ISOT 2014

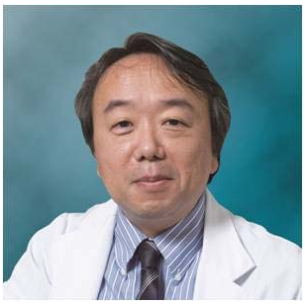
13th Congress of the International Society of Ocular Toxicology

Entering an era of novel research in ocular toxicology

October 9-12, 2014

Kanazawa Bunka Hall / Ishikawa, Japan

WELCOME MESSAGE



Hiroshi Sasaki, MD, PhD
President, ISOT 2014

Dear colleagues,

It is with great pleasure that we host the 13th Congress of the International Society of Ocular Toxicology. We are grateful to the committee members for giving us this wonderful opportunity. Indeed, we are honored to be involved in the preparations.
Since this is the first ISOT Congress to be held outside of the Euro-American sphere, we were concerned there would be few participants from overseas, but we have received 43 registrations from overseas and 55 from Japan as of the end of July. For 4 days from the afternoon of October 9 to the morning of October 12, the program will include 65 abstracts; 1 keynote speech, 47 oral presentations and 17 posters. The keynote speech will be presented by Prof. Frederick T Fraunfelder, Oregon Health and Science University, with the title “An overview of how ISOT was started, how it progressed and it’s possible future”, including a review of the history of ISOT and some future options. We expect that many ophthalmologists and researchers, who have not attended previous ISOT congresses, will participate in the ISOT 2014 and present new research related to ocular toxicity in a fresh dimension. The ISOT 2014 theme might be described as “*Entering an era of novel research in ocular toxicology*”.
As Kyoto, Kanazawa is also an ancient capital of Japan. It is an acclaimed historical city that regularly attracts both foreign and domestic tourists. We have planned some events including sightseeing, traditional Japanese Noh theater, tea ceremony, and others to ensure you enjoy Kanazawa during the congress period. You can also enjoy city walking before, during, and after the congress, as Kanazawa is a small city enabling one to stroll around for pleasurable sightseeing. In addition there are abundant outlets where you may enjoy seafood, good Japanese sake, beautiful Japanese-style confectionery, and best traditional crafts in Kanazawa.

On behalf of the ISOT, I welcome your participation.

See you all in Kanazawa.

Yours sincerely,

GENERAL INFORMATION

Venue
Kanazawa Bunka Hall
15-1 Takaoka-machi, Kanazawa, Ishikawa 920-0864, JAPAN

Official Language
English is the official language of the congress.

Registration and Information Desk
Registration and information desk opens on the 2nd floor of Kanazawa Bunka Hall as below;

From 10:00 a.m. on October 9 (Thursday), 2014
From 8:30 a.m. on October 10 (Friday), 2014
From 8:00 a.m. on October 11 (Saturday), 2014

Registration Fee (per person)

Payment in U.S. dollars

Category	On-site
Participants	US$ 400
Residents	US$ 150
Students	US$ 100
Health Professionals*	US$ 150
Exhibitors	US$ 300
Accompanying Guests	US$ 150

Payment in Japanese yen

Category	On-site
Participants	40,000 JPN Yen
Residents	15,000 JPN Yen
Students	10,000 JPN Yen
Health Professionals*	15,000 JPN Yen
Exhibitors	30,000 JPN Yen
Accompanying Guests	15,000 JPN Yen

*Health Professionals: Nurses, Pharmacologists (non-researchers), Orthoptists

Congress Bag and Name Badge
Upon registering you will receive a congress bag containing your personal name badge. Your name badge is your entrance ticket to all sessions and you are asked to wear it throughout the congress. Should you misplace your badge, a replacement can be obtained at the registration and information desk.

Certificate of Attendance — A certificate of attendance will be available at the registration and information desk.

Points for Japanese Ophthalmic Specialists — Japanese ophthalmologists may acquire scientific points valid for the ophthalmic specialist of the Japanese Ophthalmological Society, at the desk next to the registration and information desk.

Lunches — Lunches are included in the registration fee and are served at the restaurant "ポロ (PoLo)" on the 2nd floor in *Kanazawa New Grand Hotel - Annex*.

Coffee Breaks — Drink fees for coffee breaks are included in the registration fee and are served in the poster session room on the 2nd floor.

Internet Facilities — Free internet access is available in the lobby areas of the 2nd and 3rd floors, main hall and sub hall on the 3rd floor, poster session room on the 2nd floor throughout the congress period.

Information for Presenters

Oral Presentation — The speakers preview room is situated next to the main hall on the 3rd floor and opens from 10:00 a.m. on October 9 (Thursday) and from 8:00 a.m. on other days.
We strongly advise all speakers to give a copy of your presentation data (PowerPoint), on USB flash memory or CD-ROM or DVD, to the engineers in the preview room at least a day before your presentation, to avoid any technical problems. Please be present in the preview room no later than 1 hour before the start of your session.

Posters — Posters will be presented in the poster session room on the 2nd floor. All presenters are asked to set up your poster in the afternoon of October 9 (Thursday). Posters will be continuously presented throughout the congress.
As poster presenters have 3 minutes for oral presentation of a few slides in the main hall on October 10 (Friday) from 3:30 to 4:21 p.m., we strongly advise all presenters to give a copy of your presentation data (PowerPoint), on USB flash memory or CD-ROM or DVD, to the engineers in the preview room on October 9 (Thursday), to avoid any technical problems. Please be present in the preview room no later than 1 hour before the start of your poster oral presentation. After your poster oral presentation, poster discussion will be held in front of your poster on the 2nd floor.

Exhibition — Machine exhibition is located at the sub hall on the 3rd floor.

Credit Cards Credit cards cannot be used at the congress hall (including registration). Commonly accepted cards may be used in hotels, shops, and restaurants. Credit cards: Visa, MasterCard, AMEX, etc.

Banking Facilities Several banks have facilities within one block of the congress hall. Opening times are Monday to Friday from 9:00 a.m. to 3:00 p.m.

Social Program

October 9, Thursday

17:00 - 18:00 Committee Meeting at the "カトレア(Cattleya)" on the mezzanine (M2) floor in *Kanazawa New Grand Hotel - Palace*
* Committee members should arrive by 17:00.

18:30 - 21:00 Get Together at the "相生(Aioi)" on the 4th floor in *Kanazawa New Grand Hotel - Main building*
* All participants, please be sure to get together in this reception, which is included in the registration fee.

October 10, Friday

10:30 - 15:00 Tea Ceremony at the Tea Ceremony Room "閑清庵(Kanseian)" on the 1st floor in *Kanazawa Bunka Hall*
* Only applicants who are interested.

October 11, Saturday

10:30 - 12:00 Tea Ceremony at the Tea Ceremony Room "閑清庵(Kanseian)" on the 1st floor in *Kanazawa Bunka Hall*
* Only applicants who are interested.

12:20 - 12:30 Business Meeting in the main hall on the 3rd floor in *Kanazawa Bunka Hall*

14:00 - 18:00 Outing
(Please meet in the main lobby (front desk) of *Kanazawa New Grand Hotel - Main building* by 13:45.)

19:00 - 21:00 Banquet at the "銀扇(Ginsen)" on the 5th floor in *Kanazawa New Grand Hotel - Palace*
* All participants, please attend this banquet, which is included in the registration fee.

PROGRAM OVERVIEW

	Oct. 9 (Thu)	Oct. 10 (Fri)	Oct. 11 (Sat)	Oct. 12 (Sun)
8:00			8:00~ **Registration Desk Open**	
		8:30~ **Registration Desk Open**	**8:30~ SESSION 6: AMD**	
9:00		**9:00~ SESSION 3: Cornea and contact lens studies**	9:40~10:00 Coffee Break	**9:00~ SESSION 8: ERG and vitreoretinal disease**
10:00	10:00~ **Registration Desk Open** (2nd floor, Kanazaw a Bunka Hall)	10:25~10:50 Coffee Break	**10:00~ SESSION 7: Phototoxicity, environmental electromagnetic radiation**	10:25~10:45 Coffee Break
11:00		**10:50~ SESSION 4: Lens related studies**	**11:40~ KEYNOTE SPEECH**	**10:45~ SESSION 9: Anti-glaucoma therapeutics**
12:00		12:30~13:45 Lunch (“ポロ(PoLo)”, 2nd floor, KNGH – Annex)	12:20~12:30 **Business Meeting**	11:40~11:45 **Closing Remarks**
13:00	13:00~ **Opening Remarks**		12:30~13:40 Lunch (“ポロ(PoLo)”, 2nd floor, KNGH – Annex)	
	13:10~ SESSION 1: Supplement & drug toxicity	**13:45~ SESSION 5: Drug-related toxicity in eye**		
14:00			14:00~18:00 **Outing**	
	14:50~15:15 Coffee Break			
15:00	**15:15~ SESSION 2: Preservative related studies**			
		15:30~16:21 Poster Oral Presentation		
16:00		**16:30~17:30 Poster Discussion** & Coffee Break		
17:00	17:00~18:00 **Committee Meeting** (“カトレア(Cattleya)”, M2 floor, KNGH - Palace)	Free Evening		
18:00				
	18:30~21:00 **Get Together** (“相生(Aioi)”, 4th floor, KNGH - Main building)		19:00~21:00 **Banquet** (“銀扇(Ginsen)”, 5th floor, KNGH - Palace)	
19:00				
20:00				
21:00				

10:30~ 15:00 Tea Ceremony *

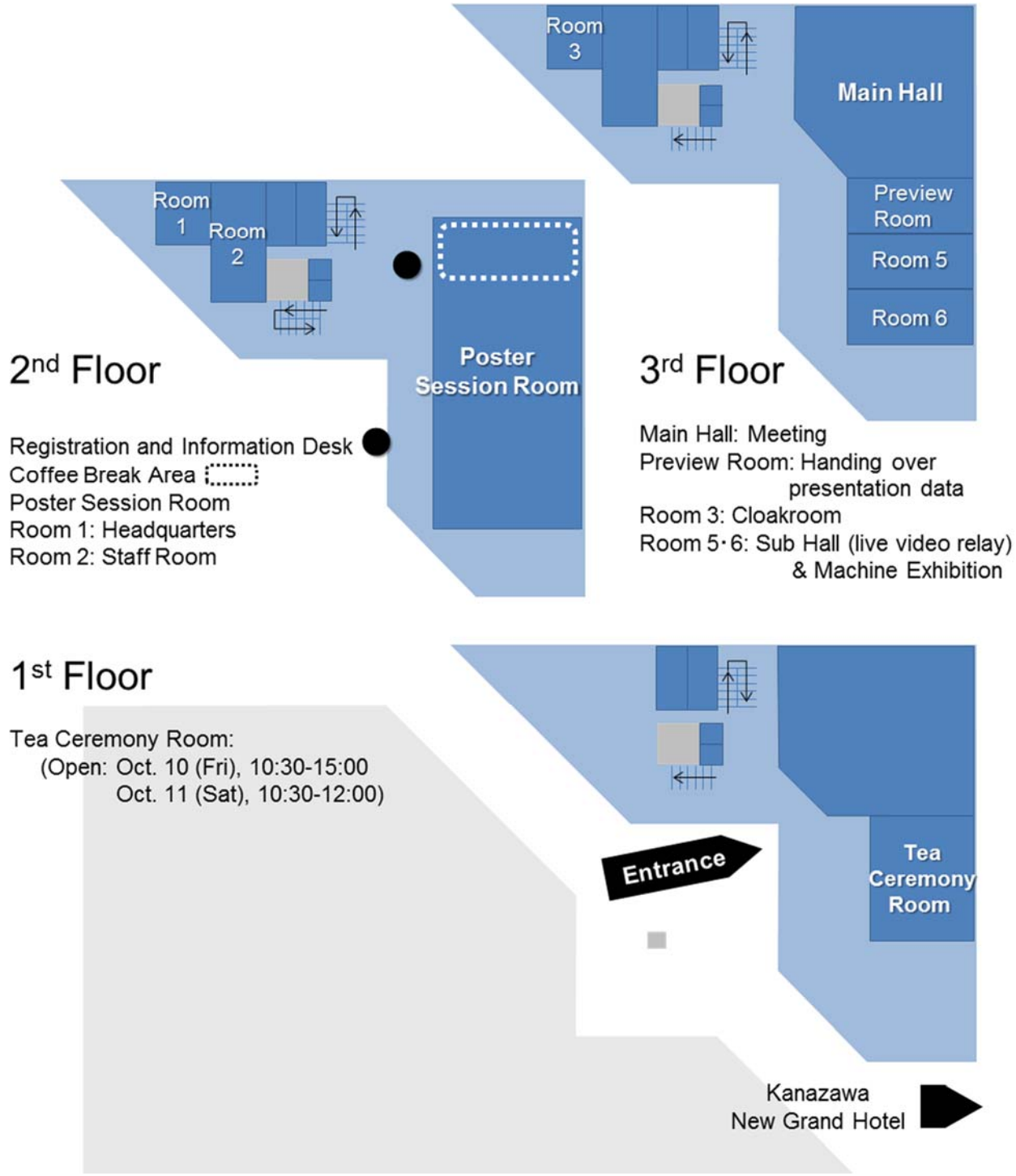
10:30~ 12:00 Tea Ceremony *

KNGH = Kanazawa New Grand Hotel

* Only applicants who are interested

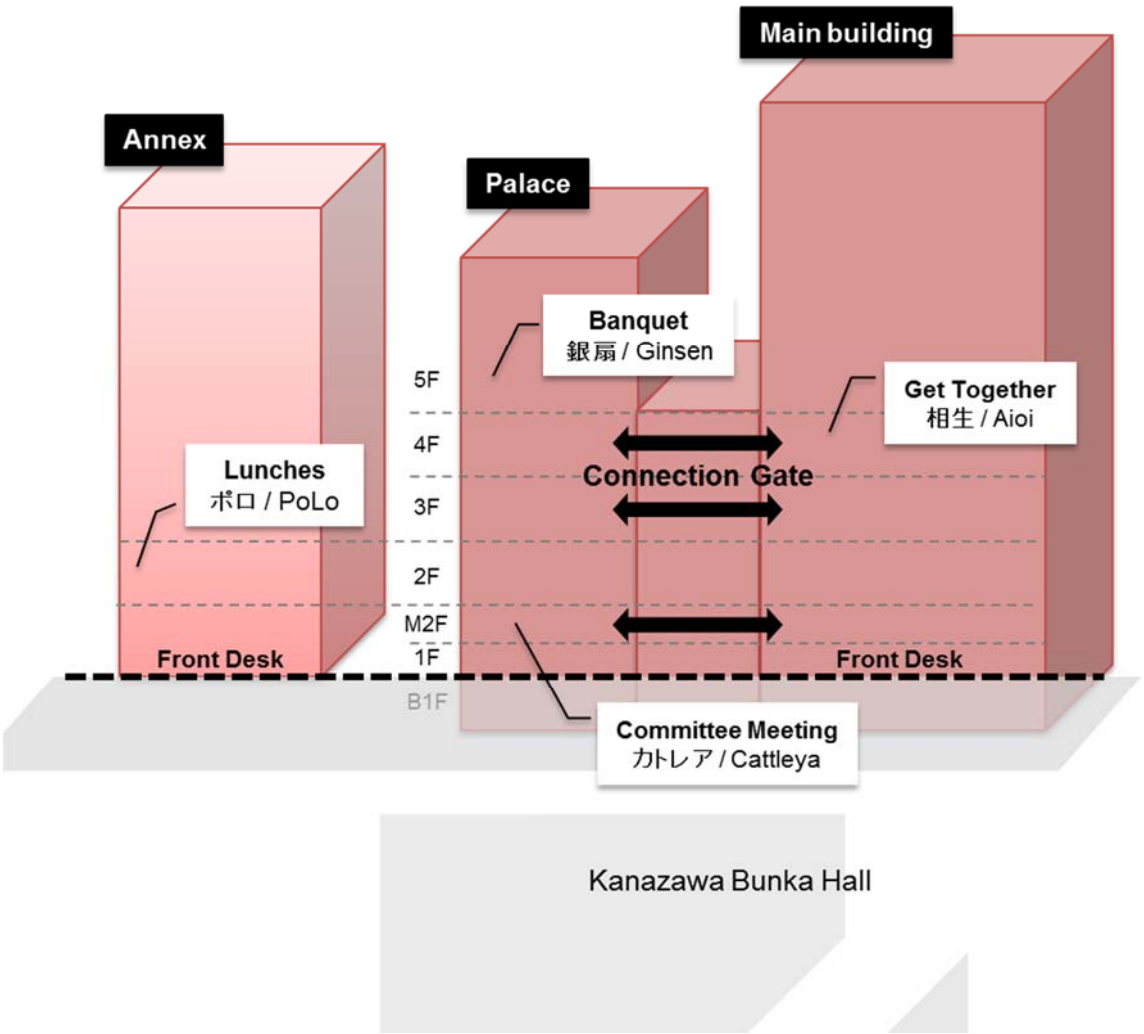
CONGRESS VENUE / EXHIBITION

Kanazawa Bunka Hall



Kanazawa New Grand Hotel *Main building, Palace and Annex*

Committee Meeting:	“カトレア/Cattleya” on the mezzanine (M2) floor, Palace 17:00-18:00 on Oct. 9 (Thu)
Get Together:	“相生/Aioi” on the 4th floor, Main building 18:30-21:00 on Oct. 9 (Thu)
Lunches:	“ポロ/PoLo” on the 2nd floor, Annex 12:30-13:45 on Oct. 10 (Fri) 12:30-13:40 on Oct. 11 (Sat)
Outing:	Meeting point at the main lobby (Front Desk), Main building 14:00 on Oct. 11 (Sat)
Banquet:	“銀扇/Ginsen” on the 5th floor, Palace 19:00-21:00 on Oct. 11 (Sat)



SCIENTIFIC PROGRAM

October 9 (Thursday)

