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Efficacy of Sodium Channel Inhibitors as Anticonvulsants in the Rat MES Assay is Predictive of the Therapeutic Plasma Concentration in Humans

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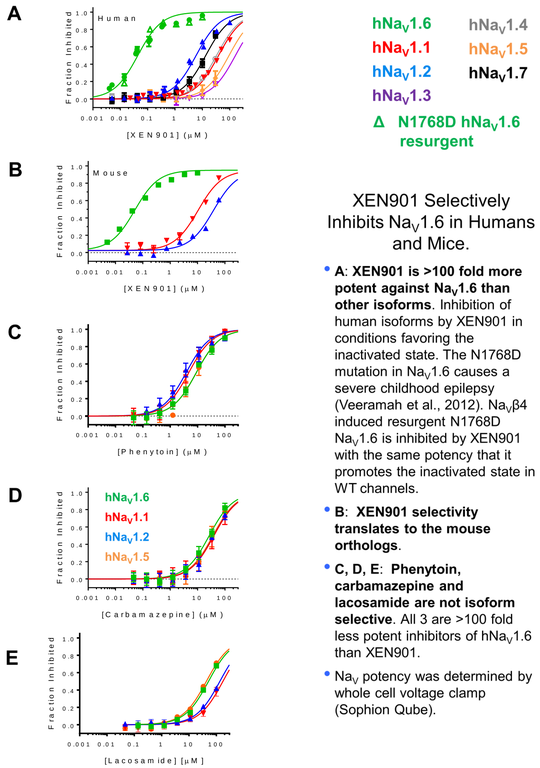
INTRODUCTION

- Sodium channel inhibitors are widely used as antiepileptic drugs (AEDs) but seizure control is often inadequate at maximally tolerated doses.
- Currently available Na_V AEDs are nearly equipotent inhibitors of isoforms widely expressed in the CNS ($Na_V1.1$, $Na_V1.2$, $Na_V1.6$).
- Loss of function mutations in $Na_V1.1$ cause Dravet Syndrome, indicating that $Na_V1.1$ is the dominant Na_V channel in inhibitory GABAergic neurons (Catterall et. al., 2010). Thus, inhibition of $Na_V1.1$ is likely counter-productive for treatment of epilepsy.
- Reduced expression of $Na_V1.6$ reduces seizures in epilepsy mode (Blumenfeld et al., 2009; Makinson et al., 2014; Wong et al., 2018)
- XEN901 is highly selective for inhibition of $Na_V1.6$.
- We explored whether plasma levels in the Maximal Electroshock Stimulus assay correlate with therapeutic plasma concentration for nonselective sodium channel inhibitors, thereby enabling an estimate of the necessary concentration of XEN901.

Selective Inhibition of $Na_V1.6$

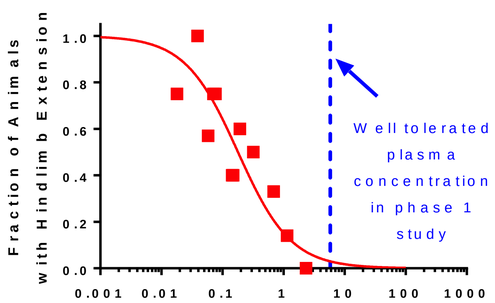


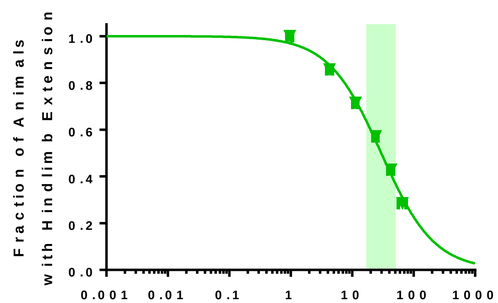
XEN901 Selectively Inhibits $Na_V1.6$ in Humans and Mice.

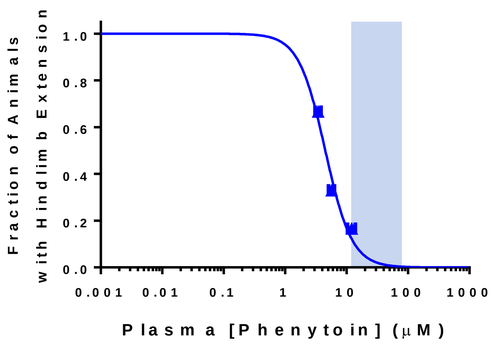
- **A**: **XEN901 is >100 fold more potent against $Na_V1.6$ than other isoforms**. Inhibition of human isoforms by XEN901 in conditions favoring the inactivated state. The N1768D mutation in $Na_V1.6$ causes a severe childhood epilepsy (Veeramah et al., 2012). $Na_V\beta4$ induced resurgent N1768D $Na_V1.6$ is inhibited by XEN901 with the same potency that it promotes the inactivated state in WT channels.
- **B**: **XEN901 selectivity translates to the mouse orthologs**.
- **C, D, E**: **Phenytoin, carbamazepine and lacosamide are not isoform selective**. All 3 are >100 fold less potent inhibitors of $hNa_V1.6$ than XEN901.
- Na_V potency was determined by whole cell voltage clamp (Sophion Qube).

