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ORIGINAL ARTICLE

Mathematical evaluation of melatonin secretion in hypoxic ischemic encephalopathy

Justyna Paprocka [1], Marek Kijonka [2], Damian Borys [3], Ewa Emich-Widera [1], Piotr Wojcieszek [4], Maria Sokół [2]

1 Department of Paediatric Neurology, School of Medicine in Katowice, Medical University of Silesia in Katowice, Poland
2 Department of Medical Physics, Maria Skłodowska- Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Poland
3 Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland
4 Brachytherapy Department, Maria Skłodowska- Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Poland

Correspondence to: Justyna Paprocka
Department of Pediatrics and Developmental Age Neurology, School of Medicine in Katowice, Medical University of Silesia, 40-752 Katowice, Medyków 16, Poland
tel.: 48606415888; fax: 48322071615; e-mail: justyna.paprocka@interia.pl

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Abstract

BACKGROUND: Hypoxic-ischemic brain injury is caused by a cascade of molecular reactions and mechanisms concerning calcium influx, free radical formation, free iron accumulation, nitric oxide production, apoptosis activation.
THE AIM OF THE STUDY: The authors access the endogenous melatonin diurnal secretion in the hypoxic-ischemic group and compare the results with the results obtained for children with epilepsy (EG) and children from the comparison group (CG).
MATERIAL AND METHODS: The hypoxic-ischemic encephalopathy group (HIE) consists of 9 prematurely born children at the mean age of 6.5 years, all diagnosed with epilepsy and suffering because of sleep problems. The parameters of the melatonin secretion model obtained for this group were interpreted and compared with data from the patients with epilepsy (n=78) and the children from the non-epileptic group (n=38). The melatonin level was assessed by a radioimmunoassay method.
RESULTS: Among the model parameters, the phase shift of melatonin release is one that strongly differentiates the HIE and CG groups (p-value = 0.001157). DLMOon50 and both offset parameters: DLMOoff50 and DLMOoff25 are helpful in distinguishing HIE from CG, whereas DLMOoff50 is found to differentiate statistically two epileptic groups: HIE and EG.
CONCLUSIONS: The characteristic feature of the HIE melatonin secretion is delayed melatonin phase release and shift of the DLMO parameters to the later morning hours. HIE secretion is even more disturbed than in EG. Our mathematical modelling of circadian melatonin cycle facilitates statistical analysis of the patients' hormone levels offering a set of parameters that enable objectification of the secretion description.

INTRODUCTION

Perinatal hypoxia occurs with an incidence of 2-6/1000 term births (Morales et al 2011). The neurological consequences of perinatal hypoxia-ischemia may be visible in 50-75% of children as cerebral palsy, epilepsy, developmental delay, hyperactivity disorder. This acute disordered brain function is known as neonatal encephalopathy (NE) – the second most frequent preventable cause of childhood neuro-disability worldwide (Lawn et al 2014). Its psychosocial and economic consequences for families and society are profound. Hypoxic-ischemic (HI) events, probably most frequent around the time of birth or in the first days after birth, constitute a major contributor to neonatal morbidity and mortality. The prognosis of a hypoxic-ischemic insult to the term newborn depends on the degree of the encephalopathy (Tataranno et al 2015).

Hypoxic-ischemic brain injury is the result of a deprivation of oxygen and glucose in the neural tissue. Brain damage following a perinatal HI is an evolving process involving various cellular and molecular processes leading to the cell death (Tataranno et al, De Cerio et al 2013). Due to high utilization of oxygen, not adequate antioxidant defense and high amount of easily oxidizable fatty acids, the brain is especially sensitive to free radical injury.

Hypoxic-ischemic brain injury is caused by a cascade of molecular reactions and mechanisms concerning calcium influx, free radical formation, free iron accumulation, nitric oxide production, apoptosis activation (Zhao et al 2016). The studies of injured preterm brain proved the involvement of the white matter and the cortical and subcortical grey matter manifesting as focal and/or diffuse lesions. Still little is known about the exact time of hypoxemia needed for the hypoxia to occur, and still specific biomarkers of perinatal hypoxia are lacking.

Melatonin (MLT) – acting as a direct scavenger – is able to remove singlet oxygen, superoxide anion radical, hydroperoxide, hydroxyl radical and the lipid peroxide radical. A substantial strong body of evidence now supports that melatonin is neuroprotective for acute hypoxic-ischemic perinatal brain injury, mediated via its anti-oxidant, anti-apoptotic, and anti-inflammatory properties (Hassell et al 2015).

Hypoxic-ischemic encephalopathy (HIE) comprises abnormalities of consciousness, muscle tone, autonomic control. The relationship between stage of HIE (I-III) and clinical presentation is reflected by Sarnat Grading Scale (Sarnat et al 1976). The reported rate of epilepsy following NE due to hypoxic-ischemic encephalopathy ranges from 9% to 33% (Glass et al 2011). Hypoxic ischemic encephalopathy and epilepsy in neonates were found to increase the risk of epilepsy in older children (Banani et al 2013).

