The Association for Ocular Pharmacology and Therapeutics

AOPT 2013 Scientific Meeting

Alicante, Spain | February 7-10, 2013

Association for Ocular Pharmacology and Therapeutics

11th Scientific Meeting

February 7-10, 2013
Alicante, Spain

Sponsored by
Association for Ocular Pharmacology and Therapeutics
Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas

ACKNOWLEDGEMENTS

The Organizing Committee and the AOPT Board wish to offer very special thanks to the following sponsors for their generous support and educational grants for the 11th Scientific Meeting of the Association for Ocular Pharmacology and Therapeutics.

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INNPACTO

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Our special thanks to PIKOLINOS naturally good **and Ángeles Gallar**

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WELCOME

Welcome to Alicante and the 11th Scientific AOPT Meeting. This year marks the 20th anniversary of the Association for Ocular Pharmacology and Therapeutics, AOPT. Its origins date back to an inaugurating Ocular Pharmacology Symposium organized by founding members Hitoshi Shichi and George Chiou in Novi, Michigan in 1993. The goal was, and remains to this day, to bring together scientists (basic, preclinical, clinical) from academia and the pharmaceutical industry and eye care professionals who share an interest in ocular pharmacology and the treatment of ophthalmic diseases and to promote an exchange of scientific information. The biennial AOPT meetings provide an international forum for close interaction with colleagues and friends who share a passion for finding new treatment solutions for patients with vision related problems and diseases. These personal interactions strengthen collaborations and friendships or lead to new ones.

With these goals in mind the program spans many aspects from basic and clinical pharmacology and overcoming hurdles of drug delivery to demonstrating and monitoring clinical success with biomarkers, imaging and other technologies – leading to new therapeutic strategies and approaches. I hope you will find the program stimulating, learn new things, walk away with new ideas, make new contacts while strengthening existing ones, and forge new relationships and collaborations.

I want to take this opportunity to express my sincere thanks to the local organizing committee, chaired by Juana Gallar, for all their efforts and countless hours spent in putting together an exciting meeting at a fabulous venue.

More information on previous meetings and the society can be found at www.aopt.org and elsewhere in this program book. If you are not a current member of AOPT I encourage you to sign up or renew your membership.

Achim Krauss
AOPT President

WELCOME

It is our great pleasure to welcome you to the 11th Scientific Meeting of the Association for Ocular Pharmacology and Therapeutics (AOPT), in the city of Alicante, Spain.

The Biennial Meeting of AOPT is a special opportunity to check the state of the art in ophthalmic pharmacology and therapeutic intervention, and an excellent opportunity to share face-to-face with world leading researchers and other specialists your latest research findings and experience. AOPT meetings present top scientists from academia and the industry, and eye care professionals from around the world with the latest scientific achievements and research in the field of ocular pharmacology and therapeutics. This meeting also promotes the participation of young scientist and some of them have been invited to speak in the sessions, giving them the opportunity of presenting their work.

The AOPT 2013 scientific program includes the Keynote Lecture presented by Dr. Anand Swaroop, eleven oral sessions and two poster sessions which address topics in the ultimate advances in ocular pharmacology and therapeutics. We have done our best to prepare a stimulating scientific program, covering different fields: *Drugs and Ocular Blood Flow*, *New Strategies for Neuroprotection and Regeneration in Glaucoma*, *Eye Development and Myopia*, *Pharmacokinetics and sustained drug delivery*, *Novel Drugs and Devices for IOP lowering*, *Imaging and Therapy Monitoring*, *New treatments for Retinal Disease*, *Clinic Meets Research: Aspects of Translational Science*, *Biomarkers and Therapies for Regeneration*, *New Ophthalmic uses for existing drugs* and *Corneal and Ocular Surface Pharmacology and Therapeutics*.

We wish all of you a very productive and enjoyable meeting, and we hope that you enjoy also our city of Alicante, its weather and its excellent cuisine.

The AOPT 2013 Organizing Committee

CSIC

INSTITUTO DE NEUROCIENCIAS

COMMITTEES

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GlaxoSmithKline, USA

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Oliver Zeitz, MD
Bayer HealthCare, Germany

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Instituto de Neurociencias
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San Juan de Alicante, Spain

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Instituto de Neurociencias
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Facultad de Medicina
Universitat de Barcelona-IDIBAPS
Barcelona, Spain

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Christopher Paterson, University of Louisville, USA
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Ganesh Prasanna, Novartis Institute of Biomedical Research, USA
Herbert Reitsamer, Paracelsus Medical University, Austria
Daniel Stamer, Duke University, USA
Olaf Strauss, Charite University Medicine Berlin, Germany
Carol Toris, University of Nebraska Medical Center, USA
Richard Stone, Univ. Pennsylvania School of Medicine, USA
Christine Wildsoet, UC Berkeley School Optometry, USA
Thomas Yorio, North Texas Eye Research Institute, USA
Oliver Zeitz, Bayer HealthCare, Germany

Association for Ocular Pharmacology and Therapeutics
11th Scientific Meeting

Target Audience

This program is designed for principal investigators, scientists, medical professionals, technicians, and ophthalmic-care providers.

Objectives

● Discuss new developments in medical topics focusing on issues specific to ophthalmic pharmacology
● Review ophthalmic treatment paradigms to enhance management of patients with chronic ophthalmic conditions such as glaucoma, age-related macular degeneration, diabetes, myopia and ocular surface disease
● Expand the scope of care to encompass contemporary laboratory investigations with potential patient-care implications

Mission Statement

The **mission** of the Association for Ocular Pharmacology and Therapeutics (AOPT) is to serve as a global forum for the exchange of information about ocular pharmacology to meet the needs of vision scientists and eye care professionals for the advancement of vision research and the treatment of ophthalmic disorders worldwide.
The **vision** for this organization is to be recognized throughout the world as the premier scientific society and forum for pharmacology of the eye. AOPT uses innovative techniques for publication and dissemination of research findings through its official journal, the Journal of Ocular Pharmacology and Therapeutics, and biennial meetings. AOPT has a diverse leadership and membership and is recognized for its collegiality, responsiveness, and community spirit.

The **mission** of the Universidad Miguel Hernández (UMH) is to serve society by providing higher education, research and technology transfer, as well as top quality services that meet the expectations and demands of the community, and actively collaborate in the socio-economic development of the region. UMH is also dedicated to delivering a comprehensive education to the students, facilitating job placement, and enabling and fostering the professional development of the members of the university community.
The **goals** of UMH include to stand out as a prestigious, high-quality and socially respected educational and research institution, which actively contributes to economic, social and cultural development, and to provide, within the framework of the European High Education Area, a range of top-quality degrees tailored to the students' needs and providing them with a comprehensive education, thus enabling them to their transition right into a professional career and facilitating their continuing education and long life learning.
The AOPT 2013 Scientific Meeting represents an opportunity of postgraduate education for physicians, pharmacists and other health care providers that maintain or produce changes in knowledge, skills, attitudes or behaviors to nurture and enable the optimum provision of health care.

Accreditation

The Universidad Miguel Hernández designates this educational activity for a maximum of 2.75 ECTS.

AOPT MEETINGS

The Association for Ocular Pharmacology and Therapeutics has sponsored the following meetings:

Meeting	Date	Location	Organizer
Tenth Meeting	February 17-20, 2011	Ft. Worth, Texas	Thomas Yorio Abbot Clark
Ninth Meeting	February 18-21, 2009	Salzburg, Austria	Herbert Reitsamer
Eighth Meeting	February 9-11, 2007	San Diego, CA	John Liu
Seventh Meeting	February 3-5, 2005	Catania, Sicily, Italy	Filippo Drago
Sixth Meeting	February 1-4, 2003	Kona, HI	Peter Kador
Fifth Meeting	November 2-5, 2000	Birmingham, AL	Jimmy Bartlett
Fourth Meeting	January 28-31, 1999	Irvine, CA	Achim Krauss
Third Meeting	October 22-24, 1997	Bethesda, MD	Peter Kador
Second Meeting	August 15-17, 1996	Los Angeles, CA	David Lee
First Meeting	January 26-29, 1995	New Orleans, LA	Herb Kaufman
Ocular Pharmacology Symposium	August 8-10, 1993	Novi, MI	Hitoshi Shichi

AOPT MEMBERSHIP INFORMATION

The bylaws of the association establish four classes of membership in AOPT: Regular Members, Associate Members, Contributing Members, and Emeritus Member.

- REGULAR MEMBERS. Regular Members are individuals demonstrating a genuine interest in or making significant contribution to ocular pharmacology and therapeutics. This may be evidenced by a) scientific publications; b) attendance at pharmacological, ophthalmological, optometric, or visual science meetings; c) direct involvement in research. A candidate for membership completes the online membership form and pays the appropriate membership dues. Membership is for two years. A subscription to the Journal of Ocular Pharmacology and Therapeutics is optional.
- ASSOCIATE MEMBERS. Associate Membership is for predoctoral and postdoctoral students. A candidate for this membership must have a pre-doctoral, or post-doctoral student status, and must complete the online membership form and pay the appropriate membership dues.
- CONTRIBUTING MEMBERS. Contributing Membership is restricted to corporations, associations, and individuals who support the objectives of AOPT but do not satisfy the requirements of Regular Membership or individuals elected to membership in any class who voluntarily choose to become Contributing Members. A candidate for contributing membership completes the online membership form and pays the appropriate membership dues.
- EMERITUS MEMBERS. Any Regular Member may make a written request to the Treasurer that his/her membership be transferred to that of an Emeritus Member. The request is subject to approval of the membership committee. Emeritus Members have all the rights and privileges of Regular Members, except those of voting and holding elective office.

Membership application form is available at the Association for Ocular Pharmacology and Therapeutics website (http://www.aopt.org/membership/apply/?Membership-Application). AOPT is now accepting membership dues for the 2013-2014 period.

Official Journal

The Journal of Ocular Pharmacology and Therapeutics (JOPT), published by Mary Ann Liebert, Inc., publishers (140 Huguenot Street, 3rd Floor, New Rochelle, NY 10801), is the official Journal of AOPT. A substantially reduced subscription rate for this journal (electronic format) is an optional membership benefit, as indicated above.

AOPT Information

Information about the AOPT membership or any other matters related to the Association can be obtained using the on-line contact available at http://www.aopt.org/aboutus/contact.

AOPT 2013 KEYNOTE LECTURE

The AOPT 2013 Keynote Speaker is Dr. **Anand Swaroop**, Chief, Neurobiology-Neurodegeneration and Repair Laboratory, National Eye Institute (NEI), National Institutes of Health (NIH), Bethesda, MD, USA. The title of his lecture will be "***Cell and Gene-Based Treatment of Retinal Neurodegenerative Diseases***".

Anand Swaroop

Anand Swaroop obtained his Ph.D. at the Indian Institute of Science in Bangalore, India, and postdoctoral training at Yale University. He joined the University of Michigan in 1990 as assistant professor in Ophthalmology and Human Genetics. He became full professor in 2000 and Harold F. Falls Collegiate Professor in 2003. In 2007, Dr. Swaroop joined the National Eye Institute as Senior Investigator to establish a new program in retinal development and knowledge-based treatment paradigms for retinal degenerative diseases.

He has received several honors, including the Board of Director' s award from The Foundation Fighting Blindness, the Harrington Senior Scientific Award from Research to Prevent Blindness, and Distinguished Faculty Lectureship Award from the University of Michigan Medical School.

Dr. Swaroop has authored over 200 peer-reviewed research papers that have appeared in journals such as the *Nature Genetics,* the *New England Journal of Medicine* and the *Proceedings of the National Academy of Sciences.* He has also served as a reviewer of scientific publications for major journals, including *Science* and *Cell*.

Anand Swaroop leads the "Neurobiology Neurodegeneration & Repair Laboratory" of the NEI. The goal of his research team is to develop novel treatment modalities for blinding retinal diseases based on a fundamental understanding of genetic defects and/or biological pathways underlying differentiation, homeostasis, aging and disease pathogenesis.

Dr. Swaroop is investigating how stem cells develop into nerve cells (particularly photoreceptors) in the retina, how these neurons connect to each other, and how they become dysfunctional or die during aging or in disease conditions. His goal is to use this understanding to develop new treatments for blinding retinal conditions. His research highlights include:

- Uncovered several **genes and variants that increase the susceptibility for age-related macular degeneration (AMD)**. Through genetic and genome-wide association studies, major biochemical pathways have been revealed and may be targets for future AMD treatments.
- **Identified genes for several types of retinitis pigmentosa**, a group of inherited retinal degenerative diseases and **the gene for a common form of Leber congenital amaurosis**, a childhood blinding disease. Currently involved in identifying disease mechanisms and developing treatment paradigms using gene therapy, cell-based therapy, and small molecule screening.
- Discovered how **certain genes and molecules control the development of light-sensitive retinal cells** from stem/progenitor cells in the eye.

For more information, visit the Neurobiology Neurodegeneration & Repair Laboratory website.

GENERAL INFORMATION

Meeting Venue

Hotel Spa Porta Maris
Plaza Puerta del Mar, 3
03002 Alicante, Spain
Tel:+34 965147021
Fax: +34 965216945
www.hotelspaportamaris.com

Dates

February 7-10, 2013.

Language

The official language of the AOPT 2013 meeting is English.

Welcome Reception

The Welcome reception will be held Thursday February 7, 2013 at 18:00 at the Meeting Venue (Poster-exhibit area).

Opening Remarks

The official opening of the meeting will be held Friday February 8 at 8:00 at the Meeting Room.

AOPT Dinner

The AOPT Dinner, open to all registered participants, will be held Saturday February 9 at 20:00 at Darsena Restaurant, located at the *Alicante Marina*, 6 East Pier, 03001 Alicante.
Since 1961, the Darsena is specialized in Spanish rice dishes, and pioneered the introduction of a new term into the Spanish culinary culture: *Arrocería* (restaurant specialized in rice dishes). The Darsena offers excellent traditional Alicante cuisine, including many rice dishes together with exceptional fresh fish and shell fish from the local fish markets.
Additional tickets for the AOPT Dinner can be purchased from the registration desk for 60€/person until Saturday, February 9 at 15:00 hours.

AOPT Business Meeting

The AOPT Business Meeting will be held on Friday, February 8 from 12:00 to 12:45 hours. All AOPT members are encouraged to attend.

On-site Registration Desk and Exhibition Opening Hours

Thursday, February 7: 16:00 – 18:00
Friday, February 8: 07:30 – 19:00
Saturday, February 9: 07:30 – 19:00
Sunday, February 10: 07:30 – 12:00

Name Badges
All participants are kindly requested to wear their name badges throughout the meeting.

Clothing
Clothing is informal for all occasions.

Certificate of Attendance
Certificates of attendance will be provided in the delegate bag.

Internet Access
Free WIFI connection is provided at all SPA PORTA MARIS and SUITES DEL MAR rooms and other areas of the hotel.

Liability and Personal Insurance
The AOPT 2013 Organizers cannot accept liability for personal accidents or loss of or damage to private property of participants and accompanying persons. Participants are recommended to take out their own personal travel and health insurance for their trip in Alicante.

Safety and Security
We kindly request you not to leave bags, suitcases or backpacks unattended at any time during the meeting.

AOPT 2013 Meeting Secretariat
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INFORMATION FOR PRESENTERS

Oral Presentations

Presenters using Powerpoint (or equivalent software) presentation should bring it on memory stick (USB connected), CD or DVD, and load it in the designated computer between 7:30-8:15 (morning sessions) or 13:00-14:00 (afternoon sessions).
Presenters combining Powerpoint and video films are requested to double-check their presentations before the sessions to be sure they work properly.
Please note that PCs in the session hall will be provided with Office 2010.
Macintosh will be also available. Alternatively, MAC users can bring their own computer and a VGA adaptor.

Poster Presentations

Posters will be on display in two sessions. Posters must be mounted by 10:00 on the day of their presentation, and must remain on display until the end of the day (19:00 hours). Posters presenters are requested to stand by their posters for informal discussion during the designated poster session and during the coffee breaks on the day of presentation. Posters left after the end of the sessions each day will be removed and discarded. AOPT 2013 is not responsible for poster materials left at meeting's end.

Poster Session 1: Friday, February 8, 17:45–19:00 - **Poster Numbers:** P1.01 to P1.16
Topics
Corneal and ocular surface pharmacology and therapeutics
Imaging and therapy monitoring (cornea)
Pharmacokinetics and sustained drug delivery (cornea)
Poster Session 2: Saturday, February 9, 17:45–19:00 - **Poster Numbers:** P2.01 to P2.16
Topics
Clinic meets research: aspects of translational sciences
Drugs and Ocular blood flow
Imaging and therapy monitoring
New ophthalmic uses for existing drugs
New strategies for neuroprotection and regeneration in glaucoma
New treatments for retinal disease
Novel drugs and devices for IOP lowering
Pharmacokinetics and sustained drug delivery

Disclosures

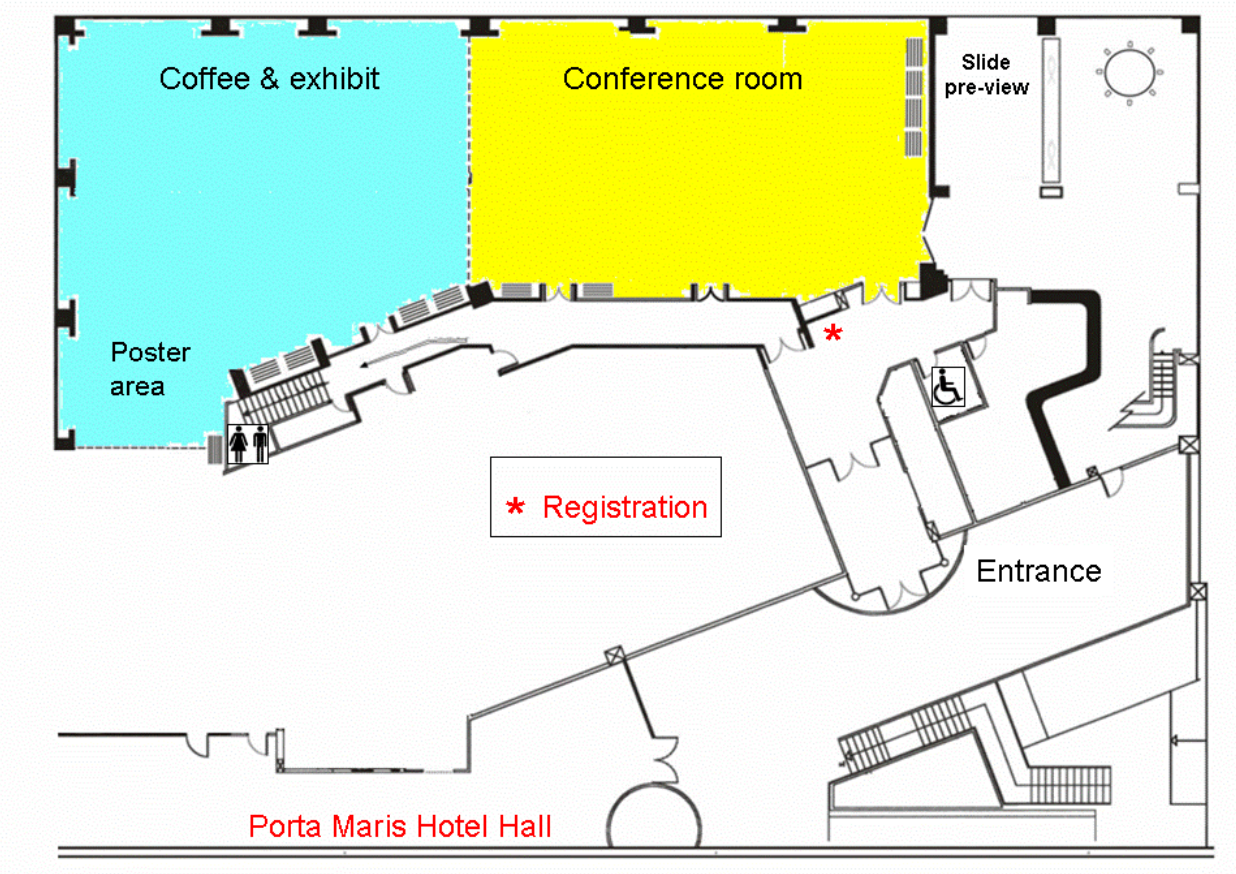
All Commercial Relationships must be indicated on a slide of the presentation and on the posters, even if they were not indicated at abstract submission.

Recording Policy

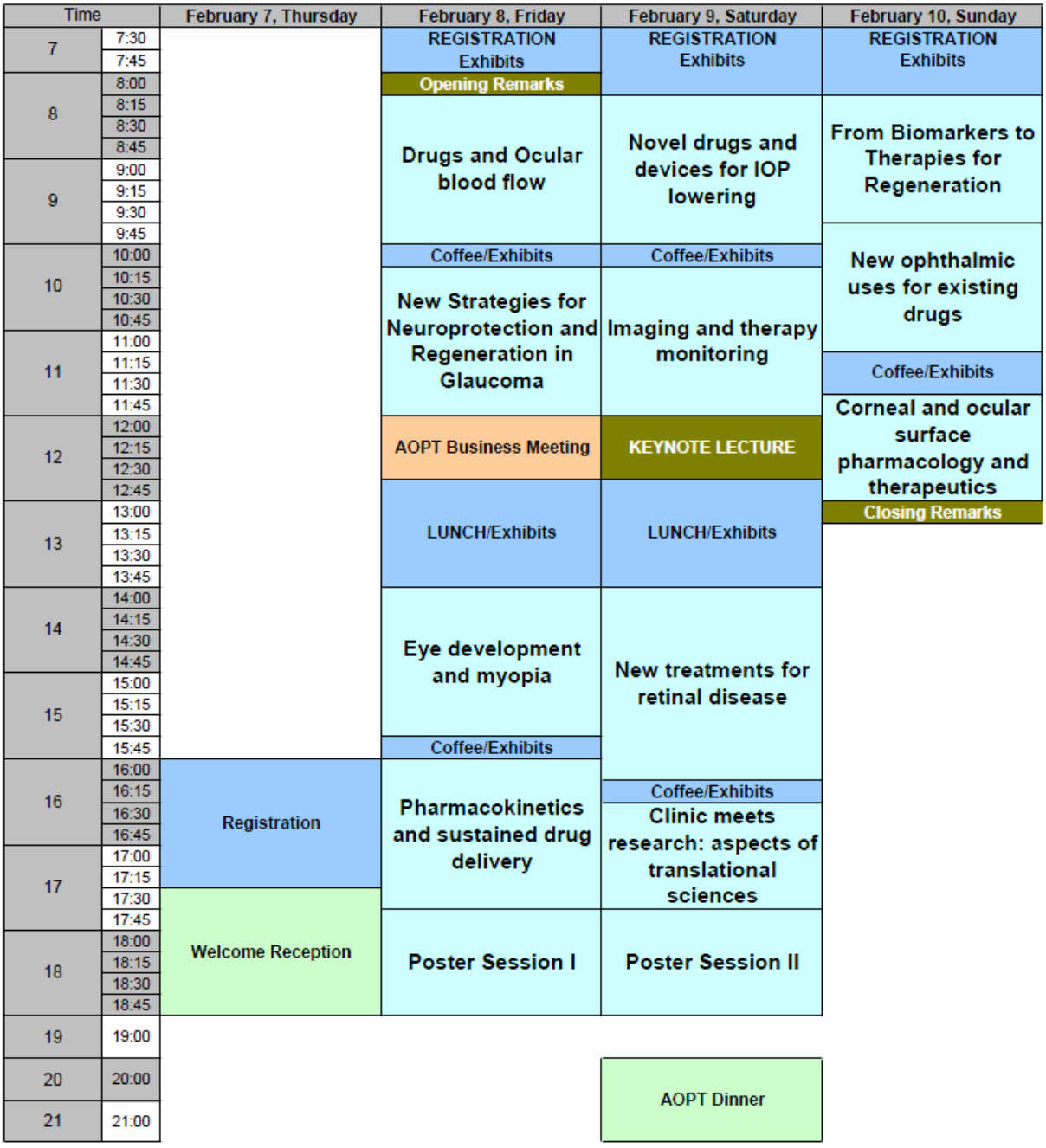
Recording (photographing, audiotaping, or videotaping) any presentation or poster is PROHIBITED, except by AOPT agents for official purposes or by authors who want to photograph their own poster presentations.

