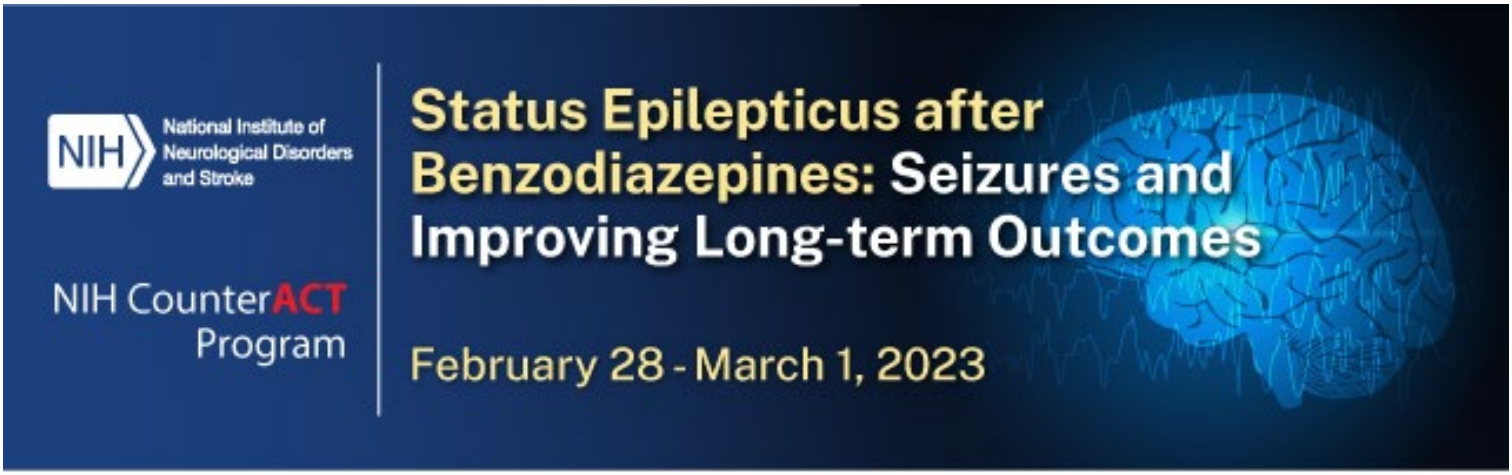

Abstracts

Poster abstracts are organized alphabetically by presenter's last name.

The virtual poster session will be held in Gather Town on February 28, 2023 at approximately 4:00 PM EST.

For program and poster session questions, please contact RefractoryEpilepsyWorkshop@nih.gov

Blood-Brain Barrier Impairment is Seizure-Dependent in a Male Rat Model of Acute Organophosphate Intoxication

Poster Number: 1

PN Bernardino[1], PM Andrew[1], AS Luo[1], JA MacMahon[1], Y Tsai[1], PJ Lein[1]

[1] *Department of Molecular Biosciences, University of California, Davis, CA*

Presenting Author: Pedro Negri Bernardino

Acute intoxication with organophosphate (OP) cholinesterase inhibitors commonly manifests as cholinergic crisis. Current standard of care targets cholinergic symptoms to prevent death and mitigate acute seizures, but does not prevent long-term neurologic consequences. Our previous work demonstrated that blood-brain barrier (BBB) leakage is observed in various brain regions as early as 6 h post-acute OP intoxication and persists up to 7 d post-exposure (DPE). While seizures and BBB impairment are causally linked in multiple neurologic diseases, it is unknown if OP disruption of BBB function is secondary to seizure activity. To address this outstanding question, we used a rat model of acute intoxication with the OP diisopropylfluorophosphate (DFP). Adult male rats were administered DFP, followed 1 min later by atropine sulfate and 2-pralidoxime, and midazolam at 40 and 50 min post-DFP. Behavior seizures were scored and animals classified as low responders (minimal seizure behavior) or normal responders (robust seizure behavior). Tissue was collected at 1-, 3-, and 7- DPE, and BBB leakage was assessed by quantification of albumin immunofluorescence. A separate cohort of animals was monitored for electrographic seizures post-DFP exposure. A subgroup of instrumented animals received a high dose of MDZ 30 min before DFP exposure to prevent occurrence of seizures – confirmed electrographically – independent of cholinergic intervention. Initial results show low-responders presented a significant lower albumin leakage in the piriform cortex and amygdala than normal responders at 7 DPE. Lower albumin leakage was observed at 3 DPE in several brain regions of a low-responder animal compared to normal responders. Rats receiving MDZ 30 min before DFP did not show seizure activity prior to tissue collection. These data suggest that OP-associated BBB impairment is primarily driven by seizure activity. Further investigation will seek to confirm these results and determine if BBB function is affected by OP exposure, independent of seizures.

