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Topiramate plus Cooling for Hypoxic-Ischemic Encephalopathy: A Randomized, Controlled, Multicenter, Double-Blinded Trial

Antonio Nuñez-Ramiro[a] Isabel Benavente-Fernández[b] Eva Valverde[c] Malaika Cordeiro[c] Dorotea Blanco[d] Hector Boix[e] Fernando Cabañas[f] Mercedes Chaffanel[g] Belén Fernández-Colomer[h] Jose Ramón Fernández-Lorenzo[i] Julia Kuligowski[j] Begoña Loureiro[k] Maria Teresa Moral-Pumarega[l] Antonio Pavón[m] Angel Sánchez-Illana[j] Inés Tofé[n] David Hervás[o] Ana García-Robles[i] Anna Parra-Llorca[a] Maria Cernada[a] Juan Martinez-Rodilla[j] Sheila Lorente-Pozo[j] Roberto Llorens[p] Remedios Marqués[q] Máximo Vento[a, j] on behalf of the Hypotop Study Group

[a]Division of Neonatology, University and Polytechnic Hospital La Fe, Valencia, Spain; [b]Division of Neonatology, University Hospital Puerta del Mar, Cádiz, Spain; [c]Division of Neonatology, University Hospital La Paz, Madrid, Spain; [d]Division of Neonatology, University Hospital Gregorio Marañón, Madrid, Spain; [e]Department of Neonatology, University Hospital Vall d'Hebrón, Barcelona, Spain; [f]Division of Neonatology, University Hospital Quirónsalud Madrid, Madrid, Spain; [g]Division of Neonatology, Regional University Hospital Málaga, Málaga, Spain; [h]Division of Neonatology, Central University Hospital of Asturias, Oviedo, Spain; [i]Division of Neonatology, University Hospital Complex of Vigo, Vigo, Spain; [j]Health Research Institute La Fe, Valencia, Spain; [k]Division of Neonatology, University Hospital Cruces, Bilbao, Spain; [l]Division of Neonatology, University Hospital 12 de Octubre, Madrid, Spain; [m]Division of Neonatology, University Hospital Virgen del Rocío, Sevilla, Spain; [n]Division of Neonatology, University Hospital Reina Sofía, Córdoba, Spain; [o]Department of Biostatistics, Health Research Institute La Fe, Valencia, Spain; [p]Department of Radiology, University and Polytechnic Hospital La Fe, Valencia, Spain; [q]Departament of Pharmacy, University and Polytechnic Hospital La Fe, Valencia, Spain

Keywords
Hypoxic-ischemic encephalopathy · Seizures · Hyperexcitability · Seizures · Anaerobic metabolism · Oxidative stress biomarkers

Abstract

Background and Objectives: Therapeutic interventions to improve the efficacy of whole-body cooling for hypoxic-ischemic encephalopathy (HIE) are desirable. Topiramate has been effective in reducing brain damage in experimental studies. However, in the clinical setting information is limited to a small number of feasibility trials. We launched a randomized controlled double-blinded topiramate/placebo multicenter trial with the primary objective being to reduce the antiepileptic activity in cooled neonates with HIE and assess if brain damage would be reduced as a consequence. ***Study Design:*** Neonates were randomly assigned to topiramate or placebo at the initiation of hypothermia. Topiramate was administered via a nasogastric tube. Brain electric activity was continuously monitored. Topiramate pharmacokinetics, energy-related and Krebs' cycle intermediates, and lipid peroxidation biomarkers were determined using liquid chromatography-mass spectrometry and MRI for assessing brain damage. ***Results:*** Out of 180 eligible patients 110 were ran-

A complete list of non-author contributors appears in the Appendix.

E-Mail karger@karger.com
www.karger.com/neo

Prof. Máximo Vento, MD, PhD
Division of Neonatology and Health Research Institute La Fe
University and Polytechnic Hospital La Fe
Avenida Fernando Abril Martorell 106, ES–46026 Valencia (Spain)
E-Mail maximo.vento@uv.es

domized, 57 (51.8%) to topiramate and 53 (48.2%) to placebo. No differences in the perinatal or postnatal variables were found. The topiramate group exhibited less seizure burden in the first 24 h of hypothermia (topiramate, $n = 14$ [25.9%] vs. placebo, $n = 22$ [42%]); needed less additional medication, and had lower mortality (topiramate, $n = 5$ [9.2%] vs. placebo, $n = 10$ [19.2%]); however, these results did not achieve statistical significance. Topiramate achieved a therapeutic range in 37.5 and 75.5% of the patients at 24 and 48 h, respectively. A significant association between serum topiramate levels and seizure activity ($p < 0.016$) was established. No differences for oxidative stress, energy-related metabolites, or MRI were found. ***Conclusions:*** Topiramate reduced seizures in patients achieving therapeutic levels in the first hours after treatment initiation; however, they represented only a part of the study population. Our results warrant further studies with higher loading and maintenance dosing of topiramate.

