation as evidence of diabetes mellitus. Another example of this was the OHTS that concluded that diabetes had a protective effect for the progression of OAG [17,18], but the patients with DR were excluded from the analysis and the diagnosis of diabetes was based only on self-report. Thus, these results should be interpreted with caution.

In four population-based studies that examined the association of diabetes mellitus with the prevalence of OAG [62-65], three found a positive association between the two diseases [62-64], but the Baltimore Eye Surgery failed to find a significant association [65]. But, again only self-presentation was used to ascertain that diabetes was present [65]. In the Beaver Dam Eye Study, diabetic subjects were determined by a history of diabetic treatment, increased glycosylated hemoglobin levels, or increased blood glucose levels [62]. The Beaver Dam Eye Study concluded that patients with older-onset diabetes had a higher risk for

| Association with Prevalence | Association with Incidence | Association with Progression |
|---|---|---|
| Klein et al. [62] (+) | Ellis et al. [67] (-) | AGIS Investigators [23] (+) |
| Tielsch et al. [65] (-) | Le et al. [47] (-) | Leske et al. [24] (-) |
| Dielemans et al. [63] (+) | Kim et al. [69] (+) | Gordon et al. [17] (-) (protective) |
| Mitchell et al. [64] (+) | Welinder et al. [66] (+) | |

(+) = Positive results that showed association between diabetes and open angle glaucoma. (-) = Negative results that showed the relationship between diabetes and open angle glaucoma. AGIS= Advanced Glaucoma Intervention Study

**Table 2:** Summary of clinical studies for the relationship between diabetes and OAG.

OAG (odds ratio = 1.68) [62]. In the Rotterdam Study, diabetes mellitus was diagnosed by serum glucose level or glucose tolerance test [63], and the investigators suggested that a risk of OAG in eyes with high IOP was increased more than three times in patients with diabetes than without diabetes [63]. In the Blue Mountains Eye Study, the presence of diabetes was confirmed by self-presentation or blood glucose levels [64]. The investigators of this study suggested that diabetes increased the risk of having OAG more than two times without a selection bias [64].

Welinder et al. conducted a population-based case-control study and found that diabetes mellitus was associated with a 1.8-fold increased risk of medically-treated glaucoma. The increased risk was independent of duration of the diabetes or the level of glycemic control. The relative effect associated with diabetes was greatest among persons <60 years, possibly due to the lower baseline glaucoma risk in younger individuals. They also reported that the effect was greater in men than in women [66].

Taking these findings together, it appears that diabetes mellitus increases the risk of OAG significantly. In addition, more reliable results were obtained when the diagnosis of diabetes was more exact.

However, two long-duration cohort studies failed to confirm an association of the incidence of OAG with diabetes mellitus [47,67]. In the first study, the authors reported that a detection bias contributed to the failure to confirm the association between diabetes and glaucoma [67]. The latter study did not present any information on the definition of diabetes that was used [47]. Recently however, Kim et al. suggested that systemic vascular factors and IOP play significant roles in the pathogenesis of NTG. The results of their study demonstrated that the disease severity of the affected eye, severity of diabetes mellitus, previous cerebro vascular accidents, and IOP ≥14 mm Hg in both eye were associated with bilateral eye involvement in NTG [68]. Except for studies that did not study a representative group of patients with diabetes [17,18], the results of most studies indicated that diabetes mellitus is a significant risk for the progression of OAG [23,24].

There is important evidence from both clinical and animal studies that support a significant association of diabetes mellitus with the progression of OAG. First, diabetic patients have significantly higher IOPs than non-diabetic patients [69], and the IOP of a diabetic patient increases significantly as the level of glycosylated hemoglobin A1c increases [69]. Our clinical data shows that the IOP of eyes of patients with poor control diabetes (HbA1c ≥ 8.0%) was significantly higher than the IOP of eyes of patients with mild control diabetes (HbA1c ≤ 6.0%) (16.6 ± 2.4% vs. 15.5 ± 2.5 mmHg) [69]. In the Tajimi Study, the mean IOP of patients with glaucoma and control subjects are 15.4 mmHg, 14.5 mmHg, respectively. Thus, in Japanese patients, only a 1.0 mmHg difference of IOP is critical for development of glaucoma. The higher IOPs may impose a stress on the RGCs and their axons in diabetic patients, because physical stress on the lamina cribrosa by high pressure may cause axonal damage including the disturbance of axonal transport followed by a reduction of neurotrophic factors delivered to the RGC bodies through retrograde axonal transport.