Association Between Neuronal Hyperexcitability and Astrocytic Kir4.1 Potassium Channels Downregulation in Diabetic Female Mice

Poster Number: 2

Luis A. Rojas-Colón[3], Miled Maisonet-Nieves[3], Christian J. Malpica-Nieves[1], David E. Rivera Aponte[1], Serguei S. Skatchkov[1,2], Misty J. Eaton[1], Miguel P. Méndez-González[1,3,4]

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Presenting Author: Luis A. Rojas-Colón

Epilepsy is one of the most common neurological disorders in the U.S. Diabetics are at increased risk of suffering from seizures, increasing their morbidity and mortality. According to the 2022 National Diabetes Fact Sheet, more than 37.3 million adults have diabetes. Association between epilepsy and diabetes is accepted by the medical community but the pathophysiology remains to be elucidated. One of the factors that may contribute to epileptiform activity is the accumulation of extracellular potassium in active synaptic areas. Astrocytes are cells that provide support, deliver nutrients to neuronal circuits, and maintain extracellular ion balance utilizing a wide variety of channels and transporters. The inwardly rectifying potassium channel 4.1 (Kir4.1) located in astrocytes surrounding synapses largely carries out the process of potassium buffering. Kir4.1 channel mutations have been described in human epilepsy. The present study aims to determine if the downregulation of functional astrocytic Kir4.1 channels occurs in the brains of type 2 diabetic female mice and if this reduction affects hippocampal neuronal hyperexcitability. The mRNA and protein levels of the Kir4.1 channel were significantly downregulated by 40% each in the hippocampus of diabetic db/db mice when compared to their genetic db/+ heterozygous controls (non-diabetic). Furthermore, using whole-cell patch clamp recording in ex-vivo hippocampal brain slices, we determined the electrophysiological properties of CA1-pyramidal neurons. We found no significant difference in membrane potential values from CA1-pyramidal neurons of diabetic female mice compared to non-diabetics under ACSF control conditions. Moreover, we found an increase in the percent of CA1-pyramidal neurons firing and action potential firing frequency in diabetic mice when compared with non-diabetics in both ACSF and ACSF containing the pro-convulsant 4-aminopyridine. Overall, our data suggest that the downregulation of the Kir4.1 channel may affect neuronal electrophysiological properties under a hyperglycemic environment as seen in the type 2 diabetic db/db mouse model.

Preventing Long-Term Brain Damage After Soman-Induced Status Epilepticus in Rat Models Applicable to the Pediatric and Infant Populations: Significant Protection by Tezampanel Combined with Caramiphen but not by Midazolam Treatment

Poster Number: 3

T. Figueiredo[1], V. Aroniadou-Anderjaska[1,2], V. Pidoplichko[1], J. Apland[3], M. Furtado[1], K. Rossetti[1], M.Braga[1,2]

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Presenting Author: Taiza H. Figueiredo

Acute exposure to nerve agents induces a peripheral cholinergic crisis and severe status epilepticus (SE), which can cause death or long-term brain damage. The FDA-approved anticonvulsant for the treatment of nerve agent-induced SE is diazepam, and, recently, midazolam (MDZ) was also approved as a better alternative. However, clinical and animal data show that the antiseizure effects of both benzodiazepines are only transient, and neuroprotective efficacy is minimal. To provide preclinical data pertinent to the protection of preschoolers, infants and newborns, we compared the antiseizure and long-term neuroprotective effects of treating soman-induced SE with MDZ versus tezampanel (LY293558; an AMPA/GluK1-kainate receptor antagonist) in combination with caramiphen (CRM; an NMDA antagonist), in immature rats, 21-, 12-, and 7-day old; the anticonvulsants were administered 1 h after soman exposure. In all three ages, the total duration of SE within 24 h after soman exposure was significantly shorter in the LY293558+CRM groups compared with the MDZ groups. Neuronal degeneration was substantial in the MDZ-treated groups but absent or minimal in the groups treated with LY293558+CRM. Loss of neurons and interneurons in the basolateral amygdala and the CA1 hippocampal area was significant in the MDZ-treated groups but virtually absent in the LY293558+CRM groups. Atrophy of the hippocampus and the amygdala occurred only the MDZ groups. Neuronal/interneuronal loss and atrophy of the amygdala and hippocampus deteriorated over time, from 1 month to 6 months post-exposure. Increased anxiety was found only in MDZ groups. Spontaneous recurrent seizures developed in the MDZ groups, deteriorating over time, but a small percentage of rats from the LY293558+CRM groups also developed seizures. These results suggest that brain damage may be long-lasting or permanent if nerve agent-induced SE in pediatric victims is treated with midazolam at a delayed timepoint after SE onset, while treatment with tezampanel and caramiphen can provide full neuroprotection.