Introduction

Hypoxic-ischemic encephalopathy (HIE) occurs in approximately 1.5 cases per 1,000 live births and is one of the leading causes of neonatal death and adverse long-term neurological outcome [1]. Neuron viability is intimately reliant on a continuous blood-borne supply of both oxygen and glucose to avoid ATP exhaustion and subsequently cells swelling, apoptosis, and necrosis [2, 3].

Therapeutic hypothermia (TH) protocols have led to a significant reduction in mortality and improved neurocognitive outcomes; however, still a considerable number of infants will die or suffer severe disabilities. Therefore, complementary approaches to TH are being explored [4]. Excitotoxicity identified by amplitude-integrated EEG (aEEG) has been associated with neuronal death and adverse outcomes [5, 6]. Seizures increase both brain metabolic demand and the release of excitatory amino acids, leading to exacerbated neuronal injury as shown in MRI and follow-up studies [7]. Hence, there is considerable interest as to whether anticonvulsants could enhance hypothermic neuroprotection. Topiramate (TPM) is an anticonvulsant drug that inhibits the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors, while it potentiates γ-amino butyric acid (GABA) [8]. Experimental studies in piglet, rat, and mouse models of hypoxia-ischemia have shown that TPM reduces brain damage and improves neurobehavior, offering a dose-dependent and long-lasting neuroprotection [8]. Pilot studies in the newborn period have described the efficacy of TPM as an antiseizure drug and suggest a synergistic action of TPM in combination with hypothermia. However, these were safety trials with a small number of patients that did not include follow-up and therefore did not provide sufficient evidence on the dose regime employed [9–11]. We hypothesized that prophylactic TPM would reduce seizure activity and as a consequence enhance neuronal survival, leading to improved outcomes.

Population and Methods

Study Design

This is a randomized, controlled, double-blinded, placebo versus TPM multicenter clinical trial performed in newborn infants with a diagnosis of HIE and performed between June 2014 and December 2016, and registered as EudraCT No. 2011-005696-17 under the acronym "Hypotop."

Eligible patients were newborn infants with perinatal asphyxia evolving to HIE and requiring cooling therapy (online suppl. file, Table S1; see www.karger.com/doi/10.1159/000499084 for all online suppl. material). Encephalopathy classification included evaluation of consciousness, activity, posture tone, primitive reflexes, and autonomic system (online suppl. file, Table S2) [12]. The primary outcome of the study was to reduce seizure activity during hospitalization. Secondary outcomes included a reduction in mortality during hospital stay, severity of brain damage assessed with MRI, and oxidative stress. We also analyzed TPM side-effects and pharmacokinetics, and energy-related metabolites. Power calculation was based on the reduction of seizure activity and not on the neuroprotective effect. Our aim was to reduce the epileptic activity from a median of 40% assessed in the participating centers to 25%. Considering the incidence of HIE, loss to recruitment, and mortality, 48 patients per group were needed to achieve a power of 90% and a confidence interval of 95% with an alpha error of 0.05 for a period of 24 months.

Randomization was performed using sealed envelopes. TPM and placebo vials contained 30 mL of solution with a TPM concentration of 5 mg/mL. An initial dose of 1 mL/kg (5 mg/kg) and a maintenance dose of 0.6 mL/kg/day (3 mg/kg/day), each for 5 days, were administered via nasogastric tube.