In addition, the RGC damage induced by stress caused by mechanical injury or high IOPs may be enhanced under more severe diabetic conditions [58,70]. Thus, it has been hypothesized that once apoptosis of the RGCs is induced by glaucomatous stress in diabetic patients, the apoptosis may be enhanced by diabetic stress. In addition, visual fields defects may progress faster in OAG patients with diabetes than in OAG patients without diabetes. The results of a recent study

supported this hypothesis; Kim et al. suggested that both systemic vascular factors increased the susceptibility of RGCs [68]. In addition, a higher IOP can cause a pressure-induced stress of RGC axons which can play a significant role in the pathogenesis of NTG [69]. Further clinical studies with an exact definition of diabetes and OAG need to be performed to confirm the associations of OAG with diabetes mellitus.

## Retinal Vein Occlusion As Risk Factor For Open Angle Glaucoma

The association between retinal vein occlusion (RVO) and OHT or glaucoma has been recognized since the beginning of the 20th century [71] (Table 3). Case-control studies showed that patients with a central RVO in one eye were more frequently associated with a history of glaucoma or OHT in the fellow eye [72,73].

Barnett et al. have come to the conclusion concerning the relationship between RVO and glaucoma. They studied the incidence of RVO in a large, prospectively followed sample of ocular hypertensive individuals [74]. In this multicenter, randomized clinical trial of 1636 patients under topical IOP-lowering medication had a mean follow-up period of 9.1±2.7 years. Twenty-three OHTS participants had RVOs during the follow-up period for a cumulative incidence of only 1.4%. The incidence of RVO in patients with OHTS was comparable to that reported from population-based studies.

Although RVO was found more frequently in the observation group than in the medication group which is consistent with previous studies implicating elevated IOP as a risk for RVO, the difference was not significant.

Consistent with earlier studies, older age and larger horizontal cup-to-disk ratio at baseline were significant predictive factors for the development of RVO [74].

Prata et al. also found that RVO occurred more frequently in patients with the exfoliation syndrome, the most common identifiable cause of OAG worldwide [75]. The reason why an RVO occurred most often in the affected or more severely affected eye in patients with exfoliation syndrome could possibly be attributed to a combination of factors. Structural vascular abnormalities documented in eyes with exfoliation syndrome could be involved [76-78]. Hyperhomocysteinemia which is a major risk factor for vascular diseases, including venous thrombosis, was reported to be more common in exfoliation syndrome and exfoliation OAG patients than in healthy controls in several studies [79-82].

It has been shown in several studies that there is a strong correlation between glaucoma and RVO because of the association of optic disk cupping with distortion of the retinal vessels at the disk, predisposing the vein to occlusion [83-85]. Other studies suggest that the significant association of OAG and RVO may be a manifestation of a common underlying vascular abnormality, such as systemic hypertension [86,87]. Kim et al. suggest that RVO and OAG may share common systemic

| Association with Prevalence | Association with Incidence | Association with Progression |
|---|---|---|
| Verhoeff [71] (+) | Barnett et al. [74] (-) | Kim et al. [88] (+) |
| EDCCSG [72] (+) | Prata et al. [75] (+) | Bonomi et al. [56,86] (+) |
| | Yoo et al. [85] (+) | Simons et al. [87] (+) |

EDCCSG – Eye Disease Case-Control Study Group
(+) = Positive results that showed association between RVO and OAG
(-) = Negative results that showed the relationship between RVO and OAG

**Table 3:** Summary of clinical studies for the relationship between RVO and OAG.

risk factors reflecting a common pathogenic mechanism [88]. They found that the RNFL thinning, especially in the inferior-temporal and superior-temporal sectors in the fellow eyes, was more prominent in a subgroup of patients >60 years old. It has been suggested that arterial stiffness and atherosclerosis may explain both RVO and thinning of the RNFL. The patients with thinner RNFL in the contralateral eye had the same IOP as the controls which suggested the possibility that OAG and RVO shared a common vascular abnormality [88].