MEETING FLOOR PLAN

Ground Floor Layout



PROGRAM AT A GLANCE



Time		February 7, Thursday	February 8, Friday	February 9, Saturday	February 10, Sunday
7	7:30		REGISTRATION Exhibits	REGISTRATION Exhibits	REGISTRATION Exhibits
	7:45				
8	8:00		Opening Remarks		
	8:15		Drugs and Ocular blood flow	Novel drugs and devices for IOP lowering	From Biomarkers to Therapies for Regeneration
	8:30				
	8:45				
9	9:00				
	9:15				
	9:30				
	9:45				
10	10:00		Coffee/Exhibits	Coffee/Exhibits	New ophthalmic uses for existing drugs
	10:15		New Strategies for Neuroprotection and Regeneration in Glaucoma	Imaging and therapy monitoring	
	10:30				
	10:45				
11	11:00				Coffee/Exhibits
	11:15				
	11:30				Corneal and ocular surface pharmacology and therapeutics
	11:45				
12	12:00		AOPT Business Meeting	KEYNOTE LECTURE	
	12:15				
	12:30				
	12:45				Closing Remarks
13	13:00		LUNCH/Exhibits	LUNCH/Exhibits	
	13:15				
	13:30				
	13:45				
14	14:00		Eye development and myopia	New treatments for retinal disease	
	14:15				
	14:30				
	14:45				
15	15:00				
	15:15				
	15:30				
	15:45		Coffee/Exhibits		
16	16:00	Registration	Pharmacokinetics and sustained drug delivery	Coffee/Exhibits	
	16:15			Clinic meets research: aspects of translational sciences	
	16:30				
	16:45				
17	17:00				
	17:15				
	17:30	Welcome Reception	Poster Session I	Poster Session II	
	17:45				
18	18:00				
	18:15				
	18:30				
	18:45				
19	19:00				
20	20:00			AOPT Dinner	
21	21:00				

SCIENTIFIC PROGRAM

Friday, February 8, 2013

Session **O1: Drugs and Ocular Blood Flow**

Moderators: **Jeff Kiel**, University of Texas Health Science Center at San Antonio, USA
Malinda Fitzgerald, Christian Brothers University/UTCHS, USA

8:15	O1-01	DOPPLER OCT FOR THE QUANTIFICATION OF RETINAL BLOOD FLOW IN HUMANS Leopold Schmetterer, Medical University of Viena, Austria
8:35	O1-02	OPTIC NERVE HEAD AUTOREGULATION Doreen Schmidl, Medical University of Vienna, Department of Clinical Pharmacology, Austria
8:55	O1-03	EFFECTS OF FLUPENTHIXOL ON OCULAR BLOOD FLOW AND INTRAOCULAR PRESSURE IN THE RABBIT. Jeffrey Kiel, UTHSCSA, USA
9:15	O1-04	THE ROLE OF SEROTONERGIC NEURONS OF THE PARAMEDIAN RAPHE IN REGULATING CHOROIDAL BLOOD FLOW IN RATS Malinda E.C. Fitzgerald, Christian Brothers University/ UTCHS, USA
9:35	O1-05	EPISCLERAL VENOUS PRESSURE (EVP) AND INTRAOCULAR PRESSURE (IOP) RESPONSES TO CENTRAL ELECTRICAL STIMULATION IN THE RAT Clemens Strohmaier, Ophthalmology/Paracelsus University, Austria
9:55	O1-C	Conclusions

Session **O2: New Strategies for Neuroprotection and Regeneration in Glaucoma**

Moderators: **Neville Osborne**, Oxford University, UK and FIO Spain
Abbot Clark, University of North Texas Health Science Center, USA
Shahid Husain, Medical College of South Carolina, USA

10:15	O2-01	OPTIC NERVE REGENERATION AND RETINAL GANGLION CELL PROTECTION IN ANIMAL MODELS OF TRAUMATIC NERVE INJURY AND GLAUCOMA Larry Benowitz, Children's Hospital Boston/Harvard Medical School, USA
10:55	O2-02	SIGMA-1 RECEPTOR-MEDIATED NEUROPROTECTION OF RGCS. Thomas Yorio, UNT Health Science Center, North Texas Eye Research Institute, USA
11:15	O2-03	HYDROGEN SULPHIDE: A POTENTIAL SUBSTANCE FOR THE TREATMENT OF GLAUCOMA Neville N. Osborne, Fundación de Investigación Oftalmológica, Instituto Oftalmológico Fernández-Vega, Oviedo 33012, Spain and Nuffield Laboratory of Ophthalmology, Oxford University, Oxford, UK United Kingdom
11:35	O2-04	DELTA OPIOID-RECEPTOR AGONISTS PROTECT RGCS AND OPTIC NERVE AXONS FROM GLAUCOMATOUS INJURY Shahid Husain, Medical University of South Carolina, USA
11:55	O2-C	Conclusions

Session **O3: Eye development and myopia**

Moderators: **Richard Stone**, Univ. Pennsylvania School of Medicine, USA
Christine Wildsoet, UC Berkeley School Optometry Center for Eye Disease & Development, USA

14:00	O3-01	MYOPIA- THE BIG ELEPHANT IN THE ROOM Jost Jonas, Ophthalmology Universitätsmedizin Mannheim, Germany
14:22	O3-02	THE CENTRAL ROLE OF THE RETINA IN EYE GROWTH AND REFRACTION Ian Flitcroft, Children's University Hospital, Dublin Ireland
14:44	O3-03	SCLERAL MICRO-RNA SIGNATURES IN ADULT AND FETAL EYES AND POSSIBLE ROLES IN OCULAR GROWTH REGULATION Ravikanth Metlapally, UC Berkeley, USA
15:06	O3-04	7-METHYLXANTHINE FOR PREVENTION OF EYE ELONGATION AND MYOPIA PROGRESSION IN MYOPIC CHILDREN Klaus Trier, Trier Research Laboratories, Denmark
15:28	O3-C	Panel

Session **O4: Pharmacokinetics and sustained drug delivery**

Moderators: **Uday Kompella**, University of Colorado Anschutz Medical Campus, USA
Rocio Herrero-Vanrell, Universidad Complutense de Madrid, Spain

16:00	O4-01	CONTROLLED DRUG DELIVERY TO THE BACK OF THE EYE BY NONINVASIVE METHODS Ashim Mitra, University of Missouri-Kansas City, USA
16:18	O4-02	ROLE OF DENDRIMER-DRUG CONJUGATES IN SUSTAINED INTRA-OCULAR DRUG DELIVERY Raymond Iezzi, Ophthalmology, Mayo Clinic, USA
16:36	O4-03	NEW APPROACHES IN OCULAR HYPOTENSIVE TOPICAL TREATMENTS BASED ON BIOADHESIVE POLYMERS. Vanessa Andrés-Guerrero, Complutense University of Madrid, Spain
16:54	O4-04	NANOTECHNOLOGY FOR DRUG AND GENE DELIVERY Uday Kompella, University of Colorado Anschutz Medical Campus, USA
17:14	O4-05	MICROSPHERES FOR THE TREATMENT OF CHRONIC DISEASES AFFECTING THE BACK OF THE EYE Rocio Herrero-Vanrell, Complutense University of Madrid, Spain
17:32	O4-C	Panel

Poster Session 1- **P1**

17:45-19:00

Moderators: **Adolfo Aracil**, Universidad Miguel Hernández-CSIC
Illés Kovács, Semmelweis University, Hungary

P1-01 TREATMENT OF NEUROTROPHIC KERATOPATHY WITH NICERGOLINE
So-Yoon Kim, Department of Ophthalmology and Visual science Uijeong, Republic of Korea

P1-02 EFFECTS OF IL-6 AND IL-10 ON AN IN VITRO MODEL OF CORNEAL WOUND HEALING
Isabel Arranz-Valsero, IOBA-University of Valladolid, Spain

P1-03 CARBON MONOXIDE ACCELERATES WOUND HEALING AFTER CORNEAL EPITHELIAL INJURY
Giuseppina Marrazzo, University of Catania, Italy

P1-04 CORNEAL INNERVATION AND EPITHELIAL WOUND HEALING IN SP AND ALPHA-CGRP KNOCKOUT MICE
Kamila Mizerska, Universidad Miguel Hernández-CSIC, Spain

P1-05 TEAR SECRETION INDUCED BY CARBACHOL IS MEDIATED BY Ap4A RELEASE
Begoña Fonseca Vazquez, Universidad Complutense de Madrid, Spain

P1-06 SYL1001: A NEW TREATMENT FOR OCULAR PAIN ASSOCIATED TO DRY EYE SYNDROME BASED ON RNAi TECHNOLOGY: SAFETY AND TOLERANCE.
Tamara Martinez, Sylentis, Spain

P1-07 LONG-TERM EYE DRYNESS ENHANCEMENT OF CORNEAL COLD-THERMORECEPTIVE NERVE TERMINALS FIRING IS ASSOCIATED TO CHANGES ON VOLTAGE-GATED Na+ CURRENTS
Xavier Gasull, Universitat de Barcelona-IDIBAPS, Spain

P1-08 CORNEAL COLD NERVE ACTIVITY IN DRY EYE IS MODULATED BY CYCLOSPORINE A.
Carolina Luna, Universidad Miguel Hernández-CSIC, Spain

P1-09 SODIUM CHANNEL BLOCKERS MODULATE CORNEAL SENSORY NERVES ACTIVITY IN INTACT AND INJURED CORNEAS.
Susana Quirce, Universidad Miguel Hernández-CSIC, Spain

P1-10 CHANGES IN SENSORY NERVE ACTIVITY OF THE OCULAR SURFACE UNDER ALLERGIC CONJUNCTIVITIS ARE MEDIATED IN PART BY TRPA1 CHANNEL
M. Carmen Acosta, Universidad Miguel Hernández-CSIC, Spain

P1-11 ATOPIC DERMATITIS IS A RISK FACTOR FOR INTRACORNEAL RING SEGMENT EXTRUSION
Waldir Neira, University of Helsinki, Finland

P1-12 Ap4A INCREASES CORNEAL EPITHELIAL PERMEABILITY
Ana Guzman-Aranguez, UCM, Spain

P1-13 EFFECT OF CYCLODEXTRIN ON THE PENETRATION OF DICLOPHENAC SODIUM THROUGH HUMAN AMNIOTIC MEMBRANE
Laszlo Marsovszky, Semmelweis University, Hungary

P1-14 POLYVINYL CAPROLACTAM-POLYVINYL ACETATE-POLYETHYLENE GLYCOL GRAFT COPOLYMER NANOMICELLE AS A POTENTIAL OCULAR DRUG DELIVERY SYSTEM FOR CYCLOSPORINE A
Haoran Jiang, Shandong Eye Institute, China

P1-15 LIPOSOMAL FORMULATIONS CONTAINING VITAMIN C FOR DRY EYE TREATMENT. PRELIMINARY STUDIES.
María Angela Caballo González, Complutense University of Madrid, Spain

P1-16 LACOSAMIDE DECREASES THE HYPEREXCITABILITY OF CORNEAL COLD THERMOSENSITIVE NERVE TERMINALS IN EXPERIMENTAL DRY EYE
Illés Kovács, Semmelweis University, Hungary

Saturday, February 9, 2013

Session **O5: Novel drugs and devices for IOP lowering**

Moderators: **Carol Toris**, University of Nebraska Medical Center, USA
Xavier Gasull, Universitat de Barcelona-IDIBAPS, Spain

8:15	O5-01	MICROIMPLANTS IN GLAUCOMA SURGERY - THE AQUESYS SHUNT Herbert Reitsamer, SALK/PMU, Austria
8:35	O5-02	EFFECTS OF INVESTIGATIONAL COMPOUNDS ON INTRAOCULAR PRESSURE & MECHANISM OF IOP REDUCTION IN MONKEY EYES Janet Serle, Mount Sinai Medical Center, USA
8:55	O5-03	QUANTITATIVE DIFFERENCES AMONG NOVEL GLAUCOMA DEVICES IN A HUMAN PERFUSION MODEL Carol Toris, University of Nebraska Medical Center, USA
9:15	O5-04	MELATONIN AND ITS ANALOGUE 5-MCA-NAT POTENTIATE THE OCULAR HYPOTENSIVE EFFECT MEDIATED BY ADRENERGIC RECEPTORS: SIGNIFICANCE FOR GLAUCOMA TREATMENT Fernando Huete-Toral, Universidad Complutense de Madrid, Spain
9:35	O5-05	UNIQUE RESPONSE PROFILE OF TM CELLS TO THE NOVEL SEGRA, GW870086X Dan Stamer, Duke University, USA
9:55	O5-C	Conclusions

Session **O6: Imaging and therapy monitoring**

Moderators: **Neil Lagali**, Linköping University, Sweden
Thomas Fuchsluger, Düsseldorf University Eye Hospital, Germany

10:15	O6-01	IMAGING THE INTEGRATION OF BIOENGINEERED STROMAL IMPLANTS IN A NEW SURGICAL MODEL Neil Lagali, Linköping University, Sweden
10:35	O6-02	HIGH-RISK KERATOPLASTY-WHY DO WE NEED SYSTEMIC IMMUNOSUPRESSION? Thomas A. Fuchsluger, Düsseldorf University Eye Hospital, Germany
10:55	O6-03	FLUORESCENCE MULTI-LASER SCANNING MICROSCOPY OF THE CORNEA AND OCULAR ADNEXA: A NEW ERA FOR FUNCTIONAL CONFOCAL MICROSCOPY IN OPHTHALMOLOGY Gilles Thuret, Jean Monnet University, France
11:15	O6-04	CORRELATION BETWEEN OPTICAL COHERENCE TOMOGRAPHY AND RETINAL FOVEAL MORPHOLOGY Nicolás Cuenca, Universidad de Alicante, Spain
11:35	O6-05	γ-CYCLODEXTRIN NANOPARTICLE EYE DROPS FOR OCULAR DRUG DELIVERY Einar Stefansson, University of Iceland National Hospital, Iceland
11:55	O6-C	Conclusions

Session **O7: New treatments for retinal disease**

Moderators: **Olaf Strauss**, Charite University Medicine Berlin, Germany
Achim Krauss, GlaxoSmithKline, USA

14:00	O7-01	ANTI VEGF THERAPY - CURRENT CONCEPTS, UNRESOLVED QUESTIONS AND FUTURE DIRECTIONS Leopold Schmetterer, Medical University of Vienna, Austria
14:25	O7-02	TOPICAL PAZOPANIB EFFECT ON VEGF-INDUCED RETINAL VASCULAR LEAKAGE AND NEOVASCULARIZATION Achim Krauss, GlaxoSmithKline, USA
14:40	O7-03	DEVELOPMENT OF ANTISENSE OLIGONUCLEOTIDES FOR OCULAR DISEASE – EMPHASIS ON PRECLINICAL OCULAR PK, SAFETY AND PHARMACOLOGY AND CLINICAL TRANSLATION Husam Younis, ISIS Pharmaceuticals, USA
15:00	O7-04	NANOTECHNOLOGY-GUIDED TARGETED THERAPY FOR RETINAL VASCULAR DISEASES Ashwath Jayagopal, Vanderbilt Eye Institute, USA
15:20	O7-05	THE LOCAL RENIN-ANGIOTENSIN-SYSTEM UNDER SYSTEMIC INFLUENCE: NEW TARGETS FOR THERAPY Olaf Strauß, Charite University Medicine Berlin, Germany
15:40	O7-06	NEUROPROTECTION OF RETINAL PHOTORECEPTORS BY NORGESTREL, A SYNTHETIC PROGESTIN Francesca Doonan, University College Cork, Ireland
16:00	O7-C	Conclusions

Session **O8: Clinic meets research: aspects of translational sciences**

Moderators: **Oliver Zeitz**, Bayer HealthCare, Germany
Jesús J. Pintor, Universidad Complutense de Madrid, Spain

16:30	O8-01	CLINICIAN'S PERSPECTIVE: INTERPRETATION OF CLINICAL TRIALS AND IMPACT ON DAILY DECISION MAKING Lars Wagenfeld, University Medical Center Hamburg-Eppendorf, Germany
16:52	O8-02	DEVELOPMENT OF SYL040012, FROM PROOF OF CONCEPT TO PHASE II Covadonga Pañeda, Sylentis, Spain
17:14	O8-03	ENDPOINTS IN CLINICAL DEVELOPMENT FOR RETINAL DISEASE Oliver Zeitz, Bayer HealthCare Global Clinical Development, Germany
17:36	O8-C	Panel

Poster Session 2- **P2**

17:45-19:00

Moderators: **Laura Fernández-Sánchez**, Universidad de Alicante, Spain
Xavier Gasull, Universitat de Barcelona-IDIBAPS, Spain

P2-01 INTRAVENOUS LIPOPOLYSACCHARIDE (LPS)-INDUCED BLOOD AQUEOUS BARRIER PERMEABILITY CHANGE ASSESSED BY FLUOROPHOMETRY IN RABBITS
Tim Lam, Covance, USA

P2-02 MELATONIN ANALOGUE AGOMELATINE REDUCES INTRAOCULAR PRESSURE IN RABBIT WITH NORMOTENSIVE AND HYPERTENSIVE CONDITIONS
Alejandro Martínez Águila, UCM, Spain

P2-03 DEVELOPMENT OF SYL040012, A siRNA FOR TREATING INCREASED INTRAOCULAR PRESSURE ASSOCIATED TO GLAUCOMA
Covadonga Pañeda, Sylentis, Spain

P2-04 TRAFFICKING OF AQUAPORIN-1 MEDIATED BY Ap4A IN RABBIT NON PIGMENTED CILIARY EPITHELIAL CELLS: INVOLVEMENT OF P2Y2 RECEPTOR IN IOP RAISE
Alba Martin-Gil, UCM, Spain

P2-05 INTRACELLULAR ELECTRICAL PROPERTIES AND CHLORIDE TRANSPORT CHARACTERISTICS OF PORCINE CILIARY EPITHELIAL CELLS
Chi-Ho To, Hong Kong Polytechnic University, Hong Kong

P2-06 RETINAL P2X7 RECEPTORS IN A MURINE MODEL OF GLAUCOMA
Maria J. Pérez de Lara, Universidad Complutense de Madrid, Spain

P2-07 UNDERCORRECTION OF REFRACTIVE ERROR AND COGNITIVE FUNCTION
Jost Jonas, Medical Faculty Mannheim of the Ruprecht-Karls-University, Germany

P2-08 INTRAVITREAL BEVAZICUMAB FOR RETINOPATHY OF PREMATURITY: REFRACTIVE ERROR RESULTS
Jost Jonas, Universitätsmedizin Mannheim Medical Faculty, Germany

P2-09 NEUROPROTECTIVE EFFECT OF TUDCA ON GLIAL CELLS IN RETINAL DEGENERATION.
Laura Fernández-Sánchez, University of Alicante, Spain

P2-10 SAFRANAL, A CONSTITUENT OF CROCUS SATIVUS (SAFFRON), SLOWS RETINAL DEGENERATION AND VISION LOSS
Laura Fernández-Sánchez, University of Alicante, Spain

P2-11 ADDITIONAL NEUROPROTECTIVE EFFECTS OF NORGESTREL IN RETINITIS PIGMENTOSA: PRESERVATION OF RETINAL CYTOARCHITECTURE AND SYNAPTIC CONNECTIVITY
Violeta Gomez-Vicente, Universidad de Alicante, Spain

P2-12 A PROOF-OF-PRINCIPLE OF THE NEUROPROTECTIVE ROLE OF TUDCA ON RETINAL GANGLION CELLS
Violeta Gomez-Vicente, Universidad de Alicante, Spain

P2-13 TOLERANCE OF SUB-TENON INJECTION OF PLGA NANOPARTICLES AND MICROPARTICLES
Irene Bravo-Osuna, University Complutense of Madrid, Spain

P2-14 FREEZE-DRYING MATRICES AS PROLONGED RELEASE SYSTEM FOR INTRAVITREAL ADMINISTRATION OF BEVACIZUMAB
Susi Burgalassi, University of Pisa, Italy

P2-15 MU-PH1, A NEW MURINE MÜLLER-DERIVED RETINAL CELL LINE WITH PHOTORECEPTOR PROPERTIES
Violeta Gómez-Vicente, Universidad de Alicante, Spain

P2-16 LONG-TERM RESULTS OF THE TREATMENT WITH BEVACIZUMAB IN JUXTAPAPILLARY RETINAL CAPILLARY HEMANGIOMA
Cristina Marin-Lambies, Hospital Universitario y Politecnico La Fe Valencia, Spain

Sunday, February 10, 2013

Session **O9: From Biomarkers to Therapies for Regeneration**

Moderators: **Nicolás Cuenca**, Universidad de Alicante, Spain
Juana Gallar, Universidad Miguel Hernández-CSIC, Spain

8:15	O9-01	IMMUNOLOGICAL ALTERATIONS IN A GLAUCOMA MODEL Stephanie Joachim, Ruhr University, Germany
8:32	O9-02	PATIENTS WITH RETINITIS PIGMENTOSA PRESENT OCULAR AND BLOOD OXIDATIVE STRESS Regina Rodrigo Nicolás, Instituto de Investigación Sanitaria-La Fe, Spain
8:49	O9-03	ALPHA CRYSTALLIN-MEDIATED PROTECTION AGAINST HEAT- AND OXIDATIVE STRESS-INDUCED CELL DEATH Mark Petrash, University of Colorado, USA
9:06	O9-04	NEW PHARMACOLOGICAL CLASSES OF RBP4 ANTAGONISTS FOR INHIBITION OF PATHOGENIC BISRETINOID ACCUMULATION IN THE RETINA Konstantin Petrukhin, Columbia University, USA
9:23	O9-05	STEM CELL-BASED APPROACHES TO THE TREATMENT OF RETINAL DEGENERATIVE DISEASE Isabel Pinilla, Hospital Clinico Universitario Lozano Blesa, Spain
9:40	O9-C	Conclusions

Session **O10: New ophthalmic uses for existing drugs**

Moderators: **Peter Kador**, Univ. Nebraska Medical Center, USA
Illés Kovács, Semmelweis University, Hungary

9:45	O10-01	THIOREDOXIN ACTIVITY AND TBP2 EXPRESSION IN DIABETIC LENSES ARE LINKED TO ALDOSE REDUCTASE ACTIVITY. Marjorie Lou, University of Nebraska at Lincoln, USA
10:02	O10-02	TOPICAL ALDOSE REDUCTASE INHIBITOR KINOSTAT PREVENTS THE CLINICAL DEVELOPMENT OF CATARACT IN DIABETIC DOGS – AN UPDATE Milton Wyman, Therapeutic Vision, Inc., USA
10:19	O10-03	TRANSGENIC AK-SMAA-GFPHAR MICE SUPPORT THE PREMISE THAT ALDOSE REDUCTASE INITIATES DIABETIC RETINOPATHY Peter Kador, University of Nebraska Medical Center, USA
10:36	O10-04	EFFECT OF STATINS ON VISUAL OUTCOMES IN PRIMARY RHEGMATOGENOUS RETINAL DETACHMENTS Hubert Pham, Georgetown University Hospital/Washington Hospital Center, USA
10:53	O10-05	PROBABLE LACK OF CORRELATION BETWEEN INHIBITION OF PGE2 LEVELS AND BLOOD AQUEOUS BARRIER INHIBITION IN A RABBIT MODEL OF OCULAR INFLAMMATION. L David Waterbury, Raven Biosolutions LLC, USA
11:10	O10-C	Conclusions

Session **O11: Corneal and ocular surface pharmacology and therapeutics**

Moderators: **Maria del Carmen Acosta**, Universidad Miguel Hernandez-CSIC, Spain
Yolanda Diebold, Universidad de Valladolid, Spain

11:45	O11-01	NOVEL FORMULATION FOR DRY EYE TREATMENT BASED ON LIPOSOMES AND BIOADHESIVE POLYMERS Marta Vicario-De-La-Torre, Complutense University of Madrid, Spain
12:02	O11-02	GENE THERAPY APPROACHES TO THE CORNEAL ENDOTHELLIUM Thomas Fuchsluger, Düsseldorf University Eye Hospital, Germany
12:19	O11-03	CORNEA TISSUE ENGINEERING BASED ON NANOSTRUCTURED SCAFFOLDS AND ACELLULAR XENOGRAFTS Miguel Gonzalez-Andrades, University of Granada, Spain
12:36	O11-04	ALTERED FUNCTION OF CORNEAL NERVES IN OCULAR SURFACE PATHOLOGIES Carlos Belmonte, Universidad Miguel Hernandez-CSIC, Spain
12:53	O11-C	Conclusions

ABSTRACTS

Oral sessions

DOPPLER OCT FOR THE QUANTIFICATION OF RETINAL BLOOD FLOW IN HUMANS

Leopold Schmetterer, Medical University of Vienna, Austria, leopold.schmetterer@meduniwien.ac.at

There is a long-standing interest in measuring retinal blood flow. Although several methods have been realized to quantify retinal perfusion in the past none of these techniques made it into clinical routine. In the recent years several research teams focussed on combining the principles of optical coherence tomography (OCT) with those of Doppler velocimetry. In Fourier domain this means that the phase of the object signal has to be measured. Several techniques have been realized to measure blood flow in the retinal vasculature based on Doppler OCT. In the absence of a gold standard technique it is not easy to validate these different approaches. It is, however, possible to verify the law of mass conservation at vessel bifurcations. In addition, data during stimuli such as 100% oxygen breathing or diffuse luminance flicker may provide some insight into the reliability of the techniques. Whereas the approach of Doppler OCT is promising it is not yet ready for clinical routine application.

