Effect of Phenobarbitone on Amplitude-Integrated Electroencephalography in Neonates with Hypoxic-Ischemic Encephalopathy during Hypothermia

Poorva Deshpande[a, b]   Amish Jain[a, b]   Patrick J. McNamara[a, c]

[a]Division of Neonatology, Hospital for Sick Children, Toronto, ON, Canada; [b]Department of Pediatrics, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; [c]Department of Physiology, University of Toronto, Toronto, ON, Canada

Abstract

Background: Phenobarbitone induces suppression of cerebral electrical activity on amplitude-integrated electroencephalography (aEEG) in neonates with hypoxic-ischemic encephalopathy (HIE); however, its effect during therapeutic hypothermia (TH) has not been well characterized. ***Objective:*** To evaluate the effect of phenobarbitone on aEEG in neonates with HIE undergoing TH. ***Methods:*** Thirty-five neonates born at $\geq 35^0$ weeks gestational age (GA), who received phenobarbitone as first-line antiepileptic drug during TH for $\geq$ Sarnat stage II HIE with aEEG recordings were retrospectively studied. Background pattern, upper and lower margin voltages were characterized for a 30-min period before and 30–60 min after phenobarbitone administration. Primary outcome was presence of severely abnormal aEEG pattern after phenobarbitone administration. ***Results:*** Mean (±SD) GA and median birth weight were 38.2 ± 1.9 weeks and 3.1 (2.5–3.9) kg, respectively. Phenobarbitone (10–20 mg/kg), administered at median age 16.8 h, was associated with background pattern worsening in 19/29 (65.5%) cases. Severe background patterns were more prevalent in post- versus pre-phenobarbitone tracings (21/29 [72%] vs. 11/29 [38%]; $p = 0.01$). Presence of severe pattern versus either continuous normal voltage or discontinuous normal voltage pattern post-phenobarbitone, (20/25 [80%] vs. 3/8 [38%]; $p = 0.036$) was associated with death or moderate-to-severe injury on MRI brain. Median time to trace recovery, when measurable, was 4 h (45 min–72 h). ***Conclusions:*** Phenobarbitone induces significant suppression on aEEG in infants with HIE undergoing TH. Development of severe aEEG background patterns after phenobarbitone may unmask a population at greater risk of abnormal outcome.

© 2021 The Author(s)
Published by S. Karger AG, Basel

Background

Amplitude-integrated EEG (aEEG) is commonly employed in neonatal intensive care units (NICUs) to monitor background cortical activity, seizure management,

Poorva Deshpande
Department of Paediatrics, Staff Neonatologist
Room 19-231, Mount Sinai Hospital, 600 University Avenue
Toronto, ON M5G 1X5 (Canada)
poorva.deshpande @ sinaihealth.ca

and aid prognostication in infants with hypoxic-ischemic encephalopathy (HIE) [1–11]. Previously, presence of a severely abnormal aEEG background pattern at <6 h of age was considered to be a strong predictor for adverse neurodevelopmental outcome [2, 4]. Therapeutic hypothermia (TH), however, has shown to delay the overall recovery of aEEG in HIE patients, with recent studies demonstrating persistence of aEEG abnormalities ≥24 h of age and time to normalization of trace being better predictors of adverse outcomes [12–14].

Antiepileptic drugs (AED), such as phenobarbitone being the commonest first-line agent, are frequently used for seizure management in infants with moderate and severe HIE and are well known to induce aEEG suppression [15–17]. In clinical practice, trends in aEEG characteristics are often used as an ancillary tool to refine prognosis. Hence, accurate documentation of the degree and/or duration of drug-induced aEEG suppression may help clinicians differentiate these "iatrogenic" effects from HIE-related suppression and potentially avoid misinterpretations.

Although the suppressive effects of phenobarbitone on neonatal aEEG have been described under normothermic conditions, its impact and clinical relevance in the presence of TH remains unknown [16–18]. Therefore, the primary aim of this study was to characterize the effect of phenobarbitone on aEEG background pattern in infants with moderate and severe HIE undergoing TH. Secondary aims were to assess the effect of phenobarbitone on aEEG voltage, investigate the time to trace recovery and to study the association between aEEG suppression following phenobarbitone and severity of HIE. We hypothesized that during hypothermia, phenobarbitone causes suppression of aEEG background in >50% of tracings.

