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Neonatal seizures: controversies and challenges in translating new therapies from the lab to the isolette

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Abstract

Neonatal seizures have unique properties that have proved challenging for both clinicians and basic science researchers. Clinical therapies aimed at neonatal seizures have proven only partially effective and new therapies are slow to develop. This article will discuss neonatal seizures within the framework of the barriers that exist to the development of new therapies and the challenges inherent in bringing new therapies from the bench to the bedside. With the European Union and United States creating national collaborative project infrastructure, improved collaborative resources should advance clinical research on urgently needed new therapies for this disorder.

Keywords

Treatment; translational research; rodent models; epilepsy

Introduction

Neonatal seizures constitute one of the most common neurologic issues in the newborn period and have engendered significant study and debate among neurologists. They are unique in many facets including their pathophysiology, treatment and outcome compared to seizures later in life. Much of this debate focuses on whether intensive treatment of neonatal seizures is necessary, as their impact on outcome remains unclear. In addition, the current treatments are often ineffective in controlling the seizures acutely and do not seem to impact the development of later epilepsy. This article will discuss the evidence supporting the deleterious effects of neonatal seizures in humans and animal models, future treatments, and the challenges in moving those therapies from the lab to clinical practice.

Neonatal seizures are common, with an incidence of 1.8–3.5 per 1000 live births (Saliba *et al.*, 1999, Lanska *et al.*, 1995). However, they can be difficult to identify clinically and are challenging to differentiate from a variety of normal, poorly coordinated, neonatal movements. Continuous EEG is currently the gold standard for identifying neonatal seizures, which may be subclinical >50% of the time (Scher *et al.*, 1993). In a study comparing clinical identification of neonatal seizures by healthcare professionals with continuous EEG monitoring, only 27% of clinical seizures were correctly identified and 73% of presumed clinical seizure had no electrographic correlate - leading to overdiagnosis (Murray *et al.*, 2008).

Neonatal seizures are most commonly associated with perinatal hypoxic-ischemic encephalopathy (Tekgul *et al.*, 2006). This entity has been extensively studied and has a complicated pathophysiology that is mediated by excitatory amino acids, inflammatory

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cytokines, and free radical formation. Other common etiologies include vascular events, brain malformations, infections, and inborn errors of metabolism. Table 1 lists common etiologies associated with neonatal seizures and attempts that have been made to recreate these in rodent models for laboratory study. The etiology of the neonatal seizures is important to identify as it may have a significant effect on treatment (e.g. – pyridoxine-responsive seizures) and directly relates to outcome.

Why is the neonatal brain uniquely susceptible to seizures?

The incidence of seizures is highest in the first year of life (Hauser *et al.*, 1993) and the risk of seizures is greatest in the neonatal period (Ronen *et al.*, 1999, Saliba *et al.*, 1999). Basic science research studies suggest that, compared to the mature brain, the developing brain is more excitable. The amount of chemoconvulsant required to induce seizures in immature animal is much lower than that required for induction of seizures in adult animals (Strafstrom et al., 1992). The enhanced excitability of the developing brain can be attributed to a variety of factors including early and exuberant development of excitatory neurotransmitter systems and comparatively delayed development of inhibition (for review see Holmes, 1997; Rakhade & Jensen, 2009). The increased neural activity associated with the enhanced excitation in the immature brain is essential for numerous activity-dependent developmental processes, but it also renders the developing brain more susceptible to seizures. Glutamate is the major excitatory neurotransmitter in the CNS that mediates its action via two types of receptors, metabotropic and ionotropic. The ionotropic receptors are further subdivided into N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA), and kainic acid (KA) receptors. During early postnatal development, NMDA and AMPA glutamate receptors are transiently overexpressed as compared to the mature brain and have a subunit composition that enhances excitability. Compared to the adult brain, for example, the immature brain has higher levels of NMDA receptor (NR) 2B proteins and lower levels of NR2A proteins (Monyer et al., 1994). The NMDA receptor that contains NR2B subunit, in place of NR2A subunit, has been shown to have a longer current decay time (Flint et al., 1997).