10:00	**Registration Desk Open**		
			2nd floor, Kanazawa Bunka Hall
13:00	**Opening Remarks**		
			Hiroshi Sasaki, President, ISOT 2014
13:10	**SESSION 1: Supplement & drug toxicity**		
			Chairperson: Peter F. Kador, Eri Kubo
			* Introductory Talk: 20 minutes
13:10-13:30	*1)	*In vitro* lens culture studies: A potent research tool for assessing chemical toxicity	Peter F. Kador USA
13:30-13:50	*2)	Risk and benefit of supplement for vision and eye health	Eri Kubo Japan
13:50-14:05	3)	Effect of supplementary water contains minerals on corneal wound healing rate in ovariectomized rats	Noriaki Nagai Japan
14:05-14:20	4)	Non-toxic polymethoxyflavones for prevention of posterior capsular opacification	Yoshiki Miyata Japan
14:20-14:35	5)	Spontaneous ocular abnormalities in rats from longterm toxicity studies	Ursula Müller-Breitenkamp Germany
14:35-14:50	6)	Antioxidant effect of caffeine in the lens	Per G. Söderberg Sweden
14:50-15:15	Coffee Break		
			2nd floor, Kanazawa Bunka Hall
15:15	**SESSION 2: Preservative related studies**		
			Chairperson: Brenda J. Tripathi, Kazuhide Kawase
15:15-15:35	*1)	Cytotoxicity of ophthalmic preservatives	Brenda J. Tripathi USA
15:35-15:55	*2)	The effect of human cultured conjunctiva cells by anti glaucoma eye drops	Kazuhide Kawase Japan
15:55-16:10	3)	Ocular toxicity of benzalkonium chloride homologs compared with their mixtures and new approach to avoid corneal injury caused by preservatives	Yoshihiko Esaki Japan
16:10-16:25	4)	Benzalkonium chloride of alkyl chain length C12 is safer for corneal epithelium	Masafumi Uematsu Japan
16:25-16:40	5)	Evaluation of mucin barrier induced by benzalkonium chloride on the ocular surface Epithelium	Yuichi Hori Japan

17:00-18:00	**Committee Meeting** “カトレア(Cattleya)”, M2 floor, Kanazawa New Grand Hotel – Palace
18:30-21:00	**Get Together** “相生(Aioi)”, 4th floor, Kanazawa New Grand Hotel – Main building

October 10 (Friday)

8:30 **Registration Desk Open**

2nd floor, Kanazawa Bunka Hall

10:30-15:00 Tea Ceremony
Tea Ceremony Room “閑清庵(Kanseian)”, 1st floor, Kanazawa Bunka Hall

9:00		**SESSION 3: Cornea and contact lens studies** *Chairperson: Hong-Ming Cheng, Motozumi Itoi*	
9:00-9:20	*1)	Contact lens related ocular toxicity	Hong-Ming Cheng Taiwan
9:20-9:40	*2)	Eye complications of color soft contact lens wear	Motozumi Itoi Japan
9:40-9:55	3)	Microbial adhesion to beauty contact lenses	Pauline Cho Hong Kong
9:55-10:10	4)	Confounding corneal lesions in toxicology studies in Dutch-belted rabbits	JoAnn C.L. Schuh USA
10:10-10:25	5)	Does smoking affect corneal endothelial cell density?	Gunnar M. Zoega Iceland
10:25-10:50	Coffee Break		

2nd floor, Kanazawa Bunka Hall

10:50		**SESSION 4: Lens related studies** *Chairperson: John Sparrow, Hiroshi Sasaki*	
10:50-11:10	*1)	Human lens opacities - Retrodots	John Sparrow UK
11:10-11:30	*2)	Visual function of eyes by five types of lens opacities	Hiroshi Sasaki Japan
11:30-11:45	3)	Use of the Casey Eye Institute camera for recording lens opacity sub-types and correlation with vision quality	Peter N. Steinkamp USA
11:45-12:00	4)	The variation of oxidative levels in aluminum attached on intraocular lenses using a perfusion culture system	Rijo Hayashi Japan

12:00-12:15	5)	Data-analysis pre-study of initial lenticular findings in emergency workers at Tokyo Electric Power Fukushima Nuclear Power Plant	Hiromi Osada Japan
12:15-12:30	6)	Experimental postoperative endophthalmitis using aluminum sticking IOLs	Hiroyuki Matsushima Japan
12:30-13:45	Lunch "ポロ(PoLo)", 2nd floor, Kanazawa New Grand Hotel – Annex		
13:45	**SESSION 5: Drug-related toxicity in eye** *Chairperson: Rick W. Fraunfelder, Kazuko Kitagawa*		
13:45-14:05	*1)	Drug related immunotoxicity in ophthalmology	Rick W. Fraunfelder USA
14:05-14:25	*2)	Adverse effects of topical anti-glaucomatous agents in patients with severe dry eye	Kazuko Kitagawa Japan
14:25-14:40	3)	Dacryoendoscopic observation and incidence of canalicular obstruction/stenosis associated with S-1, an oral anticancer drug	Tsugihisa Sasaki Japan
14:40-14:55	4)	Intracameral antibiotics for the prevention of post-cataract surgery endophthalmitis	Kevin Winthrop USA
14:55-15:10	5)	Current situation of nonclinical ocular toxicity assessment in Japanese pharmaceutical industry	Masayuki Tomohiro Japan
15:10-15:25	6)	Utilizing of ocular anatomical features of laboratory animals; for the extrapolation of animal data to human	Shingo Nemoto Japan
15:30	**Poster Oral Presentation** *Chairperson: Alfred R. Wegener, Masami Kojima*		
15:30-15:33	1)	Cytotoxic effects of 4 OTC ophthalmic solutions on cultured rabbit corneal cell line	Masamichi Fukuda Japan
15:33-15:36	2)	Comparison of toxicities of moxifloxacin, cefuroxime, and levofloxacin on corneal endothelial cells in vitro	Kazuki Matsuura Japan
15:36-15:39	3)	Lens pathological change after intravitreal injection of bevacizumab	Atsushi Arimoto Japan
15:39-15:42	4)	Side-effects associated with cycloplegic myopia control in Taiwan	Ching-Ying Cheng Taiwan
15:42-15:45	5)	*In vivo* confocal microscopic observation of patients with amiodarone induced keratopathy and Fabry disease	Yasuhito Ikegawa Japan
15:45-15:48	6)	Effect of dry-eye ophthalmic solutions in intraoperative desiccation-induced corneal conjunctival damage	Norihito Gotoh Japan
15:48-15:51	7)	Decrease in corneal damage due to benzalkonium chloride by the addition of mannitol	Chiaki Yoshioka Japan

15:51-15:54	8)	Effect of travoprost on expression of EGF and corneal epithelial wound healing	Yukihisa Takada Japan
15:54-15:57	9)	The effects of lychee flower against lipopolysaccharides-treated rabbit corneal epithelial cell	Jung-Kai Tseng Taiwan
15:57-16:00	10)	Lucentis update 2014 - Posology of Lucentis in US/EU/Japan	Shigeru Kabutoya Japan
16:00-16:03	11)	Visual complaints from cycloplegic control of myopia in elementary school students in Taiwan	Ching-Chung Chen Taiwan
16:03-16:06	12)	Comparison of intraocular and ocular surface bacterial isolates and their antibiotic resistance patterns at a tertiary eye hospital in South China	Kaili Wu China
16:06-16:09	13)	Eye disorders associated with colored contact lenses: 3 case studies	Shinsuke Shibata Japan
16:09-16:12	14)	Measurement of ocular UV exposure with mannequin UV sensors	Natsuko Hatsusaka Japan
16:12-16:15	15)	Visual function of cataract sub-types	Norihiro Mita Japan
16:15-16:18	16)	Auto-fluorescence is an early sign of developing pinguecula	Naoko Shibata Japan
16:18-16:21	17)	The role of the JNK/c-Jun and P38 pathways in the protective effect of hydrogen peroxide on RGC-5 cells from serum deprivation-induced apoptosis	Wen Hua Zheng China
16:30-17:30	**Poster Discussion** & Coffee Break 2nd floor, Kanazawa Bunka Hall		
	Free Evening		

October 11 (Saturday)

8:00	**Registration Desk Open** 2nd floor, Kanazawa Bunka Hall		
	10:30-12:00 Tea Ceremony Tea Ceremony Room “閑清庵(Kanseian)”, 1st floor, Kanazawa Bunka Hall		
8:30	**SESSION 6: AMD** *Chairperson: Fridbert Jonasson, Tatsuo Hirose*		
8:30-8:50	*1)	Five-year incidence and progression of age related macular degeneration (AMD) and associated risk factors in Icelanders	Fridbert Jonasson Iceland

8:50-9:10	*2)	Treatment of neovascular age related macular degeneration (Wet AMD) with intravitreous anti-VEGF agents	Tatsuo Hirose USA
9:10-9:25	3)	Lutein and zeaxanthin supplementation reduces photo-oxidative damage to retinal pigment epithelial cells	Fu Shang China
9:25-9:40	4)	*In-vivo* imaging of laser-induced choroidal neovascularization in different rodent models	Johanna Meyer Germany
9:40-10:00	Coffee Break		
			2nd floor, Kanazawa Bunka Hall
10:00	**SESSION 7: Phototoxicity, environmental electromagnetic radiation**		
			Chairperson: Paulus T.V.M. de Jong, David H. Sliney
10:00-10:20	*1)	Risk factors for AMD; Focus on light exposure, nutrients, and medication	Paulus T.V.M. de Jong Netherlands
10:20-10:40	*2)	Retinal phototoxicity, blue light and AMD—What do we know?	David H. Sliney USA
10:40-10:55	3)	Retinal light damage as subliminal confounder in toxicity studies	Alfred R. Wegener Germany
10:55-11:10	4)	Effects of environmental factors during millimeter wave exposure	Cheng-Yu Tsai Taiwan
11:10-11:25	5)	Ocular temperature measurements during electromagnetic exposure	Masami Kojima Japan
11:25-11:40	6)	Near-infrared radiation cataract, thermal or photochemical?	Zhaohua Yu Sweden
11:40-12:20	**KEYNOTE SPEECH** ***An overview of how ISOT was started, how it progressed and it's possible future*** Frederick T. Fraunfelder, USA		
12:20-12:30	Business Meeting		
12:30-13:40	Lunch "ポロ(PoLo)", 2nd floor, Kanazawa New Grand Hotel – Annex		
14:00-18:00	**Outing** New Grand Hotel ⇒ Kanazawa Castle Park ⇒ Kenroku-en Garden ⇒ Noh Theater ⇒ New Grand Hotel		

19:00-21:00	**Banquet** "銀扇(Ginsen)", 5th floor, Kanazawa New Grand Hotel – Palace

October 12 (Sunday)

9:00	**SESSION 8: ERG and vitreoretinal disease** *Chairperson: Oliver Loget, Ying-Bo Shui*	
9:00-9:20	*1) Electroretinography in drug discovery and development	Olivier Loget France
9:20-9:40	*2) Synthetic proteoglycan mimics preserve vitreous structure and function *in vitro* and *in vivo*	Ying-Bo Shui USA
9:40-9:55	3) High glucose induces and activates toll-like receptor 4 in endothelial cells of diabetic retinopathy	Shao Bo Su China
9:55-10:10	4) Comparisons of cone electroretinograms after indocyanine green-, brilliant blue G-, or triamcinolone acetonide-assisted macular hole surgery	Shigeki Machida Japan
10:10-10:25	5) Biomechanical effects of proteoglycan degradation on vitreous and vitreoretinal structure	Benjamen A. Filas USA
10:25-10:45	Coffee Break 2nd floor, Kanazawa Bunka Hall	
10:45	**SESSION 9: Anti-glaucoma therapeutics** *Chairperson: Kazuhisa Sugiyama, Carla J. Siegfried*	
10:45-11:05	*1) The effects of topical antiglaucoma drugs on ocular surface	Kazuhisa Sugiyama Japan
11:05-11:25	*2) Oxygen “toxicity” and the anterior segment	Carla J. Siegfried USA
11:25-11:40	3) Ocular surface disorders in patients treated with BAK containing antiglaucoma drops	Ivan Georgiev Bulgaria
11:40-11:45	**Closing Remarks** *Alfred R. Wegener, Vice President, ISOT 2014*	

KEYNOTE SPEECH

KEYNOTE SPEECH

An overview of how ISOT was started, how it progressed and it's possible future

Frederick T. Fraunfelder

Casey Eye Institute, Oregon Health & Science University, USA

This will be a historic review of ISOT.

Reviewing sites of past meetings and past Presidents.

Present some options for future meetings.

ABSTRACT

In vitro lens culture studies: A potent research tool for assessing chemical toxicity

Peter F. Kador

College of Pharmacy and Department of Ophthalmology, University of Nebraska Medical Center, Omaha, NE, USA

Therapeutic Vision, Inc., Omaha, NE, USA

Objective/Purpose: The lens is the largest organ in the body that lacks a vasculature and behaves both electrically and chemically like a single cell. Therefore, the lens can serve as a sensitive indicator of chemical toxicity. The appearance of lens opacities during the toxicological phase of systemic drug development in either long-term rat, dog, and preclinical or clinical human studies can quickly result in the death of a promising drug or drug class project. Quickly identifying the specific adverse biochemical mechanism(s) of action of the candidate drug is required in order to determine whether the project can be saved.

Materials/Methods: Lenses from mouse, rat, rabbit, dog or humans can be successfully cultured *in vitro* using TC-199 / bicarbonate medium containing 30 mM fructose. Basic lens analyses require the evaluation of choline, aminoisobutyric acid (AIB), and rubidium uptake, as well as GSH and ATP levels and the activities of GR, GPx, G3PD, G6PDH, LDH, Catalase, and ER stress.

Results: Lens culture studies are useful not only for elucidating the experimental mechanism of cataract formation but also for identifying the toxicological mechanism(s) of action of how a drug can alter lens biochemistry and clarity. While lens culture studies have been conducted since the 1960's, few specific experimental details are available and an overall established procedure for conducting these studies has not been published. Cell culture techniques and tissue culture media cannot and should not directly be applied to lens culture studies and the proper evaluation of the results obtained requires expertise in classical lens biochemistry because there are significant species differences in lens biochemistry. Caution must also be used in assuming that lens viability can be significantly maintained for longer than 72 hours of culture and that lens clarity is the ultimate indicator of lens viability. Examples of the use of lens culture to elucidate the toxicological effects of drugs will be presented.

Conclusions: Lens culture studies are a powerful research tool that can rapidly identify the specific adverse mechanism(s) of action of a drug candidate as well as determine whether the parent compound or its metabolite initiates the adverse effect(s).

SESSION 1-2

Risk and benefit of supplement for vision and eye health

Eri Kubo

Department of Ophthalmology, Kanazawa Medical University, Japan

The use of dietary supplements is extremely common in advanced countries. There are now a number of different supplements for eye health on the market. There is still divided medical opinion on the use of supplements for preventing the progression of AMD and cataract.

The Age-Related Eye Disease study (AREDS) showed a beneficial effect of high doses of vitamins C, E, beta-carotene, and zinc with copper in reducing the risk of progression to advanced age-related macular degeneration (AMD) in patients with intermediate AMD or in patients with one-sided late AMD. The AREDS-2 study has shown that lutein and zeaxanthin may substitute beta-carotene because of potential increased incidence of lung cancer. However, while these supplements provide a variety of benefits, high doses may result in potentially dangerous ocular side effects. For example, we have studied the anti-cataract effects of the isothiocyanate sulforaphane (SFN). SFN is an anti-cancer compound in cruciferous vegetables, mostly commonly credited to Broccoli. It appears to have general but potent antioxidant and possible anti-inflammatory actions. In our previous studies, low dose of SFN protects cultured lens epithelial cells against H2O2 and UV-B induced stress, however high-dose of SFN induces cell death.

In this session, the risk and benefit of supplement for eye will be reviewed.

Effect of supplementary water contains minerals on corneal wound healing rate in ovariectomized rats

Noriaki Nagai[1)], Fumihiko Ogata[1)], Naohito Kawasaki[1)], Yoshimasa Ito[2)], Norio Okamoto[2)], Yoshikazu Shimomura[2)]

[1)] Faculty of Pharmacy, Kinki University, Japan

[2)] Department of Ophthalmology Kinki University Faculty of Medicine, Japan

Purposes: It is important to clarify age-related functional disorders or changes in ophthalmology. In this study, we investigated the relationship between calcium deficiency and corneal wound healing, using ovariectomized rats that received a low-calcium diet.

Materials: Female Wistar rat (normal rat) and ovariectomized Wistar rats (ovariectomized rat) were used in this study. These rats allowed free access to a diet and water. The normal rats were fed on a commercial diet (CE-2) and water, and the ovariectomized rats were fed on a low-calcium diet and purified water (PW group) or a low-calcium diet and high-mineral drinking water (CR group) for 1 month. The calcium level was determined using an Inducticely Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The corneal epithelium was removed with a BD Micro-SharpTM, and the resulting corneal wounds were dyed with 1% fluorescein solution. The wounds were monitored using a TRC-50X (Topcon) equipped with a digital camera.

Results: The amount of food intake and water consumed was similar between of PW and CR groups, and the diet and water used in this study not affect the body weight in rats. However, the calcium content of their serum and bone clearly decreased after 1 month of the combination of low-calcium diet and PW, and the calcium content of their serum and bone in PW group was approximate 0.90, 0.85-fold that of normal rats, respectively. On the other hand, the administration of high-mineral drinking water suppressed these decrease in the ovariectomized rat, the in calcium content of their serum and bone in CR group was similar to that in normal rat. The corneal wound of normal rats was approximately 50% healing at 12 hr, and approximately 80% healing at 24 hr after corneal epithelial abrasion. The corneal wounds of normal rats showed almost complete healing by 36 hr after corneal epithelial abrasion. In the PW group, the corneal healing rate of PW groups was significantly lower than in normal rat, although the delay of corneal wound healing rate was also prevented by the administration of high-mineral drinking water.

Conclusions: The present study demonstrates that calcium deficiency in menopause cause the delay in corneal wound healing. In addition, the supplementary water contains minerals potently prevents the delay of corneal wound healing. The supplement of mineral may provide an effective therapy of eye in postmenopausal patient.

