LOOKING AHEAD

SIGNALING TO P-GLYCOPROTEIN—A NEW THERAPEUTIC TARGET TO TREAT DRUG-RESISTANT EPILEPSY?

Recent evidence links epileptic seizures to upregulation of P-glycoprotein, which may, in part, be responsible for antiepileptic drug resistance.

by Anika M.S. Hartz, Sylvia Notenboom and Björn Bauer

Epilepsy is a serious neurological disorder that affects more than 60 million people worldwide. The majority of epileptic patients can be treated with antiepileptic drugs, but up to 40% of patients, more than 20 million people including 2 million children under the age of 15, do not respond well to pharmacotherapy.[1-3] The consequences for epileptic patients resistant to treatment are severe. A high incidence of uncontrolled seizures elevates the risk of brain damage and increases mortality rates.[4,5] Increasing evidence suggests that therapeutic failure in drug-resistant epilepsy is in part due to overexpression of the drug efflux transporter P-glycoprotein at the blood–brain barrier.[6-8] In this paper we review recent findings on the signaling pathway that leads to P-glycoprotein upregulation in epilepsy and that could potentially serve as a new therapeutic target to treat drug-resistant epilepsy.

P-GLYCOPROTEIN IN DRUG-RESISTANT EPILEPSY

The molecular cause for drug resistance in epilepsy is not fully understood. One theory is the multidrug transporter hypothesis that is based on the seminal observation from 1995 by Tishler et al., who showed that mRNA of *ABCB1*, the gene coding for the drug efflux transporter P-glycoprotein, is significantly upregulated at the blood–brain barrier of patients with drug-resistant epilepsy.[8] This was a critical discovery because P-glycoprotein acts as a "gatekeeper" and limits a large number of therapeutic drugs from crossing the blood–brain barrier and therefore, from entering the brain.[9]

Other groups confirmed the findings by Tishler et al. and it was suggested that upregulation of P-glycoprotein at the blood–brain barrier could prevent antiepileptic drugs from accessing the brain and cause drug resistance in epilepsy (Fig. 1).[7,10,11] Indeed, in addition to a substantial amount of in vitro evidence, recent in vivo data demonstrated that P-glycoprotein limits antiepileptic drugs from penetrating the brain. Using a drug-resistant epilepsy rat model, Volk and Loescher[12] and Potschka et al.[13] showed that animals not responding to the antiepileptic drugs phenobarbital and phenytoin exhibited a two-fold increase in P-glycoprotein expression at the blood–brain barrier compared to animals responding to treatment. Brandt et al.[14] and van Vliet et al.[15] confirmed these findings and demonstrated that inhibiting blood–brain barrier P-glycoprotein counteracted phenobarbital and phenytoin resistance, which decreased seizure occurrence in rats. This was the first in vivo proof-of-concept of the multidrug transporter hypothesis of drug-resistant epilepsy.

Additional evidence obtained from human brain tissue confirmed these findings found in animals. Marchi et al.[16] showed that patients with high blood–brain barrier *ABCB1* mRNA expression had low antiepileptic drug brain levels. Cucullo et al., using brain capillary endothelial cells from cerebral cortex biopsies from normal and drug-resistant epileptic patients, demonstrated that phenytoin permeation was 10-fold lower in drug-resistant cells but that

SUMMARY

Epilepsy affects more than 60 million people worldwide. While most patients can be treated with antiepileptic drugs, up to 40% of patients respond poorly to pharmacotherapy. This drug resistance is not well understood and presents a major clinical problem. In this short review we provide background information on one potential cause of antiepileptic drug resistance, namely, upregulation of the drug efflux transporter P-glycoprotein at the blood–brain barrier. We summarize recent findings that connect antiepileptic drug resistance with P-glycoprotein upregulation and show a mechanistic link between seizures and upregulation of this transporter. We provide an overview of results demonstrating that glutamate released during seizures signals through *N*-methyl-D-aspartate (NMDA) receptor and cyclooxygenase-2 (COX-2) to increase P-glycoprotein. In this context we discuss the NMDA receptor and COX-2 as potential therapeutic targets and provide information on current clinical trials on drug-resistant epilepsy involving blood–brain barrier efflux transporters. Finally, we provide a perspective on future research that could help improve the treatment of drug-resistant epilepsy.

