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Original Article

High-dose phenobarbital or erythropoietin for the treatment of perinatal asphyxia in term newborns

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Abstract ***Background***: The aim of this study was to compare two neuroprotective strategies to supportive care in the treatment of perinatal asphyxia.

Methods: A total of 67 term newborns with perinatal asphyxia were included and randomized into three groups: one group received supportive treatment; another group received a single dose of 40 mg/kg phenobarbital; and the third received three daily doses of 1000 IU/kg erythropoietin. The following parameters were analyzed: gestational age, birthweight, Apgar scores, cord blood pH, total serum antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA). The newborns were included in the follow-up program and examined up to 18 months of age.

Results: TAS was higher in the erythropoietin group than in the other groups. SOD and GPx were lower for infants treated with phenobarbital or erythropoietin compared to control infants. MDA was lower in the erythropoietin group compared to the other groups, although the difference was not statistically significant ($P > 0.05$). The mortality rate was lower in the phenobarbital and erythropoietin groups (both 4.6%) than in the control group (17.4%). Long-term neurologic follow up showed a high incidence of sequelae in the control group compared to the phenobarbital and erythropoietin groups. Follow-up results were better in the phenobarbital group than in the erythropoietin group for motor and cognitive function at 3 and 6 months and worse for expressive language. At 18 months, however, the differences between these two groups were not significant.

Conclusion: High-dose phenobarbital or erythropoietin along with supportive treatment has a positive influence on the outcome of newborns with perinatal asphyxia. Phenobarbital has the advantage of low cost and simplicity.

Key words erythropoietin, high-dose phenobarbital, neuroprotective strategies, oxidative stress, perinatal asphyxia.

During the neonatal period, the most important neurologic problem is hypoxic–ischemic encephalopathy (HIE), following perinatal asphyxia.

Perinatal asphyxia occurs when an antenatal, intranatal or postpartum neurologic insult or any combination of the three leads to: (i) hypoxemia (decreased oxygen flow to the fetus/newborn); (ii) hypercapnia (altered O_2/CO_2 exchange); and (iii) ischemia (inadequate perfusion of tissues and organs).[1]

Systemic hypoxia and cerebral ischemia lead to a decrease in oxygen and glucose. The consequence is anaerobic glycolysis, followed by decrease of ATP and acidosis, all of these contributing to impairment of brain function. Various intertwined mechanisms (excessive excitatory amino-acids receptors synthesis,[2] intracellular calcium accumulation, free radical generation) influence the outcome of hypoxic–ischemic injury. DNA is damaged by oxidative destruction but also by structural alterations, such as chain breakage, deletions of bases and chromosomal anomalies.[3] Moreover, reactive oxygen species modulate the transduction of cellular proliferation pathways, and an excessive accumulation can lead to cellular death through necrosis and apoptosis.[4] Reactive oxygen species can promote the expression of adhesion molecules, leading to activated granulocyte accumulation, which amplifies cellular destruction.[5]

Total serum antioxidant status (TAS), antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPx], catalase), vitamins and metabolites of lipid peroxidation, such as malondialdehyde (MDA), have been extensively used for the assessment of oxidative stress in various diseases and have proved to be useful markers of free radical damage.[6,7]

Therapeutic cooling of the whole body or the head reduces the long-term adverse effects of HIE, but many infants still die or suffer neurologic impairment despite cooling.[8–11] Therefore, newer and more effective neuroprotective therapies are urgently needed. Phenobarbital and erythropoietin are two additional therapies that might be used in addition to or instead of therapeutic hypothermia. Phenobarbital acts by suppressing the oxidative cerebral metabolism and diminishing the neuronal response to glutamate. High-dose i.v. phenobarbital, used early after the neurologic insult, lowers the cerebral metabolic rate and

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lipid peroxidation in plasma and cerebrospinal fluid, decreases the incidence of seizures and that of long-time complications, is well-tolerated and does not influence mortality.[12] Erythropoietin has been shown to have potential for ameliorating the neurological sequelae of HIE.[13,14] We therefore conducted a randomized controlled trial to examine the potential beneficial effects of phenobarbital and erythropoietin for infants with perinatal asphyxia who had signs of HIE.

