

Basic Science/Methodology

4053

A TL1 Team Approach to CNS-Localized Delivery of Glial Cell-Derived Neurotrophic Factor for Treatment of Parkinson's Disease*

Shaheen Farhadi¹, Adithya Gopinath¹, Wolfgang Streit², Gregory A Hudalla², and Habibeh Khoshbouei²

¹University Of Florida Clinical and Translational Science Institute;

²University of Florida

OBJECTIVES/GOALS: Develop a strategy to restrict GDNF diffusion at an injected CNS tissue site for dopamine neuron rescue by endowing it with binding affinity for carbohydrates that are abundant on the cell surface and extracellular matrix. **METHODS/STUDY POPULATION:** GDNF will be fused to galectin-3 (G3), a human protein that binds to β -galactoside residues of cell surface and matrix glycoproteins. We characterized the binding of G3 fusion proteins to various glycoproteins and primary human myeloid cells. We incubated G3 fusions with CNS tissue *ex vivo* to measure their binding and depth of penetration via diffusion. We next plan to administer GDNF-G3 via CNS intracranial infusion in a murine PD model and then conduct behavioral PD phenotype testing via rotarod and pole descent to compare to non-parkinsonian controls. We will further examine the effects of GDNF-G3 on degeneration using immunohistochemical examination of post-mortem brain tissue. **RESULTS/ANTICIPATED RESULTS:** Based on results from previous clinical trials of GDNF delivery, we anticipate that a successful intervention using GDNF-G3 will result in rescue of midbrain dopaminergic neurons in a murine PD model. In murine CNS tissue, we observed binding to glycans at the tissue surfaces when incubated with G3 fusion proteins *ex vivo*, suggesting GDNF-G3 will remain localized to the injection site. Next we will administer GDNF-G3 via CNS intracranial infusion in a murine PD model and assess efficacy by behavior and histopathology. GDNF-G3-mediated dopamine neuron rescue are expected to slow or reverse the progression of PD in these animal models. **DISCUSSION/SIGNIFICANCE OF IMPACT:** PD treatments focus on symptomatic relief. Standard therapies have not been efficacious in rescuing of dopaminergic neurons. GDNF-G3 administered at the site of neurodegeneration would represent a milestone on the path to treating PD pathology and address limitations of GDNF delivery.

4163

Aging and Smoking Exacerbates Post-Stroke Complement Driven Neuroinflammation

Christine Couch¹

¹Medical University of South Carolina

OBJECTIVES/GOALS: Following stroke, complement-dependent neuroinflammation exacerbates secondary injury and worsens acute and chronic outcomes. We have shown that an injury site-targeted

complement inhibitor (B4Crry), that targets specifically to the ischemic brain, inhibits complement activation leading to improved outcomes. Stroke comorbidities have been shown to promote a pro-inflammatory environment in the brain and systemically, and to exacerbate inflammatory responses after injury. We investigated the impact of age and smoking on acute outcomes after stroke and assessed whether increased complement activation contributes to the worsening outcomes with these stroke comorbidities. **METHODS/STUDY POPULATION:** Mouse brain endothelial cells (bEnd3) were exposed to hypoxia followed by exposure to serum that was derived from either cigarette smoke (CS)-exposed mice or naïve mice, and IgM and C3d deposition assessed. Adult (12 weeks) and aged (1 year) mice were subjected to 1h transient middle cerebral artery occlusion. Animals were exposed to CS for 3-6 months (5hr/day, 5days/week) by burning 3R4F cigarettes using a smoking machine. Animals were treated with B4Crry or vehicle intravenously 2h post-MCAO. Survival analysis and neurological deficit scores were performed up to 7 days. Brains were extracted for histological and molecular analyses. **RESULTS/ANTICIPATED RESULTS:** Following hypoxia, bEnd3 cells exposed to serum from CS-exposed mice had higher C3d and IgM deposition compared to naïve serum. Older and CS-exposed mice had significantly worse neurological deficits and mortality compared to younger adults post-MCAO. B4Crry reduced mortality and motor deficits in young, old and old+CS mice with a higher effect size in comorbid animals. Age and/or CS exposure resulted in larger infarct volumes, and increased levels of C3d deposition and microglial activation compared to young adults, but aged/CS animals treated with B4Crry fared comparable to young adults. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The pro-inflammatory effects of aging and smoking contribute to worse stroke outcomes, and these effects can be successfully mitigated by injury site-targeted complement inhibition.