To the authors' knowledge, this is the first study describing endogenous melatonin secretion profile in HIE children.

AIM

In this paper, we describe the endogenous melatonin secretion patterns in HIE children. The characteristics describing the diurnal hormone secretion: minimum melatonin concentration, release amplitude, phase shift of melatonin release and sleep duration as well as the dim light melatonin onset (DLMO) of melatonin secretion (being an important circadian marker) were estimated using our mathematical model of melatonin circadian secretion together with the max γ parameter that provides information about the fit and the disturbances in the shape of the fitted function relative to the physiological course. The HIE circadian rhythm parameters were compared with those obtained for the children with epilepsy and with the non-epileptic children with different medical conditions (Paprocka et al 2016, Paprocka et al 2017).

MATERIAL AND METHODS

The study was approved by the Ethic Committee of the Medical University of Silesia in Katowice. The informed written consent was taken from the parents or caregivers. The study was carried out at the Department of Pediatric Neurology, School of Medicine in Katowice, the Medical University of Silesia in Katowice.

Patients

None of the studied subjects had taken any medications affecting melatonin secretion (benzodiazepines and their agonists, fluvoxamine, caffeine, vitamin B12, non-steroidal anti-inflammatory drugs: aspirin, ibuprofen, indometacine, adrenolytics, prostaglandins inhibitors, calcium channel blockers, dexamethasone, clonidine, antidepressants) before and during the study.

The hypoxic-ischemic group (HIE)

The hypoxic-ischemic group consists of 9 children at the mean age of 6.5 years. The children were born before term (before 32 weeks' of gestation). All children were diagnosed with epilepsy with polymorphic features and suffered because of sleep problems (decreased sleep latency and sleep maintenance). The first epileptic seizures appeared in all patients within the first year of life. The mean epilepsy duration time was 6 years. The mean IQ was 47. Brain magnetic resonance confirmed periventricular leucomalacia (PVL) in all children from HIE group. EEG showed generalized epileptiform discharges.

The inclusion criteria were as follows: preterm delivery, PVL on MRI scan, symptomatic epilepsy with polymorphic seizures diagnosed in the first year of life, antiepileptic treatment limited to valproic acid and levetiracetam (to minimalize effect of antiepileptic treatment on melatonin secretion profile),neurological abnormalities (spastic tetra-, di- or hemiparesis). The clinical characteristics of the HIE patients is shown in Table 1.

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The comparison group (CG)

The comparison group was constituted of 42 non-epileptic patients (mean age was 6 years and 11 months); female to male ratio was 22:20. Among the patients facial nerve palsy (n=18, 58,3%), perineal nerve palsy (n=4, 9,5%), myopathy (n=10, 23,8%) or back pain (n=10, 23,8%) were diagnosed. All children developed normally, the family history and gestation period were uneventful. The routine laboratory test and EEG tracing were normal. All children were drug naïve. Because the abovementioned conditions are not reported as influencing endogenous melatonin secretion that is why they should not affect the results of the melatonin profiles.

Epilepsy group (EG)

The epilepsy group included 82 patients at the mean age of 6 years 6 months; female to male ratio was 44:38. The patients were reviewed for the following seizure type and syndrome: seizure frequency, age at seizure onset, electroencephalogram tracings, current and previous AEDs, seizure timing, etiology, cognitive status, family history. The type of epileptic seizures was defined following the International League Against Epilepsy Classification and Terminology. The mean duration of epilepsy was about 4.7 years (range: 2 months – 17 years). Children were born at term and exhibit polymorphic seizures, in half of the patients epilepsy was symptomatic (due to hypoxic-ischemic abnormalities). Antiepileptic drugs used were as follows: valproic acid (n= 68, 83%), levetiracetam (n= 21, 25.6%), lamotrygine (n= 24, 29,2 %).

Experimental Design

The study started at 9:00 and lasted 24 hours. Due to the higher melatonin levels present in plasma that allow greater resolution and sensitivity than sampling by urine or saliva we decided to use blood as a material for the analyses. Blood samples for serum melatonin determining were drawn 8 times at 9:00, 12:00, 15:00, 18:00, 21:00, 24:00, 3:00 and 6:00 from intravenous catheters placed in the median cubital vein before the beginning of the study. Attention was paid to maintain as much darkness as possible when the blood samples were taken at night. Therefore during night hours, blood samples were taken by dim red light. The material was collected in plastic tubes without anticoagulant agents. To determine the in-vitro plasma melatonin level we used the radioimmunoassay (RIA) method.