Clinical Methods

Cooling was performed with servo-controlled hypothermia mattresses (Criticool, MTRE Advanced Technologies, Rehovot, Israel) keeping the rectal temperature at 33.5 ± 0.5 °C during 72 h. Rewarming was performed by increasing 0.3 °C/h until 36.5 °C. Infants were monitored with aEEG/multichannel EEG during the hospital stay. Conventional EEG and consultation with an electrophysiologist/pediatric neurologist were performed at the request of the neonatologist in charge. EEG registries were evaluated by 2 blinded expert neonatologists following a pre-established scoring system [13, 14]. The total seizure time was calculated as the addition of the time (minutes) during which abnormal seizure activity in the aEEG was assessed. When electrical seizures were detected a loading dose of phenobarbital (20 mg/kg) was given. If ineffective, second and third doses (10 mg/kg) were added. Midazolam (0.05–0.02 mg/kg) was used as second choice and lidocaine (loading dose 2 mg/kg; maintenance dose 4–6 mg/kg/h) for refractory seizures [15].

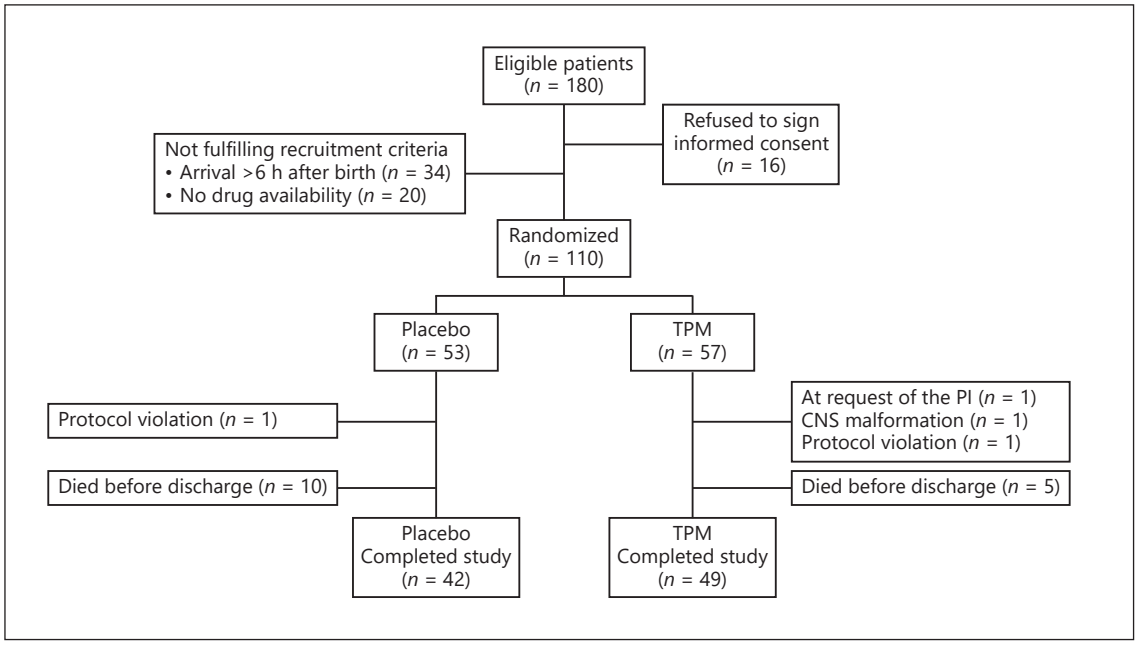

Fig. 1. Flow diagram including eligibility, recruitment, randomization and patients who completed the study in babies with birth asphyxia evolving to hypoxic ischemic encephalopathy treated with therapeutic hypothermia and randomized to topiramate (TPM) or placebo. PI, principal investigator.

The MRI protocol included 3D Gradient Echo T1-weighted MR images, coronal and axial Fast Spin Echo T2-weighted MR images, diffusion-weighted images (b0 and b1,000 values), and susceptibility-weighted imaging. MRI was interpreted using a standardized score that rated the extent and intensity of the injury in posterior limb internal capsule, basal ganglia and thalamus, white matter, and cortex [16]. Interpretation of MRI for study purposes was centralized and performed by 2 expert blinded radiologists.

Energy-related and Krebs' cycle metabolites were determined in cord and peripheral vein blood at 24, 48, and 72 h after the administration of the first dose of TPM/placebo. Plasma was stored at −80 °C. For pharmacokinetic studies serum samples were obtained at 2, 4, 8, 12, 18, 24, 48, 72, 96, and 120 h after TPM/placebo administration. Urine for oxidative stress biomarkers was collected and frozen at −80 °C. Analytical determinations were centralized at the HRI La Fe (Valencia).

