seen in wild type mice was significantly attenuated in TRPV1 KO mice. Brain CBD concentrations were consistent with those required for TRPV1 activation and desensitisation irrespective of genotype.

CBD inhibits the equilibrative nucleoside transporter 1 (ENT1), reducing adenosine reuptake. The increase in extracellular adenosine reduces hyperexcitability and neurotransmission. CBD inhibited [^{3}H] adenosine uptake into rat cortical synaptosomes at low micromolar concentrations.

Conclusions: While the precise mechanisms by which CBD exerts its anticonvulsant properties in humans remain unknown, growing preclinical evidence suggests CBD reduces neuronal hyperexcitability through a unique multimodal mechanism of action. CBD antagonises GPR55 at excitatory synapses, desensitises TRPV1 channels and inhibits adenosine reuptake.

Funding: GW Research Ltd.

doi:10.1016/j.yebeh.2019.08.009

Epilepsy & Behavior 101 (2019) 106735

Drug-drug Interaction Studies with Coadministration of Cannabidiol (CBD) and Clobazam, Valproate, Stiripentol or Midazolam in Healthy Volunteers and Adults with Epilepsy

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Background: Drug-drug interactions (DDIs) between cannabidiol (CBD) and commonly used antiepileptic drugs (AEDs) is of clinical interest since it is anticipated that CBD will be used concomitantly with other AEDs. We present a summary of current understanding of DDIs when CBD is coadministered with clobazam (CLB), valproate (VPA), stiripentol (STP) or CYP3A4 substrate.

Methods: Effects of multiple-dose CBD on steady-state pharmacokinetics (PK) of CLB, N-desmethyl clobazam (N-CLB), VPA, 2-propyl-4-pentenoic acid (4-ene-VPA) and STP, and multiple-dose CLB, VPA and STP on steady-state PK of CBD and metabolites were evaluated in healthy volunteers. Effects of multiple-dose CBD on steady-state PK of CLB, N-CLB, VPA and 4-ene-VPA were evaluated in patients with epilepsy. The effect of CBD on CYP3A4 activity was evaluated in healthy volunteers using midazolam (MDZ) as a probe. In all studies, GW Pharmaceuticals' formulation of plant-derived highly purified CBD in oral solution (100 mg/mL) was uptitrated over 10 days to 750 mg twice daily in healthy volunteers (20 mg/kg/day for a 75 kg subject) or 20 mg/kg/day in patients.

Results: Concomitant CBD had no relevant effect on CLB exposure but increased exposure to its active metabolite, N-CLB, in healthy volunteers (3.4 fold) and patients (2.6 fold). Conversely, concomitant CLB increased CBD (by 30%) and its active metabolite, 7-OH-CBD (by 47%). Concomitant CBD had no effect on VPA or 4-ene-VPA, and slightly increased exposure to STP (by 55%). Concomitant VPA or STP did not alter CBD or its metabolites. CBD had no effect on MDZ clearance. CBD demonstrated a safety profile consistent with previous randomised placebo-controlled trials.

Conclusions: Combination of CBD with CLB resulted in a bi-directional DDI that increased levels of active metabolites of both compounds. There was no evidence of a DDI between CBD and VPA, or any effect of CBD on CYP3A4 activity (MDZ). The slight increase of exposure to STP when coadministered with CBD is not expected to result in a clinically important DDI.

Funding: GW Research Ltd.

doi:10.1016/j.yebeh.2019.08.010

Epilepsy & Behavior 101 (2019) 106736

Acute reduction of the Extracellular Trans-Synaptic Protein LGI1 increases network excitability

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Autoantibodies against LGI1 have been detected in the serum of adult patients with limbic encephalitis, seizures and status epilepticus. It is not clear if the seizures are generated by inflammation due to the antibodies or through a direct effect of the antibodies on LGI1. LGI1 (Leucine Rich Glioma Inactivated 1) is a secreted trans-synaptic protein which interacts presynaptically with Kv1.1 potassium channels and ADAM23, a membrane-anchored protein with no catalytic effect. Postsynaptically, LGI1 influences AMPA and NMDA receptors through a direct link with the ADAM22 adhesion protein. Mutations in the gene encoding LGI1 lead to temporal lobe epilepsy in humans and animal models.

We, therefore, asked if an acute reduction in LGI1 was sufficient to increase network excitability and promote seizure activity.

For this purpose, we chose and validated a silencing RNA (shRNA) against LGI1. In neuronal cultures and in *ex vivo* granule cells, shRNA against LGI1 increased neuronal firing. Local field potential (LFP) of *ex vivo* slices after injection of shRNA-LGI1 in the hippocampus, revealed an increase in the facilitation of mossy fibers to CA3 pyramidal cell neurotransmission. Application of Kv1 family blocker, alpha-dendrotoxin, occluded the increased facilitation in shRNA-LGI1 injected mice.

These results indicate that an acute reduction in LGI1 is sufficient to increase neuronal network excitability. Specifically, acutely decreasing LGI1 protein affects synaptic excitability and short-term plasticity in DG-CA3 hippocampal circuitry.

doi:10.1016/j.yebeh.2019.08.011

Epilepsy & Behavior 101 (2019) 106737

Argonaute-2 sequencing of rodent status epilepticus models identifies multiple microRNA targets for seizure suppression

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Background: MicroRNAs are short noncoding RNAs that shape the gene expression landscape, including during the pathogenesis of temporal lobe epilepsy. *In vivo* deployment of oligonucleotide inhibitors, termed antagomirs, has been successful in demonstrating functional roles for several microRNAs in epilepsy models. It is unknown, however, what portion of brain-expressed microRNAs are functionally engaged or whether additional microRNAs may be targets for seizure control.

