



Wnt Signaling: The Next Step on the Road to Anti-Epileptogenic Therapies?

Canonical Wnt activator Chir99021 prevents epileptogenesis in the intrahippocampal kainate mouse model of temporal lobe epilepsy

Mardones MD Rostam KD Nickerson MC Gupta K. *Experimental Neurology*. 2024 Mar 21;376:114767.

The Wnt signaling pathway mediates the development of dentate granule cell neurons in the hippocampus. These neurons are central to the development of temporal lobe epilepsy and undergo structural and physiological remodeling during epileptogenesis, which results in the formation of epileptic circuits. The pathways responsible for granule cell remodeling during epileptogenesis have yet to be well defined, and represent therapeutic targets for the prevention of epilepsy. The current study explores Wnt signaling during epileptogenesis and for the first time describes the effect of Wnt activation using Wnt activator Chir99021 as a novel anti-epileptogenic therapeutic approach. Focal mesial temporal lobe epilepsy was induced by intrahippocampal kainate (IHK) injection in wild-type and POMC-eGFP transgenic mice. Wnt activator Chir99021 was administered daily, beginning 3 h after seizure induction, and continued up to 21-days. Immature granule cell morphology was quantified in the ipsilateral epileptogenic zone and the contralateral peri-ictal zone 14 days after IHK, targeting the end of the latent period. Bilateral hippocampal electrocorticographic recordings were performed for 28-days, 7-days beyond treatment cessation. Hippocampal behavioral tests were performed after the completion of Chir99021 treatment. Consistent with previous studies, IHK resulted in the development of epilepsy after a 14 day latent period in this well-described mouse model. Activation of the canonical Wnt pathway with Chir99021 significantly reduced bilateral hippocampal seizure number and duration. Critically, this effect was retained after treatment cessation, suggesting a durable antiepileptogenic change in epileptic circuitry. Morphological analyses demonstrated that Wnt activation prevented pathological remodeling of the primary dendrite in both the epileptogenic zone and peri-ictal zone, changes in which may serve as a biomarker of epileptogenesis and anti-epileptogenic treatment response in pre-clinical studies. These findings were associated with improved object location memory with Chir99021 treatment after IHK. This study provides novel evidence that canonical Wnt activation prevents epileptogenesis in the IHK mouse model of mesial temporal lobe epilepsy, preventing pathological remodeling of dentate granule cells. Wnt signaling may therefore play a key role in mesial temporal lobe epileptogenesis, and Wnt modulation may represent a novel therapeutic strategy in the prevention of epilepsy.

Commentary

Mesial temporal lobe epilepsy (MTLE) is the most common form of drug-resistant epilepsy.¹ Development of effective anti-seizure medications (ASMs) for MTLE is expected to be facilitated by a better understanding of epileptogenic processes. Further, understanding the mechanisms at work during epileptogenesis may suggest novel therapeutic targets that, when pharmacologically manipulated, may attenuate epileptogenesis, or halt it altogether, thus, preventing MTLE development. Since anti-epileptogenic compounds are, by definition, disease-modifying, it is anticipated that these compounds may also improve treatment of pharmacoresistant seizures in MTLE even if they fail to fully prevent epileptogenesis.^{2,3}

The Wnt signaling pathway is of potential therapeutic importance because it plays an important role in different

neurodevelopmental processes that may be pathologically co-opted during epileptogenesis. Manipulations of this pathway can result in circuitry alterations that predispose individuals to epilepsy.⁴ Accordingly, the Wnt pathway has gained interest as a target for anti-epileptogenic therapeutics. In the study by Mardones et al, the authors used an activator of canonical Wnt to study its role in epileptogenesis of MTLE in the intrahippocampal kainate (IHK) mouse model.⁵ To explore its therapeutic potential in a clinically relevant scenario, daily intraperitoneal injections of the Wnt activator Chir99021 (or vehicle) were started 3 h after SE induction and continued for up to 21 days; this scenario models treatment administration to prevent disease progression in clinical studies for newly diagnosed epilepsy following SE onset or TBI. Since it was determined that all animals receiving