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult brain. However, due to developmental differences in the chloride gradient, GABA is excitatory in immature neurons (Ben-Ari et al., 1989). Potassium/chloride cotransporter 2 (KCC2), a cation chloride co-transporter channel important in extruding chloride out of the intercellular space, does not reach mature levels until after the neonatal period (Rivera et al., 1999). The chloride transporter sodium/potassium/chloride co-transporter 1 (NKCC1) predominates in the neonatal period and actively transports chloride into the cell (Plotkin et al., 1997; Dzhala et al., 2005). When $GABA_A$ receptors (a subtype of GABA receptors) are activated in immature neurons, the neuronal membrane is depolarized and there is a net excitatory effect, unlike the inhibitory effect of activating $GABA_A$ receptors in adult brain. Depolarizing GABA currents are critically important for normal activity-dependent developmental processes including neuronal proliferation, migration, targeting & synaptogenesis (LoTurco et al., 1995; Owens et al., 1996; Ben-Ari et al., 1997; Leinekugal et al., 1997). Moreover, in comparison to the adult brain, the immature brain has lower levels of $GABA_A$ receptors (Swann et al., 1989; Brooks-Kayal & Pritchett, 1993) and smaller GABA-mediated currents (Brooks-Kayal et al., 2001). Also, early in life, the subunit composition of $GABA_A$ receptors is different than that of the adult brain and this difference in subunits makes them less sensitive to benzodiazepine augmentation (Gibbs et al., 1996; Kapur & Macdonald 1999, Brooks-Kayal et al., 2001).

Deleterious effects of neonatal seizures

Results from animal research suggest that neonatal seizures may exacerbate hypoxia-ischemia induced brain injury (Dzhala *et al.,* 2000; Wirrell *et al.,* 2001; Björkman *et al.,* 2010; but also see Towfighi *et al.,* 1999). Björkman and colleagues (2010) observed that newborn piglets that were exposed to hypoxia and had seizures had greater brain injury compared to piglets without seizures. Neonatal seizures in rats have also been shown to cause long-term neurological problems. Adult rats that experienced flurothyl-induced recurrent neonatal generalized tonic-clonic seizures do not exhibit spontaneous seizures but have reduced seizure threshold (Isaeva *et al.,* 2010). Multiple studies performed in various animal models suggest that rats develop cognitive and behavioral deficits in later life following early-life seizures (Lee *et al.,* 2001; Sayin *et al.,* 2004; Cornejo *et al.,* 2008; Kleen *et al.,* 2011). An elegant study by Cornejo and colleagues (2008) found that even a single episode of neonatal seizure in rats is sufficient to cause life-long alterations in working memory.

While basic science research would suggest that neonatal seizures can be harmful to the developing brain, there is a limited, but growing, literature supporting that this occurs in humans. Legido et al. (1991) found seizure frequency to be a strong predictor of the risk of developing a poor outcome and cerebral palsy in patients with asphyxia. However, this study did not use continuous EEG monitoring and likely underdiagnosed seizures. Similar results were found in a study evaluating neonates at risk for seizures, with the occurrence of electrographic seizures being associated with microcephaly and severe cerebral palsy (McBride *et al.*, 2000). A 2002 study attempted to correlate the severity of seizures with MRI and ^{1}H-MRS findings (Miller *et al.*, 2002). The study found significant changes in the ^{1}H-MRS spectra correlated to seizure severity suggesting that neonatal seizures in patients with hypoxia were associated with worsened brain injury. A study evaluating the utility of selective head cooling found that the presence of neonatal seizures at enrollment was associated with an unfavorable outcome at 18 months (Gluckman *et al.*, 2005). A more recent study followed neonates with hypoxic-ischemic injury and clinical neonatal seizures to assess neurodevelopmental outcome at four years of age (Glass *et al.*, 2009). They found that patients with HIE and neonatal seizures had worse motor and cognitive outcomes than those without seizures independent of the severity of their MRI findings. There is, of course, an inherent “chicken vs. egg” conundrum with studies of neonatal seizures in humans, and it is not possible to be certain that seizures “cause” additional brain injury and worsened outcome rather than being simply a marker of more severe underlying brain injury that may not be detectable by history, exam or current neuro-imaging techniques. However, with this caveat and in combination with studies in rodent models, the preponderance of the evidence seems to support a contributory role of neonatal seizures to brain injury and subsequent neurological disability.

