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mTOR Inhibition in Epilepsy: Rationale and Clinical Perspectives

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Abstract

Despite a large number of available medical options, many individuals with epilepsy are refractory to existing therapies that mainly target neurotransmitter or ion channel activity. A growing body of preclinical data has uncovered a molecular pathway that appears crucial in many genetic and acquired epilepsy syndromes. The mammalian target of rapamycin (mTOR) pathway regulates a number of cellular processes required in the growth, metabolism, structure and cell-cell interactions of neurons and glia. Rapamycin and similar compounds inhibit mTOR complex 1 (mTORC1) and decrease seizures, delay seizure development or prevent epileptogenesis in many animal models of mTOR hyperactivation. However, the exact mechanisms by which mTOR inhibition drives decreased seizure activity have not been completely determined. Nonetheless, these preclinical data have led to limited use in humans with epilepsy due to tuberous sclerosis complex (TSC) and polyhydramnios, megalencephaly and symptomatic epilepsy (PMSE) with promising results. Currently, larger controlled studies are underway using mTOR inhibitors in individuals with TSC and intractable epilepsy.

1. Introduction

Chronic epilepsy affects 1 to 4% of the general population [1,2]. First-line treatment for epilepsy is antiseizure medication. Despite the availability of over twenty approved antiseizure medications, nearly one-third of those affected continue to have seizures and fall into the category of having drug-resistant epilepsy [3]. The mechanisms of action of current antiseizure medications focus primarily on decreasing neuronal excitability through increasing inhibitory neurotransmitters, decreasing excitatory neurotransmitters and modulating ion channel permeability. The development of more effective treatments for drug-resistant epilepsy likely depends on targeting mechanisms of action that are significantly different than current antiseizure medications.

Epilepsy occurs through an extremely diverse set of genetic and acquired mechanisms. Although abnormalities in the electrophysiological properties of ion channels and

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neurotransmitter systems may represent a final common product, cell signaling pathways may act as an intermediate mechanism linking diverse etiologies of epilepsy to downstream changes in neuronal excitability that lead to seizures. The mammalian target of rapamycin (mTOR) pathway is dysregulated in a number of genetic and acquired epilepsy syndromes. Thus, mTOR modulation may represent an alternative approach to treating epilepsy than previous generations of antiseizure medications through a novel mechanism and multiple epileptogenic pathways. Furthermore, while no proven antiepileptogenic or disease-modifying therapy currently exists for epilepsy, mTOR inhibitors may also have antiepileptogenic properties to prevent epilepsy in high-risk patients.

2. mTOR physiology under normal conditions

mTOR is a protein kinase important in regulating cell metabolism, growth, structure, proliferation, and death through apoptosis and autophagy (Figure 1) [4,5]. Brain-specific roles also include regulation of synaptic plasticity and learning [6,7], neurogenesis, and dendritic and axonal morphology of neurons [8–10]. The protein is part of two larger signaling complexes, mTORC1 and mTORC2. mTORC1 is regulated by the upstream PI3K/Akt activation in anabolic states and the LKB1/AMPK inhibition in catabolic states [11], is sensitive to inhibition by rapamycin [12], and stimulates cell growth and proliferation through protein synthesis. In contrast, mTORC2 participates in the regulation of cell survival, metabolism and cell structure, including modulation of the actin cytoskeleton, soma size, dendritic growth and dendritic tiling, and is relatively insensitive to acute rapamycin treatment [13]. While mTOR is involved in regulating a diversity of physiological functions under normal conditions, dysregulation of these same mechanisms may contribute to the pathogenesis of a variety of diseases, including epilepsy [11].