Analytical Methods

Energy and Krebs's cycle metabolites were determined in 50 µL of plasma derivatized employing a two-step oximation-silylation procedure. Quantification was carried out using a 6890GC-5973N a GC-Q-MS system equipped with a HP-5MS column from Agilent Technologies (Santa Clara, CA, USA) [17]. Biomarkers of lipid peroxidation, specifically F2-isoprostanes, isofurans, neuroprostanes, and neurofurans, were determined in 600 µL of urine using an Acquity UPLC-Xevo TQS LC-MS/MS system from Waters (Manchester, UK) and a reversed phase chromatographic column as described elsewhere [18]. Results were normalized to creatinine in urine samples determined using DetectX Urinary Creatinine Detection Kits from Arbor Assays (Ann Arbor, MI, USA) following the instructions of the manufacturer after a 1:20 dilution of urine in H_2O.

TPM Solution Preparation, Stability Tests, and Quality Control of TPM

TPM was elaborated as an oral solution at a concentration of 5 mg/mL from Topamax® 100-mg tablets (Janssen-Cilag, Cologno Monzese, Milan, Italy) at the Compounding Area (Division of Pharmacy; UPH La Fe). The placebo was sterile water for injection (Grifols®, Barcelona, Spain). The concentration of TPM in the samples was determined in triplicate by LC-MS/MS using an Acquity Xevo TQ system from Waters. The stability of the preparation was assessed for 180 days and quality controls included: organoleptic and physicochemical (particles and pH) characteristics, and microbiological (thioglycolate and blood agar culture for bacteria, and liquid Sabouraud medium culture for fungi) and quantitative controls.

Pharmacokinetic Study

Plasma TPM concentrations were measured after the first dose of TPM at 2, 4, 8, 12, 18, and 24 h (before the second dose), 48 h (before the third dose), 72 h (before the forth dose), 96 h (before the fifth dose), and 120 h (before the sixth dose). Blood extractions were interrupted after 24 h of the last dose of TPM. TPM was determined in serum samples employing the QMS® TPM immunoassay from Thermo Fisher Scientific Inc. (Waltham, MA, USA) and following the manufacturer's instructions. The reliability of the method in neonates was validated in our lab in a reduced number of samples by HPLC-MS/MS. Serum concentrations of TPM obtained during the first 24 h (after

Table 1. Maternal and perinatal confounders in asphyxiated newborn infants evolving to HIE receiving TH and randomly assigned to TPM or placebo

Parameters	TPM (n = 54)	Placebo (n = 52)	Significance
Age of the mother, years	34.2±6.2	31.2±4.8	0.05
Gestational diabetes	6 (11.1)	6 (11.5)	ns
Hypertension, pre-eclampsia	5 (9.3)	3 (5.8)	ns
Maternal fever during delivery	7 (13.2)	1 (2)	ns
Chorioamnionitis	3 (5.7)	1 (2)	ns
Gestational age, weeks	39±1.6	38.7±1.8	ns
Meconium-stained amniotic fluid	22 (40.7)	15 (31.2)	ns
Preterm rupture of membranes, h			
Mean	8.5±11.6	5.7±9.8	ns
Median	2.5 (0–13.7)	0.5 (0–8)	ns
Type of delivery			
Vaginal delivery	11 (20)	5 (9.6)	ns
Instrumental delivery	13 (24)	12 (23)	ns
Emergency cesarean delivery	30 (55.6)	35 (67.3)	ns
Type of anesthesia			
Epidural	22 (66.7)	18 (60)	ns
General	11 (33.3)	12 (40)	ns
Multiple births	3 (5.8)	3 (5.8)	ns
Antenatal sentinel events	29 (53.7)	35 (67.3)	ns
Cord anomalies	3 (6.2)	3 (6.4)	ns
Uterine rupture	2 (4.2)	4 (8.5)	ns
Abruptio placentae	5 (10.4)	9 (19.1)	ns
Shoulder dystocia	12 (25)	11 (23.4)	ns
Non-reassuring fetal status	38 (71.7)	38 (77.6)	ns

Data are presented as n (%), mean ± SD, or median (IQR). No significant differences between the TPM and the placebo group for the parameters shown in the table were found. HIE, hypoxic-ischemic encephalopathy; TH, therapeutic hypothermia; TPM, topiramate; ns, not significant.

the first dose administration) were used to determine TPM pharmacokinetic parameters with the WinNonlin program (version 5.1, Pharsight Corp., Mountain View, CA, USA). The values of the following non-compartmental pharmacokinetic parameters were obtained: maximum serum concentration (C_{max}) and time to C_{max} (t_{max}), half-life ($t_{1/2}$), area under the concentration-time curve from the time of dosing to 24 h (AUC_{0-24}), and apparent oral clearance (CL/F).

