Animal Models of Drug Resistant Epilepsy Redux

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Current Era of AED Discovery

- Ushered in by Merritt and Putnam in 1938 with the discovery of phenytoin
- Employs well-characterized animal models of generalized and partial seizures
- Goal is to provide sufficient Proof-of-Concept data to support an Investigational Drug Application
Models and Efficacy
Decision tree for anticonvulsant drug testing in the NIH anticonvulsant screening project

Identification (MES & s.c. PTZ tests in mice)

- ACTIVE
  - Quantification
    - time of peak effect
    - anticonvulsant ED$_{50}$
    - neurotoxic TD$_{50}$ in rotarod test
  - Differentiation
    - hippocampal kindled rats
    - corneally kindled mice
    - audiogenic seizure-susceptible mice
    - etc

- inactive
  - 6 Hz test in mice
    - ACTIVE
    - inactive
    - Stop testing

Advanced studies
- mechanistic studies
- enzyme induction/inhibition
- proconvulsant potential
- etc

Löschner, Seizure, 2011
Existing Approach Does Identify Clinically Effective AEDs

Eslicarbazepine acetate
Felbamate
Fosphenytoin
Gabapentin
Lamotrigine
Lacosamide
Levetiracetam
Oxcarbazepine
Pregabalin
Retigabine (ezogabine)
Rufinamide
Tiagabine
Topiramate
Vigabatrin
Zonisamide
Each New AED

• Has had a **POSITIVE** impact on the treatment of the patient with epilepsy
  – improved efficacy and pharmacokinetic profile, and fewer drug-drug interactions, etc.

• However, there have been **SURPRISES**; e.g.,
  – felbamate-induced aplastic anemia and hepatic failure
  – lamotrigine-induced skin rash
  – topiramate-induced word finding difficulties
  – levetiracetam-related behavioral/psychiatric issues

• Has provided **HOPE** for the patient with uncontrolled epilepsy
Löscher and Schmidt, Epilepsia, 2011
Plus, many more AEDs in the Pipeline

- brivaracetam
- 2-deoxy-glucose
- ganaxolone
- huperzine
- NAX-5055
- propylisopropylacetamide (PID)
- ezogabine (i.e., retigabine)
- T-2000
- tonabersat
- valroceamide
- YKP-3089

Bialer et al., Epilepsy Res. 92(2-3):89-124, 2010

Who is Underserved by the Current Approach?

- The adult with highly refractory partial onset epilepsy
- The child with catastrophic epilepsy
- The elderly
- The patient with co-morbidities
Therapy Resistant Models

• Models
  – Phenytoin-resistant kindled rat
  – Lamotrigine-resistant kindled rat
  – 6 Hz “Psychomotor” seizure test
  – Post-status spontaneous seizure models
  – *In utero* methylazoxymethanol (MAM) acetate-induced heterotopia
  – In vitro brain slices from kainate-treated rats

• Properties
  – All six models display unique attributes that may model pharma-coresistant epilepsy
  – Therapy resistant seizure models are more likely to identify novel AEDs

Yet to be “truly” validated by human experience
Animal models of drug-resistant epilepsy

1. Model is *per se* resistant to AEDs

   - 6-Hz psychomotor seizure model in mice
     - At 44 mA resistance to various AEDs, except valproate, levetiracetam, brivaracetam, carisbamate, and retigabine
   - Lamotrigine-pretreated kindled rats
     - Resistance to lamotrigine, carbamazepine, phenytoin and topiramate, but not valproate, felbamate and retigabine
   - MAM*-exposed rats
     - Resistance to ethosuximide, valproate and carbamazepine

   *methylazoxymethanol, induces cortical malformation

2. Model allows selection of AED responders and nonresponders

   - Amygdala-kindling in rats
     - Responder/nonresponder selection by phenytoin
     - Resistance extends to various other AEDs, except levetiracetam
   - Post-SE models of TLE in rats
     - Responder/nonresponder selection by levetiracetam in the pilocarpine model
     - Not yet known whether resistance extends to other AEDs
     - Responder/nonresponder selection by phenobarbital in the BLA model
     - Resistance extends to phenytoin

