Hypothermia Plus Melatonin in Asphyctic Newborns: A Randomized-Controlled Pilot Study

Antonio Jerez-Calero, MD; Maria Teresa Salvatierra-Cuenca, MD; Ángela Benitez-Feliponi, MD; Carmen Elisabeth Fernández-Marín, MD; Eduardo Narbona-López, MD; José Uberos-Fernández, MD; Antonio Muñoz-Hoyos, MD

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Objectives: To investigate the effect of adding melatonin to hypothermia treatment on neurodevelopmental outcomes in asphyctic newborns.

Design: Pilot multicenter, randomized, controlled, double-blind clinical trial. Statistical comparison of results obtained in two intervention arms: hypothermia plus placebo and hypothermia plus melatonin.

Setting: Level 3 neonatal ICU.

Patients: Twenty-five newborns were recruited.

Interventions: The hypothermia plus melatonin patients received a daily dose of IV melatonin, 5 mg per kg body weight, for 3 days. General laboratory variables were measured both at neonatal ICU admission and after intervention. All infants were studied with amplitude-integrated electroencephalography and brain MRI within the first week of life. The neurodevelopmental Bayley III test, the Gross Motor Function Classification System, and the Tardieu scale were applied at the ages of 6 and 18 months.

Measurements and Main Results: Clinical characteristics, laboratory evaluations, MRI findings, and amplitude-integrated electroencephalography background did not differ between the treatment groups. The newborns in the hypothermia plus melatonin group achieved a significantly higher composite score for the cognitive section of the Bayley III test at 18 months old, with respect to the hypothermia plus placebo group ($p = 0.05$). There were no differences between the groups according to the Gross Motor Function Classification System and Tardieu motor assessment scales.

Conclusions: The early addition of IV melatonin to asphyctic neonates is feasible and may improve long-term neurodevelopment. To our knowledge, this is the first clinical trial to analyze the administration of IV melatonin as an adjuvant therapy to therapeutic hypothermia. (*Pediatr Crit Care Med* 2020; XX:00–00)

Key Words: hypoxic-ischemic encephalopathy; melatonin; neurodevelopment; neuroprotection; therapeutic hypothermia

All authors: Department of Paediatrics, San Cecilio University Hospital, Granada, Spain.

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Neonatal hypoxic-ischemic encephalopathy (HIE) is a low-prevalence pathology (1–2 per 1,000 full-term newborns), but it represents an important cause of infant morbidity and mortality and is associated with important medical, social, and legal implications.

Increasing numbers of studies are being undertaken to explain the physiopathologic mechanisms involved in brain damage by hypoxia-ischemia acidosis. At the cellular level, a significant role is played by the enzymatic activation of proteases, endonucleases, and phospholipases, by alterations to the reuptake of excitotoxic amino acids and by the formation of free radicals. Sequential primary, secondary, and tertiary energy failures are responsible for the neurologic damage (1). However, there exists a "treatment window" during which a therapeutic intervention can reduce this brain damage. The treatment window lasts approximately 6 hours, according to studies based on animal models, and during this period, the oxidative metabolism can be recovered (2). Among the current treatment resources, only moderate hypothermia has been shown to be effective in reducing mortality and improving the neurologic prognosis of these patients. The standardized protocol for neonatal therapeutic hypothermia includes a controlled decrease in body temperature to 33–34°C, maintained for 72 hours, followed by a subsequent progressive rewarming at a rate of less than 0.5°C per hour until normothermia (3–5). The neuroprotective effect of hypothermia arises from the reduction of inflammatory phenomena and oxidative stress secondary to the hypoxic-ischemic event (6, 7). No other therapy has been shown to be effective in reducing brain damage after a hypoxic-ischemic insult (8).

Investigations have been conducted into various molecules (anticonvulsants, erythropoietin, allopurinol, xenon, melatonin, cannabinoids, stem cells) that, in conjunction with therapeutic hypothermia, may improve the long-term neurodevelopmental results achieved by these neonatal patients (8, 9). Among these possibilities, melatonin has obtained promising effects (10, 11). Full-term newborns are known to present, physiologically, a transitory deficit of melatonin production, for at least 2–4 months (12–15).

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This hormone is known to regulate circadian cycles and is physiologically secreted by the pineal gland. It also performs a highly effective antioxidant, antiapoptotic, anti-inflammatory, and free radical chelating function (10, 16–19). It can readily cross the blood–brain barrier, and it can be safely administered to children with disturbed sleep patterns (20–22). Experimental evidence shows it has a neuroprotective effect after brain damage, acting both on white matter and on gray matter. Its underlying mechanisms include the activation of gabergic receptors, the chelating action of free radicals, antiepileptic effects, and action mediated by specific receptors or through the inhibition of adenylate cyclase (7, 23, 24).

