EXPERIMENTAL GLAUCOMA; ANIMAL MODELS: RODENT

P124

NEUROPROTECTIVE EFFECT AGAINST THE AXONAL DAMAGE-INDUCED RETINAL GANGLION CELL DEATH IN THE APOLIPOPROTEIN E DEFICIENT MICE THROUGH THE SUPPRESSION OF KAINATE TOXICITY

T. Nakazawa[1], M. Ryu[1], A. Shanab[1], N. Fuse[1]
[1]Department of Ophthalmology, Tohoku University Graduate School of Medicine, Sendai - Japan

Background: Apolipoprotein E (ApoE) protein played the important roles including a carrier of cholesterol, anti-oxidant, and ligand for LDL receptors. In the nervous systems, the presence of ApoE4 isoform was associated with the Alzheimer's disease. ApoE gene polymorphisms were also reported to be associated with glaucoma, however, the function of ApoE remained unclear in the retina. In this study, we investigated the roles of ApoE on the axonal damage-induced RGCs death.
Methods: Adult ApoE-deficient mice (Male, 10-12 weeks old) were used in this study. RGCs damage was induced by optic nerve crush (NC) with fine forceps for 10 seconds with or without glutamate receptor antagonists (MK801 or CNQX) 30 minutes before injury or by an intravitreal administration of Kainate (KA). Seven days later, treated retinae were harvested and the density of surviving RGCs retrogradelly labeled with fluorogold was quantified on the flat-mounted retina.
Results: ApoE protein was detected in the astrocytes and Muller cells in the retina. In the wild-type mice, NC induced the RGCs death (Control: 4085 ± 331 cells/mm2, NC: 1728 ± 170) and the RGCs death was suppressed by CNQX (3031 ± 246), but not MK801 (1769 ± 212). NC- or KA-induced RGCs death was significantly less in the ApoE-deficient mice (NC: 2396 ± 193 cells/mm2, KA: 4279 ± 471) than that in the wild-type mice (NC: 1728 ± 170, KA: 2720 ± 205).
Conclusion: These data suggest that the suppression of ApoE and KA receptors had a neuroprotective effect on the axonal damage-induced RGCs death.

P125

NEUROPROTECTIVE EFFECT OF MALTOL ON OXIDATIVE STRESSED RETINAL GANGLION CELLS

S. Hong[1], K. Park[1], C.Y. Kim[1], G.J. Seong[1]
[1]Department of Ophthalmology, Yonsei University College of Medicine, Seoul - Korea Republic

Background: Maltol (3-hydroxy-2-methyl-4-pyrone), a naturally occurring organic compound that is used primarily as a flavor enhancer, has known to have the free radical scavenging activity. It was investigated whether maltol protects the oxidative stressed retinal ganglion cells (RGCs).
Methods: For in vitro study, the primary cultured mouse RGCs were exposed to hydrogen peroxide with or without maltol. Cytotoxicity and apoptosis were determined by ATP assay and TUNEL. For in vivo study, the left middle cerebral artery (MCA) of mice was intraluminally occluded and the apoptosis of retinal cells was determined by TUNEL. Mice in the maltol group were treated once with an intraperitoneal maltol injection 30 minutes before MCA occlusion.
Results: In primary RGCs, maltol significantly attenuated the hydrogen peroxide-induced cytotoxicity from 60% to 40%, as determined by ATP assay. This observed cell damage was related to apoptotic cell death, as established by TUNEL assay. In animals, maltol treatment definitely reduced the number of TUNEL-positive retinal cells.
Conclusions: It was revealed that maltol procets the oxidative stressed RGCs in vitro and in vivo. It may offer a new neuroprotective agent for oxidative stress-related ocular diseases including glaucoma.

P126

TOLERANCE OF A POLYDIOXANONE MEMBRANE IN THE SUBCONJUNCTIVAL SPACE OF THE RABBIT EYE. POTENTIAL USE AS A DRUG DELIVERY SYSTEM AND PERSPECTIVE IN GLAUCOMA SURGERY

D. Gruber[1], E. Gardéa[1], B. Coquerel[2], S. Lefevre[2], R. Andrieu[3], A. Della Martina[3], M. Muraine[1]

[1]Charles Nicolle Hospital, Rouen - France; [2]Laboratoire Merci, Rouen - France; [3]Laboratoire Cerebel, Lausanne - Switzerland

Background: Polydioxanone (PDO) is a biocompatible polymer largely used in biodegradable sutures. It received FDA approval in cardiology for valvular surgery. Drugs such as mitomycin C (MMC) can be included in the core of the polymer during the synthesis process resulting in a sustained release drug delivery system as the PDO is biodegraded by hydrolysis. The duration of the hydrolysis can be modulated by adapting the synthesis parameters of the PDO. In this way, MMC or other drugs could be released progressively for up to several weeks. The aim of this preliminary study was to evaluate the clinical and histological tolerance of a PDO membrane in the subconjunctival space of the rabbit eye.