DATA ANALYSIS

Determining the melatonin secretion parameters

We developed and applied a mathematical model of circadian melatonin cycle to obtain the set of parameters that enable objectification of the secretion description (Paprocka et al 2016, Paprocka et al 2017). The model is based on a time-dependent melatonin function MLT(t) used in approximation of the experimental data:

$$MLT(t) = b_1 + b_2 \exp\left(-\frac{\left(\cos\left(\frac{\pi}{12}t - \frac{\pi}{12}b_3\right) - 1\right)^2}{\left(\frac{\cos\left(\frac{\pi}{24}b_4\right) - 1}{\sqrt{\ln 2}}\right)^2}\right) \quad (1)$$

Such approximation allows to characterize and determine secretion parameters such as: b_1 – a minimum melatonin concentration [pg/mL], b_2 – a melatonin release amplitude [pg/mL], b_3 – a phase shift of melatonin release [h], b_4 – a sleep duration (represented by the full width at half maximum (FWHM) of melatonin secretion model, [h]). Maximum melatonin concentration (b_{max}) is given by a sum of b_1 and b_2. The b_3 and b_4 parameters enable the DLMO characteristics to be estimated:

- DLMO onset at the 50% relative threshold,

$$DLMOon_{50} = b_3 - \frac{b_4}{2}$$

- DLMO offset at the 50% relative threshold,

$$DLMOoff_{50} = b_3 + \frac{b_4}{2}$$

- DLMO onset at the 25% relative threshold,

$$DLMOon_{25} \approx b_3 - \frac{2}{\pi}b_4 \text{ and}$$

- DLMO offset at the 25% relative threshold,

$$DLMOoff_{25} \approx b_3 + \frac{2}{\pi}b_4 .$$

To obtain information on the degree of disturbance from the bell-shaped secretion pattern (such as a triangular secretion course and the diurnal fluctuations of melatonin concentration) for each patient the max γ parameter was also calculated (Paprocka et al 2017). The max γ parameter for a physiological bell melatonin secretion should not be higher than 2. For an ideal course with a clearly marked amplitude phase and with plateau during the day-time, its value should be below 1.

The Levenberg-Marquardt algorithm and nonlinear least squares method were chosen to estimate the melatonin secretion parameters for each patient's blood collection data separately.

Statistical Analysis

In this study the model parameters obtained the in the approximation process for the HIE group were interpreted and compared statistically with the appropriate characteristics of the EG and CG groups. Since the individual group sizes differ markedly and also the groups do not meet the requirements for parametric tests (the data is not normally distributed, and variances in the individual subpopulations are not homogeneous), the

Tab. 1. The clinical characteristics of the HIE patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Family history	hypo-thyroidism	develop-mental delay	non-remarcable	non-remarcable	epilepsy, autism	non-remarcable	hypo-thyroidism	hypo-thyroidism	mental retardation
Gestation and delivery period	GII, DI, delivery by Ceasarian section, GA:32wks BW: 1980g	GI, DI, delivery by Ceasarian section, GA:31wks BW:1950g	GI, DI, delivery by Ceasarian section, GA:34wksBW: 2200g	GII, DII, delivery by Ceasarian section, GA:34wksBW: 2300g	GV, DII, delivery by Ceasarian section, GA:28wks BW:1590g	GII, DII, delivery by Ceasarian section, GA:33wks BW:2250g	GIII, DII, delivery by Ceasarian section, GA:29wks BW:1640g	GI, DI, delivery by Ceasarian section, GA:30wks BW:2000g	GII, PI, delivery by Ceasarian section, GA:30 wks BW:2370g
Psychomotor development	retarded IQ=39	retarded IQ=40	retarded IQ=60	retarded IQ=62	retarded IQ=30	retarded IQ=53	retarded IQ=52	retarded IQ=41	retarded IQ=49
Neurological examination	right hemiparesis	spastic diplegia	spastic diplegia	spastic diplegia	spastic tetraplegia	spastic diplegia	spastic tetraplegia	left hemiparesis	spastic diplegia
EEG	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity	generalized paroxysmal activity
Seizures	MS, GTCS	MS, GTCS	MS, GTCS	MS, GTCS	MS, GTCS	MS, GTCS	MS, GTCS	MS, GTCS	MS, GTCS
Antiepileptic drugs	VPA, LEV	VPA, LEV	VPA, LEV	VPA, LEV	VPA, LEV	VPA, LEV	VPA, LEV	VPA, LEV	VPA, LEV
Brain MRI	PVL	PVL	PVL	PVL	PVL	PVL	PVL	PVL	PVL

Abbreviations: G- gestation, D- delivery, GA- gestational age, wks- weeks, BW- birth weight, MS- myoclonic seizures, GTCS- generalized tonic-clonic seizures, VPA- valproic acid, LEV- levetiracetam, PVL- periventricular leukomalacia

nonparametric Kruskal-Wallis rank sum test and the Mann-Whitney-Wilcoxon test were used. The p values less than 0.05 – a predetermined significance level – were accepted as indicating that the observed result would be highly unlikely under the null hypothesis. To explore the HIE intragroup variability and the outliers, the qualitative research of the obtained estimators was also applied.

RESULTS

The HIE, EG and CG groups were homogeneous with respect to age (p = 0.1391, Kruskal–Wallis test) and intellectual development (p = 0.4181, Kruskal–Wallis test).

The model parameters of the melatonin circadian rhythm obtained for each patient from the HIE group are collected in Table 2.

