

## Department of Ophthalmology

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### General Summary

The main research interest of our department is the pathophysiology of the visual processing system. The following topics are the subjects of basic and clinical studies: cataract, neuro-ophthalmology, ocular oncology and histopathology, biochemistry, functional magnetic resonance imaging (MRI), glaucoma, electrophysiology, diabetes, vitreoretinal diseases, age-related macular degeneration, uveitis, color vision, and the cornea.

### Research Activities

#### *Cataract*

The widespread use of ultrasound technology in cataract surgery and the introduction of foldable intraocular lenses (IOLs) have allowed cataract surgery and IOL implantation to be performed through incisions of 2.4 to 3.0 mm. Surgeons are now experimenting with even smaller incisions. We began using a standard phacoemulsification and aspiration (PEA) device to perform bimanual PEA with a sleeveless phaco tip through an incision 1.2 to 1.4 mm wide. We used an irrigating hook through a side port to infuse the anterior chamber. After the lens was extracted, we could safely implant a hydrophobic acrylic single-piece IOL through a 1.8-mm incision. We are able to choose various premium IOLs, for example multifocal IOLs, toric IOLs, and yellow IOLs. We implant these new IOLs and evaluate visual functions with them.

#### *Neuro-ophthalmology*

1. Optic neuropathies caused by toxic substances, especially ethambutol, were explained in detail in the section on optic pathway disorders of *Management Manuals for Serious Adverse Effects* published by the Japan Pharmaceutical Information Center.
2. Neuro-ophthalmological pathophysiology was reviewed in a textbook for medical students on receptors for vision and the visual pathways, light reflex, near response (convergence reflex), corneal reflex, nystagmus, choked disc, optic neuropathy, and perimetry and visual field defects. An outline of general ophthalmology was prepared for a textbook for pharmacology students on visual disturbances and diagnostic approaches to eye diseases.

3. We reported a case of pediatric optic neuritis with thalamic lesions (clinically isolated syndrome), cases of optic neuritis associated with hypophysitis, pupillary responses to blue light stimulus in macular detachment using a novel computerized pupillometry, and clinical features of anti-aquaporin 4 antibody-positive optic neuritis.

#### *Ocular oncology and histopathology*

We reported rare cases of subretinal giant hematoma improved by spontaneous hemorrhage into the vitreous cavity, a mixed tumor of the lower eyelid, compressive optic neuropathy due to an intraorbital tumor associated with von Hippel-Lindau disease, and a tumor of the lacrimal drainage system.

#### *Glaucoma*

1. The purpose of treatment in glaucoma is to maintain visual function, and clear evidence for the success of treatment is a reduction in intraocular pressure (IOP). We have used eyedrops as medical treatment. Operative treatment is usually performed when glaucoma is resistant to medical treatment. On the other hand, the operation for glaucoma changes the shape of the cornea, exacerbates astigmatism, and decreases the quality of vision. Astigmatism can be divided into types that can or cannot be corrected with lenses (eyeglasses). Therefore, examining which type of astigmatism is increasing is important. A device for analyzing the shape of the cornea was recently developed and has allowed astigmatic quality to be measured in greater detail. We examine corneas with the Orbscan (Bausch & Lomb, Rochester, NY, US) and OPD-Scan (Nidek Co., Ltd., Gamagori, Japan) devices.

2. Because glaucoma requires long-term treatment with eyedrops, patient compliance is important. Beta-adrenergic receptor antagonist eyedrops have been used. Twice-daily treatment has been standard, but several types of once-daily eyedrops that can decrease IOP have recently been marketed. However, eye stimulation and foggy vision became problems, because once-daily eyedrops are gels. Some agents, such as carteolol hydrochloride durability drops (Mikelan LA, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), contain alginic acid, which leads to fewer side effects (stimulation and foggy vision). Therefore, we examined the effects of a change from twice-daily carteolol hydrochloride drops to once-daily carteolol hydrochloride durability eyedrops on IOP and the feelings of use in a patient with glaucoma. The change in eyedrops improved patient compliance and convenience, but no significant change in IOP was noted until 6 months later.

3. Numerous studies have shown that IOP measured with the subject in the sitting position is high in the morning and low in the afternoon and evening. The IOP increases by as much as 2 to 6 mm Hg when the subject, either a healthy person or a patient with glaucoma, lies flat. Recent data incorporating the concept of the habitual body position — sitting during waking hours and supine during sleeping hours — have demonstrated that peak IOP is most likely to occur at night while the subject is supine. The progression of visual field damage in normal-tension glaucoma is associated with IOP in the supine position and the magnitude of IOP elevation accompanying postural changes. It would be beneficial if treatments were available that could specifically

decrease the supine IOP, resulting in less fluctuation in IOP due to postural change. However, the agents timolol maleate, latanoprost, and brinzolamide lower IOP in both the sitting and supine positions but do not alter the response of IOP to postural change. The postural response is unaffected by trabeculectomy without mitomycin C and argon laser trabeculoplasty.