Methods: Six New Zealand White rabbits (12 eyes) were operated on under general anaesthesia: after fornix based flap and tenon dissection in all eyes, a membrane of PDO was inserted in 6 eyes whereas the subconjunctival space was left empty in the contralateral 6 eyes which served as a control. A limbal suture closed the conjunctiva in all eyes. Corticoantibiotic drops were applied twice daily until euthanasia. The conjunctivas were clinically evaluated daily and 2 animals were euthanised at day 7, day 15 and day 45. After Hematoxylin-Eosin staining a histological study was completed.

Results: Clinically, the PDO membrane remained visible under the conjunctiva for 2 weeks, associated with a moderate and transient hypervascularity of the conjunctiva. No difference was noted between the 2 groups at day 45.

Histologically, the PDO membrane and the surrounding conjunctiva were infiltrated with a few inflammatory cells compared to control at days 7 and 15. By the 45^{th} postoperative day, the PDO membrane had degraded and the cellularity returned to control level.

Conclusion: Polydioxanone is well tolerated in the subconjunctival space of the rabbit eye without significant inflammatory response during its biodegradation. This could yield to a new drug delivery system in ophthalmology, particularly in glaucoma filtering surgery while using MMC.

P127

INTRAVITREAL TRANSPLANT OF HUMAN MESENCHIMAL STEM CELLS AS A CAPABLE SOURCE OF NEURO-PROTECTIVE GROWTH FACTORS: NEW CHALLENGING APPROACH ON THE TREATMENT OF GLAUCOMATOUS NEUROPATHY

L. Scorolli[1], S.Z. Scalinci[1], G. Corradetti[1], E. Meduri[2]
[1]Department of Ophthalmology, Sant'Orsola Malpighi Hospital - University of Bologna, Bologna - Italy; [2]Faculté de Médecine - Université Catholique de Louvain, Brussels - Belgium

Background: Intravitreal injection of human mesenchimal stem cells (hMSCs) has been shown to be effective in slowing down the progression of glaucomatous degeneration in animal models . Here we studied changes in the concentration of growth factors released from human progenitors cells (hMSCs) in the vitreous cavity as well as changes in concentration of growth factors in the host retinal neurons following the intravitreal injection in a model of genetically-determined glaucoma in mice. The human mesenchimal stem cells (hMSCs), derivated from human healthy placenta at term, represent a large reserve of anti-apoptotic and anti-neoangiogenic growth factors. Moreover, the placenta is a notably source of hMSCs both due to the abundance of cells which can be recovered and to the absence of ethical problems since this is a waste product. The aim of our research was to evaluate the effectiveness of transplanting hMSCs and the functional capacity of the transplant was assessed by means of the neuroprotective, anti-apoptotic and anti-angiogenic effects of the growth factors liberated from hMSCs.
Methods: Thirteen male DBA/2J strain mice were included in this randomized experimental study, conducted by the Glaucoma Study Center of the University of Bologna (Italy). All mice enrolled were treated with a single intravitreal micro-injection of hMSCs at concentration of 50,000 cells/ µL, using a micro-injector. All mice were monitored for 5 hours postoperatively and they all received a topical treatment with antibiotic eye-drops 3 times a day for a period of 7 days postoperatively.
Results: The determination of neurotrophic growth factors having an anti-apoptotic, anti-neoangiogenic and neuroprotective role, released by hMSCs, which had been implanted in the vitreous camera, was carried out using Real Time polymerase Chain Reaction (PCR). Real time PCR showed notable levels of the expression of neurotrophic factors at the vitreous level in all mice postoperatively. Qualitative and quantitative analyses of these factors showed that the greatest peaks of concentration at the endovitreal level were reached by BDNF, CNTF and NGF. The histological evaluation was performed in order to confirm the real migration of hMSCs from vitreous to retina.
Conclusions: The increased both intravitreal and retinal concentration of neuroprotective growth factors in all mice treated with hMSCs confirm its neuroprotective activity in the glaucomatous neuropathy. This innovative approach could have significant implications in the glaucoma treatment, in association with the traditional hypotonic topical therapy, although further large long-term clinical studies are required.

