

## UPREGULATION OF MULTIDRUG RESISTANCE TRANSPORTERS IN THE EPILEPTIC BRAIN

### Limbic Seizures Induce P-glycoprotein in Rodent Brain: Functional Implications for Pharmaco-resistance

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The causes and mechanisms underlying multidrug resistance (MDR) in epilepsy are still elusive and may depend on inadequate drug concentration in crucial brain areas. We studied whether limbic seizures or anticonvulsant drug (AED) treatments in rodents enhance the brain expression of the MDR gene (*mdr*) encoding a permeability glycoprotein (P-gp) involved in MDR to various cancer chemotherapeutic agents. We also investigated whether changes in P-gp levels affect AED concentrations in the brain. *Mdr* mRNA measured by reverse transcriptase–polymerase chain reaction (RT-PCR) increased by 85% on average in the mouse hippocampus 3–24 h after kainic acid–induced limbic seizures, returning to control levels by 72 h. Treatment with therapeutic doses of phenytoin (PHT) or carbamazepine (CBZ) for 7 days did not change *mdr* mRNA expression in the mouse hippocampus 1–72 h after the last drug administration. Six hours after seizures, the brain/plasma ratio of PHT was reduced by 30%, and its extracellular concentration estimated by microdialysis was increased by twofold compared with control mice. Knockout mice (*mdr1a/b*  $\_/\_$ ) lacking P-gp protein showed a 46% increase in PHT concentrations in the hippocampus 1 and 4 h after injection compared with wild-type mice. A significant 23% increase was found in the cerebellum at 1 h and in the cortex at 4 h. CBZ concentrations were measurable in the hippocampus at 3 h in *mdr1a/b*  $\_/\_$  mice, whereas they were undetectable at the same interval in wild-type mice. In rats having spontaneous seizures 3 months after electrically induced status epilepticus,

*mdr1* mRNA levels were enhanced by 1.8-fold and fivefold on average in the hippocampus and entorhinal cortex, respectively. Thus changes in P-gp mRNA levels occur in limbic areas after both acute and chronic epileptic activity. P-gp alterations significantly affect AED concentrations in the brain, suggesting that seizure-induced *mdr* mRNA expression contributes to MDR in epilepsy.

### Overexpression of Multiple Drug Resistance Genes in Endothelial Cells from Patients with Refractory Epilepsy

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**PURPOSE:** It has been suggested that altered drug permeability across the blood–brain barrier (BBB) may be involved in pharmaco-resistance to antiepileptic drugs (AEDs). To test this hypothesis further, we measured multiple drug resistance (MDR) gene expression in endothelial cells (ECs) isolated from temporal lobe blood vessels of patients with refractory epilepsy. ECs from umbilical cord or temporal lobe vessels obtained from aneurysm surgeries were used as comparison tissue.

**METHODS:** cDNA arrays were used to determine *MDR* expression. MDR protein (MRP1) immunocytochemistry and Western blot analysis were used to confirm cDNA array data.

**RESULTS:** We found overexpression of selected MDR and significantly higher P-glycoprotein levels in “epileptic” versus “control” ECs. Specifically, *MDR1*, *cMRP1*, *MRP2*, and *MRP5* were upregulated in epileptic tissue, whereas *Pgp3/MDR3* levels were comparable to those measured in comparison tissue. The gene encoding cisplatin resistance–associated protein (*hCRA- $\alpha$* ) also was

overexpressed in epileptic tissue. Immunocytochemical analysis revealed that *MDR1* immunoreactivity was localized primarily in ECs; MRP1 protein levels also were significantly higher in epileptic tissue.

**CONCLUSIONS:** Complex MDR expression changes may play a role in AED pharmacoresistance by altering the permeability of AEDs across the BBB.

## COMMENTARY

Pharmacologically intractable epilepsy is one of the most difficult problems facing clinicians who treat epilepsy today. It has been tacitly assumed that the problem of pharmacoresistance may not be easily solved because these patients have complicated pathology, such as complex and diffuse circuit rearrangements, as well as other abnormalities that current diagnostic procedures may not be able to identify.