IHK had Racine 3–6 seizures within 2 h of the injection and demonstrated granule cell dispersion, the authors chose to start Wnt activator administration 3 h post-IHK, thereby limiting the potential confound of this compound diminishing SE. Accordingly, this design allowed the authors to conclude that strategic activation of the Wnt signaling pathway soon after the initial seizure can significantly improve later epileptic symptoms through an antiepileptogenic/disease-modifying mechanism. Observations in this study include a reduced total non-convulsive seizure duration and count in animals that received 21 days of treatment with Chir99021 compared to those that received vehicle injections. The fact that the observed reduction in seizure count applied to both the ipsilateral and contralateral (peri-ictal zone (PIZ)) hippocampus implies that the therapeutic effect of Wnt activation applies beyond the epileptogenic zone.⁵ While Chir99021's ability to reduce seizure counts during treatment is a promising observation, a true anti-epileptogenic effect would be expected to remain beyond the treatment window. Therefore, the authors tested this by observing seizures for 7 days after the final treatment. They found that animals continued to have a reduced seizure burden compared to vehicle.

Another desirable therapeutic effect of an anti-epileptogenic treatment would be the prevention of comorbid cognitive dysfunction observed in epilepsy. Thus, the authors examined the consequences of Chir99021 treatment on hippocampal and extra-hippocampal dependent learning and memory. Although the authors conclude that mice treated with the Wnt activator showed improved hippocampal memory, these results are not as unambiguous as those showing prevention of seizure-like events. Specifically, while the Chir99021 group showed a significantly increased exploration of a displaced object, they failed to show significance as a standard discrimination index. Furthermore, Chir99021 may have had a negative impact on NOR in control mice suggesting that, in the context of non-pathological conditions, Chir99021 may have deleterious effects at the doses examined. Taken together, the data presented in this regard is promising but warrants additional investigation.

Finally, the study evaluated hippocampal histological changes that occur in the IHK model and Chir99021's potential to mitigate or prevent these changes. Chir99021 failed to rescue granule cell dispersion or increased neurogenesis. However, the authors conclude that it did significantly rescue dentate granule cell proximal dendrite morphology based on a statistical evaluation of differences in primary dendrite angle in either the ipsilateral epileptogenic zone or the PIZ. Differences between vehicle- and Chir99021-treated IHK mice were observed in the PIZ where Chir99021 shifted cumulative frequency towards more acute angles similar to vehicle-treated control mice. While this result is consistent with Chir99021's potential therapeutic benefits, additional data unfortunately raises questions that obscure this conclusion; in control mice, Chir99021 also shifted the cumulative frequency of primary dendrite angles towards less acute angles seemingly identical to those observed in vehicle-treated IHK mice (although this

comparison was not statistically evaluated). These data would again suggest that, in the context of non-pathological conditions, Chir99021 may have deleterious effects at the doses examined.

Overall, this study presents Wnt signaling activation as a promising therapeutic approach for anti-epileptogenesis. Nevertheless, the exact role of Wnt signaling is yet to be determined. Previous studies have implied a bimodal role of Wnt signaling, meaning that a delicate balance between the activation and inhibition of this pathway is needed to use this approach to its full potential. The authors take a major step in this direction with the observations presented here. Further studies are still necessary to determine how long the therapeutic effects remain after the treatment is complete. It would also be interesting to determine the stage of epileptogenesis during which treatment is beneficial and the effect of missing the critical treatment window. This pathway requires a delicate balance, as such there is a possibility that activation outside of the critical window could be detrimental. In addition, a dose-response relationship is necessary. Thus, a deeper understanding of epileptogenesis, the stage at which Wnt activation is most therapeutically beneficial, and how long the therapeutic effect lasts, is crucial for leveraging this novel approach in the future.

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