What are the changes in the brain caused by neonatal seizures that may contribute to long-term neurological disability in certain patients later in life? In human patients, it is very difficult to parse out the effects of the etiology of the seizures and the effects of treatment from the effects of seizures on the developing brain. Studies conducted in animal models have identified cell death (Kadam & Dudek, 2007), aberrant synaptic connections (Grigonis & Murphy, 1994; Sogawa et al., 2001; Kadam & Dudek, 2007; Xiu-Yu et al., 2007; Rakhade et al., 2011), increases in thickness of prefrontal cortex (Kleen et al., 2011) and changes in rate of neurogenesis (McCabe et al., 2001; Liu et al. 2003; Xiu-Yu et al., 2007; for review see Porter, 2008) following neonatal seizures. In addition to structural modifications, chronic suppression of inhibitory activity (Isaeva et al., 2009), a long-term increase in excitatory activity (Isaeva et al., 2010) and modifications in subunit composition of GABA (Zhang et al., 2004a; Laurén et al, 2005) and glutamate receptors (Zhang et al.,

2004b; Silva et al., 2005; Swann et al., 2007; Cornejo et al., 2007) have been observed after the occurrence of neonatal seizures (for review see Holopainen, 2008). Persistent changes in inhibitory and excitatory pathways may explain some of the neurologic sequelae, such as increased seizure susceptibility and memory impairment, observed in later life following neonatal seizures. Cornejo and colleagues (2007) found impairment in working memory associated with alterations in synaptic plasticity, increase in intracellular levels of GluR1 subunit (reduced expression at the membrane) and reduced levels of NR2A subunit. However, currently these associations remain correlative and direct evidence that connects neonatal seizures to adverse neurological outcome in later life remains elusive.

Challenges in treating neonatal seizures

Identification of neonatal seizures

One of the limitations for clinical research in neonatal seizures is accurate identification of seizure activity. Amplitude-integrated EEG (aEEG) has become more widely available due to its ease of application (fewer recording channels and can be applied by bedside nursing) and lower cost compared to traditional EEG. Many recent studies have attempted to correlate the seizures noted on aEEG with outcome to determine if the seizures are independently associated with a worse outcome. This technique likely allows for better screening of at-risk infants, though has well documented limitations with lower seizure detection rates for brief, low amplitude, or focal seizures (Toet *et al.*, 2002, review by Tao & Mathur, 2011). In a retrospective study of patients who underwent aEEG and were found to have neonatal status epilepticus (SE), the subgroup of patients with HIE and a poor outcome had a longer duration of status epilepticus (van Rooij *et al.*, 2007). Interestingly, this did not hold true statistically for other causes supporting the idea that the HIE subgroup may be particularly at risk for injury from neonatal SE. A recent randomized study evaluated the treatment of clinical and subclinical seizures identified by aEEG in patients with HIE (van Rooij *et al.*, 2010). The study found that there was a significant correlation between the severity of brain injury on MRI and the duration of seizure activity. Treatment of subclinical seizures shortened the total duration of seizure patterns and reduced brain injury, suggesting that patients at risk for HIE should be monitored electrographically to identify and treat these subclinical seizures.

Lack or paucity of good treatment options

Effective treatment of neonatal seizures has proven challenging with studies suggesting that traditional therapies are only modestly effective. In a study comparing the efficacy of phenobarbital to phenytoin, seizure control was achieved in only about 45% of the patients following administration of the first medication (Painter *et al.*, 1999). The patients were then given the alternate medication - increasing seizure control to only about 60%. This study highlighted that 40% of patients continued to have seizures despite treatment with two conventional antiepileptic medications. Benzodiazepines have been advocated for the treatment of neonatal seizures and may be effective in refractory patients. In one study, midazolam was effective in controlling electrographic seizures in patients who had failed first-line therapy with phenobarbital or phenytoin (Castro Conde *et al.*, 2005). Lidocaine has also proven effective for neonatal seizures that have failed to respond to traditional anticonvulsant medications; however, concerns for cardiac toxicity has limited its widespread use (Malingré *et al.*, 2006). Topiramate (Glass *et al.*, 2011) and levetiracetam (Abend *et al.*, 2011) have shown some efficacy in preliminary retrospective studies. Table 2 lists neonatal seizure treatments and their presumed therapeutic targets. All of the currently available antiepileptic drugs, including phenobarbital, have been developed using adult animal models and tested clinically in adult patients. However, there are significant anatomical, electrophysiological and neurochemical differences between the developing and