Key words: retinal blood flow, OCT, imaging

OPTIC NERVE HEAD AUTOREGULATION

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Purpose: Little is known about the regulation of optic nerve head blood (ONH) flow in humans. We set out to investigate the response of ONH blood flow (ONHBF) in response to changes in perfusion pressure. In addition, we investigated mechanisms of blood flow regulation focussing on the nitric oxide (NO) system and the endothelin system. Methods: All studies were performed in healthy volunteers. Experimental changes in ocular perfusion pressure (OPP) were induced by isometric exercise and an artificial increase in intraocular pressure (IOP). ONHBF was assessed using laser Doppler flowmetry. Studies during an increase in OPP were done in the presence of a NO synthase (NOS) inhibitor or an endothelinA-receptor antagonist. Studies during a decrease in OPP were done in the presence of a NOS inhibitor. Results: The ONH vasculature showed autoregulatory capacity during both an increase and a decrease in OPP. During a combined increase in blood pressure and intraocular pressure ONHBF regulated better when blood pressure was manipulated. Administration of an NOS inhibitor significantly decreased ONH blood flow at rest ($p < 0.01$), but did not alter the pressure/flow relationship. By contrast, an endothelinA-receptor antagonist did not alter basal ONHBF, but changed the pressure/flow curve to the right. Conclusions: The ONH shows a complex pattern of autoregulation during changes in perfusion pressure. NO is an important regulator of basal vascular tone in the ONH but seems not to be involved in the regulatory processes during experimental changes in OPP.

Key words: optic nerve head blood flow, autoregulation, ocular perfusion pressure, nitric oxide, laser Doppler flowmetry

EFFECTS OF FLUPENTHIXOL ON OCULAR BLOOD FLOW AND INTRAOCULAR PRESSURE IN THE RABBIT.

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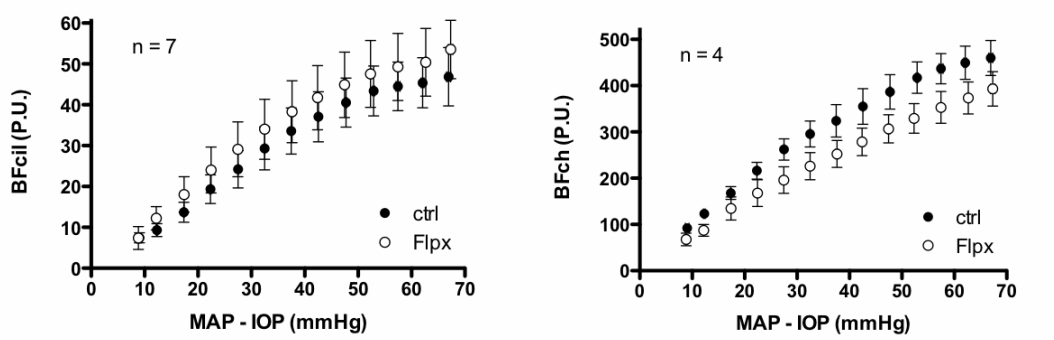
Purpose: Intravenous infusion of dopamine at 40 microliters/kg/min causes increases in ciliary and choroidal blood flow indicating the presence of dopaminergic receptors. In the present study, we evaluated endogenous dopaminergic activity using the D1-D5 antagonist flupenthixol (Flpx). Methods: In pentobarbital anesthetized New Zealand White rabbits (n=11) the following variables were measured: mean arterial pressure (MAP), intraocular pressure (IOP), and orbital venous pressure (OVP) by direct cannulation and ciliary and choroidal blood flow by laser Doppler flowmetry (BFcil and BFch, Perimed PF 5000). Occluders on the vena cava and aorta were used to manipulate MAP. The protocol entailed varying MAP over a wide range before and after Flpx (5 mg/kg, iv). Pre- and post-Flpx values and the pressure-flow curves are presented as mean ± SE. Statistical tests included paired t-tests and repeated measures ANOVA with post hoc tests as indicated. Results: Flpx caused significant decreases in MAP (71 +/- 1 to 57 +/- 3 mmHg, $p<0.01$), IOP (15 +/- 0.2 to 11 +/- 0.6 mmHg, $p<0.01$) and BFch (436 +/- 28 to 333 +/- 13 P.U., $p<0.05$); Bfcil and OVP were not significantly altered. BFch was shifted downward over most of the perfusion pressure range examined, while Bfcil tended to be shifted upwards. The rapidity and lack of recovery in the IOP response to Flpx was noteworthy. Conclusions: The results suggest that endogenous dopaminergic vasodilatory tone is present to a greater extent in the choroid than the ciliary circulation in the anesthetized rabbit model.



Support: NIH EY09702, the van Heuven endowment, Fuchs-Stiftung, Adele Rabensteiner Stiftung

Key words: Choroid, ciliary body, dopamine

THE ROLE OF SEROTONERGIC NEURONS OF THE PARAMEDIAN RAPHE IN REGULATING CHOROIDAL BLOOD FLOW IN RATS

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We have been investigating the central circuits that mediate parasympathetic control of choroidal blood flow (ChBF) via the superior salivatory nucleus (SSN) in rats. Our findings have shown that SSN neurons appear to receive blood pressure related input from the nucleus of the solitary tract, by which they maintain ChBF during times of systemic hypotension. To further investigate the inputs that regulate SSN, we used neuroanatomical and physiological approaches. In rat, we observed a high density of serotoninergic (5HT) terminals and 5HT2A receptors in SSN by immunocytochemistry. Pseudorabies virus transneuronal retrograde tracing from choroid, and ChBF recording using transscleral laser Doppler Flowmetry combined with brain stimulation both showed that neurons within the paramedian pontine raphe region (PPR), which includes the serotonergic neurons of the raphe magnus, pallidus and obscurus, project to SSN. PPR-elicited ChBF vasodilation was blocked by IV administration of the 5HT antagonist ritanserin. These data indicate PPR serotonergic regulation of ChBF via SSN. The PPR itself receives information on blood osmolarity, blood oxygen levels, blood volume, blood pressure, and skin temperature from several central cell groups, and projects to preganglionic sympathetic neurons in the spinal cord. Therefore, the PPR 5HT input to SSN may serve to integrate parasympathetic control of ChBF into sympathetic control of the systemic vasculature.

Support: NIHEY05298 (AR); Department of Ophthalmology, unrestricted grant Research to Prevent Blindness, NIH5T37MD001378(MECF);Center for Neuroscience (CL) NIH/NEI-5P30EY013080 (UTHSC core:D. Johnson) . The authors would also like to acknowledge the late Dr. Claudio Toledo as a participant at the outset of these studies.

Key words: Paramedian raphe, choroidal blood flow, superior salivatory nucleus, parasympathetic, serotonin

EPISCLERAL VENOUS PRESSURE (EVP) AND INTRAOCULAR PRESSURE (IOP) RESPONSES TO CENTRAL ELECTRICAL STIMULATION IN THE RAT

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Purpose: Histological evidence suggests a role for the central nervous system in controlling episcleral venous pressure and intraocular pressure. Based on prior studies that identified candidate regions in the brain stem, the present study sought to investigate the effect of electrical stimulation of the superior salvatory nucleus (SSN) on IOP and EVP in rats. Methods: Male Sprague-Dawley rats (n= 7, 270 – 340 g) were anesthetized using Pentobarbital Sodium (100mg/kg ip. initially, supplemented iv. as needed) and paralyzed with gallamine triethiodide(1mg/kg, iv.). The animals were artificially ventilated and the femoral artery and vein were cannulated for blood pressure measurement and drug administration. IOP was measured through a cannula in the vitreous body, EVP was measured through a micropipette in the episcleral circulation using the servonull technique. After a craniotomy was performed, a unipolar stainless steel electrode was inserted into the brainstem (10.6 mm behind Bregma, 2.2 mm lateral to the midline and 9.5 mm below the bone surface) using a stereotactic instrument. Stimulations were performed at 20Hz, 5-15 µA, 1 µs pulse duration and 100 – 2000 pulses. Results: Electrical stimulation at the SSN coordinates caused a statistically significant increase in IOP from 11.84 ± 0.83 to 13.48 ± 1.21 mmHg (p □ 0.05) and an increase in EVP from 5.55 ± 2.95 to 7.89 ± 1.79 mmHg (p □ 0.05). Mean arterial pressure and heart rate remained unaltered. Conclusion: The present study demonstrates an increase in intraocular pressure and episcleral venous pressure in response to electrical brain stem stimulation at the SSN coordinates in rats.

Key words: intraocular pressure, episcleral venous pressure, SSN stimulation, rats

OPTIC NERVE REGENERATION AND RETINAL GANGLION CELL PROTECTION IN ANIMAL MODELS OF TRAUMATIC NERVE INJURY AND GLAUCOMA

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Retinal ganglion cells (RGCs) undergo apoptotic death and cannot regenerate their axons after traumatic injury to the optic nerve or in degenerative diseases such as glaucoma. Surprisingly, however, the induction of a limited inflammatory response in the eye causes an influx of peripheral neutrophils and macrophages that secrete Oncomodulin (Ocm), a newly discovered growth factor that transforms injured RGCs into an active growth state. Regeneration induced by inflammation is amplified by elevating cAMP levels and deleting the gene for PTEN, a suppressor of signaling through the PI3 kinase pathway. When these treatments are combined, RGCs can regenerate injured axons the entire length of the optic nerve and into the brain, where they terminate in the correct central visual areas, form synapses, and partially restore visual responses. In other contexts, however, inflammation can lead to RGC death. Using mouse and rat models, we found that elevation of intraocular pressure (IOP) causes a rapid rise in the cytokine TNF-α, which activates microglia at the optic nerve head and leads to a delayed loss of RGCs. Deleting the gene for TNF-α or blocking its functions with Etanercept (Embrel), a soluble “decoy” receptor, prevents microglial activation and prevents RGC death despite continued IOP elevation. Thus, depending upon which cells become activated, the innate immune system can either promote axon regeneration or lead to RGC death. These studies may lead to the development of novel treatments for traumatic nerve damage and glaucomatous RGC loss.

Key words: retinal ganglion cells, optic nerve regeneration, inflammation, glaucoma, vision, axon pathfinding, oncomodulin

SIGMA-1 RECEPTOR-MEDIATED NEUROPROTECTION OF RGCS.

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Purpose: The role σ-1rs play in regulating activated L-type Voltage Gated Calcium Channels (L-type VGCCs) in isolated purified retinal ganglion cells (RGCs) was investigated. Methods: RGCs were isolated from P3-P7 Sprague-Dawley rats and purified by sequential immunopanning. Calcium imaging measured changes in intracellular calcium after depolarizing the cells with potassium chloride (KCl) in the presence or absence of two σ-1r agonists [(+)-SKF10047 and (+)-Pentazocine)], one σ-1r antagonist (BD1047), and one L-type VGCC antagonist (Verapamil). Co-localization studies assessed interaction of σ-1r with L-type. Results: Pre-treatment with an L-type VGCC blocker decreased calcium ion influx by 57%. Calcium imaging demonstrated that σ-1r agonists, (+)-N-allylnormetazocine hydrochloride [(+)-SKF10047] and (+)-Pentazocine, inhibited calcium ion influx through VGCCs. Antagonist treatment using BD1047 potentiated calcium ion influx and abolished all inhibitory effects of the σ-1r agonists on VGCCs. An L-type VGCC blocker (Verapamil) inhibited KCl activated VGCCs and when combined with the σ-1r agonists there was no further decline in calcium entry. Co-localization studies demonstrated that σ-1rs and L-type VGCCs are co-localized in purified RGCs. Previous studies in our lab have shown that σ-1r activation can protect RGCs from cell death following severe insults. Conclusion: These findings indicate that σ-1r agonists can inhibit KCl-induced calcium ion influx through activated L-type VGCCs in purified RGCs and that σ-1rs co-localize with L-type VGCCs implying that these two proteins are in close proximity to each other and such interactions regulate L-type VGCCs. Such actions result in a reduction in RGC death representing a potential site for neuroprotective strategies.

Key words: retinal ganglion cells, sigma receptors, VGCCs

HYDROGEN SULPHIDE: A POTENTIAL SUBSTANCE FOR THE TREATMENT OF GLAUCOMA

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Purpose. Hydrogen sulphide (H2S) is now recognised as a gaseous secondary messenger with neuroprotective and vasodilator properties. Molecules such as dithiolethiones are capable of releasing H2S, and when linked to other substances (e.g. latanoprost, aspirin - kindly provided by Dr. Piero Del Soldato, Milan, Italy) provide duel acting molecules. Methods. A comparison was made between the neuroprotective properties of latanoprost and aspirin on their own and when combined with H2S on the rat retina in situ given an ischemic insult and on cultured cells (RGC-5 cells) subjected to oxidative stress. Moreover, studies are reported on the intraocular pressure lowering influence of latanoprost on its own and when combined with H2S (ACS67).Results. ACS67 (latanoprost/H2S) was more effective at reducing intraocular pressure in the rabbit than latanoprost on its own. Moreover, ACS67 and ACS14 (aspirin combined with H2S) were more effective than latanoprost or aspirin as neuroprotective agents in the ischemic rat retina in situ and in cultured RGC-5 cells subjected to oxidative insults of glutamate/BSO or light. Interestingly, latanoprost displayed no neuroprotective properties and was toxic at fairly low concentrations. Conclusions. The present studies show that molecules capable of releasing H2S may be of value in the treatment of glaucoma where raised intraocular pressure, oxidative stress, light and ischemia are implicated to play a part in ganglion cell loss.

Key words: Hydrogen sulphide, Neuroprotection, Glaucoma, Latanoprost, Aspirin

DELTA OPIOID-RECEPTOR AGONISTS PROTECT RGCS AND OPTIC NERVE AXONS FROM GLAUCOMATOUS INJURY

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Purpose: This study was designed to determine the neuroprotective activity of δ-opioid receptor agonists for retinal ganglion cells (RGC) and optic nerve axons from glaucomatous injury. Methods: Brown Norway rats were used to elevate intraocular pressure (IOP) by injecting 50 µL of 2M hypertonic saline into the circumferential limbal veins. IOP was recorded as the average of 6-8 consecutive measurements prior to surgery (baseline IOP) and weekly after treatment, using a calibrated Tonolab tonometer. Animals were treated with δ-opioid receptor agonist, SNC-121 or SNC-80 (1 mg/kg; i.p), daily for 7 days. Pattern electroretinograms (PERG), retinal ganglion cells in flat mount, and axons were counted the 6th week, post injury. The expression of TNF-α, phospho-p38 MAP kinase, and MMP-2 were determined by Western blotting and immunohistochemistry. Results: PERG amplitudes were significantly reduced in ocular-hypertensive eyes (14.29±0.63 µvolts) when compared to normal eyes (17.04±0.72 µvolts) the 6th week, post injury. PERG deficits in hypertensive eyes were significantly improved by SNC-121 treatment (17.16±1.3 µvolts; $P<0.05$). There was a significant loss of RGCs and axons in the hypertensive eye when compared to the normal eye. The loss in RGCs and axons was fully blocked in SNC-121-treated animals by the 6th week, post injury. To dissect out the early cellular events during glaucomatous injury, optic nerves were analyzed for TNF-α, phospho-p38 MAP kinase, and MMP-2, at 3 and 7 days post injury. In ocular hypertensive eyes, TNF-α, phspho-p38 MAP kinase, and MMP-2 were increased by: 76%, 70%, 124% at 3 days, and 115%, 110%, 215% at 7 days, respectively, post injury. TNF-α, p38 MAP kinase activation and MMP-2 production were significantly ($P<0.05$) inhibited in SNC-121-treated ocular hypertensive eyes. Conclusions: These data provide concrete evidence that enhancement of δ-opioidergic-receptors by exogenous ligand provides retina neuroprotection against glaucomatous injury. Mechanistic data provide clues that TNF-α and p38 MAP kinase play key roles in the production of MMP-2, which may subsequently remodel and destabilize the optic nerve.

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Key words: Retina, Neuroprotection, Delta Opioid-Receptors, TNF-alpha

MYOPIA- THE BIG ELEPHANT IN THE ROOM

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Due to a profound increase in the prevalence of myopia, particularly in the young generation in East Asian metropolitan regions, myopia has markedly gained in importance. Since high myopia is associated with an increased risk for vision-threatening diseases such as rhegmatogenous retinal detachment, glaucomatous optic neuropathy and myopic retinopathy, research has been focused on factors associated with the development of myopia. Recent investigations revealed that the prevalence of myopia in children was associated with older age, female gender, urban region of habitation, type of attended school, and time spent outdoors versus indoors. Histologic studies on highly myopic eyes were focused on the optic nerve head and showed a marked thinning of the lamina cribrosa resulting in a steepening of the trans-lamina cribrosa pressure gradient due to the reduction of the distance between the intraocular space and the orbital cerebrospinal fluid space. These studies also revealed a stretching and thinning of the peripapillary scleral flange which as biomechanical anchor of the lamina cribrosa is of potential importance for the increased susceptibility for glaucomatous optic neuropathy in highly myopic eyes. Recent histological studies showd that highly myopic eyes (axial length ≥27mm) can show a secondary macular Bruch´s membrane defect or hole associated with complete loss of retinal pigment epithelium and choriocapillaris, and marked reduction of photoreceptors and large choroidal vessels. These defects were strongly associated with axial length and indirectly with the recently defined parapapillary gamma zone and delta zone. The myopia associated secondary macular defect in Bruch´s membrane may occur parallel to the myopia associated widening of Bruch´s membrane opening around the optic nerve head.

Key words: High Myopia, Macula, Epidemiology, Risk factors

THE CENTRAL ROLE OF THE RETINA IN EYE GROWTH AND REFRACTION

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Research over the last thirty years has revealed that the eye can regulate its growth according to the quality of the image formed on the retina. This allows the eye, under ideal circumstances, to grow so as to achieve emmetropia, the state whereby the eye is in focus for distant targets when ocular accommodation is fully relaxed. The visual control of eye inevitably requires the involvement of the retina, a conclusion that is supported by both clinical findings and animal studies. Efforts to influence eye growth so as to prevent or minimize myopia in humans have concentrated on optical manipulations of the retinal image and pharmacological manipulations of retinal function. To date pharmacological interventions using anticholinergic drugs have proved more effective than optical treatments but we still don't have an acceptable treatment to prevent or limit myopic progression. Although interest has focused on anticholinergic drugs, a wide range of retinal neurotransmitters have been implicated in the visual control of eye growth. A full understanding of the role of the retina in controlling refractive development of the eye is essential if we are to make progress in developing effective, disease modifying, treatments for myopia.

Key words: Myopia, retina, emmetropization, therapy, neurotransmitters

SCLERAL MICRO-RNA SIGNATURES IN ADULT AND FETAL EYES AND POSSIBLE ROLES IN OCULAR GROWTH REGULATION

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Purpose: Exaggerated ocular growth underlying myopia is driven by a signaling cascade originating in the retina and ultimately guided by scleral extracellular matrix remodeling. miRNAs regulate gene expression and serve as nodes of signaling networks, however, their roles in scleral remodeling are unknown. Our study aimed to identify and investigate scleral miRNAs as novel growth regulators, and thus as potential therapeutic targets. Methods: Scleral samples were obtained from normal human fetal donor eyes (24 wk), representing rapidly growing eyes, as well as normal adult donor eyes (n=6, each group). RNA was extracted and genome-wide miRNA profiling performed using the Agilent Human miRNA Microarray platform. miRNA target predictions were obtained (Microcosm, TargetScan, PicTar) and select collagen-specific miRNAs validated using Taqman® MicroRNA Assays in samples from posterior and peripheral scleral regions (n=7, each group). Microarray data were analyzed using R (http://www.r-project.org), and QPCR data with 2^-deltaCt method. Results: Human sclera expressed several miRNAs and many were differentially expressed, higher in rapidly growing fetal eyes ($p<0.01$, min $p= 6.5x10^{11}$). Increased expression of collagen-specific mir-214, let-7c, let-7e, mir-103, mir-107, and mir-98 in fetal tissue was confirmed (fold changes 1.5 to 4, $p<0.01$) in follow-up experiments. No significant differences were observed between posterior and peripheral regions, for either age group. Conclusions: The sclera expresses several miRNAs, some of which show age-related differential regulation, consistent with roles in ocular growth regulation. Future studies will focus on exploring the roles of specific scleral miRNAs and their potential as therapeutic targets for controlling ocular growth.

Key words: MiRNA, myopia, ocular growth

7-METHYLXANTHINE FOR PREVENTION OF EYE ELONGATION AND MYOPIA PROGRESSION IN MYOPIC CHILDREN

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Purpose. Animal studies and a clinical trial (ClinicalTrial.gov NCT00263471) suggest that the caffeine metabolite 7-methylxanthine works against eye elongation and myopia progression. We here present data from an extension of the trial, in which the efficacy of increasing the dosage to twice per day was tested. Methods. Axial eye elongation and myopia progression (cycloplegic) over six months were measured using Zeiss IOL-Master and Nikon Retinomax autorefractor in two treatment groups, one with an average compliance of 91% (n=95) and one with an average compliance of 55% (n=31), and compared with data from a control group (n=258). Participants were children aged 8-15 years with any degree of myopia (spherical equivalent in cycloplegia). The treatment groups received tablets containing 400 mg 7-methylxanthine; the control group received no treatment. Results. Axial eye growth was 0.081 mm in the high compliance group, 0.113 mm in the low compliance group, and 0.143 mm in the control group (p=0.000). Myopia progression was 0.094 diopters in the high compliance group, 0.160 diopters in the low compliance group, and 0.277 diopters in the control group (p=0.000). The efficacy of the treatment seems to increase with age. No side effects of the treatment were found. Conclusion. The efficacy of 7-methylxanthine treatment on eye elongation and myopia progression in myopic children increases when given more than once per day. For children older than 11 years taking the treatment twice per day, average myopia progression is close to zero and eye elongation comparable to that of emmetropic children.