This randomized clinical trial (RCT) was designed to study the feasibility and effects of IV-administered melatonin to asphyctic newborns who are under moderate hypothermia treatment (the "hypothermia plus" effect). The main study aim was to evaluate biochemical, neurophysiologic, radiological, and long-term neurodevelopmental outcomes.

MATERIAL AND METHOD

Patients

This multicenter clinical trial was conducted in newborn infants at the San Cecilio University and Virgen de las Nieves hospitals in Granada (Spain) and at the Torrecárdenas hospital in Almería (Spain) (EudraCT No. 2012- 000184-24). Twenty-five newborns, 15 and 10 respectively, were recruited to the study, between January 2015 and March 2016. The pilot study had two intervention arms: melatonin and placebo. The intervention was masked to the investigator. All patients were randomized into two groups: hypothermia plus placebo (HP) and hypothermia plus melatonin (HM) (**Fig. 1**). The newborns enrolled met international criteria for hypothermia treatment during the recruitment period. The Pharmacy Unit at the participating hospitals was responsible for treatment randomization. Thirteen newborns were assigned to the HP group and 12 to the HM group.

All newborn infants who presented indications for therapy with moderate corporal hypothermia were included in the RCT, after their parents or guardians had signed the corresponding informed consent form. The following inclusion criteria were applied: 1) gestational age greater than or equal to 36 weeks, 2) severe perinatal asphyxia, and 3) moderate-severe HIE. Severe perinatal asphyxia was considered to be present if at least one of the following conditions was met: 1) Apgar test score less than or equal to 5 at 5 minutes after birth, 2) need for resuscitation in the delivery room for more than 10 minutes by positive pressure ventilation (bag and mask or endotracheal tube), 3) pH less than or equal to 7.00 or base deficit less than or equal to –16 mmol/L within first hour, according to blood gas analyses (cord, arterial, venous, or capillary samples). Signs of moderate or severe encephalopathy were quantified by serial assessment using the modified Sarnat and Sarnat scale (25). A score greater than or equal to 6 was considered as moderate HIE and greater than or equal to 13, as severe HIE. Newborns meeting one or more of the following criteria were excluded from the clinical trial: 1) birth weight less than 1,800 g, 2) gestational age less than 36 weeks, 3) surgical pathology within the first 3 days of life, 4) serious congenital malformation located in the CNS, 5) hypoxemia (preductal oxygen saturation level < 90%) with no response to invasive mechanical ventilation, full-oxygen supplement (FIO_2=100%), and inhaled nitric oxide therapy, 6) life-threatening coagulopathy refractory to replacement treatment (plasma and/or platelet transfusion), 7) chromosomopathy, 8) postnatal age greater than 6 hours.

The patients in the HM group received a daily IV dose of melatonin, 5 mg per kg of body weight, during the first 3 days of life. The melatonin was administered by continuous IV infusion for 2 hours, beginning during the first 6 hours after birth. The newborns assigned to the HP group received the same volume of 0.9% normal saline solution, also administered by continuous IV infusion for 2 hours.

The IV vials were made of glass, had a total capacity of 10 mL, and were manufactured by Farma Mediterrania SL (Barcelona, Spain). In the vials used for the HM group, propylene glycol and macrogol were the main solvents, and the melatonin concentration was 6.5 mg/mL. The trial was approved by the appropriate Regional Ethics Committees and by the corresponding Ethics Committee at each of the participating hospitals. In addition, the mandatory authorization was obtained from the Spanish Agency for Medicines and Health Products.

The whole body hypothermia protocol used in this study was applied by experienced staff in the hospital's neonatal ICU (NICU). The protocol is similar to those previously reported in the scientific literature and is considered safe and effective in neonates with HIE (26). Cooling was achieved with a Tecotherm Neo automatic device (Tecotherm Neo; Inspiration Healthcare Ltd, Leicester, United Kingdom) and was maintained for 72 hours (target temperature: 33–34°C). The neonates were then rewarmed at 0.5°C per hour until 36.5°C was reached. Servo-controlled technology ensured that the body temperature (monitored by rectal probe every 2 s) remained stable.

A comparative biochemical and hematologic analysis was performed at two different time points (at hospital admission and after the third day of life) in order to assess the safety of the interventions. Blood samples were collected to measure metabolic parameters, main plasma ion levels and biomarkers of renal, hepatic, and hematologic function and for coagulation tests.