3. mTOR hyperactivation in genetic and acquired epilepsy syndromes

Several important upstream and downstream molecules involved in the mTOR pathway have been implicated in epilepsy syndromes (Figure 1). Perhaps best known and most rigorously studied is tuberous sclerosis complex (TSC), caused by mutations in the genes *TSC1* or *TSC2* which produce the proteins harmartin and tuberin, respectively, and normally down-regulate mTOR activity [14,15]. Individuals with this autosomal dominant syndrome develop hamartomas throughout the body, including cortical malformations and subependymal giant cell astrocytomas (SEGAs) in the brain, and frequently develop medically intractable epilepsy. Other disorders with prominent seizures and mTOR dysregulation include polyhydramnios, megalencephaly and symptomatic epilepsy (PMSE) [16]; focal epilepsy secondary to disheveled, Egl-10 and pleckstrin domain containing protein 5 (DEPDC-5) [17]; neurofibromatosis type 1 [18]; Fragile X syndrome [19]; hemimegalencephaly due to multiple mTOR pathway gene mutations [20]; and mutations in phosphatase and tensin homolog (PTEN) [21]. These syndromes share many molecular, cellular and histopathological phenotypes implicated in the development of epilepsy [22]. Furthermore, multiple animal models have been described that mimic genetic and molecular features of the conditions found in humans (Table 1).

Several animal models (Table 2) and human tissue studies of acquired epilepsy or seizures have also demonstrated mTOR hyperactivation. Preclinical models of temporal lobe epilepsy have shown mTOR hyperactivation within the hippocampus [23–27]. A model of traumatic brain injury also demonstrated hyperactivation of the mTOR pathway [28]. Hypoxic ischemic seizures in juvenile rats were associated with widespread hippocampal and neocortical mTOR hyperactivation [29], and a rat model of symptomatic infantile spasms also demonstrated mTOR hyperactivation [30]. In humans with acquired focal epilepsies associated with a variety of pathological lesions, including focal cortical dysplasia, glioneuronal tumors and hippocampal sclerosis, increased immunoreactivity against pS6 demonstrated mTOR dysregulation primarily in dysmorphic and immature cells [31–34]. In contrast, previous studies have found minimal or no mTOR dysregulation in pathology-negative epilepsy cortex [33,35,36], suggesting that mTOR activation is most closely correlated with specific pathological changes in acquired epilepsy. However, mTOR activation has also been implicated in some cases of non-lesional focal epilepsy [17]. Given the correlative nature and diversity of findings, presently it is difficult to establish the mechanisms and causal role of mTOR hyperactivation in epileptogenesis in many of these cases.

Both genetic and acquired models of seizures and epilepsy in the setting of mTOR hyperactivation have been of intense interest in recent years, as this pathway represents a possible target for rational pharmacotherapy both for suppression of seizures (antiseizure effects) and the delaying or inhibiting epileptogenesis (antiepileptogenic effects) [11]. The mechanisms by which mTOR inhibition, most commonly through the use of rapamycin, results in these effects are currently incompletely understood. Further insight into this process may be gained by examining the specific models of mTOR hyperactivation, timing of pathway alteration, treatment timing and therapeutic result. A major question is whether mTOR inhibitors are primarily effective through inhibition of gross histopathological abnormalities or direct modulation of cellular electrophysiological properties.

4. Antiseizure/antiepileptogenic mechanisms of mTOR inhibition - histopathology

mTOR inhibition may suppress, or even rescue, some of the mechanisms and phenotypes that lead to the development of epilepsy. Histological abnormalities are common in animal models of genetic epilepsies due to mTOR hyperactivation, some of which are abated following treatment with rapamycin with a subsequent delay in epileptogenesis. These data may grant insight into abnormalities in mTOR-mediated conditions critical for epileptogenesis. In mouse models of TSC, several early-treatment paradigms using rapamycin delayed epileptogenesis and altered histopathologic phenotypes such as astrogliosis, hippocampal pyramidal cell disorganization and neuronal size, suggesting these abnormalities may be crucial in seizure development and may in part explain the antiepileptogenic effects of rapamycin [37–42]. Following discontinuation of rapamycin therapy, these phenotypes at least partially return and are accompanied by progressive development of severe seizures and early death. Late treatment of TSC knockout mice with rapamycin partially decreased astrogliosis, hippocampal pyramidal cell disorganization and

brain hypercellularity and resulted in fewer seizures, suggesting the seizure phenotype is related to the severity of the histopathological findings [37].