Methods: Here we sequenced Argonaute 2-loaded microRNAs in the hippocampus from three different animal models, in two species and across multiple time-points, to identify unique and shared functional microRNA changes in experimental status epilepticus. We used this to rationally inform target microRNAs for seizure suppression and tested them using antisense oligonucleotides (antagomirs) in the mouse intra-amygdala kainate model. Finally, we used electrophysiological techniques to probe the mechanistic effects of these antagomirs in naïve rodent brain.

Results: We identified over 400 Argonaute 2-loaded microRNAs in each model and found levels of almost half changed in epilepsy. We selected microRNAs that were commonly upregulated in all three animal models and performed a systematic antagomir screen which identified anti-seizure phenotypes upon inhibition of miR-10a-5p, miR-21-5p and miR-142-5p. We assessed effects of these antagomirs on network, synaptic and biophysical properties of rodent hippocampi and identified mechanisms using a target capture sequencing assay.

Conclusions: Together, these studies provide a comprehensive cataloguing of the functional microRNA in the hippocampus and a pipeline of new targets for seizure control in experimental epilepsy. Antagomir based therapies represent a highly promising new disease-modifying therapy for epilepsy, which can suppress seizures with seemingly limited off-target effects.

doi:10.1016/j.yebeh.2019.08.012

Epilepsy & Behavior 101 (2019) 106738

Efficacy of Intranasal Allopregnanolone in a Mouse Seizure Model

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Intranasal delivery (IN) is a noninvasive, efficient and safe route for drug administration that may circumvent poor gastrointestinal bioavailability. The IN route is increasingly being investigated for drugs intended to treat neurological disorders because of the potential that drugs deposited into the nasal cavity may be transported directly to the brain along the olfactory and trigeminal nerves. Allopregnanolone (5α,3α-P), an endogenous neurosteroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors, is currently under evaluation as a treatment for status epilepticus. 5α,3α-P exerts antiseizure activity in various animal seizure models, including models of status epilepticus. 5α,3α-P protects against seizures when administered intravenously or intramuscularly, but it is not active orally. The objective of this study was to determine if 5α,3α-P has antiseizure activity when administered by the IN route.

Solutions of 5α,3α-P (15 mg/ml) were prepared in 40% sulfobutylether-β-cyclodextrin sodium salt in 0.9% saline. Seizures were induced in mice with pentylenetetrazol (PTZ; 80 g/kg IP). 5α,3α-P solution (6 & 10 mg/kg) or vehicle was administered IN 5, 10 and 15 min prior to administration of the PTZ. Animals were observed for 30 min following PTZ. The times to onset of myoclonic body twitches and clonic and tonic seizures were recorded. 5α,3α-P was considered to have antiseizure activity if it delayed the onset of seizure signs in comparison with the time of their occurrence in vehicle-treated animals.

5α,3α-P 6 mg/kg administered IN delayed the time to onset of all seizure signs with a pronounced effect on tonic hindlimb extension. At 10 mg/kg in addition to a delay in seizure signs, some animals were protected from tonic hindlimb extension and mortality. Delay in seizure signs was evidenced when 5α,3α-P was administered 5 min before PTZ but at 15 min it was less active, indicating a short acting effect.

Our results for the first time demonstrate that 5α,3α-P solubilized with a cyclodextrin excipient exhibits antiseizure activity when administered into the nose. In the model test system we used, 5α,3α-P acted rapidly to delay seizure onset and improve survival. Delivery by the IN route may allow 5α,3α-P to be conveniently and atraumatically administered by a non-medically-trained caregiver to abort ongoing seizures.

Supported by NINDS grant #1U54NS079202.

doi:10.1016/j.yebeh.2019.08.013

Epilepsy & Behavior 101 (2019) 106739

Anticonvulsant and Neuroprotective Effects of Delayed Treatment with Midazolam in a Rodent Model of Organophosphate Exposure

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Background: Exposure to organophosphates (OP) can cause status epilepticus (SE) and irreversible neural injury. Rapid control of seizure activity is important to minimize central nervous system injury and the subsequent development of neurological and behavioral disorders. Although the standard-of-care for OP-induced SE is administration of benzodiazepines, the anticonvulsant effect of these agents has been reported to decrease as the duration of SE is prolonged. However, the effect of delayed treatment with midazolam (MDZ) on electrographically recorded seizures and subsequent neuronal death resulting from OP-induced SE has not been studied quantitatively as a function of time.

Methods: Male, Sprague Dawley rats (150-200 g) were implanted with electrodes for recording of the electroencephalogram (EEG) 1 week prior to the testing. On the day of treatment, SE was induced by administration of diisopropyl fluorophosphate (DFP). At 30, 60 or 120 min after the start of SE, rats were administered MDZ (2 mg/kg). EEG was recorded for 24 hr, at which time the rats were perfused, and the brains were sectioned and labeled with Fluoro-Jade B (FJB).