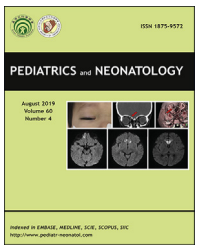
Pediatrics and Neonatology (2019) 60, 435–440

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



Original Article

Clinical significance of detecting serum melatonin and SBDPs in brain injury in preterm infants

Caixia Yan, Binghong Zhang*

Department of Neonatology, Renmin Hospital of Wuhan University, Wuhan, 430060, Hubei, China

Received Jun 17, 2018; received in revised form Sep 11, 2018; accepted Nov 21, 2018
Available online 25 November 2018

Key Words
brain injury;
melatonin;
preterm infants;
αII spectrin cleavage products

Background: To investigate the clinical values of serum melatonin and αII spectrin cleavage products (SBDPs) in assessing the severity of brain injury in preterm infants.
Methods: Sixty-four premature infants in total were selected and classified into the brain injury group (BI, n = 30) and the non-brain injury group (CON, n = 34) according to cranial imaging examination. The serum melatonin and SBDPs were detected by ELISA. All the preterm infants were received NBNA testing at 40 weeks of corrected gestational age.
Results: The levels of melatonin and SBDPs in the BI group were significantly higher than the CON group ($p < 0.05$) and the levels in the infants with severe brain injury were significantly higher than those with mild brain injury ($p < 0.05$), as well as exhibiting a negative correlation with the NBNA score at 40 weeks of corrected gestational age ($p < 0.05$).
Conclusions: Detecting melatonin and SBDPs has clinical value in diagnosing and assessing the severity of brain injury in preterm infants.

1. Introduction

With the increased survival rate of preterm infants, brain injury in preterm infants has become one of the major causes of neurodevelopmental disorders in neonates.[1] Currently, imaging is the main method for diagnosing brain injury in preterm infants. Because all imaging examinations have limitations or because the best examination timing is missed as well as other reasons, brain abnormalities cannot be precisely determined, therefore, normal findings in brain imaging do not exclude the presence of brain injury. In order to improve the quality of life of preterm infants, discovering and confirming the biomarkers of neonatal brain injury is a key step in protecting the neonatal neurodevelopment.[2] These markers may enable clinicians to screen for brain injury in preterm infants, monitor disease progression, and evaluate the

* Corresponding author. Fax: +86 2788048911.
E-mail address: cnbinghongzhang@163.com (B. Zhang).

https://doi.org/10.1016/j.pedneo.2018.11.005

effectiveness of neuroprotective strategies in clinical trials. Recent studies[3–5] have found that serum melatonin is related to the severity of nervous system diseases. Melatonin can reduce the damage of excitotoxic neurotoxin on the blood–brain barrier of newborn rats. Marseglia[5] found that the secretion of melatonin being significantly increased in children with acute brain injury, and that melatonin produced in damaged brain cells could enter the blood circulation.

Others studies[6–9] have found that serum αII spectrin breakdown products (SBDPs) are associated with neurological disease and severity, among which αII spectrin can decompose into SBDPs, including SBDP150 and SBDP145, or be degraded to SBDP120 by caspase-3. Calpain and caspase-3 are important factors that promote the apoptosis after brain injury.[6] SBDP145, SBDP150, and SBDP120 have been found to increase in varying degrees in the cerebrospinal fluid and serum of patients with severe brain trauma[7] and in animal models with brain trauma or hypoxia-ischemia,[8,9] and these factors are related to the extent of brain injury. After brain injury, SBDPs produced in damaged brain cells may enter the blood circulation. However, there are few reports about melatonin and SBDPs in preterm infants with brain injury. We speculate that melatonin and SBDPs may also increase in preterm infants with brain injury. This study examined the levels of serum melatonin and SBDPs and explored their early predictive values for brain injury in preterm infants. The study aimed to provide a favorable biological basis for the early intervention and prognosis for brain injury in preterm infants.

