

Levetiracetam versus Phenobarbital. Effects in the neurodevelopment in newborns treated for neonatal seizures: a clinical trial

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PURPOSE: To analyze if the use of Phenobarbital compared with Levetiracetam, it's associated with more neurodevelopmental problems in newborns treated for neonatal seizures. As a secondary objective identify which are the most affected areas of the neurodevelopment: cognition, socio-emotional, motor or language skills.

DESIGN: A 5 years long clinical trial administering, with double-blind and a randomized distribution of the sample, Phenobarbital or Levetiracetam for the management of neonatal seizures

KEYWORDS: Levetiracetam, Phenobarbital, Neurodevelopment

1. INTRODUCTION

Neonatal seizures are the most frequent clinical manifestation of central nervous system dysfunction in the newborn, with an incidence of 1.5-3.5/1000 in term newborns and 10-130/1000 in preterm newborns (1). Furthermore they are a major risk for death or subsequent neurological disability and can independently confer an adverse neurodevelopmental outcome in high-risk neonates (2).

Physiopathology

A seizure is a sudden, excessive, synchronous electrical discharge of a group of neurons within the central nervous system. This electrical discharge is due to depolarization of neurons, resulting from an influx of sodium ions. Negative potential across neuronal cells is maintained by an ATP (adenosine triphosphate) dependant $\text{Na}^+ - \text{K}^+$ pump. Depolarization can result from one of four mechanisms:

- 1) Decreased energy production and failure of ATP dependant $\text{Na}^+ - \text{K}^+$ pump
- 2) Excessive release of the excitatory neurotransmitter glutamate and reduced (energy dependant) uptake into cells.
- 3) Deficiency of inhibitory neurotransmitters: gamma-amino butyric acid (GABA) is the predominant inhibitory neurotransmitter in the brain. A deficiency of pyridoxine, a cofactor for GABA synthesis, will lead to reduced levels of GABA and consequently to seizures.
- 4) Hypocalcaemia and hypomagnesaemia also cause seizures as both calcium and magnesium inhibit Na^+ movement across neuronal cells.

Then as it's seen, glutamate is the major excitatory neurotransmitter in the central nervous and GABA the major inhibitory transmitter. Glutamate receptors are located at synapses, on non-synaptic sites, on neurons and on glia, and it has 3 types of receptors: NMDA receptor (N-methyl-D-aspartate), AMPA receptor (alpha-amino-3 hydroxy-5-methyl-4-isoxazolepropionic acid) and kainitine receptor. However the neonatal central nervous system has some differences related to glutamate and GABA functions. In the neonatal brain, both NMDA and AMPA receptors are over expressed and their subunit composition renders them susceptible to enhanced excitability. Moreover to compound the relative excess of 'excitability' of the perinatal brain, GABA receptor expression is low in early life. In addition, GABA receptor activation produces excitation rather than inhibition of neurotransmission. This paradoxical action of GABA in the neonate is due to age related differences in chloride homeostasis. Chloride transport is a function of two membrane pumps. In the neonate $\text{Na}^+\text{-K}^+\text{-Cl}^-$ co-transporter (NKCC1) imports large amounts of chloride into the neuron. Chloride levels within the neuron remain high because of relative under expression of $\text{K}^+\text{-Cl}^-$ co-transporter, (KCC2) which is a K^+ exporter. When the chloride permeable GABA receptors are activated, chloride flows out of the cell depolarizing it. As a result GABA activation is excitatory rather than inhibitory. With maturation, NKCC1 expression diminishes and KCC2 expression increases. GABA activation then causes chloride to flow into the cell and hyperpolarize it. Maturation of these chloride co-transporters occurs in a caudal to rostral direction with maturation of spinal cord and brain stem receptors occurring before that of the cerebral cortex (3).

Aetiology of neonatal seizures

Hypoxic-ischemic encephalopathy (HIE), intracranial haemorrhage, intracranial infection and developmental defects are responsible for 80-85% of all causes of neonatal seizures (1).

Hypoxic-ischemic encephalopathy remains the most frequent underlying cause of seizures in term infants (1,4,5). It contributes to 40-60% of seizures in term infants (1). This seizures occurring in infants with HIE may contribute to a poor neurodevelopmental outcome (6,7), and they predominantly occur within the first 24h (1,4,5).

Cerebrovascular disorders are the second most frequent cause of neonatal seizures (1,4,5). Within this group we can find intraventricular haemorrhage: grade 3 or 4 are the most significant cause of seizures in preterm in the first 3 days of life (1), large subarachnoid haemorrhage can occasionally lead to seizures that tend to occur on the second day of life (1), and large subdural haemorrhages which are associated with traumatic delivery (8).

Central nervous system infections are present in about 5-10% of babies with neonatal seizures (1), and commonly take the form of meningitis, but viral meningoencephalitis particularly due to herpes simplex virus is also a well-recognised cause (8).

