



“TOR”RENTS OF EXCITEMENT OVER RAPAMYCIN’S ANTIEPILEPTOGENIC POTENTIAL

Response of a Neuronal Model of Tuberous Sclerosis to Mammalian Target of Rapamycin (mTOR) Inhibitors: Effects on mTORC1 and Akt Signaling Lead to Improved Survival and Function. Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. *J Neurosci* 2008;28(21):5422–5432. Tuberous sclerosis (TSC) is a hamartoma syndrome attributable to mutations in either TSC1 or TSC2 in which brain involvement causes epilepsy, mental retardation, and autism. We have reported recently (Meikle et al., 2007) a mouse neuronal model of TSC in which Tsc1 is ablated in most neurons during cortical development. We have tested rapamycin and RAD001 [40-O-(2-hydroxyethyl)-rapamycin], both mammalian target of rapamycin mTORC1 inhibitors, as potential therapeutic agents in this model. Median survival is improved from 33 d to more than 100 d; behavior, phenotype, and weight gain are all also markedly improved. There is brain penetration of both drugs, with accumulation over time with repetitive treatment, and effective reduction of levels of phospho-S6, a downstream target of mTORC1. In addition, there is restoration of phospho-Akt and phospho-glycogen synthase kinase 3 levels in the treated mice, consistent with restoration of Akt function. Neurofilament abnormalities, myelination, and cell enlargement are all improved by the treatment. However, dysplastic neuronal features persist, and there are only modest changes in dendritic spine density and length. Strikingly, mice treated with rapamycin or RAD001 for 23 d only (postnatal days 7–30) displayed a persistent improvement in phenotype, with median survival of 78 d. In summary, rapamycin/RAD001 are highly effective therapies for this neuronal model of TSC, with benefit apparently attributable to effects on mTORC1 and Akt signaling and, consequently, cell size and myelination. Although caution is appropriate, the results suggest the possibility that rapamycin/RAD001 may have benefit in the treatment of TSC brain disease, including infantile spasms.

Rapamycin Prevents Epilepsy in a Mouse Model of Tuberous Sclerosis Complex. Zeng LH, Xu L, Gutmann DH, Wong M. *Ann Neurol* 2008;63(4):444–453. **OBJECTIVE:** Tuberous sclerosis complex (TSC) represents one of the most common genetic causes of epilepsy. TSC gene inactivation leads to hyperactivation of the mammalian target of rapamycin signaling pathway, raising the intriguing possibility that mammalian target of rapamycin inhibitors might be effective in preventing or treating epilepsy in patients with TSC. Mice with conditional inactivation of the Tsc1 gene primarily in glia (Tsc1^{GFAP}CKO mice) develop glial proliferation, progressive epilepsy, and premature death. Here, we tested whether rapamycin could prevent or reverse epilepsy, as well as other cellular and molecular brain abnormalities in Tsc1^{GFAP}CKO mice. **METHODS:** Tsc1^{GFAP}CKO mice and littermate control animals were treated with rapamycin or vehicle starting at postnatal day 14 (early treatment) or 6 weeks of age (late treatment), corresponding to times before and after onset of neurological abnormalities in Tsc1^{GFAP}CKO mice. Mice were monitored for seizures by serial video-electroencephalogram and for long-term survival. Brains were examined histologically for astrogliosis and neuronal organization. Expression of phospho-S6 and other molecular markers correlating with epileptogenesis was measured by Western blotting. **RESULTS:** Early treatment with rapamycin prevented the development of epilepsy and premature death observed in vehicle-treated Tsc1^{GFAP}CKO mice. Late treatment with rapamycin suppressed seizures and prolonged survival in Tsc1^{GFAP}CKO mice that had already developed epilepsy. Correspondingly, rapamycin inhibited the abnormal activation of the mammalian target of rapamycin pathway, astrogliosis, and neuronal disorganization, and increased brain size in Tsc1^{GFAP}CKO mice. **INTERPRETATION:** Rapamycin has strong efficacy for preventing seizures and prolonging survival in Tsc1^{GFAP}CKO mice.