Non-toxic polymethoxyflavones for prevention of posterior capsular opacification

Yoshiki Miyata, Tetsuta Oshitari, Arata Shimada, Hideyo Takahashi, Hideaki Natsugari, Hiroshi Kosano

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Purpose: Posterior capsular opacification (PCO) is the most frequent complication of cataract surgery. Advances in surgical techniques, intracellular lens materials, and designs have reduced the PCO rate, but is still a significant problem. Pharmacological prevention of PCO is still in the experimental stage but previous studies showed promise for finding medical treatment of PCO by targeting the survival, migration, proliferation, and transdifferentiation of residual LECs. However, the pharmacological approach has restricted their clinical use due to the risk of their toxic effects on surrounding intraocular tissues. This study investigated the effect of non-toxic polymethoxyflavones (PMFs) on the pathogenesis of PCO.

Methods: We synthesized metabolites and derivatives of nobiletin (3', 4', 5, 6, 7, 8-hexamethoxyflavone) isolated from citrus fruits. We investigated the cytotoxic effect of nobiletin and the derivatives to human lens epithelial cell line SRA01/04 by LDH analysis. Gelatin zymography was employed to evaluate the expression of proMMPs in conditioned medium from SRA01/04 cells. The cell proliferation was analyzed with the AlamarBlue analysis.

Results: First we examined the cytotoxicities of the nobiletin and the derivatives in SRA01/04 cells. Most of tested PMFs, with a few exceptions such as benzyl ethers, exerted little cytotoxic effect at 64 microM. Nobiletin was found to inhibit the production of proMMP-9. The IC50 values of nobiletin in PMA or TNF-alpha-stimulated SRA01/04 cells are 20.9±6.5 microM or 17.0±1.6 microM, respectively. Among nobiletin derivatives, 2'-hydroxylated flavone showed markedly potent inhibitory activity against proMMP-9 production in PMA-stimulated SRA01/04 cells (IC50: 0.4 microM) with extremely high selectivity (IC50 against TNF-alpha-stimulated cells: 68.0 microM). 4'-hydroxylated flavones also showed the considerable inhibitory action against proMMP-9 production in both PMA- and TNF-alpha-stimulated SRA01/04 cells. Nobiletin and the derivatives also displayed a potent inhibition of LEC proliferation.

Conclusion: MMP plays a pivotal role in LEC migration in PCO. Our results suggested that nobiletin and the derivatives may exert the anti-cataract action, especially against PCO through not only suppressing MMP activity but also inhibiting the LEC proliferation. Ongoing studies to elucidate the mechanism of action are currently underway in our laboratories.

Spontaneous ocular abnormalities in rats from longterm toxicity studies

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Objective: Provide an overview on spontaneous ocular abnormalities observed in longterm toxicity studies in rats (6 - 24 months) and distinguish them from treatment related pathologies.

Methods: Slitlamp examination and photography were performed in regular intervals under standardized conditions in in a dark room in various pharmaceutical and toxicology institutions. Pupils were dilated (0.5% atropin, tropicamid) before the examination and the investigator had no information on the treatment regimen. In mosts toxicity studies Wistar or Sprague Dawley rats of both gender were involved.

Results: Beyond 12 months the frequency of spontaneous ocular abnormalities especially in the anterior eye segment increased significantly. All types of cataract presented the most frequent finding followed by corneal pathologies and abnormalities of the iris and pupil. Spontaneous glaucomas were rarely found. Retinal and choroidal abnormalities were rarely found, the most frequent changes detected were degenerative retinal vessel occlusions.

Conclusion: In toxicity studies in rats up to 1 year, non-treatment related spontaneous ocular lesions were rarely found. As the number of spontaneous lesions increased markedly beyond 16 months study duration, careful ocular examination in mydriasis is mandatory to be able to discern between treatment related and spontaneous ocular abnormalities in toxicity studies. Slitlamp photography is the method of choice for documentation of these findings.

Antioxidant effect of caffeine in the lens

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Purpose: To clarify if: 1) Topically applied caffeine protects against ultraviolet radiation (UVR)-induced oxidative stress in the lens. 2) Macroscopically elucidate potentially toxic effects of topically applied caffeine and the time evolution for penetration of caffeine to the lens and the blood, respectively, after topical application of caffeine.

Materials and methods: 1) Albino Sprague-Dawley rats (6 weeks) were divided into one group that was treated topically with 72 mM aqueous solution of hydroxymethylcellulose, 0.9 %, and a control group that was treated with the vehicle only on both eyes. Both groups were subdivided into five subgroups that were exposed in vivo to incrementing doses of UVR. The animals were sacrificed at 1 week after exposure and the threshold dose of UVR was estimated as Maximum Tolerable Dose-2.3:16. 2) Caffeine 72 mM dissolved in 0.9 % aqueous solution of hydroxymethylcellulose was applied topically to both eyes of Sprague-Dawley rats and the animals were sacrificed in the interval 30-120 min. The concentration of caffeine in the lenses and in the supernatant of blood was measured with HPLC with UVR detection. Another group of rats were topically treated with caffeine on both eyes, in incrementing concentrations in the interval 0.72-72 mM, dissolved in 0.9 % aqueous solution of hydroxymethylcellulose. The animals were sacrificed 30 min. after topical application. The concentration of caffeine in the lenses and in the supernatant of centrifuged blood was measured.

Results: 1) Topical treatment with caffeine increased the threshold dose from 4.6 kJ•m-2 in the placebo group to 5.7 kJ•m-2 in the caffeine treated group. This corresponds to a protection factor (PF) of 1.2. 2) No toxic effects were macroscopically observed. Topical administration of 0.72 mM caffeine induces an increase before 30 min. and then an exponential decrease in the time interval 30-120 min with a time constant (1/e) of 125 min. while the blood concentration increases linearly in the same time interval. The concentration of caffeine achieved in the lens and the blood, respectively, is proportional to the concentration of caffeine applied topically in the investigated interval of caffeine applied.

Conclusions: The antioxidant caffeine, topically applied, protects against photooxidative damage in the lens caused by in vivo exposure to UVR. Topically applied caffeine penetrates to the lens and blood.

SESSION 2-1

Cytotoxicity of ophthalmic preservatives

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Purpose: Many topical ophthalmic drugs contain preservatives to enhance the shelf life of the product. However, because these preparations contact the corneal epithelium in full strength and are usually used repeatedly, preservatives have been implicated in iatrogenic corneal disease. We evaluated the toxicity of preservatives commonly incorporated in topical ophthalmic solutions by using a sensitive cell culture model of human corneal epithelium *in vitro*.

Methods: Primary cultures of epithelium were initiated by scraping cells from the corneal surface of freshly enucleated human cadaver eyes. At confluence (usually achieved within 7 days), the cell monolayers were exposed to single dose of preservative incorporated in the culture medium at the concentration present in marketed ophthalmic solutions. The dynamic response of the epithelial cells was monitored by phase-contrast microscopy and continuous time-lapse videomicrography. After fixation in formalin or glutaraldehyde, the cultures were processed for light microscopy or scanning electron microscopy, respectively.

Results: Exposure to 0.01% benzalkonium chloride induced immediate cell retraction and the cells died within 2 hours. Chlorobutanol (0.5%) caused immediate cessation of cell movement and mitotic activity and, after 2 hours exposure, formation of cytoplasmic blebs followed by cell death. Thimerosal (0.001%) also elicited marked cell retraction with cell death and lysis occurring within 9 hours of exposure. Cells exposed to hydrogen peroxide (30 to 60 ppm) exhibited a dose-/time- dependent response consisting of marked cell retraction, cessation of mitosis and cell movement, development of optically-dense spots that evolved into optically-lucent membranous vesicles on the cell surface, and cell death occurred within 4 to 8 hours of exposure. Sorbic acid (0.1%) suppressed cell movement and mitosis but cell death was not evident after 24 hours exposure. Epithelial cultures exposed to Dymed (polyaminopropyl biguanide, 0.005%) showed cytoplasmic retraction and minimal changes in cell movement, but no cell death occurred after 24 hours. Compared to control cultures, incubation of epithelial monolayers with Polyquad (0.001%, Alcon) showed normal cell movement and proliferation by mitosis.

Conclusions: Our results demonstrate that different preservatives used in ophthalmic solutions induce different reactions in human corneal epithelial cells. The cellular responses varied from extreme cytotoxicity and cell death to no discernible changes as evidenced by normal cell activity and mitotic activity. We advocate that, as new preservatives continue to be developed, our model of human corneal epithelium *in vitro* should be used to evaluate their potential cytotoxic effects.

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SESSION 2-2

The effect of human cultured conjunctiva cells by anti glaucoma eye drops

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Purpose: Glaucoma is a chronic disease in ophthalmology and life-long treatment is needed. Glaucoma patients often receive a multi-drug combination therapy. However, multi-drug combination therapy can cause many eye disorders. We studied the inhibitory effects of anti-glaucoma ophthalmic solutions on the proliferation of cultured human conjunctiva cells. Materials: 1 No BAK study with Travatanz® (Travoprost +SofziaTM), Travatan® (Travoprost +BAK0.005%), Xalatan® (Latanoprost +BAK0.02%) and Less BAK study with new Tapros® (Tafulprost + 0.001%BAK), old Tapros® (BAK0.005%) , Xalatan® (Latanoprost +BAK0.02%), Travatanz® (Travoprost +SofziaTM) in prostaglandin analogues, 2 New preservation study with Duotrav® (with POLYQUAD®), Xalacom® (Latanoprost +Timolol +BAK0.02%) in combination solutions, 3 Good additive study with Rysmon® TG (Timolol +thermo-setting gel), Timoptol®XE (Timolol +ion-activated gel) in long acting β-adrenergic antagonists and the effects of vehicle solution study with vehicle + BAK and vehicle only.

Methods: Cultured human conjunctival cells were planted in 96-well plates. These drugs were diluted with the medium so that the final concentration became 1/30, 1/100 and 1/300, and 10μL of each solution was administered 24 hours later. Experiment 1: Impacts on the morphology of human conjunctiva cells. The cell morphology in each well was checked under a phase-contrast microscope. Experiment 2: Impact on the activity of human conjunctiva cells. The diluted drugs were put into the wells after the cells had been incubated for 24 hours. After another 24 hours, the cells were allowed to react with WST-8 for 90 minutes, and fluorescence intensity was measured by absorption spectrophotometer. The resulting fluorescence intensities were calculated and compared via unpaired t-test.

Results: 1 No BAK study: Travatanz® showed significantly higher values than Travatan® and Xalatan®. Less BAK study: New Tapros® showed significantly higher values than old Tapros®, Xalatan® and Travatanz®. 2 New preservation study: Duotrav® showed significantly higher values than Xalacom®. 3 Good additive study: Rysmon® TG showed significantly higher values than Timoptol®XE. The effects of vehicle solution study: Vehicle only showed significantly higher values than vehicle + BAK and the cell activity increased concentration-dependently in vehicle only.

Conclusions: BAK may perform worth action for a culture conjunctiva cells. However, small amount of BAK (0.001-0.005) may have less influence on cultured conjunctiva cells. Some additives may cause better influence for a culture conjunctiva cells.

These results showed we have to consider about, type of the preservation, amount of the preservation and additive of the eye drop when using anti-glaucoma eye drops.

SESSION 2-3

Ocular toxicity of benzalkonium chloride homologs compared with their mixtures and new approach to avoid corneal injury caused by preservatives

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Benzalkonium chloride (BAK) has strong antimicrobial activity and has been widely used as a preservative for many kinds of ophthalmic solutions. However, BAK may on occasion cause corneal disorders in some patients clinically. Recently, the ocular toxicity of BAK has been investigated based on *in vitro* and *in vivo* studies.

Several studies were performed in rabbits and monkeys in order to evaluate the *in vivo* ocular toxicity of BAK homologs with C-12 or C-14 alkyl chain length (C12-BAK, C14-BAK), a BAK mixture consisting of different homologs with alkyl chain lengths ranging from 8 to 18 carbon atoms, and vehicles of ophthalmic solutions containing a BAK mixture. C12-BAK, C14-BAK and the BAK mixture were administered 10 times a day in rabbits at concentrations of 0.001 to 0.03% that range from generally used concentrations in commercial ophthalmic solutions to excessive concentrations. 0.01% C12-BAK was administered 7 times a day for 39 weeks in rabbits. 0.005% and 0.01% BAK mixture-containing vehicles were administered up to 8 times a day, for up to 52 weeks in rabbits and monkeys. As a result, BAK in concentrations up to 0.01% showed no toxicity in the eye ball and its accessory organs after repeated instillations. Also, C12-BAK showed weaker acute ocular toxicity (ocular irritation) than C14-BAK or the BAK mixture after 10 instillations. Our results suggest that BAK is still useful within the appropriate concentration because of its ocular safety and C12-BAK, rather than a BAK mixture, as a preservative in ophthalmic solutions has the potential to reduce the incidences of corneal injury caused by the preservative.

In addition, the new approach to avoiding corneal injury caused by preservatives will be introduced. There are some alternative chemical substances to reduce the toxicity but it is difficult to completely avoid this as long as a chemical substance is used as a preservative. On the other hand, preservative-free bottles prevent the contamination by bacteria with a mechanical structure. It is one of the promising alternatives to relying on chemical preservatives.

Benzalkonium chloride of alkyl chain length C12 is safer for corneal epithelium

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Purpose: To evaluate acute corneal epithelial toxicity induced by benzalkonium chloride (BAC) homologs with different alkyl chain lengths using an *in vivo* electrophysiological method, and to evaluate the toxicity of an ophthalmic solution containing C12 BAC.

Methods: BAC homologs with C12, C14, and C16 alkyl chain lengths were used at concentrations of 0.0025%, 0.005%, and 0.01%, respectively. Cytotoxicity of BAC homologs on the normal rabbit corneal epithelial cells was examined by using a WST-1 assay. Corneal transepithelial electrical resistance (TER) was measured in living Japanese white rabbits. TER changes were evaluated after a 60-second exposure to these BAC homologs. Morphological changes in corneal epithelium after exposure to the BAC homologs were examined using scanning electron microscopy. The antimicrobial activity of BAC homologs against Escherichia coli was also assessed. Corneal TER and morphological change by DIQUAS containing 0.075% C12 BAC was also examined.

Results: All BAC homologs caused cytotoxicity and corneal barrier dysfunction in a concentration-dependent manner. However, the degree of corneal toxicity differed among the BAC homologs. Based on cytotoxicity and TER measurement, C12-BAC caused the least corneal impairment followed by mixed BAC/C16-BAC and C14-BAC. Scanning electron microscopy images indicated an intact corneal epithelium after exposure to 0.005% C12-BAC, whereas 0.005% C14-BAC damaged the epithelium. There were no remarkable differences noted in the antimicrobial activity among the BAC homologs. DIQUAS showed less corneal toxicity compared to 0.075% mixed BAC.

Conclusions: Acute corneal epithelial toxicity induced by BAC homologs depends on the alkyl chain length. Thus, the use of C12-BAC instead of commercially available BAC is potentially safer for patients undergoing ophthalmological pharmacotherapy.

Evaluation of mucin barrier induced by benzalkonium chloride on the ocular surface epithelium

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Purpose: To evaluate the effect of the mucin barrier on the ocular surface epithelia induced by benzalkonium chloride (BAK) using a human conjunctival epithelial (HCjE) cell line.

Methods: HCjE cells were grown to confluence in serum-free medium and switched to DMEM/F12 with 10% fetal bovine serum for 5 days to promote differentiation and express membrane-associated mucins. The cells then were exposed to 0.001%, 0.005%, and 0.01% BAK for 1 minute. BAK cytotoxicity on the HCjE cells was examined using a WST-1 assay. Quantitative real-time polymerase chain reaction (PCR) was performed to investigate the gene expression of membrane-associated mucin MUC16 mRNA. To evaluate the mucin-associated ocular surface protection, a rose bengal uptake assay was performed.

Results: The WST-1 assay showed that cell viability values after 1-minute exposure of 0.001%, 0.005%, and 0.01% BAC were 94.2%, 90.8%, and 86.2%, respectively, meaning that the cytotoxicity resulting from these exposures was mild. Nevertheless, MUC16 mRNA expression decreased significantly to 0.65, 0.43, and 0.33, respectively ($p < 0.005$, ANOVA). The rose bengal uptake assay showed that exclusion of the dye was reduced significantly in cells with all BAK concentrations ($p < 0.005$, ANOVA).

Conclusions: These results suggested that even short exposure to BAK down-regulates MUC16 expression and disrupts the mucin barrier for the ocular surface epithelial cells.

SESSION 3-1

Contact lens related ocular toxicity

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In the context of contact lens wear, lens related ocular toxicity can be from the lens itself, the add-on elements such as cosmetic lens pigments, contaminants on or in the lens, and the chemical components of various contact lens solutions.

Contact lens related ocular toxicity, while rarely devastating unless infectious keratitis is involved, often causes issues ranging from discomfort, dryness, neovascularization, to inflammation that ultimately may result in shortened wear time, even total discontinuation of lens wear.

Since contact lens wear essentially creates an hypoxic environment for the cornea, the development of high Dk/t lens materials now provides patients with a wide array of silicone hydrogel and GP lenses. A more recent trend away from extended wear and high Dk/t, while emphasizing daily disposables and wear comfort, has seen the appearance of water-gradient hybrids. However, it should be noted that it is still unclear at what Dk/t level and when the shift from aerobic to anaerobic glycolysis in, e.g., corneal epithelium, take place.

The popularity of cosmetic lenses has also seen lenses with pigments printed directly onto the surface of, rather than the safer, albeit more complicated embedment into the lens. Surface pigments dislodge readily, and if they are not FDA-approved, the pigments may also cause allergic reaction leading to toxic keratopathy.

Allergy to the contact lens, in fact to protein deposits on the lens, in the form of GPC is well-known. The incidence of GPC has been largely reduced with improvement in lens surface treatments plus better cleaning regimens. Cleaning and storage solutions have also long ago eliminated thimerosal from the formulation; although the patients may still be sensitive to certain components in one solution, necessitating a change to another. Typically, patients who have developed corneal infiltrates also are users of multi-purpose solutions. They also tend not to replace lens cases that are cultured positive of rare bacteria such as Delftia, Stenotrophomonas, and Achromobacter. Not only the improper care of lens caddies can be a source of problem, the use of saliva and tap water as cleaning agents are not unheard of in economically challenged areas.