Cortical malformations account for 5-9% of all causes of neonatal seizures (5). The more widespread malformations include tuberous sclerosis, focal cortical dysplasia, hemimegalencephaly, lissencephaly, subcortical band heterotopia, periventricular nodular heterotopia, schizencephaly and plimicrogyria (8).

The 15-20% that rest are caused by transient metabolic causes as hypoglycaemia, hypocalcaemia/hypomagnesaemia, hypernatraemia/hyponatremia, or inborn errors of metabolism (8).

Types and diagnosis of seizures

Four main types of seizures are recognised (9):

- Subtle: include oro-facial manifestations such as eye deviation, eyelid blinking, sucking, chewing and lip smacking. Limb movements described as swimming, boxing or cycling can also indicate subtle seizure activity. Apnoeic episodes can be due to seizures. There can be difficulty distinguishing subtle seizures from jittering, an extremely common normal phenomenon in newborns. Jittering does not involve the face, is markedly stimulus sensitive, and ceases when the limb is held. The autonomic nervous system changes of a seizure, such as tachycardia or hypertension, are never seen in jittering (9).
- Clonic: involve one limb or one side of the face or body jerking rhythmically at a frequency of 1–4 times/s. Clonic seizures in the neonate can have more than one focus or migrate. They are often a clue to an underlying focal lesion but they can be due to a metabolic cause. Infants are not usually unconscious during this kind of seizures (9).
- Tonic: Sustained posturing of the limbs or trunk, or deviation of the head or eyes are the usual manifestations (9).
- Myoclonic: tend to occur in the flexor muscle groups (9).

For a correct diagnosis we'll have to perform the following steps:

- **Detailed clinical examination**: we have to perform it in all infants with suspected seizures. The neurological examination should include an assessment of the level of

consciousness, tone, gaze, body-posture, tendon, cranial nerve and newborn reflexes. The general body examination focuses on finding indications for an underlying disease/condition. The skin is inspected for bleeding and bruising, eruptions, birth-marks (for example in the face, indicating phacomatosis). Cardiac examination includes checking for murmurs, including auscultation over the fontanelle as AV malformations can be diagnosed this way. Abdominal examination focuses on intra- abdominal masses (liver, spleen, kidneys)(10).

- **Initial laboratory work-up:** this one will be focused on finding those conditions where an immediate treatment is mandatory either to save the life of the infant, or to minimize the ongoing brain damage and thereby reduce any subsequent neurodisability in surviving children. It is important to prioritize laboratory tests directed at the recognition of metabolic disturbances and infections that, if untreated, can lead to permanent brain damage. Hypoglycaemia is readily identified using point-of-care techniques, and the rapid start of i.v. glucose can be rapidly achieved. In addition, serum concentrations of sodium, potassium, calcium and magnesium should be analysed. Blood-gas analysis will indicate whether there is a lactic acidosis that would warrant further metabolic screening. If initial laboratory testing is negative a more thorough investigation should take place aiming to rule out rare causes of neonatal seizures (10).
- **Neurophysiologic diagnosis and monitoring:** Seizures in the newborn are notoriously difficult to diagnose as clinical signs may be very subtle or completely absent (1). In addition, there is a considerable mismatch between clinical and electrographic diagnosis of neonatal seizure (11). Neonates can make jerky movements that may be misinterpreted as seizures and may receive multiple antiepileptic drugs (AEDs) over many days, become sedated, cannot breast feed and remain in hospital longer than necessary because of this imprecision in diagnosis. Other babies are under treated and spend hours in status epilepticus. EEG monitoring is the only reliable method available for the detection of neonatal seizures (12). In view of this diagnostic complexity, some argue that diagnosis of neonatal seizures should not be based on clinical observation alone. As EEGs are difficult to obtain round the clock on the neonatal unit, initial diagnosis and treatment is based on clinical observation. Several centres are now using amplitude integrated EEG (aEEG) as a readily available bedside tool. This device uses a single or dual channel EEG and acute variations in spectral width to detect seizures. Several reports show that aEEG detects approximately 75% of seizures detected by

conventional EEGs (3).

- **Neuroimaging:** Cranial ultrasound is readily available in most modern NICUs and offers a rapid bedside tool with the possibility of identifying many intracranial pathologies (13,14). A cranial ultrasound is routine management in the investigation of neonatal seizures. It should be carried out as soon as possible after the first occurrence of seizures and will help in early diagnosis of many underlying causes including intraventricular haemorrhage, arterial stroke, malformation and infections. Magnetic resonance imaging (MRI) is the 'gold standard' in the examination of the newborn brain and will reveal most brain pathology (14,15).