Methods

We conducted a prospective randomized study of 67 term neonates with perinatal asphyxia, admitted from 1 January 2010 to 30 September 2011 to the Cuza-Voda Clinical Hospital of Obstetrics and Gynecology Neonatal Intensive Care Unit (NICU) in Iasi, Romania. Perinatal asphyxia was diagnosed using the criteria of the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists,[15]and newborns were included when at least three of the four criteria were met. The study was approved by the hospital's Ethics Committee, and informed consent was obtained from the infants' mothers prior to cesarean section or in the first hour after birth. Preterm infants (with gestational age <37 weeks), infants with major congenital malformations, and infants with hemolytic disease due to Rh incompatibility were excluded from study.

The following data were collected and analyzed: birthweight, gestational age, Apgar scores at 1, 5 and 10 min, cord blood pH, red blood cell activity of antioxidant enzymes (SOD, GPx), TAS and MDA. TAS was analyzed using the ABTS® technique (Boehringer Mannhein, Germany) and RANDOX reactants (Randox Laboratories, Crumlin, County Antrim, Northern Ireland). SOD was measured by the degree of formazan inhibition using RANSOD kits (Randox Laboratories) with control serum.[16] GPx was measured using RANSEL reactants through the Paglia and Valentine method.[17] MDA was analyzed using thiobarbituric acid reaction.[18] The samples were collected at 4, 24, 48, and 72 h and at 7 days of life, and measured using a Beckmann spectrophotometer. Neurologic clinical examination was performed as soon as possible after birth and periodically thereafter, noting the presence or absence of neurologic abnormalities (of muscle tone and inborn reflexes) and their duration. In addition, the presence or absence of seizures throughout the admission was noted on clinical examination and confirmed on amplitude-integrated electroencephalography. Treatment was started as soon as the diagnosis was confirmed. All subjects were included in the Cuza-Voda follow-up program, examined at discharge using the Amiel-Tison assessment and at 3, 6, 9, 12, 18 months, using Bayley Infant Scales of Development, edition II.

The infants were randomly assigned to supportive treatment (oxygen, volume expanders, inotropes, diuretics, antibiotics), a single dose of i.v. phenobarbital, 40 mg/kg, during the first 4 h after birth plus supportive treatment, or s.c. erythropoietin, 1000 IU/kg per day, for the first 3 days plus supportive treatment. Infants were allocated to treatment groups by unblinded, random-draw, numerical assignment. No infant was treated with whole-body or head cooling, because these therapies were not available during the period of study.

The data were analyzed using SPSS V.19.0. (SPSS, Chicago, IL, USA). Descriptive statistics were used to express characteristics and tendencies of studied parameters. The independent variables – treatment-derived differences among parameters – were analyzed using ANOVA test, for normal frequency distribution. In other cases, the non-parametric Kruskal–Wallis test was used, based on the analysis of attributed ranks. Statistical significance was defined as $P < 0.05$.

Results

Of 9302 term infants admitted to the Cuza-Voda Clinical Hospital of Obstetrics and Gynecology during the 21 months of the study, 67 (0.72%) were diagnosed with perinatal asphyxia (Fig. 1). This rate is similar to the incidence reported worldwide (0.1–0.8%[19,20]). These infants comprised 3.8% of all infants admitted to the Cuza-Voda NICU. All 67 infants were enrolled in the study: 23 were randomized to the control group (supportive care), 22 to phenobarbital, and 22 to erythropoietin.

The enrolled newborns had a mean gestational age of 40.4 weeks and a mean birthweight of 3278 g. The treatment groups were homogenous (Table 1). Apgar scores at 1 min were between 3 and 4.1 and had a mean of 3.6. As resuscitation continued, Apgar scores at 5 min rose slightly to a mean of 5.3, and at 10 min – 6.6. Low cord blood pH confirms the diagnosis of perinatal asphyxia (7.00–7.09).

Antioxidant enzymes (SOD and GPx) were lower for infants treated with phenobarbital or erythropoietin compared to control infants (Fig. 2). When compared to reference values for healthy term newborns (SOD, 216–310 U/L; GPx, 4171–9881 U/L), the high values in the control infants suggested the existence of oxidative stress following perinatal asphyxia.

The TAS was higher, although not significantly so ($P < 0.5$), in the phenobarbital and erythropoietin groups, compared to the control group (Fig. 2). The mean reference values for TAS in healthy term newborns are 1.20–1.30 mmol/L.