Biochemical and Molecular Characterization of the Most-Common SLC13A5-Epilepsy Causing Missense-Mutations

Poster Number: 4

Valeria Jaramillo-Martinez[1], Vadivel Ganapathy[2], Ina L. Urbatsch[2,3]

[1]Translational Neuroscience and Pharmacology [2]Cell Biology and Biochemistry [3]Center for Membrane Protein Research, Texas Tech University Health Sciences Center, Lubbock, TX

Presenting Author: Valeria Jaramillo-Martinez

The sodium-coupled citrate transporter (NaCT) is a plasma membrane transporter, which is energized by an inwardly directed electrochemical sodium gradient. It mediates the symport of sodium and the carboxylate citrate into cells. NaCT is expressed in the liver, testis, brain, bone, and teeth, where citrate plays key roles in the synthesis of neurotransmitters, cholesterol, and fatty acids, the generation of energy, and teeth/bone mineralization. In humans, loss-of-function mutations in SLC13A5, the NaCT gene, cause early infantile epileptic encephalopathy type-25 (EIEE25, SLC13A5-Epilepsy), which leads to epilepsy, impaired speech, limited motor skills, developmental delay, and tooth defects. Currently, there is no treatment for EIEE25. Recently, the cryo-electron microscopy structure of the human NaCT was solved in an inward-facing conformation. This was an important advancement in the NaCT field, paving the way for a better understanding of the structure-function relationships for this clinically important transporter. We classified 22 NaCT missense disease-causing mutations based on their localizations in the 3D structure. Class I mutations interfere with the transport function by blocking the elevator-type mechanism for substrate translocation. Class II mutations cause defects in protein folding and protein trafficking to the cell surface, which may be corrected by small molecule therapeutics. As there are not NaCT-specific antibodies, we expressed WT and the mutants with specific epitopes to facilitate detection, which didn't interfere with the presentation of the mutant phenotypes. The Class I mutations C50R, T142M, and T227M displayed protein and surface expression levels similar to WT. Class II mutants G219R, S427L, and L488P showed significantly decreased protein expression and no plasma membrane expression. Both classes displayed diminished transport activity. These experiments have brought us one step closer to understanding the defects of disease-causing mutations at the molecular level, allowing us to begin dissecting NaCT trafficking pathway(s).

Predictors and Prognosis of Refractory Status Epilepticus Treated in Intensive Care Unit at Mulago Hospital

Poster Number: 5

Mark Kaddumukasa[1], Martin. N Kaddumukasa[1], Jane Nakibuuka[1], Stephen Matovu[1]

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Presenting Author: Mark Kaddumukasa

Objective: To assess risk factors and prognosis in patients with refractory status epilepticus (RSE).

Methods: We retrospectively analysed all patients admitted with a diagnosis of status epilepticus (SE) on the neurological unit of the Mulago Hospital. The clinical features of those with refractory status epilepticus (RSE) were compared with non-RSE (NRSE).

Results: A total of 60 patients fulfilled our criteria of SE. Of these 30% were refractory to first line anticonvulsants. The mean age of patients with SE was 43.4 (SD 20) years. Missing antiseizure medications was significantly more often associated with NRSE ($p<0.001$) among people with epilepsy. Hypoglycemia and hypoxia within the first 24 hours after onset of status activity were associated with RSE ($p<0.05$). Among those with refractory status epilepticus a longer duration of seizure activity ($p<0.001$), aspiration pneumonia and acute respiratory distress syndrome were associated with the need for mechanical ventilation.

Conclusions: Refractory status epilepticus to first line anticonvulsant drugs in common in Uganda. Drug non-adherence is the leading factor associated with markedly poor outcome. Prevention of this most severe form of SE should be emphasized to people with epilepsy and their health care providers.

Thrombin Mediates Seizures following Cortical Injury

Poster Number: 6

Jaideep Kapur[1,2], Tamal Batabyal[1], Tanveer Singh[1], Suchitra Joshi[1]

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Presenting Author: Jaideep Kapur

The neurobiological mechanisms underlying acute seizures or SE following cortical insult are unknown. We used a cobalt-homocysteine model of SE, in which cobalt was implanted in the supplementary motor cortex, and 16hr later homocysteine was injected. The animals experienced few discrete seizures which evolved into continuous seizures lasting several hours. The animals were comatose at the end of SE and EEG showed a burst-suppression pattern; all the animals died within 24 hr. The seizures were refractory to diazepam (10, 30, 100 mg/kg) administered after the onset of continuous seizure activity. A high dose of diazepam (300 mg/kg) stopped the seizures but also caused the death of the animals. Evans blue staining and western blotting revealed the presence of thrombin and albumin in the brain parenchyma indicative of blood-brain barrier damage. Infusion of thrombin (5, 10, or 20 U) in the supplementary motor cortex triggered seizures within 25.53 ± 5.6 minutes, whereas that of albumin (0.4 mM of BSA in 5 µl of ACSF) triggered seizures after 9.165 ± 3.06 hr in 40% of animals. Pretreatment of animals with thrombin inhibitor α-NAPAP (0.75 mg/kg i.p.) improved survival (α-NAPAP: 80% vs control: 45%), shortened SE duration (α-NAPAP : 76 mins vs control: 150.5 mins, p = 0.016, Mann-Whitney test), and reduced edema. PAR-1 blocker SCH79797 (30, 100, and 1000 µg/kg, i.p.) also improved survival (SCH: 100% vs control: 45%). Thrombin-evoked neuronal Ca2+ activity was measured in cortical slices using GCaMP7 fluorescence. Thrombin (5 U/ml)-induced Ca2+ spikes were partially suppressed by NMDA receptor antagonist APV and completely eliminated by SCH79797 (1 mM). Thrombin activation and extravasation mediated seizures and cerebral edema following cortical injury. Thrombin inhibitors could be a potential therapy in patients with seizures or SE following neocortical injury.

