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FULL-LENGTH ORIGINAL RESEARCH

Topiramate concentrations in neonates treated with prolonged whole body hypothermia for hypoxic ischemic encephalopathy

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Summary

Purpose: **Therapeutic hypothermia reduces mortality and neurologic impairment in neonates with hypoxic–ischemic encephalopathy. Topiramate exerts a neuroprotective effect in asphyxiated neonatal animal models. However, no studies have investigated the association of hypothermia and topiramate, because topiramate pharmacokinetics during hypothermia and the optimal administration schedule are unknown. The influence of hypothermia on topiramate pharmacokinetics was evaluated in asphyxiated neonates treated with prolonged whole-body hypothermia and topiramate.**

Methods: **Thirteen term newborns were treated with mild or deep whole body hypothermia for 72 h; all received oral topiramate, 5 mg/kg once a day for the first 3 days of life, and seven had concomitant phenobarbital treatment. Topiramate concentrations were measured on serial dried blood spots.**

Results: **Topiramate concentrations were within the reference range in 11 of 13 newborns, whereas concentrations exceeded the upper limit in 2 of 13, both newborns on deep hypothermia. Topiramate concentrations reached a virtual steady state in nine newborns, for whom pharmacokinetic parameters were calculated. Values of topiramate maximal and minimal concentration, half-life, average concentration, and area under the time—concentration curve resulted in considerably higher values than those reported in normothermic infants. With respect to normothermic infants, time of maximal concentration was mildly delayed and apparent total body clearance was lower, suggesting slower absorption and elimination. Pharmacokinetic parameters did not differ significantly between infants on deep versus mild hypothermia and in those on topiramate monotherapy versus add-on phenobarbital.**

Conclusion: **Most neonates on prolonged hypothermia treated with topiramate 5 mg/kg once a day exhibited drug concentrations within the reference range for the entire treatment duration.**

KEY WORDS: **Newborn, Asphyxia, Neuroprotection, Pharmacokinetics.**

Recent trials have demonstrated that mild hypothermia (MH), started within 6 h after birth and protracted for 48–72 h, can significantly improve survival and reduce neurologic impairment in neonates with hypoxic—ischemic encephalopathy (HIE) (Eicher et al., 2005; Gluckman et al., 2005; Shankaran et al., 2005). MH can be obtained

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by selective head or whole-body cooling (rectal or esophageal temperature 33–34°C). A preliminary study showed also that deep hypothermia (DH) (temperature 30–33°C) is similarly safe and neuroprotective (Compagnoni et al., 2008). Recently, an interim advisory statement proposed support for the introduction of therapeutic hypothermia in the recommendations of the International Liaison Committee on Resuscitation (Hoehn et al., 2008).

It is not currently known whether neuroprotective drugs can further improve the beneficial effects of hypothermia. Topiramate (TPM) is an antiepileptic drug with multiple mechanisms of action, including glutamate-receptor inhibition (Shank et al., 2000; Guerrini & Parmeggiani, 2006). TPM demonstrated neuroprotective properties in vitro and in animal models in vivo (Angehagen et al., 2005; Sfaello et al., 2005; Noh et al., 2006) and was recently proposed as an innovative neuroprotective therapy for ischemic stroke (Yang et al., 1998; Lee et al., 2000; Edmonds et al., 2001; Follett et al., 2004; Liu et al., 2004; Schubert et al., 2005; Costa et al., 2006; Noh et al., 2006) and neonatal hypoxic–ischemic cerebral injury (Choi & Kim, 2007). However, the association of hypothermia and TPM for the treatment of HIE has not been investigated.

Hypothermia affects the pharmacokinetics of several drugs (Tortorici et al., 2007). In order to plan future trials designed to evaluate the possible neuroprotective effects of combined topiramate and hypothermia, we studied the pharmacokinetics of TPM in newborns treated with hypothermia. Thirteen neonates with HIE were treated with hypothermia and oral TPM administrations, with serial determinations of plasma TPM concentrations.

