Early postnatal mTOR inhibitor treatment in a mouse model of TSC with epilepsy delays onset of hyperexcitability, epilepsy, and mortality

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**BACKGROUND**

Tuberous Sclerosis Complex (TSC) is characterized by brain malformations and severe epilepsy in up to 90% of patients. Brain malformations exhibit mTOR dysregulation and are associated with areas of aberrant neuronal hyperexcitability. mTOR inhibitors are promising therapeutic agents, predicted to function as anti-epileptogenic drugs. However, clinical studies demonstrate that mTOR inhibitors function as anticonvulsants or antiepileptostatics, indicating seizure-generating mechanisms remain intact. To explore potential mechanisms of mTOR inhibitor-resistance we treated a mouse model of TSC that exhibits severe seizures with the mTOR inhibitor RAD001 (Everolimus) and tracked EEG activity during treatment. We have also begun pilot experiments using the octuple whole-cell patch technique in cortical slices from KO mice to study circuit behavior.

**PURPOSE**

The purpose of the study is to determine if early postnatal treatment with an mTOR inhibitor of a mouse model of TSC with forebrain deletion of Tsc2 gene rescues seizures.

**METHODS**

Mice with conditional forebrain deletion of the Tsc2 gene in post-mitotic excitatory neurons [Wild type, (WT) NEX-Cre/+/Tsc2\(^{+/+}\) Knockout (KO), NEX-Cre/+/Tsc2\(^{-/-}\) were treated with vehicle or 60mg/kg RAD001 by intraperitoneal route every 2 days starting on postnatal day 8 (PS). Video electroencephalography (vEEG) activity was recorded (Natus Nicolet) starting on P10 for 3hrs/day pre-weaning and continuously post-weaning. Offline EEG analysis of epileptiform and seizure activity was carried out using LabChart v8 (ADInstruments). Octuple patch recordings were obtained from slices using Quadro EPC 10 amplifiers, PatchMaster software (HEKA), and custom-written Matlab-based programs to determine connectivity and synchrony.

**RESULTS**

vEEG recordings revealed Tsc2 KO mice treated with the mTOR inhibitor RAD001 beginning early in postnatal development exhibit a significant delay in development of seizures from P12 to approximately P50. During inhibitor treatment there is a seizure-free interval of normal EEG activity followed by an incremental rise in epileptiform activity leading up to the appearance of seizures by P50 which is markedly delayed compared to the untreated group. In multi-whole cell patch recordings, we found excitatory neurons from Tsc2 KO mice have increased connectivity and pyramidal cell lacking Tsc2 exhibit increased spontaneous firing, which is different from WT.

**CONCLUSION**

Our results in NEX-Tsc2 mutant mice suggest that, as in TSC humans, RAD001 is suppressing seizures without a long-lasting disease modifying effect. Examination of aberrant circuit behavior may help reveal mechanisms of seizure recurrence. The delayed onset of epileptiform activity and seizures in this mouse model during treatment provides an opportunity to explore circuit deficits associated with epileptogenesis and drug resistance in TSC. The goal of these studies is to identify candidate mechanisms underlying epileptogenesis in TSC and drug resistance using these techniques to provide novel therapeutic targets.

**REFERENCES**

Crowell, et al. (2016) Complex neurological phenotype in mutant mice lacking Tsc2 in excitatory neurons of the developing forebrain. eNeuro. DOI: 10.1523/EINEURO.0046-15.2015

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