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);

iii. by eliminating some high Hz, and all lower, measurements;

With these adaptations, we devised a prospective, randomized, crossover, controlled iv 2h study using frequent measurements of the evoked Photo-paroxysmal EEG Response (PPR) as a pharmacodynamic (PD) efficacy endpoint. We conducted an *intra*-patient comparison of three PD metrics (time to effect-time to peak effect-magnitude of effect), in adult photosensitive epilepsy patients, time 0-2h, post-15-min zero-order infusion LEV 1500 mg versus equipotent BRV 100 mg, on two separate occasions, in random, crossover, double-blind fashion (n = 8 patients).

Results: We adapted 'The Model' such to be able to elicit data to compare the *rapidity* of effect of two similar AEDs given *intravenously*. The adaptation of 'The Model' has worked in the first patients being investigated (comparative AED EEG data generated).

Conclusion: Adaptation of the standard "Photosensitivity Model" should allow the determination of differences (if it exists) in time to CNS entry (effect) of i.v. infusion of two nearly identical AEDs. Data obtained in such a manner could help SE treatment algorithms.

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Possible epigenetic regulatory effect of dysregulated circular RNAs in epilepsy

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Background: Circular RNAs (circRNAs) involve in the epigenetic regulation and its major mechanism is the sequestration of the target micro RNAs (miRNAs). We hypothesized that circRNAs might be related with the pathophysiology of chronic epilepsy and evaluated the altered circRNA expressions and their possible regulatory effects on their target miRNAs and mRNAs in a mouse epilepsy model.

Methods: The circRNA expression profile in the hippocampus of the pilocarpine mice was analyzed and compared with control. The correlation between the expression of miRNA binding sites (miRNA response elements, MRE) in the dysregulated circRNAs and the expression of their target miRNAs was evaluated. As miRNAs also inhibit their target mRNAs, circRNA–miRNA-mRNA regulatory network, comprised of dysregulated RNAs that targets one another were searched. For the identified networks, bioinformatics analyses were performed.

Result: Forty-three circRNAs were dysregulated in the hippocampus (up-regulated, 26; down-regulated, 17). The change in the expression of MRE in those circRNAs negatively correlated with the change in the relevant target miRNA expression ($r = -0.461$, $P < 0.001$), supporting that circRNAs inhibit their target miRNA. 333 dysregulated circRNA–miRNA-mRNA networks were identified. Gene ontology and pathway analyses demonstrated that the up-regulated mRNAs in those networks were closely related to the major processes in epilepsy. Among them, STRING analysis identified 37 key mRNAs with abundant (≥4) interactions with other dysregulated target mRNAs. The dysregulation of the circRNAs which had multiple interactions with key mRNAs were validated by PCR.

Conclusion: Dysregulated circRNAs might have a pathophysiologic role in chronic epilepsy by regulating multiple disease relevant mRNAs via circRNA — miRNA — mRNA interactions.

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Two Russian cases of malignant migrating partial seizures of infancy due to KCNT1 mutations

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Background: Malignant migrating partial seizures of infancy (MMPSI) or Coppola-Dulac syndrome is severe form of epileptic encephalopathy developing migrating multifocal status epileptic of polymorphic seizure types. This epileptic syndrome has heterogenic etiology including autosomal dominant mutations in gene KCNT1 encodes a sodium-activated potassium channel. OMIM genetic classification for this type of MMPSI is early infantile epileptic encephalopathy, type 14 (EIEE14; 614959).

Methods: DNA sequencing - panel "Hereditary epilepsy" (Next Generation Sequencing on platform IlluminaNextSeq 500, USA) was done for two Russian girls with MMPSI. Diagnose was verified by clinical observation with dynamical video-EEG monitoring investigation ("Encephalan-Video" RM-19/26 "Medicom MTD", Russia). 1,5 Tl MRI (Siemens, Germany) revealed no dysplastic changes.

Results: In two unrelated Russian girls with MMPSI - M.V., 3 years and 3 month old and T.V., 9 month old were newly identified de novo mutations in KCNT1 gene. Girl T.V. has renowned mutation in chromosome 9: 138651532G>A with amino acid substitution Gly288Ser (OMIM: 608167.0010). The girl M.V. has previously not described mutation in 12 exome KCNT1 gene (chr9:138656907C>T, rs752514808) with amino acid substitution Arg356Trp. Mutations were confirmed by Sanger sequencing. Girl M.V. had seizure onset at the age of 4 month with seizures of behavior arrest and tonic versive. Girl T.V. developed seizures at 4,5 months in the manner of behavior arrest and ophthalmo-clonic seizures with hyperemia of face. Both the girls had further developing typical clinical and EEG characteristics of MMPSI. T.V. was resistant to valproates and hormone therapy, aggravation on levetiracetam, oxcarbazepine and barbiturate (pagluferalum-1), but with positive effect to combination of topiramate and benzodiazepine (nitrazepamum). M.V. demonstrated resistance to valproates, lamotrigine, topiramate, levetiracetam, oxcarbazepine, ethosuximide, zonisamide, benzodiazepines and hormone therapy, with weakly positive effect to barbiturate (pagluferalum-1) and rufinamide treatment was started.

Conclusions: KCNT1 is a major disease-associated gene for the MMPSI phenotype. All the children with pharmacoresistant epileptic encephalopathy need complex investigations including dynamic video-EEG monitoring, high quality neuroimaging, but also genetic investigation. Next Generation Sequencing (NGS) methodics - panel