mature brain. Therefore, immature brain may respond very differently than the adult brain to both injury and treatment. As described above, in comparison to the adult brain, GABA as an inhibitory system in early life is less well developed and even has an excitatory activity that may explain the poor efficiency of phenobarbital and diazepam (which act by augmenting $GABA_A$ receptor activity) to treat neonatal seizures. Another important factor to be considered while treating neonatal seizures (in fact any disease), that is often neglected, is the gender of the patient. A recent study demonstrated that the hyperpolarizing reversal potential of GABAergic postsynaptic currents appear earlier in female than in male rat hippocampus and neonatal seizures had very different immediate effects on the GABAergic system in animals of different gender (Galanopoulou, 2008). These differences may affect the efficacy of GABAergic drugs as well as later neurologic outcomes.

Risk of treatment

A major consideration for physicians treating neonates with seizures is the potential for deleterious effects of seizure treatment on the developing brain. Several studies have shown that *in utero* exposure to certain antiepileptic drugs (AEDs) can increase the risk of cognitive dysfunction later in life (Meador et al., 2009, for review see Bromley et al., 2009). In children with seizures treated with AEDs, the effects of AED treatment on cognition can be difficult or impossible to differentiate from those of the seizures and/or underlying cause of the epilepsy; although, in the well-known study of children with febrile seizures randomized to placebo or phenobarbital, Farwell and colleagues demonstrated a persistent decrease in the mean IQ of the phenobarbital treated group (Farwell *et al.,* 1990). No such placebo controlled randomized studies in humans exist that examine potential cognitive effects of AED treatment of neonates, however based on animal research there is potential risk. In rodent models, there is evidence that neonatal AED exposure, especially with older drugs such as phenobarbital, phenytoin, and valproic acid, alters a number of activity-dependent developmental processes, including neuronal gene expression, migration, differentiation and survival (for review see Marsh *et al.*, 2006).

New treatment targets and options

NKCC1 transporter inhibitors

Bumetanide, a loop diuretic, may prove to be a valuable adjunctive therapy for neonatal seizures. As described above, due to developmental differences in chloride transporter expression (higher NKCC1 levels and lower KCC2 levels in immature brain), during early development GABA has excitatory activity (Ben-Ari et al., 1989; Plotkin et al., 1997; Rivera et al., 1999; Dzhala et al., 2005). Bumetanide inhibits the NKCC1 transporter, altering the chloride gradient such that GABA channel opening is more hyperpolarizing and possibly allowing GABAergic medications to be more effective. There are currently two multicenter clinical trials evaluating the efficacy of bumetanide in neonates with hypoxic-ischemic encephalopathy.

Hypothermia

Therapeutic hypothermia has proven effective in improving outcomes in moderate to severe hypoxic-ischemic encephalopathy (Shankaran *et al.*, 2005, Gluckman *et al.*, 2005). Brain cooling likely modulates multiple neurotoxic processes including decreased cerebral metabolism, ion pump dysfunction, formation of cytotoxic edema, free radical formation, and neuroinflammation (Polderman, 2009). Limited case series suggest that it may be effective for status epilepticus in children and adults, but evidence for the efficacy of hypothermia in the treatment of seizures in neonates is limited. (Rossetti, 2011). A recent study of neonates undergoing therapeutic hypothermia found that 65% had electrographic seizures during or immediately after cooling, suggesting that hypothermia may have limited

impact on the incidence of seizures (Wusthoff *et al.*, 2011). Harbert et al. reviewed patients with focal neonatal stroke who underwent therapeutic hypothermia for neonatal encephalopathy and compared them with subjects with neonatal stroke who did not receive hypothermia. They found that none of the five patients with focal stroke and hypothermia developed seizures, while 70% of those who did not receive hypothermia developed seizures (Harbert *et al.*, 2011). While therapeutic hypothermia may have a positive impact on hypoxic-ischemic encephalopathy, its utility as a treatment option for neonatal seizures remains questionable.

