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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.

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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.

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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).

Neuropathology was assessed as the number of FJB positive cells in 10 brain regions: dorsal CA1, dorsal CA3, hilus, ventral CA1, ventral CA3, amygdala, thalamus, and the parietal, entorhinal and piriform cortices.

Results: At 30, 60 and 120 min after the start of SE, MDZ treatment significantly reduced both seizure power as well as EEG spike frequency for several hours. However, at all three time points, MDZ did not completely terminate electrographic SE and had no significant effect on neuronal death. However, when data for MDZ treatment were combined from all three delay times, a small but significant reduction in global neuronal death was detected when compared to vehicle treatment.

Conclusions: These data demonstrate that treatment of OP-induced SE by MDZ can reduce seizure intensity even when delayed by as much as 120 min. However, this treatment alone was insufficient to completely stop seizures and resulted in a minimal reduction in cell death, indicating the need for better treatment options that enhance neuronal survival following OP exposure.

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Rapid intranasal delivery of diazepam utilizing prodrug/enzyme formulations: a promising drug delivery strategy for outpatient treatment of seizure emergencies

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Background: Diazepam is effective in interrupting status epilepticus and halting acute repetitive seizures. It is imperative to achieve a therapeutic concentration of diazepam as quickly as possible, because 1) seizures become increasingly difficult to control when treatment is delayed, so a short window of opportunity exists when rescue therapy with benzodiazepines, such as diazepam, is maximally effective; and 2) the risk of life-threatening complications and permanent neuronal damage increases with prolonged seizure activity. Substantial effort and resources have been dedicated to developing a safe, rapid-acting diazepam nasal spray that can be administered in emergency situations or prophylactically by patients who experience auras. However, formulating an aqueous solution of diazepam for a nasal spray device has been challenging because the drug has very low solubility. This solubility issue can be circumvented by co-administering a hydrophilic prodrug of diazepam with a converting enzyme. Besides addressing solubility, this strategy leads to an increase in the chemical activity gradient that drives drug absorption.

Methods: A pharmacokinetic study in rats was performed. Single doses of a hydrophilic diazepam prodrug, avizafone (equivalent to diazepam at 0.500, 1.00, and 1.50 mg/kg), and a converting enzyme, human *aminopeptidase B*, were administered intranasally. Resulting diazepam concentrations were measured in plasma samples and in whole brain homogenates at time points ranging from 2 to 90 minutes.

Results: Both diazepam and a transient open ring intermediate were readily absorbed through the nasal mucosa, with first order absorption rate constants 0.122 ± 0.022 min^{-1} for the intermediate and 0.0689 ± 0.0080 min^{-1} for diazepam. For the low, medium, and high dose levels respectively, bioavailablities were 77.8 ± 6.0, 112 ± 10, and 114 ± 7 %; maximum plasma concentrations were 71.5 ± 9.3, 388 ± 31, and 355 ± 187 ng/mL; and times to peak plasma concentration were 5, 8, and 5 min.

Conclusions: Our results demonstrate that practically insoluble diazepam can be delivered intranasally with rapid and complete absorption by co-administering avizafone with aminopeptidase B. Therapy based on this aqueous drug formulation approach is expected to result in swift rescue from seizure emergencies, with an excellent safety profile.

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Novel Use of the 'Photosensitivity Model of Epilepsy' to Identify the Rapidity of Anti-Epileptic Drug (AED) CNS Penetration: Implications for Future Choice in iv Treatment of Status Epilepticus (SE)

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Background: The overall 40-70% efficacy rate for status epilepticus (SE) treatment by AEDs is not optimal; *time* required to abort seizures is key. The conventional human Phase-IIa "Photosensitivity Model in Epilepsy" has been successfully utilized to identify efficacy of single *oral* doses of potential new AEDs, including Levetiracetam-(LEV) and brivaracetam-(BRV); both suppressed EEG photosensitivity response at ≥1h. In order to assess differences in time to effect of intravenous neuroactive AEDs, the Model's procedure needs to be conducted every few minutes. The conventional 'Model' involves simultaneous, intermittent (regular, hourly intervals x12hr) photic-induced EEG + blood sampling for concurrent AED concentration. EEG measurements are time-intensive, requiring 7-10min of operational activity (3-eye conditions at separate flash frequencies [2-60 Hz]) per photic-stimulation-result. 'The Model' methodology has not yet been applied to i.v. AEDs, where EEG effect is anticipated in <30min.

Methods: The 'Model' needed to become more time efficient; we adapted it:

i. by studying AED-produced change in each volunteer-patient's EEG upper limit/threshold only (Kasteleijn-Nolst Trenité DG, Reed RC. Epilepsy Curr 2013; 13 (Suppl 1).
ii. by limiting 3 eye conditions to a "best one" (via screening photosensitivity-data);