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**PURPOSE:** Visual impairment and blindness present significant economic, social and personal burdens for millions of patients and caregivers around the world. Whole eye transplantation (WET) is a potential solution. Our lab has established a viable rodent model with promising results in syngeneic transplants. To investigate allotransplantation, successful immunosuppression is necessary. Tacrolimus monotherapy is successful in rodent VCA and has possible neuroprotective effects in the central nervous system and injured optic nerve, but its efficacy in WET is unknown. Here, we present survival of allograft WET treated with Tacrolimus monotherapy.

**METHODS:** Brown-Norway to Lewis rat transplants were performed (n=6), followed by daily intraperitoneal 1mg/kg Tacrolimus injection. Animals were examined at weeks 1, 3, 5, and 6, and compared to syngeneic transplants. Structure and blood flow of the eye and retina were studied using Optical Coherence Tomography (OCT). A retina specialist ophthalmologist performed anterior segment examination, fundoscopy, indirect ophthalmoscopy, and tonometry for intraocular pressures. Animals were sacrificed at 6 weeks. Specimens of the transplanted globe, external ear, eyelid, bone and vessel anastomoses were stained with H&E and interpreted by an ocular pathologist.

**RESULTS:** Compared to syngeneic transplants, allografts demonstrated comparable corneal thickening, retinal thinning, and blood flow in the central retinal artery and vein (OCT). Intraocular pressures were normal and comparable to syngeneic transplants. On clinical examination, both groups had mild corneal anomalies, but allografts had more frequent fundus and optic nerve ischemia (moderate). Histologically, both groups had global ocular chronic inflammation, some degree of retinal degeneration, but, in contrast to allografts, syngeneic transplants actually showed consistent degeneration of the optic nerve.

**CONCLUSION:** This is the first study of orthotopic allograft eye transplantation and immunosuppression. Compared to syngeneic transplants, allografts had increased ischemia, but less optic nerve degeneration, without signs of rejection. Overall preservation of ocular structures is an exciting first step. With this, we can begin to explore innumerable new questions in eye transplantation.

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**Study of Retinochoroidal Circulation with Fluorescein Angiography after Whole Eye Transplantation in Rodents**

**Presenter: Chiaki Komatsu, MD**

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**PURPOSE:** Approximately 39 million people worldwide are blind. Whole eye transplantation (WET) could potentially provide a viable optical system to people worldwide with irreversible vision loss. As a first step toward realizing this goal, we have developed an orthotopic model for whole eye transplantation in the rat. Given that viability of the retina is crucial to functional visual return, we evaluated the structural integrity of the retinochoroidal circulation after transplantation using fluorescein angiography (FA), which is the gold standard to evaluate retinal circulation.

**METHODS:** Brown Norway rats underwent syngeneic whole eye transplantation (n=4). At post-operative week 1, transplanted animals had ocular exams under anesthesia and wide-field FA and fundus photographs of both eyes were obtained to evaluate retinochoroidal blood flow. Ocular examinations were performed by an ophthalmologist with retina specialization to evaluate the anterior and posterior segments of the eye. We used a stereo microscope that has fluorescence imaging capability to capture fundus and fluorescein angiography images. The objective lens of the microscope is used in conjunction with a 78D Volk lens, which allows for wide-field imaging. The right eyes of 3 unoperated naïve Brown Norway rats (n=3) served as controls.

**RESULTS:** FA revealed that retinochoroidal circulation was restored in all transplanted eyes exhibiting normal choroidal background, arterial and venous filling, and no leakage from the vascular tree. These results were comparable to normal naïve eyes. In two of the transplants, retinal arteries...
were narrowed in fundus examination, fundus images and fluorescein angiography, while in the other two transplants retinal vasculature seemed similar to the control eyes. There appeared to be decreased retinal perfusion in the animals with narrowed retinal arteries as compared to controls.

**CONCLUSION:** FA results have confirmed that retinochoroidal circulation can be established after WET in a rat model. Although 2 out of 4 rats exhibited some vascular attenuation in comparison to naïve rats, all rats exhibited a normal vascular filling pattern and the absence of vessel leakage which indicates that the structural integrity of blood-retinal barriers can be maintained after WET. The etiology of vascular attenuation and presumed decrease in retinal perfusion will be investigated in future studies.

**Skin Wound Healing: The Effect of Cannabinoid CB1 Receptor Antagonism**

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**BACKGROUND:** Fibroblasts are key players for maintaining skin homeostasis and for orchestrating physiological repair. They are present in all the phases of wound healing and their phenotype and function is changed under influence of various cytokines, of which transforming growth factor beta (TGF-β) stands out. TGF-β induces fibroblasts transdifferentiation into myofibroblasts and matrix preservation and deposition.1 CB1 receptor activation has been linked with fibrosis and formation of scar tissue in various tissues as in liver and skin.2,3 In fatty acid amide hydrolase knock-out mice it has been shown that elevated levels of endocannabinoids may induce skin fibroses in a CB1 dependent manner.4 However, it is not known if cannabinoids have an effect in fibrinogenesis due to fibroblast activation or inflammation regulation. The aim of our study is to investigate whether CB1 ligands play a role in activation and differentiation of human fibroblasts.

**MATERIAL AND METHODS:** After informed consent, human skin samples were obtained from patients submitted abdominoplasty surgery. In total, samples from nine healthy patients with medium age of 37-years-old (23–51 years). Primary cultures of adult human fibroblasts were obtained from skin samples. Vimentin expression was used to confirm the presence of fibroblasts *in-vitro*. Human fibroblasts were stimulated with TGF-β (10 ng/ml) to induce fibroblasts differentiation and then treated with CB1 ligands (AM251 10 μM; ACEA 1 μM). *Trypan blue* exclusion test for cell viability evaluation was performed. Fibroblasts activation into myofibroblasts was quantified by the expression of alpha smooth muscle actin (α-SMA) using Immunocytochemistry and Western Blotting assays. The significance of differences between means was assessed by Student’s unpaired t-test. Values of P < 0.05 are considered significant.

**RESULTS:** TGF-β induces fibroblast activation, measured by a 17-fold increase in the relative protein expression of α-SMA (mean ± SEM.: 17.00 ± 6.1 in optical density). The CB1 agonist ACEA 1 μM alone did not change (mean ± SEM.: 1.1 ± 0.2) fibroblasts activation neither affected differentiation induced by TGF-β (mean ± SEM.: 21.16 ± 7.4). However, TGF-β effect was reverted in the presence of both CB1 agonist ACEA 1 μM and CB1 antagonist AM251 10 μM (mean ± SEM.: 2.1 ± 0.7). Moreover, the CB1 antagonist alone also reverts TGF-β effect (mean ± SEM: 2.2 ± 0.1). Cell viability was not modified after treatment with CB1 agonists and antagonists or stimulation with TGF-β.

**CONCLUSION:** Inactivation of cannabinoid receptor CB1 reverses fibroblasts differentiation induced by TGF-β in human fibroblasts.

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