Preclinical *In Vitro* and *In Vivo* Comparison of the K_V7 Activator XEN1101 with Ezogabine

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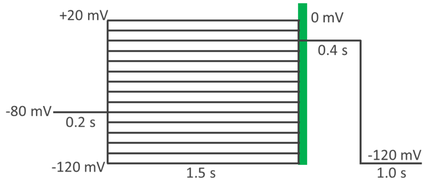
BACKGROUND

- XEN1101 is a differentiated K_V7 potassium channel modulator being developed for the treatment of epilepsy and potentially other neurological disorders.
- Ezogabine (trade names Potiga® and Trobalt™) was previously approved by the U.S. FDA for the treatment of adult focal onset seizures; however, it was withdrawn from the global market in July 2017 for commercial reasons, and no K_V7 activating drugs are currently available for the treatment of epilepsy.
- XEN1101 and ezogabine overlap in their mechanism of action; therefore, an assessment was conducted to compare their preclinical *in vitro* and *in vivo* profiles.

METHODS

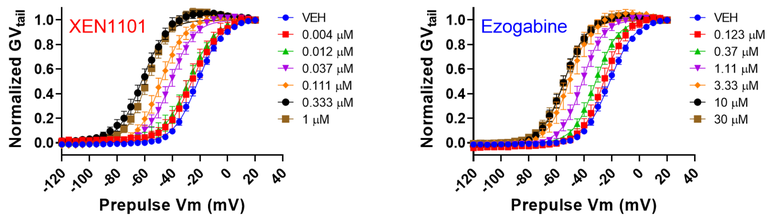
- *In vitro*, Human Embryonic Kidney cells stably expressing $K_V7.x$ were used to examine the potency of XEN1101 and ezogabine in a K^+ flux assay and whole-cell patch clamp electrophysiology.
- Electrophysiological recordings were made on a Sophion Qube-384 planar patch-clamp system. Standard intra- and extracellular solutions were used and the voltage protocol shown below was used to assess the effect of compound on the biophysics of the channel. Tail current measurements were made at the 0 mV test pulse after the conditioning prepulses.



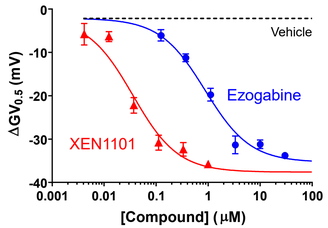
- *In vivo*, the ability of administered XEN1101 and ezogabine to prevent electrically induced seizures was evaluated in an Alternating Current Maximal Electroshock Seizure (AC-MES) mouse model.

RESULTS

Normalized GV_{tail} Curves for $K_V7.2/7.3$ Channels in Presence of XEN1101 or Ezogabine



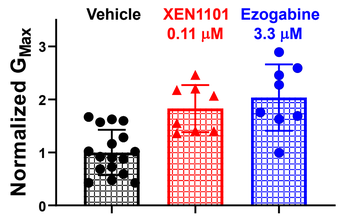
In Vitro Potency of XEN1101 and Ezogabine



Compound	K^+ Flux (WT)	$K_V7.2/7.3$ EC_{50} K^+ Flux (Pore mutant)	EP	$K_V7.2/7.3$ $\Delta V_{1/2,\ max}$ (mV)
XEN1101	0.034 µM	>30 µM	0.042 µM	-42.5
Ezogabine	0.950 µM	>30 µM	0.920 µM	-36.2

- In both K^+ flux and EP assays XEN1101 is ~20-fold more potent for potentiating $K_V7.2/K_V7.3$ heterotetramers compared to ezogabine.
- XEN1101 loses all activity when tested in a $K_V7.2$ W236L/$K_V7.3$ W265L pore mutant K^+ flux assay demonstrating that XEN1101 engages the same critical Trp236 residue in the core of the K_V channel as ezogabine.
- XEN1101 reduces the voltage threshold for channel opening ($\Delta V_{1/2,\ max}$) by ~15% more than ezogabine.