Patients and Methods

From January to August 2008, 13 full-term newborns with HIE were enrolled in two Italian neonatal intensive care units (NICUs), (A. Meyer University Children's Hospital, Florence and Carlo Poma Hospital, Mantua). Infants were treated with whole-body DH or MH, started within 6 h after birth and maintained for 72 h if the two following criteria were fulfilled: (1) Gestational age ≥36 weeks and birth weight ≥1,800 g with at least one of the following: (a) Apgar score ≤5 at 10 min; (b) persisting need for resuscitation, including endotracheal intubation or mask ventilation for more than 10 min after birth; (c) acidosis (pH ≤7.0 and/or base deficit ≥−16 mmol/L in umbilical cord blood or arterial, venous, or capillary blood) within 60 min from birth; (2) moderate to severe encephalopathy consisting of altered state of consciousness (irritability, lethargy, stupor, or coma) and ≥1 of the following signs: (a) hypotonia, (b) abnormal reflexes including oculomotor or pupillary abnormalities, (c) absent or weak suctioning, (d) clinical seizures. Exclusion criteria were congenital abnormalities, viral infections, or encephalopathy different from HIE. Outborn patients were initially cooled to 35°C at the birth hospital, avoiding heating and using ice packs during the transfer to neonatal intensive care unit (NICU). In NICUs, infants allocated to treatment with DH were cooled to rectal or esophageal temperature of 30–33°C, whereas newborns allocated to treatment with MH were cooled to 33–34°C. In one center (Mantua) hypothermia was induced by applying ice packs to head and body. Rectal temperature was continuously monitored by a probe connected to cardiomonitor (Viridia 24C, Hewlett-Packard Company, Palo Alto, CA, U.S.A. or Infinity Delta, Dräger Medical System, Telford, PA, U.S.A.) for simultaneous measurement with respiratory rate, heart rate, blood pressure, and oxygen saturation. In the other center (Florence) hypothermia was obtained by a cooling blanket (Blanketrol III, Hyper-Hypothermia System; Cincinnati Sub-Zero, Cincinnati, OH, U.S.A.). An esophageal probe was inserted, and esophageal temperature was lowered to 33.5°C by the blanket's servomechanism. Skin temperature was monitored on the abdominal wall with a skin probe by the radiant warmer thermal sensor or a temperature-monitoring unit (Mon-a-therm; Mallinckrodt Medical, St Louis, MO, U.S.A.). After 72 h of hypothermia, newborns were gradually rewarmed to 36.5–37°C over the following 6–12 h (0.5°C/h). In newborns treated with a cooling blanket, the automatic control setpoint was increased by 0.5°C per hour.

Vital signs were monitored continuously and daily determinations of liver–renal function tests, coagulation tests, and blood cell count were performed. Hematocrit was determined using an automated Coulter counter (Beckman-Coulter ACt.8, High Wycombe, United Kingdom). Blood-gas measurements were corrected for body temperature. Hypotension, defined as mean arterial blood pressure <40 mm Hg, was treated with single or repeated normal saline boluses, and in the case of persisting hypotension, dopamine, dobutamine, or norepinephrine was administered. Seizures were treated with phenobarbital (PB) (loading dose 20 mg/kg, followed by maintenance dose, 2.5 mg/kg every 12 h). A cerebral magnetic resonance (MR) scan was performed within the first week of life. The study protocol was approved by the research ethics committee of Carlo Poma Hospital, Mantua, and A. Meyer Hospital, Florence, Italy. Written informed consent for participation and publication was obtained from both parents of involved newborns.

TPM (Topamax; Janssen-Cilag, Cologno Monzese, Milan, Italy) was administered by orogastric tube as enteric-coated granules mixed with water, at the dosage of 5 mg/kg/day, starting from the beginning of hypothermia, once a day for the first 3 days of life for a total amount of three doses for each neonate. This sche-

dule was arbitrarily chosen, assuming that hypothermia would not prevent TPM absorption, but rather reduce TPM clearance.