Brain function was evaluated using amplitude-integrated electroencephalography (aEEG) from admission until the finalization of the rewarming phase (Olimpic Brainz; Natus Medical Incorporated, Pleasonton, CA). This continuous-monitoring method, based on filtered and compressed electroencephalography, evaluates electrocortical background activity and the presence/absence of seizures. The NICU neonatologists who interpreted the aEEG patterns were masked to the treatment groups. In this context, five relatively simple background patterns have been described: continuous normal and discontinuous normal voltage traces are associated with better

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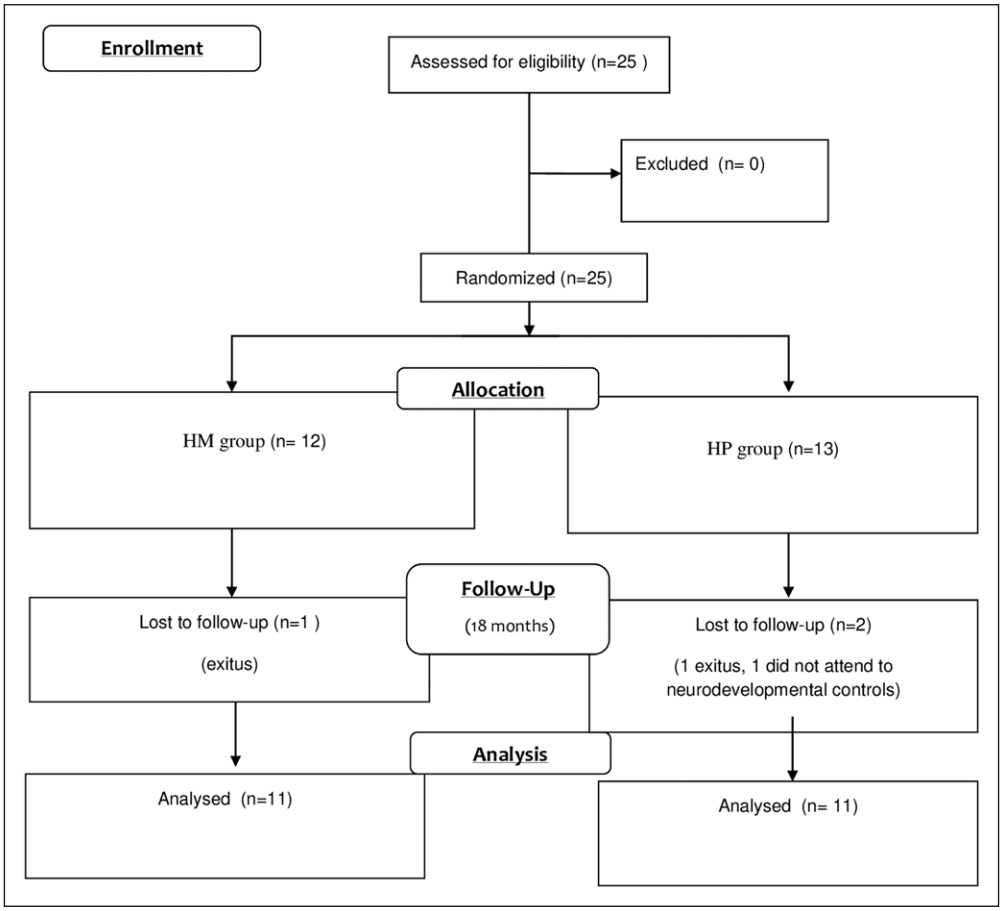

Figure 1. Flow diagram of the study. HM group = hypothermia plus melatonin group, HP group = hypothermia plus placebo group.

long-term neurologic outcomes. In contrast, patients with persistent abnormal aEEG background (burst suppression, low voltage, or inactive pattern) are at high risk of death or severe handicap (27, 28).

At 1 week of life, the neonates were all transported to the radiology unit for MRI, using the Siemens Magnetom 1.5-T device (Siemens, Munich, Germany). All were assessed by an experienced pediatric radiologist, who was blind to the treatment groups. The posterior limb of the internal capsule (PLIC) and the basal ganglia and thalami were evaluated. Abnormalities were classified as (1) mild, if focal lesions involving the posterior lentiform nuclei or ventrolateral nuclei of the thalami were seen and (2) moderate-severe, if the PLIC was affected or widespread abnormalities were seen in the thalami or in all regions of the basal ganglia. White matter images were graded as (1) normal/minimal, indicating no abnormalities or only minimal injuries affecting a small focal area; (2) moderate-severe: if subcortical or larger areas of abnormality were seen, hemorrhage, infarction, or loss of gray/white matter differentiation (29).