Investigating Soluble Epoxide Hydrolase as a Therapeutic Target for Mitigating the Chronic Neurotoxicity of Acute Diisopropylfluorophosphate (DFP) Intoxication

Poster Number: 7

JA MacMahon[1], P Andrew[1], P Bernardino[1], DA Bruun[1], BD Hammock[2], PJ Lein[1]

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Presenting Author: Jeremy MacMahon

Organophosphates (OPs) are compounds found in chemical weapons and pesticides, which cause over 300,000 casualties per year. Many OPs cause toxicity via inhibition of acetylcholinesterase, leading to cholinergic crisis, status epilepticus (SE), and death. Current medical countermeasures reduce mortality; however, they are insufficient to prevent chronic neurotoxic effects, including development of spontaneous recurrent seizures (SRS). One explanation is that current standard of care does not target cellular stress responses triggered by acute OP poisoning. Here, we test the hypothesis that OP-induced neuroinflammation contributes to neurologic sequelae. We used a rat model of acute intoxication with DFP to assess whether the soluble epoxide hydrolase inhibitor, TPPU, which reduces pro-inflammatory lipids, mitigates SRS. Adult male rats were exposed to DFP (3.25-3.75 mg/kg, s.c) and scored for seizure severity using seizure behavior criteria for 4 h following DFP exposure. Animals were assigned to short-term or long-term cohorts, wherein short-term animals received daily i.p. injections of TPPU (1 mg/kg, i.p.) or an equal volume of vehicle (VEH) starting at 1 d post- exposure (DPE) for 7 d. Long-term animals received equivalent doses starting at 21 DPE when SRS had been established and were continuously monitored 24 h a day via implanted EEG telemetry until 35 DPE. TPPU treatment in short-term animals resulted in significantly decreased Iba-1+/CD68 immunoreactivity, biomarkers of activated microglia, in the CA1 and dentate gyrus in a time-dependent manner. Long-term animals treated with TPPU had significantly reduced incidence of SRS during the 14-day treatment period. All TPPU treated animals showed improved weight recovery and faster interaction with enrichment compared to VEH. These observations identify TPPU as a potential antiepileptic and neuroprotective therapeutic. Additionally, this study points towards the importance of further investigating the role cellular stress responses play in the neuropathology associated with OP exposure. Supported by the NIH CounterACT Program (NS079202).

1400W, A Selective iNOS Inhibitor Mitigates Early Neuroinflammation and Nitrooxidative Stress in DFP-Induced Short-Term Neurotoxicity in the Rat Model

Poster Number: 8

Nyzil Massey[1], Suraj S. Vasanthi[1], Manikandan Samidurai[1], Nikhil S. Rao[1], Meghan Gage[1] and Thimmasepttappa Thippeswamy[1]

[1] *Department of Biomedical Sciences, College of Veterinary Medicine, Iowa State University, Ames IA*

Presenting Author: Nyzil Massey

Chemical nerve agent (CNA) exposure at high concentrations leads to cholinergic toxidrome and status epilepticus (SE). SE increases the production of ROS/RNS, neuroinflammation, and neurodegeneration. 1400W is a novel small molecule and an inhibitor of iNOS and has been shown to effectively reduce ROS/RNS generation. In this study, we investigated the early effects of 1400W, as a disease modifying agent in the rat DFP model. Sprague Dawley rats (8 weeks) were administered with DFP (4mg/kg, s.c.) or vehicle immediately followed by atropine sulfate (2mg/kg, i.m.) and 2-PAM (25mg/kg, i.m.) to control the peripheral effects of AChE inhibition. Telemetry devices were implanted prior to DFP exposure to monitor EEG. Behavioral SE severity was scored on a modified Racine for 60 minutes. Midazolam (MDZ, 3mg/kg, i.m.) was administered 1hr post-DFP to limit mortality. Animals were then randomly allotted to experimental groups with matched SE severity, and treated with vehicle or 1400W (10 mg/kg or 15 mg/kg for 7 or 14 days. 1400W treatment (10 mg/kg and 15 mg/kg for 15 days) significantly reduced the number of microglial and astroglial cells compared to DFP + vehicle in different regions of the brain.

1400W treatment (10 mg/kg and 15 mg/kg for 15 days) showed a promising trend in reducing the number of FJB positive cells in different regions of the brain. There were no significant differences in the epileptiform spike rate and seizures per day between the any treatment groups in mixed sex cohorts and between males and females. 1400W significantly reduced nitrooxidative markers (nitrite, ROS, GSH:GSSG) and proinflammatory cytokines/chemokine in serum (IL10, TNFα, IL-6, and MCP1). The 15 mg/kg for two weeks, rather than for a week, was largely effective, however, 10 mg/kg for a shorter period had limited benefits.