Study design

TPM concentrations were measured on dried blood spots (DBS) by liquid chromatography tandem mass spectrometry (LC-MS/MS assay), as described previously (la Marca et al., 2008). Values were then corrected by hematocrit, obtaining plasma values. Each value from DBS was divided by (100−hematocrit)/100.

Plasma TPM concentrations were evaluated for the first nine patients (four with MH and five with DH), after 48 h of hypothermia, before the administration of the third dose of TPM ($T_{48\ h}$) and at $T_{48.5}$, T_{49}, $T_{49.5}$, T_{50}, T_{52}, T_{54}, T_{56}, T_{60}, T_{64}, T_{68}, and T_{72}. Three newborns were co-treated with PB. To obtain a more detailed TPM profile, in the following four patients (all treated with MH), plasma TPM concentrations were measured at the beginning of hypothermia, before the first dose of TPM (T_0), and at $T_{0.5}$, T_1, $T_{1.5}$, T_2, T_4, T_6, T_8, T_{12}, T_{16}, T_{20}, and T_{24} (before the second dose), at T_{26}, T_{30}, T_{34}, T_{40}, and T_{48} (before the third dose), and at $T_{48.5}$, T_{49}, $T_{49.5}$, T_{50}, T_{52}, T_{54}, T_{56}, T_{60}, T_{64}, T_{68}, and T_{72}. All of these four newborns were also on PB.

TPM concentration assessment

In adult patients with normal renal function, oral TPM reaches steady state in about 4 days (Tidwell & Swims, 2003). However, our patients were treated with hypothermia and TPM for only 72 h. Therefore, TPM was considered to have reached ''virtual'' steady state if its concentrations at T_{72} were within the concentration at T_{48} ± 10%. For newborns who reached TPM virtual steady state, the following pharmacokinetic parameters were derived by a noncompartmental method (WinNonlin Prof, version 4.0.1; Pharsight, Mountain View, CA, U.S.A.): maximal plasma concentration (C_{max}), minimal plasma concentration (C_{min}), time of peak concentrations (T_{max}), area under plasma concentration–time curve from 0–24 h ($AUC_{0–24}$) which was calculated using the linear trapezoidal rule for TPM. Half-life ($T_{1/2}$) was derived from the slope β of the log-linear phase. The average plasma concentration (C_{avg}) during the 24-h dosing interval was calculated from the quotient $AUC_{0–24}/24$. Apparent oral clearance (CL/F) was calculated according to the following equation: CL/F (ml/kg/h) = TPM daily dose (mg/kg)/[$AUC_{0–24}$(mg/L/h)] × 1,000.

Statistical analysis

The trial was designed as a pilot study with limited recruitment of neonates. Means and standard deviations of TPM concentrations were calculated for all newborns, whereas pharmacokinetic parameters were calculated for newborns who reached the steady state. Data were then compared between newborns treated with MH or DH and between newborns with or without PB co-treatment. Differences in variables between the groups were first analyzed with analysis of variance (ANOVA) (threshold $p = 0.05$) and, when a significant difference was found in a variable, groups were compared using the Student *t*-test (total threshold $p = 0.05$).

Results

Thirteen newborns, 3 female and 10 male, were enrolled (Table 1). Mean age at inclusion was 4.0 ± 1.7 h. Mean temperature of outborn newborns at arrival in NICUs was 34.8 ± 0.5°C. Five newborns were treated with DH (temperature range 29.7–33.7°C) and eight with MH (temperature range 32.7–34.5°C) (Fig. 1). TPM was administered at 4.3 ± 1.3 h. Seven newborns were treated with PB for seizures starting at 4.5 ± 1.0 h.

TPM plasma concentrations, obtained by converting measurements on DBS corrected with actual hematocrit, were recorded in all newborns and plotted in a manner distinguishing between newborns in DH or MH (Fig. 2), and between newborns receiving PB or not (Fig. 3). Newborns treated with MH and cotreated with PB showed lower TPM concentrations, although no statistically significant differences were observed. The coefficient of variability (CV) of TPM values was significantly higher in the DH group than in the MH group ($p < 0.005$).