Key words: Myopia, Medical treatment, 7-methylxanthine

CONTROLLED DRUG DELIVERY TO THE BACK OF THE EYE BY NONINVASIVE METHODS

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Ocular drug delivery remains a challenging task due to its restrictive barrier functionalities. These include blood ocular barrier, efflux pumps, dynamic and static membrane barriers. Diseases affecting the posterior segment of eye need therapeutic drug concentrations to improve vision. Nanotechnologies that would minimize the frequency of dosage by administrating controlled release therapeutic formulations through less or non-invasive routes are highlights of this presentation. Role of nanoparticles and nano-micelle formulations for posterior segment drug delivery, associated formulation challenges and recent efforts to address those challenges will be presented. Normal intraocular fluid flow gradient is from vitreous to aqueous, which makes compounds that can be successfully delivered to the anterior chamber following topical dosing virtually non-permeable to the vitreous cavity. The challenges associated with topical delivery to the back of the eye and recent efforts to address this issue will be discussed. Evolution of novel tailor-made pentablock polymers and thermosensitive gel to address challenges associated with conventional nanoparticle formulation for ocular drug delivery will be another highlight of this presentation. Advantage of dual phase polymeric delivery system comprises of continuous biocompatible gel phase and a discontinuous particulate (nanoparticles) phase to minimize burst release of drugs. Utilization of pentablock copolymers for the controlled and non-invasive delivery can act as a promising platform for ocular delivery of therapeutic macromolecules, which can minimize the side effects associated with the frequent intravitreal injections.

Key words: Controlled Drug Delivery, Noninvasive Methods, Eye

ROLE OF DENDRIMER-DRUG CONJUGATES IN SUSTAINED INTRAOCULAR DRUG DELIVERY

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Purpose: To review the pharmacokinetics and pharmacodynamics of dendrimer-drug conjugates for the treatment of retinal degenerative disease using multiple in-vivo animal models. Methods: Intravitreous injections of G4-OH dendrimer-drug conjugates were performed during the early/middle stages of retinal degeneration in three in-vivo animal models. Retinal biodistribution was assessed using epi-fluorescence microscopy in animals that received dendrimer-FITC (D-FITC) and dendrimer-Cy5.5 (D-Cy5.5). After injection with the neuroprotectant, dendrimer-fluocinolone acetonide (D-FA) anatomic and functional assessment of the retina was performed. Thin-section histology was used to quantify the thickness of the outer nuclear layer as a function of retinal eccentricity from the optic nerve. Immunohistochemical staining for retinal microglia was performed in retinal cryosections and wholemounts to characterize patterns and degree of retinal neuroinflammation. Finally, electroretinography was performed to measure retinal function, with and without dendrimer-drug therapy. Results: Royal College of Surgeon Rats as well as S334-ter, lines 4 received intravitreous injections of dendrimer-drug conjugates (D-Cy5.5, D-FITC and D-FA). Biodistribution studies showed that D-FA was present within retinal photoreceptors, RPE and microglia for up to 1 month. Longer time-points were not tested. D-FA resulted in a statistically significant reduction in microglial cell counts. This reduction with a statistically significant preservation of photoreceptor cell counts and ERG amplitudes. Conclusions: After intravitreous injection, G4-OH dendrimers localize within photoreceptors, RPE and microglia for at least 1 month. When conjugated to the neuroprotective steroid, fluocinolone acetonide, they suppress retinal neuroinflammation and protect retinal photoreceptors resulting in preservation of the electroretinogram.

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Key words: nanotechnology, neuroprotection, retina

NEW APPROACHES IN OCULAR HYPOTENSIVE TOPICAL TREATMENTS BASED ON BIOADHESIVE POLYMERS.

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The treatment of hypertension associated to glaucoma requires repeated administrations of topical hypotensive formulations that generate changes in the ocular tissues, usually related to the toxic effect of the drug. Other components, such as preservatives, can also be involved. The combination of a poorly-tolerated active agent and a preservative might have a synergic effect detrimental to the patient tolerance. New trends in glaucoma therapy are directed towards the discovery of new active substances with higher therapeutic efficacy and longer effects. The use of polymers able to increase the efficacy of the drug (higher effect, lower instillations) and the improvement of the already commercialized formulations, in terms of efficacy and tolerance, are of great interest. It has been suggested that melatonin is able to influence the intraocular pressure (IOP) rhythm. Our studies showed that topical application of 5-MCA-NAT (100 µM), a melatonin receptor agonist, produced a clear reduction in IOP (18%) after a single administration in rabbit eyes. The addition of bioadhesive polymers that are protective of the eye, increased the hypotensive activity of 5-MCA-NAT up to 30%, and the effect lasted around 7 hours. We also demonstrated that the in vitro tolerance of a traditional hypotensive agent (timolol) was significantly improved with bioadhesive polymers, maintaining the hypotensive effect of the drug in rabbits. The use of new molecules and the employment of polymers able to enhance the therapeutic effect and tolerance of formulations, are necessary to be taken into consideration.

Support: Research group UCM 920415, MAT 2010-18242, RETICS RD07/0062 and IdISSC.

Key words: Glaucoma, melatonin, 5-MCA-NAT, timolol maleate, intraocular pressure, bioadhesive polymers, tolerance

NANOTECHNOLOGY FOR DRUG AND GENE DELIVERY

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A key hurdle in clinical translation of therapeutic agents intended for eye diseases is their delivery to the target eye tissues. To date, there are no topically applied therapeutic agents approved for back of the eye diseases. Additionally, sustained topical delivery of therapeutic agents from an eye drop and sustained protein drug delivery within the eye are unmet medical needs. To address these challenges, our investigations focused on applying nanotechnology and novel drug delivery systems for treating eye diseases. Our studies indicated that functionalized nanoparticles can enhance back of the eye drug and gene delivery. Further, we designed hybrid dendrimer-nanoparticle formulations for sustained delivery of anti-glaucoma drugs. In addition, we developed nanoparticle in microparticle technologies for sustaining protein drug delivery for 4 months in the eye. This presentation will summarize nanotechnology applications in the eye, with a focus on the above new developments.

Support: NanoTrans Technologies, Inc.; Commercial relationships/Conflicts of interest: Pending patents (University of Colorado Denver and University of Nebraska Medical Center).

Key words: Nanotechnology, Nanoparticles, Microparticles, Drug Delivery

MICROSPHERES FOR THE TREATMENT OF CHRONIC DISEASES AFFECTING THE BACK OF THE EYE

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Pathologies affecting the back of the eye often represent visual impairment and blindness. Repeated intravitreal injections are usually employed to achieve effective levels of the drug in the target site. However, frequent intraocular injections are associated with adverse effects. Intraocular drug delivery systems (implants and microparticles) have been developed to release the active substance in a controlled fashion for long periods of time. Depending on their size, the devices can be implanted through a relatively large surgical incision or through a smaller tissue perforation. Biodegradable microspheres (1-1000µm size) are emerging therapeutic approaches for the treatment of vitreoretinal diseases as they are able to release the active substance during weeks or months. Furthermore they can be injected as conventional suspensions avoiding surgical procedures and disappear from the site of injection after delivering the drug. Among the biodegradable polymers employed to prepare microspheres poly(lactic) acid and poly (lactic-co-glycolic) acid (PLGA) are the most popular. Microspheres intended for the treatment of chronic pathologies affecting the posterior segment have been injected by intravitreal or periocular route. These therapeutic systems can be loaded with one or more active substances resulting of special interest in the treatment of multifactorial diseases. Microspheres have been already employed to help retinal repair. Tolerance is a critical issue for intraocular therapy. Preliminary results have been shown good tolerance after injection of PLGA microspheres in humans.

Support: MAT 2010-18242, Research Group UCM 920415 GR35/10-A and European Project PANOPTES.

Key words: Microspheres, drug delivery, vitreoretinal diseases

MICROIMPLANTS IN GLAUCOMA SURGERY - THE AQUESYS SHUNT

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Purpose: To determine the safety and effectiveness of the Aquecentesis procedure in reducing intraocular pressure in patients with Glaucoma, 143 patients with poorly controlled intraocular pressure were implanted with the device. Patients were followed for up to 12 months and their outcomes for mean IOP, mean IOP change, % decrease in IOP and number of medications prescribed were recorded. Methods: All patients underwent the minimally-invasive Aquecentesis procedure which was performed by two experienced surgeons. The surgery was performed as the primary procedure, or in combination with cataract surgery. Clinical ophthalmic examinations were performed at the preoperative visit and on postoperative day 1, weeks 1 and 2 and months 3, 6, 9 and 12. The exams included assessments of IOP, Anterior Chamber OCT imaging, visual acuity, visual fields and assessment of any complications. Effectiveness was evaluated by comparing the baseline intraocular pressure values to postoperative values (mean IOP changes, % of drop). The change and/or reduction of anti-glaucomatous medications from the preoperative to postoperative periods were also determined. Results: Preoperative mean IOP values were 24.1 (±5.7) mmHg for the group. Postoperative mean IOPs in mmHg were: 21.8 at 1 week, 17.8 at 1 month, 14.6 at 6 months and 13.7 at 12 months. The mean decrease in IOP (mmHg) was -2.5 (-11% reduction) at week 1, -4.3 (-16% reduction) at month 1, -9.4 (-38% reduction) at month 6 and -5.6 (-27% reduction) at month 12 (n=6). Anti-glaucomatous medications were reduced from a preoperative mean of 3.1 medications to a mean of 0.2 medications postoperatively. Conclusions: The Aquecentesis procedure appears to be a minimally invasive and effective surgical approach to the control of intraocular pressure in this group of glaucoma patients. The mean IOP was reduced 27% from baseline after 12 months. Medications were reduced from a mean of 3.1 preoperatively to a mean of 0.2 at 12 months. Additional long-term effectiveness data will be presented. This method may prove be an effective alternative to traditional surgeries in Glaucoma patients, with and without cataract surgery.

Key words: Glaucoma, Aqueous, shunt, IOP, surgery

EFFECTS OF INVESTIGATIONAL COMPOUNDS ON INTRAOCULAR PRESSURE & MECHANISM OF IOP REDUCTION IN MONKEY EYES

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Purpose: Describe studies of topical application of investigational IOP lowering agents in monkey eyes with laser-induced unilateral glaucoma, and mechanism of IOP reduction in normal monkey eyes. This model has been predictive of efficacy, side effects and mechanism in subsequent clinical trials. Methods: 5-day dosing studies were performed in glaucomatous monkey eyes with investigational agents; SPP 301 (endothelin antagonist), SPP 635 (renin inhibitor), DNB-001 (ion channel modulator). Glaucomatous eyes were dosed (25µl x 2) twice daily for 5 consecutive days. IOP was measured hourly for 6 hours for one baseline day, one vehicle-treated day, and treatment days 1,3, and 5. Normotensive monkeys were used for tonographic outflow facility (C) and aqueous flow measurements (F) prior to and following administration of 0.6% AR-12286 (Rho kinase (ROCK) inhibitor) and 0.04% AR-13324 (ROCK) inhibitor and inhibitor of the norepinephrine transporter (NET). Results: SPP 301 0.3% reduced ($p<0.05$) IOP, with maximum reduction 7.1 ± 1.3 mmHg (21%) and duration at least 6 hrs. SPP 635 0.4% reduced IOP, ($p<0.05$) with maximum reduction 8.0 ± 1.3 mmHg (25%) and duration at least 18 hrs. DNB-001 10% reduced IOP ($p<0.05$), with maximum reduction 5.6 ± 1.0 mmHg (19%) and duration at least 18 hrs. Single-dose administration of 0.6% AR-12286 decreased IOP 20%, increased C 39%, and did not alter F in drug-treated eyes ($p<0.05$). Single-dose administration of 0.04% AR-13324 decreased IOP 25%, increased C 53%, and reduced F 20% in drug-treated eyes ($p<0.05$). Conclusion: Several new classes of compounds appear promising for the treatment of glaucoma.

Key words: Glaucoma, non-human primate, ocular hypertensive model, ocular hypotensive investigational agents, pharmacology

QUANTITATIVE DIFFERENCES AMONG NOVEL GLAUCOMA DEVICES IN A HUMAN PERFUSION MODEL

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Purpose: This study compares changes in outflow facility of three novel glaucoma devices in an ex vivo model of perfused human anterior segments. Methods: Human eyes with no history of ocular problems were studied within 48 hours of death. Anterior segments were mounted on fixtures connected to a perfusion system. Baseline measurements of outflow facility were followed by measurements after device implantation or sham procedure. Statistical tests were paired and unpaired two-tailed t-tests. Implants studied were 8 mm and 15 mm intracanalicular scaffolds (Hydrus™ Aqueous Implant, Ivantis, Irvine, CA) and trabecular micro-bypass stents (iStent® Glaukos, Laguna Hills, CA). Eyes with one 8 mm Hydrus Implant were compared with paired controls and with eyes containing two iStent implants. Results: All implants significantly increased the outflow facility compared to baseline and paired controls ($p<0.04$). The 8 mm Hydrus exhibited a significantly ($p=0.04$) greater percent increase in outflow facility than two iStent implants. Outflow facility increased by 72% and 90% with the 8 mm Hydrus Implant, 92% with the 15 mm Hydrus Implant and 36% with two iStent implants. Conclusions: Bypassing the trabecular meshwork and scaffolding Schlemm's canal allows fluid to flow directly into collector channels thus reducing outflow resistance and decreasing intraocular pressure. While the Hydrus Aqueous Implant showed a greater percent increase in outflow facility than two iStent Implants, both provide effective ways to increase outflow facility in human eyes ex vivo.

Key words: Outflow, glaucoma, pressure

MELATONIN AND ITS ANALOGUE 5-MCA-NAT POTENTIATE THE OCULAR HYPOTENSIVE EFFECT MEDIATED BY ADRENERGIC RECEPTORS: SIGNIFICANCE FOR GLAUCOMA TREATMENT

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Purpose: To investigate the effect of melatonin and its analogue 5-MCA-NAT, on β2/α2A-adrenergic receptor mRNA and protein expression in rabbit ciliary epithelial cells. Also, we have studied their combined effect with Timoftol® and Alphagan®.Methods:Changes in ADRB2 and ADRA2A expression in non-pigmented ciliary epithelial cells (NPEsv) treated with melatonin or 5-MCA-NAT (both 100nM), were determined by RT-qPCR and immunocytochemical (ICC) assays. For ICC experiments, NPEsv cells were incubated with anti-ADRA2A or anti-ADRB2-FITC. New Zealand rabbits were used for IOP studies and treated with Melatonin, 5-MCA-NAT (100µM, 10µl), Alphagan®, Timoftol® (40µl), or saline + 1% DMSO (control), and IOP were measured during 6 hours. The following days, rabbits, treated with melatonin or 5-MCA-NAT the first day, were instilled with Alphagan® or Timoftol® once daily for up to four days. IOP was measured by a Tonovet® tonometer. Results:RT-qPCR and ICC assays revealed a β2-adrenergic receptor down-regulation and α2A-adrenergic receptor up-regulation in cells. Timoftol®, in rabbits pre-treated with melatonin or 5-MCA-NAT, evoked an additional IOP reduction of 14% or 17% compared with Timoftol® alone. Alphagan® hypotensive action presented an additional IOP reduction of 29% or 39% in animals pre-treated with melatonin or 5-MCA-NAT compared with Alphagan® alone. Additionally, a sustained potentiating effect of a single dose of 5-MCA-NAT was seen in rabbits treated with Alphagan® (16 %) once daily for up four days. Conclusions:Melatonin and 5-MCA-NAT potentiate the ocular hypotensive action mediated by adrenergic receptors, mainly by up-regulation of α2-adrenergic receptors.

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Key words: Melatonin, 5-methoxycarbonylamino-N-acetyltryptamine adrenoceptors, ocular hypertension, glaucoma

UNIQUE RESPONSE PROFILE OF TM CELLS TO THE NOVEL SEGRA, GW870086X

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Purpose: Glucocorticoid (GC)-induced glaucoma is a major side-effect of traditional GCs used to treat a variety of systemic and ocular maladies. Ocular hypertension responsible for GC-induced glaucoma is due to alterations in conventional outflow homeostasis. The present study was designed to evaluate a novel selective GC receptor agonist (SEGRA), GW870086X in two different in vitro models of the human conventional outflow pathway. Methods: Primary cultures of human trabecular meshwork (TM) cell monolayers were treated with dexamethasone (DEX), prednisolone (PRED) or GW870086X for 5 days and then assayed for cellular expression and secretion of fibronectin, myocilin, tissue plasminogen activator (tPA) and/or matrix metalloproteinase-2 (MMP-2). In parallel, TM cell monolayers on permeable filters treated for 5 days with GCs were assayed for changes in hydraulic conductivity. Results: All three GCs increased fibronectin and myocilin secretion in a concentration-dependent manner ($p<0.05$). In addition, DEX increased cellular fibronectin and both DEX and PRED significantly increased cellular myocilin ($p<0.0001$), while GW870086X did neither. Interestingly, DEX and PRED significantly decreased tPA expression ($p\leq0.01$), while GW870086X had the opposite effect and increased tPA expression in a concentration-dependent manner ($p=0.01$). For MMP-2, only DEX treatment consistently decreased secretion ($p<0.01$). In a functional assay, only PRED treatment significantly decreased hydraulic conductivity of TM cell monolayers ($p<0.05$). Conclusion: All three GCs induced differential responses from TM cells. While the novel SEGRA, GW870086X increases fibronectin and myocilin secretion similar to two traditional GCs, effects on the matrix degradation enzymes, MMP-2 and tPA, differed significantly; suggesting that GW870086X affects the balance of matrix deposition and degradation more favorably than either DEX or PRED. Consequently, effects on conventional outflow homeostasis may also be dissimilar.

Key words: Aqueous humor, Glaucoma, Glucocorticoid, Outflow Facility

IMAGING THE INTEGRATION OF BIOENGINEERED STROMAL IMPLANTS IN A NEW SURGICAL MODEL

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Purpose: to evaluate the performance of a femtosecond laser-enabled intrastromal surgical model for testing the integration of bioengineered corneal stromal implants. Methods: an Intralase iFS femtosecond laser was used to cut 100 mircon thick discs of mid-stromal tissue from corneas of 15 rabbits. In a control group, stromal discs remained in place while in remaining groups, discs were removed and implants engineered from porcine collagen were inserted, with 300 (hyper-swelled) or 100 (compacted) micron thickness. Implants were monitored in vivo for 8 weeks postoperatively, using photography, optical coherence tomography, and confocal microscopy. Bio-integration was examined ex-vivo by immunohistology and electron microscopy. Results: tissue extraction and insertion was successful in all cases, with minimal impact on epithelium, endothelium, or subepithelial nerves. Healing was rapid, suture-free and irritation-free. Implants were retained in all corneas at 8 weeks. Hyper-swelled implants thinned partially over time but retained clarity, while compacted implants and control allografts retained shape, thickness, and clarity. Interface haze formed early as implants attached to the surrounding stroma. Some cell invasion of implants was noted, and the detailed collagen structure of implants and interface regions could be analyzed. Conclusions: femtosecond laser-assisted intra-stromal implantation of bioengineered materials is a viable and useful model for screening candidate materials for eventual corneal transplantation. Pure stromal integration characteristics can be investigated with this model, separate from the additional challenges of implant retention and epithelialization.

Key words: femtosecond laser, keratoplasty, imaging, bioengineering

HIGH-RISK KERATOPLASTY-WHY DO WE NEED SYSTEMIC IMMUNOSUPRESSION?

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Corneal engraftment is the most frequently performed transplantation worldwide. However, fraft failure is a considerable challenge following corneal transplantation. This talk will present different strategies to prevent corneal grafts from failure by use of systemic immunosuppression.

Key words: corneal transplantation, keratoplasty, immunosuppression

FLUORESCENCE MULTI-LASER SCANNING MICROSCOPY OF THE CORNEA AND OCULAR ADNEXA: A NEW ERA FOR FUNCTIONAL CONFOCAL MICROSCOPY IN OPHTHALMOLOGY

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Purposes. In vivo confocal microscopy (IVCM) is a routine investigation for the ocular surface in reference centres. It provides high resolution pseudo histology. Nevertheless its unique laser source and imaging principle (reflectance) only provide morphological information. Multi-laser and fluorescence laser scanning microscope add a supplementary dimension by allowing the use of fluorescent markers liable to provide specific functional data. Methods. Ex vivo animal and human corneas, healthy volunteers and patients were successively examined using the multilaser vivascope 1500 (Lucid Inc, NY, MAVIG Gbmh, Germany) equipped with 3 lasers (488, 658, 785nm) and the corresponding emission (Em) filter sets. For each excitation (Ex) wavelength (λ), 3 observation modes were available: reflexion (all λ), pure reflectance (λEx = λEm), fluorescence (3 specific band pass). Ex vivo, all corneal layers were analysed without preparation and after topical application of Fluoresceine (F) and Indocyanine green (ICG) and of numerous other molecules. Topical instillation and of intraveinous injection of F and ICG were analysed in healthy volunteers and in patients. Results. Using reflexion and reflectance, the 3 Ex λ gave complementary structural informations with the highest resolution obtained at 488nm. Topical markers helped identify specific cell population and intracellular structures. Intraveinous ICG was inefficient whereas fluo provide highly contrasted conjunctival images. Conclusions. IVCM coupled with fluorescence opens a new era in the clinical imaging of the ocular surface and probably more largely in Ophthalmology. A new semeiology remains to be learned

Key words: Confocal, microscopy, fluorescence, ocular surface, cornea, in vivo, laser, biomarker

CORRELATION BETWEEN OPTICAL COHERENCE TOMOGRAPHY AND RETINAL FOVEAL MORPHOLOGY

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Optical coherence tomography (OCT) is a main tool in clinical practice. Its introduction has revolutionized imaging tests in ophthalmology and made OCT irreplaceable for studying and diagnosing specific diseases as well as for taking therapeutic decisions and making post-surgery functional assessments. Since the use of spectral domain OCT (SD-OCT) has become widespread we have become acquainted with terms such as hyperefringence lines and their correlation with the retina layers specially the four layers observed in the outer retina. The first hyperefringent layer is the external limiting membrane. The second said IS-OS junction is no such layer. Instead, it is a line formed by the ellipsoids of the cones located in the outermost portion of the inner photoreceptor segment. Ellipsoids are full of mitochondria and these membranous structures, which in the cone are arranged in a particular manner with a longer and thinner morphology, could be the origin of the hypereflective line. At the level of the apical portion of the RPE cells, the epithelial cell establishes its contacts with cones by means of apical extensions, forming sheaths that cover the outer cone segment which has been defined as the “contact cylinder“. The third layer represents these sheaths of the RPE cell extensions with the outer portion of the outer segments, while the fourth layer represents the complex between of the RPE and Bruch´s membrane. Other important layer is the Henle fiber layer that corresponds to the axons of the cones and Müller cells processes. The correlation of hypereflective lines with their corresponding retinal structures in different diseases is a challenge that would help to improve the diagnosis and prognosis of retinal diseases.