Recent studies provide new optimism. A class of proteins has been identified that appears to mediate multidrug resistance (MDR). These MDR proteins have previously been studied in the context of cancer, where the resistance to chemotherapeutic agents is a large clinical problem.

A recent study by Rizzi et al. (1) revealed new aspects of MDR expression and function that further support a role for MDRs in epilepsy. Their experiments focused on one of the MDRs called P-gp, a permeability glycoprotein, which is a pump that is located at the plasma membrane. They demonstrate that this protein is upregulated after seizures, and its function is indeed related to pharmacoresistance (2). In some ways one wonders why P-gp has not been studied in neurobiology before, given its location is highly concentrated at the blood–brain barrier, where it is expressed primarily by endothelial cells and the end feet of astrocytes. It is actually more effective against neuropharmacologic agents than against drugs acting on the periphery.

The expression of P-gp is controlled by the *MDR1* and *MDR3* genes in humans, and *mdr1a*, *mdr1b*, and *mdr2* genes in rodents. Of these genes, *MDR1* (human) and *mdr1a* and *1b* (rodents) expression leads to MDR. Particularly intriguing is that the mRNA for *MDR1* is increased in tissue from epilepsy patients who are medically intractable (3,4), sometimes because of cortical malformations (5) or tuberous sclerosis (6). This provides a potential molecular mechanism for pharmacoresistance.

Using mice, Rizzi et al. (2) provide strong support for a key role of P-gp in pharmacoresistant epilepsy. In the first series of experiments, mice were injected with kainic acid and seizures were monitored electrographically. Reverse transcriptase–polymerase chain reaction (RT-PCR) showed that

*mdr1* mRNA was increased in hippocampus after seizures. This is a remarkable result: seizures induce a MDR protein. Yet this was a transient effect, so the relation to human epilepsy was not clear. However, in other experiments with animals having chronic seizures, elevated *mdr1* mRNA also was found in hippocampus. These data suggest that the very seizures occurring in humans with epilepsy may be responsible, at least in part, for medical intractability. Furthermore, if seizure-induced expression could be stopped, antiepileptic drugs (AEDs) might be far more effective. If ever there were a rationale for gene therapy, this would be it.

Although the hippocampus was the major focus of attention because receptors for the convulsant that was used (kainic acid) are concentrated in hippocampus, the entorhinal cortex also was studied. Interestingly, *mdr1* mRNA levels were even higher in that structure. It will be interesting to determine whether other areas of the brain are affected, perhaps allowing one to pinpoint MDRs to a specific brain area or, conversely, clarify that it is a more global issue. In this regard, it is interesting that the authors mention the cerebellum as an area that seems to be unaffected by the kainic acid–treatment paradigm. The authors suggest that the relative lack of *mdr1* upregulation in the cerebellum may be the reason that side effects involving functions attributed to the cerebellum can be such a problem during AED therapy: the brain area that mediates side effects (cerebellum) would be expected to reach a high drug level, but MDR proteins would impair the delivery of AED to the site controlling seizure activity (presumably hippocampus in this case). Exactly how MDR proteins can finely tune drug levels in different parts of the brain will be an important issue for future studies.

The next logical question might be whether gene deletion can restore AED levels. Perhaps foreseeing that question, secondary experiments were conducted with mice that had *mdr1a/b* deleted. In these experiments, prolonged treatment with AEDs was examined over the course of 7 days. In the knockouts, the levels of brain phenytoin and carbamazepine were indeed higher than in wild-type mice. Perhaps just as interesting, plasma levels were not significantly different. These results suggest that targeting *MDR1* in humans could potentially improve AED therapy. Of importance, of course, is whether *mdr1a/b* deletion has any untoward effects on the brain or periphery, and this was not indicated by the authors. The usual concerns of developmental abnormalities or upregulation of compensatory proteins in any knockout merit some caution as well. Still, these results may be some of the most exciting for AED development in a long time.

by Helen Scharfman, Ph.D.

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