Among the 13 newborns, 9 (69.2%) reached the virtual steady state; 3 were treated with DH and 6 with MH, 4 received PB and TPM, whereas 5 were treated with TPM only. Pharmacokinetic parameters in DH or MH treated newborns (Table 2) did not show significant differences, although lower $AUC_{0–24}$ (p 0.096), lower C_{avg} (p 0.096), and higher $T_{1/2}$ (p 0.080), in DH group were observed. Newborns who were co-treated with PB exhibited significantly lower C_{min} than those on TPM alone (p 0.032), and lower concentrations of C_{max} (p 0.060), $AUC_{0–24}$ (p 0.068), C_{avg} (p 0.068), $T_{1/2}$ (p 0.113), and higher CL/F (p 0.078) (Table 2). Parameter differences did not reach statistical significance, probably because of the small sample size. The subset of newborns who did not reach the steady state showed at T_{72} TPM concentrations that were 33.6% (±4.6%) higher than that measured at T_{48}.

No adverse effects attributable to TPM were identified, and none of the patients were prematurely discontinued because of limiting adverse events during association therapy of TPM and hypothermia. Twelve of 13 treated infants survived. A cerebral MR scan was performed at 5.3 ± 1.8 days and results were normal in six newborns (46.2%). Two newborns showed isolated white matter lesions; one exhibited isolated basal ganglia lesions; three

Table 1. Clinical and laboratory characteristics at birth (mean ± SD)

	All newborns (n = 13)	Mild hypothermia (n = 8)	Deep hypothermia (n = 5)	p-value	With PB (n = 7)	Without PB (n = 6)	p-value
Male, n (%)	10 (76.9)	6 (75)	4 (80)	0.426	6 (85.7)	4 (66.6)	0.230
Gestation, d, mean ± SD[a]	280.8 ± 7.0	282.1 ± 6.6	278.8 ± 7.9	0.214	283.1 ± 5.6	278.1 ± 7.9	0.107
Birth weight, g, mean ± SD	3,516 ± 661	3,532 ± 455	3,490 ± 973	0.458	3,832 ± 629	3,146 ± 519	0.029
Cesarean section, n (%)	6 (46)	4 (50)	2 (40)	0.376	4 (57.1)	2 (33.3)	0.217
Stained amniotic fluid, n (%)	6 (46)	4 (50)	2 (40)	0.376	4 (57.1)	2 (33.3)	0.217
Apgar Index, 1 min, mean ± SD	3.0 ± 2.5	2.0 ± 2.0	4.4 ± 2.6	0.043	3.1 ± 3.0	2.7 ± 1.9	0.372
Apgar Index, 5 min, mean ± SD	3.8 ± 2.5	3.1 ± 2.5	5.6 ± 2.1	0.047	3.7 ± 3.1	4.5 ± 2.1	0.304
Crib score, mean ± SD	3.7 ± 1.4	3.3 ± 0.7	4.4 ± 2.1	0.085	3.7 ± 1.9	3.7 ± 0.5	0.478
Arterial pH, mean ± SD	6,933 ± 0.16	6.967 ± 0.14	6.843 ± 0.21	0.140	6.942 ± 0.23	6.922 ± 0.06	0.428
Arterial BE, mEq/L, mean ± SD	−18.2 ± 6.3	−17.9 ± 7.6	−18.8 ± 1.6	0.420	−16.9 ± 6.0	−20.0 ± 7.0	0.239
Arterial lactic acid, mmol/L, mean ± SD	12.8 ± 6.8	13.4 ± 5.3	11.6 ± 7.8	0.410	15.9 ± 6.6	9.7 ± 7.6	0.146
H beginning, mean ± SD	4.0 ± 1.7	3.9 ± 1.5	4.2 ± 2.3	0.379	3.7 ± 1.8	4.3 ± 1.7	0.272
Survival rate at 6 months, n (%)	12 (92)	8 (100)	4 (80)	0.111	6 (85.7)	6 (100)	0.121
Treatment with mild hypothermia, n (%)	8 (61.5)				5 (71.4)	3 (50)	0.236
Treatment with phenobarbital, n (%)	7 (54)	5 (62)	2 (40)	0.236			

BE, base excess; CRIB, Clinical Risk Index for Babies; PB, phenobarbital; SD, standard deviation.
[a]Calculated from maternal menstrual history, obstetrical data, or by Ballard's score.