Statistical analysis

HIE group vs CG group

The melatonin secretion parameters obtained for the HIE patients were compared with those for the CG group. The Mann-Whitney U test was applied and the results are presented in Table 3.

As seen from the comparison (Table 3), the HIE and CG groups differ statistically significantly in respect of the phase shifts of melatonin release (b_3) and three DLMO characteristics: $DLMOon_{50}$, $DLMOoff_{50}$ and $DLMOoff_{25}$. In the HIE group the melatonin secretion peak is markedly phase shifted towards later hours as compared to CG (Figure 1).

Both melatonin offset parameters in the HIE vs. CG comparison are statistically highly significant: p < 0.005 for DLMOoff25 (Figure 2) and DLMOoff50 (Figure 3). Whereas in case of the onset parameters only DLMOon50 is significant at the p level of 0.05 (Figure 4).

HIE group vs EG group

Furthermore, the HIE group was also compared to the epilepsy EG group and the results of the Mann-Whitney U test are presented in Table 4.

Both epileptic – HIE and EG – groups differ statistically significantly (p less than 0.05) from one another only with respect to $DLMOoff_{50}$ – it is increased in the hypoxic ischemic encephalopathy group (Figure 5).

HIE intragroup qualitative analysis

Due to a small sample size (9 cases) and apparent diversity of the melatonin secretion profiles in HIE, the intragroup qualitative analysis was performed (Figure 6). In patients 1-4 the maximum melatonin levels are much higher (from 256.6 to 344.2 pg/mL, the average value of 303.9 pg/mL) than in patients 6-9 – in this subgroup the range of melatonin night-time amplitude values is between 68.1 and 100.2 pg/m, the average value being 89.6 pg/mL (Table 2). In case of patient 5, the secretion profile is the most deviated (pseudo immature circadian clock with probably poorly developed nocturnal melatonin synthesis): the amplitude is the lowest (9.35 pg/mL), whereas the phase shift of melatonin release (8.19 h) is the highest (Table 2, Figure 6).

The shapes of the melatonin secretion HIE profiles are evidently disturbed: max γ is higher than 2 in three cases

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Tab. 2. The estimates of the MLT(t) parameters for the HIE group

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Minimum melatonin concentration, b_1 [pg/mL]	14.65	13.96	7.14	6.63	3.12	5.92	2.33	2.62	2.54
Melatonin release amplitude, b_2 [pg/mL]	241.95	330.24	307.94	293.03	6.24	84.99	97.03	97.55	65.54
Phase shift of melatonin release, b_3 [h]*	3.73	4.18	2.07	1.98	8.19	3.16	3.07	2.92	2.87
Estimated sleep duration, b_4 [h]*	10.47	7.46	6.16	6.41	3.45	5.36	9.28	8.13	10.69
Maximum melatonin concentration, $b_1 + b_2$ [pg/mL]	256.60	344.20	315.08	299.67	9.36	90.91	99.36	100.17	68.07
$DLMOon_{50}$ [h]*	22.50	0.45	22.99	22.78	6.47	0.48	22.44	22.85	21.53
$DLMOoff_{50}$ [h]*	8.97	7.91	5.15	5.19	9.92	5.84	7.71	6.98	8.22
$DLMOon_{25}$ [h]*	21.06	23.43	22.15	21.9	5.99	23.75	21.17	21.74	20.07
$DLMOoff_{25}$ [h]*	10.4	8.94	6.0	6.07	10.39	6.58	8.99	8.09	9.68
Max gamma	3.34	1.68	0.4	0.4	1.97	0.27	2.28	1.27	5.14

*Time in decimal scale

(patients 1, 7 and 9) and higher than 1 but lower than 2 also in three cases (patients 2, 5 and 8). For patients 3, 4 and 6 the max γ values are normal (<1) (Table 2).

DISCUSSION

This study examined the endogenous melatonin secretion in nine children (of a mean age of 6.5 years) with hypoxic-ischemic encephalopathy, all diagnosed with epilepsy. The secretion patterns were mathematically modeled and the circadian rhythm parameters were compared with the appropriate characteristics for the patients with epilepsy (EG) and the comparison group (CG) – the latter consisted of non-epileptic children.

The present study demonstrated that the secretion of melatonin in HIE children is significantly disturbed. No statistical difference between the max γ values for the HIE vs EG and HIE vs CG comparisons was found. For each group, the max γ value is less than 2, but higher than 1 (1.6810, 1.0240 and 1.8190 for HIE, EG and CG, respectively). Thus, the shape disturbances of the circadian melatonin profiles are present also in HIE, as in the other groups (Paprocka et al 2017).