Treatment

Although immature brain is more prone to seizures, it is more resistant to post seizure damage than the mature brain. However, evidence from several animal models and a few studies on human infants have shown that prolonged and recurrent seizures have widespread effects, which are deleterious to the developing brain. Specifically, animal studies have shown that seizures result in reduced density of dendritic spines in hippocampal pyramidal neurons, delayed neuronal loss, decreased neurogenesis, synaptic reorganization and changes in hippocampal plasticity (3), and although the prognosis of neonatal seizures has improved over the past several decades, approximately one third of survivors are still left with neurologic sequelae including motor deficits, mental handicap, and epilepsy (1).

According to this data and the difficulty to diagnose a seizure episode many neonatologists have decided to treat seizures by the "rule of 3": more than three seizures per hour or if any one seizure lasts more than 3 minutes (3) and even though a 2004 Cochrane report concluded that there was little evidence to support the use of any Antiepileptic Drug (AED), these are currently used in the neonatal period to treat seizures (16).

First-generation AEDs, such as phenobarbital and phenytoin, are the drugs of first (and second/third) choice because of extensive clinical experience (17). Phenobarbital is a GABA agonist and it controls 70% of clinical seizures and 50% of electrical seizures, it has a long half-life (2-4 days) and enters CSF rapidly. The loading dose is 20mg/kg and achieves therapeutic levels (20-40mg/litre) in the serum within a short time. And phenytoin acts reducing electrical conductance in neurons by stabilizing sodium channels, having as side effects hypotension and arrhythmias that reach to a constant cardiac monitoring (3). However they have limited clinical effectiveness and potential neurotoxicity (17). Several studies support this idea and have been used as a reference for further investigations about the neurotoxicity of antiepileptic drugs.

A study performed in 2002 by P. Bittigau revealed that phenobarbital, phenytoin and other antiepileptic drugs as diazepam, clonazepam, vigabatrin, and valproate cause apoptotic neurodegeneration in the developing rat brain at plasma concentrations relevant for seizure control in humans. To determine whether AEDs exert neurotoxic effects in the developing rat brain, they injected rats i.p. with phenytoin, phenobarbital, pentobarbital, diazepam, clonazepam, vigabatrin, or valproate on postnatal day 7 (P7) and analyzed their brains 24 h later. Phenytoin (10–50mg/kg) produced widespread neurodegeneration on P7 and the neurotoxic action in the forebrain was dose-dependent. Phenobarbital (20–100 mg/kg) and diazepam (5–30 mg/kg) caused widespread apoptotic neurodegeneration in the brains of rats on P7. Neurotoxic effects were reproduced by pentobarbital (5 or 10 mg/kg) and clonazepam (0.5–4mg/kg) in 7-day-old rats and the threshold doses for triggering apoptotic brain damage were 40mg/kg for phenobarbital, 10mg/kg for diazepam, and 0.5mg/kg for clonazepam. Finally valproate (50–400 mg/kg on P7) or vigabatrin (50, 100, or 200 mg/kg twice daily on 3 consecutive days starting on P5) elicited apoptotic neurodegeneration in the developing rat brain in a dose-dependent manner. The threshold dose for valproate was 50 mg/kg and resulted in a peak valproate plasma concentration of 80 µg/ml, which rapidly declined within 8 h and 100 mg/kg for vigabatrin given twice daily on 3 consecutive days. They reported that major AEDs cause sensitive neurons to undergo apoptotic death in the developing rat forebrain and for phenobarbital, they found that plasma concentrations between 25 and 35 µg/ml over a 12-h period triggered apoptotic neurodegeneration in infant rats (17), a concentration easily achieved when phenobarbital was given to human infants (3). This is crucial during the period when the proapoptotic effect of AEDs coincides with the brain growth spurt that is, for humans, the third trimester of gestation until several years after birth (17).

In 2010 a second study performed by P. A. Forcelli PhD and based on Bittigau's conclusions, compared the effects of acute (on P7 or P14) or chronic (daily treatment from P7 to P14) use of phenobarbital during the period between P7 and P14. They presented preliminary findings on striatal synaptic function, development of reflexes, and longer-term behaviours that may prove useful in detecting functional impairment associated with AED-induced neurotoxicity. As a result they found that adult animals that had been exposed to phenobarbital as pups between P7 and P14 exhibited impaired fear conditioning, impaired sensory-motor gating, and altered anxiety-like behaviour (18).