Combination Therapy for Benzodiazepine-Resistant Status Epilepticus

Poster Number: 9

A. Mehra[1], S. Joshi[1], J. Kapur[1,2]

[1] University of Virginia Department of Neurology, VA [2] University of Virginia Brain Institute, VA

Presenting Author: Arnav Mehra

Objective: To test drug treatment of neocortical injury-induced status epilepticus (SE) with the goal of terminating seizures and preventing mortality. Methods: We evaluated the seizure-terminating efficacy of diazepam (10 mg/kg, intraperitoneal) in cobalt-homocysteine injury-induced neocortical SE at multiple time points. This model of severe SE ends in a coma, burst suppression, and death. We also created a dose-response curve to determine the ED50 for seizure termination when administered after 10 minutes of continuous seizures. Blood-brain barrier (BBB) breakdown and cerebral edema were measured using western blotting and the wet-dry method for water content. Finally, we investigated whether a combination of diazepam and thrombin inhibitor, α-NAPAP (0.75 mg/kg, intraperitoneal), terminated seizures and improved survival. Results: During SE, there are discrete seizures followed by continuous seizure activity. No further seizures occurred when diazepam was administered after 1 or 2 discrete seizures. However, it was ineffective when administered 4 minutes after the onset of constant seizures (n = 5 in each cohort). We further tested whether higher doses of diazepam were effective. The median effective dose, ED50, of diazepam was 163 mg/kg when injected after 10 minutes of continuous seizures. Regardless of seizure termination, all diazepam-treated mice had suppressed EEG and remained comatose until the end of the experiment. Western blot analysis showed thrombin leakage into the ipsilateral frontal cortex of mice in SE, indicating BBB breakdown. A combination of diazepam and thrombin antagonist, α-NAPAP, blocked further seizures, prevented burst suppression, and improved survival (4 out of 5). The combination of diazepam and α-NAPAP also reduced cerebral edema. Significance: We have shown that injury-induced neocortical SE is associated with BBB damage and cerebral edema and rapidly becomes benzodiazepine resistant. A combination therapy of diazepam and thrombin antagonist, α-NAPAP, can serve as a novel therapeutic approach to refractory SE.

The Effects of NADPH Oxidase Inhibitor, Mitoapocynin in a Rat Organophosphate Toxicity (DFP) Model of Epilepsy

Poster Number: 10

Christina Meyer[1], Nikhil Rao[1], Manikandan Samidurai[1], Beatriz Pereira[1], Meghan Gage[1], Megan Gard[1], Suraj S. Vasanthi[1], Marson Putra[1], Thimmasettappa Thippeswamy[1]

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Presenting Author: Christina Meyer

Rationale: Oxidative stress can be a promoter or consequence of seizures. Upon brain insult, the membrane bound NADPH oxidase produces reactive oxygen species in excess. The imbalance between oxidants and antioxidants spurs neuroinflammation and hyperexcitability. Thus, the oxidative stress pathway can be a therapeutic target for epileptic seizures. Mitoapocynin (MITO) is a NADPH oxidase inhibitor that targets the mitochondria. Notably, MITO has never been tested in an animal model of epilepsy. Furthermore, the dosing regimens and pharmacokinetics (PK) of MITO in rat models have not been established. In this study, we investigated the effects of MITO (10mg/kg) on the serum nitrooxidative stress markers and cytokines, as well as neurodegeneration and microgliosis, in a rat DFP model of epilepsy.

Methods: Adult Sprague Dawley rats were used to test efficacy of MITO in DFP experiments. To observe the systemic distribution of MITO in the animals, healthy male rates (7-8 weeks, N=8) were given a single dose of MITO (10mg/kg) orally. Blood samples were collected at 1hr and 3hr and hippocampal tissue at 3hr. The drug concentration levels in the sera and the hippocampi were detected via LC-MS. To induce status epilepticus (SE), the randomized mixed-sex cohorts of rats (9-10 weeks, N=24) were challenged with DFP (4mg/kg, s.c.) and immediately (~1 min) injected with 2-PAM (25mg/kg, i.m.) and atropine sulfate (2mg/kg, i.m.) to reduce mortality. The behavioral seizures were scored for an hour and midazolam (MDZ, 3mg/kg, i.m.) was administered to control SE. One hour post-MDZ, the animals were treated orally with either vehicle or MITO (10mg/kg, twice a day for 3 days and once a day for 4 days). All animals were euthanized with pentobarbital (100mg/kg, i.p.). To examine the peripheral effects of DFP and MITO, nitrite, ROS, and glutathione assays as well as key cytokines (IL-1β, IL-6, IL-10, TNF-α, & MCP1) were measured. The brain immunohistochemistry (IHC) was performed to measure neurodegeneration and microgliosis.

Results: MITO was detected in both serum (1hr, 1939.09pg; 3hr, 87.06pg) and hippocampal tissues (1hr, 502.62pg; 3hr, 18.01pg) suggesting the MITO's serum and brain concentrations are reasonable and it crosses the blood-brain-barrier. MITO treatment mitigated the DFP-induced oxidative stress markers (nitrite, ROS, glutathione) and the proinflammatory cytokines IL-1β, IL-6, and TNF-α levels. However, there were no differences in IL-10 and MCP1 in the serum. In DFP groups, the 10mg/kg MITO dosing regimen was not enough to mitigate DFP-induced neurodegeneration and microgliosis.