COMMENTARY

Rapamycin, as its name suggests, had humble beginnings as an antifungal agent. It lost favor among infectious disease specialists as a result of its immunosuppressive side effect; however, this effect was useful to transplant surgeons in preventing

organ rejection, so the drug remained on the market. Meanwhile, molecular biologists were assessing resistance of fungi to the growth-inhibitory properties of certain antibiotics and determined that there is a serine–threonine protein kinase in *Saccharomyces cerevisiae* (i.e., Brewer’s yeast) that, if mutated, confers resistance to rapamycin. The investigators creatively named this kinase target of rapamycin (TOR) (1). Mammalian studies subsequently revealed that humans have a similar kinase; the mammalian analog, hence, was named “mammalian target

of rapamycin" (mTOR). Rapamycin's efficacy appeared to be dependent on its ability to inhibit TOR.

TOR is a phosphatidylinositol kinase-related kinase involved in the second-messenger intracellular cascade that regulates protein synthesis. Studies of TOR suggest that all eukaryotes have this protein, but yeast have two TOR proteins and higher eukaryotes possess only one (2). Biochemical isolation of *TOR1* and *TOR2* from yeast resulted in the identification of two TOR protein complexes, TORC1 and TORC2. The TORC1 complex is the rapamycin-sensitive one and regulates cell growth. The TOR pathway is activated upstream by a number of major signals, including growth factors and insulin (which activate TOR), as well as by energy state and environmental stress (which inhibit TOR signaling). In mammals, these effects appear to be mediated via the tuberous sclerosis complex (TSC) proteins, TSC1 and TSC2—heterodimers that, when phosphorylated, negatively regulate mTOR signaling. Without TSC1 or TSC2, the mTOR pathway runs amuck, inducing excessive cell growth and proliferation. It has been determined that mutation of *TSC1* or *TSC2* genes accounts for tuberous sclerosis, a disease in which patients develop hamartomas in multiple organ systems. In the brain, the effects can be devastating, with mental retardation, epilepsy, and developmental difficulties being prominent features (see the Basic Review article by Crino in this issue of *Epilepsy Currents*). Because rapamycin inhibits TOR, one might expect it to be useful to patients who have excessive TOR activation that is due to mutation of *TSC* genes.

Advances in genetic engineering have resulted in the development of new animal models of disease utilizing mice with induced select genetic deletions or mutations (3). However, introduction of such genetic changes often turns out to be embryonically lethal or results in compensatory up- or down-regulation of other gene products, confounding interpretation of the resultant phenotype. Conditional knockouts therefore have been favored in recent years. In these animals, representing the second generation of genetic mouse models, targeted techniques are used to allow tissue-specific control of gene function (4). The two studies reviewed here both utilize a conditional allele of *Tsc1* in combination with various brain-specific cre recombinase alleles to produce, in one case, a neuronal *Tsc1* knockout mouse model, and in the other, an astrocytic *Tsc1* knockout mouse line.

The neuronal *Tsc1* knockout mouse develops brain pathology, including enlarged dysplastic ectopic neurons, persistently reduced myelination, and high expression of phospho-S6 (a protein downstream of mTORC1). Phenotypically, the mice exhibit hyperactivity, tremulousness, seizures, poor weight gain, and a median survival of 33 days. Many of these clinical and pathological features approximate those found in patients with tuberous sclerosis (5,6). In a series of elegantly designed studies