AMPA receptor modulators

AMPA receptors are constructed from GluR1-4 subunits. Different combinations of the subunit produce AMPA receptors with distinct function. For example, the presence of GluR2 subunits reduces permeability of AMPA receptors to calcium ions (Bochet *et al.*, 1994). The immature rodent and human brain have a higher number of AMPA receptors that lack GluR2 subunits and gate greater amounts of calcium (for review see Jensen, 2002, Talos *et al.,* 2006a,b). Post-translational modifications, such as alternative splicing of the AMPA receptor subunits, also affects AMPA receptor functional properties. Alternative splicing of AMPA receptor subunits generates *flip* and *flop* isoforms of the subunits (Rogawski *et al.*, 1999). The AMPA receptor that has subunits with the flip configuration desensitizes at much slower rate than the AMPA receptors whose subunits are in the flop configuration (Rogawski *et al.*, 1999). The immature brain has higher flip/flop ratio than the adult brain, which might contribute to the hyperexcitability of the developing brain (Monyer *et al.*, 1991; for review see Dingledine *et al.*, 1999). In fact, in a neonatal rat model of hypoxia-ischemia, Jensen and colleagues (1995) observed that the AMPA receptor antagonist NBQX effectively blocked acute seizures, whereas GABA agonists such as phenobarbital were ineffective in stopping seizures. Similarly topiramate, which has been shown to block AMPA receptor activity, was found to be effective in suppressing hypoxia-ischemia induced neonatal seizures in rats (Koh & Jensen, 2001). In a more recent study, pretreatment of neonatal rats with talampanel, a noncompetitive antagonist of AMPA receptors, was found to be effective in preventing the development of seizures during hypoxia exposure (Aujla *et al.*, 2009). These studies suggest that AMPA receptor antagonists may prove an effective treatment for neonatal seizures.

Potassium channel openers

Potassium channels play a uniquely important role in controlling excitability in the developing brain because of the lower levels of GABAergic inhibition. Mutations in genes encoding KCNQ2 and KCNQ3 subunits of voltage gated potassium channels cause benign familial neonatal convulsions (BFNC), a genetic epilepsy syndrome (Singh *et al.*, 2003). One of the interesting characteristics of BFNC is that the seizures begin in the first week of life and usually spontaneously remit after a few weeks or months. This suggests that the potassium channels play a particularly critical role in controlling hyperexcitability during the neonatal period and early infancy. A view further supported by a study in rodents that demonstrated that the blockade of KCNQ2/3 channel activity in early development results in development of severe epilepsy; whereas, blockade of KCNQ2/3 channel activity during adulthood results in a much milder phenotype (Peters *et al.*, 2005). These observations suggest that a potassium channel opener can be a highly effective way to enhance inhibition and treat neonatal seizures. A recent study showed excellent efficacy of flupirtine, a potassium channel opener, in treating neonatal seizures in rats (Raol *et al.*, 2009). Flupirtine, unlike diazepam and phenobarbital, completely blocked neonatal seizures induced by chemoconvulsants and when administered 15 minutes after rats had developed continuous seizures effectively stopped electrographic and behavioral seizures.

Hurdles in development of novel treatments and challenges in bringing a drug to the clinic

Bringing a new effective therapeutic treatment to market is an expensive and prolonged process with multiple challenges. The ideal goal is to have a firm understanding of the pathophysiologic processes leading to disease and tailor treatments to modify or block these processes. While our understanding of neonatal seizures continues to grow, translational research has proven difficult. Many of the features that make the neonatal brain uniquely susceptible to seizures, such as differences in the chloride gradient that diminish the inhibitory effects of GABA and exuberant expression of glutamate receptors that enhance excitability, are critical for driving normal activity-dependent developmental processes. Thus, treatments that target these mechanisms to reduce seizures have the potential to produce deleterious effects on normal neurocognitive development that must be carefully monitored (and somehow differentiated from those of the underlying brain injury and seizures themselves). Essential to our ability to establish therapies for neonatal seizures that are both safe and effective are improved animal models. Current models of neonatal seizures are overly simplistic and largely unrepresentative of the typical etiologies of seizures in human neonates. Further, many of the most concerning potential outcomes from neonatal seizures, such as neurobehavioral and neurocognitive abnormalities of language and executive function, are poorly assessed in most rodent models. In order to improve translational research, better models and better methods of assessing cognitive and behavioral outcomes after neonatal seizures need to be identified.