In patients with primary open-angle glaucoma or normal-tension glaucoma, we evaluated the postural change in IOP following trabeculectomy with mitomycin C. By means of a pneumatonometer, IOP was measured after the subject had been in the sitting position for 5 minutes and after the subject had been in the supine position for 10 minutes. The sitting IOP and the 10-minutes supine IOP were  $10.2 \pm 3.3$  mm Hg and  $13.7 \pm 4.5$  mm Hg, respectively. The difference between 10-minutes supine IOP and the sitting IOP ( $\Delta\text{IOP}_{10\text{min}}$ ) was  $3.43 \pm 1.8$  mm Hg ( $p < 0.05$ ). There was a significant correlation between sitting IOP and  $\Delta\text{IOP}_{10\text{min}}$  ( $r = 0.66$ ,  $p < 0.0001$ ). The lower the sitting IOP was, the lower  $\Delta\text{IOP}_{10\text{min}}$  was.

#### *Functional neuroimaging*

Patients with glaucoma were examined to confirm several structural changes in the visual pathway by means voxel-based morphometry. The chiasm was evaluated with 3-dimensional T1-weighted images, and the structure of the optic radiation was evaluated with diffusion tensor imaging. Both types of images were acquired with a magnetic resonance scanner. Ten patients with glaucoma and 10 age-matched healthy volunteers were recruited. A significant decrease in signal strength was observed with voxel-based morphometry in areas corresponding to the optic chiasm and the optic radiation in patients with glaucoma. Thus, these results suggest that structural changes occur in the visual pathway of the intracranial structures as well of the eyes in patients with glaucoma.

#### *Developmental functional abnormality*

Binocular summation on the visual cortex was explored with postoperative functional magnetic resonance imaging (fMRI) in 2 patients with strabismus. One patient, in whom central stereopsis was preserved, demonstrated binocular summation at the foveal projection area and a peripheral 4-degree projection area. Another patient, who lacked central stereopsis, demonstrated binocular summation not at the foveal projection area. This result suggests that cortical suppression of the foveal projection area was present in the latter patient.

#### *Visual neuropsychology*

##### 1. Review articles on visual psychology and neuro-ophthalmology

We wrote review articles that summarized contemporary topics about visual information processing in the primary visual cortex (V1), plasticity in the visual cortex, photophobia, visual experience during dreaming, and cortical visual prosthesis.

##### 2. Follow-up report for plasticity in the adult human V1

We extended the measurement to subjects with retinitis pigmentosa. Our results were the same as in patients with macular degeneration; there was no large-scale remapping

in the adult human V1. Our results support vision-restoring therapies that rely on the stability of the human V1.

### 3. Publishing 3 papers in international journals

Three of our research studies previously reported here have been published: objective perimetry with fMRI (*Experimental Neurology*; impact factor=3.9); 2 temporal channels in human V1 identified with fMRI (*NeuroImage*; impact factor=5.7); and evaluation of subjective color sense after cataract surgery from the super early state (15 minutes after the removal of an eye patch) (*Journal of the Optical Society of America*; impact factor=1.9).

### *Vitreoretinal diseases*

We have used a 23-gauge and 25-gauge transconjunctival vitrectomy system for treating macular holes, epiretinal membranes, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease surgical trauma and improve patient comfort postoperatively. The 25- and 23-gauge instrumentation is effective for a variety of vitreoretinal surgical indications. Although the infusion and aspiration rates of the 25- and 23-gauge instruments are lower than those of the 20-gauge high-speed vitrectomy system, the use of 25- and 23-gauge transconjunctival vitrectomy system may effectively reduce operative times for selected patients who do not require the full capability of conventional vitrectomy.

To evaluate the clinical efficacy of a 7-mm IOL (Eternity®, Santen Pharmaceutical Co., Ltd., Osaka, Japan) for combined pars plana vitrectomy, phacoemulsification, and IOL implantation, we observed the visibility of the retina during vitrectomy and measured the depth of the anterior chamber preoperatively and postoperatively with a tomographic scanner (Pentacam, Oculus Optikgeräte GmbH, Wetzlar, Germany)

We are planning to evaluate the changes in regular and irregular corneal astigmatism after 25-gauge and 23-gauge transconjunctival sutureless vitrectomy.