As expected, the HIE and CG groups differ in respect of the phase shift (b_3) of melatonin release (p-value = 0.001157). Interestingly, the melatonin release occurs in the HIE group even later than in EG, but the difference between the appropriate HIE and EG values is

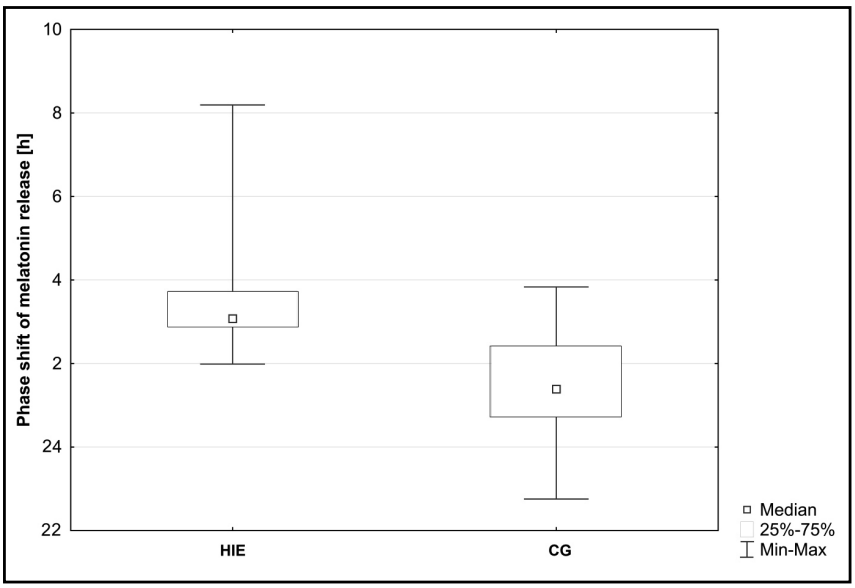

Fig. 1. Box Plot of the phase shift of melatonin release (b3) for the HIE and CG groups.

Tab. 3. The results of Mann–Whitney U test for the comparison of the CG and HIE groups. The results marked in bold are statistically significant with p <0.05.

	Median of the HIE group	Median of the CG group	p-value
Minimum melatonin concentration [pg/mL]	5.9197	6.1613	0.477630
Melatonin release amplitude [pg/mL]	97.5481	142.5104	0.520785
Phase shift of melatonin release [h]	3.0742	1.3878	**0.001157**
Estimated sleep duration [h]	7.4644	7.0774	0.520785
Maximum melatonin concentration [pg/mL]	100.1723	152.1448	0.498969
$DLMOon_{50}$ [h]	22.8504	22.0977	**0.019458**
$DLMOoff_{50}$ [h]	7.7124	5.2036	**0.002703**
$DLMOon_{25}$ [h]	21.9022	21.3282	0.079808
$DLMOoff_{25}$ [h]	8.9362	6.0692	**0.005130**
Max gamma	1.6810	1.8190	0.761441

non-significant (p-value = 0.067384). In EG, in turn, the melatonin secretion peak is markedly phase shifted towards later hours as compared to CG (Paprocka et al 2016; Paprocka et al 2017). Thus, the HIE melatonin secretion is even more disturbed than in EG, indicating that for both epileptic groups the patients' clinical-etiological and therapeutic profiles are of importance. Ruiz-García *et al.* (Ruiz-Garcia et al 2002), who compared three epileptic groups – idiopathic, cryptogenic and symptomatic, stressed this issue and pointed the importance of the epilepsy duration time as a factor influencing the neuronal damage and therapeutic efficiency. Cryptogenic and symptomatic epilepsies appear earlier than idiopathic epilepsies, and longer seizure duration could lead to structural and microbiological changes in other than epileptogenic zone brain areas. Also the biochemical CSF studies confirm the role of such factors such as etiology, seizure type and duration (Tumani et al 2015). In case of our epileptic groups, the mean duration of epilepsy was longer for HIE (6 years) than for EG (4.7 years). When taking into account the mean age of the groups (6.5 years for HIE and 5.5 years for EG) the epilepsy duration parameter can be calculated as a duration to age ratio – it equals 0.92 for HIE and 0.85 for EG.

The dim light melatonin onset (DLMOon), the point in time when melatonin levels begin to rise in the evening, and the dim light melatonin offset (DLMOoff), the point in time when melatonin levels diminish in the morning, are two commonly derived circadian phase markers. DLMOon is claimed to be the most reliable circadian phase marker, more reliable than the DLMOoff and phase markers derived from the core body temperature rhythm (Klerman et al 2002). However, in case of our study, $DLMOon_{50}$ and both offset parameters: $DLMOoff_{50}$ and $DLMOoff_{25}$, revealed to be of statistical importance distinguishing HIE from CG. Moreover, only $DLMOoff_{50}$ is found to differentiate statistically