Methods

Study Design

This retrospective cohort study was conducted at the NICU of the Hospital for Sick Children, Toronto, over a 2-year period when aEEG recordings were archived and phenobarbitone was the first-line AED used. The study was approved by the Institutional Research Ethics Board and parental consent requirement was waived.

Inclusion and Exclusion Criteria

All infants with Sarnat stage II (moderate) or III (severe) HIE, born at gestational age ≥35^0 weeks, who received treatment with intravenous phenobarbitone for clinical or electrical seizures while undergoing TH, and had aEEG recorded during phenobarbitone administration were considered for inclusion. Only the first episode of seizure treatment after admission to our unit

Table 1. National Institute of Child Health and Human Development (NICHD) scoring system for classifying brain injury on MRI for infants with HIE

Score	Injury pattern observed on brain MRI
0	No injury seen
1A	Minimal cerebral lesions without involvement of BGT, ALIC, or PLIC, and no watershed area infarction
1B	More extensive cerebral lesions but without BGT, PLIC, or ALIC involvement or watershed area infarction
2A	Any BGT, ALIC, or PLIC involvement or watershed area infarction, but without any other cerebral lesions
2B	Any BGT, ALIC, or PLIC involvement or watershed area infarction, and additional cerebral lesions
3	Cerebral hemispheric devastation

HIE, hypoxic-ischemic encephalopathy; BGT, basal ganglia or thalamus; ALIC, anterior limb of the internal capsule; PLIC, posterior limb of the internal capsule.

was considered for analysis. Infants who may have had a prior phenobarbitone dose in the community before admission were included. Infants who received AEDs other than phenobarbitone, including lorazepam as first treatment after admission, and those who had seizures after completion of rewarming were excluded. Tracings with impedance >10 ohms or where time of phenobarbitone administration was not marked were also excluded.