With progress in contact lens design and manufacturing process complemented with professional clinical care, contact lens related ocular toxicity is largely avoidable; nonetheless, it can still be subverted by patient incompliance. Patient education will therefore always play an indispensable role in successful contact lens wear.

Eye complications of color soft contact lens wear

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In Asian countries, color contact lenses are getting considerably more popular. At the same time, there is an increased number of eye disorders from wearing them. There are various types of eye disorders caused by color contact lenses. There are disorders caused by wearing soft contact lenses, relatively common disorders seen by wearing color contact lenses, and unique disorders caused by color contact lenses. One distinctive disorder associated with using color contact lenses is damage to the corneal epithelium caused by color pigment exposure on the cornea. A disorder that is relatively more common to color contact lenses is corneal epithelium damage, believed to be caused by uneven lens surface due to the use of color pigment. Color contact lenses have uneven surfaces to a lesser or greater degree, as compared to regular clear contact lenses. If the unevenness is overly rigid, it is easy to cause corneal epithelium disorder. Also, there are eye disorders caused from hypoxia due to the low water content of color contact lenses. Acute disorders are corneal edema, superficial punctate keratitis, and limbal redness. Chronic disorders are corneal neovascularization, corneal endothelium disorder, corneal thinning, and corneal warpage. There are fairly common disorders associated with tight fitting of color contact lenses. Many color contact lenses use low water content HEMA materials, and their lens diameters are large, with only one type of base-curve available. As a result, the lenses fit tightly to the eye's curvature, which can lead to conjunctiva staining, limbal redness, and corneal epithelium disorders. For the future, we also expect that various more contact lenses will be imported from overseas and we will need to be aware of the possibility of eye disorders caused by toxic pigment.

Microbial adhesion to beauty contact lenses

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Purpose: To determine if beauty contact lenses (BCL) with surface pigments facilitate microbial adhesion.

Material: Fifteen brands of BCL purchased from optical, non-optical retail outlets, and via the internet.

Method: Five lenses of each brand of BCL were subjected to a standardized rub-off test to first confirm the location of the pigments. The surface of each BCL was gently rubbed with a wetted cotton bud for a maximum of 20 rubs. Part I: Five lenses of each brand of BCL were then incubated in Pseudomonas aeruginosa overnight and viable counts of adhered bacteria on each lens were determined by the number of colony-forming units (CFU) on agar media. Part II: Bacterial adhesion to three brands of BCL and their clear counterparts (ie. Clear contact lenses of the same material and water content) were compared. (Of these three brands of BCL, two passed the rub-off test and one failed.)

Results: Of the BCL tested, 13 brands failed the rub-off test and these lenses showed higher P. aeruginosa adhesion. The two brands of BCL which passed (ie. did not have pigments that detached with the rub-off test) showed at least six times less bacterial adhesion. Compared to their clear counterparts, the levels of bacterial adhesion to BCL which did not have surface pigments were not significantly different, whereas for the BCL which have surface pigments, the levels of adhesion were significantly higher than its clear counterparts.

Conclusion: Significantly higher levels of bacterial adhesion were found on BCL with surface pigments.

Confounding corneal lesions in toxicology studies in Dutch-belted rabbits

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Objective/Purpose: Corneal dystrophy and other spontaneous corneal abnormalities can present as confounding lesions in animal toxicology studies. This presentation will detail two contrasting case studies where corneal dystrophy and other corneal changes were appropriately and inappropriately identified in Dutch belted rabbits, and how the difference in prestudy identification affected the outcome of the toxicology studies.

Materials and Methods: Two 28-day toxicology studies to evaluate topical application of novel ocular therapeutics in 4-month old male and/or female Dutch-belted rabbits were compared. In Study A, a male only study (n=40), 6% (15/250) of the animals ordered from the vendor were excluded for pre-existing corneal opacities. In Study B, male (n=40) and female (n=40) rabbits were enrolled from a lot of 168 rabbits without screening for spontaneous ocular changes. Ophthalmological and histopathological changes were evaluated at study termination. In Study A, findings of corneal opacity and trauma were not treatment-related. In Study B, corneal lesions were considered to be treatment- and dose-related, despite the lack of ophthalmologic findings in previous toxicology studies. A retrospective review of the data was undertaken to evaluate the cause of the corneal lesions in both studies.

Results: Failure to exclude pre-existing corneal dystrophy prior to study initiation and failure to properly characterize other corneal changes arising during the treatment phase of a toxicity study artificially increased the incidence and severity of treatment-related corneal lesions associated with a novel topical ocular therapeutic. Considerable time and expense was required to characterize these confounding ocular changes prior to continuance of the drug development program. True corneal dystrophy occurs as a congenital finding in Dutch belted rabbits, but rarely in other test species. In addition to corneal dystrophy, corneal opacities, mostly due to trauma or rarely pre-Descemet's membrane opacity, were retrospectively identified in these studies. Fluorescein retention, originally considered ulceration, could not be confirmed histologically as an adverse finding in Study B.

Conclusions: Failure to exclude pre-existing corneal dystrophy or properly characterize other corneal lesions at study termination can adversely impact nonclinical toxicology study results by inclusion of confounding lesions in ocular toxicology studies. The current criteria for evaluating corneal lesions in animals are not robust enough to definitively diagnosis corneal dystrophy from many other corneal changes. Unless evaluated and properly characterized, corneal changes in animals in toxicology studies should not be attributed to dystrophy.

Does smoking affect corneal endothelial cell density?

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Purpose: To assess the effect of smoking on the morphology of the corneal endothelium and corneal pachymetry in healthy corneas and in the presence of primary central cornea guttata in relation to 7-year follow-up.

Methods: The Reykjavik Eye Study is a population based prospective study. The participants were examined in 1996, 2001 and 2008. The participants underwent specular microscopy (2001 and 2008) and a standardized eye examination (1996, 2001 and 2008) in addition to answering a questionnaire on previous lifestyle, including smoking history, and health (1996).

Results: The 95% confidence interval for the mean endothelial cell density (ECD) in the absence of cornea guttata (CG) at baseline was 2491±46 and 2486±59 at 7-year follow-up for non-smokers. Similar results were found for non-smokers with (CG) and “ever-smokers” without CG. ECD in those with smoking history and presence of cornea guttata was 2289±133 at baseline and 2259±122 at follow-up. Reduction of ECD for “ever smokers” with CG at baseline was found (CI(0.95) -158±134), compared with (CI(0.95) -95±140) for non-smokers. Higher number of "packyears" was associated with an increased loss of endothelial cells in a multivariate model.

The 95% confidence interval for mean corneal pachymetry (µm) increases from baseline to follow-up in healthy corneas (11±4 vs 7±4, non-smokers vs “ever-smoker”). No change in pachymetry was found for corneas with CG.

Conclusions: Smokers with cornea guttata have a higher reduction of ECD compared to non-smokers with cornea guttata.

Human lens opacities – Retrodots

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Retrodots are among the several ‘minor’ human cataract morphological subtypes. They occur in the anterior and posterior peri-nuclear ‘deep’ lens cortex and are seen in biomicroscopic slit lamp retro-illumination as irregular droplet like disturbances in the red reflex. Their internal refractive index is higher than the surrounding lens material which causes them to behave optically as small positive refractive elements within the deep cortex. In polarised optical viewing systems they are difficult to distinguish, appearing as pale patches against the background red reflex. In direct slit-beam illumination they are virtually invisible, a feature which differentiates them from the more peripherally located cortical flake opacities. Retrodots are often associated with nuclear opacification and tend to be under recognised because they are relatively difficult to identify unless specifically looked for during clinical examination of the lens. Retrodots have an adverse effect on vision which is additional to and independent of other co-existing lens abnormalities. The underlying aetiology of lens retrodots remains unclear.

In this introductory talk these features will be described and illustrated in terms of their clinical appearance, their frequency of occurrence, their possible aetiology and their impact on vision.

Visual function of eyes by five types of lens opacities

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There are several risk factors for lens opacities, including systemic steroid-posterior subcapsular cataract (PSC), smoking-nuclear cataract (NUC) and ultraviolet radiation-cortical cataract (COR). Among subtypes, retrodots (RD) often co-exist with NUC and waterclefts (WC) develop COR indicating RD/NUC and WC/COR may be pairs sharing risk factors. Prevalence of these cataracts increases with aging and each sometimes causes visual impairment requiring surgery. Most epidemiological studies investigated prevalence, incidence, progression, and risk factors of the three main types, but few of RD and WC. Moreover, very few examined relationships between severity of these five types of cataract and visual function in detail. If cataracts develop as side effects of drugs, environmental factors or lifestyles, defining their clinical significance and risk factors is important to inform their effect on visual function.

In this study, 1116 eyes of 977 subjects examined for cataract surgery in Kanazawa Medical University hospital or from Monzen Eye Study were enrolled. COR, NUC and PSC were graded according to WHO classification system. COR was categorized CEN+/- by opacity within/outside the central 3 mm diameter area of the pupil. WC was similarly categorized WC (CEN+/-). RD was classified into four grades by quantity and area of RD in the 3 mm diameter area: grade1, less than 5 RD; grade 2, less than 25% of area; grade 3, less than 50%; and grade 4, 50% or more. Types and grades of cataract were judged by one observer. Effect of cataract on visual function was evaluated by best corrected visual acuity (BCVA), 25% daytime contrast acuity (DCA) and 25% evening contrast acuity (ECA). Spherical equivalent (SE) and lens power (LP) were examined to study the effect on refractive power. Contrast vision was measured using CAT2000 (NEITZ).

Compared with transparent lenses aged 60s, BCVA was significantly worse with COR (CEN+), NUC grade1 and over, PSC grade 2 and over, RD grade 2 and over, and WC (CEN+); DCA and ECA were significantly worse with NUC grade 1 and over, COR (CEN+) grade 2 and over, PSC grade 2 and over, RD4 (for DCA), RD3 and over (for ECA) and WC (CEN+); and LP increased with NUC grade 2 and over and RD grade 3 and over, and decreased with WC (CEN+) ($p<0.05$).

There is a relationship between severity, type of opacity, and BCA, DCA, ECA and LP. This information is very useful to evaluate the impact of each type of cataract and their risk factors on visual function.

Use of the Casey Eye Institute camera for recording lens opacity sub-types and correlation with vision quality

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Purpose: To demonstrate to physicians and surgeons the utility of the Casey Eye Institute camera for revealing subtle lens changes including retrodots and to determine the correlation between retrodot density and vision degradation.

Patients: 26 male and 40 female subjects presenting with various lens opacities, including retrodots, (a representative subtype of lens opacity) visited the Department of Ophthalmology, Kanazawa Medical University Hospital from April to June, 2013. Their ages and sex distribution were 36-88 (71.1±9.4) years and 26/40=male/female, respectively.

Methods: Retro-illumination photography under maximal mydriasis was performed with the Casey Eye Institute (CEI) camera system. The CEI camera system comprises an optical reflex housing paired with a consumer-grade digital camera with pop-up flash. Flash illumination passing through the reflex housing is redirected coaxial to the camera objective lens, resulting in "red-reflex" retro-illumination of the anatomical lens. Patients were positioned in a chinrest and the camera system was attached to an adjustable stage. Focus was set to the iris plane. A supplemental convex lens provided additional magnification of the subject. The visual acuity of each patient was taken and recorded in logMAR which was then correlated through Point Spread Function analysis with % retinal image contrast. A higher % contrast indicated less retinal masking from light scattering of lens opacities, and lower % contrast suggested more undesirable, vision-degrading retinal masking. Of 112 eyes photographed, 26 showing primarily retrodots were analyzed. Image analysis in all cases was done with Adobe Photoshop®.

Results: To demonstrate the effect of the opacity subtypes locating inside the central 3-mm pupillary area on vision, patients were divided into two groups: one with the total opacity area of >15% of the total pupillary area, and the other, <15% of the total area. Point Spread Function analysis showed that in both groups, there was a nearly linear trend associating less retinal masking (i.e., higher % retinal image contrast) with higher visual acuity. The >15% group was characterized by both lower visual acuity and higher retinal masking, i.e., more vision deterioration, than the <15% group. And all differences between these two groups were statistically significant ($P<0.05$).

Conclusions: Our results have shown a close association between the subtype opacities with vision deterioration. Retro-illumination photography with our system therefore can yield crucial information useful for decision-making prior to cataract surgery.

The variation of oxidative levels in aluminum attached on intraocular lenses using a perfusion culture system

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Purposes: A type of delayed endophthalmitis has been reported in some cases after implantation of intraocular lens (IOL) made by HOYA Corporation. The aluminum (Al) adhered on loop surface of these IOL was presumed to be the cause. As one of the procedures for elucidating the possible mechanism of this endophthalmitis, IOLs with intentionally attached Al were cultured in conditions near the human anterior chamber and the variation of oxidative levels were investigated.

Methods: Solution of Al powder was dripped to IOLs (NY-60, HOYA Corp.) and two amounts of Al, 0.2 and 4.0 μg Al/IOL, were attached on the optical area of IOLs. IOLs with Al were cultured in our original double-faced perfusion culture system with our composite aqueous humor for 2 months. The effluent was collected with time from the main chamber which IOLs with Al were cultured. Total amounts of hydroperoxides (TH) were measured with modified Free dROMs test as an indicator of oxidation.

Results: After the culturing, remaining of Al was confirmed in all IOLs with Al. In the IOLs of 0.2 μg of Al/IOL, the residual Al consisted 0.008-0.033% of the IOL optical area. The levels of TH in effluent from each IOL with Al were fluctuated with time and several peaks were revealed. The variations of TH were different in levels and intervals in each IOL.

Discussion and conclusion: A high proportion of re-inflammation was reported as a characteristic of the delayed endophthalmitis occurred among implantations of IOLs with Al. This re-inflammation was dispersed in severity, intervals and numbers of recurrences. Depressed function of trabecular meshwork had been reported to be induced by reactive oxygen species. The results of this study indicated the possibility that oxidation may take parts in inducing the re-inflammation in this delayed endophthalmitis.

Data-analysis pre-study of initial lenticular findings in emergency workers at Tokyo Electric Power Fukushima Nuclear Power Plant

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Purpose: As 17% of Chernobyl clean-up workers assessed for cataract 12 years after radiation exposure had posterior subcapsular cataracts, exposure to lower levels than considered hazardous, may cause opacification in crystalline lens. International Commission on Radiological Protection proposed a threshold dose of 0.5 Gy for ocular tissue injury adding that future studies may elucidate this judgment. We examined the crystalline lens of emergency workers at Fukushima I Nuclear Power Plant, Tokyo Electric Power Company (TEPCO), damaged by the Great East Japan Earthquake in March 2011. Going forward we will examine correlations among exposure dose, lenticular findings, and Lens transparency property (LTP) using a database of external exposure dose, managed by TEPCO and the Ministry of Health Labour and Welfare.

Methods: Of 900 workers at Fukushima Nuclear Power Plant exposed to 50 mSv and over, eyes of 330 (aged 41.4±10.3 yrs) were examined in 2013. Subjects comprised males, 54 (108 eyes) aged 20s, 80 (160 eyes) aged 30s, 108 (216 eyes) aged 40s, 84 (168 eyes) aged 50s, and 4 (8 eyes) aged 60s. Unexposed controls comprised eyes of 231 volunteer males (aged 40.9 ± 7.9 yrs) in the medical service industry with no history of occupational radiation exposure. Lenses were examined using a Scheimpflug camera (Nidek EAS-1000) and one doctor assessed cataracts from illumination and slit images. LTP was assessed by analysis of backward scattered light intensity and thickness in each lenticular layer recorded.

Results: In exposed and unexposed subjects, prevalence of cortical cataracts, CEN cortical cataracts, nuclear cataracts, posterior subcapsular cataracts, retrodots, and vacuoles were 1.51% and 0%, 0.45% and 0%, 0% and 0%, 0% and 0%, 0% and 0.43%, and 2.27% and 6.93%, respectively. LTP by age group in exposed and unexposed subjects was 140.0±14.9 and 129.8 ±8.1,171.8±21.2 and 167.4±20.1, 231.0±38.5 and 224.5±31.2, and 300.9±53.1 and 316.4 ±41.4 in 20s, 30s, 40s, and 50s, respectively. Neither LTP nor prevalence of cataract differed between exposed and unexposed subjects.

Conclusions: Although we found low dose radiation exposure had not caused any noticeable deterioration in visual function three years after exposure, there remains the possibility that sub-type cataracts such as vacuoles, potential primary lesions of posterior subcapsular cataracts, have been seeded to grow much later. A longer term analysis with access to a trusted database is advised.

Experimental postoperative endophthalmitis using aluminum sticking IOLs

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Purpose: Several soft endophthalmitis were reported using iMics 1 (251, HOYA) in Japan. In this study, the effect of sticking alminium on intraocular lens (IOL) was tested experimentally.

Methods: No alminium sticking control IOLs (251) by inspection (C group), small amount alminium sticking IOLs (A group) and 0.13μg/IOL alminium were deliberately deposited IOLs (D group) were prepared. Twelve Japanese white rabbits were anesthetized and these IOLs were implanted into one eye after phacoemulsification. The endoophthalmitis were evaluated using MacDonald-Shadduck methods.

Results: Corneal score (day 2) in C, A and D groups were 0.1, 0.1, 1.0 ($P<0.01$) respectively. Conjunctival score (day 4) were 0.1, 0.1, 1.0 ($P<0.01$) respectively. Iris score (day 35) were 1.5, 2.3, 2.3.

Conclusions: Small amount of sticking alminium on IOLs caused inflammations after phacoemulsification and IOLs implantation.

SESSION 5-1

Drug related immunotoxicity in ophthalmology

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A summary of case reports from the worlds literature, spontaneous reporting databases, and the National Registry of Drug-Induced Ocular Side Effects (www.eyedrugregistry.com) will be presented.