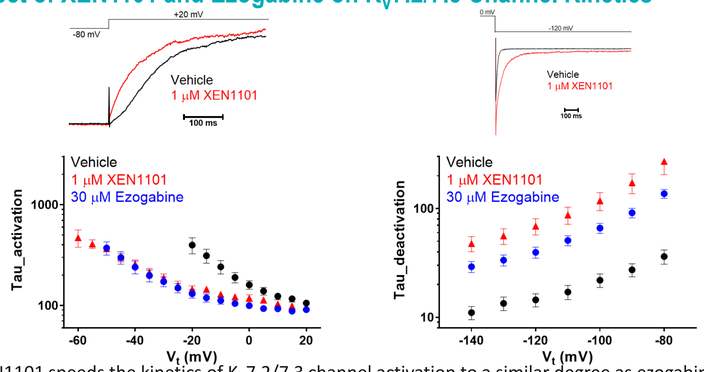
XEN1101 and Ezogabine Increase Normalized G_{max}



Compound	ΔG_{max} (Fold)
XEN1101	1.83
Ezogabine	2.03

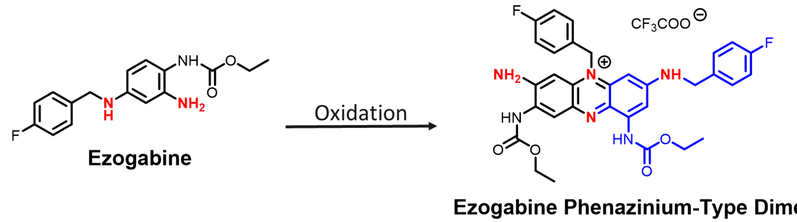
- XEN1101 and ezogabine both demonstrated a concentration-dependent increase in G_{max} that was maximal at ~2-fold increase in the conductance magnitude.

Effect of XEN1101 and Ezogabine on $K_V7.2/7.3$ Channel Kinetics



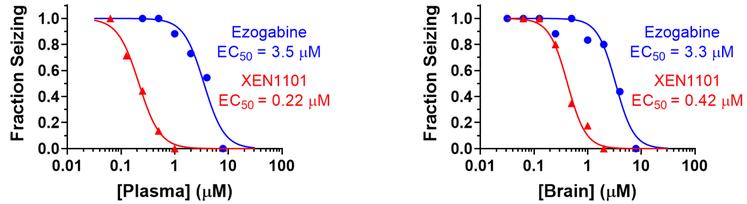
- XEN1101 speeds the kinetics of $K_V7.2/7.3$ channel activation to a similar degree as ezogabine.
- Deactivation of $K_V7.2/7.3$ channels is slowed ~2-fold more by XEN1101 than ezogabine, enhancing its ability to reduce hyper excitability.

Discoloration by Ezogabine Due to Dimer Formation



- Ezogabine can form a number of dimeric species, including highly-coloured phenazinium-type dimers[1], which have been implicated in the pigmentary abnormalities observed with long-term ezogabine exposure.
- Ezogabine has a secondary aniline function, which is key to forming phenazinium-type dimers.
- XEN1101 instead has a tertiary aniline at the corresponding position and this key structural difference prevents XEN1101 forming analogous dimers.

Efficacy of XEN1101 and Ezogabine in the Mouse AC-MES Assay



- In line with the *in vitro* data, XEN1101 requires ~15-fold less plasma and ~8-fold less brain exposure than ezogabine for half-maximal activity in an AC-MES mouse model. Data is binned by plasma and brain concentrations.

CONCLUSIONS

- Preclinical *in vitro* and *in vivo* comparison of XEN1101 with ezogabine demonstrates that XEN1101 has a similar mechanism of action to ezogabine but potentially offers substantial improvements:
  - More potent modulation of $K_V7.2/K_V7.3$;
  - Slows deactivation of $K_V7.2/7.3$ channels to a greater degree, enhancing its ability to reduce hyper excitability;
  - More potent anti-seizure activity in preclinical models;
  - No pigmented dimers and no predicted discoloration liability.
- XEN1101 is a novel chemical entity that has a strong rationale as a potential anti-seizure medication providing a mechanism of action that is not currently available for the treatment of epilepsy.
- A Phase 2b clinical trial is underway to evaluate the clinical efficacy, safety, and tolerability of XEN1101 administered as adjunctive treatment in approximately 300 adult patients with focal epilepsy.

[1] Groseclose et al. *Chem. Res. Toxicol.* 2019, 32:294-303.

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