Long-term neurologic and neurodevelopmental assessment was carried out using a previously validated test. A psychologist (always the same person) from our pediatric clinical management unit, one of the Early Care team, applied the Bayley III test (30) to each patient, obtaining scores for cognitive development, language, and gross and fine motor skills. The personnel conducting the neurodevelopment tests was blinded to the interventions. The newborn's mobility and movement capacity was assessed in two ways. First, we obtained the Gross Motor Function Classification System (GMFCS) classification, which evaluates gross motor skills and overall functional capacity, on a scale ranging from I to V (31, 32). The Tardieu scale was then used. This instrument is less commonly employed, but in our view, it is more complete, since it takes into account general motor skills, manual activity and language, and the overall impact on functional capacity (33).

Statistical Analysis

All data were analyzed using SPSS (Statistical Package for the Social Sciences, Chicago, IL) version 20. Metabolic and hematologic laboratory analyses were performed for both arms of the intervention, which were compared using the Friedman test. The aEEG and MRI findings for the groups were compared by the chi-square test.

Neurologic and psychomotor development was assessed using the Bayley III test and by inspection of the scores obtained on the GMFCS and Tardieu scales, at 6 and 18 months old. Comparison studies were performed using Mann–Whitney and Wilcoxon nonparametric tests on the Bayley test score and by applying Fisher exact test to the GMFCS and Tardieu scores. All differences were considered significant at p values less than 0.05.

RESULTS

Of the 25 newborns recruited initially, two (one in each group) died in the first month of life and another (in the HP group) did not attend the reviews for neurodevelopmental assessment. Thus, a total of 22 patients (11 in each group) were followed up for 18 months. Of these, 14 were male, and 8 were female. Nine presented moderate HIE, and 13 had severe HIE (Sarnat ≥13 points).

The characteristics of the patients are presented in **Table 1**. There were no significant differences between the groups in the following variables: weight and height at birth, gestational age, cord blood arterial pH, Apgar score at 1 and 5 minutes of life, hours of life on starting hypothermia treatment,

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TABLE 1. Clinical Characteristics of the Newborns

Clinical Characteristics	Hypothermia Plus Melatonin Group ($n = 12$) (Mean ± SD)	Hypothermia Plus Placebo Group ($n = 13$) (Mean ± SD)	p
Birth weight (g)	3.057 ± 514.24	2.973.85 ± 660.25	0.86 NS
Gestational age (wk)	38.49 ± 1.71	39.45 ± 1.72	0.22 NS
Height (centimeters)	50.73 ± 2.05	49.96 ± 2.39	0.56 NS
Cord blood pH	6.98 ± 0.15	6.96 ± 0.14	0.63 NS
Apgar score at 1 min of life	3.55 ± 2.46	3.77 ± 2.52	0.69 NS
Apgar score at 5 min of life	5.91 ± 1.97	5.42 ± 2.39	0.78 NS
Start hypothermia therapy from birth (hr)	3.95 ± 2.49	4.92 ± 2.56	0.25 NS
Sarnat-Sarnat Score	12.72 ± 7.57	20.69 ± 10.67	0.09 NS
Lower blood pH at NICU admittance	7.17 ± 0.13	7.15 ± 0.16	0.31 NS
Arterial pressure (mm Hg)	51.7 ± 11.24	48.42 ± 15.22	0.41 NS
Length of stay in the NICU (d)	11.35 ± 4.37	11.08 ± 4.25	0.53 NS

NS = not significant.
$p < 0.05$ was considered significant.

severity of HIE, lowest pH in blood gas analyses, or length of stay in the NICU. Accordingly, the groups were considered to be homogeneous.

Both at NICU admission and on the third day of life, blood and plasma parameters related to the safety of the intervention were compared. The laboratory evaluations performed included blood cell count, hemoglobin, plasma ions, osmolarity, urea, creatinine, bilirubin, creatine kinase, lactate dehydrogenase, transaminases, and coagulation. In this regard, there were no statistical differences between the HM and HP groups (**Tables 2** and **3**).

The MRI findings did not differ significantly between the HIE groups in white matter, basal ganglia, or thalami abnormalities. At NICU admission, initial aEEG background and presence of seizure activity did not differ between the treatment groups (**Table 4**). After application of the hypothermia protocol, when normothermia were achieved, aEEG background and the prevalence of neonatal seizure activity did not differ significantly between the HM and HP groups.

The Bayley III test results for neurodevelopment showed that the newborns in the HM group achieved a significantly higher composite score for the cognitive scale at the age of 18 months ($p < 0.05$). However, there were no statistical differences in the cognitive scale at 6 months or for the other items of neurologic development (language and motor skills) at 6 and 18 months although the difference for language skills at 18 months presented values very close to statistical significance ($p = 0.057$) (**Table 5** and **Fig. 2**).