Lipid peroxidation measured by MDA in plasma 7 days after the initial hypoxic insult was high in all of the patient groups compared to the normal range for healthy term newborns (1.17–1.32 μmol/L; Fig. 2).

There was a descending trend in abnormal findings on neurologic examination. At birth all of the infants had clinical neurologic abnormalities, and this aspect persisted at 6 and 12 h; after

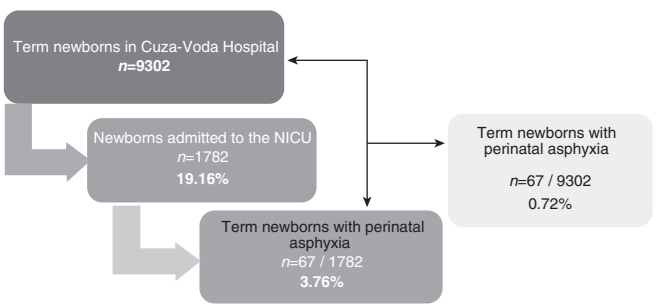

Fig. 1 Incidence of perinatal asphyxia. NICU, neonatal intensive care unit.

Table 1 Parameters evaluated at birth

	Treatment	Mean	Mean		SD	SEM	Min	Max	Q25	Median	Q75
			–95%	+95%							
Gestational age (weeks)	Supportive	40.3	39.8	40.8	1.56	0.25	36	43	40	40.5	41
	Phenobarbital	40.0	39.3	40.8	1.27	0.34	37	42	40	40	41
	Erythropoietin	40.7	39.9	41.6	1.53	0.4	36	42	40	41	42
	Mean – all groups	40.4	39.6	41.1	1.9	0.4	36	43	40	41	42
Birthweight (g)	Supportive	3365	3165	3565	609	99	2500	5000	2900	3300	3650
	Phenobarbital	3093	2725	3460	637	170	1700	4100	2800	3075	3400
	Erythropoietin	3230	2824	3636	733	189	1360	4900	2950	3240	3400
	Mean – all groups	3278	3121	3435	643	79	1360	5000	2900	3240	3650
Apgar 1 min	Supportive	3.9	3.2	4.6	2.13	0.35	1	7	2	3.5	6
	Phenobarbital	2.7	1.5	3.9	2.05	0.55	0	7	1	3	4
	Erythropoietin	3.7	2.3	5.1	2.53	0.65	1	7	1	4	6
	Mean – all groups	3.6	3	4.1	2.22	0.27	0	7	1	3	6
Apgar 5 min	Supportive	5.8	5.2	6.3	1.79	0.29	1	9	4	6	7
	Phenobarbital	4.4	3.1	5.6	2.17	0.58	1	8	3	4.5	6
	Erythropoietin	5.1	4.1	6.2	1.88	0.49	3	8	3	5	7
	Mean – all groups	5.3	4.8	5.8	1.95	0.24	1	9	4	6	7
Apgar 10 min	Supportive	7	6.5	7.5	1.50	0.24	2	9	6	7	8
	Phenobarbital	5.9	4.7	7.2	2.20	0.59	2	9	4	6.5	8
	Erythropoietin	6.3	5.3	7.3	1.80	0.46	3	9	5	7	8
	Mean – all groups	6.6	6.2	7	1.76	0.21	2	9	5	7	8
Cord blood pH	Supportive	7.08	7.01	7.14	0.14	0.03	6.90	7.39	6.97	7.10	7.17
	Phenobarbital	6.98	6.83	7.14	0.18	0.06	6.66	7.27	6.89	6.97	7.10
	Erythropoietin	6.93	6.77	7.09	0.19	0.07	6.53	7.18	6.89	6.95	7.03
	Mean – all groups	7.02	7.00	7.09	0.15	0.02	6.53	7.39	6.96	7.04	7.14