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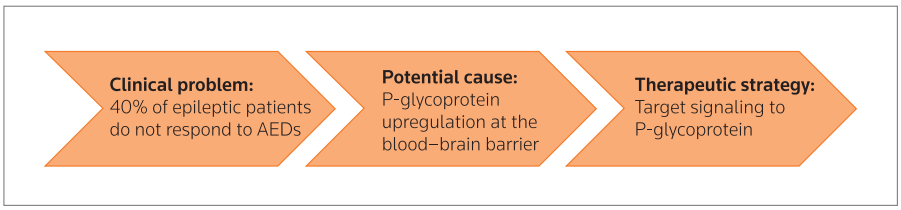

Figure 1. Drug-resistant epilepsy: clinical problem, potential cause and novel therapeutic strategy. AED, antiepileptic drug.

inhibiting P-glycoprotein significantly increased phenytoin permeation.[17] In agreement with this, Luna-Tortos et al. showed that transport of phenytoin and other antiepileptic drugs was mediated by human P-glycoprotein.[18]

These studies underline that in drug-resistant epilepsy, antiepileptic drugs have restricted access to the brain, at least in part, due to P-glycoprotein upregulation at the blood–brain barrier. They also indicate that modulation of P-glycoprotein transport activity may enhance brain distribution of some antiepileptic drugs, which could be used as a therapeutic strategy in drug-resistant epilepsy.[13,15,19,20]

THERAPEUTIC STRATEGIES TO OVERCOME P-GLYCOPROTEIN IN DRUG-RESISTANT EPILEPSY

Two strategies are currently available to modulate P-glycoprotein transport activity at the blood–brain barrier. The first one is direct inhibition of transporter function; the second one targets the signaling pathways that control P-glycoprotein expression and function.

Direct inhibition of P-glycoprotein

The Ca^{2+}-channel blocker verapamil was the first compound that was found to inhibit P-glycoprotein function and has since been used in countless in vitro and in vivo animal studies to overcome drug resistance.[21] Regarding epilepsy, few clinical case reports exist where co-administration of verapamil was used to improve seizure control with antiepileptic drugs in patients who were unresponsive to various combinations of drug therapies.[22-24] Encouraged by such studies, two clinical trials, using verapamil and the β-blocker carvedilol to inhibit P-glycoprotein-mediated drug resistance, have recently been initiated.[25,26] While verapamil and carvedilol are FDA approved for arrhythmia and congestive heart failure, respectively, and are readily available, neither drug is a highly specific or potent P-glycoprotein inhibitor.[27,28] Presumably high plasma concentrations will be needed to effectively inhibit overexpressed P-glycoprotein in drug-resistant epilepsy with either drug. While no drug–drug interactions or toxic side effects due to verapamil were observed in the clinical cases mentioned above,[22-24] there is a potential for such risks, e.g., cardiotoxicity, as was observed in clinical trials using verapamil in cancer patients with P-glycoprotein-mediated drug resistance.[29] Depending on the outcome of these trials, more potent and selective P-glycoprotein inhibitors, such as valspodar (PSC-833), tariquidar (XR-9576), laniquidar (R-101933) and zosuquidar (LY-335979), which have been tested for various multidrug-resistant cancer types, might be potential treatment options for future trials.

Targeting signaling to P-glycoprotein

A second strategy to increase brain levels of antiepileptic drugs in drug-resistant epilepsy is to target and interrupt the signaling pathway(s) that lead(s) to P-glycoprotein upregulation (Fig. 1). Such a strategy would allow controlled and selective opening of the barrier and provide a "window-in-time" during which antiepileptic drugs could be delivered to the brain with minimal disturbance of barrier function. This approach, however, requires detailed mechanistic knowledge of the link between drug-resistant epilepsy and P-glycoprotein upregulation at the blood–brain barrier. We are just beginning to understand the regulation of blood–brain barrier function on a molecular level and recent reports suggest a complex, context-dependent regulatory network that controls P-glycoprotein expression and transport activity.[9] In the following, we discuss a recently identified signaling pathway that leads to P-glycoprotein upregulation at the blood–brain barrier in epilepsy.