Study Setting

Our center is an outborn quaternary NICU, where infants with suspected HIE are referred from community hospitals after initial stabilization. When appropriate, TH is commenced pre-transfer either by the community physicians or the neonatal transport team or in the NICU after admission, in all cases according to our standardized guideline. Typically, TH is commenced at age <6 h and continued for 72 h. All infants undergoing TH also receive aEEG monitoring for the duration of treatment, starting soon after admission until completion of rewarming or death/withdrawal of life sustaining treatment, whichever occurred earlier. During the study period, aEEG was recorded using single-channel Olympic CFM 6000 monitor (Natus Medical Incorporated, San Carlos, CA, USA) with electrodes at P3-P4 location or BRM2 BrainZ monitor (BrainZ Instruments, New Zealand) with electrodes at C3-C4 and P3-P4 locations, using hydrogel electrodes, and archived electronically on our hospital's server. Phenobarbitone, given intravenously at a dose of 10, 15, or 20 mg/kg over 20 min was our first-line AED and the timing was marked on the aEEG monitor by the bedside nurse. The decision to treat, whether for a clinical and/or electrical seizure, as well as the choice of dose of phenobarbitone, was at the attending physician's discretion. Conventional 1-h EEG was obtained at some point during the infant's admission period. For
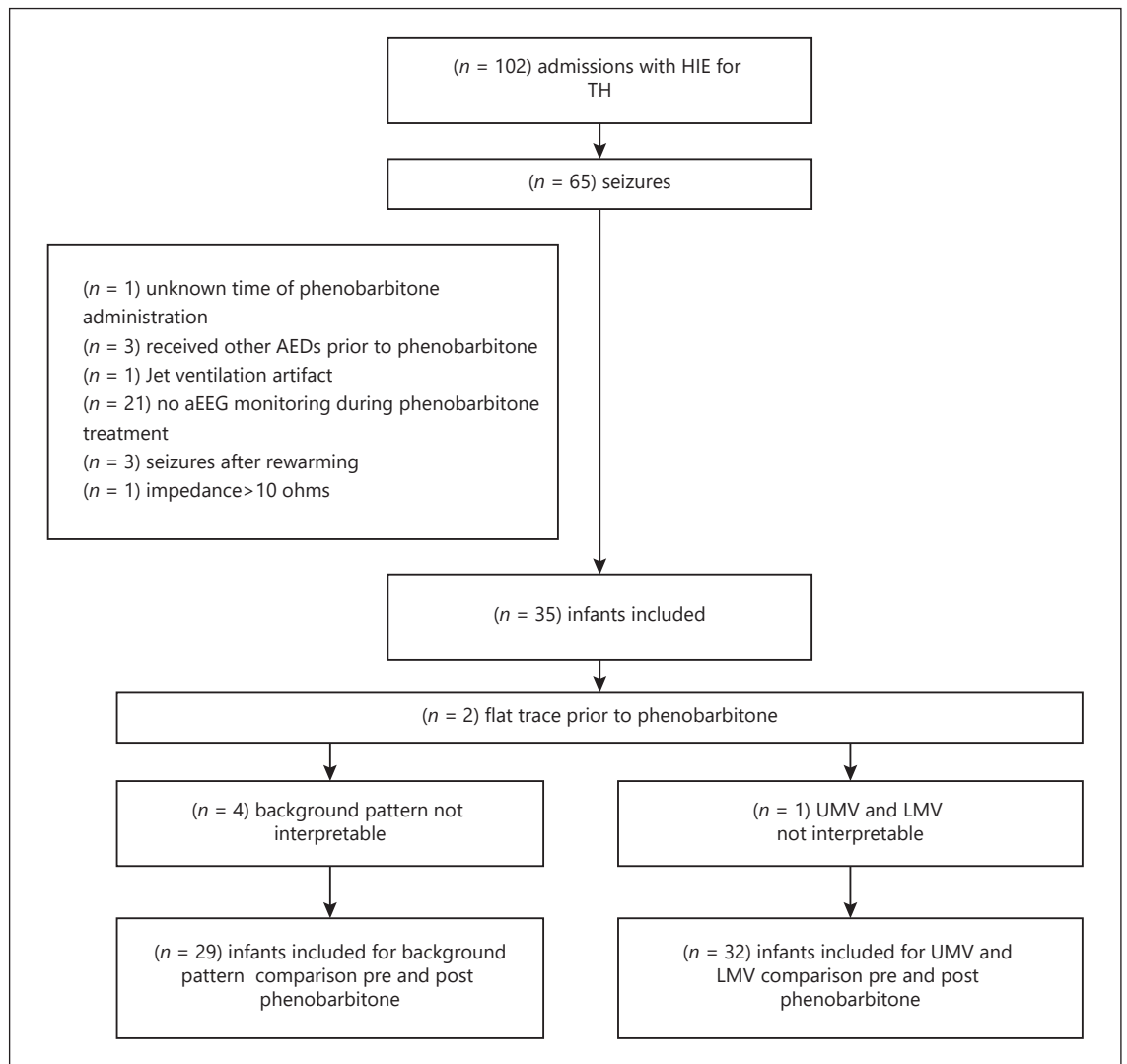
Fig. 1. Eligibility and analysis criteria. HIE, hypoxic-ischemic encephalopathy; TH, therapeutic hypothermia; aEEG, amplitude-integrated electroencephalography; AEDs, anti-epileptic drugs; FT, flat trace; LMV, lower margin voltage; UMV, upper margin voltage.

the study period, full montage continuous EEG monitoring was undertaken only for patients with intractable seizures needing midazolam infusion, in consultation with our institute's pediatric neurology team. Brain MRI was performed between days 3 and 5 after birth for all surviving infants treated with TH for HIE and reported by our institute's pediatric neuroradiologists.

Data Collection

Patients who underwent TH for HIE were identified from our unit's computerized database, and their health records were reviewed to determine eligibility. Clinical data were collected for demographics and perinatal history, Sarnat stage of HIE, timing of TH initiation and rewarming, presence of liver dysfunction, phenobarbitone dose and age at administration, concomitant use of opioid sedatives and outcomes (mortality and results of brain MRI,

whenever available). Only the first episode of phenobarbitone administration after admission to the NICU and initiation of aEEG monitoring was included for analysis.