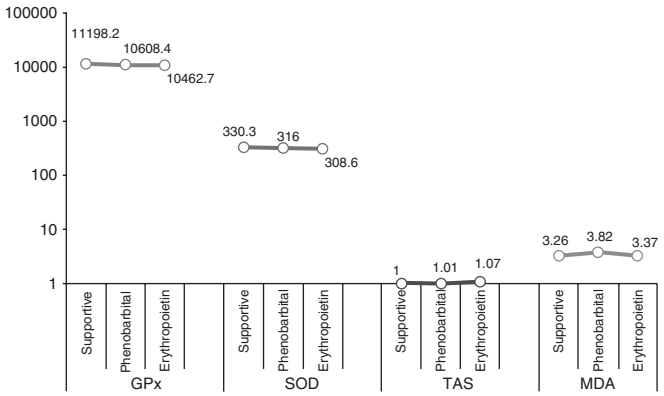

Fig. 2 Assessment of antioxidant enzymes. GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TAS, total serum antioxidant status. Reference values: GPx, 4171–9881 U/L; MDA, 1.17–1.32 μmol/L; SOD, 216–310 U/L; TAS, 1.20–1.30 mmol/L.

72 h neurologic disorders were found in only 53.7% of the newborns (Table 2). There was a higher incidence of seizures during the first 12 h after perinatal asphyxia (Table 2).

Four deaths occurred in the control group (17%); one (5%) in the phenobarbital group; and one (5%) in the erythropoietin group.

Treatment correlation with long-term neurologic outcome took into account the presence of various items during the clinical follow-up examination: motor disabilities, and disorders of receptive language, expressive language, and cognitive development (Fig. 3).

Discussion

Decrease of plasma activity of antioxidant enzymes among the infants treated with erythropoietin is due to the known role of erythropoietin in preventing oxidative stress and decreasing lipid peroxidation.[21,22] Phenobarbital decreases cellular metabolic rate and oxidative stress, favoring the decrease of antioxidant enzyme

Table 2 Incidence of neurologic abnormalities and seizures

Age at examination	No. infants with abnormal examination	Supportive therapy *n* (%)	Phenobarbital *n* (%)	Erythropoietin *n* (%)	No. infants with seizures	Supportive therapy *n* (%)	Phenobarbital *n* (%)	Erythropoietin *n* (%)
At birth	67	23 (34.3)	22 (32.8)	22 (32.8)	1	0 (0)	1 (1.5)	0 (0)
6 h	67	23 (34.3)	22 (32.8)	22 (32.8)	10	3 (4.5)	3 (4.5)	4 (6)
12 h	67	23 (34.3)	22 (32.8)	22 (32.8)	11	3 (4.5)	5 (7.5)	3 (4.5)
24 h	52	20 (29.9)	17 (25.4)	15 (22.4)	6	1 (1.9)	3 (5.8)	2 (3.8)
48 h	52	20 (29.9)	17 (25.4)	15 (22.4)	6	0 (0)	3 (5.8)	3 (5.8)
72 h	43	19 (28.4)	14 (20.9)	10 (14.9)	4	1 (2.3)	1 (2.3)	2 (4.7)
>72 h	36	17 (25.4)	13 (19.4)	6 (9)	1	0 (0)	0 (0)	1 (2.8)

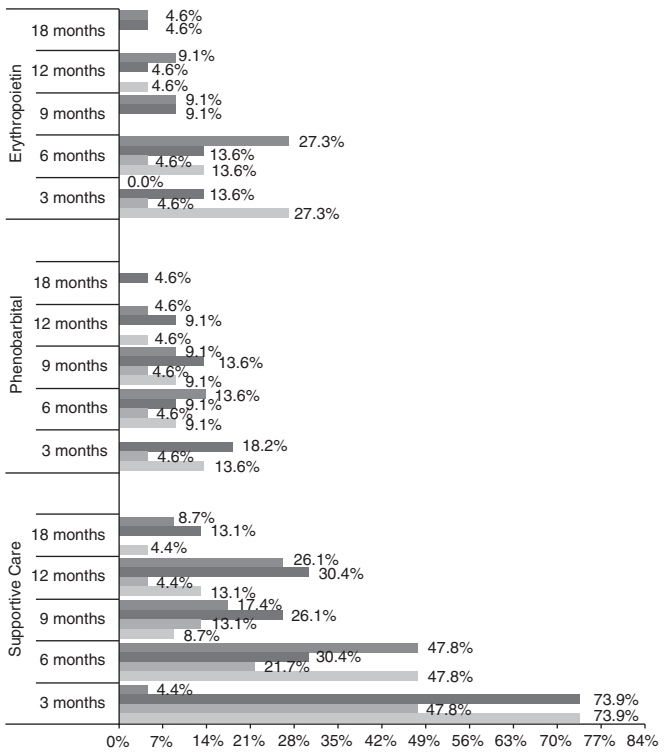

Fig. 3 Results of Bayley II assessment. ■, cognitive development; ■, expressive language; ■, receptive language; ■, motor score.