Statistical Analysis

This was an intention to treat study. Data were summarized using the mean and standard deviation or median and interquartile range in the case of continuous variables and using relative and absolute frequencies in the case of categorical variables. The association between the medication group (TPM/placebo) and total convulsion time was assessed using the Wilcox-Mann-Whitney test. Differences in survival were assessed with the log-rank test and differences in seizure activity between groups were assessed using an ordinal regression model including an interaction between the treatment group and time of hypothermia. A marginal effects plot was generated to ease the interpretation of the results of the ordinal regression model. A logistic regression model was used to test for differences in risk of seizure between groups during the study. A marginal effects plot was generated to ease the interpretation of the results of the ordinal regression model. Association between serum TPM levels along the first 48 h and seizure activity was assessed using N-way partial least squares. p values <0.05 were considered statistically significant. All statistical analyses were performed using R (version 3.4.3).

Results

Figure 1 shows the recruitment diagram. A total of 49 in the TPM group and 42 in the placebo group completed the study. No differences in maternal or perinatal circumstances were observed (Table 1, 2). Mortality was greater in the placebo group (placebo, n = 10 [19.2%] vs. TPM, n = 5 [9.2%]; p < 0.123) but did not reach significance (Fig. 2).

Table 2. Neonatal characteristics, resuscitation maneuvers, and clinical status upon arrival in the NICU of asphyxiated newborn infants evolving to HIE and randomly treated with hypothermia plus TPM or placebo

Parameters	TPM (n = 54)	Placebo (n = 52)	p value
Birth weight, g	3,250±720	3,100±770	ns
Length, cm	51±3	50±3	ns
Head circumference, cm	34.4±1.5	34.2±2	ns
Male	30 (55.7)	28 (54)	ns
Apgar score 1 min	2 (1–2)	1 (0–3)	ns
Apgar score 5 min	4 (2–5)	3 (2–4)	ns
Apgar score 10 min	5 (3–7)	5 (4–6)	ns
Transferred from birth hospital	36 (68)	41 (79)	ns
Positive pressure ventilation	53 (98.2)	51 (98.1)	ns
Intubation in delivery room	47 (88.7)	49 (94.2)	ns
Chest compressions	26 (48.1)	30 (58.8)	ns
Epinephrine	20 (37)	29 (55.8)	ns
Maximum FiO_2	0.85±0.26	0.80±0.32	ns
Cord blood			
pH	6.98±0.2	6.93±0.2	ns
pCO_2, mm Hg	71.1±34.4	68.8±30	ns
Base deficit, mmol/L	14±5.1	15±9.8	ns
Lactate, mmol/L	12.9±1.5	14.3±1.6	ns
Temperature at arrival in NICU			
Inborn babies (n = 28), °C	34.6±1.46	35.1±1.2	ns
Outborn babies (n = 78), °C	33.7±1.1	33.3±1.4	ns
Moderate encephalopathy	37 (68.5)	33 (63.5)	ns
Severe encephalopathy	17 (31.5)	19 (36.5)	ns
Time after birth to initiate active hypothermia, h			
Inborn babies	2.46 (1.6)	2.66 (1.9)	ns
Outborn babies	4.94 (1.4)	4.76 (1.2)	ns
Death before discharge	5 (9.2)	10 (19.2)	ns

Data are presented as n (%), mean ± SD, or median (IQR). No significant differences for the parameters of the TPM of placebo groups reflected in the table were found. HIE, hypoxic-ischemic encephalopathy; TPM, topiramate; ns, not significant.

No significant differences for incidence, duration, and characteristics of seizure activity were assessed between groups (Table 3). The incidence of seizure activity in the TPM group in the first 24 h after birth was lower than in the placebo group but did not reach statistical significance (TPM vs. placebo: 14 [25.9%] vs. 22 [42%]; $p <$ 0.221). No differences between groups for duration of seizure or seizure characteristics could be assessed. Moreover, no difference in the number of drugs needed to control seizure activity was established (online suppl. file, Fig. S1). Finally, the difference in risk of seizure activity between the TPM and placebo groups was non-significant (odds ratio 0.8 with a 95% confident interval of 1.747).