Assessment of aEEG

Tracings for a duration of 30 min prior to (baseline) and between 30 and 60 min after phenobarbitone administration were reviewed and categorized by one of the 2 investigators (PD and AJ), who had >7 years of clinical experience in interpreting neonatal aEEG. For consistency between the 2 aEEG devices, only the P3-P4 traces were used for trace analysis. For each time period, the worst background pattern and lowest upper margin voltages (UMV) and lower margin voltages (LMV) were recorded. The UMV and LMV were determined visually by drawing a line across the uppermost and lowermost dense part of the tracing, respec-

Table 2. Perinatal and clinical characteristics of the study cohort (*n* = 35)

Female gender	16 (46%)
Gestational age, weeks	38.2±1.9
Birth weight, g	3,085 (2,510, 3,900)
Known sentinel event[α]	10 (29%)
History of fetal distress[β]	28 (80%)
Caesarean delivery	23 (66%)
Intubation at birth	32 (91%)
Chest compressions	19 (54%)
Apgar score at 5 min	3 (0, 9)
Cord pH	6.9±0.2
Base deficit	−16.9±6.9
Persistent pulmonary hypertension	5 (14%)
Hypotension – requiring treatment	16 (46%)
Prior phenobarbitone before admission	20 (57%)
Clinical seizures only	10 (29%)
Dose of phenobarbitone for the episode studied	
20, mg/kg	24 (69%)
15, mg/kg	1 (3%)
10, mg/kg	10 (29%)
Age at phenobarbitone administration, h	16.8 (5.8, 62.9)
Liver dysfunction	22 (63%)
Severe injury on MRI	16 (46%)
Mortality	10 (29%)

Data are presented as percentage, mean ± SD, or median (range) as appropriate. [α] Include placental abruption, prolonged labor, and shoulder dystocia. [β] Defined as documentation of fetal heart decelerations, tachycardia, or abnormal variability. [γ] Rest were either electrical or electroclinical.

Fig. 2. Changes in aEEG background pattern before and after phenobarbitone in 31 infants. Background pattern could not be determined in 4 infants due to prolonged seizure activity prior to phenobarbitone administration. aEEG, amplitude-integrated electroencephalography; CNV, continuous normal voltage; DNV, discontinuous voltage; BS, burst suppression; CLV, continuous low voltage; FT, flat trace.

tively. Whenever background pattern changed after phenobarbitone, tracings were further reviewed either until it recovered to baseline status or until completion of rewarming, whichever occurred first. For the former, time to trace recovery was calculated.

Definitions

Background pattern was categorized according to the classification previously described by Hellström-Westas [19]. Continuous normal voltage (CNV) was considered a normal background, discontinuous normal voltage (DNV) as moderately abnormal, and burst suppression (BS), continuous low voltage (CLV), or flat trace (FT) as severely abnormal. UMV was categorized as > 25 µV, 10–25 µV, 5–10 µV, or <5 µV, and LMV as >5 µV, 3–5µV, or <2 µV. A "clinically significant" change after phenobarbitone was defined a priori based on (i) background pattern: CNV to any other pattern or DNV to BS/CLV/FT or BS/CLV to FT or (ii) changes in UMV: 10–25 µV to <10 µV or >5 µV to <5 µV or (iii) changes in LMV: >5 µV to <5 µV or 3–5µV to <2 µV. Brain MRI findings were scored from 0 to 3, as per the National Institute of Child Health and Human Development system (Table 1) [20]. For this study, adverse outcome was defined as death or moderate-to-severe injury on MRI (score ≥2 A). Furthermore, liver dysfunction was defined as alanine transferase >52 Units/L and/or aspartate transferase >70 Units/L as per our laboratory normal values.
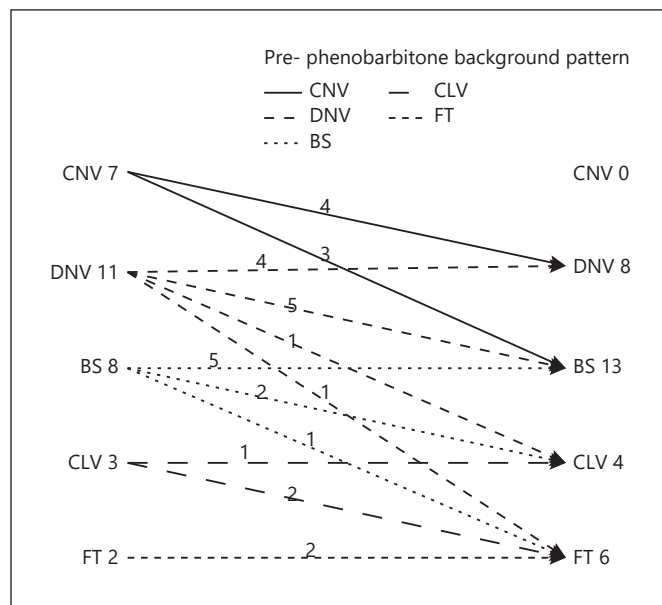
Study Outcomes