SIGNALING LEADING TO P-GLYCOPROTEIN UPREGULATION

While many studies have shown that P-glycoprotein is upregulated at the blood–brain barrier in drug-resistant epilepsy, the cause and mechanism leading to transporter upregulation remains to be elucidated. Recent results by our group and others have contributed to identifying one signaling pathway that seems to connect seizure activity with increased blood–brain barrier P-glycoprotein expression and transport activity (Fig. 2).

Seizures and glutamate release

One hallmark of epileptic seizures is neuronal and glial release of high amounts of glutamate, the major excitatory neurotransmitter in the brain.[30-32] It has been reported that normal interstitial glutamate concentrations of 0.2–0.5 µM can transiently increase up to 10–100 µM following a seizure.[33-36] In this regard, Zhu and Liu have demonstrated that exposing brain capillary endothelial cells to 100 µM glutamate mediated P-glycoprotein upregulation.[37] In agreement with this we have found glutamate induced P-glycoprotein expression and transport activity in isolated brain capillaries from rat and mouse ex vivo.[6] We also demonstrated that glutamate microinjections into the hippocampus of rats increased P-glycoprotein expression at the blood–

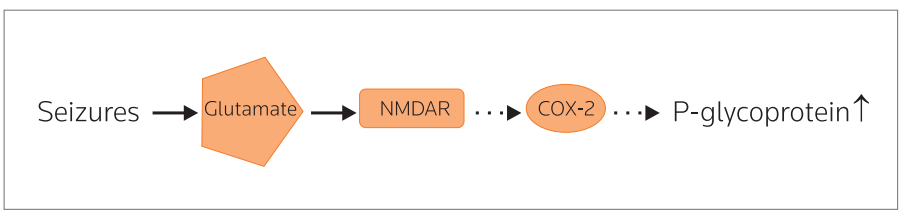

Figure 2. Proposed signaling mechanism that leads to P-glycoprotein upregulation in epilepsy. Glutamate released during seizures signals through the *N*-methyl-D-aspartate receptor (NMDAR) and cyclooxygenase-2 (COX-2) to increase P-glycoprotein at the blood–brain barrier.[6,53]

brain barrier in vivo. These findings indicate that seizure-induced glutamate release is one critical factor that contributes to P-glycoprotein upregulation at the blood–brain barrier in epilepsy.

Glutamate signaling through the *N*-methyl-D-aspartate receptor

Extracellular glutamate in the brain exerts its effects through membrane receptors, such as the *N*-methyl-D-aspartate (NMDA) receptor. Accordingly, excessive glutamate release during epileptic seizures and glutamate activation of NMDA receptors significantly contribute to epilepsy pathophysiology, including excitotoxic damage and neuronal death.[38,39] Consistent with glutamate signaling through the NMDA receptor, Zhu and Liu showed in cultured brain capillary endothelial cells that blocking the NMDA receptor with the specific receptor antagonist dizocilpine (MK-801) abolished glutamate-mediated P-glycoprotein upregulation.[37] We made the same observation in rat brain capillaries. We also detected protein expression of the NMDA receptor subunit NR1 in brain capillaries which has previously been shown by other groups in cultured brain endothelial cells.[6,40,41] Moreover, Bankstahl et al. recently demonstrated in rats, that using dizocilpine to block the NMDA receptor abolished seizure-induced P-glycoprotein upregulation at the blood–brain barrier in vivo.[42] These results strongly indicate that glutamate signals P-glycoprotein upregulation by acting through NMDA receptors expressed in plasma membranes of brain capillaries.