MRI was performed in 41 patients of the placebo (78.8%) and 48 patients of the TPM group (88.9%). MRI was informed as abnormal in 58.5% of the placebo and 56.3% of the TPM group with no differences in the degree of severity.

No differences for lactate and pyruvate were found during the study period (online suppl. file, Table T1 and Fig. S2A). Similarly, plasma succinate and malate exhibited a similar metabolite profile (online suppl. file, Fig. S2B). No differences for urinary lipid peroxidation biomarkers determined after rewarming were found (online suppl. file, Fig. S3).

From a total of 105 patients enrolled in the pharmacokinetics study, data of 52 patients on TPM were retrieved. The mean serum concentrations showed an increase as the dosage regimen progressed and a steady-state was apparently reached with the fourth dose, since practically the same minimum serum concentration (C_{min}) was obtained at 96 and 120 h (Fig. 3). The TPM pharmacoki-

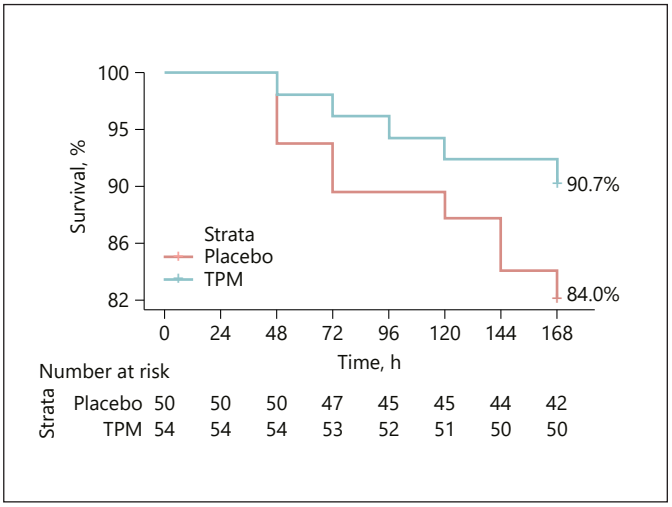

Fig. 2. Kaplan-Meier plot describing the evolving survival rate of patients with HIE randomized to prophylactic TPM or placebo and treated with TH. Mortality differences did not reach statistical significance. Time is expressed in hours after the initiation of hypothermia.

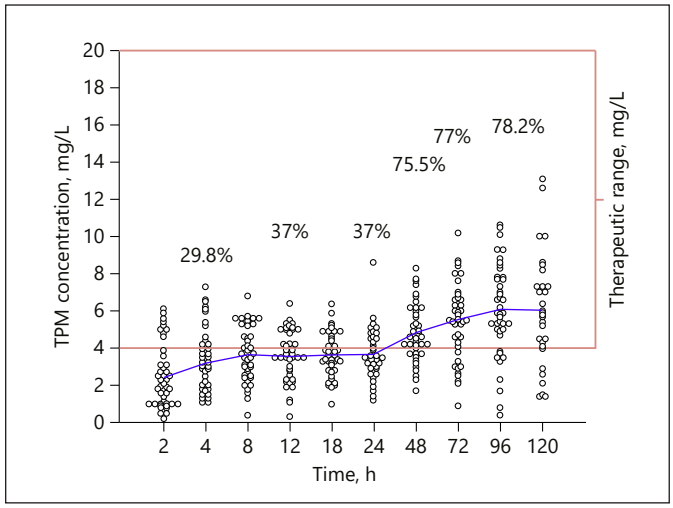

Fig. 3. TPM serum concentrations versus time curves (mean ± SD) obtained in newborn infants administered with an initial dose of 5 mg/kg followed by a maintenance dose 3 mg/kg/day.

Table 3. Incidence, duration, and type of seizures registered using continuous aEEG monitoring in asphyxiated babies evolving to HIE treated with TH and randomized to TPM or placebo