Presence of new-onset, severely abnormal background patterns after phenobarbitone was considered the primary outcome. Secondary outcomes included: (i) changes in UMV and LMV after phenobarbitone compared to baseline, (ii) time to trace recovery, where applicable, (iii) association between pre- and post-phenobarbitone aEEG characteristics and death or moderate-to-severe injury on brain MRI.

Statistical Analysis

Data are described as frequency (percentage), mean (standard deviation), or median (range), as appropriate. The frequency of aEEG patterns pre- and post-phenobarbitone as well as their association with adverse outcome of death or moderate-to-severe MRI injury was analyzed using Fisher's exact test. Time to trace recovery was described in hours and compared between infants with Sarnat stage II versus stage III HIE, with or without liver dysfunction and phenobarbitone dose <20 mg/kg versus 20 mg/kg using Wilcoxon signed-rank test. Furthermore, interobserver reliability was tested on 20 tracings (10 pre- and 10 post-phenobarbitone) from 10 randomly selected subjects using Cohen's kappa statistic, which was 0.92 and 0.93, for categorization of background pattern and LMV, respectively.
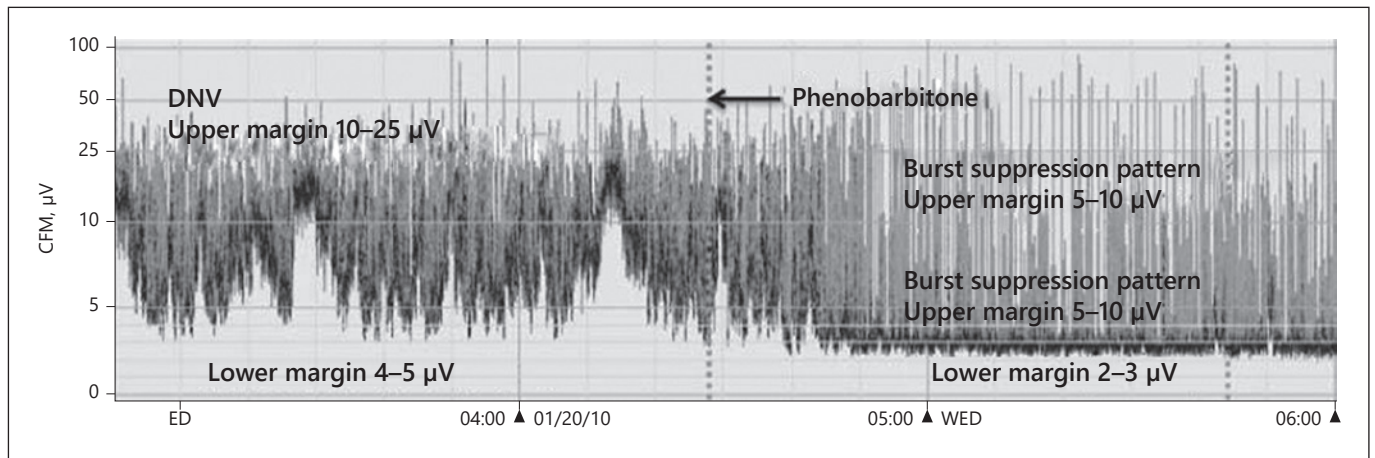
Fig. 3. Example of an aEEG tracing illustrating effect of phenobarbitone administration to an infant with HIE and seizures while receiving therapeutic hypothermia. The left side of the tracing shows a discontinuous background pattern (UMV between 10 and 25 μV and LMV at 4–5 μV) with repetitive seizures. The dotted line in the middle part of the tracing indicates intravenous administration of phenobarbitone at 20 mg/kg over 20 min. Following this, the background changed to a BS pattern with a drop of both UMV and LMV to 5–10 μV and 2–3 μV respectively. aEEG, amplitude-integrated electroencephalography; HIE, hypoxic-ischemic encephalopathy; UMV, upper margin voltage; LMV, lower margin voltage; BS, burst suppression; DNV, discontinuous normal voltage.