2. Materials and methods

2.1. Materials

Sixty-four preterm infants in total with suitable gestational ages (at 27–34 weeks of gestational age and weighing 1–2 kg) hospitalized in the NICU of Renmin Hospital of Wuhan University from January 2016 to July 2017 were collected, and 30 children who met the diagnostic criteria of preterm brain injury[10–13] were divided into the brain injury group (BI), while the 34 children without brain injury were assigned to the control group (CON). There was no statistical significance in the birth weight, gestational age, gender, Apgar scores and mode of delivery between the two groups. Children were excluded if brain injury was caused by genetic metabolic disorders, severe congenital malformations, bilirubin encephalopathy, hypoglycemic encephalopathy, central nervous system infections, other specific central nervous system diseases, or if they had slightly enlarged ventricles. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Wuhan University. Written informed consent was obtained from all participants' guardians.

2.2. ELISA

All the enrolled preterm infants were transferred to NICU within 30 min after birth and 3 ml of femoral venous blood sampled from the 4th day after birth at 9–11 a.m. Blood was centrifuged at 3000 r/min for 10 min, and the serum was stored at −80 °C for further testing. ELISA kit was used the detection of melatonin, and the detection of SBDPs was conducted using human serum SBDP120 and SBDP145 ELISA Kit (Wuhan Huamei Biological Co., Ltd).

2.3. Imaging of brain injury in premature infants and grades

2.3.1. Imaging examination of brain in preterm infants

All premature infants underwent first skull ultrasound within 3 days of birth and were reviewed once every other week until discharge. However, for children with suspected intracranial lesions, brain MRI was further performed within 1 week to 1 month of birth to confirm the presence of brain injury. Brain injuries in preterm infants were divided into the following[10,11] (1) intracranial hemorrhage–intraventricular hemorrhage (IVH), subarachnoid cavity, cerebellum, basal nucleus and pontine hemorrhage; (2) periventricular leukomalacia (PVL); and (3) others, such as cerebral infarction. In the early stage of cerebral infarction, the infarct sites were strongly echoed; at the late stage showed low echo or anechoic in MRI was evident, which is the gold standard of cerebral infarction.

2.3.2. IVH, PVL grades

IVH grade I: single or bilateral subependymal stroma hemorrhage; grade II: subventricular hemorrhage through ependyma, causing intracerebral hemorrhage but no ventricle enlargement; grade III: intraventricular hemorrhage with ventricle enlargement; grade IV: intraventricular hemorrhage accompanying ventricle hemorrhagic infarction. Grades I–II were mild while grades III–IV were severe IVH.[12] PVL grade I: the local echo around the ventricle was enhanced continuously or over 7 days, and no cystic cavity appeared later; PVL grade II: local echo enhancement around the ventricle was enhanced and then changed to the local small capsule; PVL grade III: extensive echo enhancement around the ventricle, followed by a broad cystic change; PVL grade IV: extensive echo enhancement around the ventricles of the ventricles and subcortical superficial white matter, followed by a change in the periventricular and subcortical superficial white mass diffuse cystic cavity. Grades I–II were mild and grades III–IV were severe. If IVH and PVL are consistent, the grade should be the more serious category.[13]

2.4. Neonatal behavioral neurological assessment (NBNA) testing

All the preterm infants underwent NBNA testing at 40 weeks of corrected gestational age.[14] The NBNA scoring included five major items: behavioral ability, passive muscle tone, active muscle tone, primary reflexes, and general assessment, Scoring included 20 sub-items, with 2 points assigned to each item and a total of 40 points.

2.5. Statistical analysis

SPSS version 21.0 statistical software was used for statistical analysis. The measurement data were expressed as

$\bar{x}\pm$SD. The intergroup comparison of general conditions used the t test or χ^2 test, and ANOVA was used among three groups. The comparison of the mean between two groups used the two independent-sample LSD-t test. The receiver operating characteristics (ROC) curve was used to analyze the sensitivity and specificity of melatonin on preterm brain injury. The Pearson correlation analysis was used for correlation analysis, and the proximity of correlation coefficient r to 1 indicated the stronger correlation. $P < 0.05$ was considered as statistical significance.

3. Results

3.1. Comparison of general conditions

This study included a total of 64 premature infants, including 37 boys and 27 girls. The average birth weight was 1586 ± 23.8 g, and the average gestational age was 32.03 ± 2.04 weeks. According to the brain imaging findings, 34 patients were divided into Group CON and 30 patients were divided into Group BI. Nineteen children in group BI had IVH, including 15 cases with mild IVH and 4 cases with severe IVH; 11 children in group BI had PVL, including 9 cases of mild PVL and 2 cases of severe PVL. There was no significant difference in the gender, birth weight, and gestational age between the two groups ($P > 0.05$, Table 1).