by Meikle et al., the *Tsc1* neuronal model was used to test the efficacy of early treatment of these mice with rapamycin and the rapamycin derivative RAD001. The data are very carefully and systematically analyzed, and a combination of genetic, histologic, biochemical, and pharmacokinetic studies are presented. After demonstrating substantial penetration of rapamycin into the CNS, with increased penetration in younger mice, the investigators determined that an appropriate dose for the studies was 6 mg/kg every other day, and treatment was begun at postnatal day 7–9 (P7–P9). The therapy dramatically prolonged life span, with over 90% of animals surviving to at least 80 days and most beyond 100 days; there was a reduction in tremor and other abnormal motor behaviors as well. Pathologically, myelination improved and cell enlargement was reduced in the rapamycin-treated animals, but the dysplastic features and migration defects of the neurons appeared unchanged, probably because these developmental aspects are completed before age P7. Importantly, when treatment with rapamycin was terminated at P30 (i.e., after only 23 days of treatment), the beneficial effects persisted, with continued phenotypic improvement as well as median survival of 78 days.

Lacking in the Meikle data is electrophysiological studies of seizures accompanying tuberous sclerosis complex. In fact, it is not clear how the Meikle group distinguished the whole body tremors they observed from seizures. Fortunately, an unrelated investigation on rapamycin's effect on seizures was published by Zeng, almost simultaneously with Meikle's publication. Like Meikle et al., Zeng and colleagues performed genetic and histologic studies, but they also collected electrophysiological data, employing video-EEG monitoring to compare rapamycin- and vehicle-treated knockout mice. Their studies used the astrocytic *Tsc1* conditional knockout mouse, in which astrogliosis develops throughout the brain beginning at 3 weeks of age, with the neocortex and hippocampus being most severely affected. These animals have disorganization in the pyramidal cell layer of the hippocampus and reduced astrocytic expression of the glutamate transporters Glt-1 and GLAST, effects that may have epileptogenic sequelae. Phenotypically, these animals develop seizures, progressive encephalopathy, interictal EEG abnormalities, and premature death (7).

Importantly, Zeng et al. compared initiation of treatment at P14 (prior to onset of neurological abnormalities) to initiation at 6 weeks of age (late treatment, after clinical seizures appear). Their data support the benefits of early treatment with rapamycin, which was successful both in preventing astrogliosis and increasing Glt-1 expression, toward control levels. While seizures were routinely detected in the knockout mice by 1–2 months of age, no seizures were recorded in the early-treatment group (which was monitored up to 17 weeks). The progressive EEG changes were prevented, and

the decreased feeding accompanying encephalopathy was reduced in rapamycin-treated animals, although weight gain was somewhat better in vehicle-treated normal controls than in rapamycin-treated controls, suggesting rapamycin itself may have some systemic gastrointestinal side effects. While all untreated knockout mice died by 4 months of age, over 90% of the treated mice survived for at least 6 months. Regrettably, in contrast to the data presented in the preceding paper, seizures in the early treatment group did appear as soon as treatment ceased, indicating that continued long-term treatment would be necessary to sustain the benefits. However, the bigger surprise here was that late treatment was not entirely ineffective. In fact, in epileptic knockout animals in which treatment was initiated only at 6 weeks of age, seizures were dramatically suppressed, EEG abnormalities were reduced, and survival was markedly improved (i.e., no deaths while on treatment). Furthermore, although astrogliosis was not reversed, it appeared to be stopped in its tracks.

The potential for clinical application of these findings is expedited by the fact that rapamycin is an agent that is already FDA approved and available. In fact, its use has already expanded beyond immunosuppression for transplant patients, with rapamycin and rapamycin-derivatives gaining popularity as anticancer agents (8). Clinicians must proceed with caution, of course, since the agent has not been routinely used in children, and it may severely impact normal growth and development. Cognitive side effects also might be predicted, since the mTOR pathway participates in synaptic transmission (9) and plasticity (10). Nevertheless, considering the limited options for a child with tuberous sclerosis, rapamycin may offer, for the first time, a means to prevent epilepsy—the dream of every epileptologist and epilepsy researcher. With continued studies using these animal models, the future of epilepsy research and

the potential for prevention (the only true cure for epilepsy) are bright indeed.

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