Lack of good animal models

Since the discovery of the anticonvulsant activity of phenytoin in cats (Putnam & Meritt, 1937), experimental models of seizures and epilepsy have played a tremendously important role in improving our understanding of the disease process and discovering newer treatment options. A good animal model that replicates all of the important aspects of the disease it is modeling is the most important tool required in the fight against disease. A good animal model of an epilepsy disorder will accurately replicate the etiology of the disorder, the age of onset of the disorder, the seizure phenotype, the EEG characteristics and the long-term consequences of the disorder (Sarkisian, 2001; Stafstrom *et al.*, 2006). As mentioned earlier in this review and as pointed out in the report from the National Institute of Health (NIH) workshop about models of epilepsy and epileptogenesis, the existing programs for antiepileptic drug discovery have aimed at identifying therapies for the adult, rather than the pediatric, population (Stables *et al.,* 2002). The developing brain is not a smaller version of the adult brain; therefore, to identify the most effective therapeutic intervention strategy, it is imperative to target age-specific mechanisms and test new therapies in age-specific disease models. In recent years, the NIH and the epilepsy research community has put a great emphasis on the development of model systems specific for pediatric epilepsies (Stables *et al.*, 2002; Stafstrom et al., 2006). As a result, a significant increase in the research activity directed towards the development of newer models for childhood epilepsies has occurred, which is evident by three newly proposed animal models of infantile spasm in as many years (Marsh *et al.,* 2009; Price *et al.,* 2009; Scantlebury *et al.,* 2010). As shown in Table 1, there are some animal models that replicate the etiology of human neonatal seizures, however many (such as common chemoconvulsant models such as kainate and flurothyl) do not, but rather model general phenomena of excitotoxicity. In addition, most of the animal models of neonatal seizures have not been validated in terms of the presence of electrographic seizures (which often do not have predictable behavioral correlates) and the development of epilepsy in later life has not been established in many of these models. It is extremely challenging to characterize behavioral or electrographic seizures in newborn animals due to their smaller size (mouse and rat pup) and due to the fact that the newborns cannot be kept separate from

the mother for a very long time. Moreover, almost all of the available animal models are created using animals that are normal and do not have any brain pathology, which is in contrast to what is observed in the majority of human patients (for review see Lombroso, 2007). The other challenge in developing a good neonatal seizure model is identifying an age in the animal that accurately correlates with the human neonate (for review see Avishai-Eliner et al., 2002; Watson et al., 2006). Studies of synaptogenesis, neuroanatomy, metabolism and neurotransmitters receptors expression suggest that the first year of human life is roughly equivalent to 7–14 days of life in the rat. However, the inter-species age correlation may vary depending on the specific developmental factor considered for comparison. A comparison of total brain weight gain as a percentage of adult weight suggest that a 5 to 7 day old rat is equivalent to a human newborn (Dobbing & Sands, 1979), whereas, a comparison of development of hippocampus between human and rat suggest that the first week of life in rats might be comparable to the third trimester of gestation in a human (Avishai-Eliner et al., 2002). Cortical glutamate decarboxylase activity in 7.4 to 9 days old rat is comparable to a 40 week post-conceptional human (Romijn et al., 1991), whereas, comparison of electrical activity recorded using aEEG suggest that 10-day old Wistar rat brain is equivalent to a new born (Tucker et al., 2009). Further, the developmental changes in the brain often vary between two strains of the same species. For example, the developmental changes in AMPA receptor subunit expression in the cortex occurs 2–3 days earlier in Sprague-Dawley rats than in the Long-Evans rats, which could be due to differences in the gestation period between the two strains (Talos et al., 2006b).