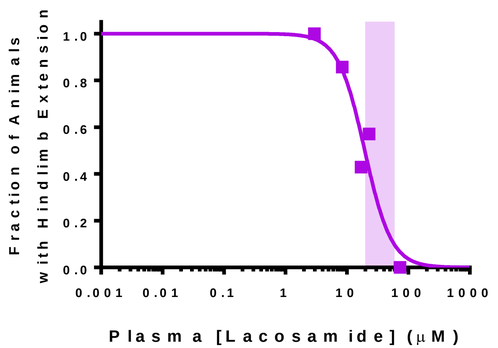
Maximal Electroshock Seizure (MES) Model

- Highly predictive of clinical efficacy of Na_V inhibitors against human seizures.
- Clinically effective concentration of nonselective Na_V inhibitors at 1-3 times EC_{50} in rat MES assay.
- XEN901 is well tolerated at a plasma concentration 32-times higher than the EC_{50} in the rat MES assay.



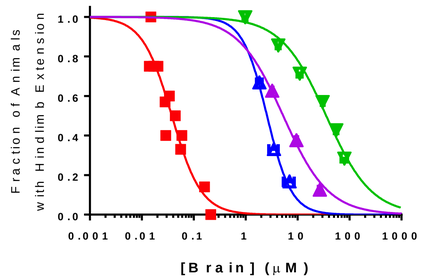


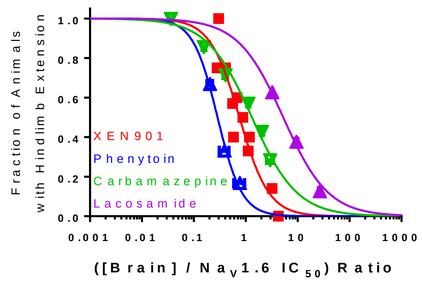




- Efficacy in the rat MES Assay is plotted vs measured plasma concentrations for XEN901, Carbamazepine, Phenytoin, and Lacosamide. The vertical dotted line in the plot for XEN901 corresponds to the highest plasma concentration achieved in a phase I study with healthy humans, achieved at a well tolerated dose.
- The shaded areas on the graphs for Carbamazepine, Phenytoin, and Lacosamide correspond to reported effective plasma concentrations for human patients.
- Binary seizure readout – animals are monitored for presence/absence of tonic-clonic seizure.
- Seizures were induced by a constant current stimulus applied to cornea of 150 mA, consisting of 0.5 ms pulses delivered at 60 Hz for 200 ms.

Potency in MES Assay Correlates with Inhibition of $Na_V1.6$ in Brain





- ***Upper panel***: Efficacy in the MES assay increased with brain concentration of compounds.
- ***Lower panel***: Dividing the brain concentration by the in vitro potency on $Na_V1.6$ results in close overlay of all curves.
- IC_{50} concentration *in vivo* corresponds to a brain concentration approximately 0.3-5 fold the IC_{50} for $Na_V1.6$ *in vitro*. The greater potency of XEN901 in the MES assay can be accounted for by the greater potency for inhibition of $Na_V1.6$ in the brain.
- After the MES assay, animals were euthanized and plasma and brain concentrations were measured by mass spectrometry. All points represent results of 5-8 animals in a single study group. Error bars on the x-axis are standard errors of the mean but in many cases are smaller than the symbols.

CONCLUSIONS

- Clinical efficacy of nonselective sodium channel inhibitors is achieved at 1- 3 times the EC_{50} in the rat MES assay.
- Nonselective sodium channel inhibitors are effective at approximately the same multiple of IC_{50} for $Na_V1.6$ as the selective inhibitor, XEN901.
- $Na_V1.6$ inhibition is likely the primary driver of the efficacy of non-selective Na_V inhibitors in the rat MES assay.
- The rat MES assay suggests that therapeutically effective plasma concentrations of XEN901 may be attained in humans without toxicity.