Figure 1.
Temperature profile of newborns treated with deep or mild hypothermia.
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showed combined basal ganglia, thalamic and cortical injuries; and a more diffuse pattern of brain injury (basal ganglia, thalami, cortex, and white matter) was observed in one infant.

Discussion

TPM has demonstrated neuroprotective properties in neuronal cultures exposed to oxygen-glucose deprivation (Noh et al., 2006) or excitotoxic glutamate or kainate concentrations (Angehagen et al., 2005), and in adult rodent models of transient global cerebral ischemia (Yang et al., 1998; Lee et al., 2000; Edmonds et al., 2001). Intravenous, intraperitoneal, and oral TPM, alone or combined with hypothermia, also reduced hypoxic–ischemic cerebral injury in newborn animals in a dose-dependent manner, with a neuroprotective dose ranging from 5–200 mg/kg, usually in single administration (Lee et al., 2000; Edmonds et al., 2001; Liu et al., 2004; Schubert et al., 2005; Noh et al., 2006). TPM was also demonstrated to exert neuroprotective effects against periventricular leukomalacia (Follett et al., 2004). TPM neuroprotective properties have been attributed mainly to glutamate alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-kainate receptor inhibition (Follett et al., 2004; Kaminski et al., 2004; Koh et al., 2004; Angehagen et al., 2005; Sfaello et al., 2005), but also to blockade of Na^+ channels (Zona et al., 1997), high voltage-activated calcium currents (Costa et al., 2006), carbonic anhydrase isoenzymes (Dodgson et al., 2000), and mitochondrial permeability transition pore (MPTP) (Kudin et al., 2004). The neuroprotective mechanism of combined hypothermia and TPM in HIE is still uncertain, but might rely on additive or synergistic action of TPM on hypothermia effects, such as glutamate reuptake preservation and inhibition of nitric oxide synthase, oxidative stress, and apoptosis (Edwards et al., 1995; Thoresen et al., 1997; Liu et al., 2004).

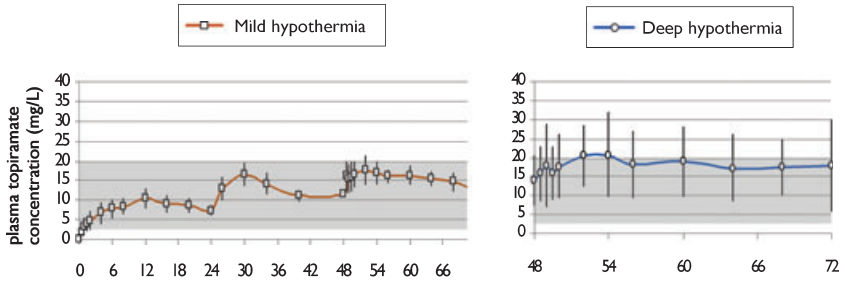

Figure 2.
Plasma TPM concentrations in newborns treated with deep or mild hypothermia. Gray area represents the reference range. *Epilepsia* © ILAE

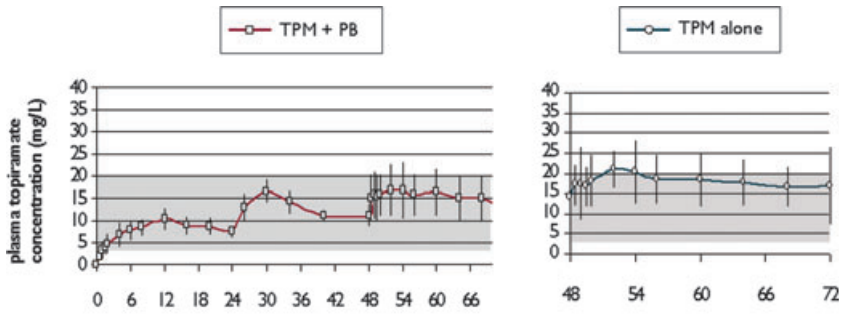

Figure 3.
Plasma TPM concentrations in newborns co-treated with PB or with TPM alone. Gray area represents the reference range. *Epilepsia* © ILAE