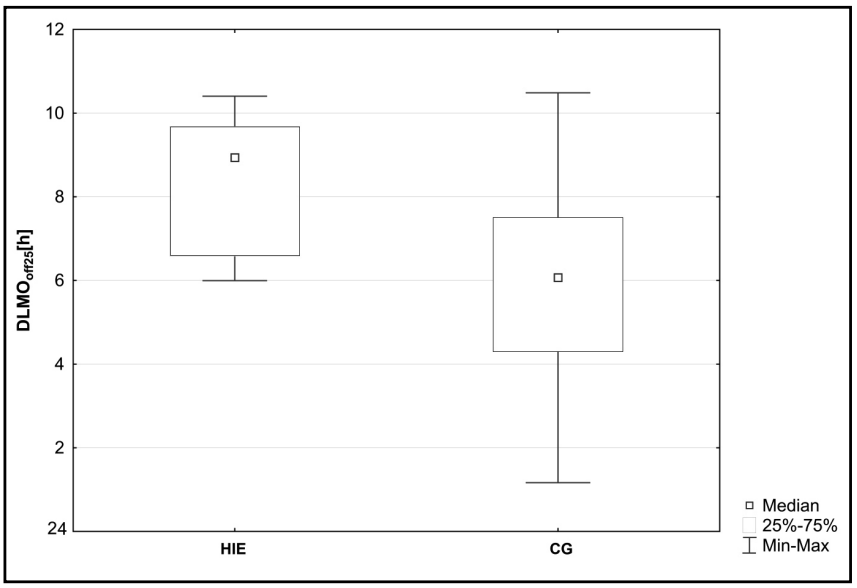

Fig. 2. Box Plot of the DLMOoff25 parameter for the HIE and CG groups.

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Tab. 4. The results of Mann–Whitney U test for the comparison of the EG and HIE groups. The results marked in bold are significant with p <0.05.

	Median of the HIE group	Median of the EG group	p-value
Minimum melatonin concentration [pg/mL]	5.9197	5.8466	0.657275
Melatonin release amplitude [pg/mL]	97.5481	116.3419	0.719573
Phase shift of melatonin release [h]	3.0742	2.3417	0.067384
Estimated sleep duration [h]	7.4644	7.3289	0.882543
Maximum melatonin concentration [pg/mL]	100.1723	117.3876	0.800006
$DLMOon_{50}$ [h]	22.8504	-0.9851	0.553790
$DLMOoff_{50}$ [h]	7.7124	5.8341	**0.035660**
$DLMOon_{25}$ [h]	21.9022	-2.0097	0.568092
$DLMOoff_{25}$ [h]	8.9362	6.6973	0.057893
Max gamma	1.6810	1.0240	0.484947

two epileptic groups: HIE and EG, of various etiologies (the p-value = 0.035660). It is now recognized that after neonatal hypoxic-ischemic incidents the brain injury process lasts for weeks to years after the initial insult – thus, it opens up new possibilities for therapy. Currently, there are no established therapies for the treatment of HI injuries (Cilio&Ferriero 2010). Seizures are commonly associated with HIE – although the majority of cases are controlled with first- or second-line therapy, many infants develop status epilepticus, requiring multiple anticonvulsants (Aicardi 2008). To diminish the neurological sequelae from HI brain injury and prevent brain injury and long-term neurodevelopmental impairment, effective neuroprotective strategies are urgently required. Although many antioxidant agents have been shown to be protective in animal models of hypoxia-ischemia, only a few substances have been used in pilot studies for newborns. Recently, interest has grown in the neuroprotective possibilities of melatonin (Alonso-Alconada et al 2013). Melatonin is a powerful endogenous antioxidant with a high level of biosafety and multiple neuroprotective actions, as confirmed in animal studies (Carloni et al 2008; Cetenkaya et al 2011; Ozyener et al 2012; Yawno et al 2017) and in neonates with hypoxic-ischemic encephalopathy (Aly et al 2015). It penetrates the brain and organelles (Acuna-Castroviejo et al 2001) and is safe for humans (Rybakowski et al 1995) and animals (Jahnke et al 1999). It reduces oxidative stress and inflammatory cell recruitment and glial cells activation in cerebral cortex after neonatal HI damage (Balduni et al 2012). In animal studies, melatonin administration before and after hypoxia was found to induce positive effects on the immature rats' brains (Carloni et al 2008) and to prevent oxidative stress (Signorini et al 2009). In the study performed on children after severe traumatic brain injury (TBI) high increase in serum endogenous melatonin levels was observed – it was interpreted as a protective response

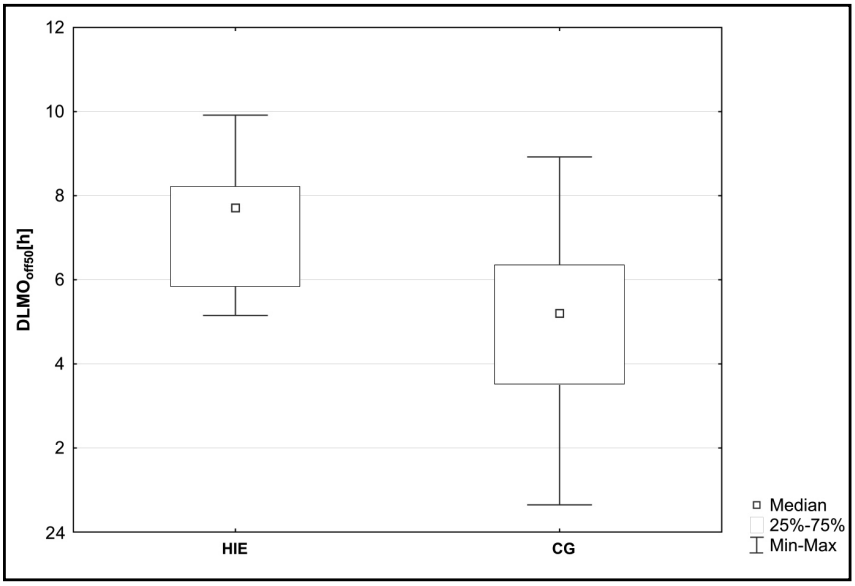

Fig. 3. Box Plot of the DLMOoff50 parameter for the HIE and CG groups.