Preclinical studies

Drug development involves three major steps: (1) basic science research, (2) preclinical studies and, (3) clinical trials. Basic science research is required to discover a physiological target that can be manipulated to modify the disease. Preclinical studies help with identification of a compound that can modulate the target. Preclinical studies also determine pharmacological and toxic properties of the test compound in animal models. Translating treatments from the rodent to the human can be particularly challenging as there are significant differences between the species that may affect the utility of the treatment, such as differences in metabolism or toxicity. Translation of neonatal treatments can be especially challenging given the pharmacodynamic differences between adults and neonates (for review see Stephenson, 2005). Funding is, of course, always a substantial challenge. In US, the main source of funding for basic science and preclinical research is the NIH. Private foundations, such as Citizens United for Research in Epilepsy (CURE) and other state and federal programs, also provide vital support for the drug discovery research. For preclinical development and early clinical trials, NIH provides resources such as the Rapid Access to Interventional Development (RAID) pilot program, Small Business Innovative Research (SBIR) program, Small Business Technology Transfer (STTR) program, Rapid Access to Preventive Intervention Development (RAPID) program, and UO1 grant program. However, in recent years research funding has become more scarce due to reductions in the NIH budget. Moreover, because of the economic downturn, private foundations have seen reductions in donations. Limitations on the amount of funding affects the development of new drugs in multiple ways, including the ability to accurately translate rodent study data for human use. For example, calculating an effective dose of a drug that does not have any side effects in human based on the data from rodents is not only complicated, it can also be inaccurate (Reagan-Shaw *et al.,* 2008). If a drug is found to be effective in treating a disease in a rodent model, it would be ideal to test its effects in a primate model before its use in the humans. However, primate research is very expensive and involves complex ethical questions.

Clinical trials

Clinical trials in children and neonates are complicated by ethical, physiologic, pharmacologic, neurodevelopental and economic concerns (Kern, 2009). Consent, beneficence, confidentiality and equipoise are important considerations in the design of all trials, but may be particularly challenging in neonates who are often critically ill. The varied physiologic and pharmacologic responses and interactions of medications in neonates are difficult to extrapolate from adult studies and critical phase I and II trials are often lacking, making clinical trials difficult to formulate safely. There are economic concerns for drug manufacturers when medications have a limited market – particularly in small affected populations or rare diseases and with short treatment periods (most neonates are treated for seizures for a period of only a few weeks). Companies often cite the limited market for some of these drugs and the relatively poor return on investment in performing trials in children. This process of bringing a drug to market typically takes between 8 and 12 years and is estimated to cost $403 million of dollars per drug (DiMasi *et al.*, 2003). Typically only one of five thousand to ten thousand compounds actually becomes approved for marketing. A recent study found that only 8% of CNS drugs that entered clinical testing were successful in achieving clinical approval, the lowest of all therapeutic classes (DiMasi *et al.*, 2010). There has been an increasing complaint from drug manufacturers that there are limited affected patients available for studies in the United States and Europe. Sponsors have found it challenging to find sufficient US investigators and subjects and have been conducting more trials overseas in central Europe and Asia (Krall *et al.*, 2011). This can be particularly problematic for studies in children and neonates for diseases that have a low prevalence in the population. In the absence of adequate support from the pharmaceutical industry, funding from governmental agencies such as NIH will be critical. Due to their relatively low prevalence, adequate clinical trials for neonatal seizures will require involvement of multiple sites and a long follow-up period of 5–10 or more years will be needed to adequately assess neurodevelopmental outcome, making such studies both expensive and logistically complex.

Possible solutions for bringing drugs to market

Childhood cancer trials have been enormously successful at lowering mortality rates for relatively rare childhood cancers through a network of national and multinational research groups. Neonatal seizures, while relatively common, may benefit from a similar framework for amassing patients and funneling promising projects through a series of academic centers with the expertise to conduct high-quality research in a collaborative way. The European Union and United States have created programs with the goal of streamlining research and drug approval. In the European Union, the Innovative Medicine Initiative (IMI) and the European Seventh Framework Programme (FP7) are building collaborative research efforts between academic centers as well as commercial biomedical partners. For example, The Treatment of Neonatal seizures with Medications Off-patent: evaluation of efficacy and safety of bumetanide (NEMO) trial is funded through the FP7 program. This study will evaluate the efficacy of bumetanide in neonates following hypoxic-ischemic injury. A similar trial is underway in the United States, with both of these trials striving toward a successful transition from bench to bedside with government sponsored support.