Compared with the association of diabetes mellitus with OAG, the association of RVO with OAG seems to be stronger. One possible reason is that the definition of RVO is more accurate than that of diabetes mellitus, and ophthalmologists can make a diagnosis of RVO more easily. However, further clinical studies are needed to confirm the association of RVO with OAG.

## Neuroprotective Therapies For Patients With OAG, DR, and RVO

Because there are very few clues on the exact mechanism that causes RGC death and axonal degeneration in DR and RVO in humans, the development of neuroprotective therapies must be based on evidence obtained from animal glaucoma models.

Although the IOP is the only modifiable risk factor for glaucoma, good IOP control alone is not always sufficient to preserve vision and the visual field. It is known that there may already be significant damage to the RGCs before functional visual decrease is detected. Also, patients may continue to lose RGCs even when the IOP has been normalized [89].

RGC death and axonal degeneration are common pathological changes in patient with DR, RVO, and OAG. Thus, therapies aimed at protecting and regenerating RGCs are necessary for a complete therapy regime for patients with DR, RVO and OAG. However, even if the apoptosis of RGCs could be completely inhibited, other cellular death events such as necrosis or autophagy may be induced under chronic stress in eyes with RGC neuropathy. Thus, the main therapies should be the reduction of the causes of the neuronal stress such as an improvement of hemokinesis, glycemic control, and reduction of the IOP.

The main cause of stress for RGCs and their axons in glaucoma patients is an elevation of the IOP, and thus the first choice of glaucoma therapy should be IOP-lowering therapies. Neuroprotection implies the use of drugs or chemicals to slow down whatever causes the vision decrease independent of the IOP. Neuroregeneration refers to anything that stimulates the re-growth of injured RGC axons [90]. Thus, neuroprotective therapies should be combined with IOP-reducing therapies as the optimal approach for the complete management for the patients with glaucoma. There are many neuroprotective agents for glaucoma treatment such as brimonidine [91], neurotrophic factors [92], erythropoietin [93], and Cop-1 [94].

### Anti-apoptotic agents

Several pathogenic mechanisms have been proposed that induce the apoptotic death of RGCs. These include reduced levels of neurotrophic factors, deprivation of cytokines to neurons, altered intracellular calcium levels, presence of reactive oxygen species, and excitotoxicity due to raised extracellular levels of certain neurotransmitters and neuromodulators [95-96]. The mitochondria- and caspase -dependent cell death pathway are known to be associated with neuronal cell death in diabetic retinas [59]. Brimonidine [97] and caspase inhibitors [98] use may block the apoptotic process.

### N-methyl-D-aspartate (NMDA) receptor antagonists

It is known that the activation of NMDA receptor leads to the opening of associated ion channels in the neurons and the entry of extracellular calcium and sodium. Glutamate-mediated neuronal toxicity is due to the influx of extracellular calcium, which acts as second messenger that activates a cascade of reactions, leading to neuronal cell death by excitotoxicity [99]. To protect RGCs from excitotoxic cell death, the removal of synaptic glutamate is necessary. Hence, using NMDA antagonists would be an efficient way to prevent RGC loss. Memantine is a promising neuroprotective agent and has been approved for use on patients with moderate to severe Alzheimer`s disease by the US Food and Drug Administration [100,101]. Memantine is a NMDA receptor blocker and may prevent excitotoxic cell death of damaged RGCs [100,101].

Memantine has undergone phase 3 trials, the most advanced clinical trial, for neuroprotective therapy in glaucoma patients. The complete results of this study have not been published although parts of the results are presented on the home page of the Glaucoma Research Foundation. According to the Memantine Update, there was no significant benefit in the memantine-treated group compared to that in the placebo group. Although patients with higher doses of memantine had a significantly slower progression of glaucoma than patients with low dose of memantine, the study failed to meet the primary endpoint.