### *Electrophysiology*

We are recording electroretinograms (ERGs) to evaluate retinal function for hereditary retinopathy, retinal dystrophy, and macular disease. The ERG waveforms compound the responses from various retinal cells, such as ganglion, amacrine, bipolar, and photoreceptor cells, which are recorded as a single wave pattern. In addition, we perform the examination with 4 types of recording system, such as the Ganzfeld stimulator, multifocal stimulation, color stimulation, and focal macular stimulation. In Ganzfeld stimulation, we record separate responses from cone and rod cells of the retina according to an international protocol. The multifocal stimulator, which reflects cone function, can record the responses of each separate element in 61 areas of the central 30 degrees of the posterior pole and compare with visual field examination. The color ERG records each response to separate long- and middle-wavelength and short-wavelength cones. We have recently obtained a focal macular stimulator. This stimulator can record the retinal function of the central 5, 10, and 15 degrees and can effectively search for unidentified conditions, such as occult macular dystrophy, causing visual disturbance.

In the future, we will evaluate the waveforms recorded from these ERG stimulators and analyze them further with personal-computer programs. Moreover, as we extract single waveforms from retinal cells of a specific type, we will be able to investigate retinal disorders at the cellular level.

#### *Diabetic retinopathy section*

We perform subtenon injections of triamcinolone acetonide for outpatients with diabetic macular edema. After injection, a decrease in macular retinal thickness can be observed with optical coherence tomography, but in some cases macular edema recurs 3 months after injection. For cases in which triamcinolone acetonide injection has no effect, we perform vitrectomy. The transconjunctival microincision vitrectomy is performed with a 23-gauge trocar system. The 23-gauge system is used to make a scleral incision that does not need to be sutured. The advantages of such small incisions include decreased postoperative inflammation and decreased surgical stress.

The vulnerability of retinal ganglion cells in diabetes mellitus has been observed in diabetic animal models and in patients. We are evaluating retina function by recording ERGs in patients with diabetes in whom retinopathy is absent on ophthalmoscopy. We measured photopic negative response (PhNR) among wave patterns obtained in cone ERGs and examined the correlation between the PhNR and the duration of diabetes. We are measuring the thickness of the nerve fiber layer with optical coherence tomography and are investigating the correlation between the thickness of the nerve fiber layer and PhNR amplitude or implicit time or both.

#### *Uveitis*

1. A novel therapy with a chimeric antibody against tumor necrosis factor alpha for Behçet disease

Intravenous infliximab significantly decreased the frequency of ocular attacks and improved visual acuity. In addition, we believe that intraocular surgery can be performed effectively and safely to improve vision in patients receiving infliximab therapy for Behçet disease.

2. Intravitreal bevacizumab for idiopathic choroidal neovascularization

We assessed the long-term visual and anatomical outcomes and safety of intravitreal injection of bevacizumab for idiopathic choroidal neovascularization. The long-term results suggest an encouraging efficacy and safety of intravitreal bevacizumab for idiopathic choroidal neovascularization.

3. Evaluation of S-cone sensitivity with SITA-SWAP in patients with Vogt-Koyanagi-Harada syndrome

We investigated macular function with Swedish Interactive Thresholding Algorithm-short-wavelength automated perimetry in patients with Vogt-Koyanagi-Harada syndrome. Our results suggest that the measurement of the sensitivities of the short-wavelength-sensitive cones (S-cones) with short-wavelength automated perimetry is a sensitive method for evaluating visual recovery in this syndrome.

*Macular degeneration*

1. Endothelin (ET)-1 is a potent vasoconstrictor peptide whose levels are increased in diseases associated with vascular dysregulation. The pathogenesis of neovascular age-related macular degeneration (nAMD) is poorly understood but may involve vascular dysregulation and vasoconstriction of the nutrient vessels supplying the choroid. To clarify the possible pathophysiological role of ET-1 in the development of nAMD, we examined plasma ET-1 levels in patients with nAMD. We found increased plasma levels of ET-1 in patients with nAMD. Our data suggest that elevated plasma ET-1 is an important risk factor for nAMD and that an ET receptor antagonist might offer a new therapeutic approach to this disease.

2. Intravitreal pegaptanib for nAMD

We assessed the short-term visual and anatomical outcomes and safety of intravitreal injection of pegaptanib for nAMD. The short-term results suggest an encouraging efficacy and safety of intravitreal pegaptanib for nAMD.

