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Effect of Phenobarbital on Nitric Oxide Level in Term Newborn Infants with Perinatal Asphyxia

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ABSTRACT

Objectives: Perinatal asphyxia (PA) is very significant in perinatal medicine due to the involvement of the central nervous system. This study was conducted to investigate the biochemical, clinical, and paraclinical changes associated with phenobarbital administration in neonates with PA. ***Methods***: In this prospective, case-control study, 30 neonates with PA in two groups of 15 each (case and control) were investigated. The case group received 20 mg/kg intravenous phenobarbital within six hours of birth, and the control group did not receive phenobarbital. Serum concentrations of nitric oxide (NO) were measured at enrollment and one week after birth in the two groups. Clinical, electroencephalography, and magnetic resonance imaging findings of the two groups were compared. ***Results:*** At enrollment, the two groups did not differ in clinical severity, seizure incidence, or NO concentration. After one week, NO concentration was significantly lower in the case group ($p < 0.050$), but there was no significant difference in other variables between the two groups. ***Conclusions:*** Early administration of phenobarbital in term neonates with PA could protect them against encephalopathy.

Perinatal asphyxia (PA) is associated with dangerous metabolic problems as a result of temporal interruption of oxygen availability.[1] Brain injury due to PA is a common reason for severe long-term neurological deficiency in infants.[2] Clinically, this condition is referred to as hypoxic ischemic encephalopathy (HIE). HIE is the main cause of morbidity and mortality in approximately 2% and 60% of full-term and premature newborns, respectively.[3] HIE is a serious birth complication in full-term infants, and there are few available therapeutic approaches to prevent it.[4,5] Fetal hypoxia or ischemia is likely to manifest in newborns as encephalopathy, and may lead to motor or mental disability and even death.[6–8] During HIE, free radicals are produced (by phospholipase activation) within mitochondria[9] causing damage to the brain by attacking membrane fatty acids.[10,11]

HIE activate nitric oxide (NO). Cerebrospinal fluid NO levels rise after asphyxia with an increase in HIE intensity within one to three days.[12] NO mediates the cytotoxic activity of macrophages and induces cerebral edema.[13] Early neuronal NO and late inducible NO may trigger cerebral edema, ischemia and, subsequently, cell mortality.[13,14] Neuronal cell death due to HIE happens due to quick cell death by glutamate receptor activation. This leads to increased sodium entry followed by a passive influx of chloride ions disrupting the electrochemical gradient. This is followed by a net movement of water into the cells causing them to swell and burst (lyse).[2] Therapeutic mechanisms aim to inhibit the activity of harmful cell processes including reducing the release of nitric oxide, free radicals and excitatory amino acid neurotransmitters, glutamate, and the induction of genes decreasing neuronal cell death.[15,16]

Phenobarbital is a neuroprotective agent.[17] It works by reducing the cerebral blood flow with less edema, lowering the cerebral metabolic rate and lipid peroxidation in plasma and cerebrospinal fluid, scavenging free radicals, and depressing glutamate response within the brain.[18] A high dose of phenobarbital can have a positive influence on the outcome of newborns with perinatal asphyxia, but larger studies are required to confirm the present data.[19]

Since very few studies on the impact of phenobarbital in infants with PA and the debilitating effects of this complication have been conducted,

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we sought to evaluate the effect of phenobarbital on NO levels in term neonates with PA.

METHODS

In this prospective, case-control study, 30 neonates with PA in two groups of 15 each (case and control) were studied. The study protocol was registered in the Iranian Registry of Clinical Trials (2014090919101N1).

The inclusion criteria consisted of full-term infants with a gestational age ≥ 37 weeks, an Apgar score ≤ 5 at 10 minutes after birth or the need for resuscitation within 60 minutes after birth, and acidosis (base deficit ≥ 16 mmol/L, pH < 7), moderate to severe encephalopathy (lethargy, stupor, or coma) or hypotonic and abnormal reflexes or clinical seizures.[20] All infants with maternal diabetes mellitus, twin gestation, and congenital abnormalities of the central nervous system, chorioamnionitis, chromosomal abnormalities, congenital infections, and intrauterine growth restriction were excluded from the study.[21]

The infants in the case group received intravenous phenobarbital (20 mg/kg) over 60 minutes within the first six hours of birth and had their heart rate, oxygen saturation, and respiration continuously monitored. Neonates in the control group did not receive phenobarbital but received similar care.

Blood samples were obtained to measure NO levels shortly after their birth and before any intervention from all infants. This process was repeated at 14 days in both groups. NO levels were determined by spectrophotometry as described by Miranda et al.[22]

Blood samples were centrifuged for 10 minutes at 1000 rpm. Serum was separated and stored at - 20°C. Plasma samples were deproteinized with ethanol to reduce turbidity. The nitrate and nitrite of stable NO metabolites, a reliable estimate of NO output in vivo, were measured to determine NO levels. These anions were detected calorimetrically by Griess reagent. The mixture was maintained at room temperature for 30 minutes following nitrate reduction to nitrite with vanadium. The absorbance was measured at 540 nm by a double-beam spectrophotometer (Shimatzu UV-PC 1601; Shimatzu, Kyoto, Japan). A standard curve was obtained by plotting absorbance against concentration (μmol/L).

Electroencephalography (EEG) was performed at enrollment and repeated at two/three weeks of age using a digital computerized apparatus (Neurofax EEG-9000; Nihon Kodhen, Tokyo, Japan). The neurologist who interpreted all EEG results was blinded to the clinical data and treatment group.