One possible reason for the failure of memantine in the clinical trials may be the limitations of neuroprotection for RGC apoptosis in glaucoma patients. RGCs have large somas and long axons that run to the lateral geniculate nucleus in the brain. Once the axons degenerate, the degenerated axons cannot regenerate because of the environment surrounding the axons, e.g., gliosis derived from astrocytes and debris from oligodendrocytes, impede axonal regeneration. Thus, even if a RGC body could be rescued from irreversible damage by a neuroprotective agent, the loss of visual function cannot be restored after axonal degeneration has occurred. But the analysis of the memantine use is continuing in the hope that some other molecule will prove successful at protecting and/or restoring damaged RGCs [90].

### Glutamate antagonists

Excessive amounts of glutamate, which causes excitotoxic cell death of RGCs, have been detected in the vitreous of glaucoma patients [102]. The amino acid glutamate is an essential neurotransmitter in the central nervous system and the retina. Glutamate concentrations higher than the physiological levels are toxic to the neurons, and the degree of toxicity depends on the duration and magnitude of the increase. Excitotoxic neuronal injury is a self-perpetuating cascade of events caused by a continuous activation of ionotropic glutamate receptors [103,104]. However, an elevated glutamate level in the vitreous could not be confirmed in patients with glaucoma in more recent studies [105-108]. Thus, it is still not determined conclusively that excitotoxic cell death of RGCs is involved in the pathogenesis of glaucomatous optic neuropathy.

Riluzole, a glutamate regulator still in preclinical investigations, has been approved by the FDA for amyotrophic lateral sclerosis [109,110], and should be considered as a possible neuroprotectant for patients with glaucoma.

### Neurotrophic factors

It has been found that endogenous neurotrophic factors can function as neuroprotective agents [111-113]. Various studies in

experimental animal models have shown that neurotrophic factors, especially brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF), neurotrophin-4 (NT-4), and citicoline can increase the number of RGCs that survive after the optic nerve is injured [114-117].

BDNF is a member of the nerve growth factor family of neurotrophins which also includes nerve growth factor (NGF), NT-3, and NT-4 [117]. In a recent study, Coassin et al. found that NGF is over-expressed in experimental glaucoma, but not to an extent sufficient to support RGC survival. Their results illustrated the potential importance of multiple neurotrophin signaling pathways for RGC survival [118].

A potential survival enhancing role for glial cell line-derived neurotrophic factor (GDNF) was investigated by Jiang et al. In retinas treated with GDNF spheres, the authors reported a decrease in the nerve head cupping, increase in the nerve fiber layer thickness, and increase in the inner plexiform layer thickness. The degree of RGCs and axons survival was significantly increased by about 50%, while glial activation appeared to be less (p<0.001). These observations led the authors to conclude that sustained GDNF delivery had a significant neuroprotective effect to injured RGCs in rats [119].

Another potential neuroprotective drug is 5'-diphoshocoline (CDP-choline) or citicoline, which is prescribed for brain injury, Alzheimer`s disease, and Parkinson`s disorders in Japan and Europe [120]. Citicoline is an intermediate in the synthesis of phosphatidylcholine which is one of the major phospholipids of membranes of cells in the central nervous system (CNS). After cells are damaged, exogenous citicoline participates in the synthesis of the phospholipids of cell membranes and stabilizes the intracellular conditions of neuronal sells. Our earlier study showed that citicoline had a neuroprotective effect on damaged RGCs and supported neurite regeneration in vitro [111]. Other groups demonstrated the protective effects of citicoline for RGCs in vivo animal models [121,122]. A more recent study showed that intramuscular injections of citicoline significantly improved the electroretinograms and visual evoked cortical potentials in glaucoma patients during an 8-year follow-up period [123]. As a neuroprotectant, citicoline requires high doses and a relatively chronic exposure for retinal neuronal protection. Thus, only oral administration may not be sufficient for neuroprotective therapies of glaucoma patients. In some patients with NTG, visual field defects continued to progress even when the IOPs was kept in the low-teens level. Such patients must continue receiving non-operative therapies with full medications or undergo trabeculectomy for reducing the IOP below 10 mmHg. In these cases, citicoline treatment may be an option other than IOP-lowering therapies.