Conclusions: At the tested dose of 10mg/kg, MITO curtailed inflammation and oxidative stress in the periphery but not in the brain. Moreover, concentrations of the drug in the brain remain low. This implies that to achieve modifying effects, the MITO dosing regimen needs to be optimized to increase the drug concentration in the brain.

Acknowledgment: This study is funded by the NIH/NINDS CounterACT program (R21 NS120916-01).

GABAergic Abnormalities of Focal Neocortical Status Epilepticus are Rescued by Partial Activation of TrkB Receptors

Poster Number: 11

Perez-Ramirez[1], M. B., Parada[1], I., Yang[1], T., Buckmaster[1,2], P., Longo[1], F. M., Prince[1], D. A

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Presenting Author: Maria-Belen Perez-Ramirez

Rationale: Epilepsy progression mechanisms following focal neocortical status epilepticus (FSE) are not fully understood, and there are no available antiepileptogenic treatments. Results in SE models show functional and structural abnormalities in interneuronal populations leading to a malfunction of the inhibitory system. The maintenance of interneurons depends on trophic support regulated by the activation of TrkB receptors (TrkB-Rs) by brain-derived neurotrophic factor. We hypothesized that a single episode of FSE induces abnormalities in inhibitory neurons and inhibitory synaptic connectivity and that treatment with a partial agonist of TrkB-Rs: PTX BD4-3 (BD, Adams et al., 2020) would rescue the observed abnormalities. Methods: We induced a single episode of FSE by epidural application of gabazine and 4-AP (150μM) over the right somatosensory cortex of anesthetized mice. FSE was identified with EEG recordings and contralateral focal myoclonic activity. After ~2hr, behavioral seizures were terminated (i.p. diazepam, 5-10 mg/Kg). BD treatment (50mg/kg/day) or vehicle was given via i.p. for 7 days, starting 1 hour after diazepam injection. Ten days after FSE, confocal images of VGAT/Gephyrin and stereological counts of parvalbumin (PV)-IR neurons were obtained in cortical layer V sections. We measured the frequency of sIPSC and mIPSC on pyramidal neurons in vitro. Local field potential recordings were used to determine the incidence of epileptiform activity in vitro. Statistical significance was tested with Wilcoxon test ($p < 0.05$). Results and Conclusions: FSE induced morphological and electrophysiological alterations in the major neocortical GABAergic network. The decreases in VGAT/Gephyrin colocalization and sIPSC and mIPSC frequency suggest a reduction in inhibitory synapses and inhibitory synaptic transmission. Other evidence of interneuronal damage includes decreases in PV neuronal density. BD treatment rescued the inhibitory synapses, reduced PV neuronal density, and decreased the evoked epileptiform field potentials. These results emphasize a potential role for TrkB activation as an antiepileptogenic treatment following SE.

iNOS Inhibitor, 1400W, Suppresses Soman-Induced Long-Term Epilepsy Associated-Neuropathology: Structural and Functional Magnetic Resonance Imaging Studies

Poster Number: 12

Marson Putra[1], Manikandan Samidurai[1], Nikhil Rao[1], Meghan Gage[1], Christina Meyer[1], Suraj S. Vasanthi[1], Sridhar S. Kannurpatti[2], Thimmasettappa Thippeswamy[1]

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Presenting Author: Marson Putra

Organophosphate nerve agent (OPNA) intoxication leads to long-term brain dysfunctions. The ineffectiveness of current treatments creates a need for a more effective treatment. Previously, we discovered that 1400W, iNOS inhibitor, improves epilepsy, and seizure-induced brain pathology in a rat model of OP intoxication. In this soman (GD) OPNA model, we used structural and functional Magnetic Resonance Imaging (MRI) to determine brain abnormalities and the impact of 1400W treatment utilizing MRI outcomes. Mixed-sex Sprague Dawley rats were intoxicated with soman (GD, 132 µg/kg, s.c. 1.2 x LD50), followed by HI-6 (125 mg/kg, i.m.) and atropine sulfate (2 mg/kg, i.m.). The animals had status epilepticus (SE) for ~40 minutes before midazolam was given (3 mg/kg, i.m.). An hour later, the animals were treated with vehicle (Veh) or 1400W (20 mg/kg, i.m. daily for two weeks). 10 wk post-GD exposure, the animals were imaged in 7 Tesla scanner for Spoiled gradient recalled echo (SPGR) T1-weighted, fast imaging employing steady-state acquisition (FIESTA) T2-weighted, and T2*-weighted functional MRI using the blood oxygen level-dependent (BOLD) contrast. Signal intensities and resting-state functional connectivity (RSFC) were compared between groups. T1 decreases (atrophy) in the rostral hippocampus, and amygdala of GD+veh animals was significantly attenuated in the GD +1400W group. 1400W treatment significantly lowered GD-induced FIESTA-T2 enhancements in the hippocampus, amygdala, and piriform cortex. Network connectivity in the hippocampus, cortex, and thalamus measured by RSFC signal was significantly reduced in GD+veh group, whereas GD+1400W group demonstrated significant improvement except in the thalamus. Both T1 and T2 analyses robustly identify unique brain abnormalities associated with epilepsy. 1400W rescues soman-induced T1 and FIESTA-T2 intensity changes. 1400W protects the loss of hippocampal and cortical network. Overall, this study highlights the ability of structural and functional MRI modalities to capture the pathologies of soman-induced neurotoxicity and the neuroprotective effects of 1400W.

