The role of immune system in RSE: preclinical perspectives

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Disclosure

Nothing to disclose
Learning Objectives

• to understand the role of immunity and inflammation in status epilepticus and in epilepsy

• to describe preclinical data on candidate new targets for novel therapies
Is activation of the immune system a common pathogenetic mechanism?

**Experimental precipitating events of SE to study the role of immune system:**

- Chemoconvulsants/electrical stimulation
- Febrile status epilepticus (immature rodents)
- Systemic/CNS inflammation+ SE

**Table 1. Etiology of status epilepticus**

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From Treiman, ref. 105, with permission.
IL-1 Receptor/Toll-like Receptor signaling

Pathogen associated molecular pattern

Microbial-clearance and killing pathways
Apoptotic and necrotic cell-death pathways

Inflammatory mediators

Innate immune system

Sterile inflammation

Status epilepticus
Brain injury
Spontaneous Seizures

Cytokines, Chemokines
mTOR, Complement cascade
Cell adhesion Molecules
Metalloproteases
Cox-2

Neuronal hyperexcitability
Structure of this presentation

Relevant information to design pharmacological intervention

• **Temporal evolution** of the inflammatory process after the first injury

• **Cell types** expressing the inflammatory mediators and responding to them

• **Adult vs immature** brain

Pharmacological intervention

• Intervention **during status epilepticus** to block unremitting seizures

• Intervention **post-SE** to resolve long-term consequences
Inflammatory gene response following SE induced in adult rodents

**Soman**

- **IL-1β**
- **Piriform Cortex**

- mRNA level (normalized to HPRT)

- **Time following soman exposure**
  - 30min
  - 1h
  - 2h
  - 6h
  - 24h
  - 48h
  - d7
  - control

**Electrical SE**

- **Hippocampus**

- **De Simoni et al, 2000**

- **IL-1β**
- **IL-1Ra**

- Different triggers but common features
  - Rapid onset
  - Long lasting
  - Lack of resolution

*Dhote et al, 2007; see also Svensson et al, 2001; Williams et al, 2003*
SE-induced epilepsy in adult rodents

SE induces inflammation

Granulocytes
Macrophages

Latent period (epileptogenesis)

Inflammation outlasts acute seizures

BBB damage

Inflammation persists in epileptic tissue

Ravizza et al, 2008; Marcon et al, 2009; see also Voutsinos-Porche et al, 2004; Dhote et al, 2007; Kutykin-Teplyakov et al, 2009
FEBRILE SE induced by hyperthermia in P14 mice

- IL-1β induced in astrocytes in the absence of cell loss

- Inflammation persists only in epileptic animals (~30%)

Seizure threshold depends on IL-1β

Dubé et al, Ann Neurol, 2005
Heida et al, Epilepsia, 2005

Dubé et al, J Neurosci, 2010
Effects of **pre-existing inflammation** in infant rats on SE

Inflammation in early post-natal development increases SE-induced neuronal cell loss

In adulthood, rats are more susceptible to SE induction and show increased SE-related cell loss

Auvin, Mazarati and Sankar, 2007

Galic and Pittman, 2008; Riazi and Pittman, 2008; Riazi et al, 2010
Human brain: common inflammatory molecules in different etiologies

Activation of IL-1R/TLR signaling

IL-1β (neurons & glia)

mTLE

IL-1R1 (neurons & glia)

HMGB1 (astrocytes & microglia)

TLR4: neurons & astrocytes

RAGE: astrocytes & vessels

Crespel et al, 2002; Boer, Crino et al, 2008; 2010, 2012
Innate immune mechanisms are activated in microglia, astrocytes, neurons during status epilepticus in experimental models & in human epilepsy.