### T-588

Several studies showed that t-588 (R(-)-1-(benzo [b] thiophen-5-yl)-2-[2-(N, N-diethylamino) ethoxy] ethanol hydrochloride) had neuroprotective powers against RGC death [124,125] by elevated IOP and optic nerve crush in the rat. T-588 was developed to treat the dementia associated with Alzheimer disease [126]. Although the molecular mechanisms of T-588 are not fully understood, enhancement of the neuronal transmitter system, such as acetylcholine and noradrenaline, is considered to be one possible mechanism [127]. Investigations showed that repeated treatment with T-588 at doses of 3 to 30 mg/kg enhanced RGC survival without affecting the IOP in an elevated IOP rat model. In addition, T-588 prevented the death of RGCs in an optic nerve crush model of the rat [125].

### Antioxidants

Free radicals are a byproduct of oxidative metabolism. The high metabolic activity of retinal tissues renders RGCs especially vulnerable to oxidative stress. Free radicals interfere with macromolecular cellular constituents of the cells and further lead to derangement of protein breakdown, lipid peroxidation and nucleic acid degeneration, resulting in cell death. To counteract this, ocular tissues have highly efficient antioxidant mechanisms that include the superoxide dismutase-catalase system, ascorbic acid and reduced glutathione [104]. Antioxidants such as vitamins C and E may reduce RGC death caused by NMDA-induced toxicity [128,129]. Gingko biloba (EGb761), apart from increasing blood flow, has also been found to have a free radical scavenger property. Its extract is also known to preserve mitochondrial metabolism and enhance ATP production in various tissues [104].

### Gene therapy

A gene therapy approach in which a mutated gene is replaced or inactivated, or in which a new gene is introduced, could provide a new and more effective way of targeting the disease. Given the results of recent laboratory studies utilizing gene therapy techniques to lower intraocular pressure and to provide neuroprotection and the continued development of tissue-specific vectors, it seems that a new generation of treatments for glaucoma should be attempted [130]. It was shown that a single intramuscular injection of recombinant adeno-associated virus carrying EpoR76E (rAAV2/5.CMV.EpoR76E) protected the RGCs in a mouse model of glaucoma without inducing polycythemia. This systemic treatment not only protected the RGCs somata located within the retina, it also preserved axonal projections within the optic nerve, while maintaining the hematocrit within normal limits [131].

### Stem cell therapies

Stem cell transplantation is another promising method being studied for many neurodegenerative diseases. Stem cells are thought to exert neuroprotective effects by generating neurotrophic factors, modulating matrix metalloproteinases, and alter the CNS environment that may promote endogenous healing [104,132]. Research on stem cell mobilization and the possible neuroprotective contribution of granulocyte-colony stimulating factor (G-CSF) showed that G-CSF was strongly expressed by the RGCs, thereby providing neuroprotection in neurodegenerative diseases [133].

### Challenge to neuroprotective therapies for DR and RVO

Although there are very few clues on the exact mechanism that causes RGC death and axonal degeneration in DR and RVO in humans, we have been challenging to establish neuroprotective therapies for DR. In case of RVO, few suitable animal models are available. Zhang et al. indicated that in laser-induced RVO in rat models, significant ganglion cell layer cell loss is associated with the pathogenesis of RVO [134]. On the other hand, Ameri et al. suggested that the natural course of experimentally created RVO in rabbits was different from that in human [135]. Thus, it still seems to be difficult to obtain the basic evidence for the neuroprotective therapies for RGC death in RVO. However, from the clinical findings, RGC neuropathy should be associated with the pathogenesis of RVO and the association between RVO and glaucoma is stronger than that between diabetes and glaucoma (Tables 2 and 3). Thus, neuroprotective therapies for RGC neuropathy of glaucoma and DR may be applicable for patients with RVO.

We have evaluated the early changes in the thickness of the macula and RNFL by Stratus OCT in patients with early stage diabetes [136].