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Key words: Optical coherence tomography, Fovea, confocal microscopy, retinal layers

γ-CYCLODEXTRIN NANOPARTICLE EYE DROPS FOR OCULAR DRUG DELIVERY

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Purpose: We have developed an ocular drug delivery platform with cyclodextrin nanoparticles in an eye drop suspension. The eye drops can deliver drugs to the retina and also deliver anterior segment drugs more effectively than conventional eye drops resulting in either more intense treatment or fewer applications. We will discuss this broadly. In this abstract we test this drug delivery platform with dorzolamide g-cyclodextrin (gCD) nanoparticle eye drops for intraocular pressure (IOP) control and irritation and compare with Trusopt®. Methods: Self aggregating gCD nanoparticle eye drops containing 3% dorzolamide were given once a day (QD) and compared to Trusopt® given three times a day (TID) in a prospective randomized single masked cross-over trial over 24 hours. Seventeen subjects with IOP over 18 mmHg were recruited. IOP was measured with an Icare tonometer pro®. Results: The nanoparticle eye drops QD and Trusopt® TID lower IOP and there was no statistically significant difference in IOP between the two groups. At peak (4 hours) the IOP reduction from baseline was 3.8 ± 2.6 mmHg (18%, $p < 0.05$) in the nanoparticle eye drop group and 3.1 ± 3.7 mmHg in the Trusopt® group (14%, $p < 0.05$, $p = 0.97$ between groups). At trough (24 hours) the IOP reduction was 1.4 ± 2.8 mmHg (6%, $p > 0,05$) in nanoparticle eye drops group and 1.5 ± 2.0 mmHg (7%, $p > 0.05$) in Trusopt® group ($p = 0.23$ between groups). Burning sensation in the nanoparticle eye drop group on the scale 1-100 was 12 ± 15 and in Trusopt® eyes 37 ± 30 ($p = 0.0038$). Visual acuity and conjunctival hyperemia did not differ between the two groups. Conclusions: Dorzolamide cyclodextrin nanoparticle eye drops QD lower IOP and the effect seems comparable to Trusopt® given TID. The nanoparticle eye drops are well tolerated and more comfortable than Trusopt®.

Key words: Trusopt, dorzolamide, γ-cyclodextrin, nanoparticle, intraocular pressure

ANTI VEGF THERAPY - CURRENT CONCEPTS, UNRESOLVED QUESTIONS AND FUTURE DIRECTIONS

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The introduction of Anti VEGF drugs has revolutionized the treatment of choroidal neovascularization (CNV) secondary to age-related macular degeneration. In the recent years the indication of these class of drugs was extended towards macular edema. Currently, three drugs are approved for intravitreal use (pegaptanib, ranibizumab, aflibercept). In addition, bevacizumab, is used off label in many countries because of prizing issues. Only recently several randomized trials including CATT, IVAN and MANTA have compared the efficacy and safety of ranibizumab and bevacizumab in patients with CNV. Several pharmacological differences between these drugs have been identified, but the clinical importance of these issues is not entirely clear. Unresolved questions remain with regard to the identification of non-responders, pharmacokinetics of the drugs and the systemic inhibition of VEGF.

Key words: VEGF, age-related macular degeneration, pharmacology

TOPICAL PAZOPANIB EFFECT ON VEGF-INDUCED RETINAL VASCULAR LEAKAGE AND NEOVASCULARIZATION

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Purpose: To test the effect of the tyrosine kinase inhibitor pazopanib on vascular leakage and neovascularization (NV) in the retina. Methods: In rho/VEGF mice with subretinal NV, VEGF-induced vascular permeability was determined by measuring [3H]mannitol retina:lung (RLLR) and retina:renal leakage ratios (RRLR). In rabbits, the effect of intravitreal, topical, and systemic pazopanib on VEGF-induced leakage was tested by vitreous fluorophotometry. Results: In mice, oral pazopanib (40 mg/kg BID) reduced RLLR (0.84 to 0.58, p=0.0014) and RRLR (0.55 to 0.30, p=0.0018) in VEGF-injected eyes. After intraocular injection of VEGF in both eyes, topical pazopanib (10mg/ml TID for 14 days) reduced RLLR (0.85 vs. 0.56, p=0.001), RRLR (0.44 vs. 0.28, p=0.0075), and immunoreactive albumin in the retina compared to fellow eye controls. Treatment of one eye of rho/VEGF mice with 10 mg/ml, but not 5 mg/ml, pazopanib TID reduced the mean area of subretinal NV compared to fellow eyes (0.0055 vs. 0.0025 mm2, p=0.020). In rabbits, intravitreal pazopanib suppressed VEGF-induced fluorescein leakage, but topical (10mg/ml QID or 12mg/ml BID) had no significant effect. Systemic administration of pazopanib by osmotic pump with or without 10mg/ml drops TID also failed to suppress VEGF-induced leakage. Conclusions: Administration of pazopanib topically or systemically suppressed retinal vascular leakage in mice, but not rabbits. These data suggest differences in the blood-retinal barrier (BRB) of mice and rabbits and that penetration through the outer BRB may be needed for topically administered drugs to exert effects in the retina.

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Key words: Pazopanib, retina, leakage, neovascularization

DEVELOPMENT OF ANTISENSE OLIGONUCLEOTIDES FOR OCULAR DISEASE – EMPHASIS ON PRECLINICAL OCULAR PK, SAFETY AND PHARMACOLOGY AND CLINICAL TRANSLATION

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The safety and efficacy of antisense oligonucleotides (ASOs) following systemic delivery (e.g. subcutaneous injection) has been well documented for multiple disease targets and therapeutic indications. The utility of antisense technology for ocular disease following intraocular administration is an active area of investigation. In fact, the first ASO approved for human use was for the treatment of CMV retinitis following intravitreal injection. Moreover, an ASO targeting human C-raf kinsase is currently in Phase 2 trials for the treatment of diabetic macular edema. The objective this paper is to provide an overview of the preclinical pharmacokinetics, safety and pharmacology of the ASO class of drugs following intraocular administration. The preclinical to clinical translation of the pharmacodynamic and safety properties of ocular administered ASOs will also be discussed. Progress has been made in understanding the ocular cell types that readily uptake ASO drug molecules, and, hence, where antisense pharmacology can be employed to reduce RNA. One of the important attributes of the ASO class of drugs is the relatively long half-life and subsequent duration of action in the eye relative to small molecules or biologic, which enables less frequent treatment. ASOs have successfully targeted multiple mRNA transcripts in the retina that have been implicated in angiogenesis, inflammation, and/or cell proliferation in several animal models (rodents, rabbits, pigs and non-human primates).

Key words: Antisense oligonucleotides, diabetic macular edema, C-raf kinase, intravitreal injection, mRNA targeting

NANOTECHNOLOGY-GUIDED TARGETED THERAPY FOR RETINAL VASCULAR DISEASES

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Cell adhesion molecules (CAMs) are markers of inflammation expressed on retinal endothelial cell surfaces in a broad spectrum of ocular vascular diseases, including retinal neovascularization, and therefore constitute potential targets for promoting homing, binding, and internalization of nanoscale imaging and therapeutic agents. We have developed a series of nanocarriers targeted against CAMs which can bear imaging or therapeutic payloads and deliver them to the cytoplasm of dysfunctional endothelial cells. The goal of this study was to demonstrate the utility of CAM targeted nanocarriers for site-specific delivery of antiangiogenic siRNAs in two animal models of retinal neovascularization. CAM targeted nanocarriers bearing VEGFR2 siRNAs were synthesized and characterized to determine optimal size, surface charge, and encapsulation efficiencies. Cytotoxicity, delivery efficiency, and functional knockdown of several molecular targets were determined in retinal microvascular endothelial cells. Biodistribution and efficacy of nanocarriers in animal models of laser-induced choroidal neovascularization and oxygen-induced retinopathy were analyzed. CAM targeted nanocarriers were capable of specific targeting of ICAM-1 and VCAM-1 on inflamed retinal endothelial cells in vitro, and triggered release of siRNA following internalization was observed. Specific targeting of neovascular endothelial cells was observed in both animal models of vascular disease, using CAMs on neovessel endothelial cells as a portal for delivery of therapy. Knockdown of molecular targets via siRNAs was achieved in vitro and in vivo without adverse effects on cell and tissue function. CAM targeted nanocarriers are a promising framework for the delivery of diverse imaging and therapeutic payloads to diseased retinal endothelial cells in vivo.

Key words: Nanotechnology, drug delivery, angiogenesis

THE LOCAL RENIN-ANGIOTENSIN-SYSTEM UNDER SYSTEMIC INFLUENCE: NEW TARGETS FOR THERAPY

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Purpose. Local renin-angiotensin systems (RAS) are often under influence of systemic RAS. Since the local RAS of the retina is known to contribute to a variety of retinal degenerations such as age-related macular degeneration or diabetic retinopathy we aimed the investigation of systemic influences on the retinal RAS. Methods. Analysis of RAS gene expression by means of qPCR, renin protein was analyzed by immunohistochemistry or measurement of protease activity. Analysis of cAMP and Ca2+-signaling in cultured RPE cells. Results. Staining of mouse retina sections against the angiotensin-receptor type-1 (ATR1) revealed expression in the RPE and ganglion cells. Water deprivation as well as AngII infusion decreased the renin expression in the RPE. The latter was blocked by systemic administration of the ATR1 blocker losartan. In contrast, systemic administration of ACE inhibitor enalapril led to an increase in the renin expression. Systemic application of isoproterenol also increased the renin expression in the RPE. The underlying signal pathway of AngII to suppress renin expression involves increases in intracellular free Ca2+ and activation of TRPV2 ion channels. The pathway to stimulate renin expression involves increases in intracellular cAMP. Conclusions. As part of the blood retina barrier the RPE is able to react on changes in the systemic RAS via stimulation of ATR1 at the blood side of the epithelium. As a response the RPE changes in the intraocular RAS by renin secretion to the retina side. The cellular regulatory mechanisms of renin expression involve Ca2+ as a negative regulator and cAMP as a positive regulator.

Key words: retinal pigment epithelium, renin-angiotensin system, beta-adrenergic stimulation

NEUROPROTECTION OF RETINAL PHOTORECEPTORS BY NORGESTREL, A SYNTHETIC PROGESTIN

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Retinal degenerations such as Retinitis Pigmentosa remain difficult to treat given the diverse array of genes responsible for their aetiology. Rather than concentrate on specific genes, our focus is on identifying therapeutic avenues for the treatment of retinal disease that target general survival mechanisms or pathways. Norgestrel is a synthetic progestin commonly used in hormonal contraception. Here, we report a novel neuroprotective function for Norgestrel in degenerating mouse retinas in vivo. Dosing with Norgestrel protects photoreceptor cells from undergoing apoptosis in two distinct models of retinal degeneration; the light damage model and the Pde6b(rd10) model. Photoreceptor rescue was assessed by analysis of cell number, structural integrity and function. Improvements in cell survival of up to 70% were achieved in both disease models, indicating that apoptosis had been halted or at least delayed with a significant preservation of ONL structure. Animals also showed significantly improved ergs indicating preservation of visual function. The mechanism by which Norgestrel mediates its function is by its ability to stimulate Muller Glial cells to produce FGF which in turn mediates the protective effect on photoreceptors. (In previous publications we demonstrated that FGF is a neuroprotective agent for photoreceptors). In summary, our results demonstrate significant protection of photoreceptor cells which may be attributed to Norgestrel mediated activation of endogenous survival pathways within the retina.

Key words: Photoreceptors, neuroprotection, retinitis pigmentosa

CLINICIAN'S PERSPECTIVE: INTERPRETATION OF CLINICAL TRIALS AND IMPACT ON DAILY DECISION MAKING

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Large clinical studies influence the clinical decision making every day. Besides the primary and secondary endpoints planned in the study design, many other conclusions are drawn from exploratory post-hoc findings . Generally, clinicians are responsible for the patients' wellbeing and, therefore, they are particularly interested in patient-relevant outcomes, i.e. visual function in ophthalmology rather than morphology. However, in various cases these interpretations are influenced by the readers expectations and wishes. The CATT results e.g. show that a monthly dose regimen is slightly more effective than prn but it is commonly interpreted that prn treatment can be tailored for the individual patient. A similar situation can be found for the question wether to use ranibizumab or bevacizumab. Another good example is the HARBOR trial, where the primary analysis has failed to show non-inferiority of prn dosing versus scheduled monthly dosing. Depending on the view-point, clinicians may still conclude that prn is efficacious by doing informal cross-study comparisons. Sometimes clinicians tend to base their decisions even on small trials or case series. A good example is the EVEREST trial, a three-arm study with ~60 patients enrolled in the PCV subtype of neovascular AMD. Although not powered adequately and only small differences between the arms, the results guide clinicians in the treatment of PCV. The presentation will give an impression on how data from clinical studies can help the clinician to draw conclusions. Clinical decision making depends on the educational background and the individual experiences, and, therefore, the same data can be interpreted very differently by different physicians.

Key words: clinical studies, study endpoints, study interpretation

DEVELOPMENT OF SYL040012, FROM PROOF OF CONCEPT TO PHASE II

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Purpose: To review the development of SYL040012, a siRNA for the treatment of glaucoma and increased intraocular pressure (IOP) from the early discovery phases to clinical aspects. Methods: SYL040012 was the first siRNA to reach clinical trials in Spain. In the present talk we will review the development process that has been carried out in order to reach the current phase II. siRNAs are target designed but chemically synthetized therefore the development process had to be tailored for this specific compound. Results: Validation of the target was performed in vitro and in vivo, thereafter pilot and regulatory toxicology studies were performed in two relevant animal models. Phase I studies were performed in healthy subjects and in individuals with higher than normal IOP. Phase II efficacy studies are currently on-going. Conclusions: Guidelines had to be adapted for this specific compound that even though classified as a chemical compound shares some similarities with biologicals. A tailored program was developed in collaboration with regulatory agencies to ensure that the appropriate studies were performed.

Key words: SiRNA, development, glaucoma

ENDPOINTS IN CLINICAL DEVELOPMENT FOR RETINAL DISEASE

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Background: Retinal drug therapy was introduced by demonstrating maintenance of vision defined as avoidance of 15-ETDRS-letter loss in pivotal trial settings. This work investigates implications of the outcomes of current treatment standards on future study endpoints for novel therapies. Methods: 1) Pivotal phase 3 studies for drug therapy in neovascular AMD have been reviewed and evaluated in regards to their endpoints and outcome. 2) Dry AMD studies have been reviewed for applicable endpoints. Results: 1) Wet AMD: In pegaptanib approx. 70% of patients were counted as responders maintaining vision. Ranibizumab and VEFG Trap-Eye increased this responder rate to approx. 95%. Improvement of vision by 15 letters has been reported to be 6% for pegaptanib and increased to 30 to 40% with ranibizumab and VEGF Trap-Eye. Morphological endpoints such as central retinal thickness and CNV lesion size were investigated as additional endpoints in these studies, but no correlation between vision and morphology has been established. 2) In dry AMD vision changes are very slow. The FAM study and others suggests that growth of geographic atrophy lesion size might be appropriate. Discussion: Visual acuity endpoints were valuable for introduction of treatments for wet AMD. Future therapies will face ceiling effects. Endpoints related to improvement of vision require focusing on a patient population with impaired vision at baseline. Anatomic outcome measures have so far not shown reliable correlations with visual outcomes in wet AMD, while in dry AMD evidence is emerging that increase of geographic atrophy area might be an appropriate endpoint variable.

Conflict of interest: Employment at Bayer Pharma AG, Berlin, Germany

Key words: Clinical Trial Design, Endpoints, Visual Acuity, Retinal Disease

IMMUNOLOGICAL ALTERATIONS IN A GLAUCOMA MODEL

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Purpose: Glaucoma is characterized by death of retinal ganglion cells (RGCs), but its cause is still unknown. Over the past decades evidences obtained from experimental studies supported the role of auto-antibodies in glaucoma. These antibodies might trigger an immune mediated RGC loss. Methods: Rats were immunized with optic nerve homogenate (ONA) or S100 protein in Freunds Adjuvans and Pertussis Toxin. The control group (CO) received sodium chloride. After 14 and 28 days RGC density was quantified via Brn3a-antibody (Santa Cruz). To evaluate the activation of the complement system retina sections were stained with C3 (Cedarlane) and membrane attack complex (MAC; Biozol). Astrocytes were quantified using GFAP (Millipore) and microglia with Iba1 (Wako). Cell counts or GFAP area evaluation were performed using Image J Software, followed by statistical analysis. Results: No change in the density of the RGCs could be observed in the immunized animals compared to CO after 14 days (ONA: p=0.9; S100: p=0.8), but after 28 days there was a significant RGC loss (ONA: p=0.0005; S100: p=0.005). In the ONA group, MAC was significantly increased in the ganglion cell layer (GCL) after 14 and 28 days (14 d.: p=0.02; 28 d: p=0.004). A significant difference regarding the GFAP staining intensity could also be observed in the ONA group (p=0.00003). In the S100 group, no difference in MAC staining could be seen either at 14 or 28 days. In contrast, the total number of C3+ cells was increased after 14 days (p=0.0002). GFAP staining showed no difference in the S100 group. In all groups only few microglia could be detected. Conclusions: Our data suggest that immunization with ocular antigens leads to RGC death, but the mechanisms seems antigen dependent. The complement seems to be involved in apoptosis of RGCs in ONA immunized animals. Secondary glial changes were also noted in this group. The slow dissolution of RGCs in animals with autoimmune glaucoma is comparable to slow progressive cell loss in glaucoma patients, thus making this a useful model to develop neuroprotective therapies.

Key words: Glaucoma, complement system, glia, autoimmunity

PATIENTS WITH RETINITIS PIGMENTOSA PRESENT OCULAR AND BLOOD OXIDATIVE STRESS

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Purpose: Retinitis Pigmentosa (RP) is a common form of hereditary retinal degeneration characterized by loss of retinal photoreceptor cells and constitutes the largest single cause of blindness in the developed world. It has been suggested that after rod death, cone die due to several causes including oxidative damage, depletion of trophic factors and release of toxic factors by apoptotic rods and surrounding cells. Oxidative stress probably plays a major role in the pathogenesis of RP. We evaluated whether RP is associated with oxidative damage in eye, and whether the damage is reflected in peripheral blood. We also investigated the relation between visual function and oxidative stress in these patients. Methods: Forty-one patients diagnosed with typical RP (non-syndromic RP) and fifteen patients diagnosed with Usher syndrome were included in this study. We included sixty individuals' without oxidative stress-related disease, including thirteen patients diagnosed with cataract as controls. Aqueous humour and peripheral blood were collected to measure several oxidative stress markers such as superoxide dismutase (SOD) activity, total antioxidant capacity or thiobarbituric acid reactive substances (TBARS) formation (as indicator of malonyldialdehyde formation). Ophthalmic test included were best-corrected visual acuity and visual field. Results: MANCOVA revealed that RP induces oxidative stress on eye and also an imbalance redox status in blood independent of sex and visual impairment. Patients with RP present low total antioxidant capacity including reduced superoxide dismutase (SOD) activity in aqueous humour. In blood RP also induces lower SOD activity, and higher TBARS formation (indicator of lipid peroxidation) than in control situation. Conclusions: Our study demonstrated that RP is associated with oxidative damage on eye and this oxidative damage is also reflected in peripheral blood.

Key words: Retinitis pigmentosa, Oxidative stress, Antioxidant supplements

ALPHA CRYSTALLIN-MEDIATED PROTECTION AGAINST HEAT- AND OXIDATIVE STRESS-INDUCED CELL DEATH

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Purpose: Loss of α-crystallins is associated with a variety of diseases, including cataracts and multiple degenerative disorders caused by protein aggregation. We hypothesize that delivery of therapeutic levels of α-crystallins may be a useful strategy to block the progression of such diseases. The purpose of the current study was to assess the uptake and ability to protect against stress-induced cell death of various forms of α-crystallin. Methods: Five types of recombinant α-crystallins were purified from bacterial expression systems: Wild type forms of αA- and αB-, and forms encoding a cell penetration peptide (gC peptide or TAT peptide): gC-αB, TAT-αB, gC-αA. The human lens epithelium-derived cell culture line HLE-B3 was pretreated with α-crystallins, followed by insult of thermal (45^{o}C, 60 min) or oxidative (add to 0.04 mM H2O2, incubate 24h) stress. The protective effect of pretreatments with α-crystallin was measured using a calcein viability assay. Controls for α-crystallin treatments included incubations with a non-crystallin recombinant protein, and vehicle controls. Results: All α-crystallins conferred protection to HLE-B3 cells in these stress models. Under conditions that induced ~100% cell death by exposure to H2O2, pretreatment with WT-αB or gC-αB resulted in >80% survival; TAT-αB treatment gave ~60% protection. WT-αA and gC-αA also afforded protection, but at lower levels (~40-50%). Similarly, all αB variants afforded significant protection against heat-induced cell death, but the αA-crystallins were comparatively less effective. Conclusions: This study provides evidence that exogenously-administered α-crystallins can protect eukaryotic cells from death induced by oxidative and heat stress.

Support: NIH Grant RC1EY20361 and T35EY021455

Key words: Crystallin, retinal disease, cataract, neuroprotection

NEW PHARMACOLOGICAL CLASSES OF RBP4 ANTAGONISTS FOR INHIBITION OF PATHOGENIC BISRETINOID ACCUMULATION IN THE RETINA

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Excessive accumulation of lipofuscin is associated with pathogenesis of atrophic AMD and Stargardt disease. Pharmacological inhibition of the retinol-induced interaction of Retinol-Binding Protein 4 (RBP4) with transthyretin (TTR) in the serum may decrease the uptake of serum retinol to the retina and reduce formation of lipofuscin bisretinoids. We evaluated in vitro and in vivo properties of two new classes of non-retinoid RBP4 antagonists. RBP4 binding potency, ability to antagonize RBP4-TTR interaction and compound specificity were compared for the representatives of two new classes and for the prototypic RBP4 antagonist fenretinide. Specificity of compounds was confirmed in in vitro assays probing the compound effect on activity of protein targets capable of binding different types of retinoids. The in vivo effect of compound administration on serum RBP4, visual cycle retinoids, lipofuscin bisretinoids, and retinal visual function was evaluated using a combination of biochemical and electrophysiological techniques. We documented significant reduction of serum RBP4 in response to compound administration which induced partial depletion of visual cycle retinoids and significant inhibition of bisretinoid accumulation in the mouse model of enhanced lipofuscinogenesis while no significant changes in kinetics of dark adaptation after the photobleach were evident following long-term compound dosing. Ongoing medicinal chemistry optimization targets identification of novel analogs with optimized potency and improved pharmacokinetic characteristics

Key words: age-related macular degeneration, Stargardt disease, RBP4, lipofuscin, bisretinoids, visual cycle

STEM CELL-BASED APPROACHES TO THE TREATMENT OF RETINAL DEGENERATIVE DISEASE

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Purpose: to compare cell fate and differentiation potential of human retinal progenitors generated from different stem cell sources over time. Methods: Human embryonic stem cells (hESC) (H1 and H9 lines) and human induced pluripotent stem cells (iPSC) derived from either fibroblast IMR-90), or T-lymphocyte-derived (TiPSC-5) were used to generate optic vesicle-like structures following a stepwise differentiation process. Highly enriched populations of optic vesicle (OV) stage retinal progenitors from each line were manually separated from forebrain progenitor populations at day 20 and allowed to differentiate for up to an additional 120 days. The sequence and timing of expression of markers indicative of retinal development were determined via immunocytochemistry over a total time course of 130 days. Results: Both hES and iPS cells are able to generate optic vesicle-like structures which contain a population of proliferating neuroretinal progenitor cells. These cells differentiate into multiple neuroretinal cell types and express synaptic markers in a manner that is similar to that seen during human retinal development. More specifically, markers of ganglion cell differentiation were observed initially, followed by the appearance of photoreceptor and retinal interneuron markers. Conclusions: Both hESc and iPS cells can generate retinal cells types and thereby can facilitate the study of human retina development and disease and provide a donor cell source for iPS cell-based retinal studies.

Support: Instituto de Salud Carlos III RETICS RD12/0034/0010, FIS PS0901854, BAE (12/00090), Fundación Gangoiti

Key words: Regenerative Medicin, Stem cells, iPS cell, hES cell, retinal progenitor

THIOREDOXIN ACTIVITY AND TBP2 EXPRESSION IN DIABETIC LENSES ARE LINKED TO ALDOSE REDUCTASE ACTIVITY.