3.2. Comparison of serum melatonin and NBNA score

As shown in Table 2, the comparison of serum melatonin and NBNA score between Group MBI/SBI and Group CON exhibited statistical significance ($P < 0.05$).

3.3. Analysis of correlation of serum levels of melatonin, SBDP120, and SBDP145 with NBNA score

The melatonin and NBNA score was negatively correlated, with the correlation coefficient $r = -0.518$, and the difference was statistically significant ($P < 0.05$); SBDP120 and SBDP145 were negatively correlated with NBNA score, with the correlation coefficient $r = -0.56$ and -0.46, respectively, and the differences were statistically significant ($P < 0.05$, Fig. 1).

3.4. Diagnostic value of melatonin, SBDP120, and SBDP145

The analysis of the ROC revealed that the area under curve (AUC) of melatonin in diagnosing brain injury in preterm infants was 0.76, with 95% confidence interval (95%CI) as (0.635, 0.886), with cut-off value of 69.5 pg/ml, sensitivity of 86.7%, and specificity of 68.4% ($P < 0.05$). The AUCs of SBDP120 and SBDP145 in diagnosing brain injury in preterm infants were 0.97 and 0.77, with 95% CI as (0.635, 0.886) and (0.664, 0.891), with cut-off values of 1.55 ng/ml and 1.35 ng/ml, sensitivities of 86.1% and 96.7%, and specificity of 96.7% and 85.3%, respectively ($P < 0.05$, Fig. 2).

4. Discussion

The term 'preterm cerebropathy' includes not only brain injury,[10–13] such as intraventricular hemorrhage (IVH), but also complex disturbances toward late development of the infant brain. In preterm infants, the most common sources of brain injury are intracranial hemorrhage (IVH) and PVL. In addition, 20%–25% of infants with birth weight less than 1500 g will develop IVH, and nearly 60% of newborns with low birth weight will suffer from hypoxic-ischemic injury. Therefore, lifelong neurodevelopmental disorders occur frequently in preterm infants. Innovative methods for preventing or reducing brain injury in preterm infants need to identify biomarkers that can identify the risk of brain injury in infants, monitor the progress of the injury, and assess the effectiveness of clinical neuroprotective trials.[15]

The melatonin level changed with circadian rhythm based on the sleep and waking cycle since no cycle has been established in infants, the Results of melatonin testing would be reliable.[16,17] Studies have shown that the

Table 1 Comparison of general conditions between Con and BI groups ($\bar{x}\pm$SD).

Group	n	Boy	Girl	Birth weight(g)	Gestational age (weeks)	Apgar score		Delivery	
						1 min	5 min	Vaginal	Uterine-incision
Con	34	19	15	1599 ± 235	31.1 ± 2.03	7.0 ± 1.02	8.8 ± 0.72	15	19
BI	30	18	12	1573 ± 241	30.9 ± 2.04	7.1 ± 1.16	8.7 ± 0.79	16	14
t/x^2		0.11		0.443	0.238	−0.369	0.481	0.063	
P		0.8		0.660	0.813	0.714	0.633	0.803	

Table 2 Comparison of serum melatonin, SBDP120, SBDP145, and NBNA score among the three groups ($\bar{x}\pm$SD, ng/ml).

Group	n	Melatonin (pg/ml)	SBDP120 (ng/ml)	SBDP145 (ng/ml)	NBNA score
COn	34	66.42 ± 9.34	1.07 ± 0.33	0.99 ± 0.36	37.20 ± 1.12
MBI	24	72.71 ± 8.99*	2.19 ± 0.59*	1.30 ± 0.39*	35.75 ± 1.78*
SBI	6	94.5 ± 10.07*Δ	4.63 ± 0.88*Δ	2.67 ± 0.36*Δ	32.0 ± 1.41*Δ
F		24	137	52	36
P		0.00	0.00	0.00	0.00

Compared with CON group, $^*P < 0.05$; Compared with MBI group, $^{\Delta}P < 0.05$.