Comparisons were also made of the results obtained for the motor assessment scales of the GMFCS and Tardieu scales, also referring to newborns 6 and 18 months old. Finally, Fisher exact test was applied, comparing the less severe categories (I and II) versus those most severely affected (III, IV, and V). According to these tests, with respect to the severity of motor dysfunction, there were no statistically significant differences between the two treatment groups (Table 5).

DISCUSSION

The reduction of body temperature (hypothermia) is currently the only scientifically accepted means of alleviating neurologic sequelae in perinatal HIE. Its use in level 3 NICUs has enabled a major improvement in the results achieved. However, many newborns still experience significant and irreversible consequences after a severe hypoxic-ischemic insult. Accordingly, it remains essential to conduct further research in this field and to discover new neuroprotective drugs (34–36).

Our pilot study suggests that HM treatment could be considered a safe intervention in newborns. The main plasmatic ion levels and several metabolic, renal, hepatic, and hematologic parameters were similar between the HM and HP groups after rewarming (on the third day of life).

The results obtained suggest that promising changes may be achieved in the long-term neurodevelopment outcomes of neonates with HIE. Thus, the mean scores recorded in the cognitive field, measured with the Bayley III test, at 18 months old, were improved in HM group. At this stage of life, cognitive development involves crucial functions such as perceptual and temporosequential orientation, and learning capacity, attention, and memory. In our study, however, there were no statistical differences for the Cognitive scale at the age of 6 months or for the other items of neurologic development (language and motor) at either 6 or 18 months. Similarly, no differences were found between the groups according to the GMFCS and Tardieu motor assessment scales.

In the search for a molecule with high neuroprotective capacity, controlled models of brain damage in experimental

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animals have been examined in various clinical trials. A recent RCT observed a decrease in inflammatory cytokines in rats subjected to cerebral ischemia-reperfusion following the oral administration of melatonin (37). In newborn rats given intracerebral injections of ibotenate, melatonin administration has been shown to reduce the extent of periventricular white lesion lesions and to improve learning capacity (38, 39). A systematic review and meta-analysis of more than 400 adult rodents reported a 43% reduction in stroke infarct size following melatonin treatment (40). Robertson et al (41) published an interesting study of newborn asphyctic pigs, according to which melatonin associated with body hypothermia significantly improved the metabolism in the basal encephalic ganglia compared with the animals treated only with hypothermia.

In 2015, Aly et al (42) performed the only clinical trial performed to date in which melatonin was added to cooling therapy versus conventional treatment with hypothermia alone. Forty-five full-term newborns were recruited (30 with HIE and 15 healthy controls). The HIE infants were randomized into a hypothermia group (n = 15) and a HM group (n = 15). The melatonin administration schedule was five daily enteral doses of melatonin (10 mg kg^{-1}). The HM group presented fewer white matter abnormalities, according to MRI, fewer seizures, and had improved survival without neurologic or developmental abnormalities at 6 months old. Although the clinical study model is similar, and the results present a similar pattern, there are important methodological differences between this study and our own, regarding, for example, the administration route and the dose of melatonin supplied. In order to ensure more stable plasma melatonin concentrations, our clinical protocol stipulated the IV route. This view was taken in the consideration that oral drugs could be poorly absorbed in asphyxiated newborns, who are at high risk of gastrointestinal injury (inflammation, bleeding, vomiting) and multisystemic failure. Our results are in line with those obtained in earlier preclinical and clinical

TABLE 2. General Laboratory Parameters Measured at NICU Admission, Before Intervention (First 6 hr of Life); Comparison and Significance Level