4512

Allopregnanolone Dose Finding for Status Epilepticus Treatment by Pharmacokinetic-Pharmacodynamic Modeling using Quantitative EEG in Dogs

Edward "Ned" Patterson¹, Irene Vuu², Dorota Zolkowska³, Chun-Yi Wu³, Ilo Leppik⁴, Greg Worrell⁵, Vaclav Kremen⁵, and James Cloyd⁴

¹University of Minnesota CTSI; ²University of Minnesota College of Veterinary Medicine; ³University of California Davis Medical School; ⁴University of Minnesota College of Pharmacy; ⁵Mayo Clinic

OBJECTIVES/GOALS: Allopregnanolone (ALLO), a modulator of GABA_A receptors, may be useful as a treatment for human and canine benzodiazepine-refractory status epilepticus (SE). Our objective was to develop a pharmacokinetic-pharmacodynamic (PKPD) model relating ALLO plasma concentrations to electroencephalographic (EEG) effects in dogs. **METHODS/STUDY POPULATION:** Four healthy dogs and one dog with epilepsy that had implanted intracranial electrodes were utilized. ALLO doses ranging from 1-6 mg/kg were administered IV over 5 min. EEG data were collected during four IM doses (1-2 mg/kg). Blood samples were collected up to 6 hr following dosing. ALLO concentrations were measured using

*Blue Ribbon Awardee; †Gold Ribbon Awardee

© The Association for Clinical and Translational Science 2020. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

HPLC-MS/MS. Power density was determined in EEG bands using a custom algorithm. A two-compartment link PKPD model was developed to describe the relation between ALLO plasma concentration and change in EEG power in the alpha, beta, delta and theta bands. RESULTS/ANTICIPATED RESULTS: ALLO caused a rapid increase in absolute power density in all EEG bands measured (1-4, >4 - 8, >8 - 12, >12 - 25, and >25 - 100 Hz). The onset of effect was rapid (1-3 min) and demonstrated by frequency band and dose analysis. Concentration-EEG data were best fit by a two-compartment PK model and sigmoidal Emax PD indirect link model. The beta frequency band was most sensitive, showing increases in power at the lowest ALLO concentrations. The EC50 concentration for the beta frequency was ~270 ng/mL. The EC50 values for effects on the other frequency bands were ~500-700 ng /mL. In conclusion, IV ALLO causes a rapid effect on EEG that can be used to determine minimal plasma concentrations associated with target engagement. DISCUSSION/SIGNIFICANCE OF IMPACT: Dose selection for future clinical trials will use the effective concentrations determined here in conjunction with studies in animal status epilepticus models. Studies are planned in client owned dogs with epilepsy to evaluate clinical efficacy in dogs and as nonclinical proof-of-concept evidence supporting translational studies in people. CONFLICT OF INTEREST DESCRIPTION: Michael Rogawski and Dorota Zolkowska are named as inventors on patent applications claiming use of neuroactive steroids including allopregnanolone and ganaxolone in the treatment of status epilepticus.

4395

alpha-Synuclein Induced Reactive Gliosis[†]

Sean David Carey¹, Sarah Alshawi², Mondona McCann³, and Kathleen Maguire-Zeiss⁴

¹Georgetown - Howard Universities; ²Georgetown University, Department of Biology; ³GUMC, Interdisciplinary Program in Neuroscience; ⁴GUMC, Department of Neuroscience