3. Photodynamic therapy alone versus photodynamic therapy combined with intravitreal bevacizumab for nAMD without polypoidal choroidal vasculopathy in Japanese patients

We compared 3-month results of 2 treatments — photodynamic therapy (PDT) with verteporfin alone or combined with intravitreal bevacizumab — for patients with nAMD but excluding patients with polypoidal choroidal vasculopathy (PCV), who were presumed to have AMD. After 3 months, PDT combined with intravitreal bevacizumab for Japanese patients with AMD without PCV appeared to be more effective and required fewer treatments than PDT alone.

4. PDT with verteporfin for PCV with good visual acuity

We evaluated the efficacy of PDT with verteporfin in Japanese patients with PCV and good visual acuity. The results show that PDT is effective for patients with PCV and good visual acuity.

*Biochemistry*

1. We evaluated the anti-inflammatory effects of intravenously administered methoxy polyethylene glycol- (D, L-lactide) (PLA-PEG) stealth nanoparticles encapsulating betamethasone phosphate (BP) on experimental autoimmune uveoretinitis in Lewis rats. Systemically administered BP-stealth nanoparticles reduced the expression of inflammatory cytokines and vascular endothelial growth factor in the retina and choroid. In conclusion, systemically administered BP-stealth nanoparticles should be useful for treating macular edema and choroidal neovascularization in uveitis.

2. We investigated the properties of the immunocytochemical findings of retinal degeneration in a transgenic rabbit with a Pro347Leu rhodopsin mutation. By 12 weeks of age in the transgenic rabbits, there was already a substantial loss of rods and activation of Müller cells. At the same time, rod bipolar cell processes grew into the inner and outer nuclear layers. Rod bipolar cell processes lengthen during photoreceptor degeneration in rhodopsin Pro347Leu transgenic rabbits. These findings may demonstrate inner retinal remodeling associated with photoreceptor degeneration.

### *Color vision defects and genetic analysis of retinal diseases*

1. We performed genetic testing of a woman with X-linked red-green color vision deficiency and deuteranomalous trichromacy. A 22-year-old woman (proband) and her parents were included in this study. A red-green color vision defect was diagnosed with a Nagel type I anomaloscope. Whether the color vision defects were of a mild or severe form was determined with the Farnsworth Panel D-15 test. Genotypes of L and M visual pigment genes were determined with the polymerase chain reaction. The difference in the peaks of absorbance ( $I_{\max}$ ) between the first 2 visual pigment genes was calculated. The proband and her father were found to have deuteranomalous trichromacy (mild form). Her mother was an obligate carrier of deuteranomaly because she had normal color vision. Molecular genetic analysis revealed that the proband had 2 distinct M-L hybrid genes. The first 2 expressed pigments from each X-chromosome gene array which differed in  $I_{\max}$  by 4 nm and 8 nm, respectively. Our results suggest that it is possible to determine the genotype of a female proband with deuteranomaly when both genetic and color vision testing are performed in other family members.
2. We performed clinical and molecular genetic analysis of various inherited retinal diseases, such as retinitis pigmentosa and macular and cone dystrophies. We identified causative mutations in these diseases. To clarify disease haplotypes, haplotype analysis with mutations was compared between family members and control subjects.
3. We investigated the involvement of various genetic factors in Japanese patients with AMD, which is a common cause of blindness in elderly persons of industrialized countries. Over 500,568 single nucleotide polymorphisms of the entire genome were genotyped with the Affymetrix Human Mapping Arrays (Affymetrix, Santa Clara, CA, USA) and TaqMan assay (Applied Biosystems, Inc., Carlsbad, CA, USA). We are now analyzing candidate single nucleotide polymorphisms in Japanese patients with AMD.

### *Cornea*

The cornea group at The Jikei University chooses the most appropriate corneal surgery by discussing the various options with each patient. We perform Descemet's stripping automated endothelial keratoplasty for corneal endothelium dysfunction.

We have adapted several new treatments for all corneal diseases, for example dry eye, corneal infection, corneal injury, hereditary corneal disease, allergic corneal disease, and keratoconus. Automated lamellar therapeutic keratectomy, in which a microkeratome is used to make a lamellar flap, was performed for several patients with corneal opacity. We found that automated lamellar therapeutic keratectomy enables earlier suture removal and induces less astigmatism than does conventional lamellar keratoplasty.

We studied the clinical outcomes of secondary implantation of iris-clip IOLs for aphakic eyes 5 years postoperatively. Clinically significant complications were not found with specular microscopy or laser flaremetry.

### **Publications**

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