Laboratory Parameter	Hypothermia Plus Melatonin Group (n = 12) (Mean ± SD)	Hypothermia Plus Placebo Group (n = 13) (Mean ± SD)	p	Significance
Sodium (mmol/L)	135 ± 4	136 ± 5	0.86	NS
Potassium (mmol/L)	4.3 ± 0.6	4.1 ± 1.0	0.31	NS
Chlorine (mmol/L)	100 ± 4	100 ± 4	0.79	NS
Calcium (mmol/L)	2.3 ± 0.2	2.4 ± 0.2	0.22	NS
Magnesium (mmol/L)	2.2 ± 0.1	2.0 ± 0.3	0.8	NS
Osmolarity (mmol/kg)	265 ± 8	271 ± 8	0.57	NS
Urea (mmol/L)	5.1 ± 1.7	4.6 ± 1.3	0.34	NS
Creatinine (µmol/L)	84.9 ± 27.4	86.6 ± 15.9	0.42	NS
Creatine kinase (µkat/L)	21.2 ± 15.6	27.4 ± 11.5	0.5	NS
Lactate dehydrogenase (µkat/L)	28.2 ± 16.1	27.03 ± 24.03	0.5	NS
Aspartate amino transferase (µkat/L)	2.37 ± 0.55	3.59 ± 0.90	0.61	NS
Alanine aminotransferase (µkat/L)	1.44 ± 0.73	1.84 ± 0.82	0.64	NS
Gamma glutamyl transferase (µkat/L)	2.25 ± 0.32	2.09 ± 0.58	0.34	NS
Direct bilirubin (µmol/L)	8.9 ± 4.1	8.7 ± 2.7	0.9	NS
Indirect bilirubin (µmol/L)	42.3 ± 26.2	30.4 ± 12.8	0.35	NS
Prothrombin (proportion of 1.0)	0.51 ± 0.13	0.39 ± 0.20	0.11	NS
International normalized ratio	1.66 ± 0.30	2.48 ± 1.86	0.37	NS
Leukocytes (×10^9/L)	24.7 ± 13.5	19.0 ± 4.4	0.26	NS
Neutrophils (×10^9/L)	15.7 ± 7.0	10.7 ± 3.3	0.1	NS
Platelets (×10^9/L)	227 ± 60	185 ± 66	0.13	NS
Hemoglobin (g/L)	155 ± 15	163 ± 22	0.63	NS

NS = not significant.
$p < 0.05$ was considered significant.

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TABLE 3. General Laboratory Parameters Measured After Intervention (72 hr of Life); Comparison and Significance Level

Laboratory Parameter	Hypothermia Plus Melatonin Group ($n = 12$) (Mean ± SD)	Hypothermia Plus Placebo Group ($n = 13$) (Mean ± SD)	p	Significance
Sodium (mmol/L)	137 ± 4	135 ± 5	0.20	NS
Potasium (mmol/L)	3.9 ± 0.8	4.0 ± 0.8	0.71	NS
Chlorine (mmol/L)	102 ± 5	102 ± 10	0.66	NS
Calcium (mmol/L)	2.24 ± 0.27	2.38 ± 0.16	0.23	NS
Magnesium (mmol/L)	1.09 ± 0.17	1.08 ± 0.15	1	NS
Osmolarity (mmol/kg)	283 ± 11	272 ± 8	0.19	NS
Urea (mmol/L)	16.35 ± 8.53	10.54 ± 6.84	0.34	NS
Creatinine (µmol/L)	73.37 ± 39.78	64.53 ± 43.32	0.42	NS
Creatine kinase (µkat/L)	7.53 ± 6.16	8.52 ± 5.42	0.57	NS
Lactate dehydrogenase (µkat/L)	20.77 ± 11.19	45.39 ± 28.39	1	NS
Aspartate amino transferase (µkat/L)	1.54 ± 0.56	1.39 ± 0.58	0.91	NS
Alanine aminotransferase (µkat/L)	0.77 ± 0.81	2.76 ± 2.61	0.60	NS
Gamma glutamyl transferase (µkat/L)	2.28 ± 1.8	1.32 ± 0.44	0.28	NS
Direct bilirubin (µmol/L)	8.89 ± 3.93	10.26 ± 5.13	0.49	NS
Indirect bilirubin (µmol/L)	92.87 ± 61.23	63.28 ± 30.79	0.18	NS
Prothrombin (proportion of 1.0)	0.72 ± 0.12	0.66 ± 0.22	0.42	NS
International normalized ratio	1.26 ± 0.14	1.41 ± 0.32	0.37	NS
Leukocytes ($\times 10^9$/L)	10.7 ± 9.2	9.2 ± 3.9	0.69	NS
Neutrophils ($\times 10^9$/L)	8.0 ± 6.7	6.1 ± 4.1	0.47	NS
Platelets ($\times 10^9$/L)	169 ± 87	127 ± 58	0.5	NS
Hemoglobin (g/L)	137 ± 27	158 ± 23	0.82	NS

NS = not significant.
$p < 0.05$ was considered significant.

studies, and suggest that melatonin, added to therapeutic hypothermia, could offer synergistic neuroprotective effects. Nevertheless, further RCTs are needed to provide more information in this respect and to determine appropriate management guidelines.

Recent trials in newborns have evidenced a reduction in various inflammatory biomarkers (cytokines, tumor necrosis factor, malondialdehyde, nitrites/nitrates) in asphyctic newborns and ventilator-dependent preterm infants (43–46). In other serious neonatal pathologies, such as sepsis, the administration of melatonin as an adjuvant therapy is associated with improved clinical and laboratory outcomes (44, 47, 48). Furthermore, in processes related to pain and neonatal inflammation, Gitto et al (49) showed that melatonin reduces levels of proinflammatory and anti-inflammatorycytokines (interleukin [IL]–6, IL-8, IL-10, and IL-12).

