Current Literature

In Basic Science

Prophylaxis for Post-Traumatic Epilepsy: Can Your Kinase Do That?

Rapamycin Attenuates the Development of Posttraumatic Epilepsy in a Mouse Model of Traumatic Brain Injury.

Guo D, Zeng L, Brody DL, Wong M. *PLoS One* 2013;8:e64078. doi:10.1371/journal.pone.0064078.

Posttraumatic epilepsy is a major source of disability following traumatic brain injury (TBI) and a common cause of medically-intractable epilepsy. Previous attempts to prevent the development of posttraumatic epilepsy with treatments administered immediately following TBI have failed. Recently, the mammalian target of rapamycin complex 1 (mTORC1) pathway has been implicated in mechanisms of epileptogenesis and the mTORC1 inhibitor, rapamycin, has been proposed to have antiepileptogenic effects in preventing some types of epilepsy. In this study, we have tested the hypothesis that rapamycin has antiepileptogenic actions in preventing the development of posttraumatic epilepsy in an animal model of TBI. A detailed characterization of posttraumatic epilepsy in the mouse controlled cortical impact model was first performed using continuous video-EEG monitoring for 16 weeks following TBI. Controlled cortical impact injury caused immediate hyperactivation of the mTORC1 pathway lasting at least one week, which was reversed by rapamycin treatment. Rapamycin decreased neuronal degeneration and mossy fiber sprouting, although the effect on mossy fiber sprouting was reversible after stopping rapamycin and did not directly correlate with inhibition of epileptogenesis. Most posttraumatic seizures occurred greater than 10 weeks after TBI, and rapamycin treatment for one month after TBI decreased the seizure frequency and rate of developing posttraumatic epilepsy during the entire 16 week monitoring session. These results suggest that rapamycin may represent a rational treatment for preventing posttraumatic epilepsy in patients with TBI.

PI3K-Akt Signaling Activates mTOR-Mediated Epileptogenesis in Organotypic Hippocampal Culture Model of Post-Traumatic Epilepsy.

Berdichevsky Y, Dryer AM, Saponjian Y, Mahoney MM, Pimentel CA, Lucini CA, Usenovic M, Staley KJ. *J Neurosci.* 2013;33:9056–9067.

mTOR is activated in epilepsy, but the mechanisms of mTOR activation in post-traumatic epileptogenesis are unknown. It is also not clear whether mTOR inhibition has an anti-epileptogenic, or merely anticonvulsive effect. The rat hippocampal organotypic culture model of post-traumatic epilepsy was used to study the effects of long-term (four weeks) inhibition of signaling pathways that interact with mTOR. Ictal activity was quantified by measurement of lactate production and electrical recordings, and cell death was quantified with lactate dehydrogenase (LDH) release measurements and Nissl-stained neuron counts. Lactate and LDH measurements were well correlated with electrographic activity and neuron counts, respectively. Inhibition of PI3K and Akt prevented activation of mTOR, and was as effective as inhibition of mTOR in reducing ictal activity and cell death. A dual inhibitor of PI3K and mTOR, NVP-BEZ235, was also effective. Inhibition of mTOR with rapamycin reduced axon sprouting. Late start of rapamycin treatment was effective in reducing epileptic activity and cell death, while early termination of rapamycin treatment did not result in increased epileptic activity or cell death. The conclusions of the study are as follows: (1) the organotypic hippocampal culture model of post-traumatic epilepsy comprises a rapid assay of anti-epileptogenic and neuroprotective activities and, in this model (2) mTOR activation depends on PI3K-Akt signaling, and (3) transient inhibition of mTOR has sustained effects on epilepsy.

Commentary

Among the major goals for epilepsy research is discovering a means of altering the disease progression of acquired epilepsy. To this end, animal and cellular models have been developed to identify outcome measures associated with the development of temporal lobe epilepsy (TLE), post-traumatic epilepsy (PTE), and other types of seizure disorders that are induced or acquired, rather than genetic in etiology. Animal epilepsy models, such as electrically or chemically induced status epilepticus (SE) and traumatic brain injury provide valuable information about key features of brain plasticity that result in TLE and PTE by producing postinjury cellular changes associated with the eventual development of spontaneous seizures (i.e., epileptogenesis). The overarching thesis is that an injury (broadly defined) triggers a cascade of events that, following a seizure-free “latent period,” eventually leads to altered neural function underlying the increased propensity for seizure expression that defines epilepsy.

The candidate cellular “trigger” for epileptogenesis investigated by the Staley and Wong labs is the protein kinase, mammalian target of rapamycin (mTOR). mTOR activation and subsequent protein translation is associated with cell death or survival, axon outgrowth and synaptic reorganization, altered ion channel and receptor expression, and neurogenesis—cellular events in the hippocampal formation that are also associated with development of acquired epilepsy. Recent evidence from a number of animal model studies showed that inhibition of mTOR signaling is variably effective in suppressing eventual development of epilepsy, some of its cellular correlates, or both. Since mTOR is activated by seizures and after traumatic brain injury, inhibiting the mTOR pathway represents a logical and reasonable prospect for preventing the induction of one or more epileptogenic processes and inhibiting postinjury epilepsy development.