Time elapsed from the beginning of hypothermia, h	Placebo group (n = 52)					TPM group (n = 54)				
	seizures		type of seizure (% total seizures)			seizures		type of seizure (% total seizures)		
	present, n (%)	total duration, min[1]	isolated, %	repeated, %	status, %	present, n (%)	total, duration, min[1]	isolated, %	repeated, %	status, %
0–24	22 (42)	12.01	47.5	38.0	14.2	14 (25.9)[a]	14.79[a]	37.2[a]	37.2[a]	24.8[a]
24–48	6 (12)	6.95	50.0	16.7	33.3	8 (14.8)[a]	10.47[a]	30.0[a]	50.0[a]	20.0[a]
48–72	3 (6.4)	2.36	66.7	0.0	33.3	3 (5.8)[a]	6.58[a]	25.0[a]	75.0[a]	0.0[a]
72–96	2 (4.4)	0.59	50.0	50.0	0.0	3 (5.9)[a]	0.48[a]	40.0[a]	60.0[a]	0.0[a]

n, number of infants that had seizure activity; %, percentage of babies with seizures among survivors. [a] Non-significant versus placebo group. aEEG, amplitude-integrated EEG; HIE, hypoxic-ischemic encephalopathy; TH, therapeutic hypothermia, TPM, topiramate.
[1] Addition of seizure activity expressed in minutes assessed by aEEG from all the patients pertaining to the group.

netic parameters are summarized in Table 4. All patients receiving TPM were within the safety ranges [19]. However, serum therapeutic levels (TL) of 5–20 mg/L, as previously established by Patsalos et al. [19], were reached in 17/46 patients (36.9%) at 24 h, 37/49 patients (75.5%) at 48 h, 37/48 patients (77%) at 72 h, and 36/46 patients (78.2%) at 96 h (Fig. 4). Notably, the association between serum TPM levels in the first 48 h of TH and seizure activity showed a statistically significant association (p = 0.016). However, the sample size did not allow precise inference (95% confidence intervals or specific p values) of this complex non-linear relationship to be performed.

Discussion

TPM in experimental models has displayed neuroprotective effects [8] and has been safely used in the neonatal period [9–11]. One of the most compelling benefits of TH relies on the attenuation of post-depolarization release of excitatory amino acids [20]. Recently, the use of TPM (10 mg/kg/day) for 3 days during TH in patients with moderate to severe HIE did not reduce mortality or improve neurodevelopmental outcome at 18–24 months; however, it significantly reduced seizure activity [11]. Glass et al. [9] have also described the efficacy of TPM using similar

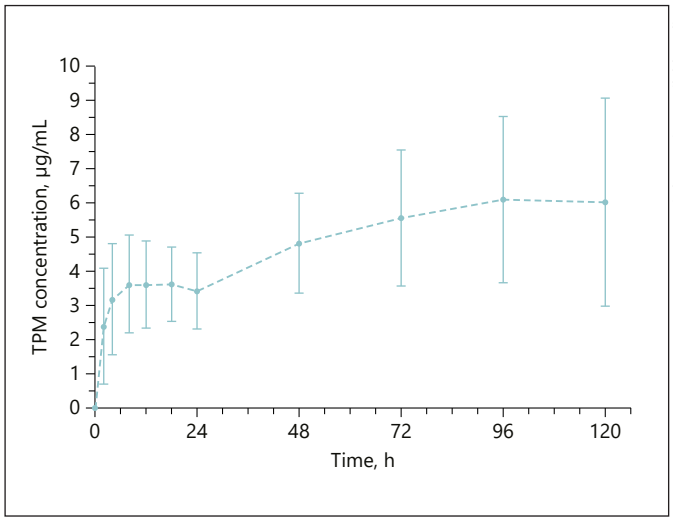

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Fig. 4. Serum levels of TPM from the initiation of therapy until the 5th day after birth (2 days after concluding hypothermia). TL are indicated by red horizontal lines between 4 and 20 mg/L. The percentage of patients who reached TL at 4, 12, 24, 48, 72, and between 96 and 120 h after the initial dose is shown.

Table 4. Pharmacokinetic parameters of TPM in newborn infants administered with an initial dose of 5 mg/kg

Parameter	Mean ± SD
C_{max}, µg/mL	4.2±1.4
t_{max}, h	9.8±5.5
$t_{1/2}$, h	54.1±23.6
AUC_{0-24}, µg × h/mL	77.8±25.6
CL/F, mL/kg/h	19.7±12.4