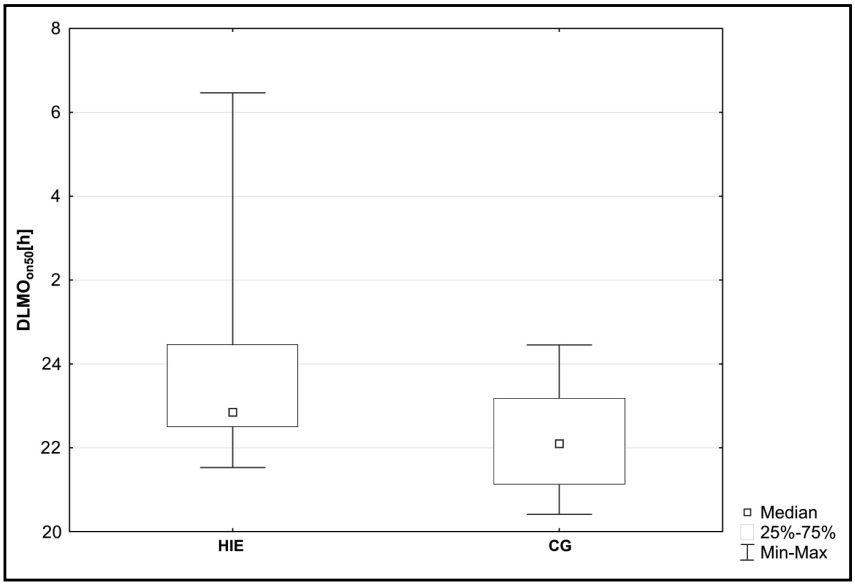

Fig. 4. Box Plot of the DLMOon50 parameter for the HIE and CG groups.

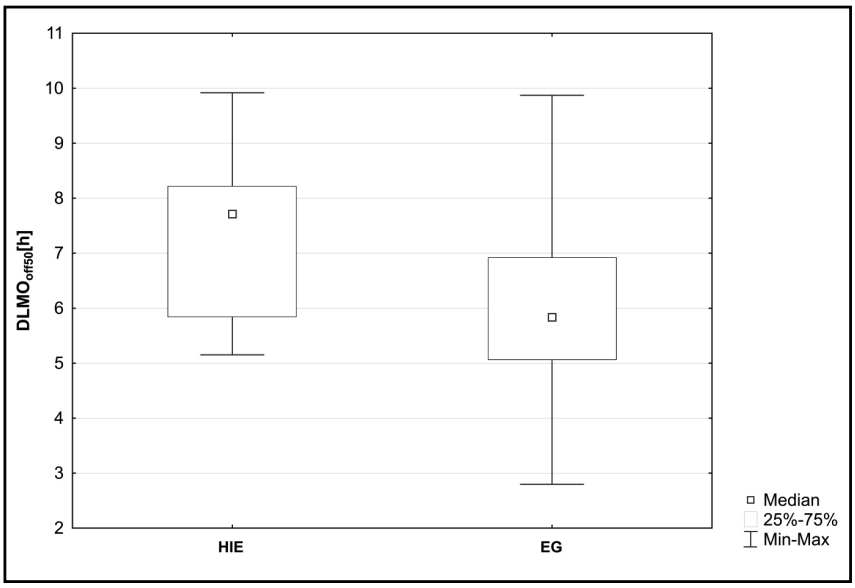

Fig. 5. Box Plot of the DLMOoff50 parameter for the HIE and EG groups

to oxidative stress and/or inflammation due to severe head injury, a "natural protection" operating in children and disappearing in parallel with the overall decline in melatonin levels described later in life (Marseglia et al 2017).

At the moment, however, neither the mechanisms behind the disturbed melatonin secretion rhythm are completely known, although environmental factors, primarily including light stimulus, are clearly involved, nor the neuroprotective mechanisms for melatonin are completely understood. Although investigators have suggested several neuroprotective mechanisms, including antioxidant activity, activation of GABAergic pathways, and antiepileptic effects, several lines of evidence suggest that the neuroprotective effects of melatonin are largely mediated by specific melatonin receptors and through adenylate cyclase inhibition (Husson et al 2002; Deniz et al 2016).