The Wong and Staley groups approached this issue from two different perspectives. Guo et al. inhibited mTOR in mice that underwent controlled cortical impact (CCI) as a trigger for PTE development. This model recapitulates several cellular and behavioral outcomes of other TLE models, including postinjury cell loss, ion and receptor reorganization, mossy fiber sprouting in the dentate gyrus, synaptic reorganization, and delayed development of spontaneous seizures in a subset of animals (1–3). Treating CCI-injured mice with the mTOR inhibitor, rapamycin for 4 weeks post injury reduced injury-induced cell death, suppressed mossy fiber sprouting temporarily, and reduced the incidence of spontaneous seizure development up to 4 months post injury. The effectiveness of rapamycin treatment in suppressing seizure development and cellular changes associated with epileptogenesis is inconsistent across different models of acquired epilepsy (4, 5). Cell death, synaptic reorganization, axon sprouting, and seizures are typically much more robust in SE-induced TLE than in models of epilepsy resulting from trauma, and mechanisms underlying the changes may also be different across models. Even within the CCI-injury model, outcome measures vary considerably among investigators, possibly owing to differences in injury parameters, injury location, damage resulting from the trauma, species, or strain (1, 3, 6). At least some of this variability appears to have been minimized in the present study, resulting in

a relatively narrow range of seizure onset latency. That seizures were not as prevalent, at least in terms of the percentage of injured mice that developed epilepsy after rapamycin treatment, suggests disease-altering effects of mTOR inhibition shortly after CCI injury. Other parameters were measured mainly in a semiquantitative manner and suggested that at least some of the cellular plasticity associated with epileptogenesis may have been suppressed or delayed by the treatment. Given the variably long delay to spontaneous seizure onset reported elsewhere in the CCI model (7 weeks to 6 months) (1, 2) and the finding of delayed epilepsy development in other models with rapamycin treatment, the authors acknowledge that a truer assessment of epilepsy prevention might require a longer seizure-monitoring period. Even so, the lower incidence of epilepsy development in rapamycin-treated mice 4 months after injury suggests the possibility that activation of the mTOR pathway shortly after the injury participates in the etiology of PTE over time.

The mTOR pathway is complex, with multiple mechanisms of activation and even more complexity in its mode of effect. The work of Berdichevsky et al. aimed to identify mTOR activators by treating slice cultures with rapamycin or other regulators of mTOR function to modify in vitro correlates of epileptogenesis (e.g., epileptiform electrical activity, lactate hypermetabolism, cell loss, axon sprouting) that occur naturally and on an accelerated time scale in the slice culture preparation. Similar to Guo et al., but unlike some other animal models of TLE, transient inhibition of mTOR with rapamycin or blockade of kinases that regulate mTOR (i.e., PI3K or Akt) reduced the severity of several outcome measures. In the slice culture preparation, cellular changes can be quantified over days (versus weeks to months in animals), providing a tool for rapid assessment of pathway-specific mechanisms underlying several correlates of epilepsy progression. Debate about the relationship of the slice culture preparation to PTE notwithstanding, the cellular outcomes of these experiments are consistent with the hypothesis that mTOR signaling triggers a cascade of events that may underlie expression of seizure-like electrographic and cellular metabolic behavior and that the PI3K-Akt-dependent activation of mTOR is necessary to trigger epileptogenic changes.

Conclusions made in both articles provide important information that may guide development of disease-modifying treatments for prevention of PTE based on mTOR inhibition after injury. Rapamycin is an immunosuppressant, and long-term use of the drug can adversely alter cognitive and metabolic functions, side effects that might limit its utility for suppressing epileptogenesis after brain injury. On the other hand, hormone, nutrient, and secreted growth factor systems activate PI3K endogenously, and their effects on epileptogenesis might be better understood in the context of their actions on mTOR activity. Further, if short-term mTOR inhibition after brain injury is proven to be sufficient to suppress long-term PTE, the negative side effects of rapamycin may be less prominent. Because rapamycin effects in other epilepsy models is variably effective in controlling epileptogenesis, additional and more quantitative assessment of the long-term effects of mTOR suppression on epilepsy development in this and other animal models of brain injury should clarify the efficacy

of rapamycin. Aside from effects on seizure disorders, implications of mTOR inhibition for short- and long-term functional recovery after brain injury are not fully appreciated. mTOR inhibition reportedly promotes neuroprotection and improves some functional outcomes after brain injury. To the contrary, activation of PI3K-dependent pathways (including mTOR) improves posttrauma recovery by augmenting neurogenesis and synaptic reorganization, both of which are associated with epileptogenesis. If treatments aimed at inhibiting mTOR are to be effective in preventing or altering the progression of post-traumatic epileptogenesis, effects on other consequences of brain injury should also be considered.

The cellular outcomes of mTOR pathway activation are numerous and include effects on cell survival, proliferation, ion channel expression, neurogenesis, and neurite outgrowth, to name some. The causative contribution, if any, of these factors to epileptogenesis is not known. Identifying cellular and molecular alterations in brain function that are causative (versus correlative) for epilepsy development remains critical for developing treatments selective for PTE prevention based on modulation of the mTOR pathway or any other potential trigger of cellular plasticity. Together, the work by the Staley and Wong groups represents a step toward PTE prevention by helping to define epileptogenesis-related pathways and features of mTOR inhibition after head injury. A number of treatments have been developed to suppress seizures once epilepsy has been established, but treatments to prevent epilepsy development after brain trauma have not been developed successfully. Preventing brain trauma-induced triggering of a cascade of cellular or

molecular events might represent a roadmap for prevention of acquired epilepsy.

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