activity.[12] TAS was higher in the erythropoietin group than in the other groups. This could represent the effort of antioxidant systems to counterbalance free radical injury, generated by hypoxia–ischemia. Also, TAS may be greatly influenced by other medications used during the first 72 h after perinatal asphyxia.

Lipid peroxidation is a slow process, and it is a good reflection of oxidative stress generated by both perinatal asphyxia and oxygen therapy. Because lipids are key components of cellular membranes, their peroxidation leads to severe disruptions of membrane structure and function. The degree of lipid peroxidation can be measured by the levels of MDA in plasma and cerebrospinal fluid,[23] because MDA is very persistent in plasma. It results from the peroxidation of fatty acids with three or more double links and it is the cause of cross-linking and polymerization of membrane components.

Malondialdehyde was lower in the erythropoietin group compared to the other groups, although the difference was not statistically significant ($P > 0.05$). This could be explained by the various models of neonatal hypoxia, which showed that in doses ranging from 1000 IU/kg to 30 000 IU/kg, erythropoietin has anti-apoptotic and anti-inflammatory effects in the acute post-injury period, with neurogenic and vasculogenic effects in the recovery period.[23–31]

The dynamics of the parameters involved in antioxidant defense were more pronounced in the erythropoietin group than in the other groups, but the differences between the phenobarbital and erythropoietin groups were not statistically significant.

The descending trend in neurologic abnormalities of muscle tone and reflexes, as well as seizures suggests the recovery of cerebral metabolism following the early transient failure of cerebral blood flow.

The mortality rate was lower in the phenobarbital and erythropoietin groups (both 4.6%) than in the control group (17.4%; $\chi^2 = 7.26$, $P = 0.0087$, 95% confidence interval). This may be explained by the facilitation of antioxidant defense mechanisms through the use of phenobarbital or erythropoietin.

Long-term neurologic follow-up showed a high incidence of sequelae in the control group compared to the phenobarbital and erythropoietin groups. In the control group, delay in the achievement of motor and expressive skills prevailed (each 73.9%) at 3 months but became less frequent over time, as described by Ment *et al*.[32] At 18 months, delayed cognitive development (8.7%) and expressive language (13.0%) were more frequent. Receptive language delays became less frequent when the same infants were tested repeatedly over time, and by 18 months, receptive language was normal in all subjects.

In the phenobarbital group, expressive language delays were found in only 18.2% of infants at 3 months, a significantly lower rate than in the control group. Receptive language delays were constant in this group until 9 months of age (9.1%) but absent by 12 months.

In the erythropoietin group, motor development was significantly affected at 3 months (27%), although at the final evaluation only cognitive and expressive language disorders existed (each in 4.6% of infants). Receptive language disorders were very rare and were absent by 9 months of age.

Follow-up results were better in the phenobarbital group than in the erythropoietin group in the fields of motor and cognitive function at 3 and 6 months and worse for expressive language. At 18 months, however, the differences between these two groups were not significant. The present results regarding the efficacy of high-dose phenobarbital during the immediate recovery period are consistent with the work of Gathwala *et al*., who noted decreased oxidative stress after phenobarbital.[12] The present study shows that high-dose phenobarbital improves long-time neurologic outcome, as shown by Hall *et al*.[33]

Conclusions

To our knowledge, this is the first study to compare the efficacy of high-dose phenobarbital and erythropoietin for the neuroprotection of infants with encephalopathy following perinatal asphyxia. High-dose phenobarbital or erythropoietin along with supportive treatment has a positive influence on the outcome of newborns with perinatal asphyxia. phenobarbital also has the advantage of low cost and simplicity. Unlike therapeutic cooling, phenobarbital can easily be given in any NICU, even in low- or modest-resource countries. The present study was limited by its small size and the lack of blinding of the caregivers and examiners. Larger studies are needed to confirm or refute the present findings.

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