INTRODUCTION

Epilepsy and anti-epileptic drug resistance epilepsy

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. About 50 million people worldwide suffer from epilepsy; it is usually controlled but cannot be cured with medication, although surgery may be considered in difficult cases. Despite advances in anti-epileptic drug (AED) therapy and epilepsy surgery in recent years, AED-resistant epilepsy remains a major clinical problem. The consequences of uncontrolled epilepsy can be quite severe and include shortened lifespan, excessive bodily injury, neuropsychological impairment and social disability. An important characteristic of AED-resistant (refractory) epilepsy is that most affected patients are resistant to several, if not all, AEDs, even though these drugs act by different mechanisms.

HYPOTHESIS

Minocycline: A Reasonable Choice for Combination Therapy in Management of Pharmacoresistant Epilepsy

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ABSTRACT

About 30% of epileptic patients do not respond to the usual antiepileptic drugs (AEDs). There is accumulating evidence demonstrating that multi-drug transporters such as P-glycoprotein (P-gp) are over-expressed in capillary endothelial cells and astrocytes in epileptogenic brain tissue, resulting in impaired AED access to the site of action. It has also been indicated that glutamate release is critically involved in the over-expression of P-gp at the blood brain barrier (BBB). Glutamate signaling via the N-methyl-D-aspartate (NMDA) receptor is coupled to elevation of Ca²⁺, generation of intracellular reactive oxygen species (ROS) and activation of cyclooxygenase-2 (COX-2), and increased protein expression and transport activity of P-gp in isolated rat brain microvessel endothelial cell (BMEC) capillaries. In addition, conditions that generate ROS have been shown to increase P-gp expression in cells derived from liver and kidney. Minocycline, a second-generation tetracycline, has been reported to exert neuroprotective effects over various experimental and clinical models. It has been indicated that minocycline inhibits P-gp. The neuroprotective effects of Minocycline have been reported to involve antioxidant systems, prevention of the activation of Ca²⁺-dependent intracellular pathways, a marked decrease in glutamate release, and blockade of inflammation pathway inhibiting molecules such as COX-2 and inflammatory mediators such as reactive oxygen species (ROS) and Prostaglandin E2 (PGE₂). Therefore, it is hypothesized that minocycline is a reasonable choice for combination therapy in management of pharmacoresistant epilepsy.

Key Words: Minocycline; Pharmacoresistant Epilepsy; P-glycoprotein; Antiepileptic Drug
resistance (MDR) gene, located on the luminal membranes of BMECs.\textsuperscript{10} Because of its transmembrane location, P-gp functions as an efflux transporter that limits cellular uptake of drugs and toxins from the BBB into the brain. Therefore, P-gp plays an important role in the integrity of BBB and protects the brain from many exogenous toxins and sudden changes in the levels of cerebral transmitters.\textsuperscript{11} Seizures are known to increase the expression of drug efflux transporters in the BBB\textsuperscript{12} and it has been shown that some AEDs are substrates of P-gp.\textsuperscript{13,14} Recent experiments on animal models of epilepsy have shown that brain uptake of antiepileptic drugs can be significantly improved by co-administration of tariquidar, a selective and potent inhibitor of the P-gp drug efflux pump.\textsuperscript{12} Over-expression of such transporters in epileptogenic tissues is likely to reduce the amount of drug that reaches the epileptic neurons, which could possibly explain AED-resistance epilepsy.

Glutamate, Reactive oxygen species, P-glycoprotein and AED-resistant epilepsy

Excessive glutamate release during epileptic seizures and its signaling via N-methyl-D-aspartate (NMDA) receptors is a major contributor to the pathophysiology of epilepsy. In the epileptic brain, activation of the NMDA receptor and subsequent downstream events contribute to excitotoxic damage and loss of neurons.\textsuperscript{15,16} An experiment on isolated rat brain capillaries showed that extracellular glutamate at same concentration found in seizure signals increases P-gp in this tissue. Moreover, NMDA receptor antagonists counteract over-expression of P-gp in hippocampus microvessels, strongly indicating that glutamate release is critically involved in the over-expression of P-gp in the BBB.\textsuperscript{17} Activation of the NMDA receptor by glutamate is coupled to elevation of Ca\textsuperscript{2+} and generation of intracellular reactive oxygen species (ROS).\textsuperscript{18} Conditions that generate ROS have been shown to increase P-gp expression in cells derived from liver\textsuperscript{19} and kidney.\textsuperscript{20} In line with this suggestion, the ROS scavenger N-acetyl-cysteine prevented the glutamate-induced increase in P-gp.\textsuperscript{11} On the other hand, ROS is the link between seizure-induced glutamate release and over-expression of P-gp. The intermediate steps between ROS and P-gp over-expression are unknown at this point, but are likely to involve PGE\textsubscript{2} signaling and activation of downstream effectors that initiate transcription of MDR1, the gene encoding P-gp.\textsuperscript{22} Preventing expression of the P-gp transporter in the epileptic brain by blocking the NMDA receptor and COX-2 and ROS inhibition has the potential to improve the brain penetration and efficacy of AEDs that are P-gp substrates. This approach could allow a therapeutic brain level to be achieved with lower drug doses, which would decrease potential adverse side effects. Such a strategy could also help to over-

![Fig. 1 Brief summary of seizure-induced production of COX-2 and PGE\textsubscript{2}](image-url)