Table 2. Mean ± SD topiramate pharmacokinetic profiles of newborns who reached the virtual steady state. Data are plotted that distinguish between newborns in DH or MH and between newborns receiving PB or not

	All newborns (n = 9)	Newborns with DH (n = 3)	Newborns with MH (n = 6)	p-value	Newborns with PB (n = 4)	Newborns without PB (n = 5)	p-value
C_{max}, mg/L, mean ± SD	17.96 ± 4.2	17.87 ± 6.4	18.71 ± 3.2	0.219	15.38 ± 5.3	19.87 ± 1.9	0.060
C_{min}, mg/L, mean ± SD	10.35 ± 2.5	10.54 ± 3.2	10.77 ± 1.9	0.199	8.70 ± 2.9	11.67 ± 0.9	0.032
T_{max}, h, mean ± SD	3.80 ± 2.2	4.00 ± 1.1	4.08 ± 2.7	0.333	3.13 ± 2.4	4.40 ± 2.2	0.216
AUC_{0-24}, mg/L/h, mean ± SD	343.2 ± 72.2	318.1 ± 101.6	366.2 ± 48.1	0.096	302.4 ± 89.7	375.8 ± 37.4	0.068
C_{avg}, mg/L, mean ± SD	14.29 ± 3.0	13.25 ± 4.2	15.26 ± 2.0	0.096	12.60 ± 3.7	15.66 ± 1.6	0.068
$T_{1/2}$, h, mean ± SD	35.58 ± 19.3	48.82 ± 4.6	29.03 ± 23.8	0.080	26.46 ± 17.7	42.88 ± 19.1	0.113
CL/F, ml/kg/h, mean ± SD	15.42 ± 4.6	15.72 ± 7.3	13.87 ± 1.9	0.084	17.92 ± 6.2	13.42 ± 1.4	0.078

DH, deep hypothermia; MH, mild hypothermia; PB, phenobarbital; C_{max}, maximal plasma concentration; C_{min}, minimal plasma concentration; T_{max}, time of peak concentrations; AUC_{0-24}, area under plasma concentration-time curve from 0 to 24 h; C_{avg}, average plasma concentration; $T_{1/2}$, half-life; CL/F, apparent oral clearance.

Although perinatal HIE is the single most important cause of serious brain injury in newborns, to date no clinical study has been published to prove TPM efficacy and evaluate combined TPM and hypothermia treatment in newborns with HIE.

TPM exhibits high affinity/saturable low-capacity binding to erythrocytes (Patsalos et al., 2008), and this peculiarity contributes to a different pharmacokinetics of TPM when determined in plasma or in whole blood (Shank et al., 2005). Considering that hematocrit influences significantly the plasma/whole-blood distribution of TPM (Gidal & Lensmeyer, 1999), conversion from DBS to plasma values was made according to hematocrit. TPM determination by LC-MS/MS on DBS, converted to plasma concentration, provides values comparable to those obtained on heparinized plasma (la Marca et al., 2008).

However, TPM pharmacokinetic determinations presented some limitations. First, the steady state was approximated. Second, in neonates, peculiarity of gastrointestinal pH, emptying time, enzymatic activities, splanchnic blood flow pattern, and increased total body

water/fat ratios (Kearns et al., 2003) considerably influence drug absorption and distribution, whereas physiologically poor renal function can influence TPM clearance (Morselli et al., 1980). Third, a small amount of TPM is metabolized by hydrolysis, hydroxylation, and glucuronidation, which are markedly enhanced by enzyme-inducing antiepileptic drugs (AEDs) (Perucca & Bialer, 1996), although drug-metabolizing enzymes show poor activity in newborns (Perucca, 2006).