doses; however, increasing the dose to 25 mg/kg/day did not increase TPM efficacy. Notwithstanding, the information regarding the use of TPM to control seizure activity in the newborn period at the time when our study was planned was very limited. Hence, there was only one safety trial performed in Italy with a small number of patients and without follow-up [10]. Moreover, the authors confirmed that: "Long-term effects on cognitive functions of TPM administration in early life remain to be assessed" [10]. In addition, TH lowers the activity of the hepatic enzymatic complex CYP3A4 which metabolizes TPM and increase above TL [21]. Given the lack of experience in the newborn period and the lack of information regarding long-term effects of TPM, we decided to lower the loading (5 mg/kg) and a maintenance dose (3 mg/kg/day) for a total of 5 days to avoid undesirable and unexpected side-effects. Interestingly, only 36.9% of our patients reached a serum TL in the first 24 h of hypothermia, and 75.5% at 48 h during the time of maximal seizure activity. However, there was a significant correlation between serum level and reduction of seizures in the first 48 h, revealing that probably earlier achievement of TL would have rendered TPM more efficacious in seizure control. The half-life and apparent oral clearance of TPM calculated in this study (Table 4) using serum concentrations obtained after the first dose were similar to the values reported using the concentrations obtained at a "virtual" steady state using 5 mg/kg once a day during 3 days [22]. The TPM group exhibited non-significantly lower mortality (9.2%) than the placebo group (19.2%). In animal models of cerebral ischemia, TPM reduced the severity of cerebral damage either alone or with hypothermia [9]. Neuronal death after ischemia has been associated with changes of the AMPA receptor, massive entry of Ca^{2+}, and subsequently an increase in the generation of free radicals and apoptosis [23]. However, TPM did not significantly reduce selective lipid peroxidation biomarkers and/or MRI brain damage.

Under anaerobic conditions pyruvate is diverted to the formation of L-lactate, which reflects the duration and intensity of hypoxia [24]. Hypothermia reduces the cerebral metabolic rate by 5–8% for every 1 °C reduction of core temperature [25]. Asphyxiated babies showed increased plasma L-lactate, pyruvate, acetoacetate, and β-hydroxybutyrate before the initiation of TH; however, the concentration of metabolites decreased after a few hours of TH revealing that cooling favored a rapid return to normalized metabolic status [17]. An increased succinate concentration is associated with severe tissue hypoxia, especially in the myocardium [26]. Of note, for the first time in newborn babies we have shown that hypothermia rapidly reduced succinate levels, speeding up the normalization of Krebs' cycle metabolites.

Our study has limitations. First, mortality was not a primary outcome and the number of babies was not adjusted to this endpoint. Therefore, the tendency towards reduction in mortality in the TPM group should be cautiously interpreted. Second, it can be argued that the dose of TPM employed was low as compared to that employed by Filippi et al. [10]. We chose to avoid toxicity or unknown side-effects using TPM dosing in the lower range. As a consequence, a substantial number of babies were below therapeutic efficacy in the first 48 h (Fig. 4).

We conclude that TPM reduced seizure activity and mortality; however, it did not reach statistical significance. TH rapidly reduced succinate levels speeding up

the normalization of Krebs' cycle metabolites. The use of higher loading and maintenance doses of TPM and especially the availability of parenteral TPM warrant further studies.

Appendix

Non-Author Contributors in the Hypotop Trial

J.F. Ferreira, MD (University Hospital Virgen del Rocio, Sevilla); P. Jaraba-Caballero, MD (University Hospital Reina Sofía, Córdoba); M.M. Serrano-Martín, MD (Regional University Hospital Málaga, Málaga); C. Fernández, MD, Y. Castilla, MD (University Hospital Vall d'Hebrón, Barcelona); M.A. Caballero, MD, M. López-Azorín, MD (University Hospital Quironsalud Madrid, Madrid); L. Sánchez, MD (University Hospital La Paz, Madrid); M. Arriaga, MD, M.L. Franco, MD (University Hospital Gregorio Marañón, Madrid); M.J. Martínez-González, MD, C. Iniesta-Oskariz, MD (University Hospital Cruces, Bilbao); S.P. Lubián-López, MD (University Hospital Puerta de la Mar, Cádiz); Elena Bergón-Sendín, MD, María del Carmen Pérez-Grande (University Hospital 12 de Octubre Madrid); I. Izquierdo, MD, A. Gimeno, MD, M. Gormaz, MD, R. Escrig, MD, M. Cernada, MD, M. Aguar, MD, E. Torres, MD (University and Polytechnic Hospital La Fe, Valencia).

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Statement of Ethics

The study protocol was approved by the Comité de ética e investigación medica (CEIm) of the University and Polytechnic Hospital La Fe for all the study sites. Parents/tutors of all patients signed informed consent forms.

Disclosure Statement

The authors declare no conflicts of interest.

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