All infants at 21 days old were transported to the magnetic resonance imaging (MRI) unit when they were in a clinically stable condition. They were accompanied by a pediatrician and monitored with pulse oximetry throughout the procedure. Chloral hydrate (40 m/kg) was administered orally to sedate neonates during the procedure.

Continuous variables were expressed as mean±SD and categorical variables as number and frequency. The continuous variables were tested for normal distribution using the Kolmogorov test. The data were analyzed by SPSS Statistics (SPSS Statistics, Chicago, US) using the Chi-square test (for comparisons between the two groups), exact Fisher's test (for categorical variables), independent *t*-test or Mann-Whitney test (for continuous variables), and paired *t*-test (for comparison between NO levels during the intervention). A *p*-value < 0.050 was considered significant.

Table 1: Infant characteristics in case and control groups.

Variable	Case	Control	*p*-value
Male gender, n (%)	8 (53.3)	8 (53.3)	1.000
Birth weight, g	3252±333	3311±334	0.636
Gestational age, weeks	39.1±0.9	39.2±0.9	0.624
Mothers age, years	25.6±2.9	25.3±3.3	0.506
1 minute Apgar score	2.1±0.8	2.3±0.7	0.567
5 minute Apgar score	6.3±1.1	6.4±1.1	0.870
Natural vaginal delivery, n (%)	7 (46.7)	8 (53.3)	0.715
HIE (grade 1), n (%)	7 (46.7)	7 (46.7)	1.000

RESULTS

Of the 30 infants in this study, 16 (53.3%) infants were male. The gestational age was 38–41 (39.1±0.9) weeks. The mean age of mothers was 25.6±2.9 (20–34) years [Table 1]. There was no difference between the two groups in gender, birth weight, gestational age, mothers' age, Apgar score, delivery status, and HIE grade.

Differences in resuscitation, convulsion, bradycardia, meconium, EEG, MRI, and hospitalization were not statistically significant in the two groups [Table 2]. NO levels at day one were not significantly different between the two groups, but after one week it was significantly lower in the case group. NO decreased in both groups after one week, but the decrease was significantly higher in the case group.

DISCUSSION

The administration of 20 mg/kg of phenobarbital decreased serum NO concentration in full-term neonates with HIE, but had no effect on clinical, EEG, and MRI changes. Infants with HIE have higher NO levels compared with healthy control infants. In our study, NO at day one was not significantly different between the case and control group. NO level has been correlated with the intensity of cerebral damage in infants with HIE.[23,24] However, in the present study, the intensity of HIE was not high. In our study, NO concentration decreased significantly within one week in infants with HIE who received intravenous phenobarbital, which suggested that phenobarbital improves the production of endogenous NO.

NO synthase (NOS) catalyzes NO synthesis, the oxygenation of arginine in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) to form nitric oxide, citrulline, and $NADP^+$.[20] NO plays an important role in the pulmonary systemic and cerebral vasodilation and is produced in response to increased intracellular calcium by endothelial NOS in endothelial cells and by neuronal NOS in astrocyte and neurons. A NOS inducible isoform produces NO in response to cellular stress, which causes neuronal damage when converted into secondary reactive nitrogen species facilitating nitrosylation reaction and nitration.[25]

In our study, use of phenobarbital was not associated with development of normal EEG compared with the control group. In addition, clinical seizure was not decreased significantly in the case group. These results were inconsistent with the results of a previous study where high dose phenobarbital in term newborns with severe PA was associated with a 27% reduction in the incidence of seizures.[18] In our study, asphyxia was not severe in all neonates.

Phenobarbital is believed to exert a neuroprotective effect partially by reducing brain metabolism and oxygen intake.[26] The vasoconstrictive effect of phenobarbital declines cerebral edema[27] and reperfusion injury after the acute phase of asphyxia.[28] However, few clinical trials of barbiturates in asphyxiated newborns in developed countries have shown marginal or no beneficial results and reported remarkable hemodynamic adverse effects.[29] In a study, early phenobarbital use was associated with a three-fold increase in the incidence of later seizures in term newborns with PA.[30]

Table 2: Outcomes variables in the case and control groups.

Variable	Case	Control	*p*-value
Resuscitation	12 (80.0)	12 (80.0)	1.000
Convulsion	3 (20.0)	5 (33.3)	0.682
Bradycardia	7 (46.7)	5 (33.3)	0.710
Meconium	5 (33.3)	3 (20.0)	0.682
Normal EEG	12 (80.0)	9 (60.0)	0.427
Normal MRI	11 (73.3)	12 (80.0)	1.000
Hospitalization, days	8.6±1.74	10.1±2.4	0.081
NO1 (at day one)	15.3±1.3	14.9±1.4	0.353
NO2 (after one week)	12.9±1.3	14.6±1.3	0.002
NO1-NO2	2.5±0.5	0.3±0.3	< 0.001

EEG: electroencephalography; MRI: magnetic resonance imaging; NO: nitric oxide.

CONCLUSION

Our study suggested that phenobarbital exerted its neuroprotective effects by reducing the formation and antagonizing the toxicity of free radicals mediated by NO, but it had no effect on seizure, duration of hospitalization, clinical findings, and changes in EEG and MRI.

Disclosure

The authors declared no conflicts of interest. This study was a research project with ethical approval no. 92-8-5, and was funded by Research and Technology Deputy of Shahrekord University of Medical Sciences (grant no. 1393-01-90-2038).

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