Fluoroquinolones, bisphosphonates, vaccines, and statins all can cause ocular immunotoxic reactions.

Recognition of these rare side effects and prompt withdrawal of offending agents will lessen ocular morbidity.

Adverse effects of topical anti-glaucomatous agents in patients with severe dry eye

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Purpose: Among ophthalmic solutions, anti-glaucomatous agents sometimes induce ocular surface lesions, as well as systemic side effects. In patients with dry eye, the ocular surface is more easily damaged than in normal eyes. We examined the eyes of patients with severe dry eye that were treated for glaucoma.

Patients and Methods: Nine patients (8 female) average age 68.3 yrs with severe dry eye treated with anti-glaucomatous topical agents in outpatient clinic for dry eye in Kanazawa Medical University Hospital underwent optical examinations. Ocular surface damage revealed by staining; fluorescein (cornea), lissamine green (nasal / temporal bulbar conjunctiva), was graded according to van Bijsterveld's scoring system. Corneal epithelial barrier function was evaluated in 1 case using a Scheimpflug camera system equipped for fluorescein photography of tear film and cornea.

Results: Three patients had anti-SS-A/Ro antibody positive Sjögren's syndrome and another 3 had anticentromere antibody positive primary Sjögren's syndrome (J Rheumatol. 2001; 28: 2238-44). The remaining 3 had no autoimmune disease nor took drugs that caused dry eye. Van Bijsterveld's scores were 4 to 9 in severer dry eyes though 6 of these had already received punctal occlusion therapy. All patients used eye drops for dry eye. Prostaglandins were usually the first choice, but they could not be continued in 6 cases, even if they didn't contain benzalkonium chloride. Beta-blockers and carbonate dehydrate inhibitors were also related to corneal damage in some cases. Scheimpflug imaging revealed, severely defective corneal barrier function which was not observed in cases with epithelial lesion due to only dry eye without using anti-glaucomatous agents as a control. In 2 patients whose both (upper and lower) puncta were occluded, the toxicity of topical agents was very potent so both were treated with auto-serum eye drops and temporary discontinuance of anti-glaucomatous agents. The retention of drugs in cul-de-sac and/or fragility of the ocular surface in eyes requiring double unilateral occlusion might be the cause of severe ocular surface disorder.

Conclusions: The toxicity of anti-glaucomatous agents did not seem to be solely due to benzalkonium chloride but also other elements of drugs including main gradients and other additives. If both puncta are occluded in a patient, we must pay attention to the elongation of contact time with the agent because of reduction of tear fluid turnover.

Dacryoendoscopic observation and incidence of canalicular obstruction/stenosis associated with S-1, an oral anticancer drug

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Purpose: To report dacryoendoscopic observations and the incidence of lacrimal obstruction/stenosis associated with S-1, an oral anticancer drug.

Design: Retrospective, nonrandomized clinical trial.

Methods: A total of 52 patients (41 men, 11 women; age, 42-93 years) who were prescribed the anticancer drug S-1 were studied. Patients who suffered eye complaints following S-1 treatment underwent ophthalmic examination, probing and lacrimal irrigation. Patients whose tear meniscus was high or had abnormal lacrimal irrigation were evaluated by dacryoendoscopy.

Results: Overall, 5 of 52 S-1-treated patients (9.6%) experienced lacrimal passage stenosis/obstruction. One patient had punctal stenosis, and 4 patients had canalicular obstruction/stenosis. The onset of epiphora ranged from 2 to 8 months (4.4 ± 2.2 months, mean ± SD) after the initiation of chemotherapy.

Conclusions: Patients receiving S-1 treatment should be evaluated for potential lacrimal disorders, particularly canalicular obstruction/stenosis. Dacryoendoscopic observation is effective for the diagnosis of this side effect.

Intracameral antibiotics for the prevention of post-cataract surgery endophthalmitis

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Purpose: To compare the effectiveness of intracameral (IC) cefuroxime and moxifloxacin prophylaxis.

Methods: Retrospective cohort study of 150,086 Kaiser Permanente members, 2005-12, 118 with confirmed endophthalmitis. Key variables were validated. The logistic regression analysis adjusted for age, year of surgery, ocular and systemic comorbidity, posterior capsular rupture, and topical antibiotic.

Results: Risk of endophthalmitis while using IC cefuroxime was 0.43 per 1,000. The odds of endophthalmitis with IC moxifloxacin compared with IC cefuroxime was 1.16 (CI, 0.51-2.65).

Conclusion: Cefuroxime and moxifloxacin are effective intracameral agents. No important difference in effectiveness between intracameral cefuroxime and moxifloxacin for preventing phacoemulsification-related endophthalmitis.

Current situation of nonclinical ocular toxicity assessment in Japanese pharmaceutical industry

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Ocular toxicology assessment of new drugs is considered to be global common problem, as well as in Japan. However, relationship between ocular toxicologists in Japan and in other countries have been insufficient. In this presentation, we will introduce current situation of pharmaceutical industries, regulatory agency and academia around ocular toxicity assessment in Japan.

Since the eye is a very unique organ, a lot of specific knowledge is required for conducting of eye examinations and nonclinical ocular toxicology assessment of new drugs. However, veterinary schools in Japan never have the ophthalmology department, and a biggest problem in eye examinations of nonclinical toxicity study was an education and training system of animal eye examiner in the past. The Japanese society of comparative and veterinary ophthalmology (JSCVO) led an education and training for the past three decades, and basic eye examination skills have been widely spread. In addition, the JSCVO established a certification system for diplomates in 1990, and more than 20 diplomates have been certified as a fundamental ophthalmologist. In most of toxicity studies conducted in Japan, these diplomates conducted eye examinations. Therefore, eye examination skills have been extremely improved compared with the past.

On the other hand, study directors of toxicity study (toxicologist) do not always have knowledge for ophthalmology. Insufficient communication between ophthalmologist and toxicologist might mislead risk assessment of ocular toxicity.

Authors of this paper formed the Ocular Toxicity Forum in 2012 for the purpose of enhancement of interaction between ophthalmologists and toxicologists in Japan. Most members of this forum are ocular toxicologists who have skills and experiences both for ophthalmology and toxicology belonging to regulatory agency, pharmaceutical companies and nonclinical CROs. We coordinated workshops in the annual meeting of the Japanese Society of Toxicology in 2013 and 2014, and published several review articles.

In conclusion, further close collaboration among global ocular toxicologist is necessary, and we would like to share common global issues about ocular toxicology assessment.

Utilizing of ocular anatomical features of laboratory animals; for the extrapolation of animal data to human

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Purposes: In the preclinical studies for ophthalmic drugs, laboratory animals are usually administered excessive drug because the same volume of formulation as human is dosed to the animals. Therefore, quantitative data of the ocular comparative anatomy is necessary to extrapolate the influence of the drug to human. However, there is less information easily to compare among the species.

In particular, several ophthalmic drugs for posterior segment with intravitreal injection have been developed in recent years. The species differences of ocular anatomical features of posterior segment are common concern for preclinical research.

In this study, we investigated ocular anatomical features of the animals frequently used for the development of ophthalmic drugs in order to improve the precision of extrapolation to human clinical use.

Materials and Methods: We used eyeballs of rabbits, dogs and monkeys. Animals were not purchased or euthanized for the purpose of this study. The eyeballs used were all obtained from the purpose-bred animals ethically euthanized. We measured the parameters of eyeball, the lens and vitreous (axial length, thickness, weight or the volume).

Results: In comparison among these 3 species, the size of the eyeball was found to be the largest in dogs, followed by monkeys and rabbits. The size of the vitreous was the same order of the eyeball size. But the relative value of the vitreous size against the eyeball size in each species was found to be the largest in monkeys, because of the different order of lens size.

In comparison with human, the eyeball and vitreous sizes were relatively small in animals except for the lens size of rabbits and dogs. The ratio were 0.39, 0.82 and 0.46-fold against human value (as 1) for eyeball weight, 1.71, 2.48 and 0.53-fold for lens weight, and 0.34, 0.73 and 0.55-fold for vitreous volume in rabbits, dogs and monkeys respectively.

Based on anatomical parameters, we calculated exposure ratios when animals and human were given sample solution of the same concentration with intravitreal injection. The result of calculation indicated that there is exposure ratio of up to approximately 3-fold in the vitreous body between human and animals merely due to their anatomical differences.

Conclusions: This study indicated that evaluating the preclinical studies and extrapolating from animals to human for the development of ophthalmic drugs can be improved by integrating anatomical species differences into the evaluation.

SESSION 6-1

Five-year incidence and progression of age related macular degeneration (AMD) and associated risk factors in Icelanders.

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Purpose: To investigate the incidence, progression and associated risk factors for AMD in persons aged 50 years and older (Reykjavik Eye Study I & II) and in persons 67 years and older (AGES Reykjavik I & II).

Patients and Methods: In the Reykjavik Eye Study we used a population sample of persons 50 years and older at baseline, 1045 persons examined with gradable fundus photographs in at least one eye, and in the Age, Gene/Environment Susceptibility Reykjavik Study, also a population sample, we examined 5272 persons 67 years and older, with gradable fundus photographs in at least one eye. We used standardized fundus photography and grading and standardized questionnaires in both studies. The follow-up studies were performed five years after the baseline study. The eye with the more severe lesion was used in the analysis.

Results: In the Reykjavik Eye Studies there was a significant annual increase in the risk over 5 years for all AMD phenotypes. Being married rather than divorced or widowed and moderate alcohol consumption seems to decrease drusen formation whereas smoking and rare consumption of diatery fibre rich vegetables and meat increased the risk.

In the AGES studies, the 5-year incidence of early and late AMD combined among those <70 years at baseline was 6.9% and 31.3% among those aged >80 years at baseline. Both late forms of AMD, GA and exudative AMD were similarly common. Among 563 persons with early AMD at baseline 128 (22.7%) had progressed to late AMD over 5 years.

Age, high HDL cholesterol, higher BMI and female sex as well as certain AMD phenotypes were associated with elevated risk of AMD. Mortality was not associated with AMD except for those 83 years and older where signs of late AMD were associated with increased risk of mortality (OR= 1.8, 95% confidence interval, 1.2-2.6).

Conclusion: Certain AMD phenotypes, age, smoking, plasma HDL cholesterol, elevated BMI are associated with AMD risk. Poor diet may also be associated with AMD.

SESSION 6-2

Treatment of neovascular age related macular degeneration (Wet AMD) with intravitreous anti-VEGF agents

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Treatment of neovascular age related macular degeneration (wet AMD) has been changed dramatically since 2004 when FDA first approved Pegaptanib (Macugen). It is anti Vascular Endothelium Growth Factor (VEGF) agent which is used intravitreously. However, Pegaptanib's dominance was short lived. Ranibizumab (Lucentis) and Bevacizumab (Avastin) were found more effective in treating wet AMD. The anti-VEGF treatment appears to have almost replaced thermal laser, Photodynamic therapy (PDT) and surgical treatments for wet AMD now.

Bevacizumab (Avastin), Ranibizumab (Lucentis) and Aflibercept (Eylea) are the major players for treatment of wet AMD at present time.

Many clinical trials of these three drugs for wet AMD have been reported concerning their efficacies and the risks. Head-to-head comparison studies between Avastin and Lucentis disclosed that both were equally effective in reducing the thickness of the macula with improvement of vision in patients with wet AMD and their risks of death, myocardial infarction, stroke were slightly higher in Avastin than Lucentis but the difference was insignificant. The cost difference of Lucentis and Avastin is significant, the former being much more expensive. The author will discuss the follows:

Drug to choose, the treatment protocol, judgment of treatment effects, options of the further treatment of the poor responders, the risk factors of the poor responders, the treatment and follow-up periods.

The anti VEGF treatment for wet AMD is aimed to stop blood supply from the neovascularization that develops in wet AMD thus making the macula dry. Although subretinal neovascularization is an important mechanism with which wet AMD develops, it may be only one of the important parts of the disease processes. The course of the disease may be modified by treatment but process itself may continue after the successful initial treatment.

Several attempts have been tried now for improving the intraocular drug delivery system such as implant or eye drops or improving the treatment effects such as combined treatment of Anti-VEGF with Anti Platelet-Derived Growth Factor (PDGF) agent or Fovista, or with photodynamic therapy (PDT) or with radiation therapy rather than Anti-VEGF agent mono-therapy at present time.

Anti-VEGF treatment for wet AMD will stay at present time until next new method develops in future.

SESSION 6-3

Lutein and zeaxanthin supplementation reduces photo-oxidative damage to retinal pigment epithelial cells

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Purpose: Oxidative damage and excessive inflammatory responses are etiologically related to the pathogenesis of age-related macular degeneration (AMD). Epidemiologic studies suggest that insufficient dietary lutein and zeaxanthin intake or lower serum zeaxanthin levels are associated with increased risk for AMD. The objective of this work is to test the protective effects of lutein and zeaxanthin against photo-oxidative damage to retinal pigment epithelial cells (RPE) and oxidation-induced changes in expression of inflammation-related genes.

Methods: To mimic lipofuscin-mediated photo-oxidation *in vivo*, cultured ARPE-19 cells were allowed to accumulate A2E, the major fluorophore and photosensitizer of lipofuscin, for 10 days and then cultured in the presence or absence of 10 μM lutein or zeaxanthin for an additional 3 days. The cells were then exposed to blue light for 10 min, followed by 6 h recovery in normal medium. Levels of oxidized proteins were determined by Western Blot. The levels of mRNA and secreted proteins of MCP-1, IL-8 and complement factor H (CFH) were determined by real-time RT-PCR and ELISA, respectively. Proteasome activity in the cells was measured using fluorogenic peptides as substrates.

Results: Supplementation of lutein or zeaxanthin to cultured ARPE-19 cells resulted in accumulation of lutien or zeaxanthin in the cells. The concentrations of lutein and zeaxanthin in the cells were 2-14-fold of that detected in the medium, indicating that ARPE-19 cells actively uptake lutein or zeaxanthin. Exposure of A2E-containing RPE to blue light resulted in a 2-fold increase in levels of oxidized proteins and 40-60% decrease in proteasome activity. Exposure of A2E-containing RPE to blue light also resulted in 50-80% decrease in expression of CFH and MCP-1 and ~ 20-fold increase in expression of IL-8. The photo-oxidation-induced changes in expression of MCP-1, IL-8 and CFH were similar to those caused by chemical inhibition of the proteasome, suggesting that inactivation of the proteasome is involved in the photo-oxidation-induced alteration in expression of these inflammation-related genes. Incubation the A2E-loaded RPE with lutein or zeaxanthin prior to blue light exposure significantly attenuated the photo-oxidation-induced inactivation of the proteasome and photo-oxidation induced changes in expression of MCP-1, IL-8, and CFH.

Conclusion: These data indicate that lutein or zeaxanthin modulates inflammatory responses in cultured RPE in response to photo-oxidation. Protecting the proteasome from oxidative inactivation appears to be one of the mechanisms by which lutein and zeaxanthin modulate the inflammatory response and AMD-related pathogenesis.

In-vivo imaging of laser-induced choroidal neovascularization in different rodent models

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Purpose: New technologies and devices for retinal imaging allow better visualization of the tissues at the posterior pole of the eye *in-vivo* with improved resolution and enhanced image contrast. During recent years, *in-vivo* imaging of rodent eyes in disease models has been increasingly performed using confocal scanning laser ophthalmoscopy (cSLO). Our studies demonstrate imaging of laser-induced choroidal neovascularization (CNV) in mice and rats for better and more refined diagnostic of the disease model.

Methods: A cSLO as well as a fundus camera were used for *in-vivo* imaging in rodents. A new formulation of indocyanine green (ICG, ICG/HS 15) was developed in order to improve chemical stability and fluorescence efficacy. In addition, different fluorescence labelled antibodies were created and applied intravitreally in rats for molecular imaging. *In-vivo* imaging was performed in pigmented rodents that had undergone argon laser photocoagulation to induce CNV. Retinal uptake and fluorescence following intravitreal injection were recorded.

Results: *In-vivo* imaging before dye application showed well-defined retinal lesions. cSLO imaging allowed visualisation of the inner and outer retina as well as the choroid. Immediately following intravenous injection of ICG/HS 15, a strong fluorescence was visible in the retinal vasculature and within the laser lesions. Pixel intensity in the retinal vessels, laser lesions and in the background was higher for ICG/HS 15 compared to conventional ICG. Fluorescence labelled markers were also immediately visible following intravitreal injection showing a strong fluorescence in the vitreous. Twenty-four hours after injection an accumulation of the marker-conjugate was observed in the laser lesions. Furthermore, multiple fluorescent spots were detected mainly in the retinal cell layers. Over time, a continuous decrease of fluorescence intensity was observed for all markers.

Conclusion: The spatio-temporal kinetics of different fluorescent dyes and markers following intravenous and intravitreal injection can be studied using *in-vivo* cSLO imaging methods. Strong accumulation within the laser lesions was observed for all markers and dyes tested. Laser-induced CNV in rodents combined with cSLO imaging and injection of customized dyes (ICG/HS15) are versatile tools for ocular toxicology studies and the development of new treatment regimes human CNV.

Risk factors for AMD; Focus on light exposure, nutrients, and medication

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The strongest risk factors world-wide for aging macula disorder (AMD), when we leave out the genetic interactions, are age, cataract surgery and smoking. It is unlikely at present time that UV light exposure is responsible for the association between AMD and cataract surgery due to the blocking filters in implant lenses that were introduced around 1980. However, also blue and visible light may be harmful to the retina due to reduction of lipofuscin autofluorescence and to disruption of the retinal pigment epithelium. Blue light filters have been added to around 25% of IOLs since 1990. Their rationale has been challenged and they may reduce scotopic and circadian photoreception. The latter is noteworthy especially since blue light sensitive ganglion cells were discovered in 2002 in the retina, responsible for the circadian clock. The lecture will cover the evidence for light-mediated associations with AMD including interactions with nutrients or antioxidants and commonly used medications.

Retinal phototoxicity, blue light and AMD —What do we know?