P128

A NEW ACUTE ATTACK GLAUCOMA ANIMAL MODEL WITH INTRACAMERAL INJECTION OF AN OPHTHALMIC VISCOSURGICAL DEVICE

Y.S. Lau[1]
[1]Eye Institute, The University of Hong Kong, Hong Kong - Hong Kong

Background: Acute angle closure glaucoma (AACG) is an ocular emergency and sight -threatening disease in which the intraocular ocular pressure (IOP) rises suddenly due to blockage of aqueous humor outflow. It can cause permanent loss in visual acuity and visual field. In animal study, the well-established model to study AACG is by fluid infusion and by adjusting the bottle level, a high IOP can be induced in a few seconds. However, there is no blockage of aqueous outflow and the pressure rise is unrealistically fast. To mimic human AACG, we suggest to use an ophthalmic viscosurgical device, which is injected intracamerally to block the aqueous outflow. The IOP is allowed to increase naturally inside the eyeball.
Methods: 5 male gerbils of age 4 months were included in this study. Healon 5 (AMO, Santa Ana, CA, USA) was injected into the anterior chamber (AC) of the right eye of the animal via a 30 g needle. When the AC was fully filled with Healon 5, the wound was dried and sealed with cyanoacrylate instant adhesive. IOP was allowed to build up and maintained at ≥ 40 mmHg for ≤ 2 h. The pressure was released manually by a corneal puncture followed by adhesive removal. Photopic ERG was performed preoperatively and at day 3, 7 and 28 postoperatively. The animal was sacrificed on day 28 and the retinas from both eyes were collected for flat mounting and sectioning. Retinal thickness and viable cell count in the ganglion cell layer were compared between the operated eye and the contralateral eye.
Results: After injection, the mean IOP change from baseline was +60.4 ± 6.69 mmHg. The mean maximum IOP registered was 75.2 ± 6.22 mmHg and the mean time to achieve peak IOP was 141 ± 37.65 min. The mean duration of attack (i.e. IOP ≥ 40 mmHg) was 123 ± 6.71 min. The mean rate of increase in IOP before the attack was 0.57 ± 0.19 mmHg/min. When compared to preop, the change in mean a-wave, b-wave, photopic negative response (PhNR) and oscillatory potentials (OPs) amplitude at D28 were -42%, -42%, -25% and -29% ($p = 0.006$) respectively, in the attacked eye. There were 21.78% and 15.31% ($p = 0.041$) less neurons in the superior and inferior retina of the attacked eye than the contralateral eye. The nerve fibre layer (NFL) thickness in the attacked eye was 17.95% and 3.71% thinner than the contralateral eye at the superior and inferior optic nerve head respectively.
Conclusion: Intracameral injection of Healon 5 can successfully induce a transient acute IOP rise in gerbil. By blocking the aqueous outflow, it resembles the condition observed in human AACG. Glaucomatous changes such as reduction in visual function, neuron count and NFL thickness are observed. Our animal model proves to be a new model for human AACG.

P129

ELEVATED PRESSURE INDUCES DRP-1 MEDIATED MITOCHONDRIAL FISSION AND INCREASES REACTIVE OXYGEN SPECIES IN RETINAL GANGLION CELLS

W. Ju[1], K. Kim[2], G.A. Perkins[2], R.N. Weinreb[1]
[1]Department of Ophthalmology, [2]Department of Neuroscience, University of California San Diego, La Jolla - USA

Purpose: To determine whether elevated pressure triggers Drp1-mediated mitochondrial fission and alters mitochondrial bioenergetics in cultured primary retinal ganglion cells (RGCs) and glaucomatous DBA/2J mice that spontaneously develop elevated intraocular pressure (IOP).
Methods: IOP in the eye of glaucomatous DBA/2J mice was measured and *in vitro* primary RGCs were exposed to elevated hydrostatic pressure. The expression and cellular distribution of Drp1 protein was assessed by immunohistochemistry, immunocytochemisrty or Western blot. Mitochondrial structural changes were assessed by conventional electron microscopy (EM) and the 3D technique of electron tomography. Oxygen consumption and cytochrome c oxidase (COX) activity were measured using a Clark electrode. Cell viability was measured by MTT assay and reactive oxygen species (ROS) was measured using 2´,7´-dichlorodihydrofluorescein diacetate (DCFH-DA, 10 µM).
Results: Drp1 protein expression was significantly increased by 1.29 ± 0.04-fold (n = 4 retinas per pool, $p < 0.05$) in the retinas of 9 month-old glaucomatous DBA/2J mice. Immunohistochemistry analysis showed that Drp1 immunoreactivity was increased in the ganglion cell layer of 9 month-old glaucomatous DBA/2J mice, compared with 3 month-old DBA/2J mice. Using EM analysis, 3 month-old DBA/2J mice showed the classical elongated tubular mitochondria of various lengths in RGC soma and axons of the nerve fiber layer (NFL). In contrast, 9 month-old glaucomatous DBA/2J mice showed the small rounded mitochondria with matrix swelling in RGC soma and axons of the NFL. Elevated hydrostatic pressure induced mitochondrial fission in primary cultured RGCs. Mitochondrial lengths were significantly decreased but the number of mitochondria, normalized to the total area occupied by somas in each image, was significantly increased in pressurized RGCs, compared with non-pressurized RGCs. There was no difference in mitochondrial volume density. Both respiratory capacity and oxidative phosphorylation were compromised in the retinas of 9 month-old glaucomatous DBA/2J mice, compared to the retinas of 3 month-old DBA/2J mice. Elevated hydrostatic pressure significantly increased ROS production in primary RGCs by 1.8 ± 0.2-fold ($p < 0.05$), compared with non-pressurized RGCs.
Conclusions: These results demonstrate that elevated pressure triggered Drp1-mediated mitochondrial fission and bioenergetics alterations in RGCs during glaucomatous neurodegeneration. Based on these observations, we propose that increased Drp1 protein expression may contribute to a distinct mitochondrial dysfunction-mediated RGC death in glaucomatous retina. These results suggest that inhibition of Drp1 expression may provide new strategies to protect RGCs against glaucomatous neurodegeneration.