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Purpose: Cataracts in diabetic rats are associated with osmotic stress that is amelioreated by aldose reductase inhibitors (ARIs). Studies suggest that oxidative stress is involved; however, its source or mechanism is unknown. Thioredoxin (Trx) controls cellular redox homeostasis and its bioavailability is negatively regulated by the intrinsic protein TBP2. Here, we investigate whether oxidative stress in diabetic lens is linked to hyperglycemia / aldose reductase activity that can alter TBP-2 expression and compromise Trx activity. Methods: Lenses from streptozotocin-induced diabetic rats treated with/without ARI (AL1576) and nondiabetic rats were collected after 3 and 8 wks of diabetes and analyzed for GSH levels and Trx activity. Trx and TBP-2 expression levels were determined with specific anti-Trx and anti-TBP-2 antibodies. Results: Blood glucose was > 400 mg/dL in all diabetic rats. In the untreated diabetics, lenses appeared hazy after 3 wks, and developed moderate to severe opacities by 8 wks. Lenses from ARI-treated diabetic and nondiabetic rats remained clear. Lenses from untreated diabetic rats showed a >80% reduction in GSH and >35% reduction in Trx activity at both 3 and 8 wks while TBP-2 expression increased ~4-fold by 8 wks and Trx expression remained unchanged. No change in GSH levels, Trx activity, or upregulation of TBP-2 expression were observed in lenses from ARI-treated rats. Conclusions: Diabetic lenses show up-regulated TBP-2 with concurrent suppression of Trx activity that can contribute to the oxidative stress. ARI protection suggests that osmotic stress may induce oxidative stress to accelerate lens opacification.

Support: NIH EY10595. No conflict of interest.

Key words: Cataract, oxidation, redox, aldose reductase inhibitor

TOPICAL ALDOSE REDUCTASE INHIBITOR KINOSTAT PREVENTS THE CLINICAL DEVELOPMENT OF CATARACT IN DIABETIC DOGS – AN UPDATE

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Purpose: A majority of dogs develop bilateral cataracts within 1 year after diagnosis of diabetes mellitus (DM). Here, we update the results of a randomized progressive double-masked placebo control pilot study initiated in 2007, where topical KinostatTM significantly reduced cataracts in diabetic dogs. Methods: Dogs were evaluated by board certified veterinary ophthalmologists at the time of enrollment, then monthly for the first 3 months, and at 6-month intervals thereafter for the life of each dog. Throughout this time period KinostatTM was administered TID by their owners. Results: As presented in Vet. Ophthlamol. 13:363-8, 2010, the cataract score after 12 months in the placebo group significantly increased with 7 out of 12 dogs developing mature cataracts, 1 developing cortical opacities and 1 developing equatorial vacuoles. In contrast, the cataract score in the KinostatTM group was significantly lower with only 4 out of 28 dogs developing mature cataracts, 2 developing cortical opacities, and 7 developing equatorial vacuoles. Although the average life-span of a diabetic dog is 3 years, 4 of the original 28 dogs treated with KinostatTM remained alive after 5 years with no signs of diabetic cataracts. Of these dogs, 2 entered the study with no signs of osmotic cataracts while the other 2 entered the study with osmotic vacuoles that subsequently reversed. Conclusion: These results demonstrate that continuous treatment with topical aldose reductase inhibitor likely not only delays diabetic cataract formation, but prevents the need for surgery in diabetic dogs. The implication of similar treatment in juvenile diabetics will be discussed.

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Key words: Cataract, diabetes, aldose reductase inhibitor

TRANSGENIC AK-SMAA-GFPHAR MICE SUPPORT THE PREMISE THAT ALDOSE REDUCTASE INITIATES DIABETIC RETINOPATHY

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Purpose. Demonstrate that AR plays a central role in the onset of diabetic retinopathy.Methods. A colony of transgenic C57BL mice expressing green fluorescent protein (SMAA-GFP), human aldose reductase(SMAA-hAR) or both (SMAA-GFP-hAR) in vascular tissues under the control of smooth muscle alpha actin promoter have been established and crossbred with diabetic C57BL/6-Ins2Akita/J (AK) mice to produce naturally diabetic offspring of AK-SMAA-GFP or AK-SMAA-GFP-hAR. Aldose reductase inhibitors (ARIs) were administered in chow. Results. The presence of GFP and hAR in retinal capillary pericytes was confirmed by confocal microscopy. AK-SMAA-GFP-hAR mice had higher sorbitol and VEGF levels compared to AK-SMAA-GFP mice, which were normalized by ARI. AK-SMAA-GFP-hAR mice also showed induction of retinal growth factors IGF-1, bFGF and TGFβ, as well as signaling changes in P-Akt, P-SAPK/JNK, and P-44/42 MAPK which were normalized by ARIs. Histological evaluation of isolated retinal capillaries from 18 week old AK-SMAA-GFP-hAR mice also demonstrated increased loss of nuclei/capillary length and a significant increase in the percentage of acellular capillaries present. These changes were not observed in similar mice treated with ARI.Conclusion: Diabetic mice expressing hAR in their retinal vascular tissue demonstrate capillary pathology and growth factor and signal expression associated with DR. These changes were not observed in similar mice treated with AR, supporting the premise that AR in these transgenic mice plays a central role in DR.

Support: NIH EY016730. No conflict of interest.

Key words: Diabetes, retinopathy, animal model, aldose reductase inhibitor

EFFECT OF STATINS ON VISUAL OUTCOMES IN PRIMARY RHEGMATOGENOUS RETINAL DETACHMENTS

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Purpose: Statins have been theorized to aid in neurologic recovery after a stroke or traumatic brain injury through anti-inflammatory and neuroprotective mechanisms. By viewing rhegmatogenous retinal detachment (RRD) as a form of ischemic insult similar to a stroke with the addition of mechanical shearing forces similar to a traumatic brain injury, it can be hypothesized that statins may have similar neuroprotective effects and may result in improved visual acuity after RRD repair. The purpose of this study is to evaluate whether visual and anatomic outcomes following retinal detachment are improved in individuals on long-term statin therapy following surgical repair of primary RRDs. Methods: This is a retrospective, consecutive, interventional case series. All eyes underwent repair of rhegmatogenous retinal detachment (RRD) with either a standard 3-port pars plana vitrectomy (PPV) or a scleral buckling procedure (SB). Eyes with previous retinal surgery, giant retinal tear, grade C or higher proliferative vitreoretinopathy, or any other ocular comorbidity that might affect visual acuity were excluded. The main outcome measure studied was final best-corrected visual acuity (BCVA) as a function of ongoing statin use at the time of surgery. Secondary outcome measures included change in pre- to post-operative BCVA and rates of redetachment. Results: 160 eyes of 159 patients met inclusion criteria, with a mean follow-up length of 20.1 months (range 1-108) and with 45 patients using statins (28%). In those using statins, pre-operative logMAR BCVA 0.756 (20/114) improved to 0.196 (20/31); those not taking statins improved from 0.518 (20/66) to 0.231 (20/34) post-operatively. Overall, 77.5% of patients obtained 20/40 or better final BCVA (82% statins vs. 73% non-statins). While the data tended to suggest overall better visual outcomes and an increased improvement in BCVA from pre- to post-operatively in those taking statins, there was no statistically significant correlation found when controlling for confounders, such as pre- and post-op lens status, age and type of procedure performed ($p>0.10$). Moreover, there was no difference in rates of redetachment during the follow-up period (statins 4.4%, non-statins 3.5%; $p > 0.10$). Conclusions: Statins do not correlate with improved visual or anatomical outcomes after repair of primary RRD with either SB or PPV.

Key words: Statins, Rhegmatogenous retinal detachment, Visual outcomes, Scleral buckle, Pars plana vitrectomy, Neuroprotective, Anti-inflammatory

PROBABLE LACK OF CORRELATION BETWEEN INHIBITION OF PGE2 LEVELS AND BLOOD AQUEOUS BARRIER INHIBITION IN A RABBIT MODEL OF OCULAR INFLAMMATION.

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Purpose: In a model of ocular inflammation, several topical NSAIDs were simultaneously tested for inhibition of PGE2, and for blood aqueous barrier breakdown following LPS administration. The purpose of the study was to determine if inhibition of PGE2 concentrations was correlated with inhibition of the breakdown of the blood aqueous barrier following the dosing with topical NSAIDs. Methods: Topical dosing of suspensions (0.1% FR122047, 0.1% trans-resveratrol) or solutions (0.1% amfenac, 0.09% bromfenac, 0.1 % diclofenac, 0.4% ketorolac) of NSAIDs were administered to one eye of New Zealand White Rabbits (n=6). Rabbits received LPS (Salmonella enterica typhimurium, 10 µg/kgm), and fluorescein isothiocyanate-dextran (FITC-dextran), M.W. = ~70,000, 30 mg/kgm, iv, at 1 hour). FITC-dextran was used to determine the blood aqueous leakage. At 2 hours after topical dosing , aqueous humor samples were simultaneously analyzed for PGE2 and FITC-dextran concentrations. Results: All test drugs significantly inhibited PGE2 concentrations in the aqueous humor. The degree on PGE2 inhibition ranged from ~ 50 to 97%, and the inhibition of FITC inhibition ranged from 7 to 98%. Amfenac was more effective in inhibiting FITC-dextran accumulation than PGE2 concentrations (97% vs 68% respectively). FR122047, trans-resveratrol, and diclofenac were active in lowering PGE2, but did not significantly affect FITC-dextran concentrations. Both ketorolac and bromfenac were equally active with 2 hour dosing, but with 12 hour pre-dosing, only ketorolac was active in suppressing FITC-dextran. Conclusions: Taking into account COX selectivity and potency, there did not appear to be a correlation between PGE2 and FITC-dextran inhibition suggesting different sites of action for both effects.

Key words: NSAIDS, PGE2, Blood aqueous barrier, inflammation, LPS

NOVEL FORMULATION FOR DRY EYE TREATMENT BASED ON LIPOSOMES AND BIOADHESIVE POLYMERS

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Dry eye syndrome (DES) is a highly frequent disease affecting the ocular surface. Current treatments involve artificial tears directed to minimize the signs and symptoms of DES. The use of pharmaceutical nanosystems (liposomes) dispersed in a solution of bioadhesive polymers directed to replace a disturbed preocular tear film might have therapeutic benefits for dry eye patients. In this work, we have developed an unpreserved artificial tear based on liposomes composed by phosphatidylcholine, cholesterol and α-tocopherol. Liposomes (186.3 ± 12.2 nm) are dispersed in a hypotonic aqueous solution of hyaluronic acid and trehalose. The novel ophthalmic formulation was characterized in terms of physico-chemical properties: pH (7.4 ± 0.7), surface tension (37.5 ± 4.9 mN/m), osmolarity (202.6 ± 1.9 mOsm) and viscosity (3.0 ± 4.8 mPa•s). Tolerance studies were performed “in vitro” (immortalized cell lines of cornea and conjunctiva) and demonstrated viability values higher than 80% at different exposure times. Instillation of the artificial tear in rabbits did not produce any alteration in the clinical signs observed in conjunctiva, cornea and lids. TBUT measurements were carried out before treatment (9.25 ± 2.3 seconds) and 2 minutes (11.25 ± 2.1 seconds) and 30 minutes (12.7 ± 2.4 seconds) after the instillation of the liposomal formulation in humans. Experiments to determine the antiinflammatory activity of medroxyprogesterone-loaded liposomes are ongoing using an “in vitro” model of ocular surface inflammation.
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Key words: Dry eye syndrome, Preocular tear film, Artificial tears, Liposomes, Bioadhesive polymers, Medroxyprogesterone

GENE THERAPY APPROACHES TO THE CORNEAL ENDOTHELLIUM

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Purpose: To present and discuss gene therapy approaches to target corneal endothelium during storage and after transplantation. Methods: Viral and non-viral vector approaches will be presented to transduce endothelial cells in order to increase corneal cell survival during storage and after transplantation by anti-apoptotic gene transfer. Results: Endothelial cell survival can be significanlty enhanced by this gene therapy treatment. Conclusion: Corneal endothelium is a very suitable cell layer for gene therapy approaches. Further translational steps will be discussed.

Key words: gene therapy, viral vectors, apoptosis, corneal storage, transplantation

CORNEA TISSUE ENGINEERING BASED ON NANOSTRUCTURED SCAFFOLDS AND ACELLULAR XENOGRAFTS

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Purpose: The generation of a corneal substitute in the laboratory has emerged as a necessity because of the inconveniences related to corneal transplantation. To accomplish this challenge, tissue engineering appears as promising science whose aim is to generate artificial tissues and organs that can replace damaged tissues and organs in the human body. Here, two models of tissue engineered corneas developed by our research group are presented. Methods: Both models are based on the combination of corneal cells and a scaffold. Corneal epithelial cells and keratocytes were obtained from the sclerocorneal limbus donated by human cadavers. The nanostructured fibrin-agarose model was developed by seeding these cells into an artificial matrix generated with a mixture of agarose and human fibrin. The acellular xenograft model was created by applying a decellularization process to pig corneas, which were finally recellularized with human corneal cells. Once we developed both models, histological and optical analyses were carried out. Results: We observed in both models a well-organized stroma with collagen fibers and proteoglycans. The keratocytes proliferated and spread across the corneal matrix. Immunohistochemical analyses demonstrated the differentiation and expression of specific corneal proteins. Optical analyses revealed the high transparency level that presented both models. Conclusions: All these results suggest that corneal substitutes made by tissue engineering show similar characteristics to human cornea. Thus, artificial corneas could represent a promising treatment for many corneal diseases.

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Key words: Cornea, Tissue engineering, Regenerative medicine, Decellularization

ALTERED FUNCTION OF CORNEAL NERVES IN OCULAR SURFACE PATHOLOGIES

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The eye surface is richly innervated by sensory nerve fibers originated at trigeminal ganglion neurons. They have been functionally classified as mechano-nociceptors, which react only to mechanical forces; polymodal nociceptors that respond to mechanical forces but also to heat, chemical irritants and endogenous inflammatory mediators, and cold receptors which exhibit a background impulse activity whose frequency increases markedly upon moderate cooling. Differences in transduction capacity among ocular sensory fibers depend on the expression of different transduction channels. Their selective stimulation in humans evokes sensations of specific quality with a variable component of unpleasantness. Ocular sensory fibers also contribute to basal and reflex neural regulation of tearing and blinking and to trophic maintenance of the cornea and conjunctiva. During inflammation or following nerve injury, activity of ocular sensory nerve fibers is markedly altered due to sensitization and long-term changes in their ion channel expression. In pathological processes primarily characterized by local inflammation (allergic kerato-conjunctivitis, actinic keratitis), polymodal nociceptors become sensitized, while cold receptor activity is depressed. In ocular surface pathologies that involve nerve damage (photorefractive surgery, dry eye), polymodal nociceptors are sensitized while cold thermoreceptors exhibit an augmented spontaneous activity and enhanced responsiveness to cold stimuli. Differences in nerve activity of the various functional classes of sensory fibers innervating the cornea and conjunctiva in different pathological conditions reflect changes in the expression and activity of ion channels present in their nerve terminals and explain the qualitative differences of the unpleasant sensations evoked by the different pathological processes affecting the ocular surface.

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Key words: ocular surface, cornea, ocular inflammation

ABSTRACTS

Posters

TREATMENT OF NEUROTROPHIC KERATOPATHY WITH NICERGOLINE

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Purpose: To determine the effect of nicergoline in patients with neurotrophic keratopathy. Methods : This is prospective, noncomparative, interventional study. The study included 27 eyes of 24 patients with neurotrophic keratopathy who were unresponsive to conventional therapy. Patients were treated with 10mg of oral nicergoline twice daily for at least 2 weeks. Slit lamp examination, photography, corneal fluorescein dye testing, Cochet-Bonnet corneal sensitivity and best-corrected visual acuity (BCVA) were performed prior to and after treatment. Results: In 23 eyes (85%), the epithelial defect healed completely after 7 to 30 days of treatment with nicergoline (mean, 15.6±8.8days). The epithelial defect persisted in 4 eyes (15%). The mean corneal sensitivity before and after treatment with nicergoline was 20.5±8.5 mm and 30.2±10.8 mm, respectively ($p<0.001$). The BCVA (log MAR units) was significantly improved from 1.083 ± 0.56 to 0.83 ± 0.59 ($p<0.001$). Conclusions: Nicergoline may help patients with neurotrophic keratopathy in whom conventional treatment has failed.

Key words: Cornea, Corneal sensitivity, Epithelium, Neurotrophic keratopathy, Nicergoline

EFFECTS OF IL-6 AND IL-10 ON AN IN VITRO MODEL OF CORNEAL WOUND HEALING

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Purpose: Corneal healing process under inflammatory conditions is not fully understood. We aimed at determining the effect of an inflammatory (IL-6) or anti-inflammatory (IL-10) environment and a mixture of both on corneal wound healing in an in vitro scratch assay. Methods: Human corneal epithelial (HCE) cells were cultured until confluence. The monolayer was mechanically wounded using a sterile 20µl-tip. Cells were exposed to IL-6 (10ng/ml), IL-10 (20ng/ml) or IL-6+IL-10 in 1%FBS-supplemented culture medium. Wound healing in the presence or absence of these cytokines was measured immediately after cytokine exposure (T0) and after 4, 8, and 24h. At least 4 images of the scraped area per well per time interval were captured and wound width measured. Three independent experiments were performed, with triplicates. For the statistical analysis a two-factor design of experiment method was applied. Levene test was used to contrast equality of variances. Results: Scraped areas had an initial width of 570.57±75.82µm. Rate of wound closure was different depending on cytokine exposure. At T0 and after 4h, wound in IL-6-exposed cells was significantly smaller than that of cells exposed to the other conditions. Wound width in IL-6- or IL-10-exposed cells, but not in IL-6+IL-10-exposed cells, was significantly smaller than that of control cells after 8h. Wounds were closed after 24h regardless the experimental condition. Conclusions: IL-6 accelerates wound healing in HCE cells in an in vitro scratch assay. The combination of IL-6 and IL-10 does not improve cell migration and wound healing; rather, it seems to inhibit their isolated effects.

Key words: corneal epithelium, wound healing, cytokines

CARBON MONOXIDE ACCELERATES WOUND HEALING AFTER CORNEAL EPITHELIAL INJURY

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Purpose: Corneal epithelial debridement is accompanied by a pronounced inflammatory response that mediates ocular damage but is also essential for the tissue reparative process. Carbon monoxide (CO) can exert potent anti-inflammatory effects in animal and cell culture models. CO-releasing molecules (CORMs) are a new class of drugs able to release small amounts of CO in biological systems. We performed a dose-ranging study to assess the effects of topical instillation of CORM-A1 on the wound healing rate and the inflammatory response after epithelial removal. Methods: The corneal epithelium of rats was removed using alcohol-assisted debridement and a CORM-A1 solution at different concentrations (1-100 µM) was applied to the ocular surface four times a day. Re-epithelialization was monitored daily after injury using fluorescein staining and a slit-lamp. Corneal levels of HO-1, TNF- α, and Nrf-2 were assessed by real- time PCR. Results: The 100 µM solution of CORM-A1 significantly accelerated the wound healing rate at 12 hours after injury whereas the 1µM, 10µM didn't show any significant effect. Real- time PCR showed a significant increase of the mRNA level of HO-1, TNF- α and Nrf-2 in the cornea of animals treated with the 100 µM CORM-A1, no differences were observed in the other groups of treatment compared to vehicle. Conclusions: Increased expression of HO-1, TNF-α and Nrf-2 by CORM-A1 provides a mechanism that modulates inflammation accelerating wound closure; pharmacologic amplification of this system may constitute a novel strategy to promote wound repair after corneal injury.

Key words: Cornea, wound healing, carbon monoxide

CORNEAL INNERVATION AND EPITHELIAL WOUND HEALING IN SP AND ALPHA-CGRP KNOCKOUT MICE

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Purpose. To analyze the morphological differences of corneal innervation in knockout mice for Substance P (Tac1 KO) and for alpha calcitonin gene-related peptide (alpha-CGRP KO) and their corresponding wild type (WT) strain. The corneal epithelial wound healing was also studied in these animals. Methods. For the morphology studies both eyes of Tac1 KO (n=9), alpha-CGRP KO (n=8) and WT mice (n=6) were dissected at 8 months of age, fixed in paraformaldehyde and cryoprotected in gradients of sucrose. Whole-mount corneas were incubated with neuronal class III b-tubulin primary antibody and subjected to the procedure of ABC staining technique with diaminobenzidine. Corneas were photographed with a Neurolucida microscope and drawn using a camera lucida, and images were analyzed with image processing software. For the wound healing study, 2 mm of diameter chemical wounds were performed in the corneal epithelium of Tac1 KO (n=6), alpha-CGRP KO (n=7) and WT animals (n=12). The diameter of the epithelial wound was measured in a masked fashion every 12 hr by staining the ocular surface with fluorescein. A double-masked vehicle-controlled assay was performed to study the effect on wound healing of exogenous CGRP on alpha-CGRP KO (n=5) and WT animals (n=6). The Epithelial Migration Rate (EMR) and the Estimated Time of Healing (ETH) were calculated. Results. Tac1 KO and alpha-CGRP KO mice showed the 49% and 85% respectively of the subbasal nerve density observed in WT. The EMR for the alpha-CGRP KO and their WT was respectively 4.7±1.6 and 32.8±7.7 µm/h ($p<0.01$, n=7), and for the Tac1 KO and their WT, 11.4±4.1 (n=6) and 32.1±2.1 (n=5) µm/h respectively ($p<0.01$). The ETH was longer in the KO mice than in the corresponding WT animals: 230.6±61.8 vs. 56.7±10.4 h (alpha-CGRP, $p<0.01$), 141.8±31.9 vs. 39.3±4.0 h (Tac-1, $p<0.01$). Exogenous application of alpha-CGRP delayed the healing in WT animals in comparison with vehicle-treated mice (EMR: 13.16±2.14 vs. 29.8±2.63 µm/h, respectively, $p=0.001$), and failed to accelerate the healing rate in alpha-CGRP KO corneas (9.36±1.04 vs 19.92±5.33 µm/h, CGRP-treated vs. vehicle, respectively). Conclusions. The percentage of decrease in subbasal nerve density in Tac1 KO and alpha-CGRP KO animals is similar to the percentage of the SP and CGRP positive neurons previously described in the bibliography for control animals. The impairment of the wound healing process may be related to the absence of the corresponding neuropeptides, as well as to the reduction of corneal sensoy input.

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Key words: corneal innervation, wound healing, SP, CGRP

TEAR SECRETION INDUCED BY CARBACHOL IS MEDIATED BY Ap4A RELEASE

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Purpose: To investigate if the cholinergic stimulation by carbachol on tear secretion is a direct process or if it is also mediated by purinergic mechanisms. Methods: Experiments were performed in New Zealand male rabbits. The amount of tear secretion was measured with Schirmer test, and then analysed by a HPLC protocol, in order to study the nucleotide levels. Animal eyes were instilled with carbachol (a cholinergic agonist), pirenzepine, gallamine and 4-DAMP (muscarinic antagonists), suramin and reactive blue 2 (purinergic antagonists), and a P2Y2 receptor siRNA. Results: Tear secretion increased with the instillation of carbachol, approximately 84% over control values 20 minutes after the instillation and so did Ap4A and ATP release. When we applied carbachol in the presence of muscarinic antagonists, tear volume only increased 4% with atropine, 12% in case of pirenzepine, 3% with gallamine, and 8% with 4-DAMP. In the presence of carbachol and purinergic antagonists tear secretion was increased 12%. (All values compared to basal tear secretion) Analysing tear secretion induced with carbachol in presence of a P2Y2 receptor siRNA, we found that tear secretion was diminished 60%. The inhibition of tear secretion in the presence of carbachol and purinergic antagonists or P2Y2 siRNA, occurred with no apparent change in the tear concentration of Ap4A. Conclusions: These experiments demonstrated the participation of Ap4A in lacrimal secretion process.