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Despite many laboratory research studies over several decades indicating the potential role of light—particularly short-wavelength light—in the etiology or promotion of age-related macular degeneration (AMD), there is little of epidemiological evidence supporting this link. This has led to considerable controversy over this expected link. Light levels employed in laboratory studies were significantly greater than the normal physiological range of environmental retinal illumination. Hence, it is difficult to extrapolate to the human condition of chronic exposure. Furthermore, most laboratory experiments have used rod-only animals and the relevance to the cone-rich macula of primates and humans must be questioned. Were there flaws in the epidemiological estimates of retinal exposure? Most published epidemiological studies do not show a statistically significant link of light levels and AMD, but these studies have questionable estimates of dose. There may also be an age factor in phototoxic susceptibility, and either elderly exposure to light or childhood exposure to UV (when the crystalline lens transmits some UV) have been proposed age ranges of interest. Whatever the answers to these questions, eye protective sunglasses may serve as good insurance against this possible link.

SESSION 7-3

Retinal light damage as subliminal confounder in toxicity studies

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Objective: Characterization of subliminal retinal light damage due to inappropriate light exposure conditions in longterm toxicity studies in albino rats.

Methods: Serial histopathological sections of albino rat eyes from various stages of longterm drug toxicity studies were examined for abnormalities of retinal morphology. The eyes had been fixed in Davidson solution and were stained with hematoxilin-eosin. Light microscopic examination has been performed using an Olympus microscope, but the examiner was unaware of the assignment to treatment groups.

Results: Various stages of retinal damage could be classified ranging from slightly increased loss of single photoreceptors to destruction of larger areas of the photoreceptor layer including the outer nuclear layerceptor to complete loss of the photosensitive layers of the retina. The damage always started on the temporal side of the optic disc and than extended to the periphery. The border between damaged and undamaged areas mostly presented as a narrow transition zone.

Conclusion: Retinal light damage is very difficult to detected in albino rats *in vivo* with an ophthalmoscope. It can occur in longterm toxicity studies simply due to inappropriate lighting conditions in the animal facilities. This can cause severe interpretation problems when the compound tested is suspected to have its own phototoxic potential. Due to the optical conditions in the rat eye - extremely large lens compared to the diameter of the eye ball - the light damage starts in the area of highest light concentration, temporal to the optic disk, and than extends towards the periphery. In albino rats the effect is independent of the pupil size whereas in pigmented rats the extension of the damage is depending on the pupil size.

SESSION 7-4

Effects of environmental factors during millimeter wave exposure

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Purpose: In recent years, some people experience millimeter wave (MMW) exposure in different environments, for which there is a paucity of data on potential biological effects. Although it has been demonstrated that MMW are absorbed by surface tissue, such as skin and cornea, there is insufficient evidence to determine whether environment factors including relative humidity, airflow and temperature play a role. As it is considered that relative humidity and airflow might have some influence on heat transport and evaporation, the present study was designed to investigate 75 GHz MMW band exposure to rabbit eye under different conditions of relative humidity and airflow.

Materials and Methods: Pigmented rabbits (N=30, Dutch-Belted, 12-15 week-old) were exposed unilaterally to 50-200 mW/cm^2 75 GHz MMW band for 5 min by lens antenna. Microencapsulated Thermochromic Liquid Crystal (MTLC) is 20-30 micrometer particles which change color with surrounding temperature. In order to record aqueous humor convection and change of MTLC color in the anterior chamber, MTLC was injected into the anterior chamber. Room temperature during MMW exposure was maintained at 24±2ºC by air-conditioner. Relative humidity was controlled by a humidifier and a dehumidifier. Airflow was maintained at 0.5 m/s by a commercial fan and airflow measurement device.

Results: During 75 GHz 100 mW/cm^2 MMW exposure, the anterior chamber just behind the cornea showed slight change of MTLC color at 2 minutes exposure without fan. At the same time point, no color change occurred with fan. Change to red and yellow of MTLC in the upper part of anterior chamber indicated temperature elevation. At 4 minutes, the border of lens surface showed blue, indicating heat at the cornea was transported to the lens. In contrast, with airflow there was no color change in the anterior chamber during MMW exposure.

No color change occurred in low humidity environments during 50 mW/cm^2 exposure. In contrast, color change occurred in the anterior chamber at 50 seconds exposure to 50 mW/cm^2 in high humidity environment. Then the entire anterior chamber showed increased temperature during the exposure was prolonged.

Conclusion: Environment humidity and environment airflow are important factors in heat transfer in eyes exposed to MMW. The possibility of controlling airflow and humidity may have a great impact on MMW exposure. The system we developed is useful to obtain data and quickly evaluate outcomes of MMW exposure to eye.

Ocular temperature measurements during electromagnetic exposure

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Purpose: To clarify difference of ocular damage mechanism between millimeter wave (MMW) and infrared (IR), ocular temperature changes in rabbit eyes during exposure to 40 or 95 GHz MMW or IR-A (808 nm) were compared with 3 different temperature measurement methods.

Material and methods: Pigmented rabbits (Dutch, 12-15 week-old) were unilaterally exposed to MMW (40 or 95 GHz: 200-300 mW/cm^2) or IR-A (808 nm: 1.0-2.0 mW/cm^2). Their ocular conditions were checked before and after exposure with a slit-lamp microscope. Ocular temperature during MMW exposure was measured with a fluoroptic thermometer (probe method) and a thermography camera. Microencapsulated thermochromic liquid crystals (MTLC) are 20-30 micron size particles, the color of which changes as the surrounding temperature changes, and MTLC 0.2% solution was injected into the anterior chamber of each rabbit eye.

Results: Ocular damage induced by 40 and 95 GHz MMW differed with 95 GHz being more severe in both cornea and crystalline lens. Corneal surface temperature by thermography during 40 and 95 GHz exposure showed that 95 GHz induced higher temperature than 40 GHz. Corneal surface temperature measured by thermography was well correlated with observed corneal damage, especially corneal epithelial cell damage. In contrast, cornea and lens temperature measured by probe method during 40 and 95 GHz exposure showed that 40 GHz induced exposure was higher than that of 95 GHz. Anterior chamber temperature distributions during MMW or IR were elucidated by MTLC. Heat induction by MMW and IR markedly differ. That is, MTLC color change under IR exposure was near the exposed iris. In contrast, under both 40 and 95 GHz MMW, MTLC color tone change started at the area of the anterior chamber located just behind the cornea.

Conclusion: The mechanism of heat cataract induced by MMW and the IR is different. The frequency and the thermal transport absorption characteristics of the ocular tissue are important factors in cataractogenesis. The MTLC temperature measurement method is a powerful tool to elucidate ocular temperature transport and temperature distribution in the anterior chamber.

SESSION 7-6

Near-infrared radiation cataract, thermal or photochemical?

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Purpose: 1) To estimate the just above threshold exposure time for the near IRR exposures in the seconds time domain at a constant irradiance of 197 W/cm^2 within the pupil, and the time evolution of lens damage after the estimated threshold exposure. 2) To determine the temperature time evolution in the eye during an 8 s in vivo threshold exposure to 197 W/cm^2 at 1090 nm, and the heat diffusion associated with exposure. 3) To investigate if 1090 nm IRR induces cataract photochemically considering irradiance exposure time reciprocity

Materials and Methods: Albino rats, 6-weeks old, were anesthetized. The pupils of both eyes were dilated with tropicamide, 5 mg/ml. Five minutes after pupil dilation, the animals were unilaterally exposed to 1090 nm IRR within the pupil. Temperature was recorded in selected positions of the eye with thermocouples. At the planned post-exposure time, the animal was sacrificed and the lenses were extracted for measurements of forward light scattering and macroscopic imaging.

Results: The in vivo exposure to 197 W/cm^2 1090 nm IRR required a minimum exposure time of 8 s for cataract induction. There was a 16 h delay between exposure and light scattering development in the lens. The same radiant exposure was found to cause a temperature increase of 10 ˚C at the limbus and 26 ˚C close to the retina. The rate constants estimated for the increase of temperature induced by the exposure was on the order of 10 times higher than those estimated for decrease of temperature after the end of the exposure. The in vivo exposure to 96 W/cm^2 1090 nm IRR with an exposure time up to 1 h resulted in an average temperature elevation of 7 ˚C at the limbus with the cornea humidified and no significant light scattering was induced at one week after exposure.

Conclusions: An in vivo exposure to 197 W/cm^2 IRR at 1090 nm within the pupil for 8 s induces cataract with a time delay. This threshold exposure for cataract induction causes a temperature rise of 10 ˚C at the limbus of the rat eye. If the exposure is limited within the pupil and the irradiance is limited to provide an equilibrium temperature rise of 7 ˚C at the limbus, no cataract develops within 1 hr of exposure (0.35 MJ/cm^2). Thus exposure of the transparent media of the eye to IRR at 1090 nm causes thermal cataract if the irradiance is high enough to produce a temperature rise above 7˚C but there is no support for photochemically induced cataract. The current data strongly suggest that cataract development without other damage to the eye after exposure to near IRR is due to temperature increase caused by IRR absorption in the iris.

Electroretinography in drug discovery and development

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Discovery investigations followed by non-clinical research toxicology studies are required for molecule screening as well as NCE's (New Chemical Entities) and CC (Clinical Candidates) selection before and after EIH (Entry Into Human). One of the most important points to consider is the choice of the non-rodent species which should have electrophysiology characteristics, anatomy (including the eye) and metabolic assimilation of the drug under investigation as close as possible to that of Human. It is important to use objective means od comparing these characteristics.

Not only classical drugs like NCE's but also other entities or certain eye-care products applied locally or systemically can reach the back of the eye, and potentially affect retinal function justifying the use of electroretinography (ERG) for this kind of development too.

Electrophysiological investigations (including ocular investigations like ERG) allow obtaining measurable and quantifiable data, which can be used for research purpose in order to compare the ERG profile in different species, to select the closest to human and/or to assess and to quantify the effects of NCE's and CC's on the retinal function. For research of ocular toxicity, it is important to obtain objective standardized measurements of retinal function, like those provided with the use of ERG.

This kind of investigations was historically and primarily aimed to assess the photoreceptors. ERG was shown to confirm coherence between scotopic/photopic ERG b-waves and anatomical rod/cone ratio determined histologically for various species.

However, in addition to rods and cones, nowadays, there are several other retinal structures and cells which can be assessed by ERG. As an example, flicker ERG can be used for testing lateral inhibition from horizontal and amacrine cells in various experimental animal species. ERG allows investigating the consequences of anatomical differences between the specificity of the target species (most often human (except for veterinary drugs), devoid of TL (tapetum lucidum)) and the selected experimental species. The TL can also be studied with ERG. As an example, it has been shown, in beagle dogs with one eye devoid of a TL, that for all eyes short-latency ERG components (a-b-wave complex) are within the normal range in amplitude, peak time and morphology. However, immediately following the b-wave, the large negative component (most pronounced in the scotopic conditions) recorded from the normal eye is absent in signals obtained from the TL-free eyes. The short-latency ERG responses are not modified by the absence of TL. In contrast, it has been considered that the post b-wave component, present in photopic and scotopic ERG could represent the electrophysiological evidence of some rebound effect (re-processing of the ERG within the neural retina). The increased amplitude of this post

b-wave negative response evoked in scotopic conditions could suggest a modification of the reflectivity of the TL during the dark adaptation.

When the ERG is expected to be affected by the studied NCE or CC, because of data obtained in preliminary studies, it might be relevant to select the closest model, including for the rodent species, for the part of the ERG which is involved. The rat or mouse scotopic responses are similar to those of humans but their photopic ERGs do not include a-waves and their b-waves have much higher amplitudes when compared to those of humans. On the contrary, the Guinea pig scotopic ERG is also different from that of human but the cone response is almost identical. Consequently, taking into consideration amplitude, frequency content and ERG morphology, the cone ERG of the Guinea pig is considered to be the most relevant rodent model for mimicking human photopic ERG. Thus, if human photopic ERG is expected to be affected, the Guinea pig should be used as a model rather than the rat or the mouse.

In conclusion, the ERG measurement is definitely important for research of ocular toxicity. Therefore findings related to species physiological and/or anatomical particularities, similarities or differences (for each ERG part respectively) or recording conditions - lightness or darkness, conscious or anaesthetized - have to be taken into consideration and precisely documented. For example, when anesthesia is used, not only the direct effect of aesthetics and other drugs (sometimes pancuronium avoiding globe bascule) but also those of removing diet on the day before anesthesia (potentially inducing hypoglycemia) have to be taken into consideration.

Synthetic proteoglycan mimics preserve vitreous structure and function *in vitro* and *in vivo*

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Purpose: The vitreous is a non-regenerative tissue that undergoes slow liquefaction with age. Currently, causes for vitreous degeneration are not well understood and there is no treatment for vitreous degeneration, except removal of the vitreous gel (vitrectomy) and replacement with saline solution. The goal of this study is to investigate a possible new therapy to delay vitreous degeneration or preserve the structure of the vitreous gel, thereby reducing the chance of damage to the retina and lens that caused by vitreous degeneration.

Materials and Methods: The key molecules of the vitreous gel are hyaluronic acid and type II collagen. We designed three synthetic proteoglycan mimics that specifically bind to either hyaluronic acid or/and type II collagen. First, we developed the enzymatically-degraded vitreous degeneration model *in vitro* and tested how each mimic reacted with fresh bovine vitreous. A shear rheometry was used to test the viscoelastic stiffness of vitreous. Quantification of morphological changes in vitreous ultrastructure was performed by a novel technique of deep-etch electron microscopy (DEEM). 18 adult albino rabbits were used for injection of the mimics to evaluate toxicity and stability as compared to BSS control in the fellow eye. *In vivo* observations included slit lamp, IOP and ISO inflammation score standard. Retinal histological examinations were also performed.

Results: *In vitro* studies show that trypsin treatment of bovine vitreous significantly reduced the viscoelastic stiffness and increased liquefaction. Addition of mimics to this trypsin-treated vitreous resulted in maintain of viscoelastic stiffness and liquefaction similar to control vitreous. DEEM analysis showed preservation of vitreous structure by mimics without formation of fiber bundles. *In vivo* studies in rabbit eyes confirmed safety of mimic injections. Histological study showed no changes in retinal morphology. The rheometry data revealed that the mimics could maintain normal values of viscoelastic stiffness for at least one month *in vivo*.

Conclusions: This study provides a potential novel approach to safely maintain or even restore the vitreous structure and physical properties for the protease-degraded or age-related vitreous degeneration.

High glucose induces and activates toll-like receptor 4 in endothelial cells of diabetic retinopathy

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Objective: Hyperglycemia-induced inflammation causes the dysfunction of blood vessels, and Toll-like receptor 4 (TLR4) plays a key role in inflammation-induced angiogenesis. However, the impact of TLR4 on the pathogenesis of diabetic retinopathy (DR) is poorly understood. In this study, we examined the expression of TLR4 in retinal vascular endothelial cells of patients with DR and diabetic mice, and explored the role of TLR4 in mediating inflammatory responses by human microvascular endothelial cells (HMEC-1) under high-glucose condition.

Methods and Results: The expression of TLR4 in retinal vascular endothelial cells of patients with proliferative diabetic retinopathy and diabetic mice induced by streptozotocin was examined using immunofluorescence. HMEC-1 were cultured and the expression of TLR4, MyD88 and Interleukin-1beta (IL-1beta) was examined under high-glucose condition. Endothelial cells with MyD88 silencing and antagonist of TLR4 as well as peritoneal macrophages from TLR4 deficient mice were used to study the effect of activated TLR4 on inflammation induced by high-glucose treatment. We observed that TLR4 was detected in CD31-labled human retinal vascular endothelia and its expression was markedly increased in fibrovascular membranes from DR patients and in retinal vascular endothelial cells of diabetic mice. The expression of TLR4, MyD88 and IL-1b was enhanced by high glucose in cultured HMEC-1 and the expression of TLR4 and IL-1beta was inhibited by TLR4 siRNA knock-down and TLR4 antagonist.

Conclusions: Our results revealed that hyperglycemia induced overexpression and activation of TLR4 in endothelial cells. This effect may lead to inflammatory responses contribute to the pathogenesis of diabetic retinopathy.

Comparisons of cone electroretinograms after indocyanine green-, brilliant blue G-, or triamcinolone acetonide-assisted macular hole surgery

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Purpose: To compare the function of retinal ganglion cells (RGCs) using the photopic negative response (PhNR) of cone electoretinograms (ERGs) in patients who had undergone indocyaine green- (ICG-), brilliant blue G- (BBG-), or triamcinolone acetonide (TA)-assisted internal limiting membrane (ILM) peeling during macular hole (MH) surgery.

Methods: Forty-eight eyes of 48 patients with a macular hole were randomly divided into those undergoing ICG-, BBG-, or TA-assisted vitrectomy (n=16 for each group). Full-field cone ERGs were recorded before and 1, 3, 6, 9, and 12 months postoperatively. ERG recordings were made using red stimuli on a blue background. The amplitudes and implicit times of the a- and b-waves and the amplitudes of the OPs and PhNRs were measured. The mean deviations (MDs) of standard automated perimetry and the best-corrected visual acuity (BCVA) were measured. The circumferential retinal nerve fiber layer (RNFL) thickness was evaluated by SD-OCT.

Results: All macular holes were closed with a significant improvement of the BCVA and MD without differences among the groups. There was no significant difference between the pre- and postoperative RNFL thickness. The implicit times of the a- and b-waves were significantly prolonged, and the ΣOPs amplitude was significantly decreased postoperatively in all groups. These ERG changes were not significantly different among the groups. The postoperative PhNR amplitudes were significantly lower in the ICG group than in the BBG or TA group.

Conclusions: The results indicate that the PhNR may detect subclinical impairments of RGCs caused by the possible toxic effect of ICG. This finding adds to the data that BBG and TA may be safer than ICG for use during MH surgery.

SESSION 8-5

Biomechanical effects of proteoglycan degradation on vitreous and vitreoretinal structure.

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Objective: Enzymatic degradation of structural proteoglycans (e.g. ocriplasmin injection) is emerging as a clinical tool for the treatment of symptomatic vitreoretinal adhesion. Not often considered or understood in this procedure, however, is how enzymatic degradation affects the vitreous body outside the immediate vitreoretinal interface. Hence, the purpose of this study was to determine how enzymatic degradation of proteoglycans affects the gross structure of the vitreous body.

