StEPing “EP2” to Prevent Status Epilepticus–Induced Mortality and Inflammation

Inhibition of the Prostaglandin Receptor EP2 Following Status Epilepticus Reduces Delayed Mortality and Brain Inflammation.

Prostaglandin E2 is now widely recognized to play critical roles in brain inflammation and injury, although the responsible prostaglandin receptors have not been fully identified. We developed a potent and selective antagonist for the prostaglandin E2 receptor subtype EP2, TG6-10-1, with a sufficient pharmacokinetic profile to be used in vivo. We found that in the mouse pilocarpine model of status epilepticus (SE), systemic administration of TG6-10-1 completely recapitulates the effects of conditional ablation of cyclooxygenase-2 from principal forebrain neurons, namely reduced delayed mortality, accelerated recovery from weight loss, reduced brain inflammation, prevention of blood–brain barrier opening, and neuroprotection in the hippocampus, without modifying seizures acutely. Prolonged SE in humans causes high mortality and morbidity that are associated with brain inflammation and injury, but currently the only effective treatment is to stop the seizures quickly enough with anticonvulsants to prevent brain damage. Our results suggest that the prostaglandin receptor EP2 is critically involved in neuroinflammation and neurodegeneration, and point to EP2 receptor antagonism as an adjunctive therapeutic strategy to treat SE.

Commentary
A substantial body of literature indicates that inflammation plays a key role in mediating seizure-induced brain injury and epileptogenesis leading to a quest for novel inflammatory mediators as therapeutic targets for epilepsy. Cyclooxygenase-2 (COX-2) is among several key inflammatory factors known to promote seizure-induced brain inflammation. It is rapidly induced by seizures in select brain regions and known to promote seizure-induced neuronal loss, leukocyte infiltration, astrogliaosis, microglial activation, and breakdown of the blood–brain barrier (BBB). COX-2 has been explored as a therapeutic target for neuroprotection in epilepsy using a variety of approaches (1). While global inhibition of COX2 by genetic or pharmacologic approaches was complicated by its early protective versus delayed deleterious role in seizure-induced brain injury, a greater clarity was observed in a conditional knock-out mouse in which the COX-2 gene was selectively deleted postnatally in forebrain neurons specifically upregulating COX-2 after seizures. Forebrain-specific conditional COX-2 knock-out mice demonstrated delayed neuroprotection, decreased release of inflammatory mediators, and BBB permeability after seizures (2). Prostaglandin E2 (PGE2) is a major product of COX-2 in the brain and can activate four G-protein coupled receptors (GPCRs): EP1, EP2, EP3, and EP4. Whereas, PGE2 is considered a crucial mediator of COX-2–induced events following seizures, what specific class of prostanoid receptors mediates seizure-induced inflammation and neuronal death is unknown and the subject of investigation by Jiang et al. (3). Of the four receptors, EP2 receptor is expressed in both neurons and glia, and its activation is thought to promote inflammation and neurotoxicity in animal models of several neurodegenerative diseases. However, EP2 activation by PGE2 has been shown to be neuroprotective after ischemia and to promote spatial learning (3, 4).

In an effort to elucidate EP2’s functions, Jiang et al. have previously utilized a high-throughput cell-based time-resolved fluorescence resonance energy transfer (TR-FRET) assay to identify selective allosteric potentiators of the human EP2 receptor, which conferred neuroprotection against NMDA-induced excitotoxicity in cultured hippocampal neurons (5). These initial studies accompanied by the observation that EP2 activation has some pathological consequences such as potentiation of inflammatory responses, allowed them to hypothesize that pharmacologic blockade of the PGE2/EP2 signaling might represent an innovative approach to mitigate delayed inflammation and neuronal damage induced by prolonged status epilepticus (SE). In earlier studies, the group developed a brain-permeable small molecule EP2 antagonist (TG4-155) that completely suppressed the induction of COX-2 mRNA in cultured microglia by EP2 activation and significantly reduced hippocampal neuronal injury in mice following pilocarpine-induced SE. However,
this molecule had a relatively short half-life and a low brain to plasma ratio (3). In the current study, Jiang et al. overcame the unfavorable pharmacokinetic properties of TG4-155 with a novel compound, TG6-10-1, making significant headway in their efforts to develop a more potent EP2 antagonist for the therapeutic attenuation of SE-induced neuronal damage and associated morbidities. TG6-10-1 possessed a superior pharmacokinetic profile for in vivo use coupled with high potency in the low nanomolar range for the EP2 receptor. Of importance, administration of TG6-10-1 four hours after SE demonstrated a wide variety of protective effects on neuroinflammation, mortality, and neurodegeneration following pilocarpine-induced SE. Notably, these effects were revealed without exerting an acute anticonvulsant effect (i.e., modifying SE).

Jiang et al. determined the potency of TG6-10-1 in C6G-EP2 cells by evaluating its effects using the TR-FRET assay, similar to their previous studies in which PGE2-induced cAMP accumulation in C6G cells overexpressing human EP2 receptor was monitored. The results indicated a competitive mechanism of antagonism by TG6-10-1 for the EP2 receptor coupled with a 300-fold selectivity for the EP2 receptor compared with human EP3 and EP4 receptors. Additionally, it had negligible off-target effects in vitro. TG6-10-1 displayed a plasma half-life of approximately 1.6 hours and a brain to plasma ratio of 1.6 after systemic administration in mice, which was a significant improvement from its predecessors. Following the in-vitro studies, the authors proceeded to test the effect of TG6-10-1on EP2-receptor inhibition on pilocarpine-induced SE in C57BL/6 mice. Administration of the compound four hours after SE caused a significant increase in survival compared with the vehicle group, improving 1-week survival from 60 to 90% in addition to accelerating the recovery of lost weight after SE. Animals that were treated with the compound showed normal behavior 4 days after SE compared with vehicle-treated animals. These results reveal the beneficial effects of the EP2 antagonist on survival, weight loss, and improved functional recovery after pilocarpine-induced SE.