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Key words: Carbachol, Nucleotides, Tear Secretion

SYL1001: A NEW TREATMENT FOR OCULAR PAIN ASSOCIATED TO DRY EYE SYNDROME BASED ON RNAi TECHNOLOGY: SAFETY AND TOLERANCE.

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Purpose: Sylentis is developing a siRNA for treating ocular pain associated to dry eye: SYL1001. SYL1001 targets TRPV1, an ocular nociceptor present in the cornea that transmits pain stimuli. SYL1001 decreases TRPV1 in animal models and reduces pain related behaviour in an animal model of capsaicin induced eye pain. Preclinical regulatory studies indicated that the compound was safe and in 2011 Sylentis initiated clinical trials. Methods: Efficacy and toxicology studies were performed in animal models. In phase I clinical trial SYL1001 was administered into one eye of 30 healthy voluntaries. In the first period a single dose of product was administered to ensure safety; in the second period repeated administrations of two doses were assayed. Results. In vitro and in vivo studies demonstrated that administration of SYL1001 reduced TRPV1 levels and ocular pain in rabbits. 28-day toxicology studies in rabbits and dogs showed that the drug was systemically and locally well tolerated and that the compound did not reach the blood-stream. Phase I results indicated that the compound was well tolerated and safe.Conclusions. SYL1001 reduces TRPV1 mRNA levels and response to ocular pain in animals. Preclinical toxicology studies and phase I trial showed that SYL1001 was locally and systemically well tolerated after single and multiple administrations. These results suggest that SYL1001 is a good candidate for treating of ocular pain associated to dry eye syndrome and maybe other related pathologies. SYL1001 is currently undergoing a phase Ib clinical trial in subjects suffering ocular pain.

Key words: siRNA, TRPV1 antagonists, chronic pain.

LONG-TERM EYE DRYNESS ENHANCEMENT OF CORNEAL COLD-THERMORECEPTIVE NERVE TERMINALS FIRING IS ASSOCIATED TO CHANGES ON VOLTAGE-GATED Na+ CURRENTS

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Purpose: Impaired tear secretion reduces ocular surface wetness and evokes unpleasant eye dryness sensations whose peripheral origin remains unclear. Alterations on corneal sensory neuron firing properties and cellular ionic mechanisms involved in these changes were studied in a guinea pig dry eye model (DED). Methods: Exorbital main lacrimal gland was removed to reduce basal tearing. Ongoing spontaneous activity (34ºC) and responses to cooling down to 20ºC and heating up to 50ºC were recorded in corneal cold thermosensitive nerve terminals 4 weeks after. Na+ currents were recorded from dissociated trigeminal ganglion corneal cold neurons from control and DED animals. Results: Lacrimal gland removal reduced tear secretion rate by 80% ($p=0.01$). Ongoing firing frequency of corneal cold sensitive nerve terminals at 34ºC and peak firing frequencies evoked by cooling ramps to 20ºC were higher in DED than in control corneas (13.2±1.0 vs. 10.3±0.8Hz, and 31.1±1.8 vs. 25.3±1.8Hz; $p<0.01$). Cold threshold was reduced in DED terminals (-2.2±0.1 vs. -3.1±0.2ºC, $p<0.01$). TTX-sensitive INa activated around -40mV with maximal currents at -30mV in DED and -20mV in controls (V0.5=-42.8±1.9 and -29.7±2.8mV respectively, $p<0.01$). Current amplitude was larger in DED at -40mV (-63.8±18.2 vs. -6.0±5.0pA/pF; $p<0.01$). TTX resistant INa activated maximally at -20mV (DED) and -10mV (control). Current amplitudes at -20mV were -69.5±13.8 and -50.9±17.7pA/pF, respectively (V0.5=-29.0±2.4 vs. -21.9±3.0mV, $p<0.05$). Conclusions: Long-term ocular dryness increases the excitability of cold thermoreceptors, which appears to be mediated in part by a leftward shift in INa activation. Enhanced activity of corneal cold sensory terminals may be responsible of dryness sensations accompanying DED in humans.

Key words: Cornea, Dry eye, Sensory neurons, thermoreceptors, Sodium channels, ocular surface

CORNEAL COLD NERVE ACTIVITY IN DRY EYE IS MODULATED BY CYCLOSPORINE A.

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Although long-term treatment of dry eyes (DE) with topical cyclosporine A (CYA) improves integrity of the corneal surface epithelium and ameliorates ocular dryness sensations and hyposensitivity, it is unclear if these effects are secondary to an antiinflammatory action of the drug or to a direct effect on corneal nerves. We evaluated the effect of the application of 50µM CYA on blinking and tearing and on the electrical activity of corneal termoreceptors in control and in DE animals which main lacrimal gland had been removed surgically 4 weeks before. The isolated cornea was mounted in a recording chamber superfused with physiological saline at 34°C. Spontaneous and evoked activity in response to thermal stimulation (temperature changes in bath solution from 34ºC down to 20°C or up to 50°C) were recorded using conventional electrophysiology. CYA significantly increased blinking and tearing immediately after its instillation in control animals, but recovered 20 min after. CYA decreased the increased response to cold in DE (maximal response to cold: 28.4±5.4 imp/s control; 32.7±1.5 imp/s DE; 23.0±4.2 imp/s DE+CYA; $p<0.05$, t-test). The altered excitability and thermosensitivity of cold fibers found in DE is attributable to an altered expression by primary sensory neurons, of ion channels involved in sensory transduction of cold stimuli. The reduction by CYA of the enhanced responsiveness to cold of corneal nerve terminals in DE suggests that the drug affects ion channels involved in the development of cold hyperexcitability.

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Key words: corneal nerves, corneal sensation, dry eye

SODIUM CHANNEL BLOCKERS MODULATE CORNEAL SENSORY NERVES ACTIVITY IN INTACT AND INJURED CORNEAS.

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Na+ channels are necessary for the depolarization and activation of sensory nerves. We evaluated the effect of different Na+ channel blockers on sensory nerves from intact and injured corneas, testing their analgesic effects. In anesthetized guinea-pigs, a 4mm-diameter mid-stromal corneal flap was cut in one eye using a custom-made microkeratome. At 3-5 days the whole eye or the isolated cornea were mounted in a recording chamber superfused with physiological solution at 34°C. Corneal electrical activity was recorded from cold thermoreceptors, mechano-nociceptors and polymodal nociceptors, using conventional electrophysiology. Spontaneous (SA) and evoked activity by thermal (bath solution temperature changes from 34ºC down to 20°C or up to 50°C), mechanical (von Frey hairs or pipette electrode pressure) and chemical stimulation (98% CO2, 30s gas pulse) were tested in intact and lesioned corneas, before and after treatment with Na+ blockers (lidocaine, carbamazepine, phenytoin, amitriptyline, lamotrigine and gabapentine). In injured corneas cold nerve terminals showed higher SA, lower cold threshold and enhanced response to cooling; nociceptors showed transitory increase of SA and enhanced responses to mechanical and chemical stimuli. The tested drugs reduced the SA and cold-evoked activity of cold nerve terminals and the response to CO2 of polymodal nociceptors in both intact and lesioned corneas, while mechanical threshold was not affected. The increased activity observed in injured corneas seems to be due to an enhanced expression of TTX-resistant Na+ channels, so Na+ blockers are potential tools to attenuate abnormal activity in regenerating nerves, thus reducing spontaneous pain and allodynia after corneal lesions.

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Key words: dry eye, corneal nerves, corneal sensitivity

CHANGES IN SENSORY NERVE ACTIVITY OF THE OCULAR SURFACE UNDER ALLERGIC CONJUNCTIVITIS ARE MEDIATED IN PART BY TRPA1 CHANNEL

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Purpose. The aim of this work was to study in a guinea pig model of allergic conjunctivitis, the changes in spontaneous and stimulus evoked activity of the different functional classes of corneal sensory nerves. Methods: Ovalbumin (OVA) sensitization was induced by i.p. injection of 100µg OVA+20mg Al(OH)3 in 1ml PBS. On day 14, a 10µl drop of 10% OVA in PBS was applied to each eye. Tearing, blinking and scratching movements to the eyes were measured after 1 to 5 exposures to the allergen in different days. Electrical activity was recorded after 1 or 4-5 treatments with the allergen. Spontaneous and stimulus-evoked activity was analysed in control and allergic eyes. Thermal stimulation (cooling the bath solution from 34ºC to 20°C or heating up to 52°C), mechanical stimulation (von Frey hairs) and chemical stimulation (98% CO2 for 30s) were tested. In some experiments, allergic eyes were pre-treated with the TRPA1 blocker HC-030031. Results. After the allergic challenge tearing and blinking were significantly increased, mechano-nociceptors decreased significantly their mechanical threshold and the response to chemical stimulation of polymodal nociceptors was increased. By contrast, the on-going activity and peak frequency values of cold nerve terminals were significantly lower. Pre-treatment with HC-030031, decreased significantly blinking and reverted the majority of the alterations evoked by the allergic challenge. Conclusions. Allergic challenge of the eye produces changes in the activity of corneal nerves which may contribute to the hypersensitivity and discomfort sensations evoked from the eye surface in allergic conditions. TRPA1 channel is responsible, at least in part, of the effects on nerve activity under the allergic challenge.

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Key words: corneal innervation, allergic conjunctivitis, TRP channels

ATOPIC DERMATITIS IS A RISK FACTOR FOR INTRACORNEAL RING SEGMENT EXTRUSION

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Purpose: To describe five atopic dermatitis (AD) and keratoconus patients who developed corneal melting several months after intrastromal corneal ring segment (ICRS) implantation. Methods: Retrospective review of all patients with a history of AD and who developed corneal melting following ICRS implantation. Results: All five patients had as a common factor a history of AD. The patients had no history or biomicroscopical findings of ocular compromise at the time of ICRS implantation. No intra- or early postoperative complications were present. Previous to the development of corneal melt all patients complained of worsening of their AD. All patients presented with corneal ulcers over the ICRS and in 4/5 the ulcer was located in the inferior ICRS. One patient presented initially with a corneal melt in the superior ICRS, yet 8 months later the patient presented with similar ulcer at the inferior ICRS. The time of ICRS extrusion varied between 6 months to 58 months. All corneal cultures were negative, and all patients become asymptomatic after ICRS were removed. Conclusion: We suggest that patients with history of AD and keratoconus are in a risk to develop cor-neal melt after ICRS implantation. The triggering factor may be a worsening of the AD that leads to an unbalanced response of proteolytic enzymes in the cornea leading to tissue destruction. We recommend that patients with history of AD/AKC treated by ICRS implantation should be informed about this com-plication and followed closely.

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Key words: Atopic keratoconjunctivitis, atopic dermatitis, complication, corneal melting, intracorneal ring segments

Ap4A INCREASES CORNEAL EPITHELIAL PERMEABILITY

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Purpose. The corneal epithelium is sealed with intercellular tight junctional complexes and it provides a defensive barrier preventing the entrance of potentially harmful substances but also limiting helpful ocular drug delivery. The purpose of this study was to determine the effects of the diadenosine polyphosphate Ap4A on corneal barrier function. Methods. Human corneal epithelial cells were treated with Ap4A (100 µM) for 5 minutes. After nucleotide removal, cells were incubated and harvested to different times for tight junction protein analysis by western blot. Transepithelial electrical resistance (TEER) measurement was used to evaluate barrier permeability in cells without treatment and cells exposed to Ap4A. In in vivo assays the hypotensor compound 5-methoxycarbonylamino-N-acetyltryptamine (5-MCA-NAT) (10 µM) was topically applied to New Zealand rabbit eyes alone or 2 hours after Ap4A pre-treatment. The presence of 5-MCA-NAT in the aqueous humour was assessed by high-performance liquid chromatography and its effect on intraocular pressure was measured using a Tonopen® tonometer. Results. Treatment with Ap4A induced a significant decrease in tight junction proteins levels as compared to control cells. Concomitantly, TEER values were dramatically reduced at 2 and 4 hours (% reduction: 68 and 52%, respectively). The presence of 5-MCA-NAT in the aqueous humour was 2.75 higher when Ap4A was previously instilled and its hypotensive effect was also increased. Conclusions. Treatment with Ap4A increased corneal barrier permeability by modifying tight junction protein levels and function. Ap4A application could be useful to improve ocular drug delivery and consequently therapeutic effectiveness.

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Key words: Dinucleotides, cornea, barrier permeability, drug delivery

EFFECT OF CYCLODEXTRIN ON THE PENETRATION OF DICLOPHENAC SODIUM THROUGH HUMAN AMNIOTIC MEMBRANE

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Purpose: Cyclodextrins can form complexes with hydrophobic molecules and act as true carriers to deliver drug molecules to the surface of biological membranes. Aim of the study was to investigate permeability of amniotic membrane (AM) to diclophenac sodium containing eye drops with or without cyclodextrin. Methods: Cryopreserved AM pieces on cellulose acetate filter membranes were mounted in the previously established vertical Franz-diffusion cell system equipped with autosampler. In vitro release of two commercially available eyedrops containing 0,1% diclophenac sodium was examined. Voltaren Ophtha CD (VO) (with hydroxypropyl gamma cyclodextrin, Novartis Hungary Kft.) and Uniclophen (UN) (Unimed Pharma, Bratislava, Slovakia) without cyclodextrin) were compared. Drug release was determined by quantitative absorbance measurement carried out with a high performance liquid chromatography (HPLC). Porafil filter membranes without AM served as controls. Results. Gradual increase of concentration was noted up to two hours with both solutions (24.62% for VO and 24.84% for UN of baseline concentrations $P>0.05$). Seven and half hour after instillaton we detected 28.21% and 27.12% of baseline concentrations respectively $P>0.05$). At 2,5 hours, higher percentage of UN penetrated AM, whilst at 7,5 hours higher portion of penetrated diclophenac could be detected with VO. Conclusion: Penetration of diclophenac sodium via cryopreserved AM is not affected by cyclodextrin after 2,5 hours, however significant barrier function of AM could be measured in the earlier period.

Key words: amniotic membrane, cyclodextrin, diclophenac

POLYVINYL CAPROLACTAM-POLYVINYL ACETATE-POLYETHYLENE GLYCOL GRAFT COPOLYMER NANOMICELLE AS A POTENTIAL OCULAR DRUG DELIVERY SYSTEM FOR CYCLOSPORINE A

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Purpose: To investigate whether the polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PVCL-PVA-PEG) nanomicelle had a potential as ocular drug delivery system for cyclosporine A. Methods: Cyclosporin A PVCL-PVA-PEG nanomicelle (CSA-NM) was prepared by thin film method. The formulation were characterized in terms of size, Pdl, zeta potential, morphology, drug loading and stability using dynamic laser scattering, transmission electron microscopy, and high-pressure liquid chromatography, respectively. Cytotoxicity by MTT and cellular uptake were performed on human corneal epithelial cell line (HCECs). Ocular irritation tests were tested on rabbit eyes. Results: CSA-NM had characters with size 73.14 ± 24.42nm, Pdl 0.067 and Zeta -6.7mV, while the blank NM were 78.03±19.31nm, 0.221 and -2.4mV, respectively. The CSA-NM was stability for 4 weeks in PBS solution at 25ºC. In MTT results, up to 48hrs, PVCL-PVA-PEG had no cytotoxicity in its unimer solution and its micelle solution (< 5mg/ml), when concentration is higher than 5mg/ml, some low cytotoxicity was observed (IC50 = 14.02 mg/m, while the Pluronic F127, another widely used graft copolymer in ocular drug delivery system, used as a compare in this research, had IC50 4.28mg/ml) . During the ocular irritation tests in rabbit eyes, no ocular damage or clinically abnormal signs were observed in the cornea, conjunctiva, or iris, indicating that the CSA-NM had excellent ocular tolerance. Further investigation of CSA-NM is now under performing in our lab. Conclusions: These findings indicate that PVCL-PVA-PEG nanomicelle may be a promising delivery system for ocular topical use of CSA.

Key words: polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer, nanomicelle, cyclosporine A

LIPOSOMAL FORMULATIONS CONTAINING VITAMIN C FOR DRY EYE TREATMENT. PRELIMINARY STUDIES.

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Purpose. Due to the complex and dynamic composition of the tear film there is no effective treatment for dry eye. Currently, artificial tears are considered as treatment of choice, although they do not include all components of natural tears. Natural tears contain vitamin C (100-300 µg/mL).This antioxidant agent protects the ocular surface and promotes tear production. As vitamin C is highly unstable in aqueous solutions, the use of derivatives such as palmitoyl-L-ascorbic acid (PAA) could be employed. The aim of this work was to prepare liposomal formulations for topical administration with a composition similar to natural tears capable of stabilizing the lipid film and improving tears quality. Methods. Four liposomal formulations (F1, F2, F3 and F4) were prepared according to the solvent evaporation technique. Lipid bilayer of liposomes was composed by phosphatydilcholine (PC), cholesterol, vitamin E (8:1:0.8 mg/mL) and PAA. PAA concentration for F1 and F3 was equivalent to 300 µg/mL of ascorbic acid and 100 µg/mL for F2 and F4. An isotonic aqueous phosphate solution was employed to disperse F1 and F2 while a hypotonic solution was used for F3 and F4. Final PC concentration was 40 mg/mL for all formulations. Osmolarity, pH and particle size were assessed. In vitro cytotoxicity was evaluated in human conjunctival and corneal cells at different exposure times (15 min, 1 h and 4 h). Results. All liposomal formulations showed unimodal size distribution and the mean diameter size was less than 200 nm. pH values were nearly neutral for all preparations. As expected, osmolarity values were 289.1±1.5 mOsm/L and 211±3.5 mOsm/L for isotonic and hypotonic formulations respectively. Cytotoxicity assays showed good tolerance in conjunctival and cornea cells for four formulations (cell viability>80%) at all exposure times. Conclusions. Both isotonic (F1 and F2) and hypotonic (F3 and F4) liposomal formulations with palmitoyl-L-ascorbic acid present suitable properties for topical administration in the eye. Cytotoxicity studies resulted well tolerated for ocular surface cells.

Key words: Dry eye, Vitamin C, Liposomal formulations

LACOSAMIDE DECREASES THE HYPEREXCITABILITY OF CORNEAL COLD THERMOSENSITIVE NERVE TERMINALS IN EXPERIMENTAL DRY EYE

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Purpose: Our previous results with cyclosporine A (CSA) suggested a direct effect of CSA on hyperexcitability of membrane sodium currents (see posters by Gasull et al. and Kovacs et al.) which is a characteristic feature of corneal cold receptors in experimental model of dry eye. Lacosamide is an anti-epileptic drug that is also used for the treatment of painful diabetic neuropathy acting through voltage-gated sodium channels. This work has evaluated the effects of acute application of lacosamide on the electrical activity of corneal cold nerve terminals in lacrimo-deficient guinea pigs. Methods: Four weeks after unilateral surgical removal of the main lacrimal gland in guinea pigs, corneas were excised and superfused in vitro at 34 ºC for extracellular electrophysiological recording of nerve terminal impulse activity of cold-thermosensitive nerve terminals. The characteristics of the spontaneous and the stimulus-evoked (cooling ramps from 34º to 20 ºC) activity before and in presence of lacosamide (concentration) and lidocaine (concentration) were compared. Results: Cold nerve terminals (n=19) recorded from dry eye corneas showed significantly enhanced spontaneous activity and cold response as well as reduced cold threshold to cooling ramps compared to terminals from control animals (n=18). Both lidocaine and lacosamide decreased spontaneous activity significantly (by -5.02±1.46 imp/s, $p<0.001$ and by -4.29±0.90 imp/s; $p<0.01$, respectively). Temperature threshold before drug application (1.71±0.15 ºC) was modified by lidocaine (2.91±0.34 ºC; $p=0.005$) but not by lacosamide (1.66±0.21 ºC; $p>0.05$). Application of lidocaine and lacosamide resulted in a significantly lower peak response frequency (4.71±3.32 imp/s and 14.01±2 imp/s respectively) compared to values before drug application (21.24±1.5 imp/s; $p<0.001$). Conclusions: Lacosamide decreased significantly both enhanced spontaneous activity and response to cold of corneal cold receptors, however its effect was slightly less than lidocain showed. These results suggest that lacosamide may have a role in reducing hyperexcitability of corneal cold receptors during long term ocular dryness by acting directly on voltage-gated sodium channels.

Key words: Cold receptor, Dry eye, Sodium channel

INTRAVENOUS LIPOPOLYSACCHARIDE (LPS)-INDUCED BLOOD AQUEOUS BARRIER PERMEABILITY CHANGE ASSESSED BY FLUOROPHOMETRY IN RABBITS

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Purpose: To validate the use of fluorophotometry for assessing blood aqueous barrier permeability after intravenous lipopolysaccharide (LPS) injection in rabbits. Methods: The procedure described by Cousin, et al (1982) was modified. Briefly, New Zealand White rabbits were given fluorescein isothiocyanate (FITC)-labeled dextran (70 KD) intravenously, followed by lipopolysaccharide (LPS; 2 μg/kg) intravenously. Approximately 2 hours later, fluorophotometry was performed to assess fluorescence in the anterior chamber. Immediately after the scan, animals were euthanized and plasma, aqueous humor and vitreous humor were collected for assessment of fluorescence using the same fluorophotometer. One group of animals was administered topical prednisolone acetate twice daily for 3 days prior to administration of FITC-dextran and LPS. The other group was administered topical saline as control. Results: There was a significantly ($P<0.001$; Student's t-test; n=8) reduced level of fluorescence in the anterior chamber in the prednisolone-treated group when compared to the saline control group at 2 hours after intravenous LPS administration (~62% inhibition when measured in vivo and ~48% inhibition when measured in the harvested aqueous humor). There was good correlation of fluorescence from in vivo measurements and from harvested aqueous humor measurements ($R2 =0.96$; linear regression). Conclusions: A procedure was validated for using fluorophotometry for assessing blood aqueous barrier permeability change induced by intravenous administration of LPS.

Key words: Fluorophotometry, blood aqueous barrier, FITC-dextran

MELATONIN ANALOGUE AGOMELATINE REDUCES INTRAOCULAR PRESSURE IN RABBIT WITH NORMOTENSIVE AND HYPERTENSIVE CONDITIONS

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Purpose: In the search for new compounds to reduce intraocular pressure (IOP), we have found that agomelatine, a melatonin analogue, can reduce IOP being interesting for the treatment of ocular hypertension and glaucoma. Methods: New Zealand rabbits were used for IOP studies. Agomelatine, formulated in isotonic saline (1% DMSO) was tested at different concentrations from 10-12 M to 10-4 M. It was applied to the cornea at a fixed volume of 10 µl in the treated eye and the contralateral received the same volume of saline + 1% DMSO. IOP was measured with a TonoVet® tonometer (Tiolat Oy). To induce the hypertensive condition, animals were placed in Trendelenburg position (prone - 80º head down). Luzindole, Prazosin and 4PPDOT were used as antagonists of melatonin receptors (at 100µM). Results: Agomelatine (10µl 100µM) reduced IOP 20.8 ± 1.4 % (n=18) with a maximal effect 180 minutes after the compound application in normotensive condition and 68.8 ± 5.7 (n=8) in a hypertensive condition. Concentration-response curve presented a pD2 value of 9.7 ± 0.3 (EC50 0.19 nM). The effect of agomelatine was partially antagonized by 4PPDOT (MT2 antagonist, 10µl 100µM) and prazosin (MT3 antagonist, 10µl 100µM) (85.6 ± 1.6 % and 87.2 ± 1.9 %, n=18 respectively.) Agomelatine effect in normotensive condition was comparable to Xalatan® and Alphagan® and no so effective as Trusopt® or Timolol®. Conclusions: These results suggest the use of agomelatine for the treatment of those ocular conditions, which involve an abnormal raise of intraocular pressure.