The neuroprotective effect of melatonin, improving the neonatal outcome, is expected when melatonin is administered along with hypothermia treatment (for infants >35weeks' gestation period and birth weight >1800g). Such combination – leading to the augmented hypothermic neuroprotection – has recently been applied in animal studies (Robertson et al 2013). Among the studies conducted in humans, Aly *et al.* (Aly et al 2015) found – in a prospective trial of 45 newborns treated with hypothermia alone and with hypothermia and melatonin – that administration of MLT was associated with decreased seizures per EEG and decreased

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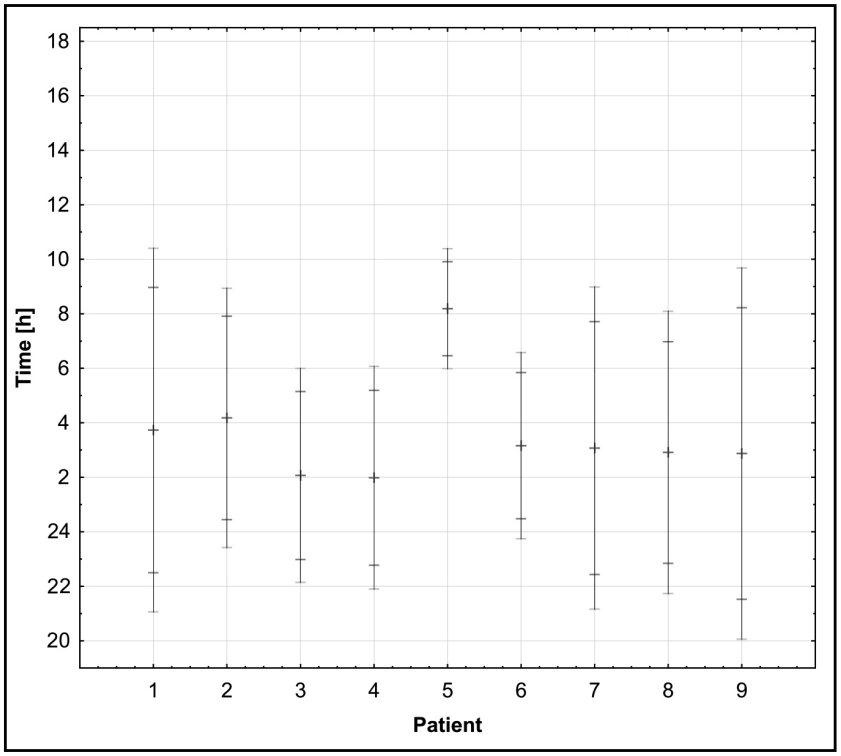

Fig. 6. Melatonin secretion characteristics for the HIE group (black line – melatonin profile; red cross – phase shift of melatonin release; orange marks – $DLMOon_{50}$ and $DLMOoff_{50}$; yellow marks – $DLMOon_{25}$ and $DLMOoff_{25}$; max γ for the consecutive patients: (1) 3.3410, (2) 1.6810, (3) 0.3959, (4) 0.3969, (5) 1.9730, (6) 0.2684, (7) 2.2800, (8) 1.2650, (9) 5.1370

white matter abnormalities on MRI after 2 weeks of age and improved survival without neurological or developmental abnormalities at 6 months of age.

In spite of the significant research progress in hypoxic-ischemic encephalopathy over the last 2 decades, the information on endogenous melatonin secretion in children who have experienced severe HI episodes, especially in low gestational age, is still limited. In view of the possible applications of MLT in novel protective therapies, such knowledge is simply necessary. Being a potential therapeutic agent that might augment protection from hypothermia melatonin should be carefully tested to design safe treatment strategies in infants, as there are increasing preclinical findings from immature rodents that many neuroinhibitors can impair brain development and that particular combinations of drugs that were individually harmless can exacerbate neurodegeneration in the developing brain (Cilio et al 2010).

The small sample size of the HIE group and the diversity of the internal melatonin profiles are the major drawbacks of our study. It may be expected that increasing the HIE group could lead to a more generalized insight into the endogenous melatonin patterns. Also antiepileptic treatment may influence the results.

On the other hand, our study was designed to compare the MLT secretion in HIE group to two others groups: EG and CG. This was done using our mathematical modeling of the secretion processes. We believe that such comparisons are more objective, since the comparison results are presented in the form of a set of the model parameters being easy to obtain, compare and collect within a database.

CONCLUSIONS

Characteristic feature of melatonin secretion in the HIE group is a delayed sleep phase syndrome (delayed melatonin phase release and the shift of the DLMO parameters to the later morning hours). Disturbance of the secretion profile shape indicate that for both epileptic groups – HIE and EG – the patients' clinical-etiological and therapeutic profiles are of importance.

Melatonin is claimed to be safe and beneficial in protecting neonatal brains from perinatal HIE; however, in order to implement it in the infant long-lasting treatment, the endogenous melatonin patterns should be known.

Our mathematical modeling of circadian melatonin cycle facilitates analysis of the patients' melatonin profile offering a set of parameters that enable objectification of the secretion description.

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CONFLICT OF INTERESTS

Authors declare no conflict of interest

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