Beyond TSC and other genetic mTORopathies, histopathological abnormalities are also implicated in the pathogenesis of acquired epilepsies due to brain injury and may be mTOR-dependent. Mossy fiber sprouting remains a controversial potential mechanism for epileptogenesis in animal models of acquired epilepsy. Treatment with rapamycin has been linked to decreases in mossy fiber sprouting [43] and decreased seizures in some studies [23,24,27,44,45]. However, other studies have noted rapamycin-induced reduction of mossy fiber sprouting did not decrease the occurrence of pilocarpine-induced spontaneous seizures in mice [26,46]. In fact, treatment with rapamycin after the development of seizures did not decrease mossy fiber sprouting or seizures [25]. The difference between these results may lie in dose-response, treatment timing or method of deletion. In addition to mossy fiber sprouting, neuronal death and neurogenesis may contribute to epileptogenesis and are reversed by rapamycin under some conditions, but this is also controversial [24].

Other histologic abnormalities appear less crucial in the development of seizures in mTOR-related animal models of epilepsy due to the presence in seizure-free animals or absence in animals with seizures. Cellular dysplasia and migrational abnormalities were present in seizure free-animals following treatment with rapamycin, suggesting these findings may not be crucial target for antiepileptogenic therapy [38]. In STRADA-deficient mice, prenatal treatment with rapamycin decreased cortical migrational abnormalities [47]. However, post-natal rapamycin has been shown to decrease seizures and prevent epilepsy in a small cohort of children with PMSE, a STRADA deficiency syndrome. These data suggest the mechanism by which seizures occurring in the presence of STRADA deficiency are suppressed is unrelated to migrational abnormalities, as children with PMSE would have pre-existing neuronal migrational abnormalities prior to treatment with rapamycin. Furthermore, while mTOR signaling impacts dendritic growth and complexity [13], abnormal dendritic processes do not appear sufficient to produce epilepsy. Decreased dendrite density and complexity has been noted in seizure-free animals treated with rapamycin [8,9,38,39,48].

Recent data suggest histopathological abnormalities are not required for epileptogenesis in TSC-related animal models of mTOR hyperactivity. Abs and colleagues deleted *Tsc1* in adult mice, which resulted in epileptogenesis without acute histopathological abnormalities [49]. The authors noted decreased threshold to induce late phase long-term potentiation in the Schaffer collateral pathway and increased excitability of hippocampal CA1 neurons, possible mechanisms for the prevalence of seizures in these animals. Importantly however, this is a model with postnatal hyperactivation of mTOR, which may not fully recapitulate the human conditions. In light of this evidence, the effect of mTORC1 and rapamycin on cellular signaling, neuronal excitability and long term potentiation may be more significant than the contribution from histopathological abnormalities to epileptogenesis.

5. Antiseizure/antiepileptogenic mechanisms of mTOR inhibition – electrophysiology, cell structure and cell-cell interactions

Similar to conventional antiseizure medications, it is possible that rapamycin directly modulates the electrical activity of neurons. However, a number of studies find minimal acute effects of rapamycin on neuronal excitability. First of all, rapamycin has limited acute effects in standard anticonvulsant drug screening assays, such as the pentylenetetrazole and maximal electroshock threshold tests [50]. In addition, the effect of mTORC1 inhibition with rapamycin on resting neuronal membrane potential likely plays little role in its antiseizure effects. Neurons from mice with increased mTOR activity and seizures show typical passive neuronal membrane properties [49]. Furthermore, passive neuronal membrane properties of wild type mice are not altered by rapamycin [51,52]. Thus, the resting membrane properties likely do not factor into seizure development or treatment in the TOROpathies. However, increased mTORC activity decreases threshold to induce late phase long-term potentiation in the Schaffer collateral pathway and increased excitability of hippocampal CA1 neurons, both processes that may contribute to epileptogenesis [49]. The modulation of late phase long-term potentiation and synaptic excitability by rapamycin likely involves direct regulation of synaptic proteins [7,49,51,53]. However, unlike conventional antiseizure medications that typically bind directly to synaptic receptors and channels, rapamycin probably affects neuronal excitability indirectly by regulating the translation and expression of voltage-gated ion channels [49,54,55] or neurotransmitter transporters and receptors [29,56–58].

