Inhibiting inflammation in epilepsy

By Tim Fulmer, Senior Writer

A team of Italian and Dutch academics has identified two proinflammatory targets in the brain—toll-like receptor 4 (TLR4) and high mobility group box 1 (HMGB1)—that help drive epileptic seizures in mice.¹ **HMGBiotech s.r.l.**, which is associated with one of the researchers, has already begun developing brain-penetrant HMGB1 antagonists. Another Italian biotech, **Bio3 Research s.r.l.**, is now evaluating the potential to use its cardiovascular HMGB1 inhibitor in epilepsy.

Most marketed epilepsy drugs target ion channels and receptors on the surface of neurons to suppress the rapid and excessive neuronal firing that triggers a seizure. However, several labs have shown that epilepsy is often associated with inflammation, which in some cases can be the primary driver of disease.

Inflammation can originate in the CNS, the periphery or some combination of the two.²

As a result, researchers have focused on two different anti-inflammatory strategies in epilepsy: either blocking systemic inflammation processes that can disrupt the blood brain barrier (BBB) and cause CNS dysfunction or blocking CNS inflammation directly.

Annamaria Vezzani, head of the laboratory for experimental neurology at **Mario Negri Institute for Pharmacological Research**, and colleagues have focused on the latter approach.

Previously, they identified a proinflammatory pathway that was upregulated during seizures in the brains of rodents with epilepsy.^{3,4} Activation of the pathway was dependent on MYD88 (myeloid differentiation primary response gene 88), a cytosolic protein that interacts with cell-surface receptors, including toll-like receptors (TLRs), to transmit proinflammatory signals to the nucleus of microglia, astrocytes and endothelial cells.

In their latest work, the researchers hypothesized that antagonizing TLRs might block or at least reduce the severity of epileptic seizures triggered by activation of the pathway. To test this hypothesis, the group focused on TLR4, which is expressed in the brain and is known to be involved in bacterial endotoxin-induced seizures in mice.^{5,6}

Mice with kainic acid-induced epileptic seizures had greater TLR4 expression in the hippocampus than control animals. Higher TLR4 further correlated with greater hippocampal expression of HMGB1, an endogenous TLR4 ligand that is released by injured tissue.

To confirm the findings in humans, the researchers looked at surgical hippocampal tissue from patients with drug-resistant temporal lobe

epilepsy and saw that expression of both TLR4 and HMGB1 was higher than that in control hippocampal tissue.

Next, Vezzani and colleagues tested whether antagonizing HMGB1 or TLR4 would reduce the duration and frequency of seizures in epileptic mice.

Intracranial injection of an HMGB1 antagonist called BoxA significantly delayed the onset of acute seizures and reduced seizure frequency compared with injection of vehicle ($p < 0.01$).

Similarly, the TLR4 antagonist lipopolysaccharide (LPS) delayed acute seizure onset and reduced seizure frequency compared with vehicle ($p < 0.01$).

Compared with baseline, BoxA and LPS also significantly reduced the number and frequency of seizures in a mouse model of chronic seizures ($p < 0.01$).

The study was co-led by Vezzani and Marco Bianchi, a professor of molecular biology at **San Raffaele University** who founded HMGBiotech. The findings were published in *Nature Medicine*.

Targeting HMGB1 via the BBB

Bianchi told *SciBX* that BoxA is not an ideal therapeutic candidate because it requires intracranial injection and has a short serum half-life. HMGBiotech is thus focused on developing HMGB1-targeting compounds that cross the BBB and don't require direct CNS delivery.

The company also will consider partnering with other research groups to work on projects that target HMGB1 in epilepsy and undisclosed indications, Bianchi said.

Bio3 could be one such partner. The company owns IP covering the composition of matter and use of antibody mimics and HMGB1 fragments (including BoxA) that bind and antagonize HMGB1 to treat cardiovascular disorders.

Bio3 and partner **Protelica** (formerly ProtElix Inc.) are designing and developing antibody mimics that target HMGB1. Their lead compound, R2F8, will soon enter preclinical development to prevent arterial stenosis and restenosis.

Bio3 president and CEO Francesco Paolo Pilato told *SciBX* that based on the *Nature Medicine* findings, "Bio3 Research and Protelica are now considering the use of R2F8 or its analogs to treat epilepsy. To the best of our knowledge, no IP exists covering HMGB1 antagonists as therapeutics in epilepsy. We therefore see an opportunity to extend the use of R2F8 to this additional indication."

According to Bianchi, the epilepsy findings are not patented. He added that HMGBiotech owns a patent "on targeting HMGB1 to increase or decrease antigenicity in the context of autoimmune diseases, including lupus." Bianchi and colleagues have also published on the role of HMGB1 in the pathogenesis of inflammatory disorders, including systemic lupus erythematosus (SLE).^{7,8}

Similar to targeting HMGB1, getting TLR4-targeting molecules into the CNS "would be a major challenge," said Greg Elson, senior scientist and project leader at **NovImmune S.A.** "Delivery of mAbs to the brain via noninvasive techniques is probably not a current option."