Table 3. Characteristics of background aEEG traces at baseline and post-phenobarbitone

aEEG characteristics	Baseline N (%)	Post-phenobarbitone N (%)	p value
Background pattern[α]			
Severely abnormal trace (BS/CLV/FT)[β]	11/29 (38)	21/29 (72)	*0.004*
Continuous	7/29	0/29	*0.01*
Discontinuous	11/29 (38)	13/29 (24)	0.6
Upper margin <10 μV[γ]	10/32 (31)	16/32 (50)	0.2
Upper margin <5 μV[γ]	2/32 (6)	10/32 (31)	*0.001*
Lower margin <5 μV[γ]	24/32 (75)	32/32 (100)	*0.005*
Lower margin <3 μV[γ]	9/32 (28)	22/32 (69)	*0.005*

aEEG, amplitude-integrated electroencephalography; BS, burst suppression; CLV, continuous low voltage; FT, flat trace. Valies in italics indicate *p* < 0.05. [α] Pre-phenobarbitone background pattern could not be ascertained for 4 infants due to presence of seizures at onset of aEEG recording. [β] Excluding 2 infants with baseline flat trace. [γ] Pre-phenobarbitone upper and lower margin voltage could not be classified for 1 infant due to seizures at onset of aEEG recording.

Results

A total of 35 infants with HIE, 18 with Sarnat stage III and 17 with stage II, satisfied the inclusion criteria (Fig. 1). The cohort characteristics are listed in Table 2. Two infants demonstrated FT even before phenobarbitone, and were not included in further analysis. Four tracings had pre-seizure recording duration <15 min, where background pattern could not be ascertained, including 1, where LMV and UMV could not be ascertained. In comparison to baseline, post-phenobarbitone tracings demonstrated higher frequency of severely abnormal patterns and UMV and LMV below pre-defined thresholds (Table 3). None of the traces demonstrated CNV pattern after phenobarbitone administration (Table 3). A clinically significant change

in background pattern was seen in 19/29 (65%) (Fig. 2, 3) and in UMV and/or LMV in 14/32 (44%) infants. There was no difference within the subgroups of infants with clinical and electrical seizures with respect to clinically significant change in the background (7/19 vs. 3/10, $p = 1$) or any clinically significant change (7/25 vs. 3/7, $p = 0.7$).

Fourteen of the 19 traces recovered to baseline status prior to exposure to any further AED. The median (range) time to trace recovery was 4 (0.75–72) hours. Four traces remained suppressed until the end of aEEG recording and 1 infant was given another AED before trace recovery. Time to trace recovery did not differ between infants with Sarnat stage II versus III HIE (4.38 [1–72] vs. 3 [0.75–9] hours; $p = 0.40$), with versus without liver dysfunction (2.75 [1–9] vs. 5.7 [0.75–72] hours; $p = 0.22$), or phenobarbitone dose <20 mg/kg versus 20 mg/kg (1.8 [0.75–72] vs. 5.67 [2–10] hours; $p = 0.10$). For 6 infants, where the study episode was the only exposure to phenobarbitone and therefore not proceeded by any AED in the community, median (range) time to trace recovery was 4 (2–10) hours.

Death or moderate-to-severe MRI injury occurred more frequently in those with severely abnormal post-phenobarbitone background pattern versus CNV/DNV (20/25 [80%] vs. 3/8 [38%]; $p = 0.036$). No association was seen between adverse outcome and pre-phenobarbitone severely abnormal background pattern versus CNV/DNV (8/11 [72%] vs. 11/18 [61%]; $p = 0.7$).

Discussion

In this study, we found that phenobarbitone treatment for seizures in neonates with HIE undergoing TH is characterized by important changes in various clinically relevant aEEG parameters including background pattern and voltage margins. We also provide data on time to trace recovery, where applicable, that clinicians may be able to apply while interpreting aEEG in the context of HIE and TH. Furthermore, we found an association between aEEG patterns 30–60 min post-treatment but not pre-treatment, with adverse outcome of death or moderate-to-severe brain injury.

