



Primum Non Nocere: Are Seizure Medications Safe in Neonates?

Neonatal Exposure to Antiepileptic Drugs Disrupts Striatal Synaptic Development.

Forcelli PA, Janssen MJ, Vicini S, Gale K. *Ann Neurol* 2012;72:363–372.

OBJECTIVE: Drug exposure during critical periods of brain development may adversely affect nervous system function, posing a challenge for treating infants. This is of particular concern for treating neonatal seizures, as early life exposure to drugs such as phenobarbital is associated with adverse neurological outcomes in patients and induction of neuronal apoptosis in animal models. The functional significance of the preclinical neurotoxicity has been questioned due to the absence of evidence for functional impairment associated with drug-induced developmental apoptosis. **METHODS:** We used patch-clamp recordings to examine functional synaptic maturation in striatal medium spiny neurons from neonatal rats exposed to antiepileptic drugs with proapoptotic action (phenobarbital, phenytoin, lamotrigine) and without proapoptotic action (levetiracetam). Phenobarbital-exposed rats were also assessed for reversal learning at weaning. **RESULTS:** Recordings from control animals revealed increased inhibitory and excitatory synaptic connectivity between postnatal day (P)10 and P18. This maturation was absent in rats exposed at P7 to a single dose of phenobarbital, phenytoin, or lamotrigine. Additionally, phenobarbital exposure impaired striatal-mediated behavior on P25. Neuroprotective pretreatment with melatonin, which prevents drug-induced neurodevelopmental apoptosis, prevented the drug-induced disruption in maturation. Levetiracetam was found not to disrupt synaptic development. **INTERPRETATION:** Our results provide the first evidence that exposure to antiepileptic drugs during a sensitive postnatal period impairs physiological maturation of synapses in neurons that survive the initial drug insult. These findings suggest a mechanism by which early life exposure to antiepileptic drugs can impact cognitive and behavioral outcomes, underscoring the need to identify therapies that control seizures without compromising synaptic maturation.

Commentary

One of the most challenging questions in the neonatal intensive care unit (NICU) is how neonatal seizures should be treated. Currently used drugs work approximately half the time – good news for some patients but not for others (1). Medications designed for a physiologically older age group (i.e., non-neonates) may be less effective in neonates due to differences (compared with older patients) in pharmacokinetics (e.g., absorption and elimination). Just as important, the role played by GABA (traditionally considered to be an inhibitory neurotransmitter) is less clear in neonates (2). At the same time, drugs currently used to treat seizures in neonates may carry a risk of adverse effects in the developing brain, including cell death and abnormal behavior and development (3, 4). These effects need to be considered against the risks of ongoing seizure activity. In general, the longer the exposure to a drug, the greater the risk of adverse reactions. But can one dose of a medication make much of a difference? Forcelli et al. explore this question and conclude that even a single

exposure to certain seizure medications can have a prolonged adverse effect. There may however, be safer alternatives.

Forcelli et al. studied electrophysiological and morphologic changes in medium spiny neurons (MSNs) from the striatum of rat pups after exposure to seizure drugs on postnatal day 7 (P7) or P10. The authors chose this cell population because of their prior work showing neurons in this region are susceptible to cell death after seizure drug exposure (3). MSNs receive input from both excitatory glutamatergic and inhibitory GABAergic neurons and integrate both cortical and subcortical signals before producing activity that impacts motor function, including motor learning and memory (5).

The authors studied phenobarbital, phenytoin, and lamotrigine. Phenobarbital is the most commonly used seizure medicine in neonates, and phenytoin is a widely-used second choice (6). Lamotrigine has been shown to be relatively safe during pregnancy but is not typically used for acute treatment of neonatal seizures because of the need for gradual dose titration, which can take 2-3 months (due to the risk of Stevens-Johnson syndrome), well beyond the neonatal period (lamotrigine used in this study was supplied by GlaxoSmithKline, Research Triangle Park, NC, which also provided funding for the Forcelli study) (7). Although the lower dose of phenobarbital used in this study (37.5 mg/kg in rats vs 20-40 mg/kg typically



used in human neonates) results in blood levels similar to neonates undergoing hypothermia treatment for neonatal encephalopathy (20–40 mg/L), the higher dose (75 mg/kg) results in blood levels seen only rarely (8). The dose of phenytoin used (50 mg/kg) is significantly higher than used in the NICU (20 mg/kg) but the resulting blood levels are similar (10 mcg/ml) (1, 9). Despite the approximate concordance with steady-state blood levels, one question is whether the magnitude of a harmful effect from a single high loading dose might be overestimated. The expected blood levels (based on doses used here) of phenobarbital and phenytoin were shown previously to induce cell death (9).