Materials and Methods: Fresh bovine vitreous was injected with 300 µL phosphate buffered saline (sham control) or purified trypsin (1 mg/mL) to enzymatically digest proteoglycans overnight. To determine changes in mechanical properties, the vitreous was isolated for viscoelastic analysis on an AR-G2 rheometer with cleated parallel plate geometry (20mm diameter). To determine changes in collagen fiber macrostructure, injected eyes were embedded in agarose and sectioned (≈1.5 cm thick) with a razorblade for quantitative polarized light imaging (QPLI). For microstructural analysis, samples were characterized using quick freeze deep-etch electron microscopy (DEEM). All data are reported as mean +/- SD with a minimum of four samples used for each set of experiments.

Results: Proteoglycan digestion via trypsin injection significantly decreased the elastic storage modulus (G'), or the 'stiffness' of the vitreous body by approximately half (G'PBS = 8.6+/2.4Pa; G'Trypsin = 5.4 +/- 2.1Pa). QPLI indicated average optical retardation (δ; a measure of collagen fiber anisotropy, or 'alignment') was significantly greater in trypsin-treated vitreous relative to controls (δPBS = 5.9+/1.0; δTrypsin = 9.1 +/- 1.0). In enzymatically digested samples, collagen fibers were mostly aligned perpendicular to the optic disk (δODTrypsin = 14.4+/1.8), an anatomical region predisposed to tractional vitreoretinal adhesion during aging. High magnification DEEM images supported these results showing a significant increase in fiber alignment and loss in circular globules (presumed proteoglycans) in trypsin-treated vitreous relative to controls.

Conclusions: Enzymatic digestion of proteoglycans reduces the elastic stiffness of the vitreous body and aligns remaining collagen fibers, with highest fiber anisotropy occurring near the optic disk. These results suggest that symptomatic vitreoretinal adhesions could worsen from the tensile forces generated by concurrent gel shrinkage and increased perpendicular collagen fiber alignment if proteoglycan digestion does not completely release the vitreous from the retina. Moreover, if proteoglycan-digesting enzymes are injected into intact vitreous (e.g., as may occur in the off-label use of enzymes for the treatment of diabetic retinopathy-related conditions), our results predict this treatment may predispose the patient to the development of localized vitreoretinal tractions.

The effects of topical antiglaucoma drugs on ocular surface

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Long-term use of topical antiglaucoma drugs is often associated with ocular surface toxicity, such as allergic reactions, dry eye symptoms, subconjunctival fibrosis, and increased rate of filtrating surgery failure due to fibrotic bleb. In the United States, a cross-sectional study evaluated that 59% of glaucoma patients reported dry eye symptoms in at least one eye. Topical antiglaucoma drugs are associated with reduction of tear film break-up time and basal tear secretion; decrease of goblet cell density; development of subconjunctival fibrosis most likely related to an increase in inflammatory cells.

In this symposium, I would like to review the recent literatures including our research regarding the effects of topical antiglaucoma drugs on ocular surface and discuss the factors contributing ocular surface toxicity and how to resolve the problems. Long-term treatment with benzalkonium chloride (BAK)-containing topical antiglaucoma drugs appears to be the main contributor to corneal toxicity in a dose-dependent manner. Topical beta-blockers also appear to contribute to corneal toxicity more than topical carbonic anhydrase inhibitor or prostaglandin analogs. Among topical antiglaucoma drugs, a BAC concentration of 0.001% or lower or non-BAC preservative sofZia was suggested to be the least toxic to the ocular surface. In animal experiments, subclinical corneal epithelial damages, such as loss of microvilli, caused by topical antiglaucoma drugs containing BAK were observed by scanning or transmission electron microscopy. Preservative-free therapy is now available for a wide range of active compounds, although there are still some misconceptions regarding their appropriate use. For patients treated topically for glaucoma or ocular hypertension, a rough estimate could be about one-fifth patients may need treatment with topical intraocular pressure reducing agents that are free from preservatives. Recently, corneal protective eye drops, usually prescribed for dry eye symptoms, may be effective for subclinical corneal epithelial damage caused by topical antiglaucoma drugs.

Oxygen “toxicity” and the anterior segment

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Purpose: Previous studies suggest oxidative stress plays a role in the development of cataract and glaucoma. Following pars plana vitrectomy, studies have demonstrated an increased risk of open angle glaucoma, accelerated by cataract extraction. To further understanding, we measured pO2 and its distribution in patients and monkey undergoing intraocular surgery.

Methods: A reference group of patients undergoing primary cataract and/or glaucoma surgery with no prior history of intraocular surgery were recruited and provided informed written consent for the study. We performed these same studies on rhesus monkeys (ages 19-21 years) and obtained trabecular meshwork for histopathological analysis. To map pO2 (mmHg) in the anterior chamber (AC), an Oxylab pO2TM optical oxygen sensor was introduced via 30-gauge needle corneal paracentesis. In all patients, pO2 was sampled in the AC angle, mid-AC, and near corneal endothelium. In pseudophakes or those undergoing cataract extraction, the probe was also introduced into the posterior chamber (PC; between the lens and peripheral iris) and near the anterior lens surface.

Results: In the reference group, pO2 was highest adjacent to the cornea decreasing near the lens in a linear gradient. Intermediate levels of pO2 occurred in the AC angle and low levels in the PC. Following vitrectomy, pO2 significantly increased in the PC and in AC angle. Following vitrectomy plus cataract extraction, pO2 also increased in the PC and the AC angle. Measurements in our monkey model followed the same patterns as the human subjects, thereby demonstrating that a non-human primate is an excellent model for our studies to examine evidence of oxidative stress in the trabecular meshwork and alterations of the extracellular matrix of the trabecular meshwork.

Conclusions: Our *in vivo* measurements provide the first comprehensive description of oxygen gradients in human and monkey eyes. pO2 at the anterior lens surface and PC were substantially lower than other species. The effect of vitrectomy and lens extraction on pO2 in the PC and AC angle suggest an important influence of both vitreous and the natural lens in regulation of oxygen distribution/metabolism within the eye. Exposure to high pO2 following vitrectomy and subsequent cataract extraction may represent the oxidant source for oxidative damage to the trabecular meshwork. This protective role of the natural lens and the vitreous requires further study. In addition, pO2 correlates with other important risk factors for the development of open angle glaucoma- African-American race and central corneal thickness.

Ocular surface disorders in patients treated with BAK containing antiglaucoma drops

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Glaucoma is a chronic disease, which requires long-term medical therapy, often involving multiple ophthalmic medications. The use of eye drops with preservatives has led to a high prevalence of ocular surface disease (OSD) in medically treated patients with glaucoma.

Purpose: The aim of this presentation is to demonstrate typical cases of patients with dry eye disease and glaucoma who have stopped their medical treatment because of side effects.

Materials and methods: We present three cases of patients who received glaucoma medications containing benzalkonium chloride (BAK). All patients had history of dry eye disease which was not diagnosed and treated. They presented at the clinic with complaints of irritation, pain, photosensitivity and foreign body sensation. The patients underwent full eye examination including Shirmer test, TBUT, visual field test and OCT of RNFL. We stopped all BAK containing medications and the patients were given preservative-free antiglaucoma eye drops.

Results: BAK containing glaucoma agents had led to severe ocular surface disease in all patients. Corneal staining with fluorescein demonstrated punctate keratitis with epithelial erosions, causing progressive intolerance. This lead to poor compliance to the antiglaucoma therapy resulting in deterioration of intraocular pressure control and progression of the disease in two patients. Switching the medications to preservative-free agents was effective and well tolerated since all patients complaints diminished.

Conclusion: The use of glaucoma medications containing BAK is associated with a number of ocular symptoms including OSD and dry eye syndrome. This leads to a marked decrease in the compliance and a reduction in the quality of patient care and control of glaucoma. Dry eye syndrome, ocular allergy, meibomian gland dysfunction should be carefully evaluated before prescribing anti glaucoma treatment. In such cases preservative-free drops should be considered as first option. This would lead not only to an enhancement of the quality of life of patients, but also to improvements in glaucoma control.

POSTER

POSTER 1

Cytotoxic effects of 4 OTC ophthalmic solutions on cultured rabbit corneal cell line

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Purpose: The effect of 4 over the counter (OTC) ophthalmic solutions for rabbit corneal epithelium was evaluated on a cultured rabbit corneal cell line (SIRC).

Methods: Cultured SIRC (2x105 cells) incubated for 5 days were exposed to the 4 ophthalmic solutions A, B, C or D for 0~15 minutes and viable cells were counted, Surviving cells were counted by a Coulter counter, and 50% cell death time (CDT50;min.) was calculated. These effects are fatigue, haze, itch, conjunctiva hyperemia of eyes, including the eye disease prevention.

Results: CDT50 was more than 60 minutes with A, 11.5 minutes with B, 7.7 minutes with C, 11.6 minutes with latanoprost ophthalmic solution, and 51.0 miuntes with travatanz ophthalmic solution.

Conclusion: The cytotoxic effects of the 4 OTC ophthalmic solutions for rabbit corneal epithelium were greatly different with each OTC. The differences in the cytotoxic effects were largely due to the BAK concentrations in each of the ophthalmic solutions.

POSTER 2

Comparison of toxicities of moxifloxacin, cefuroxime, and levofloxacin on corneal endothelial cells *in vitro*

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Purpose: To evaluate and compare the toxic effects of moxifloxacin, cefuroxime, and levofloxacin on human corneal endothelial cells *in vitro*. In addition, to determine the safe intracameral concentrations for these antibiotics.

Methods: Human corneal endothelial cells (HCEn) in culture were exposed to moxifloxacin, cefuroxime, and levofloxacin at concentrations up to 2000 µg/ml. Evaluation of membrane damage was determined by ethidium homodimer-1 (EthD-1) uptake, and cell viability by intrinsic esterase activity. The inhibitory effects of the three antibiotics on the constitutive secretion of interleukin-6 (IL-6) by HCEn cells was determined by ELISA.

Results: The acute effects (6 h) of the three antibiotics on membrane damage and cell death were dose-dependent for moxifloxacin and levofloxacin (≥500 µg/ml). For cefuroxime, membrane damage was not observed at 6 h, and only slight damage was detected at 24 h at concentrations ≥500 µg/ml. The half maximal inhibitory concentrations (IC50) on cell viability of moxifloxacin, levofloxacin, and cefuroxime were 487 µg/ml, 578 µg/ml, and 1600 µg/ml, respectively. The inhibitory effects of the three antibiotics on the constitutive secretion of IL-6 were observed at ≥15.6 µg/ml for all indicating that the antibiotics can impair the secretion of the protective cytokine even at low concentrations.

Conclusions: Moxifloxacin at >500 µg/ml causes damage of the cell membranes of corneal endothelial cells, and even higher concentrations decrease the cell viability. Considering the lower minimum inhibitory concentration for inhibiting 90% growth by moxifloxacin, we recommend intracameral moxifloxacin at ≤500 µg/ml for prophylactic use.

POSTER 3

Lens pathological change after intravitreal injection of bevacizumab

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Purpose: To study lenticular change after intravitreal injection of bevacizumab (Avastin ®) for vitreoretinal disease.

Subjects and Methods: Subjects comprised 17 eyes of 12 patients (average age: 67.3±14.7 years old) administered Avastin for the first time in August to December 2007, and healthy controls. We graded lens opacity (WHO classification) by slit-lamp examination and measured light scattering intensity (LSI) with anterior segment analyzer (EAS-1000) before and after one, 4, 8, 12, and 24 weeks of administration of Avastin.

Results: No progression of cataract was seen in eyes administered Avastin. After one week, only one control had progression of posterior subcapsular cataract. There was no significant increase in LSI of each lenticular layer by EAS-1000.

Conclusions: No progression of lens opacity was observed after administration of Avastin.

POSTER 4

Side-effects associated with cycloplegic myopia control in Taiwan

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Purpose: Using topical cycloplegics for the control of school myopia has a long history in Taiwan. While the physiological side-effects of cycloplegia are well-known, its impact on visual functions is much less understood. The latter is addressed in the present study.

Methods: 1,067 students of ages ranging from 7 to 12 years old with no eye diseases and also otherwise healthy participated in the study. In addition to examination on pupillary responses to light, habitual near visual acuity, manifest refraction, and best corrected visual acuity, a questionnaire on visual complaints was also completed by students and their parents.

Results: 63.8% never used cycloplegics whereas 21.4% were long-term past users and 14.7%. present users. The highest usage was seen in Grades 5 (20.5%), 6 (17.8%), and 2 (17.1%). Among the cycloplegic users, 31.9% showed a decrease of myopia in the low myopia group (-0.51 to -3.00D); 29.1% in the mild myopia group (-3.01D to -6.00D), and 14.3% in the high myopia group (worse than -6.01D). A lag in optical correction was also noted: while 40.2% of the current cycloplegic users wore prescription spectacles, 43.8% had no optical correction at all.

Comparing to the non-users, cycloplegic users had more photophobia complaints during outdoor activities (OR=6.575, CI=3.699~11.687), worse habitual near visual acuity and worse best corrected visual acuity (OR=2.618~2.955), and higher risks in declining visual functions such as near point of accommodation (OR=7.379, CI=4.218~12.971), accommodation facility (OR=11.000, CI=2.996~40.386), cover test (OR=2.335, CI=1.122~4.857), near phoria (OR=1.838, CI=1.075~3.143), AC/A ratio (OR=2.698, CI=1.308~5.565), near point of convergence recovery (OR=1.958, CI=1.096~3.498), saccades (OR=2.710, CI=1.561~4.707), and stereopsis (OR=5.715, CI=3.118~10.473).

Discussion: Our results showed that while cycloplegic therapy appears somewhat effective, the visual functions were clearly altered which was accompanied by a lack of timely optical correction, little or no active protection against photophobia, and inadequate near vision care.

POSTER 5

In vivo confocal microscopic observation of patients with amiodarone induced keratopathy and Fabry disease

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Purpose: Amiodarone is a class III antiarrhythmic drug that has proven to be effective in the treatment of various types of tachyarrhythmias. Amiodarone-induced keratopathy is the most common ocular side effects and is characterized by golden-brown pigmentation and whorl-shaped opacities in the corneal epithelium. In previous reports, amiodarone keratopathy is often found in both eyes 1~3 months after administration of the drug. The patients of Fabry disease, a metabolic disorder of sphingolipids, show the same whorl-shaped corneal dystrophy, named vortex keratopathy.

To determine the mechanism of development of two types of vortex keratopathy, eyes with drug-induced amiodarone keratopathy or hereditary Fabry disease were studied by *in vivo* corneal confocal microscopy (RCM).

Methods: Eight patients (5 male and 3 female, mean age 74 years) starting an oral amiodarone regimen were examined by slit lamp microscopy and RCM regularly from the beginning of amiodarone treatment. One patient (female, age 32), who is a carrier of Fabry disease, was also examined by slit lamp microscopy and RCM.

Results: In amiodarone treated group, seven of eight patients developed vortex keratopathy, which was detected by slit lamp microscopy after an average 2 months of administration of the drug. Keratopathy was detected by RCM in four of seven patients before corneal pigmentation was detected by slit lamp microscopy. On average, RCM findings were evident in these 7 patients only after one month administration of the drug. In both the amiodarone and Fabry disease patients, RCM showed groups of epithelial cells with highly reflective deposits in cytoplasm extending from the basal to surface layers of the central cornea. However, in amiodarone keratopathy, highly reflective epithelial cells were first found at the center of the cornea and then spread to the peripheral region during therapeutic period. On the other hand, in Fabry disease, highly reflective epithelial cells were consistently observed extending from the central cornea to the basal layer of the corneal limbus.

Conclusion: RCM findings suggest that limbal stem cells with preexisting sphingo-glycolipid deposition may migrate to the central cornea in Fabry disease. In contrast, in amiodarone keratopathy, epithelial cells may take up amiodarone from the lacrimal fluid throughout the process of proliferation and differentiation of corneal epithelial cells.

POSTER 6

Effect of dry-eye ophthalmic solutions in intraoperative desiccation-induced corneal conjunctival damage

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Purpose: In this study, we evaluate that the healing effect of dry-eye ophthalmic solution in intraoperative desiccation-induced corneal conjunctival damage.

Materials and Methods: 3% diquafosol tetrasodium (Santen, Japan) (D group) and 2% rebamipide (Otsuka, Japan) (R group) were instilled to Japanese albino rabbits 4 times a day for 2 weeks preoperatively. And no pre medicated control group was also prepared (C group). Under general anesthesia, rabbit eyes were dried by lid retractor for 1 hour. The ocular surface damages were evaluated by lissamine green staining score. Periodic acid schiff reagent (PAS)-positive cell count (cells / 0.25 mm^2) in the bullar conjunctiva was measured by impression cytology.

Results: The lissamine green staining score, in C Group (1.9±0.5 points) was higher than in R Group (1.1±0.8 points) and D group (0.8±0.5 points) ($P<0.05$, Tukey-Kramar). The number of PAS-positive cells in C Group (158±29 cells) was lower than in R Group (231±35 cells, $P<0.05$) and D group (273±62 cells, $P<0.01$).

Conclusions: Preoperative medication of dry-eye ophthalmic solutions are effective to prevent Intraoperative desiccation-induced corneal conjunctival damage.

POSTER 7

Decrease in corneal damage due to benzalkonium chloride by the addition of mannitol

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Purposes: We investigated the protective effects of mannitol on benzalkonium chloride (BAC)-induced corneal damage using rat debrided corneal epithelium and a human cornea epithelial cell line (HCE-T). In addition, we demonstrated the usefulness of a new preservative system for timolol maleate (TM) eye drops consisting of BAC and mannitol.

Materials: Corneal wounds were monitored using a fundus camera TRC-50X equipped with a digital camera; eye drops were instilled into the rat eyes five times a day after corneal epithelial abrasion. The viability of HCE-T cells was calculated by TetraColor One; and Escherichia coli (ATCC 8739) were used to measure antimicrobial activity. The reducing effects on transcorneal penetration (*in vitro* study) and intraocular pressure (IOP) of the eye drops (*in vivo* study) were determined using rabbits.

