### ABSTRACT

Epilepsy is a group of neurological disorders characterized by sudden and recurrent seizures. Calcium plays a role in the mechanism of neuronal hyperexcitability. Evidence shows calcium influx via the sodium calcium exchanger (NCX), creates an imbalance of excitatory and inhibitory influences on neurons and leads to seizures.

We further evaluated the role of the NCX in the development of pentylenetetrazole (PTZ)-induced seizures. Blockade of the NCX suppresses seizures. We quantified the effectiveness of a combination of SN-6 and a non-anticonvulsant dose of diazepam (DZP) in reducing or preventing the incidence of seizures in rats.

Male Sprague Dawley rats (150-250 g; n=6-8 per group) received a vehicle, SN-6 (3 mg/kg, p.o.), DZP (2.5 and 5 mg/kg, i.p.), and a combination of DZP (2.5 mg/kg, i.p.) and SN-6 (3 mg/kg, p.o.). We tested the vehicles' preventative abilities against PTZ (60 mg/kg, i.p.) induced seizures 90 minutes after administration.

We observed the severity of seizures using the Racine's scale of seizures:

- Class 0: no response, immobility, mouth and facial automatisms
- Class 1: Myoclonic jerks, mastication
- Class 2: Clonic seizure while lying on belly
- Class 3: Bilateral forelimb clonic seizures
- Class 4: Rearing without falling
- Class 5: Generalized tonic-clonic seizures with loss of posture

In controls, 100% and 87% exhibited clonic and tonic-clonic seizures, respectively.

SN-6 (3 mg/kg, p.o.) pretreatment significantly reduced the incidence of clonic and tonic-clonic seizures both by 50% compared to controls.

At the dose of 5 mg/kg, DZP pretreatment completely suppressed PTZ-induced seizures. However, at 2.5 mg/kg DZP pretreatment alone was ineffective in suppressing PTZ-induced seizures.

A combination of DZP (2.5 mg/kg, i.p.) and SN-6 (3 mg/kg, p.o.) reduced clonic seizure incidence, completely suppressed tonic-clonic seizure, and reduced the score of seizure severity.

### METHODS AND OBSERVATIONS

- **PTZ**: Pentylenetetrazole-induced neuronal excitability and was used in convulsive therapy until the 1980's. Used to induce seizures in rats.
- **DZP**: Commonly used drug called diazepam; side effects including hyperactivity, dizziness, and muscle weakness. Seizures can worsen if DZP usage is suspended.
- **SN-6**: Commonly used drug called diazepam; side effects including hyperactivity, dizziness, and muscle weakness. Seizures can worsen if DZP usage is suspended.
- **NCX forward mode**: A membrane transporter that under normal conditions exchanges one Ca^{2+} out of a cell and three Na^{+} ions into a cell.
- **NCX reverse mode**: Under some specific conditions, NCX brings three Ca^{2+} ions into the cell and one Na^{+} ion.

### RESULTS

#### CLONIC SEIZURES

- **Control group**: 100% of animals experience clonic seizures.
- **SN-6 pre-treatment**: reduces the incidence of clonic seizures by 50%. ***P<0.0001; Chi square
- **DZP 2.5 mg/kg and 3 mg/kg SN-6**: combination reduces seizures by 71%. ***P<0.0001; Chi square

#### TONIC-CLONIC SEIZURES

- **Control group**: 88% of animals experience tonic-clonic seizures.
- **SN-6 pretreatment decreases incidence by 50% from controls. ***P<0.0001; Chi square
- **DZP 2.5 mg/kg, and combination of 2.5 mg/kg DZP and 3 mg/kg SN-6 prevent tonic-clonic seizures. ***P<0.0001; Chi square

### CONCLUSIONS

- SN-6 (3 mg/kg) and DZP (2.5 mg/kg) pretreatment alone did not completely suppress PTZ-induced generalized seizures.
- Combining SN-6 and a low dose of DZP is nearly as effective as high dose of DZP (5 mg/kg) in preventing tonic-clonic seizures.
- SN-6 pretreatment required the addition of non-anticonvulsant dose of DZP to yield more efficacy in suppressing seizures.
- SN-6 can be used as adjuvant therapy for the treatment of tonic-clonic seizures.

### REFERENCES