Löschner, Seizure, 2011
# Pharmacology of the 6 Hz Model

<table>
<thead>
<tr>
<th>AED</th>
<th>ED50 (mg/kg, i.p.) and 95% C.I.</th>
<th>22 mA</th>
<th>32 mA</th>
<th>44 mA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>9.4 (4.7 - 14.9)</td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>4.4 (2.2 - 6.6)</td>
<td>&gt;60</td>
<td>&gt;60</td>
<td></td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>86.9 (37.8 - 156)</td>
<td>167</td>
<td>&gt;600</td>
<td></td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>4.6 (1.1 - 8.7)</td>
<td>19.4</td>
<td>1089</td>
<td>(787 - 2650)</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>41.5 (16.1 - 68.8)</td>
<td>126</td>
<td>310</td>
<td>(258 - 335)</td>
</tr>
</tbody>
</table>

The LTG-kindled rat displays a pharmacological profile consistent with pharmaco-resistant epilepsy and is amenable to medium through-put screening.

<table>
<thead>
<tr>
<th>Resistant</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamotrigine</td>
<td>Valproate</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Clonazepam</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Levetiracetam</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Carisbamate</td>
</tr>
<tr>
<td></td>
<td>Retigabine</td>
</tr>
</tbody>
</table>
Hippocampal sclerosis
- Cell loss: CA1, CA3, hilus of dentate gyrus
- Dispersion of granular cells (dentate gyrus)
- Neuroplasticity: sprouting of mossy fibers

EEG Quantification
- Frequency: 7-11Hz
- Mean duration = 15-20 s
- Recurrence = 45 disch./h
- Behavioral arrest
- Rare generalizations
- No remission

Slide kindly provided by Dr. Corinne Roucard, SYNAPCELL
MTLE Pharmacology

**Resistance** to classical AEDs within safety margins

**Efficacy** of new AEDs

Slide kindly provided by Dr. Corinne Roucard, SYNAPCELL
Differences between AED-nonresponders and -responders

**Phenytoin-resistant and -responsive kindled rats**
- Resistance extends to various other AEDs, except levetiracetam
- Kindling or seizure characteristics are not different between responders and nonresponders
- Breeding studies indicate genetic differences between responders and nonresponders
- Comparison of various rat strains also indicates that genetic factors are involved in resistance to phenytoin
- No obvious difference in phenytoin’s effects on voltage-dependent Na⁺ channels in CA1
- Expression of P-glycoprotein in the blood-brain barrier is significantly higher in resistant than responsive rats

**Phenobarbital-resistant and -responsive epileptic rats**
- Resistance extends to phenytoin
- Average seizure frequency is higher in nonresponders before onset of AED treatment
- Behavioral and cognitive changes are more severe in AED resistant rats
- Hippocampal damage is only observed in resistant rats
- Diazepam-insensitive GABA₆ receptor binding in the dentate gyrus is significantly increased in resistant rats, indicating target alterations
- Complex alterations in the expression of GABA₆ receptor subunits are observed in the hippocampal formation of resistant rats
- Expression of P-glycoprotein in the blood-brain barrier is significantly higher in resistant than responsive rats

Löschner, Seizure, 2011
Emerging Models of Pharmacoresistance

• Provides a mechanism to “differentiate” anticonvulsant profile.
• Provides a platform to study mechanisms underlying pharmacoresistance.
• However, it is not clear whether inclusion of emerging models in the AED discovery process will yield more effective therapies.
• Nonetheless, it is worth the consideration.
Summary and Conclusions

• Current approach **DOES** identify clinically effective AEDs.
• Despite the success that we have enjoyed, there still remains a **SUBSTANTIAL NEED** for more effective therapies.
• There is a need to determine whether a new therapy has a potential to be **DISEASE MODIFYING**.
• There is a way forward; however, it will require an investment in time and resources.
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