Although the suppressive effect of AEDs on neonatal aEEG have previously been described, to the best of our knowledge there is no previous report on the effect of phenobarbitone on neonatal aEEG in the setting of TH studied in the clinical context [16, 18]. Shany et al. [17] described the suppressive effects of commonly used AEDs

from 191 aEEG tracings in 77 neonates, who received treatment for seizures. While 75% of patients had a diagnosis of HIE, none received TH. Each repeat AED exposure was considered as a separate episode. The authors reported worsening in background pattern, UMV, and LMV in up to 12, 35, and 32% tracings, respectively. Overall, mean (range) time to trace recovery was 2.5 (0.25–15) hours and 2.7 h for phenobarbitone. Data specifically from the HIE subgroup or clinical outcomes were not evaluated. Although there are differences in the population and AEDs used by Shany et al. [17], we noted a comparatively higher frequency of post-treatment suppressed tracings and slightly longer median time to trace recovery; however, trace recovery was noted in only half of the traces in our cohort.

The mechanism behind suppression after phenobarbitone in the setting of HIE and TH may likely be because of the following reasons: first, the severity of suppression may relate to the severity of brain injury; specifically, it is plausible that treatment with AEDs may unmask a subpopulation with increased risk of brain injury. This hypothesis is supported by our finding of an association of adverse clinical outcome and abnormal tracing only after but not before phenobarbitone administration. Second, it may be related to seizure burden, which could not be addressed in our study. Although it may be plausible that severe HIE or a high seizure burden may increase the sensitivity of the brain to the suppressive effect of phenobarbitone, our findings do not explain the mechanism behind these hypotheses. Finally, although we do not have data on plasma phenobarbitone levels, pharmacokinetic studies have demonstrated no change in phenobarbitone clearance in hypothermia versus normothermia [21, 22]. In fact, a study using simulated pharmacodynamic modeling found phenobarbitone treatment to be associated with reduced rate of transition in aEEG pattern from CNV to DNV [21]. Phenobarbitone clearance may also be impacted by liver dysfunction; however, a lack of association between hepatic dysfunction and trace suppression in our study also suggests a likely lack of role of differing pharmacokinetic profile. Our observations, however, are based on a small sample size and need further validation.

Strengths of our study include strict eligibility criteria, well-defined population, and minimizing the confounding influence of multiple drugs and escalating dosage. Limitations include retrospective design and small sample size, which prevented us from accounting for the independent effect of confounders such as severity of HIE, seizure burden, dose of phenobarbitone, and concomitant use of

Deshpande/Jain/McNamara

opioids. Second, we were unable to correlate degree of aEEG suppression with plasma phenobarbitone levels due to limited and variable testing. Furthermore, though we did not use automated quantification of aEEG upper and lower margins, the categorization of margins we used makes the results clinically applicable. Finally, although we found an association between severity of aEEG suppression and adverse short-term outcomes, long-term neurodevelopmental outcome data are lacking.

In conclusion, a significant number of infants who receive phenobarbitone for seizures while undergoing TH for moderate-to-severe HIE may demonstrate clinically significant suppression in aEEG characteristics after treatment, including new-onset severely abnormal background patterns. Presence of severely abnormal aEEG patterns 30–60 min post-phenobarbitone may be a better indicator of risk of adverse outcome of death or moderate-to-severe injury on MRI brain than aEEG pattern prior to treatment. A larger prospective study that includes information on drug levels may provide more mechanistic insights. Clinicians should consider the potential suppressive effect of phenobarbitone on aEEG during decision-making and prognostication.

References

1 Bjerre I, Hellström-Westas L, Rosén I, Svenningsen N. Monitoring of cerebral function after severe asphyxia in infancy. Arch Dis Child. 1983 Dec;58(12):997–1002.

2 Hellstrom-Westas L, Rosen I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on outcome after severe birth asphyxia in full term infants. Arch Dis Child Fetal Neonatal. 1995 Jan;72(1):F34–8.

3 al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. Pediatrics. 1999 Jun;103(6 Pt 1):1263–71.

4 Toet MC, Hellström-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 1999 Jul;81(1):F19–23.

5 ter Horst HJ, Sommer C, Bergman KA, Fock JM, van Weerden TW, Bos AF. Prognostic significance of amplitude-integrated EEG during the first 72 hours after birth in severely asphyxiated neonates. Pediatr Res. 2004 Jun;55(6):1026–33.