Innate immunity cells express receptors for:
- Glutamate
- ATP (P2X7)
- Low iK⁺, high iCa²⁺ activate the inflammasome

Consequences?
- Ongoing seizures
- Neuropathology
- Spontaneous seizures
- Comorbidities

Brain Injuries
- Seizures
- Brain inflammation

Summary
Treatment options: there are available drugs in clinical use

**IL-1β has proconvulsive effects**
(Vezzani et al, BBI, 2011)

Blockade of IL-1β synthesis (**VX-765**) or IL1 receptor (**IL-1Ra/Anakinra**) reduce seizure recurrence in acute and chronic seizure models

**VX-765** in Phase IIb clinical trial in drug resistant partial epilepsy

**Anakinra** is used in rheumatoid arthritis

Vezzani et al, Curr Opin Investig Drugs, 2010
Is time of antiinflammatory intervention critical?  YES

Anakinra (IL-1ra): Vezzani et al, PNAS 2000; Epilepsia 2002
Marchi et al, Neurobiol Dis, 2011

Antiseizure effects after early intervention

Electrical SE in rats treated after 3 hours with anakinra:
Non significant contribution to the active seizure phase although neuroprotection was afforded
(Vezzani & Loscher, unpublished)

• HMGB1/TLR4
• Activation of P2X7 receptors (Faseb J, 2012)
• Complement cascade (Xiong et al, 2003; Aronica et al, 2007)

Concomitant blockade of different pathways may be required to attain optimal control of inflammation
Is the target important? COX-2 inhibition can potentiate status epilepticus

PGE2 Proconvulsant

PGF2 Anticonvulsant

Kim et al, 2007

N-Mazzacoratti et al, 1995
Post-injury COX-2 inactivation or antagonism of EP2 receptor for PGE2 results in neuroprotection

Lack of COX-2 in neurons: reduced hippocampal cell loss (Serrano et al, 2011)

CA1

Parecoxib, Polascheck et al, 2010; Celocoxib, Jung et al, 2005: neuroprotective

SC-58236 worsens outcome of SE, Holtman et al, 2010
Challenge for pharmacotherapy: disease-modifying drugs
Is brain inflammation a promising target?

- **NSAID**: celecoxib, parecoxib
- **Immunosuppressants**: fingolimod
- **Anti-integrins antibodies**: fingolimod
- **Glia activation inhibitors**: minocycline, Resveratrol

**Status epilepticus**

- **Prevention**
  - Frequency
  - Seizure duration
  - Seizure type
- **Seizure modification**
- **Cure**

**Disease or Syndrome Modification**

**Reversal of pathology**

**Co-morbidity modification**

**Inflammation**
- LPS/TLR4
- Poly I:C/TLR3

**Neurobehavioral deficits**
- Learning and memory
- Mood and behavior
- Other

*Controversial results on seizures outcome

Mazarati et al., 2010; Galic et al, 2008; Riazi et al, 2008

From A. Pitkanen, Epilepsia 2010; reviewed in Ravizza et al, Neurosci Lett, 2011
Innate immunity and inflammation contribute to the consequences of status epilepticus.

- Neuropathology
  - Spontaneous seizures
  - Comorbidities
Consequences of BBB damage

• Albumin induces synthesis of inflammatory mediators in astrocytes

• Albumin induces astrocytes dysfunction: K+ buffering and glutamate reuptake are reduced

  Friedman et al, Epilepsy Res, 2009

• Albumin increases tissue excitability and reduces seizures threshold

  Ivens et al, 2007; Frigerio et al, 2012
**Summary & Conclusions**

SE activates inflammatory pathways in brain:
- Activation of innate immune mechanisms in brain cells (glia, neurons, endothelial cells)
- Recruitment of leukocytes (*post-SE phase*)

Inflammation promotes SE:
- Acute phase and long term consequences

Inflammation contributes to:
- Neuronal hyperexcitability
- Cell loss
- BBB dysfunction
- Comorbidities

- Anti-inflammatory intervention to control unremitting seizures should be **fast**
- **Combined treatments** should be contemplated

- Delayed (post-injury) anti-inflammatory intervention **ameliorates the outcome:**
  - Survival
  - Neuropathology
  - Spontaneous seizures

**DRUGS ARE AVAILABLE FROM CNS & NON CNS INFLAMMATORY DISEASES**