Same author developed a study in 2012 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). To assess the impact of phenobarbital on functional synaptic maturation in infancy, they employed the neonatal rat as a preclinical model. In pups between postnatal day P10 and P18, the maturation of inhibitory and excitatory postsynaptic currents were examined in striatum (19), a brain region that displays reduced volume in human imaging studies of adult patients exposed to AEDs early in life (20). They compared the effects of exposure to phenobarbital to the effects of exposure to 3 other AEDs: phenytoin, lamotrigine, and levetiracetam. Phenytoin is currently a second line treatment for neonatal seizures, whereas lamotrigine and levetiracetam are newer generation AEDs that are believed to have an especially favourable safety profile. By using patch-clamp recordings and morphometric analysis of striatal medium spiny neurons (MSNs), they examined the maturation of GABAergic and glutamatergic synaptic connectivity. The examination of the maturation of striatal neuronal physiology provided the evidence that neonatal exposure to AEDs delayed or stunted the development of both excitatory and inhibitory synapse function. This results were associated with the abnormalities in motor behaviour, sensory motor gating, and memory in adult rats previously described by the same author (19).

According to these findings and results the scientific community interested in this field has been looking for new alternatives, new AED with better security profile at either short or long term; and Levetiracetam has been the one on the spotlight for his experienced good security profile and efficiency. Several studies support this theory, although we have to consider that they all had small samples. Ramantani et al. observed the anticonvulsant efficacy and safety of Levetiracetam (LEV) as a first-line AED in neonatal seizures, after excluding standard metabolic causes: 30/38 (79%) infants were seizure free under LEV at the end of the first week, and 27/30 (90%) remained seizure free at four weeks, while EEGs were markedly improved in 25/30 (83%) patients at four weeks. However some of them needed and adjunctive phenobarbital dose due to recurrency. Related to side effects LEV was tolerated extremely well in the study group, with somnolence during titration (at least partially) attributed to adjunctive phenobarbital therapy (21). Khan et al. retrospectively analyzed 22 neonates treated with intravenous levetiracetam. Fifteen patients (68%) began receiving levetiracetam because of continued seizures on phenobarbital, three (14%) because of adverse reactions to a previous antiepileptic drug, and one (5%) because of continued seizures on fosphenytoin. Three patients (14%) were initially started on levetiracetam. Their data suggested that intravenous levetiracetam could be used for the management of acute seizures in neonates. The use of intravenous levetiracetam demonstrated immediate cessation of seizures in 86% (19/22) of our study patients and concluded that intravenous levetiracetam appeared

efficacious in the management of acute seizures during the neonatal period and again, it did not result in any severe adverse events and was generally well tolerated (22) .

According to all this there's evidence (in rat studies) that treatment with phenobarbital for neonatal seizures could be related to poor neurodevelopmental outcome or influence a lowest neurodevelopmental outcome. If we keep in mind that new antiepileptic drugs, as Levetiracetam, are proved to be as effective as phenobarbital (even though the studies had a small sample) and that Levetiracetam's mechanism doesn't cause any apoptotic death or other damage to the brain; supported this by a human retrospective study (still in revision) that shows that there's a feasible association between Phenobarbital and poor neurodevelopmental outcome compared with the lack of problems with the treatment with Levetiracetam, it's encouraging us to develop a prospective study and a long-term evaluation of neurodevelopmental outcome in order to establish if Levetiracetam could be an alternative to Phenobarbital, reducing afterwards the problems related with the use of this drug and increasing the life quality and future of this babies.

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3. HYPOTHESIS

Levetiracetam has best neurodevelopmental outcome than Phenobarbital.

4. OBJECTIVE

Analyze if the use of Phenobarbital compared with Levetiracetam, it's associated with more neurodevelopmental problems in newborns treated for neonatal seizures.

Secondary objectives:

- Identify which are the most affected areas of the neurodevelopment: cognition, socio-emotional, motor or language.

5. METHODOLOGY

Study Design

A 5 years long clinical trial administering, with double-blind and a randomized distribution of the sample, Phenobarbital or Levetiracetam for the management of neonatal seizures.

The double-blind will be applied to the evaluator of the neurodevelopment status of the child, a neuropsychologist, and for the parents that need to administrate the antiepileptic drug at home, as we will explain.

Subjects of the study

Newborns admitted at the NICUs (Neonatal Intensive Care Units) and at the Hospital ward, with seizures.

Inclusion and Exclusion Criteria

Inclusion	Exclusion
Preterm newborns admitted at the NICU with a convulsive episode	Newborns that come to the NICU from other centres where they have already received a treatment
Term newborns admitted at the NICU with a convulsive episode	Newborns that won't be able to do the follow up because of bad socio-familiar support
Term newborns admitted at the Hospital ward with a convulsive episode	Newborns with a sever/terminal prognosis
Newborns treated and controlled with just 1 type of antiepileptic drug	Newborns that need more than 1 type of antiepileptic drug to control the seizure

Sample Selection

It will be a consecutive sampling as the seizure episodes are diagnosed in newborns admitted at the NICU and hospital ward. Later on after obtaining the informed consent, we will follow up, during 3 years, the neurodevelopment of these newborns.