Glutamate interactions with G-protein-coupled metabotropic glutamate receptors and NMDA-gated Ca\textsuperscript{2+} channels lead to activation of PLC and PLA\textsubscript{2}, respectively. Hydrolysis of PIP\textsubscript{2} by PLC generates DAG and IP\textsubscript{3}, whereas AA-PL hydrolysis leads to rapid accumulation of AA. Free AA-DAG can then activate PKC. Metabolism of DAG also contributes to intracellular AA pools. PLA\textsubscript{2} is capable of generating PAF (via lyso-PAF) through hydrolysis of alkyl- AA-PC. PAF could positively regulate COX-2 expression in response to seizures. PLC-Phospholipase C; PLA\textsubscript{2}-Phospholipase A\textsubscript{2}; PIP\textsubscript{2}-Phosphatidyl 4,5 bisphosphate; DAG-Diacylglycerol; IP\textsubscript{3}-Inositol triphosphate ; AA-PL-Arachidonyl-containing phospholipids; AA-PL-Arachidonyl-arachidonyl-phosphatidylcholine: AA-Arachidonic Acid; AA-PL-Arachidonyl-containing phospholipids; AA-PL-Arachidonoyl-arachidonyl-diacylglycerol; COX-2-Cyclooxygenase-2; PGE\textsubscript{2}-Prostaglandin H\textsubscript{2}; PGE\textsubscript{2}-Prostaglandin E\textsubscript{2}; PAF-1-O-alkyl-2-acetyl-glycero-3-phosphocholine (27).
come pharmacoresistance in a certain subpopulation of patients who do not respond to treatment with AEDs at all.

Minocycline

Minocycline is a second-generation, semi-synthetic tetracycline analog, a highly lipophilic molecule that easily penetrates the BBB.\(^2^8\) It is effective against Gram-positive and Gram-negative infections.\(^2^9\) Recently, Milane et al. reported that minocycline inhibits P-gp, which is known to govern the pharmacokinetics and tissue distribution of many drugs.\(^3^0\) In addition to its original antimicrobial activities, minocycline has been reported to exert neuroprotective effects over various experimental models such as cerebral ischemia, traumatic brain injury, Parkinson and Alzheimer diseases.\(^2^8\) Because of its high tolerance and excellent penetration into the brain, minocycline has been clinically tried for some neurodegenerative diseases such as stroke, spinal cord injury and Parkinson’s disease.\(^3^0\) Several intracellular signaling pathways have been implicated in the mechanism of the neuroprotective actions of minocycline. For instance, these actions have been reported to involve antioxidant systems,\(^3^1\) prevention of the activation of Ca\(^{2+}\)-dependent intracellular pathways, a marked decrease in glutamate release,\(^3^2\) blockade of the inflammatory pathway, and inhibition of molecules such as COX-2\(^2^3,2^4,2^5\) and inflammatory mediators such as ROS and PGE\(_2\).\(^3^6\)

**HYPOTHESIS**

Active inflammation has been detected not only in prototypical inflammatory epilepsy such as Rasmussen’s encephalitis or limbic encephalitis, but also in pharmacoresistant epilepsy with diverse etiologies. Furthermore, microglial activation and proliferation have been demonstrated in adult patients with chronic intractable epilepsy.\(^3^6\)

Glutamate release is critically involved in the overexpression of P-gp in the BBB via ROS and the COX-2 pathway.\(^2^1,2^2\) There is accumulating evidence that up-regulation of P-gp contributes to drug-refractoriness by an enhanced BBB efflux of antiepileptic drugs, which seems to limit drug concentrations at the target sites in the epileptic brain.\(^8\) The authors believe that in view of the neuroprotective and anti-inflammatory properties of minocycline (suppression of COX-2 expression, inhibition of microglial cell activation and attenuation of PGE\(_2\) and ROS formation)\(^3^3,3^4,3^7\) and its inhibitory effect on P-gp,\(^3^1\) it is reasonable to propose that administration of minocycline in combination with AEDs might be effective adjunctive therapy for AEDs-resistant epilepsy.

**Hypothesis evaluation**

To study the role of minocycline in multidrug-resistant epilepsy, we propose the following protocol for investigation: Minocycline is an FDA-approved drug and is known to be safe; it can be examined in patients with multidrug-resistant epilepsy. For clinical evaluation, two groups of patients who are suffering from epilepsy and are resistant to routine pharmacotherapy such as Phenytoin, Carbamazepin, Phenobarbital and Lamotrigine can be selected:

- **Group A:** Different doses of minocycline will be added to the AEDs regimen;
- **Group B:** Placebo will be added to the AEDs regimen.

This study could be time-dependent, which means that different durations of treatment can be evaluated (e.g. 30 and 60 days). The frequency of seizures and other physical examinations will be compared between these two groups.

**CONCLUSIONS**

According to the above information, minocycline shows neuroprotective effects via involvement of antioxidant systems,\(^3^1\) prevention of the activation of Ca\(^{2+}\)-dependent intracellular pathways, marked decrease in glutamate release,\(^3^2\) inhibition of transporters such as P-gp,\(^3^0\) molecules such as COX-2\(^2^3\) and inflammatory mediators such as ROS and PGE2. Taken together, the authors conclude that minocycline is a reasonable choice for combination therapy in management of pharmacoresistant epilepsy.

**REFERENCES**

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