Besides modulation of protein expression, rapamycin is a known inducer of autophagy, a critical metabolic, housekeeping function of most cells for energy retrieval and removal of damaged organelles and proteins under catabolic conditions. Impaired autophagy has also been linked to several animal and human models with abnormalities in the mTOR pathway, including humans with TSC, *Tsc1* KO mice and PTEN KO mice, with increased seizures also occurring in an mTOR-independent pathway crucial to autophagy in Atg7 KO mice [59]. While the mechanisms by which impaired autophagy may lead to epileptogenesis are unknown, they may reflect maintenance of axons [60,61], formation of balloon cells [62], or mitochondrial homeostasis [63]. Additional data in a rat model of absence epilepsy demonstrated fewer seizures following lipopolysaccharide injection due to mTOR inhibition with rapamycin, suggesting mTOR activation may modulate an inflammatory component of seizure generation [64]. Ultimately, a confluence of histopathological and cellular mechanisms may explain the heterogeneity in models of mTOR dysregulation and seizure response to rapamycin.

6. mTOR hyperactivation and rescue timing

Independent of the histopathological or cellular mechanisms involved, the timing, etiology and extent of mTOR hyperactivation and treatment conditions may be more crucial to understanding the genetic and acquired TOROpathies and epileptogenesis. Most animal studies of hyperactivation of mTOR and epileptogenesis have shown a propensity for seizures to develop following discontinuation of mTOR inhibitor therapy, especially in genetic TOROpathies in which the genetic defect is permanent (Table 1). A few exceptions

exist in the acquired epilepsy models. In an adult mouse model of traumatic brain injury following controlled cortical impact, rapamycin treatment 1 hour following and continued for 4 weeks had no effect on acute symptomatic seizures, but significantly fewer mice treated with rapamycin developed chronic post-traumatic epilepsy [28]. A rat model of acquired mesial temporal lobe epilepsy following electrical stimulation of the angular bundle developed fewer seizures following only one week of rapamycin therapy initiated 4 hours after stimulation [27]. A third study of hypoxia-induced seizures in P10 rats showed sustained antiepileptogenic effects following doses 1 day prior to and 1 hour following the event [29]. This effect of neuroprotection and antiepileptogeneis is rare but similar to the effects of levetiracetam, an antiseizure medication that binds synaptic vesicle protein 2A, when used in rat pups prior to hypoxemic injury [65]. The studies of acquired epilepsy with no difference in seizures involved no pretreatment or a more prolonged period following seizure stimulation [25,26,66]. These data suggest the antiepileptogenic effects of mTOR inhibition outlast therapy in models with acute mTOR hyperactivation when used shortly after the injury. One other example of a sustained effect occurred in a PTEN KO mouse model of treatment after the development of seizures, in which total seizure activity was decreased following discontinuation of rapamycin therapy [67]. Finally, a rat model of absence epilepsy showed a sustained decrease in the total number and cumulative duration of spike wave discharges in a pretreatment paradigm even after discontinuation of rapamycin, although the mean sustained spike wave discharge duration was similar to those in the vehicle-treated group [64]. In all other animal models of mTOR hyperactivation, continued therapy is necessary for suppression of seizures or epileptogenesis, even if the structural phenotype has been rescued.

Additional evidence for the potential antiseizure and antiepileptogenic properties of mTOR inhibition can be found in current treatments for epilepsy. The ketogenic diet, a high fat, low carbohydrate diet effective in the treatment of epilepsy, has an unknown mechanism of action, but has been shown to result in TORC1 inhibition [68]. Vigabatrin is an antiseizure medication that has demonstrated disproportional efficacy in patients with TSC which primarily exerts its antiseizure effects through an increase in gamma-aminobutyric acid (GABA), but also involves partial inhibition of the mTOR pathway [69]. These therapies have been recognized as being especially effective in individuals with TSC, potentially related to their inhibitory effects on mTOR.