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Key words: Agomelatine, glaucoma, hypertensive condition, IOP, melatonin, melatonin receptors

DEVELOPMENT OF SYL040012, A siRNA FOR TREATING INCREASED INTRAOCULAR PRESSURE ASSOCIATED TO GLAUCOMA

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Purpose: Sylentis is developing SYL040012, a siRNA for the treatment of glaucoma and increased intraocular pressure (IOP). SYL040012 targets ADRB2, a very well-known target for glaucoma. Methods: Efficacy of SYL040012 was assessed in normotensive rabbits and in a rabbit model of increased intraocular pressure. 28-day toxicology studies were performed in rabbits and non-human primates. Three clinical trials have been performed for this compound; a safety phase I trial in healthy patients, a safety trial in individuals with increased intraocular pressure and an efficacy trial that is currently on-going. Results. SYL040012 reduced ADRB mRNA levels in vitro and in vivo. 28-day toxicology studies in rabbits and non-human primates showed that the drug was systemically and locally well tolerated and that the compound did not reach the blood-stream. The results from the clinical trials showed that the compound is well tolerated in healthy volunteers and individuals with elevated IOP. The first clinical trials also indicated that SYL040012 is able to lower IOP in individuals with high IOP. The results of the phase II clinical trial that is currently on-going will assess the efficacy of this compound versus placebo. Conclusions. SYL040012 reduces ADRB2 mRNA levels and IOP in animals. Preclinical toxicology studies and phase I trials showed that SYL040012 was locally and systemically well tolerated after single and multiple administrations and that it does not reach the blood-stream. Development of this compound is currently on-going with a dose-range finding study in which efficacy of different doses of SYL040012 will be compared to placebo.

Key words: siRNA, ADRB2, increased intraocular pressure, glaucoma

TRAFFICKING OF AQUAPORIN-1 MEDIATED BY Ap4A IN RABBIT NON PIGMENTED CILIARY EPITHELIAL CELLS: INVOLVEMENT OF P2Y2 RECEPTOR IN IOP RAISE

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Purpose: To investigate the role of Ap4A acting through P2Y2 purinergic receptor in the increase of aquaporin-1 levels in NPE cell membranes and cytoplasm. Methods: Immunocytochemical studies were performed in immortalized NPE cells by means of an antibody raised against aquaporin-1 (AQP1). Cells were challenged with single doses of Ap4A, P2Y antagonists, and signalling pathway inhibitors. Time-course of AQP1 after Ap4A application was studied. Dose-response analysis was performed by assaying the agonists at concentrations ranging from 1 nM to 1 mM. Antagonists and inhibitors were pre-incubated before Ap4A was added and they were present during the dinucleotide application. Results: The application of Ap4A in NPE cells demonstrated a gradual increase in the presence of AQP1 which was time and dose-dependent. The EC50 value for Ap4A was 1.02 uM. Ap4A produced an increase in AQP1 expression which had a maximal 60 min after the dinucleotide application returning to control value in another 60 min. Concerning the distribution of AQP1, there was a significant increase of AQP1 in the plasma membrane, but its presence was increased both in the cytoplasm and in the membrane. The experiments performed with antagonist demonstrated the involvement of P2Y2 receptor in the effect of Ap4A. Experiments performed with signalling pathways inhibitors demonstrate that membrane trafficking of AQP1 is due to phosphorylation mediated by PKC. Conclusion: Ap4A acting via a P2Y2 receptor can mobilize AQP1. The increase in AQP1 expression and its trafficking could explain why IOP increase when P2Y2 receptors present on the ciliary body are activated.

Key words: Aquaporin, Nucleotide, Purinergic Receptor, Aqueous humor

INTRACELLULAR ELECTRICAL PROPERTIES AND CHLORIDE TRANSPORT CHARACTERISTICS OF PORCINE CILIARY EPITHELIAL CELLS

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Objectives: To characterize the intracellular electrical properties and chloride concentration of primary cultures of pigmented ciliary epithelial cells (PE) and non-pigmented ciliary epithelial cells (NPE) from porcine eye and the effects of transport inhibitors. Methods: The membrane potentials of primary culture of porcine ciliary epithelium were simultaneously measured by an anionic potential-sensitive fluorescent dye, bis(1,3-dibutylbarbituric acid) trimethine oxonol [DiBAC4(3)]. The effects of transporter inhibitors and ion depletion were also investigated. Results: The membrane potential of isolated NPE and PE cells were -69±0.87mV (MEAN±SEM, n=184) and -63±2.08mV (MEAN±SEM, n=105) respectively. The effects of changing extracellular Na+ concentration from 150mM to 2mM, changing extracellular Cl- concentration from 120mM to 7mM. Ouabain (Na+/K+-ATPase inhibitor) depolarized the epithelial cells whereas Diphenylamine-2-carboxylate (DPC) and IAA94 (chloride channel blocker) hyperpolarized them. However, bumetanide (Na-K-2Cl cotransporter inhibitor) showed no significant effect on membrane potential. Niflumic acid (NFA, chloride channel blocker) pretreatment induced significantly depolarization caused by low Cl-. Conclusion and discussion: The membrane potential is strongly dependent on the extracellular chloride concentration. Chloride ion replacement could induce depolarization which indicates the presence of a Cl- conductance. In general, the results from intracellular recording were consistent with an active chloride transport across the ciliary epithelium that drives aqueous humour formation.

Key words: ciliary epithelium, chloride transport, aqueous humour formation, intracellular recordings

RETINAL P2X7 RECEPTORS IN A MURINE MODEL OF GLAUCOMA

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Purpose. To investigate the rise in ATP retinal levels and P2X7 receptor expression increase in a murine model of glaucoma. Methods. DBA/2J glaucomatous mice together with C57BL/6J control mice were used along the experimentation. Animals were studied from 3-15 months of age. For the study of retinal nucleotide release retinas were dissected and prepared as flattened whole-mounts and stimulated in PBS buffer with or without 59mM KCl. Immunohistochemical and western-blot analysis were performed with antibodies against the P2X7 nucleotide receptor and GFAP. ERG recordings were performed on the right eyes of C57BL/6J and DBA/2J mice at different ages to analyze the changes in the electrophysiological response. Scotopic threshold response determines the onset of functional changes exhibited in the inner retina of the DBA/2J mice. Results. Glaucomatous mice exhibited changes in retinal ATP release as long as the pathology progressed and up-regulation P2X7 receptor. 15 months DBA/2J animals presented a basal retinal ATP level of 19.7 pmol/retina while the stimulated with KCl was 22.1 pmol/retina. There was 36 % of increase in the presence of this receptor measured both by immunohistochemical and western-blot techniques. ERG recordings in 15 months glaucomatous mice showed an important reduction in the pSTR response correlated with ganglion cell loss. Conclusions. In the development of the glaucomatous pathology in the DBA/2J mice, the increase in the presence of P2X7 receptors may contribute, with other factors, to the changes in the functionality of the retina and the concomitant death of retinal ganglion cells.

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Key words: Glaucoma, retina, scotopic threshold response, P2X7

UNDERCORRECTION OF REFRACTIVE ERROR AND COGNITIVE FUNCTION

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Background. To assess whether ocular parameters are associated with the level of cognitive function in an elderly society. Methods. The Beijing Eye Study 2011 is a population-based study performed in rural and urban regions of Greater Beijing. The study included 3469 individuals (1963 (56.6%) women) with a 9.8 years (range: 50-93 years). A detailed ophthalmic and±mean age of 64.6 medical examination was performed. The cognitive function score was measured by the MMSE (mini mental state examination) scale. Results. Cognitive function measurements were available for 3127 (90.1%) study participants (1768 (56.5%) 3.7 (median: 27; range:±women). The mean cognitive function score was 26.3 2-30). In multivariate analysis, decreasing cognitive function score was significantly associated with older age (P<0.001; standardized coefficient beta:0.14), male gender (P=0.003;beta:0.06), urban region of habitation (P=0.005;beta:0.07), lower body height (P=0.001;beta:0.07), lower level of education (P<0.001;beta:0.56), type of occupation (P=0.004;beta:0.08), higher score of psychic depression (P<0.001;beta:0.06), lower self-reported history of cardiovascular disorder (P=0.02;beta:0.07), lower best corrected visual acuity (P<0.001;beta:0.11), higher amount of undercorrection of refractive error (P=0.004;beta:0.04), and non-wearing of glasses (P<0.001;beta:0.09). Conclusions. After adjustment for age, gender, education level, type of occupation, region of habitation, body height, depression level and best corrected visual acuity, the cognitive score was significantly higher in subjects wearing glasses and simultaneously, in subjects with less undercorrection of their refractive error. Although the longitudinal cause-effect relationship could not be addressed in this cross-sectional study, the results suggest that correction of refractive errors is useful to reduce the risk of cognitive under-functioning.

Key words: Cognitive dysfunction, Alzheimer´s disease, Refractive undercorrection, Beijing Eye Study

INTRAVITREAL BEVAZICUMAB FOR RETINOPATHY OF PREMATURITY: REFRACTIVE ERROR RESULTS

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Purpose: To evaluate refractive error in infants who underwent intravitreal bevacizumab injection for treatment of threshold retinopathy of prematurity (ROP). Design: Non-randomized interventional comparative study. Methods: The study group included all infants who consecutively received a single intravitreal (0.375 mg or 0.625 mg) bevacizumab injection for therapy of threshold ROP in fundus zone I or zone II. The control group included infants who had previously undergone retinal argon laser therapy of ROP. The follow-up examination included refractometry under cylcoplegic conditions. Results: The study group included 12 children (23 eyes; mean birth weight:622±153g; gestational age:25.2±1.6 weeks) and the control group included 13 children (26 eyes; birth weight:717±197g; gestational age:25.3±1.8 weeks). Both groups did not differ significantly in birth age and weight and follow-up. At the end of follow-up at 11.4±2.3 months after birth, refractive error was less myopic in the study group than in the control group (-1.04±4.24 diopters (median:0 diopters) versus -4.41±5.50 diopters (median:-5.50 diopters);P=0.02). Prevalence of moderate myopia (17±8% versus 54±10%; P=0.02; OR:0.18 (95%CI:0.05,0.68) and high myopia (9±6% versus 42±10%;P=0.01; OR:0.13 (95%CI:0.03,0.67) was significantly lower in the bevacizumab group. Refractive astigmatism was significantly lower in the study group (-1.0±1.04 diopters versus 1.82±1.41 diopters; P=0.03). In multivariate analysis, myopic refractive error and astigmatism were significantly associated with laser therapy versus bevacizumab therapy (P=0.04 and P=0.02, respectively). Conclusions: In a one-year follow-up, a single intravitreal bevacizumab injection as compared to conventional retinal laser coagulation was helpful for therapy of ROP and led to less myopization and less astigmatism.

Key words: Retinopathy of prematurity, Bevacizumab, Myopia

NEUROPROTECTIVE EFFECT OF TUDCA ON GLIAL CELLS IN RETINAL DEGENERATION.

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Introduction: TUDCA demonstrated its neuroprotective effect in different animal models of retinitis pigmentosa (RP). The purpose of this study was to investigate changes in vascular plexus and glial cells in the retina of P23H rats, a model of RP, and to evaluate the preventive effect of the antiapoptotic agent TUDCA. Material & Methods: Homozygous P23H line-3 rats were injected with TUDCA (500 mg/kg, i.p.) weekly from 20 days to 4 months old. SD rats were used as controls. Immunoblotting and immunostaining on whole-mount retinas against different glial markers were used to study the morphology and numbers of microglia, Müller and astrocyte cells and vascular plexus. Results: At 4-month-old, the number of microglial cells in TUDCA-treated animals was smaller compared with vehicle-treated animals. TUDCA was effective protecting the loss of the vascular plexus in P23H. Astrocyte numbers increased in the P23H rat retina compared with SD, being even higher in TUDCA-treated animals. Astrocytes exhibited a deteriorated morphology in vehicle-treated animals, whereas in TUDCA-treated animals astrocytes kept a similar morphology than in control rats. Müller cells numbers remained unchanged. Immunoblot analysis showed that the treatment modifies the expression of GFAP, Connexin43, Vimentin and CRALBP compared with vehicle-treated animals. Conclusions: TUDCA prevents astrocyte, Müller and microglial cell changes in the P23H rat retina at 4 months of age and this compound could be useful for preventive treatment of RP.

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Key words: Neurodegeneration, neuroprotection, glial cells, retinitis pigmentosa, therapy, TUDCA

SAFRANAL, A CONSTITUENT OF CROCUS SATIVUS (SAFFRON), SLOWS RETINAL DEGENERATION AND VISION LOSS

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Purpose: Saffron, Crocus sativus stigma, has been used in traditional medicine for its antiapoptotic and anticarcinogenic properties. In this work, we study the effects of safranal, a component of saffron, in attenuating retinal degeneration in the P23H rat model of autosomal dominant retinitis pigmentosa. Methods: P23H line-3 rats were injected with either saline or safranal (400 mg/kg, i.p.) twice a week from P21 to P120. At P120, functional activity of the retina was evaluated by electroretinographic (ERG) recording. The effects of safranal on the number, morphology, integrity, and synaptic connectivity of retinal cells were characterized by immunofluorescence confocal microscopy. Retinal capillary network was examined after labeling with NAPDH diaphorase. Results: Administration of safranal to homozygous P23H line-3 rats preserved morphology, number and synaptic connectivity in both photoreceptors and inner retinal cells. ERG recordings showed higher a- and b-wave amplitudes under both photopic and scotopic conditions in safranal-treated versus non-treated animals. Furthermore, the capillary network in safranal-treated animals was preserved, unlike that found in untreated animals. Conclusions: These results indicate that dietary supplementation with safranal slows photoreceptor cell degeneration and ameliorates the loss of retinal function and vascular network disruption in P23H rats. This work also suggests that safranal could be potentially useful to retard retinal degeneration in patients with retinitis pigmentosa.

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Key words: Retina, retinitis, neuroprotection, electroretinogram, confocal microscopy

ADDITIONAL NEUROPROTECTIVE EFFECTS OF NORGESTREL IN RETINITIS PIGMENTOSA: PRESERVATION OF RETINAL CYTOARCHITECTURE AND SYNAPTIC CONNECTIVITY

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Purpose: Apoptosis of photoreceptors in the rd10 mouse, a model of retinitis pigmentosa, is followed by deterioration of their post-synaptic cells and loss of synaptic contacts. The anti-apoptotic compound norgestrel has been shown to delay the course of retinal degeneration in this model. The aim of this work was to evaluate norgestrel efficacy on restraining retinal cytoarchitecture disruption and synaptic connectivity impairment.Methods: Norgestrel (or vehicle) was administered intraperitoneally to rd10 mice (100 mg/kg) on alternate days, commencing on postnatal day (P)18. Mice were sacrificed on P40 and the retinal tissue was processed for immunohistochemistry. Vertical cryosections were stained with antibodies specific for photoreceptor, horizontal and bipolar cell markers, and visualized by confocal microscopy. In addition, preservation of synaptic contacts at the outer plexiform layer was evaluated.Results: Immunostaining with γ-transducin showed relatively good preservation of cone photoreceptor morphology up to P40, especially in the peripheral retina. Calbindin, GNB3 and PKCα labeling revealed improved horizontal and bipolar cell morphology in norgestrel-treated animals. Retinas from norgestrel-treated rd10 mice also exhibited stronger immunoreactivity for the synaptic ribbon components CtBP2 and bassoon, and the synaptic vesicle glycoprotein synaptophysin, when compared to vehicle-treated animals.Conclusions: Our results confirm the neuroprotective effects of norgestrel on retinal degeneration and demonstrate this compound delays retinal remodelling and loss of synaptic contacts associated with photoreceptor apoptosis. Therefore, norgestrel might be a good therapeutic candidate for the treatment of retinitis pigmentosa and related diseases.

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Key words: Photoreceptors, Progestin, rd10 mouse

A PROOF-OF-PRINCIPLE OF THE NEUROPROTECTIVE ROLE OF TUDCA ON RETINAL GANGLION CELLS

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Purpose: Although the anti-apoptotic activity of tauroursodeoxycholic acid (TUDCA) has been previously demonstrated in animal models of photoreceptor degeneration, its neuroprotective effect on retinal ganglion cells has not been evaluated. The aim of this work was to test whether systemic administration of TUDCA rescued retinal ganglion cells from apoptosis following excitotoxic damage. Methods: As a first approach, oxidative stress was induced in the RGC-5 cell line (kindly provided by Dr. N. Agarwal) after exposure to the nitric oxide donor sodium nitroprusside. Apoptosis was confirmed by TUNEL. Viability, in the presence of increasing concentrations of TUDCA, was assessed by XTT, crystal violet and calcein-AM assays. Retinal ganglion cell apoptosis was induced in Sprague-Dawley rats by intravitreal injection of N-methyl-d-aspartate (NMDA). Immunohistochemistry on whole-mount retinas was used to evaluate retinal ganglion cell survival in TUDCA- (500 mg/kg i.p.) or vehicle-treated animals. Results: When cultured in the presence of TUDCA, survival of sodium nitroprusside-treated RGC-5 cells significantly increased in a dose-dependent fashion. In vivo, TUDCA delayed NMDA-induced apoptosis of retinal ganglion cells, as revealed by Brn3a immunostaining of whole-mounts. Conclusions: Our results provide a proof-of principle of the efficacy of TUDCA as a neuroprotective factor for retinal ganglion cells, paving the way for clinical trials on glaucoma patients and other degenerative diseases coursing with retinal ganglion cell death.

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Key words: RGC-5 cell line, N-methyl-d-aspartate (NMDA), excitotoxicity, apoptosis, rat

TOLERANCE OF SUB-TENON INJECTION OF PLGA NANOPARTICLES AND MICROPARTICLES

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Purpose: To explore the feasibility of the sub-Tenon space for the administration of PLGA nanoparticles (NP) and microparticles (MP) as drug delivery systems in the treatment of diseases affecting the back of the eye. Methods: PLGA MP were prepared based on the O/W emulsion-solvent evaporation technique. NP were prepared by nanoprecipitation. 100µl of isotonic suspensions of 5mg of MP or NP were injected in the sub-Tenon space of pigmented female rabbits (2.5-3.5 kg). Experiments were performed according to the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research and in accordance with the European Communities Council Directive (86/609/EEC). Clinical evaluation was carried out by inverted image ophthalmoscopy under pharmacological midriasis and the IOP was measured by means of Tono-Pen VET. The following signs were evaluated: Conjunctival discharge, swelling and congestion, aqueous flare, light reflex, iris involvement, pannus, vitreous opacity, vascular congestion, vitreal and retinal haemorrhage, and retinal detachment. Histopathology evaluation was performed at 12 week post-injection. Results: The administration of the MP (28.7±0.1 µm) suspension provoked only slightly conjunctival congestion 24 after injection in 45% of treated eyes and also in 3% of control eyes. The administration of the NP (55,03 ± 1,56 nm) suspension produced conjunctival congestion 24 after injection in the 100% of treated eyes and in 16% of control eyes. This sign was reduced in treated eyes to 27% in the first week and disappeared four weeks after injection. No other clinical signs were observed after NP or MP injection. The IOP did not change during the 12-weeks period ($p>0.05$). The histopathology study showed the presence of macrophages and polymer material in the evaluated tissues three months after injection. Conclusions: PLGA NP and MP resulted well tolerated after sub-Tenon injection and can be considered a novel strategy for the development of drug delivery systems in the treatment of ocular diseases affecting the posterior segment.

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Key words: Sub-Tenon, Tolerance, PLGA Microparticles, PLGA Nanoparticles

FREEZE-DRYING MATRICES AS PROLONGED RELEASE SYSTEM FOR INTRAVITREAL ADMINISTRATION OF BEVACIZUMAB

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The use of intravitreal anti-vascular endothelial growth factor agents is increased dramatically over the last few years. This is due much to the successful use of off-label intravitreal bevacizumab for the treatment of exudative age-related macular degeneration, although intravitreal bevacizumab has been utilized to treat numerous ocular disorders. However, possible complications and side effects of the procedure of administration can occur. These may include eye pain, inflammation, infection, visual disturbances, but also more severe adverse events. The need to repeat the administration, even at an early date, greatly increases the risk of side effects. To reduce the number of applications, an intravitreal prolonged release system of the drug could be very useful. This work aimed at tuning solid formulations for intravitreal administration of bevacizumab to produce a sustained release of drug. The formulations consisting of polyvinylpyrrolidone and polyvinyl alcohol matrices were obtained by freeze-drying of polymeric dispersions. The matrices underwent technological characterization for evaluating their hydration ability by solvent sorption time test, and release rate of drug by dynamic dialysis. The matrices had sizes suitable for intravitreal administration, hydrated slowly, and were able to control the drug release: the matrices released an amount of bevacizumab 10-fold lower than market aqueous solution.

Key words: Bevacizumab, intravitreal, freeze-drying matrices

MU-PH1, A NEW MURINE MÜLLER-DERIVED RETINAL CELL LINE WITH PHOTORECEPTOR PROPERTIES

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Purpose: To characterize a novel cell line, derived from retinal progenitors, which could serve as an in vitro model to study photoreceptor degenerative mechanisms and to evaluate the efficacy of neuroprotective compounds. Methods: A Müller-derived cell line (MU-PH1) was isolated from adult C57BL/6 mouse retina. RT-PCR, immunoblot and immunofluorescence analyses were performed to characterize these cells. Calcium imaging allowed the determination of light-responsiveness. To establish a model for the screening of potential neuroprotective drugs, apoptosis was induced with cytotoxic agents and measured using XTT and crystal violet viability assays. Results: Spontaneously immortalized MU-PH1 cells exhibited glial-like morphology and characteristics of neural retinal progenitors, as identified by their ability to form neurospheres and the expression of neural/stem cell markers (nestin, Abcg2, α-tubulin, β-III-tubulin and Ascl1). Additionally, MU-PH1 cells expressed markers of Müller glia (vimentin, S-100, glutamine synthetase) and of differentiated retinal neurons (rhodopsin, recoverin, γ-transducin, melanopsin and cone opsins). Other markers such as CRALBP, GFAP, CD31 or CD11b were undetectable. Stimulation with 480 nm light evoked slow and fast transient calcium responses in most of the cells tested. MU-PH1s were sensitive to oxidative stress induced by sodium nitroprusside or oligomycin/rotenone. Cell death was prevented by anti-apoptotic and antioxidant compounds such as tauroursodeoxycholate and N-acetylcysteine. Conclusions: Availability of MU-PH1 cell line may provide a unique tool to study photoreceptor signalling pathways, as well as a convenient model for the screening and development of new therapeutic drugs.

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Key words: drug discovery, in vitro models, efficacy/toxicity studies

LONG-TERM RESULTS OF THE TREATMENT WITH BEVACIZUMAB IN JUXTAPAPILLARY RETINAL CAPILLARY HEMANGIOMA

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Purpose: To present the long-term results of the treatment with intravitreal(iv) bevacizumab in a juxtapapillary retinal capillary hemangioma (RCH). Methods: A 72-year old man with a juxtapapillary RCH and lipid exudation extending into the macula was treated with two monthly iv injections of 125mg bevacizumab as the only form of treatment. Best corrected visual acuity (BCVA), fundus fluorescein angiography (FFA) and optical coherence tomography (OCT) were performed at baseline and six years after treatment. Results: One month after last injection of bevacizumab, a complete regression of lipid exudates and subretinal fluid was observed . BCVA improved from 20/66 to 20/33. FFA showed a reduction of tumor leakage, with a decrease of lesion size. In the last examination, six years after treatment, the tumor remained inactive, the fundus without exudates with a dry macula and the BCVA was 20/25. Comments : Our case underlines that bevacizumab treatment alone can cause a reduction of the tumour-associated exudation, and that these results can be maintained in the long term. To our knowledge this is the longest follow-up of a patient with juxtapapillary RHC treated only with bevacizumab. Long term follow-up of other similar cases is necessary to make a conclusive statement about the bevacizumab therapy of juxtapapillary RCH.

Key words: retinal capillary hemangioma, bevacizumab, retinal exudation

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