OBJECTIVES/GOALS: Reactive gliosis is a hallmark of neurodegenerative disease and is characterized by the release of pro-inflammatory cytokines and physiologic changes to glial cells. Our work identifies a novel inflammatory glial-glia cell interaction and role for mGluR5 that has the potential to provide novel insight into the mechanisms of neurodegeneration. METHODS/STUDY POPULATION: **Cell Culture:** Mouse primary astrocytes and microglia were isolated from P0-P3 C57BL/6 or Cx3cr1^{GFP/+} mice.¹ **Treatment:** Glia were treated with oligomeric α -synuclein 1 μ g/mL or mGluR5 agonist CHPG 100 μ M.^{2,3} **ELISA:** Glia culture media was collected and analyzed according to the manufacturer. **qRT-PCR:** TaqMan[™] probes were used according to manufacturer on extracted glia mRNA. **ICC:** Microglia were labeled with 1:750 Rb x Iba1 (Wako) and 1:500 Alexa Fluor 488 Gt x Rb. **Phagocytosis Assay:** Primary glia were treated with α -synuclein or astrocyte-conditioned culture media for 24-48hrs. For treatment of microglia with conditioned media, astrocytes were washed with PBS and fresh media was added to prevent carry over of α -synuclein to microglia. The number of fluorescent microbeads per microglia was quantified. RESULTS/ANTICIPATED RESULTS: Mouse primary cortical astrocytes simulated with α -synuclein aggregates adopt a reactive A1 phenotype independent of microglial stimulation. This A1 phenotype is characterized by release of pro-inflammatory cytokines including Complement Component 3 and the monocyte chemoattractant CCL2. Reactive astrocyte media induces a phagocytic phenotype in primary mouse microglia. Along with this, α -synuclein-directed microglial phagocytosis was

attenuated with the addition of the mGluR5 agonist CHPG. DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings suggest that oligomeric α -synuclein is capable of inducing a reactive phenotype in astrocytes independent of microglia and implicate crosstalk between glia as an important mediator of inflammation and microglial phagocytosis in synucleinopathies.

4427

Angiopoietin F-domain valency determines outcome of Tie2 receptor engagement and accelerates angiogenesis in tissue regeneration

Yan Ting Zhao¹, Jorge Fallas¹, Shally Saini¹, George Ueda¹, Logeshwaran Somasundaram¹, Ziben Zhou¹, Drew Seller¹, David Baker¹, and Hannele Ruohola-Baker¹

¹University of Washington

OBJECTIVES/GOALS: Lack of blood vessels remains a major obstacle in tissue regeneration. Angiopoietin 1 and 2 modulate angiogenesis through the Tie2 receptor tyrosine kinase. Ang1 activates pAKT to promote endothelial cell survival while Ang2 antagonizes these effects. We aim to dissect the Ang/Tie2 pathway to uncover the molecular basis for these opposing effects. METHODS/STUDY POPULATION: Ang1 and Ang2 bind Tie2 via nearly identical F-domains (Fd). To investigate the molecular basis regulating the Tie2 pathway, we generated a series of computationally designed self-assembling protein scaffolds presenting F-domains in a wide range of valencies and geometries using Rosette Molecular Modeling Suite. We examined the protein kinase activation, cell migration, and blood vessel formation produced by the designed proteins in human umbilical vein endothelial cells. RESULTS/ANTICIPATED RESULTS: Two phenotypic classes were demonstrated by the number of presented F domains: scaffolds presenting 3 or 4 Fd have Ang2 like activity, upregulating pFAK and pERK but not pAKT and failing to induce cell migration and tube formation; scaffolds presenting more than 6 Fd have Ang1 like activity, upregulating the three signaling branches and enhancing cell migration and tube formation. Scaffolds with 8 or more Fd show superagonist activity, producing significantly stronger phenotypes than Ang1. These results suggest that Fd valency largely determines Ang1 vs Ang2 signaling outcomes, and our designed superagonists can outperform Ang1 in vascularization and wound healing. In *in vivo* experiments, nanoparticles displaying 60 copies of Fd produce significant revascularization in hemorrhagic brains. DISCUSSION/SIGNIFICANCE OF IMPACT: Targeting the Tie2 pathway is a new paradigm in regenerative medicine. Our designed constructs will enable us to generate high-affinity Tie2 agonists and antagonists as drugs to control angiogenesis, enabling tissue regeneration that recapitulates the biological architecture of the native tissue physiology, improving organ transplant outcome.

4425

Antibiotic-Resistant Organism Acquisition in Nursing Facility Patients

Joyce Wang¹, Marco Cassone², Kristen Gibson², Bonnie Lansing², Lona Mody², Evan Snitkin², and Krishna Rao²

¹University of Michigan School of Medicine; ²University of Michigan

OBJECTIVES/GOALS: We investigated the association between gut microbiota features in newly admitted nursing facility (NF) patients and the acquisition of vancomycin-resistant *Enterococcus* (VRE)