6 van Rooij LG, Toet MC, Osredkar D, van Huffelen AC, Groenendaal F, de Vries LS. Recovery of amplitude integrated electroencephalographic background patterns within 24 hours of perinatal asphyxia. Arch Dis Child Fetal Neonatal Ed. 2005 May;90(3): F245–51.

7 Shany E, Khvatskin S, Golan A, Karplus M. Amplitude-integrated electroencephalography: a tool for monitoring silent seizures in neonates. Pediatr Neurol. 2006 Mar;34(3): 194–9.

8 Spitzmiller RE, Phillips T, Meinzen-Derr J, Hoath SB. Amplitude-integrated EEG is useful in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischemic encephalopathy: a meta-analysis. J Child Neurol. 2007 Sep;22(9):1069–78.

9 Shah DK, Mackay MT, Lavery S, Watson S, Harvey AS, Zempel J, et al. Accuracy of bedside electroencephalographic monitoring in comparison with simultaneous continuous conventional electroencephalography for seizure detection in term infants. Pediatrics. 2008 Jun;121(6):1146–54.

10 Shankaran S, Pappas A, McDonald SA, Laptook AR, Bara R, Ehrenkranz RA, et al. Predictive value of an early amplitude integrated electroencephalogram and neurologic examination. Pediatrics. 2011 Jul;128(1):e112–20.

11 van Laerhoven H, de Haan TR, Offringa M, Post B, van der Lee JH. Prognostic tests in term neonates with hypoxic-ischemic encephalopathy: a systematic review. Pediatrics. 2013 Jan;131(1):88–98.

12 Hallberg B, Grossmann K, Bartocci M, Blennow M. The prognostic value of early aEEG in asphyxiated infants undergoing systemic hypothermia treatment. Acta Paediatr. 2010 Apr;99(4):531–6.

13 Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics. 2010 Jul; 126(1):e131–9.

14 Del Rio R, Ochoa C, Alarcon A, Arnaez J, Blanco D, Garcia-Alix A. Amplitude integrated electroencephalogram as a prognostic tool in neonates with hypoxic-ischemic encephalopathy: a systematic review. PloS One. 2016; 11(11):e0165744.

15 Bell AH, Greisen G, Pryds O. Comparison of the effects of phenobarbitone and morphine administration on EEG activity in preterm babies. Acta Paediatr. 1993 Jan; 82(1):35–9.

16 van Leuven K, Groenendaal F, Toet MC, Schobben AF, Bos SA, de Vries LS, et al. Midazolam and amplitude-integrated EEG in asphyxiated full-term neonates. Acta Paediatr. 2004 Sep;93(9):1221–7.

17 Shany E, Benzaquen O, Friger M, Richardson J, Golan A. Influence of antiepileptic drugs on amplitude-integrated electroencephalography. Pediatr Neurol. 2008 Dec;39(6):387–91.

18 Horst HJ, Of B, Af B. Burst suppression on amplitude-integrated electroencephalogram may be induced by midazolam: a report on three cases. Acta Paediatr. 2004;93(4):559–63.

19 Hellstrom-Westas L, Rosen I, de Vries LS, Greisen G. Amplitude-integrated EEG classification and interpretation in preterm and term infants. NeoReviews. 2006;7(2):e76–87.

20 Shankaran S, Barnes PD, Hintz SR, Laptook AR, Zaterka-Baxter KM, McDonald SA, et al. Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 2012 Nov;97(6):F398–404.

21 van den Broek MP, Groenendaal F, Toet MC, van Straaten HL, van Hasselt JG, Huitema AD, et al. Pharmacokinetics and clinical efficacy of phenobarbital in asphyxiated newborns treated with hypothermia: a thermo-pharmacological approach. Clin Pharmacokinet. 2012 Oct 1;51(10):671–9.

22 Shellhaas RA, Ng CM, Dillon CH, Barks JD, Bhatt-Mehta V. Population pharmacokinetics of phenobarbital in infants with neonatal encephalopathy treated with therapeutic hypothermia. Pediatr Crit Care Med. 2013 Feb; 14(2):194–202.