The dose at which melatonin should be used as a neuroprotectant is not yet known. The only information available on the clinical effects of melatonin concerns its use in the treatment of sepsis and chronic pulmonary disease, two complications that affect systems without biological barriers, similar to those observed in the CNS. Due to the blood–brain barrier, the efficient concentration of melatonin in the growing brain is not yet known. Most of the scientific papers reviewed for this study have reported neuroprotective effects of melatonin at doses ranging from 1.25 to 20 mg/kg, with which neuronal recovery improves after the hypoxic-ischemic insult (23). However, in the latter studies, the serum concentrations of melatonin used to prevent or treat sepsis or chronic lung disease were higher than the physiologic levels in the adult. These limited data suggest that neuroprotective treatment with melatonin should be viewed more as an additional treatment than as a substitution (50). In 2011, an international group of leading perinatal neuroscientists rated melatonin as the most promising of 13 neuroprotectants nearing clinical translation (51). Regarding the dose and duration of treatment, the parameters used in our clinical trial are very similar to those in comparable previous research with ventilated preterm infants, which stipulated 10 mg/kg IV administration

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TABLE 4. MRI and Amplitude-Integrated Electroencephalography Findings in the Study Groups; Comparison and Significance Level

Brain Evaluation Test	Hypothermia Plus Melatonin Group ($n = 12$), n (%)	Hypothermia Plus Placebo Group ($n = 13$), n (%)	p
MRI findings			
Basal ganglia and thalami			
Normal	7 (58.3)	7 (53.8)	0.923 (NS)
Mild abnormalities	3 (25)	3 (23.1)	
Moderate/severe abnormalities	2 (16.7)	3 (23.1)	
White matter			
Normal/minimal abnormalities	9 (75)	10 (76.9)	0.909 (NS)
Moderate/severe abnormalities	3 (25)	3 (23.1)	
Amplitude-integrated electroencephalography patterns			
Initial			
Normal background	8 (66.7)	8 (61.5)	0.968 (NS)
Abnormal background	4 (33.3)	5 (38.4)	
Seizures	5 (41.6)	4 (30.7)	
After rewarming			
Normal background	10 (83.3)	10 (76.9)	0.972 (NS)
Abnormal background	2 (16.7)	3 (23.1)	
Seizures	1 (8.3)	2 (15.4)	

NS = not significant.
$p < 0.05$ was considered significant.

TABLE 5. Neurodevelopment Bayley III Test, Gross Motor Function Classification System, and Tardieu Scales; Comparison and Significance Level

Bayley III Test Development Area	Hypothermia Plus Melatonin Group ($n = 12$) (Mean ± SD)	Hypothermia Plus Placebo Group ($n = 13$) (Mean ± SD)	p	Significance
Cog CS6	97 ± 17.19	92.78 ± 20.78	1	NS
L CS 6	100.90 ± 20.76	98.22 ± 8.29	0.65	NS
Mot CS6	97.50 ± 21.59	93.56 ± 27.52	1	NS
Cog CS18	101.25 ± 21.51	85.56 ± 17.40	0.05	S.
L CS18	95.38 ± 24.47	83.22 ± 19.23	0.057	NS
Mot CS18	96.13 ± 22.08	89.33 ± 26.12	0.347	NS
Motor classification scale and age				
GMFCS at 6 mo old			1	NS
I, II score vs III, IV, and V score				
GMFCS at 18 mo old			1	NS
I, II score vs III, IV, and V score				
Tardieu at 6 mo old			0.58	NS
I, II score vs III, IV, and V score				
Tardieu at 18 mo old			0.58	NS
I, II score vs III, IV, and V score				

Cog CS6 = Cognitive Composite Score at 6 mo, Cog CS18 = Cognitive Composite Score at 18 mo, GMFCS = Gross Motor Function Classification System, L CS6 = Language Composite Score at 6 mo, L CS18 = Language Composite Score at 18 mo, Mot CS6 = Motor Composite Score at 6 mo, Mot CS18: Motor Composite Score at 18 mo, NS = not significant.
$p < 0.05$ was considered significant. Scores at 6 and 18 mo old.