Using patch clamp recordings in acute slices from rat pups exposed to a single dose of phenobarbital, phenytoin, or lamotrigine, impaired GABAergic synaptic transmission was evidenced by lower spontaneous inhibitory postsynaptic currents (sIPSCs) and miniature IPSC (mIPSC) frequencies compared to controls at P14. This effect developed over time, as there were no differences in these parameters measured initially at P10. However, by P18 mIPSC frequency was decreased in the phenobarbital and phenytoin groups but not the lamotrigine group. Similar findings were noted when excitatory neurotransmission was studied, showing reduced frequency of miniature EPSCs (mEPSCs) at P18 after exposure to phenobarbital, phenytoin, and lamotrigine. These findings indicate that both excitatory and inhibitory neurotransmission are impaired after exposure to antiseizure drugs.

The hypothesis that these drugs were acting at the pre-synaptic level was based on the finding that IPSC and EPSC amplitudes were unaffected by drug exposure, in contrast to frequency data. The authors further explored this possibility by examining the morphology of MSN dendritic spines. Phenobarbital exposure (75 mg/kg) at P7 led to decreased spine width and increased density of immature spines at P18. Functionally, rat pups exposed to phenobarbital at P7 showed impaired performance at P25 in a reversal learning task that depends on striatum input. These data provide evidence for an electrophysiological, morphological, and functional impact of phenobarbital exposure early in life. Unfortunately, similar morphological and functional data were not reported for phenytoin, lamotrigine, or lower doses of phenobarbital, preventing the ability to determine whether these drugs have similar deleterious effects.

The authors further explored the role of antiseizure medicine-induced cell death. First, they demonstrated that not all antiseizure drugs induce pathology. Even at high doses, levetiracetam, which is being investigated for a role in the acute management of neonatal seizures, does not induce cell death. After levetiracetam exposure (400 mg/kg, nearly 5–20 times above levels used clinically) on P7 or P10, no changes were noted in IPSC frequencies measured on P14. Second, the antioxidant melatonin (20 mg/kg) prevented phenobarbital-induced caspase-3 cleavage (one marker of apoptosis, a form of programmed cell death). Melatonin also prevented the decrease in IPSC frequency on P14 noted after phenobarbital administration (at P7). The authors conclude that apoptosis is necessary for phenobarbital-induced synaptic pathology but these data also suggest a means of mitigating the effects of phenobarbital.

A number of clinically relevant questions follow from these findings. Would similar effects be noted if there was

only a single exposure of medication at doses more commonly used in the NICU? Because phenobarbital is not typically prescribed to infants who do not have some evidence of neurological illness, would potential benefits outweigh the risks in the setting of hypoxic ischemic injury or neonatal encephalopathy? Both questions were addressed in another study showing that CD1 mice treated with phenobarbital (30 mg/kg) after unilateral carotid ligation and hypoxia (at P12) have fewer seizures and a lower volume of brain atrophy, as well as a resumption of normal exploratory behavior, compared to control mice (10). Similar to the present study, higher doses of phenobarbital (60 mg/kg) afforded no benefit and may have been deleterious. Another question is whether adjunctive therapy used for neonatal encephalopathy (i.e., cooling, erythropoietin, bumetanide) alters outcomes in neonates exposed to phenobarbital. Compared with cooling alone, cooling and adjunctive phenobarbital (40 mg/kg) were associated with improved functional and pathological outcomes in rat pups that underwent a carotid ligation/hypoxia injury at P7 (i.e., the same time as those in Forcelli et al.) (11). Thus, combination therapies may act synergistically, at least in the context of neonatal encephalopathy. Finally, does drug-induced apoptosis (or other forms of cell death with potential benefit, such as autophagy) provide a “quality control” function (i.e., by eliminating dysfunctional neurons) that might be missing if non-apoptosis-inducing medications are used? Careful studies should be able to provide an answer. Confirmation of the current findings in neocortical and hippocampal neurons (i.e., other regions known to show short- or long-term pathology after neonatal insults) also would be useful to study in the paradigms used in these studies.

In the NICU, potential benefits must outweigh the risks of treatment. Given the lack of large, high-quality clinical trials to guide medication choice, as well as the ethical difficulty of performing randomized studies of adverse effects, clinicians depend to a certain degree on preclinical studies. The findings of Forcelli et al. raise awareness of the potential dangers of using some of the most commonly used medications for neonatal seizures. The value of this study is that the risk:benefit ratio of phenobarbital should be reconsidered, particularly in infants without encephalopathy or significant injury (i.e., neonatal seizures without a clear underlying cause). Other data (discussed previously) suggest infants with neonatal encephalopathy may benefit from modest doses of phenobarbital. One additional caveat is that all these data have been generated in rodent models, not humans, and important translational differences may not have been appreciated yet. Until additional clinical data regarding its adverse effects on the brain become available, the use of phenobarbital for treating neonatal seizures likely will continue worldwide because less detrimental alternatives (e.g., levetiracetam) have not been studied as thoroughly as phenobarbital. Alternative medications also may not be as available as phenobarbital in developing economies. Nonetheless, Forcelli et al. remind us to maintain a healthy skepticism about treatments we use and suggest alternatives that may make our practice safer.

by Adam L. Hartman, MD



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