Sample

Because of the lack of studies about the proportion of neurodevelopmental alterations in newborns treated with phenobarbital, we take into consideration that the proportion of newborns with seizures that suffer from neurodevelopment problems is 28%. Trying to detect a 10% less incidence of problems with Levetiracetam, accepting an alpha risk of 0.05 and a beta risk of 0.20 in a one-sided test, with a ratio between the samples equal 1 and an anticipated drop-out rate of 5%, we will need 229 people in one group and 229 people in the other group to achieve a difference statistically significant.

Multicentricity: because of our need for a big sample that cannot be achieved only for our centre, we propose a multicenter trial. To establish a good communication we will assign: a main director of the trial, a main investigator in each centre participating in the study and we will guarantee the quality of the members/staff.

- Direction: it will need to ensure that the chosen centres are appropriate, so it will visit them and it will meet the staff. The criteria that will determine if the centre becomes a participant are:
 - Research experience, which means that they have previously developed other studies or have participated in them.
 - The centre has a Neonatal ICU
 - They have a group of Neonatologists and Neuropediatricians
 - The hospital has to assume a volume of ≥ 300.000 citizens to ensure a number of deliveries big enough to achieve our sample

It has to ensure that the protocol is followed, it has to approve any modification and it has to take care of a proper distribution of it. It will establish a proper communication system between itself and every main investigator of each centre, and has to be in contact with them without any intermediary unless it's an inevitable situation. It has to be accessible for every investigator, getting informed of any incidence. It will coordinate and plan the trial and ensure that the investigators know all the necessary processes. Moreover it has to have previous experience as investigator and as a specialist in the subject, and minimum 1 publication.

- Main Investigators: they will act in name of the director of the study and they will take the responsibility to achieve everything it's established in the protocol. It will be required experience in previous researches as participants or directors, and minimum 1 publication, to be designated as main investigator.

- Study Staff: they will be chosen according to qualifications and skills related to the tasks that need to develop, plus previous experience participating in research.

It will be a unique protocol and every centre participating will be identified with a main investigator and it will have the description of their tasks. Every data that will be needed are going to be described in the protocol and the way they have to be collected too.

Variables

DEPENDANT

- Neurodevelopment alteration: according to the Bayley Test results, specified as YES or NO

INDEPENDANT

- Drug administered: Levetiracetam or Phenobarbital

COVARIABLES

- Aetiology or Disease that cause the Seizure
- Phenobarbital dose before the inclusion and randomization
- Duration of the treatment: if they don't need treatment at the moment of the hospital discharge or if they need to continue with the treatment at home, and the duration of this. This situation is determined according these criteria:
 - Severe Hypoxic-Ischaemic Encephalopathy or Neurological Injury
 - Non clinical control
 - Non electroencephalographic control/normalization

Data Collection

It will be a neonatologist the one that will diagnose the existence of seizures according to the clinical evidence. The management of the seizure will be done following these steps:

- Determine the Glucose levels to diagnose a seizure that doesn't need an antiepileptic drug.
- For ethical reasons and viability of the study, and for the need of a consecutive sampling to achieve our sample, newborns that suffer seizures will receive treatment with Phenobarbital to stop the crisis while their parents receive the Informed Consent and decide if their child is included in the study.

- Newborns whose parents accept to participate in the study will start the treatment with Levetiracetam or will continue with Phenobarbital, randomly. The dosage will be:
 - Phenobarbital: loading dose of 20mg/kg in 10-15 minutes (intravenous). We will administer additional doses of 5mg/kg until we stop the crisis (maximum dose of 40mg/kg). We will maintain a dose of 5mg/kg/24h divided in two doses a day achieving blood levels between $\leq 40\mu\text{g/ml}$ - $>10\mu\text{g/ml}$. We will follow this pattern until the newborn is included in the trial and after that, randomly, it will continue with this treatment or it will change to Levetiracetam.
 - Levetiracetam: initial dose of 10mg/kg/12h (intravenous) that results in 20mg/kg/day. In three days we will increase the doses (10mg/kg/12h per day, which results 20mg/kg/day) until we reach 30mg/kg/12h, a total amount of 60mg/kg/day.

It will be intravenous until the newborn is able to take the oral solution.

- In case of treatment failure the procedure will be: blood sample and drug levels determination. After that we will receive the results from the laboratory and in case of optimum levels we will consider that the newborn doesn't respond to only 1 drug and for security reasons it will be excluded from the study and it will initiate a treatment with Phenytoin (second line treatment). On the contrary, non optimum levels, we will administer a new dose to achieve the therapeutic levels.

Thereafter we will perform the following procedures:

1. Before the hospital discharge:
 - a. Drug used
 - b. EEG register
 - c. Neurological evaluation

These data will determine the need, as we have already explained, to continue or not the antiepileptic treatment at home.

2. After the hospital release:

If we need to continue with the treatment at home (which will be established by the criteria already mentioned) the parents will collect the medication, while the treatment is needed, at the hospital on the days they visit the neuropediatrician for the routine evaluation. They won't

know the drug they are administering, the drug will be codified to assure the blind. This will continue until the normalization of the EEG, which will determine the stop of the treatment.