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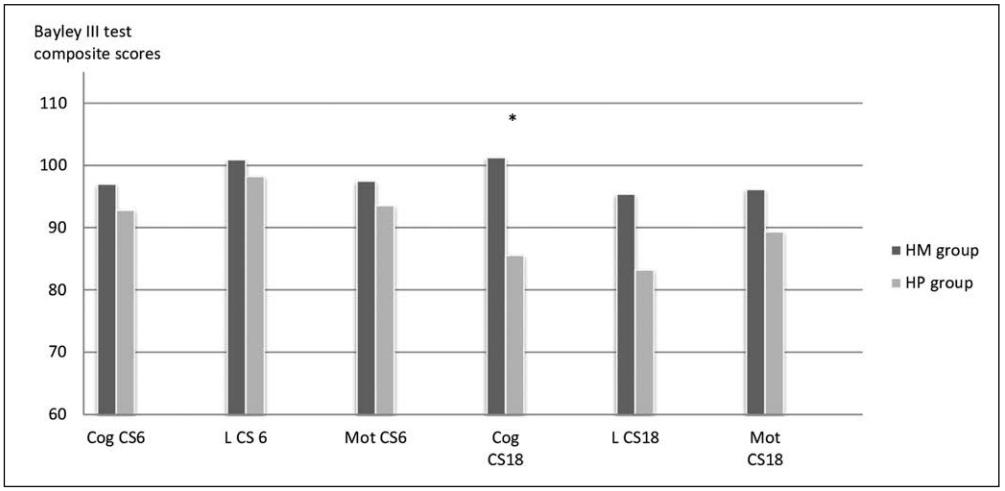

Figure 2. Neurodevelopment Bayley III test. Composite scores at 6 and 18 mo old. Comparison of two intervention arms. Cog CS6 = Cognitive Composite Score at 6 mo, Cog CS18 = Cognitive Composite Score at 18 mo, HM group = hypothermia plus melatonin group, HP group = hypothermia plus placebo group, L CS6 = Language Composite Score at 6 mo, L CS18 = Language Composite Score at 18 mo, Mot CS6 = Motor Composite Score at 6 mo, Mot CS18: Motor Composite Score at 18 mo. $p < 0.05$ was considered significant.

for 2 hours and a treatment duration of 72 hours (45). Other preliminary evidence includes clinical trials using a single oral dose of melatonin in septic neonates (20 mg per dose) within the first 24 hours of life (47, 48). In our opinion, the dose used in the present study (5 mg/kg body weight) is safe for critically ill asphyctic newborns. In pathophysiologic terms, it is logical for our intervention to begin, like hypothermia treatment, during the therapeutic window (the first 6 hr of life) and for it to be continued for 72 postnatal hours (equaling the duration of administration of the well-established protocol for hypothermia treatment). Although a longer duration of IV melatonin might be useful, we believe that greater safety is guaranteed if patients remain under intensive surveillance in the NICU. Subsequent to therapy, many of these infants are soon transferred to conventional admission areas. In addition, no recent clinical trials in newborns have been recorded that extend the duration of experimental treatment (whether oral or IV) beyond 72 hours.

The novel aspect of the present study is that it is the first RCT to be performed in newborns in which IV melatonin is administered for the treatment of HIE, as an adjuvant therapy to whole body hypothermia. On the other hand, there is a weakness, namely the low number of patients recruited ($n = 25$), due to the difficulty inherent in performing this type of study with critically ill newborns. Another limitation could be the trend ($p = 0.09$) of higher Sarnat-Sarnat scores in the HP group versus the HM group (Table 1) and its potential influence on the final outcomes. The greatest strength of the study lies in the fact that it is a multicenter, randomized, controlled, double-blind clinical trial with long-term neurodevelopmental longitudinal follow-up. The only previous clinical trial conducted in newborn infants, with treatment based on hypothermia and melatonin, only followed up the results obtained for 6 months (42), whereas in the present study, the patients were followed up until the age of 18 months.

CONCLUSIONS

Hypothermia treatment alone is known to reduce disability in newborn infants, but we remain far from achieving optimum results such that the asphyctic newborn can survive with minimal or no sequelae. In this article, we describe the first RCT in which IV melatonin was added to cooling therapy in newborns with HIE. The combined treatment was found to improve cognitive development at the age of 18 months, but it should be considered preliminary, due to the low number of newborns studied. This study supports the safety and feasibility of intervention in neonates after HIE to be studied in future RCTs.

This work was performed at San Cecilio and Virgen de las Nieves University Hospitals (Granada, Spain) and Torrecárdenas University Hospital (Almería, Spain).

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The authors have disclosed that they do not have any potential conflicts of interest.

Our research was conducted in accordance with the ethical principles set out in the World Medical Association Declaration of Helsinki. All parents gave their written informed consent to enroll their newborns. The study protocol was approved by the Clinical Research Ethics Committees of Granada and Almería.

For information regarding this article, E-mail: aejerezc@gmail.com

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