7. mTOR inhibition and clinical effects on seizures in humans

mTOR inhibitors have already proven effective and are FDA approved for treating SEGAs and kidney tumors in individuals with TSC [70–74]. In a prospective trial of everolimus, a rapamycin analog, for SEGA, nine out of 16 TSC patients also experienced a decreased in seizure frequency and 1 patient experienced an increase in seizures [71]. Another patient with TSC and drug resistant epilepsy was treated with everolimus for SEGAs and experienced complete cessation of seizures [75].

The robust animal model data and limited human experiences have led to further off-label use and current clinical trials of mTOR inhibition in genetic epilepsies. A small group of 8 pediatric patients with TSC and drug resistant epilepsy were treated with sirolimus

(rapamycin) or everolimus with minimal side effects [76]. Two patients became seizure-free at 6 months, one patient achieved greater than 90% seizure reduction, four patients achieved 50–90% seizure reduction, one patient achieved 20–50% seizure reduction and one patient had <25% seizure reduction. A prospective 12-week course of everolimus in 20 pediatric patients with TSC and drug resistant epilepsy resulted in greater or equal to 50% seizure frequency in 60% of patients [77]. A case report of a 10-year-old girl treated with 0.15 mg/kg/day rapamycin resulted in a decrease in seizure activity and associated complications of frequent seizures (frequent post-ictal hemiparesis) [78]. In a cohort of 7 patients with TSC and drug resistant epilepsy treated with everolimus, a greater than 50% reduction in seizures occurred in 2 patients and 25–100% reduction in four individuals; 1 patient discontinued treatment due to side effects [79]. Five children with PMSE were treated with sirolimus at a young age; four out of 5 experienced a dramatic decrease in seizure frequency [47]. Overall, there is accumulating evidence that mTOR inhibitors may have antiseizure effects in patients with genetic TORopathies, especially TSC, but controlled trials are still needed to provide definitive proof of the efficacy of mTOR inhibitors for epilepsy.

8. Future directions

An ongoing multicenter placebo-controlled trial of everolimus in individuals with TSC and drug-resistant focal onset seizures has been designed based on the preclinical and limited clinical data [80]. While initial data regarding possible seizure suppression effects of rapamycin is promising, perhaps a more compelling application for mTOR inhibition lies in prevention of epilepsy. Based on the mechanisms of action of rapamycin, mTOR inhibitors may have greater potential as antiepileptogenic therapy, rather than standard antiseizure treatment of drug-resistant epilepsy patients. In those patients with genetic TORopathies, this would likely involve early, chronic treatment and may be capable of delaying or suppression the development of epilepsy. In those with acquired epilepsies, such as hypoxic ischemic injury, status epilepticus-induced temporal lobe epilepsy or traumatic brain injury, treatment with mTOR inhibitors may provide sustained neuroprotective effects and inhibit the development of epilepsy even after the treatment has ceased. However, antiepileptogenic drug trials are very difficult to conduct, involving recruitment of presymptomatic at-risk patients before the onset of epilepsy and long-term follow-up through the expected onset of epilepsy. TSC represents a feasible population to plan an antiepileptogenic trial, as a subset of TSC patients are identified at a young age before the onset of seizures due to non-neurological findings and these patients are at high risk for developing epilepsy in the future [81]. Nevertheless, much clinical and preclinical work remains to further determine the optimal conditions, timing and dosing of therapy before embarking on an antiepileptogenic drug trial.

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Key Points

1. The mTOR pathway is hyperactive in some genetic and acquired epilepsies.
2. Epileptogenesis is delayed or prevented in preclinical models by inhibition of mTORC1 activity with rapamycin or an analogue, although the mechanisms have yet to be fully elucidated.
3. Early data using mTOR inhibition in patients with genetic mTOR hyperactivation and seizures has been promising and has led to current larger-scale clinical trials.