During the routine evaluation in the neuropediatrician (every 3 months during the first year, every 6 months during the second year) we will collect data about de motor development, language, cognition and socio-emotional behaviour according to the Haizea-Llevant Scale. This data will be registered as achieved or non-achieved. At the age of three a neuropsychologist will perform the Bayley test, which is designed to identify the abilities that the children have using activities that simulate games. The neuropsychologists won't know the drug used and will evaluate:

- Cognition
- Language: expressed, received
- Gross and fine motor skills
- Socio-emotional

The statistical study used for the main objective will be a logistic regression, presenting OR adjusted for the covariables already mentioned, and a multinomial regression will be used for the secondary objective, adjusted for the covariables too.

Staff

- Statistician: it will use the data for the statistical analysis.
- Neuropsychologists: in charge of the evaluation of the neurodevelopment using the Bayley Test.
- Neuropediatricians: in charge of the routine evaluations of these children, and the ones in charge to stop the treatment if they had had to continue it at home.
- Neonatologists: in charge to diagnose the neonatal seizures and authorize the treatment and determine the success of the treatment.
- Nurses: in charge to administrate the drug and to collect the data.
- Laboratory Staff: in charge to check the drug levels in the blood samples if we need a new dose for the treatment.
- Main Investigators: in charge to guarantee the quality level of the centre. They will be in touch with the Director of the Project.
- Pharmacy: in charge to prepare the drug for the treatment at home and ensure the blind for the parents with a codification.

Limitations

- The aetiology or disease that causes the seizures may disturb our results. The way we will solve it is with the randomization, equivalently distributing the samples.
- The sample we need is quite large, so this can difficult the reproduction of this study. We will solve this with the multicentricity, which will increase the population available.
- For ethical reasons and for the viability of the project we will need to administer Phenobarbital to every newborn before starting the study, so our results will be explained adjusted to the dose of Phenobarbital used.

6. ETHICAL ASPECTS

This clinical trial follows the declaration of Helsinki involving ethical principles for Medical Research involving Human subjects (last actualization October 2013).

It will be send to the ethic committees of all the centres participating in our study and has to be approved in each one of them to be performed.

It will be performed according the Spanish Laws related to clinical trials:

“Ley 29/2006 de 26 de julio, de garantías y uso racional de los medicamentos y productos sanitarios:

- *RD 223/2004 de 6 de febrero: ensayos clínicos con medicamentos*
- *RD 1591/2009 de 16 de octubre y 1616/2009 de 26 de octubre: investigación con productos sanitarios”*

And it will be registered in the EUDRA-CT.

Every subject participating in this clinical trial will be properly informed and it will sign an Informed Consent (Appendix 1 and Appendix 2). The data collected for the trial will be always treated and used anonymously preserving the confidentiality of the patients.

This clinical trial has an insurance to take the responsibility towards its members if any adverse event is suffered because of our study/intervention.

The investigators have no conflicts of interest.

7. CHRONOGRAM

The duration of this clinical trial is 5 years and will be organized according to the following phases.

1. Coordination of the centres and members of the study: 2 months. Selection of the centres that will participate in the study, checking the quality of the staff according to the needs for the trial. In addition, it will be a meeting to explain the communication system, to introduce the chiefs of the investigation, to solve all the questions and to check that the protocol has been understood and will be followed according to what's been established.
2. Sample Collection and Treatment of the Convulsive Event: 1 year. Collecting the sample in order of appearance and distributing randomly the drugs of the study. Treat the newborns and collect the variables needed.
3. Meeting nº1: 1 month. After the end of the sample collection it will be established a meeting to check the quality of the data collected in each centre and to check that the rules have been followed.
4. Follow Newborns: 2 years since birth. Every 3 months during the first year, every 6 months during the second year, as it's established for any newborn suffering from neonatal seizures. It will be checked the evolution and the neurodevelopmental status according to the standard methods (Haizea-Levant).
5. Meeting nº2: 1 month, between year 1 and 2. To check that everything it's being performed as it's established.
6. Bayley Test: 1 year. At the age of 3 years these toddlers will undergo to the Bayley Test by the Neuropsychologists, to check their neurodevelopmental status and problems. We will need a year according to the fact that we would have been collecting babies for a year and they will be 3 years old at different times.
7. Meeting nº3: 1 month. To check that the data collection has been performed according to the protocol.
8. Results: 1 year. The statistician's analysis with the data collected and results. Plus a meeting of the members of the trial to evaluate the conclusions.
9. Publication: 10 months. Write and edit the results to publish them.