Results: The corneal wound healing rate as well as cell viability were higher following treatment with 0.005% BAC solution containing 0.5% mannitol than in the case of treatment with BAC solution alone; the antimicrobial activity was approximately the same for BAC solutions with and without mannitol. In addition, the corneal wound healing rate for rat eyes instilled with commercially available TM eye drops containing 0.5% mannitol was significantly higher than that of eyes instilled with TM eye drops without mannitol. In the *in vitro* transcorneal penetration experiments, the amount of penetrated TM increased linearly for 6 h after the addition of TM eye drops with or without 0.5% mannitol into the donor chambers, and there were no significant differences in the amount of penetration between the two eye drop formulations (with or without 0.5% mannitol). On the other hand, the IOP enhancement in rabbits was induced by keeping them in a darkroom; after 5 h in the dark, the IOP of rabbits rose by 7.1-9.6 mmHg as compared with untreated rabbits (16.5 mmHg). TM eye drops containing 0.5% mannitol reduced the enhanced IOP, and the IOP reducing effects of TM eye drops both with and without mannitol were similar.

Conclusions: The present study demonstrates that BAC solutions containing mannitol are tolerated better on the rat ocular surface than the classic BAC only preservative system. In addition, mannitol does not affect the antimicrobial activity of BAC against E. coli or the corneal penetration of TM from commercially available TM eye drops. A BAC plus mannitol preservative system may provide effective therapy for glaucoma patients requiring long-term anti-glaucoma agents.

POSTER 8

Effect of travoprost on expression of EGF and corneal epithelial wound healing

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Purpose: To exmain the effects of travoprost on the expression of epidermal growth factor (EGF) and behaviors of cultured corneal epithelial cells.

Methods: Cultured human corneal epithelial cells were treated with travatanz® (Alcon) x100 diluted or travoprost (SIGMA) at the equivalent concentration. Cell proliferation was assayed by using Alamar blue method. mRNA expression of transforming growth factor beta1 (TGFb1), EGF, Vascular endothelial growth factor (VEGF), E-cadherin, and vimentin was checked by using real-time RT-PCR. Effects of the drug on epithelial sheet spreading was examined in organ-cultured rabbit cornea.

Results: Travatanz® x100 diluted or travoprost at the equivalent concentration did not affect cell proliferation. Travoprost increased EGF expression and promoted epithelial sheet movement.

Conclusions: Travoprost might promoted epithelial wound healing in corneal epithelium. *In vivo* effects of travoprost-induced EGF on epithelial barrier function is to be investigated.

POSTER 9

The effects of lychee flower against lipopolysaccharides-treated rabbit corneal epithelial cells

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Purpose: Currently bacterial keratitis treatment usually included the use of antibiotic eye drops to eliminate the bacterium, but the oxidative damage of tissue and the inflammatory response were still remaining. Therefore, this study examined the antioxidant capacity of lychee flower and whether lychee flower could balance the inflammatory response on infected corneal cells.

Methods: Rabbit corneal epithelial cells (RCEC) exposure to lipopolysaccharides (LPS) derived from Pseudomonas aeruginosa as a model of infected cornea. The cytotoxicity of medium supplemented with 150 ng/mL acetone-extracted lychee flower (ALF) on RCEC and the cell proliferation of (a) RCEC (control group); (b) RCEC treated with LPS (infected group); (c) RCEC treated with LPS and ALF (treatment group) for 24 hours were evaluated by MTT assay. The total antioxidant capacity of three groups was evaluated by Trolox equivalent antioxidant capacity (TEAC) assay.

Results: The cell proliferation of infected group is the highest among all groups ($p=0.001$), but there was no significant difference between treatment group and control group ($p>0.05$). In TEAC assay, treatment group showed the highest antioxidant capacity of all, and that in infected group was the lowest.

Conclusions: The results indicated that lychee flower could increase the antioxidant capacity and proliferation of corneal epithelial cells to against infection of Pseudomonas aeruginosa. Although the protective mechanism of lychee flower was still investigating, it might be a potential new extraction of protection on corneal infection.

POSTER 10

Lucentis update 2014 - Posology of Lucentis in US/EU/Japan

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Ranibizumab (Lucentis®) was first approved in the US on 30 June 2006 as the world's first therapeutic drug that significantly improved the mean visual acuity of patients with neovascular (wet) age-related macular degeneration (wet AMD). From this international birth day, eight years have been passed, and ranibizumab has been approved in more than 100 countries until now. During this period, ranibizumab has been established as the first line therapy for not only treatment of wet AMD, but also treatment of visual impairment due to diabetic macular edema (DME), macular edema secondary to retinal vein occlusion (RVO including branch RVO; BRVO and central RVO; CRVO), and choroidal neovascularization (CNV) secondary to pathologic myopia (PM). And research of optimal posology of ranibizumab has been continuing; the current approved posology in EU/Japan is in common agreement across the above indications on an individualized treatment regimen, namely, an initiation of once-a-month intravitreal injection to obtain the best outcome of visual acuity followed by pro re nata (PRN, as needed) regimen guided by visual acuity and/or disease activity to minimize under- or over-treatment. On the other hand, in US, once-a-month intravitreal injection is recommended for wet AMD, RVO and DME. Only for wet AMD, quarterly injection after 4 monthly injectons and PRN regimen after 3 monthly injectios are additionally mentioned in the labeling. Changes in the respective posologies of US/EU/Japan, including the latest information and future direction, are outlined.

POSTER 11

Visual complaints from cycloplegic control of myopia in elementary school students in Taiwan

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Purpose: Using topical cycloplegics for the control of school myopia has a long history in Taiwan. While the side-effects of cycloplegia, i.e., poor near vision and photophobia, are well-recognized, the impact on visual complaints and binocular functions is much less understood. Since the subjective complaints can interfere directly with academic learning, they are addressed in the present study.

Methods: 1,067 students of ages from 7 to 12 years with no eye diseases and otherwise healthy participated in the study. Ocular examination included testing of visual acuity, pupillary response to light, objective and subjective refraction, and binocular functions. For the purpose of the present study, participating students and their parents completed a questionnaire on visual complaints, adopted from CISS and COVD-QOL. Data were then analyzed for Odds Ratio (OR) as well as with $\chi 2$ Pearson by using the SPSS 18 package.

Results: Cycloplegic users were at higher risk than non-users in: (1) When viewing distance, cycloplegic users reported blurred vision (OR=11.599, CI=5.560~24.198), sore eye or eye fatigue (OR=3.658, CI=1.576~8.492), and head tilting (OR=7.482, CI=3.444~16.256). (2) In using digital devices, blurred vision (OR=4.711, CI=1.647~13.476) and head tilting (OR=6.485, CI=1.915~21.956) were also noted. (3) In addition to blurred vision (OR=14.052, CI=4.903~40.274), sore eye or eye fatigue (OR=2.928, CI=1.236~6.934), and head tilting (OR=4.205, CI=1.496~11.821), cycloplegic users also complained of double vision (OR=7.448, CI=1.029~53.894) and difficulty in completing assignment on time that required near work (OR=1.929, CI=1.031~3.610). Furthermore, cycloplegic therapy was accompanied by a lack of timely optical correction (Chi-Square Pearson R=0.096~0.266, p=.000~.035).

Discussion: Early detection and remedial action of cyclolegia-related visual complaints are both important because functional disturbances can otherwise interfere with academic learning and reduce the quality of life. Government-mandated public education of parents and teachers is imperative if cycloplegic therapy for controlling myopia in elementary school students is to be instituted.

POSTER 12

Comparison of intraocular and ocular surface bacterial isolates and their antibiotic resistance patterns at a tertiary eye hospital in South China

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Objective: Bacterial infection is a major cause of blindness around the world. This study was undertaken to compare the differences of bacterial isolates and their antimicrobial resistant patterns between intraocular and ocular surface infections at a tertiary eye hospital in South China.

Method: This is a retrospective study to evaluate the bacterial isolates and their antimicrobial resistances at Zhongshan Ophthalmic Center in south China between 2010 to 2013. Conjunctival swabs of conjunctivitis, corneal scrapes of keratitis, aqueous and vitreous samples of endophthalmitis were processed using standard microbiological procedures to identify bacterial isolates and their antimicrobial susceptibility patterns. The bacterial isolates were tested for antibiotic susceptibility to 8 antibiotics (ceftazidime, cefuroxim, cefazolin, levofloxacin, ofloxacin, neomycin, tobramycin, chloroamphenicol) using the Kirby-Bauer disc diffusion technique.

Results: Of 751 isolates, 225 were from conjunctival swabs, 398 from corneal scrapes, 36 from aqueous humor and 92 from vitreous samples. Gram-positive bacteria accounts for 78.44% of ocular surface isolates and 69.30% of intraocular infections. The first three bacteria identified were S. epidermidis (35.31%), P. aeruginosa (7.87%) and S. simulans (4.33%) in external ocular surface, and S. epidermidis (14.84%), bacillus subtilis (7.81%) and S. hominis (7.03%) in intraocular samples. Among eight antibiotics tested, resistance to neomycin was the least (8.26% of all isolates), whereas resistance to chloroamphenicol was the most (32.22%). Bacteria identified from aqueous and vitreous samples were more sensitive to levofloxacin, ofloxacin and neomycin than those from external ocular surface.

Conclusion: The bacterial spectrum and antibiotic resistance are different between intraocular and external ocular infection. Neomycin had the highest *in vitro* efficacy against bacteria identified from both intra- and external ocular infections.

POSTER 13

Eye disorders associated with colored contact lenses: 3 case studies

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Purpose: Colored contact lenses (CCL) were considered medically useful though developed and marketed as beauty products. With increased use of CCL among young women, several medical problems have arisen in Japan. We describe 3 cases of patients with eye disorders caused by CCL, treated as inpatients in our hospitals.

Subjects: Three female patients aged 22 years in average, hospitalized for CCL related eye disorders (2008 – 2013).

Results: Case 1, 21 yrs old, daily disposable CCL (Candy magic); case 2, 18 yrs old, two-week replacement CCL (2 week ACUVUE DEFINE); case 3, 27 yrs old, unknown CCL purchased from a discount shop. Corneal disorders and conjunctivitis were seen in all patients. Case 1 who had continuously worn CCL with improper lens-care, had epidemic keratoconjunctivitis in her left eye, corneal epithelium disorder in her right eye, and bilateral corneal endothelial disorders. Case 2 who had worn clear soft contact lenses before, developed corneal epithelium detachment and conjunctivitis on the second of using CCL for the first time. She used CCL properly and no bacteria was detected by conjunctival scraping culture test. Case 3 used well water as storage solution in a lens holder and had pseudodendritic keratitis. Acanthamoeba infection was suspected, though the strain was not detected, and was improved by frequent ocular instillation of chlorhexidine.

Conclusion: CCL have lower oxygen permeability than clear contact lenses and risk of scratching the cornea and conjunctiva by the colored site. Not only safety issues of CCL but also improper procedures in their use CCL by wearers are problems to addressed.

POSTER 14

Measurement of ocular UV exposure with mannequin UV sensors

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Purpose: To compare the influence of facial contour on ocular UV exposure using Asian and Western head mannequins embedded with UV sensors.

Methods: Mannequins, simulating facial contours of Western and Asian females in aged 40s, embedded with UV-AB sensors (4×4×1.5mm, sensitivity 260-400 nm) at top of head, forehead, and eyes (nasal, center, temporal) with facial reliefs (distance perpendicular to a line connecting forehead and cheek from corneal surface) of 5 mm and 10 mm for Asian and Western, respectively, were set on Kanazawa Medical University roof on April 14, 2014, and measurements in 8 azimuths were taken at each 10° from 30° to 60° of solar altitude.

Results: UV exposure dose to top of head increased with solar altitude in both mannequins. Ocular UV-AB exposure compared with exposure to top of head in Western was 5.7%, about half of that in Asian (11.5%). That of UV-B exposure (11.6%) in Asian was similar to UV-AB. Despite ocular UV-B exposure (7.9%) being slightly greater than UV-AB in Western, ocular exposure in Asian was markedly higher than in Western mannequins

Conclusions: Due to differences in facial skeletal geometry, ocular UV exposure doses and variations differ. Ocular UV exposure in Asians may be 1.5–2 fold greater than that in Westerners under high solar altitude. This difference in ocular UV exposure may be one factor why Asians have a higher prevalence of cortical cataracts than Westerners.

POSTER 15

Visual function of cataract sub-types

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Purpose: To evaluate the impact of cataract as drug side effect and anti-cataract drug effects, it is necessary to know the relationships among cataract type / grade and visual function. Since studies of visual function by cataract subtype ae few, we investigated the influence of aberration and backward light-scattering intensity (LSI) of crystalline lens on retinal image contrast in eyes with cataract subtypes.

Patients: Subjects comprised 645 eyes of 991 patients, aged mean (SD) 63.0 (±9.7) years, from Monzen Eye Study 2006 to 2013 and Kanazawa Medical University Hospital cataract surgery 2006 to 2013.

Methods: Point-spread function analyzer (PSF-1000) was used to measure retinal image contrast in 3.0 mm pupils under maximum mydriasis. Lens transparency property (LTP) was estimated from backward LSI of each layer of crystalline lens and optical distance (mm) measured, by anterior segment analysis system (EAS-1000). A wavefront analyzer (KR9000PW) was used to measure total higher-order (T-HOA) (cornea), T-HOA (ocular), coma (ocular) and spherical (ocular) in 4 mm pupil component maps. Retrodots (RD) and waterclefts (WC) were classified according to KMU cataract classification scheme.

Results and Conclusions: Retinal image contrast was significantly decreased in eyes with RD2 and over and with WC (center type) compared with eyes with transparent crystalline lens ($p<0.05$). T-HOA (ocular) was significantly increased in WC (center type) ($p<0.05$). Spherical (ocular) was significantly decreased in eyes with RD2 and over, but increased in eyes with WC (center type) ($p<0.05$). LTP was significantly decreased in eyes with WC (center type) ($p<0.05$). Factors reducing retinal image contrast differed by opacity type in crystalline lens. It was revealed that WC (center type) affected aberrations.

POSTER 16

Auto-fluorescence is an early sign of developing pinguecula

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Purposes: Our previous epidemiological surveys have shown that pinguecula and pterygium formation is closely related with ambient solar ultraviolet (UV) exposure to the eye, and that detection of early signs of pinguecula may be clinically significant. The purposes of the present study are: (1) To confirm the clinical relevance of auto-fluorescence detection by applying Ultraviolet Fluorescence Photography (UVFP) to the examination of conjunctival lesions such as pinguecula and pterygium. And (2) to conduct histopathological studies on tissues excised from UVFP-positive areas that correspond with pterygium and pinguecula, and that from one case of UVFP-negative conjunctival chalasis.

Subjects and Methods: UVFP was performed on 126 eyes of 63 volunteers (mean age 33.2 years) recruited at the Eye Clinic of Kanazawa Medical University Hospital (Group A) and 7 eyes of 6 patients with advanced pterygium and/or pinguecula and in one case, conjunctival chalasis, all underwent surgical excision (mean age 76.5 years) (Group B). HE staining and Elastic Masson staining were performed on the specimens.

Results: Auto-fluorescence in conjunctiva areas adjacent to the limbus was successfully recorded with a home-made UVFP apparatus. Among Group A, 28 of 101 eyes (27.7%) with no observable pinguecula and 25 eyes with apparent pinguecula (100%) all showed auto-fluorescence. In Group B, strong auto-fluorescence was noted within the pterygium proper, and weak punctate auto-fluorescence was also seen in the peripheral area. Characteristic histopathological findings of pterygium corresponding with the auto-fluorescence showed an increase in elastoid fibers. The increasing lamination of elastoid fibers appeared a localization marker as it was also observed in the peripheral areas of pterygium. In addition, thick elastoid fibers in the subconjunctival tissue of pinguecula was observed, in agreement with the fluorescence-positive sites. In contrast, conjunctival chalasis showed no such pathological findings.

Conclusions: The elastic fiber layers beneath conjunctival epithelium correspond well with auto-fluorescence, the latter can be regarded as an early sign of developing pinguecula. UVFP therefore appears highly useful as a clinical routine for diagnosing early pinguecula that can also be used as to mark the beginning of cumulative lifetime UV exposure to the eye.

POSTER 17

The role of the JNK/c-Jun and P38 pathways in the protective effect of hydrogen peroxide on RGC-5 cells from serum deprivation-induced apoptosis

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Purpose: To study the effect of hydrogen peroxide (H_2O_2) on the apoptosis of retinal ganglion RGC-5 cells induced by serum deprivation and the underlying mechanisms.

Methods: RGC-5 cells deprived of serum were used as a toxicity cellular model. The effect of H2O2 on the viability and apoptosis of RGC-5 cells was evaluated by MTT and Hoechst staining assays. The activation of kinases was determined by Western blotting with specific phospho-antibodies. Kinases or proteins involved were investigated by the application of kinase specific inhibitors or specific siRNA.

Result: Serum deprivation induced apoptosis of RGC-5 cells. Low concentrations of H_2O_2 (0-30 μM) blocked serum deprivation induced apoptosis of RGC-5 cells while a toxic effect was observed at higher concentration of H_2O_2 (>60 μM). Western blotting showed that 30 μM H_2O_2 significantly stimulated the activation of JNK and P38 MAPK while it had no effect on the activation of Akt, ERK1/2 and other survival kinases. Application of JNK or P38 MAPK specific inhibitors inhibited the protective effect of H_2O_2 although JNK inhibitors were more potent than inhibitors of P38 MAPK. Moreover, 30 μM H_2O_2 induced the phosphorylation of c-Jun time-dependently. Blockade of the phosphorylation of c-Jun induced by H_2O_2 or down-regulated the expression of c-Jun by siRNA attenuated the protective effect of H_2O_2 on RGC-5 cells.

Conclusion: The JNK/c-Jun and P38MAPK pathways play important roles in the protective effect of low concentrations of H_2O_2 on serum deprivation-induced injury of RGC-5 cells.

NOTES