The Effects of Fyn/Src Kinase Inhibition (Sarcatinib) on Spontaneous Seizures and Epilepsy-Associated Behavioral Deficits in a Rat Kainate Model of Temporal Lobe Epilepsy

Poster Number: 13

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Presenting Author: Nikhil Sanjay Rao

One-third of epilepsy patients are intractable to currently available anti-seizure drugs (ASD), prompting a quest to discover novel therapies for drug-resistant epilepsy. Epilepsy is often also associated with behavioral impairments. Of the several mechanisms of hyperexcitability in experimental models of epilepsy, we investigated the role of Fyn, a Src family tyrosine kinase. In this study, we investigated the impacts of Saracatinib (SAR), a Fyn/Src kinase inhibitor, on epileptic rats and epilepsy-related behavioral changes in the KA-induced temporal lobe epilepsy rat model. We observed a significant reduction of spontaneous recurrent seizures (SRS) during the 14 days of SAR treatment and a week later in epileptic rats. There was a 40% reduction in epileptiform spikes in the SAR-treated group but the difference was not significant compared to the vehicle group. In Zero Maze, the control and SAR-treated groups spent more time in closed arm than open arm, while epileptic rats spent equal time in both arms. In Fear Conditioning Test, both control and SAR-treated groups did not show significant differences in freezing time between conditioning and probe trials, while epileptic rats froze significantly longer in probe test. Interestingly, in Novel Object Recognition test, epileptic rats showed a significant positive discrimination index, at 2h post-familiarization, in contrast to the control and SAR-treated groups. In Morris Water Maze, there was significantly increased latency to the platform in both KA-treated groups relative to the control during the learning phase. Overall, our study demonstrates that SAR treatment for two weeks after the onset of epilepsy significantly reduces SRS, but only during the treatment period and a week later, indicating the anti-epileptic potential of SAR. The two-week SAR treatment demonstrated limited effect on epilepsy-induced anxiety-like behavior and cognitive deficit. These findings suggest that SAR can be a potential disease-modifying agent but may require treatment for greater than two weeks. SAR-in-diet approach for longer duration is under investigation.

Hospital EEG Capability and Associations with Interhospital Transfer in Status Epilepticus

Poster Number: 14

C. Suen[1], A. Wood[1], J. Burke[2], J. Betjemann[3], E. Guterman[1]

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Background: EEG is widely recommended for status epilepticus (SE) management. However, EEG access across the US is poorly characterized. We aimed to evaluate changes in inpatient EEG access over time and whether availability of EEG is associated with interhospital transfers for patients hospitalized with SE. Methods: We performed a cross-sectional study using the National Inpatient Sample dataset from 2012 to 2018. We identified hospitals that used continuous or routine EEG during at least one seizure-related hospitalization in a given year using ICD procedure codes and defined these hospitals as EEG-capable. We examined annual change in the proportion of hospitals that were EEG-capable during the study period and fit multivariable logistic regression models to determine whether hospital EEG capability was associated with likelihood of interhospital transfer. Results: Among 4,550 hospitals in 2018, 1,241 (27.3%) were EEG-capable. Of these, 1,188 hospitals (95.7%) were in urban settings. From 2012 to 2018, the proportion of hospitals that were EEG-capable increased in urban settings (30.5% to 41.1%, Mann-Kendall [M-K] test $p<0.001$) and decreased in rural settings (4.0% to 3.2%, M-K $p=0.026$). Among 130,580 patients hospitalized with SE, 80,725 (61.8%) presented directly to an EEG-capable hospital. However, EEG use during hospitalization varied from 8% to 98%. Initial admission to a hospital without EEG capability was associated with 22% increased likelihood of interhospital transfer (adjusted RR 1.22, [95% CI, 1.09-1.37]; $p<0.01$). Discussion: A minority of hospitals are EEG-capable yet care for a large proportion of patients with SE. Inpatient EEG use, however, varies widely among EEG-capable hospitals and lack of inpatient EEG access is associated with interhospital transfer. Given the frequency of SE and its associated morbidity, it is important to understand whether improving EEG utilization among EEG-capable hospitals and availability of inpatient EEG among EEG-incapable hospitals can improve outcomes and decrease costs to the health system.

Propofol Infusion Syndrome: A Review of Adverse Event Reports from FDA Adverse Event Reporting System (FAERS) Database.

Poster Number: 15

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Objective: We sought to analyze the Adverse drug reports of Propofol from FDA Adverse Event Reporting System (FAERS) to determine the proportion of occurrence and Outcomes of Propofol Infusion Syndrome. Methods: We have conducted a retrospective pharmacovigilance study to identify all the drug reports of propofol from the FAERS database from 1989 to September 2022. The Preferred term(PT): Propofol Infusion Syndrome [10063181] according to MedDRA was used while searching the database. As the drug names in the FAERS database are registered arbitrarily, MICROMEDEX® (Index Nominum) was utilized as a dictionary for generic names and brand names while searching the database.