Next, the authors addressed whether TG6-10-1 attenuated brain proinflammatory response after pilocarpine-induced SE. To address this, the mRNA levels of a variety of cytokines and chemokines were measured in mice receiving either vehicle or the EP2 antagonist together with markers of gliosis in the hippocampi 4 days after SE. Administration of TG6-10-1 substantially decreased SE-induced increase in inflammatory markers, which supports EP2 receptor involvement in seizure-induced brain inflammation. The compound showed significant reduction in BBB breakdown following SE. Furthermore, treatment with the EP2 antagonist significantly decreased SE-induced neurodegeneration in the CA1, CA3, and hilar regions of the hippocampus as indicated by Fluoro-Jade B staining, strengthening the link between EP2-receptor activation and neuronal death following SE.

Finally, the authors investigated whether the beneficial effects of TG6-10-1 were caused by a direct anticonvulsant effect or a true anti-inflammatory effect. Two approaches were used to rule out TG6-10-1’s ability to alter pilocarpine-induced SE. In the first approach, a pre-treatment paradigm demonstrated that the compound did not alter behavioral seizures or latency to onset of SE. A second post-treatment paradigm was employed in which the compound was administered after SE in animals monitored by continuous cortical EEG recordings. This study showed that TG6-10-1 did not change abnormal epileptiform activity over a 48-hour period or reentrance into seizure activity following recovery from pentobarbital.

Jiang et al. thus addressed an important question regarding the involvement of PGE2 in mediating seizure-induced brain inflammation and neuronal injury. Their studies highlight the role of inflammation via the COX2-PGE2-EP2 receptor pathway in the neuropathology associated with pilocarpine-induced SE. The development of a more potent antagonist with improved in-vivo pharmacokinetic profile, which could be used as an adjunctive therapy to mitigate the effects of SE on neuroinflammation and neurodegeneration as well as delayed mortality, is a crucial achievement of this study. A secondary but very critical observation was that the compound did not have any direct anticonvulsant effect, which further reinforces their hypothesis. However, this work also brings forth several important unresolved issues. Given the literature suggesting an epileptogenic role of proinflammatory cytokines and neuroinflammation, it is curious that TG6-10-1 did not prevent epilepsy development in this study despite inhibiting several key proinflammatory cytokines. Since pilocarpine results in seizures by activation of cholineric receptors and is used as a surrogate for nerve-agent toxicity, can these findings be generalized to nerve-agent neurotoxicity? Finally, since the authors noted beneficial behavioral effects in TG6-10-1–treated animals, will this translate to long-term improved cognitive function in animals treated with the compound? Answering these questions might further strengthen the case for targeting inflammation in epilepsy via EP2 activation.

The results bring to light a very significant mechanism by which COX-2 might be mediating inflammation and neuronal injury following prolonged seizures and further validate EP2-receptor inhibition as a therapeutic target. Since the long-term use of selective COX-2 inhibitors exerts adverse cardiovascular effects, the present study provides a potential avenue for the treatment of SE by EP2 inhibition. This article also exhibits a plausible mechanism by which PGE2 exerts its effects on inflammation and neurodegeneration after SE. In summary, TG6-10-1 caused a decrease in inflammation and conferred neuroprotection in addition to improving survival, accelerating recovery of weight loss, and improving functional recovery thus opening up the possibility of using EP2 inhibition as an adjunctive therapy along with benzodiazepines to treat seizure-induced neuronal injury.

By Pallavi Bhuyan, MS, and Manisha Patel, PhD

References
brain neurons is neuroprotective and dampens brain inflammation after status epilepticus. /Neurosci/ 2011;31:14850–14860.
American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.
This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.
This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships
Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.
Section #1 Identifying Information

1. Today’s Date: 1/2/14
2. First Name Manisha Last Name Patel Degree PhD
3. Are you the Main Assigned Author? ☒ Yes ☐ No
   If no, enter your name as co-author:
4. Manuscript/Article Title: StEPing “EP2” to Status Epilepticus–Induced Mortality and Inflammation.
5. Journal Issue you are submitting for: January 2014

Section #2 The Work Under Consideration for Publication
Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Grant</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consulting fee or honorarium</td>
<td>☐</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Support for travel to meetings for the study or other purposes</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Payment for writing or reviewing the manuscript</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Provision of writing assistance, medicines, equipment, or administrative support.</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Other</td>
<td>☒</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts on this study.
** Use this section to provide any needed explanation.
Section #3  Relevant financial activities outside the submitted work.
Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

<table>
<thead>
<tr>
<th>Type of relationship (in alphabetical order)</th>
<th>No</th>
<th>Money Paid to You</th>
<th>Money to Your Institution*</th>
<th>Name of Entity</th>
<th>Comments**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Board membership</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Consultancy</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Employment</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Expert testimony</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Grants/grants pending</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Payment for lectures including service on speakers bureaus</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Payment for manuscript preparation.</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Royalties</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* This means money that your institution received for your efforts.
** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4  Other relationships
Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☑ No other relationships/conditions/circumstances that present a potential conflict of interest.
☐ Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.

Epilepsy Currents Editorial Board