Thirty-one normal subjects, 45 non-DR (NDR) patients, and 24 minimal DR patients were used for the macular thickness measurements. Thirty control subjects, 45 NDR patients, and 22 minimal DR patients were used for the RNFL thickness measurements. In patients with NDR, macular was significantly thinner than that of control subjects. In patients with minimal DR, the mean RNFL thickness was significantly thinner than that of control subjects. The macula was thicker in eyes with longer duration of diabetes and the RNFL thickness was thinner in eyes with longer duration of diabetes. These results indicate that neuronal abnormalities precede vascular abnormalities in early stage diabetes and neuronal abnormalities should be gradually accumulated and progress with increase diabetes mellitus duration [136]. Van Dijk et al. indicated that the mean ganglion cell/inner plexiform layer in minimal DR patients was significantly thinner than those of age-matched controls [137]. The inner plexiform layer mainly consists of ganglion cells' dendrites. Thus, patients with early stage diabetes, RGC bodies including dendrites and axons are degenerated. These results may support the increase of the risk for OAG in diabetic patients.

We have been performing the studies to elucidate why RGC neuropathy is developing in DR patients and how RGC neuropathy is treated. We used retinas obtained from age-matched five pairs of normal and five pairs of diabetic eyes and performed immunohistochemistry of Bax, active-form caspase-9, and -3. Then, sections were co-stained with Fluoro-Jade B (FJB), degenerative neuron's marker. The numbers of Bax, caspase-9, -3, and FJB positive cells in the ganglion cell layer were counted [59]. In diabetic retinas, Bax, caspase-9 and -3 expression coexisted with FJB in the ganglion cell layer of diabetic retinas were significantly increased compared to those of retinas in control subjects [59]. The human retinal study indicates that mitochondria- and caspase-dependent cell death pathway is associated with RGC degeneration in patients with diabetes [59]. The mechanisms of RGC death in diabetic patients are, in part, common with those in RGC death of three-dimensional collagen gel retinal culture system under diabetic stress [58,114]. We have used this system to examine the effect of several neurotrophic and/or survival factors on RGC death and regeneration. We have tested BDNF, NT-4, citicoline, $VEGF_{120}$, $VEGF_{164}$, and Taurine-conjugated ursodeoxycholic acid (TUDCA). All factors showed survival effect of damaged RGCs induced by diabetic stress [114,116]. Furthermore, BDNF, NT-4 and citicoline showed regenerative effect in high-glucose-exposed rat retinas [114]. Ghazi-Nouri et al. showed that not BDNF but NT-4 was upregulated in patients with proliferative vitreoretinopathy [138]. NT-4 is an endogenous neurotrophic factor that protects retinal neurons from retinal damage and may be a suitable neurotrophic factors for treatment of DR.

Our recent study indicates that PKR-like ER kinase (PERK) and C/EBP homologues protein (CHOP) expression are associated with neuronal cell death under diabetic stress [116]. The PERK-CHOP pathway is known to be one of the major cell death pathways under chronic endoplasmic reticulum stress in retinal neurons. A recent study indicates that the PERK-CHOP pathway is associated with RGC death in experimental glaucoma model [139]. NT-4 can suppress PERK and CHOP expression and shows neuroprotective effect under diabetic stress [116]. Thus, the cell death mechanisms of damaged RGCs may be shared with glaucoma and DR and NT-4 and may be fitted with the neuroprotective therapies for both diseases.

## Summary

Although the clinical association with glaucoma and DR is not so strong and still debated by the scientific community, several basic researches support the association with both diseases. On the other hand, clinical studies strongly support the association between glaucoma and RVO. Thus, neuroprotective therapies for RGC neuropathy in glaucoma may be useful for DR and RVO. Further studies are needed to establish the neuroprotective and regenerative therapies for RGC neuropathy in glaucoma, DR and RVO.

## Conclusions

A better understanding of the risk factors that are associated with the onset and progressions of RGC neuropathy is required for a better and more complete management of DR, RVO and OAG. Although IOP-reducing therapies are the first choice for the treatment of glaucoma even when the IOP is in the conventional normal range, the existing therapies for reducing the IOP are limited. Thus, neuroprotective therapies that rescue damaged RGCs and inhibit the progression of RGC loss and axonal degeneration should be tried. Neuroprotective therapies for RGC neuropathy should be considered for the complete management of glaucoma, DR and RVO in the near future.

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