Results: From 1989 to September 2022, there were 24,953,348 adverse drug events reported in FAERS, of which 20,252(0.08%) drug reports of propofol were retrieved. Out of these 20,252 drug reports, 524 reports (2.587%) containing the Reaction term “Propofol infusion syndrome” were screened out. Excluding the missing data, the mean age of patients in reports was 32.82±0.81, 57.44% were males, and 28.05% reports were from the US. Majority of the patients were in Age group of 20-30 years (28.62%). These PRIS events were commonly reported when propofol is used for sedation (35.87%), Anesthesia (10.49%) and Status Epilepticus (10.11%). The Outcomes of PRIS events tended to be poor with 47.32% mortality. A considerable number of PRIS events have been reported to FAERS database having Propofol used along with Steroids (19.65%) and Vasopressors (17.17%) indicating a possible increased risk for developing Propofol infusion syndrome when these drugs are used together with propofol.

Conclusion: This pharmacovigilance research found that PRIS is a highly fatal condition, and it corroborates with earlier published studies, and it requires continued surveillance and further research to identify potential risk factors and newer treatment modalities.

Advantages of Diet-Incorporated Saracatinib, a Disease-Modifier, Versus Oral Gavage- Its Relevance in Chronic Epilepsy Model: Pharmacokinetics and Welfare

Poster Number: 16

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Post-seizure activation of Fyn/Src mediates neuroinflammation and neurodegeneration in experimental models of epilepsy. Saracatinib (SAR, also known as AZD0530) is a Src tyrosine kinase inhibitor and has been shown to mitigate SE-induced effects in epilepsy models upon oral gavaging (PMIDs: 34087381; 34720886). Though oral gavaging of SAR is effective, but long-term repeated dosing can be stressful as indicated by weight loss in early days and challenging in aggressive epileptic rats. Thus, a novel approach for administration of SAR is warranted in chronic epilepsy models. In this study, we incorporated SAR in rat chow for adult male Sprague-Dawley rats to achieve anticipated dose range (10-16mg/kg). Further, for comparing oral SAR gavage with SAR-in-diet, animals were divided into 3 experimental groups. In all groups, blood sampling was done at various time-points to determine serum pharmacokinetics (PK). Daily weight gain and food consumption were recorded. All animals were euthanized, hippocampal and serum samples were assessed for SAR concentrations at different time points and compared with the oral SAR. Our results showed that the rats on SAR-in-diet consumed about 3.5g/day less compared to the regular diet (14.88±0.8 vs. 18.44±0.5 grams/day). However, the average weight gain/day was not significantly different between the groups (3.167±0.3g/day vs. 2.571±0.73g/day). Importantly, we achieved the anticipated SAR dose range (10-16mg/kg). Serum SAR concentrations did not significantly vary between the animals on SAR-in-diet and orally gavaged SAR group [at 48h, 216.25 vs. 191.59 ng/mL and, at 72h, 333.43 vs 283.17 ng/mL, respectively]. SAR concentrations in the hippocampus in diet were higher than the repeated gavaging suggesting SAR-in-diet is a better approach [4th day, 238.6±143 vs 551.8±209.3ng/g and at day 7, 271.1±62.33 vs 323.4±84.61ng/g]. Overall, test drugs in diet is a translational approach and no handling-stress to animals and abates variables in experiments and are useful for disease-modifying studies such as epilepsy

KCNT1-Targeting Antisense Oligonucleotide in the Treatment of Childhood Epilepsy

Poster Number: 17

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Autosomal dominant pathogenic variants in the gene encoding the Na+-activated K+ channel KCNT1 (Slack, KNa1.1) have emerged as an important cause of epilepsy and intellectual disability (ID). These variants have been shown to increase peak potassium current magnitude and produce gain-of-function (GOF). However, the molecular mechanisms of KCNT1 channel GOF in network hyperexcitability and seizures, leading to epilepsy and ID have yet to be determined, and effective treatments are lacking. In a genetic mouse model of epilepsy expressing the Kcnt1-R455H GOF mutation, we found that both excitatory and inhibitory neurons of the cerebral cortex had increased Na+-dependent K+ (KNa) currents. Paradoxically, the intrinsic neuronal excitability was enhanced in excitatory neurons but was suppressed in inhibitory neurons. These results suggest that the Kcnt1-R455H GOF variant leads to network hyperexcitability and produces early-onset seizures by enhancing excitation in excitatory neurons and suppressing excitability in inhibitory interneurons. A novel therapeutic approach using a gene silencing antisense oligonucleotide (ASO) is being tested in the treatment of KCNT1-related epilepsy. Our preliminary data showed that KCNT1-targeting ASO suppressed the KCNT1 channel expression and reduced the Kcnt1-R455H mutation-induced increasing in KNa currents in mouse cortical neurons and patient iPSCs. Our ongoing electrophysiological, EEG and behavioral experiments aim to test if treatment with ASO attenuates the Kcnt1-R455H mutation-induced network hyperexcitability, seizures and behavioral deficits. Our study may lead to novel methods and targets of treating epilepsy and neurodevelopmental disorders in patients with KCNT1 GOF mutations.