8. CHRONOGRAM SCHEME

	YEAR 1		YEAR 2		YEAR 3		YEAR 4		YEAR 5
	January-February	March-January	February	March-January	February	March-February	March	April-February	December
Coordination of the centres and members of the study									
Sample Collection and Treatment of the Convulsive Event									
Follow Newborns									
Bayley Test									
Meeting n° 1									
Meeting n° 2									
Meeting n° 3									
Results									
Publication									

9. BUDGET

CLINICAL TRIAL BUDGET PROPOSAL			
Staff	Cost per unit/month	Number/Months	Total
Neuropsychologists	1.800 €	4 x 12	86.400 €
Statistician	2.000 €	2 x 12	48.000 €
Material	Cost per unit	Number	Total
Bayley – III Complete Kit and Screening Test Combo	814 €	4	3.256 €
EEGa	4.963 €	4	19.852 €
Levetiracetam	61,74€	500	30.870 €
Meetings			
IX Congreso Nacional de la Sociedad Española de Neurología Pediátrica	470 €	2	940 €
EPSN Congress – European Paediatric Neurology Society	750 €	2	1.500 €
Coordinators Meetings	60 €	2 people x 3	360 €
Cost of the Trips:			
• Flights			
○ National	130 €	2	260 €
○ International (EU)	150 €	2	300 €
• Accommodation	170 €	4	680 €
Publications			
Seminars in Fetal and Neonatal Medicine	3000 €		3000 €
TOTAL			195.418 €

This budget has been done according to the fact that this is a multicentric clinical trial. As we consider including 4 centres to this project, here we provide the explanation for the costs:

- **Staff:** we will need neuropsychologists that, as experts, will set the Bayley Tests to the toddlers in order to establish their neurodevelopmental status. Each one of them will be placed in the different member centres. Moreover we'll need two statisticians to analyze the data collected. Because of the fact that Levetiracetam is not the first line treatment and we will use it as a main treatment for 229 patients this will cause an extra expenditure that has to be included as an important part of the budget.
- **Material:** taking onto consideration that we won't be able to perform at certain hours of the day an EEG, we have considered buying an Amplitude Integrated EEG, which can be used by any neonatologist (not necessary a neurophysiologist) and can diagnose and show any pathologic pattern. Moreover, for each centre we'll need a Bayley Test Set¹, for each Neuropsychologist to work with.
- **Meetings:** we include in the budget two major meetings where we plan to explain our results, a national one and an international one. Taking onto consideration that it's 2 people per meeting, the flights to the national one cost approximately 130€ and international (Europe) cost 150€, which in total it's 560€. For the accommodation we propose a 4 star hotel for 4 nights for both meetings and same number of participants (2 per meeting), which will cost maximum 170€ for each Congress/person.
- **Publications:** we want to publish in two journals each one chosen according to the impact of our clinical trial, one involving the neuropaediatricians and another one involving neonatologists. One of them charges no costs to the publication so its not added in the budget; the costs of the other one (Elsevier Editorial) includes the fee to include the publications to the *Open Access* system.

¹ Bayley Test is not included in this document because of its magnitude. It's an integrated kit with games and other materials that cannot be added.

10. IMPACT OF THE PROJECT

Despite the number of studies that have proved that phenobarbital causes neurodevelopmental problems in newborns treated for neonatal seizures, this drug has still been used among children as the main treatment because of his efficacy, not always absolute, in arresting these events. However the emergence of new drugs that have proved nowadays that have no secondary effects, show up new theories and assumptions that may change the way these newborns are treated, decreasing the dangerous effects on his neurodevelopment. And among these new generation drugs, Levetiracetam has become the one around whom every studies are focused on.

At the present time there is only one retrospective study still on review from November 2013 that compares the effect between Phenobarbital and Levetiracetam on the neurodevelopment of these children. However there are lots of other studies (in rats/mice) proving that Phenobarbital causes damage or injury in their CNS. All of them insist on the need of prospective studies that compare these two drugs and prove the association clearly in order to change the way we manage neonatal seizures, to change and decrease the morbidity caused by the iatrogenic effects of the drugs we use.

Referring to the incidence of these episodes: 1-2/1000 on term newborns and 30-130/1000 premature newborns, changing the pattern of treatment and diminishing the long-term problems will entail a minor investment by the Public Health Services. In the long run this newborns won't need so many support from neuropsychologists or other health professionals because we would have decreased the level of dangerous effects that the drugs themselves can induce over the CNS of these children.

To sum up and to conclude: we will decrease the morbidity caused by the drug and therefore the health expenditure and family disruption arising from this. This results as a beneficial effect over the families, decreasing the stress and emotional suffering that comes from the neurological disabilities of a child, and over the National Health System, decreasing the investment on the needs of this population.

APPENDIX

APPENDIX 1. FULL D'INFORMACIÓ PEL PACIENT

Aquest document està destinat als pares dels nadons ingressats en el nostre centre i als que se'ls convida a participar en l'estudi sobre els efectes sobre el neurodesenvolupament per l'ús de Fenobarbital versus Levetiracetam en el maneig de les convulsions neonatals.