However, in diseases such as epilepsy, Elson said it is possible that CNS inflammation could increase BBB permeability to allow small but potentially effective quantities of a mAb to enter the brain.

NovImmune's NI-0101 is an anti-TLR4 mAb that blocks TLR4 dimerization. It is in preclinical development to treat autoimmune and inflammatory disorders, including acute and chronic lung-associated diseases.

At least three companies have small molecule TLR4 antagonists, though none is in development for neurological disorders. **GlaxoSmith-Kline plc** has CRX-526, which was in preclinical development for sepsis as of 2008.⁹ Eritoran (E5564) from **Eisai Co. Ltd.** is in Phase III testing to treat severe sepsis, and TAK-242 from **Takeda Pharmaceutical Co. Ltd.** was in Phase III testing to treat sepsis before the company discontinued development of the compound in 2009 for undisclosed reasons.

Brain or the BBB

Even if targeting inflammatory processes produces better results than approved epilepsy drugs that target aberrant neuronal activity, there remains the unanswered question of whether the inflammation originates in the CNS or elsewhere.

Compounds that target CNS inflammation, such as TLR4 and HMGB1 antagonists, could have advantages over marketed therapies which "do not have an impact on the underlying pathogenic mechanisms of epilepsy," said Jacqueline French, professor of neurology at **New York University** and director of the Clinical Trials Consortium at NYU's Comprehensive Epilepsy Center.

The *Nature Medicine* paper's approach, "like other approaches that target the adverse consequences of CNS inflammation, may have such an impact," she said.

Indeed, said French, targeting CNS inflammation "may reduce ongoing damage that is caused as each seizure occurs. Although it is not proven, there is reason to believe that inflammation leads to increased risk of seizure, which in turn leads to further inflammation, setting up a very vicious cycle."

Work by other researchers has pointed to systemic inflammation as a primary driver of epileptic seizures.

"The findings presented by Vezzani and colleagues give the impression that targeting CNS inflammation is the only anti-inflammatory strategy in development for treating and preventing epileptic seizures. However, we and others have shown that systemic inflammation leads to disruption and opening of the BBB, with subsequent loss of cerebrovascular control and development of epileptic seizures," said Damir Janigro, professor of molecular medicine at the **Cleveland Clinic Lerner College of Medicine of Case Western Reserve University**.

"Thus, according to our model, the goal is not getting anti-inflammatory therapies into the brain but into the systemic circulation, where they can reduce BBB inflammation and prevent the vascular leakiness that causes downstream CNS dysfunction and epileptic seizures," said Janigro.

Janigro has found several lines of evidence to support targeting peripheral rather than CNS inflammation to treat epileptic seizures.

In pigs, disruption of the BBB was sufficient to cause epilepsy-like seizures even in the absence of CNS pathology.¹⁰ In at least one mouse model of epilepsy, increased serum levels of the proinflammatory cytokine IL-1 β preceded the occurrence of seizures. A systemic IL-1 β antagonist reversed the seizures.¹¹

In children with refractory epilepsy, systemic corticosteroids reduced seizure frequency compared with vehicle and, according to Janigro, probably did so at least partly by reducing BBB inflammation.¹¹

At the end of the day, "it's not yet clear that both CNS and BBB inflammation are at work in all epilepsy patients—more work needs to be done to determine this. In any case, both types will need to be addressed therapeutically," said French.

Fulmer, T. *SciBX* 3(15); doi:10.1038/scibx.2010.456
Published online April 15, 2010

REFERENCES

1. Maroso, M. *et al. Nat. Med.*; published online March 28, 2010; doi:10.1038/nm.2127
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2. Lucas, S.-M. *et al. Br. J. Pharmacol.* **147**, S232–S240 (2006)
3. Balosso, S. *et al. Brain* **131**, 3256–3265 (2008)
4. Ravizza, T. *et al. Neurobiol. Dis.* **31**, 327–333 (2008)
5. Chakravarty, S. & Herkenham, M. *J. Neurosci.* **25**, 1788–1796 (2005)
6. Sayyah, M. *et al. Neuroscience* **122**, 1073–1080 (2003)
7. Urbonaviciute, V. *et al. J. Exp. Med.* **205**, 3007–3018 (2008)
8. Bianchi, M. *J. Leukoc. Biol.* **86**, 573–576 (2009)
9. Gao, H. *et al. Critical Care* **12**, 213; published online May 6, 2008; doi:10.1186/cc6862
10. Marchi, N. *et al. Epilepsia* **48**, 732–742 (2007)
11. Marchi, N. *et al. Neurobiol. Dis.* **33**, 171–181 (2008)

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