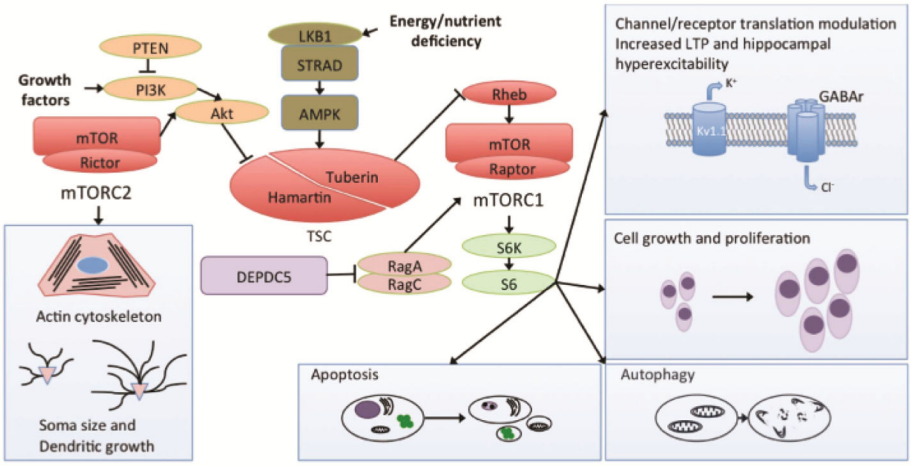

Fig. 1.

The mTOR pathway is regulated by numerous upstream pathways, typically in response to growth factors (anabolic) or energy/nutrient deficiency (cell static or catabolic). Two complexes, mTORC1 and mTORC2, then activate downstream regulators of cellular activity, including protein translation and ribosomal biogenesis. Brain-specific activity likely crucial to the development of seizures or epilepsy include regulation of cell structure, primarily through mTORC2, and mTORC1-dependent regulation of channel and receptor expression, cell growth and proliferation, autophagy and apoptosis. Rapamycin primarily inhibits the activity of mTORC1 and has little effect on mTORC2, at least under acute conditions. Abbreviations: *Akt* protein kinase B, *AMPK* AMP-activated protein kinase, *DEPDC5* DEP domain containing 5, *GABAr* gamma-aminobutyric acid receptor, *Kv1.1* potassium channel, voltage dependent 1.1, *LKB1* liver kinase B1, *mTOR* mammalian target of rapamycin, *mTORC* mTOR complex, *PI3K* phosphoinositide 3-kinase, *PTEN* phosphase and tensin homolog, *Rheb* Ras homolog enriched in brain, *S6K* S6 kinase, *TSC* tuberous sclerosis complex.

Table 1

Animal models of genetic mTOR hyperactivation and seizures and the response to treatment with an mTOR inhibitor.