Glutamate signaling through cyclooxygenase-2

Epilepsy is known to be accompanied by CNS inflammation that is reflected by elevated brain levels of the inflammatory enzyme cyclooxygenase-2 (COX-2). In this regard, COX-2 involvement in epilepsy has been demonstrated in a number of animal epilepsy models as well as in the human epileptic brain.[43-46] Consistent with this are findings showing that brain levels of prostaglandins, proinflammatory factors derived from COX, are also elevated in epilepsy.[47,48] Moreover, it has repeatedly been shown that COX-2 is a downstream target of glutamate signaling through the NMDA receptor. For example, inhibiting COX-2 abolished NMDA-receptor-mediated neuronal damage[49,50] and blocking the NMDA receptor prevented formation of the COX product prostaglandin E_2.[51] One particular important finding was made by Patel et al. who demonstrated that COX-2 activation increased P-glycoprotein expression connecting COX-2-mediated inflammation to P-glycoprotein.[52] Based on these data we hypothesized that in epilepsy COX-2 is involved in the glutamate/NMDA receptor pathway that signals P-glycoprotein upregulation at the blood–brain barrier. Indeed, celecoxib, a specific COX-2 inhibitor, blocked glutamate-mediated upregulation of P-glycoprotein in isolated rat brain capillaries, whereas a specific COX-1 inhibitor was without effect.[6] Additionally, in brain capillaries from COX-2 knockout mice, glutamate did not increase P-glycoprotein expression or transport activity. Using a rat seizure model, we also demonstrated that seizure-induced P-glycoprotein upregulation was blocked by treating rats with indomethacin, an unspecific COX inhibitor, or with celecoxib, a specific COX-2 inhibitor.[6,53] These findings are consistent with seizure-initiated glutamate/NMDA receptor/COX-2 signaling that upregulates P-glycoprotein at the blood–brain barrier in epilepsy (Fig. 2).

CLINICAL TRIALS

As mentioned above, two clinical trials aimed at direct inhibition of blood–brain barrier P-glycoprotein in drug-resistant epilepsy are currently under way.[25,26] In a recently initiated trial, multidrug-resistant protein, another blood–brain barrier efflux transporter that is upregulated in epilepsy, will be targeted with probenecid to test whether this will increase phenytoin brain levels and reduce seizure frequency in antiepileptic drug-resistant patients.[54] The outcome of these clinical trials remains to be seen and may be critical for future therapeutic approaches to treat drug-resistant epilepsy.

In addition, the findings reviewed here suggest the NMDA receptor and COX-2 could potentially be valuable therapeutic targets to interrupt and prevent seizure-induced upregulation of blood–brain barrier P-glycoprotein. Although blocking the NMDA receptor in epileptic patients has not yet been studied, several NMDA receptor antagonists have been tested for their use in epilepsy.[55,56] These clinical trials were mostly unsuccessful and this was attributed to side effects due to the drugs' narrow therapeutic window.[57] COX-2 inhibition in long-term clinical trials caused gastrointestinal, renal, cerebrovascular and severe cardiovascular side effects that eventually led to the withdrawal of several COX-2 inhibitors from the market. As a result, celecoxib is contraindicated in patients with elevated cardiovascular risk. However, celecoxib doses required to inhibit COX-2 in the brain are lower than those required in arthritis, which reduces the potential for side effects.[58] Therefore, since COX-2 inhibition has not yet been tested in epileptic patients more research is needed to test this option. Should future animal studies with selective COX-2 inhibitors prove to be successful in overcoming antiepileptic drug resistance, translation of this strategy into the clinic could be relatively rapid since multiple FDA-approved COX-2 inhibitors are available. Clearly, given the potential risks associated with COX-2 inhibition, this step would require careful selection and monitoring of patients.

Targeting transporter regulation is certainly an interesting and innovative scientific approach. However, before clinical trials in humans can be conducted, more animal experiments are required to demonstrate that NMDA receptor or COX-2 inhibition is a valid therapeutic strategy to increase brain levels of antiepileptic drugs. Moreover, other signaling proteins involved in seizure-induced P-glycoprotein upregulation may prove to be better therapeutic targets to treat drug-resistant epilepsy. Current research in our laboratory is aimed at identifying such signaling proteins.

SUMMARY AND FUTURE PERSPECTIVES

This short review summarizes recent evidence that links epileptic seizures to upregulation of the blood–brain barrier drug transporter P-glycoprotein that may, in part, be responsible for antiepileptic drug resistance. This evidence indicates that glutamate released during seizures signals through the NMDA receptor and COX-2 to increase P-glycoprotein. Therefore, inhibition of these signaling proteins could potentially be used therapeutically to interrupt seizure-induced P-glycoprotein upregulation at the blood–brain barrier, thereby increasing brain levels of antiepileptic drugs

and improving seizure control in drug-resistant epilepsy. Animal studies will show whether the NMDA receptor or COX-2 are viable therapeutic targets. Future research should focus on identifying signaling proteins up- and downstream of COX-2 that could potentially be better targets to interrupt seizure-induced P-glycoprotein upregulation at the blood–brain barrier. Such research may lead to improved treatments of drug-resistant epilepsy.

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DISCLOSURE

The authors have no conflicts of interest to declare.

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