Es divideix en:

- Informació pel participant
- Formulari de Consentiment Informat

Les convulsions neonatals són una manifestació freqüent de malfunció del sistema nerviós central en el neonat, i són un factor de risc per a mort o discapacitat neuronal conferint al infant un pobre desenvolupament neurològic, fet que inclou alteracions de la cognició, social i emocional, de llenguatge i motores.

Les convulsions resulten d'una descàrrega excessiva i sincrònica d'un grup de neurones del sistema nerviós, manifestant-se amb episodis de moviments anormals o amb senyals patològiques en electroencefalogrames.

Actualment el seu maneig es fa amb Fenobarbital, però recent estudis han suggerit que aquest farmac pot influir en el desenvolupament neurològic de l'infant, portant aquest fet a generar nous estudis en busca de nous fàrmacs. Els resultats ens han indicat que el Levetiracetam, un antiepilèptic de nova generació referit com a eficaç pel maneig de les convulsions neonatals, podria ser una bona alternativa per aquests infants, disminuint els possibles efectes secundaris del primer.

Amb aquest estudi volem determinar als 3 anys, quin es l'estat del desenvolupament dels infants tractats amb Fenobarbital o Levetiracetam per a poder presentar en un futur una alternativa a la teràpia actual.

Es tracta d'un assaig clínic que consisteix en l'administració aleatòria d'un dels dos fàrmacs (Fenobarbital o Levetiracetam) pel maneig inicial de la convulsió. L'aleatorització assegura l'assignació dels participants a un dels dos grups de l'estudi tinguent aquests la mateixa probabilitat de ser assignats a un grup o un altre.

Posteriorment l'infant serà seguit pel servei de neuropediatria en els terminis habituals per a qualsevol nadó que hagi patit un succés convulsiu en el període neonatal. Finalment als 3 anys

serà sotmés al test neuropsicològic de Bayley pels nostres especialistes, per determinar l'estat del desenvolupament neurològic i identificar-ne o no algun dèficit.

En cas que durant el quadre convulsiu es necessiti medicació de rescat pel maneig, el nadó serà sotmés a un anàlisi de sang que ens determinarà nivells de fàrmac en circulació i respecte a aquests rebrà les dosis necessàries i establertes de tractament seguint una de les dues vies següents:

- Proseguirà amb el fàrmac de l'estudi si els nivells d'aquests encara no eren els òptims.
- Rebrà el fàrmac de segona línia (Fenitoïna) en cas que els nivells ja fossin òptims essent així exclòs de l'estudi, prioritzant-ne la seva salut a la investigació.

Com s'esmenta, el fàrmac estandard per al maneig de les convulsions podria influir parcialment (a part de la patologia base) en el desenvolupament neurològic de l'infant; no obstant la teràpia alternativa es desconeix a llarg plaç algun efecte secundari, i per tant no podria descartar-se'n. En ambdós casos el seguiment exhaustiu propiciarà el maneig adequat de l'infant en cas que fos necessari.

Els convidem doncs a participar en el nostre estudi sobre el Fenobarbital i el Levetiracetam. La seva participació pot determinar un benefici directa o indirectament per a futures generacions, donant resposta a la nostra pregunta i poguent alterar el maneig futur de les convulsions neonatals.

La participació en aquest estudi es totalment voluntaria. La decisió de no incloure's no canviarà el seguiment estàndard del nostre centre i rebrà el tractament estàndard per al maneig de l'event convulsiu. Podrà abandonar l'estudi si ho considera necessari, és la seva elecció i els seus drets seran respectats.

El maneig de les seves dades serà en total confidencialitat i el seu ús serà fet només pels investigadors.

APPENDIX 2. FORMULARI DE CONSENTIMENT INFORMAT

Jo _____ i _____ com a mare/pare/tutor legal, de _____ autoritzo voluntariament la participació del meu fill/a en la investigació sobre l'ús de Fenobarbital o Levetiracetam pel maneig de les Convulsions Neonatals i el seu efecte sobre el Neurodesenvolupament, i entenc el dret de retirar-lo/la de l'estudi en qualsevol moment sense que m'afecti de cap manera el meu tracte medic.

Així mateix expresso que he llegit o m'ha estat llegida la informació sobre l'estudi per part de _____, tinguent l'oportunitat de preguntar i rebre respostes satisfactòries sobre l'estudi.

Finalment se'ns ha informat que en cas necessari, per la seguretat del nostre fill, els professionals aplicaran les mesures necessàries pel maneig de les convulsions i ens informaran de la situació.

Mare _____

Pare _____

Professional Sanitari _____

_____ de _____ del 20__

APPENDIX 3. HAIZEA-LLEVANT SCALE