Available data on TPM pharmacokinetics in normothermic neonates are limited to newborns of mothers treated with TPM and carbamazepine, in whom TPM half-life was approximately 24 h (Ohman et al., 2002), as in healthy adults (Johannessen, 1997). In infants and children TPM C_{max} appears directly related to dose, suggesting proper oral bioavailability (Glauser et al., 1999; Mikaeloff et al., 2004). Concomitant treatment with enzyme-inducing AEDs increases TPM clearance, with significantly lower C_{max}, AUC, and half-life (Glauser et al., 1999; Mikaeloff et al., 2004).

Our pilot trial demonstrates that an oral TPM schedule of 5 mg/kg, once a day, determined in most newborns plasma concentrations within the reference range of 5–20 mg/L (Johannessen & Tomson, 2006; Patsalos et al., 2008), indicating that oral TPM absorption is maintained during hypothermia. This schedule was chosen arbitrarily with the purpose of rapidly achieving plasma levels within the reference range for a short period of time, in contrast to seizure treatment schedules that aim to gradually reach the effective dosage. Hypothesizing that hypothermia would determine higher TPM plasma levels, we chose the lowest dosage among maintenance therapy schedules in infants (Glauser et al., 1999; Rosenfeld et al., 1999; Mikaeloff et al., 2004; Battino et al., 2005). Assuming a longer TPM half-life during hypothermia we chose a single daily administration.

Plasma TPM concentration during hypothermia appeared definitely higher than reported in normothermic infants treated with analog dosages (Mikaeloff et al., 2004; Battino et al., 2005). Newborns treated with DH showed higher TPM concentrations between 48 and 72 h than newborns treated with MH, with higher concentration variability, probably because of more irregular TPM absorption and elimination. TPM values exceeded 20 mg/L in only one newborn in MH, whereas values exceeded 25 mg/L in three of five newborns in DH. TPM plasma concentrations did not differ between newborns receiving TPM and PB or TPM alone, suggesting that the main cause of higher variability in the DH group was the depth of hypothermia.

Nine newborns who reached TPM virtual steady state exhibited definitely higher C_{max}, C_{min}, $AUC_{0–24}$, C_{avg}, and $T_{1/2}$ values than reported in normothermic infants treated with the same or higher dosage (Glauser et al., 1999; Rosenfeld et al., 1999; Mikaeloff et al., 2004), suggesting slower TPM elimination during hypothermia. Hypothermia significantly reduces the clearance of several drugs, including sedatives, anesthetics, and anticonvulsants (Tortorici et al., 2007). Morphine was even reported to reach potentially toxic levels in asphyxiated newborns under hypothermia (Róka et al., 2008). Reduced drug clearance during hypothermia is mainly attributable to reduced activity of the cytochrome P450 enzymatic system but also to reduced cardiac output and glomerular filtration rate of drugs mainly excreted unchanged in urines (Tortorici et al., 2007). Therefore, the reduced glomerular filtration rate, probably in combination with a physiologic immaturity of renal function during the first days of life (Kearns et al., 2003), might explain the low TPM clearance observed in our hypothermic population. We observed delayed T_{max} values with respect to normothermic infants (Glauser et al., 1999; Rosenfeld et al., 1999; Mikaeloff et al., 2004), consistent with slower TPM absorption under hypothermia. Finally, DH and PB comedication were associated with lower TPM concentrations and higher CL/F. However, these findings were not significant, probably because TPM steady state was reached in only a limited number of newborns. Larger trials are needed to determine whether higher TPM dosages are necessary in newborns under PB comedication.

In conclusion, this pilot trial identified TPM dosage that can ensure plasma values within the reference range during hypothermia. The optimal TPM schedule for neonates under hypothermia as well as the neuroprotective effects in HIE remain to be established.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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