In the United States, the NIH created the Clinical and Translational Science Awards (CTSA) to partner 60 academic centers and the private sector to facilitate utilization of scalable assets for investigator use. The NINDS has recently introduced the NeuroNEXT program to provide a standardized and accessible infrastructure to support Phase II trials in pediatric and adult neurologic diseases. This program will centralize the institutional review process, provide statistical support and assist researchers in trial design and applying for funding. This will hopefully bridge some of the gap between basic science and clinical research.

Trials looking to enroll patients with rare diseases will be able to utilize the multicenter approach to identify and enroll patients.

Neonatal seizures are heterogeneous and complex and it will require improved understanding of basic mechanisms, improved disease models, and better strategies for performing clinical trials to successfully bring effective therapies to market for clinical use. With new programs in Europe and the United States addressing some of these concerns, there will hopefully be an improved transition of new therapies from the basic science lab to the isolette.

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Table 1

Etiology of neonatal seizures and available animal models

Etiology	Animal model available
Hypoxic-ischemic encephalopathy	Yes (Jensen et al., 1991; Kadam and Dudek, 2007)
Excitotoxicity	Yes (Stafstrom et al. 1992; Lee et al., 1995; Santos et al., 2000; Isaeva et al., 2010)
Intracranial hemorrhage	Yes (Cherian et al., 2003; Alles et al., 2010, for review see Balasubramaniam and Del Bigio, 2006) but lack seizure characterization
Ischemic stroke	Yes (Comi et al., 2005; Kadam and Dudek, 2007)
Cerebral sinovenous thrombosis	No
Acute Infection • Bacterial meningitis • Viral meningoencephalitis	Yes (Leib et al., 1998) but lack seizure characterization Yes (Pedras-Vasconcelos et al., 2008; for review see Bonthius and Perlman, 2007) but lack seizure characterization
Intrauterine TORCH infection	Yes (for review see Tsutsui et al., 2005 and Schleiss, 2006) but lack seizure characterization
Metabolic abnormalities • Hypoglycemia • Hypocalcemia	Yes (Gardiner, 1980; McGowan et al., 1995; Kim et al., 2005; Zhou et al., 2008) but lack seizure characterization Yes (Yoshizawa et al., 1997; Li et al., 1997; Eyles et al., 2003; Kalueff et al., 2006) but lack seizure characterization
Cerebral dysgenesis	Yes (Backman et al., 2001; Meikle et al., Ljungberg et al., 2009; Way et al., 2009; for review see Crino, 2009) but neonatal seizures not characterized; spontaneous seizures variably observed later in life
Inborn errors of metabolism • Nonketotic hyperglycinemia • Pyridoxine-responsive seizures	Yes (Gomeza et al., 2003; Kojima-Ishii et al., 2008) but neonatal seizures not characterized; seizure susceptibility determined in later life Yes (Waymire et al., 1995; Narisawa et al., 2001)
Genetic abnormalities • Benign familial neonatal convulsions – KCNQ2 • Early infantile epileptic encephalopathy – STXBP1 – ARX	Yes (Singh et al., 2008) Yes (Verhage et al 2000) but lack seizure characterization Yes (Marsh et al., 2009; Price et al., 2009)

Table 2

Treatment of neonatal seizures

Treatment	Therapeutic Targets/Mechanism of Action
Conventional	
• Phenobarbital	Enhance GABA inhibition
• Phenytoin	Inhibit voltage-dependent sodium channels
• Benzodiazepines	Enhance GABA inhibition
• Lidocaine	Inhibit voltage-dependent sodium channels
• Pyridoxine	Cofactor for multiple enzymatic process for neurotransmitters
Off-label	
• Levetiracetam	Interacts with synaptic vesicle protein (SV2A)
• Topiramate	Multiple – modulation of AMPA, sodium, GABA channels. Carbonic anhydrase inhibitor.
• Therapeutic hypothermia	Numerous potential mechanisms
Future Therapies	
In clinical trials	
Bumetanide	Inhibits NKCC1 transporter
In preclinical development	
K^+ channel openers	Enhances activity of KCNQ2/3 type of K^+ channels
AMPA receptor blockers	Inhibits AMPA receptor mediated excitation