Model	Deletion timing	Treatment timing	Treatment response	Ref
Tsc1 induced KO	2–4 months postnatal	Rapamycin after seizure onset	Prolonged survival; no seizures during treatment	[49]
Tsc1 induced KO	2–4 months postnatal	Rapamycin immediately after deletion	No seizures developed during treatment	[49]
Tsc1 Syn-neuronal KO	Embryonic	Rapamycin treatment started at P7–P9	Prolonged survival, weight gain, absence of seizures during treatment	[38]
Tsc1 GFAP KO	Embryonic	Rapamycin treatment started at P14	No seizures developed; prolonged survival during treatment	[37]
Tsc1 GFAP KO	Embryonic	Rapamycin treatment as juvenile (6 week)	No seizures developed; prolonged survival during treatment	[37]
Tsc1 nestin KO	Embryonic	Rapamycin treatment started at P8	No seizures developed; prolonged survival during treatment	[39]
Tsc1 Emx1 KO	Embryonic	Rapamycin treatment started at P13–15	No mortality or seizures during treatment	[41]
Tsc2 GFAP KO	Embryonic	Rapamycin treatment started at P14	Fewer seizures	[40]
Tsc2 GFAP KO	Embryonic	Rapamycin E12.5 to birth (pre)	Seizures improved	[42]
Tsc2 GFAP KO	Embryonic	Rapamycin birth to P21 (post)	No seizures developed; prolonged survival during treatment	[42]
Tsc2 GFAP KO	Embryonic	Rapamycin E12.5 to P21 (combined)	No seizures developed; prolonged survival during treatment	[42]
Pten GFAP KO	Embryonic	Temsirolimus in adult mice; post-symptoms	Decreased seizures and mortality	[82]
Pten GFAP KO	Embryonic	Rapamycin in adolescent mice; post-symptoms	Decreased seizures; persisted following discontinuation of treatment	[67]
Pten GFAP KO	Embryonic	Rapamycin in adolescent mice; post-symptom; subset received several more doses	Decreased seizures and improved survival; recurrence at 10 weeks; seizures decreasing with intermittent treatment	[83]
Pten NSE KO	Embryonic	Rapamycin in adult mice following development of seizures	Decreased seizure duration and frequency	[84]
Pten induced KO	P14	Rapamycin 2–5 days post tamoxifen injection	Decreased seizures	[44]
Wag/Rij KO	Embryonic	Rapamycin in adult rats	Rapamycin decreased seizures within 30 minutes of administration. Treatment prior to development of epilepsy decreased absence seizures	[64]
STRADA KO	Embryonic	Rapamycin E15–E19 (Dams)	Did not measure seizures; histopathological findings improved	[47]

Abbreviations: *E* embryonic day, *GFAP* glial fibrillary acidic protein, *KO* knockout, *P* postnatal day, *PTEN* phosphatase and tensin homolog on chromosome 10, *STRADα* STE20-related kinase adapter alpha, *Tsc1* tuberous sclerosis complex 1 protein, *Tsc2* tuberous sclerosis complex 2 protein, *Wag/Rij* Wistar Albino Glaxo rat from Rijswijk.

Table 2

Animal models of acquired mTOR hyperactivation and seizures and the response to treatment with an mTOR inhibitor.

Model	Treatment timing	Treatment response	Ref
Pilocarpine administration in adult rats - TLE	Pre-treatment with rapamycin 3 days prior to pilocarpine	Decreased chronic seizures during treatment, gradually returning after discontinuing treatment	[23]
Kainate administration in adult rats - TLE	Pre-treatment with rapamycin 3 days prior to kainate	Decreased chronic seizures during treatment	[24]
Kainate administration in adult rats - TLE	Rapamycin 24 hours post-kainate	Decreased chronic seizures during treatment	[24]
Amygdala stimulation in adult rats - TLE	Rapamycin 24 hours post-stimulation	No effect	[25]
Pilocarpine administration in juvenile mice - TLE	Rapamycin 24 hours post-pilocarpine	No effect	[26]
Angular bundle stimulation in adult rats - TLE	Rapamycin 4 hours after status epilepticus, continued for 7days	Decreased chronic seizures during and after treatment	[27]
Adult mice; CCI	Rapamycin 1 hour after CCI; continued for 4 weeks	No effect on acute seizures; decreased PTE	[28]
Multiple acute seizure tests in adult mice	3 hours or 3 days prior to seizure induction	Variable; timing- and model-dependent	[50]
Multiple acute seizure tests in rat pups (P15) and juvenile (P55–60) rats	Pretreatment (variable)	Variable; age- and model-dependent	[85]
IS in rats prenatally treated with betamethasone, triggered with NMDA	24 hours prior to induction of spasms	No effect	[66]
IS in rats treated with doxorubicin and LPS	Following emergence of infantile spasms	Decreased spasms; no effect on other seizure types	[30]
Hypoxic seizures in rats exposed to hypoxia at P10 and triggered with kainate at P13	24 hours before and 1 hour after hypoxic event	No effect on acute seizures; decreased chronic seizures	[29]

Abbreviations: *CCI* controlled cortical injury, *IS* infantile spasms, *LPS* lipopolysaccharide, *PTZ* pentylenetetrazole, *PTE* post-traumatic epilepsy, *TLE* temporal lobe epilepsy.