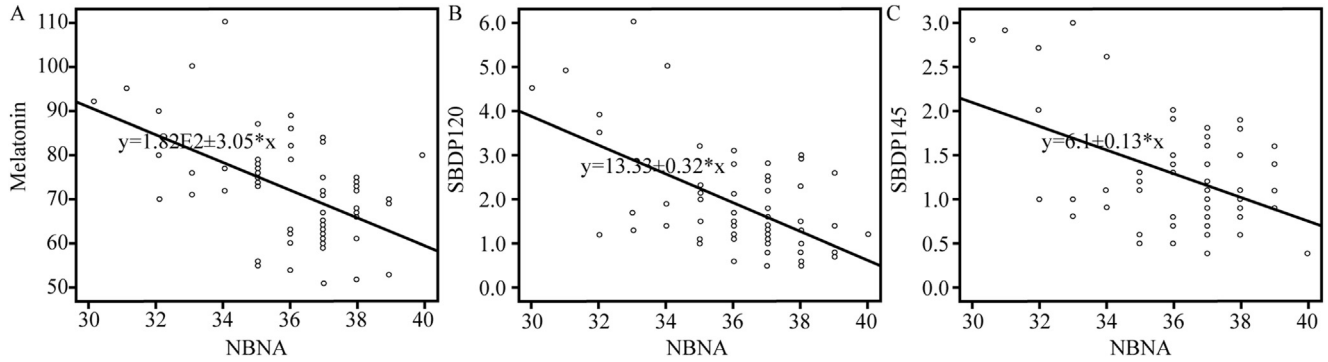


Figure 1 Scatter plot of correlation between melatonin, SBDP120, SBDP145 and NBNA score.

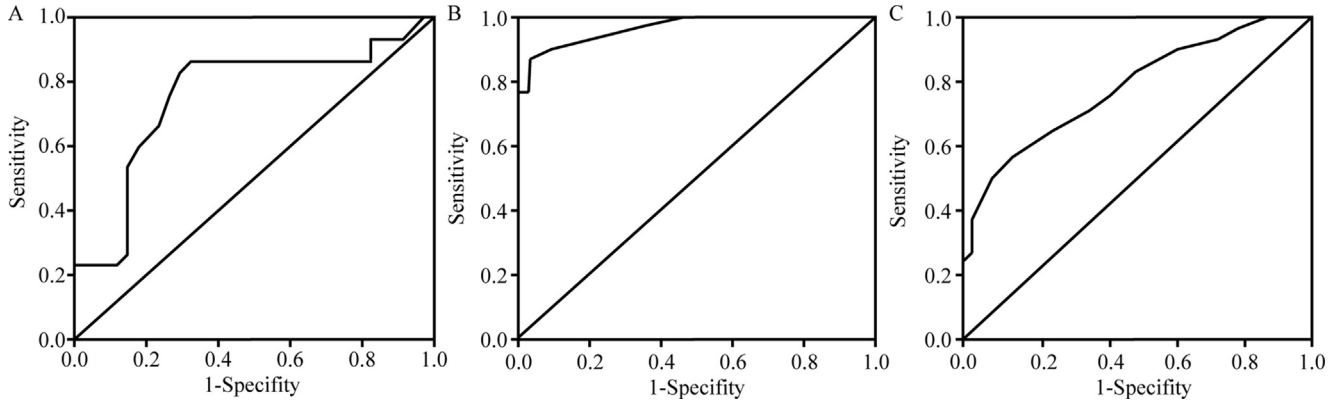


Figure 2 ROC of melatonin (A), SBDP120 (B) and SBDP145 (C).

pineal gland can release melatonin directly into the third ventricle,[5] which can then reach other brain structures through active transport and diffuse into the ependymal cells and the space around the blood vessels. High level of melatonin in the cerebrospinal fluid can provide antioxidant and anti-inflammatory protection for brain tissue. The injured brain is susceptible to oxidative damage for the following reasons: rich source of lipid peroxidation, production of reactive free radicals, increased permeability of the blood—brain barrier, as well as angioedema and microvascular injury. Previous studies[16—20] have shown that, excessive oxygen free radicals in preterm infants with brain injury may induce the cascade of inflammatory reactions, including the brain inflammation reactions, and release pro-inflammatory cytokines, thus causing apoptosis and mitochondrial dysfunction, as well as finally causing cell death.

Studies have shown that endogenous melatonin is massively produced in the cerebrospinal fluid of adults with head injury and the serum of diseases children with severe traumatic brain injury,[5,21] indicating that melatonin has antioxidant and neuroprotective effects. Studies targeting the models of brain injury[3,4,22,23] have shown that melatonin can reduce neuronal death by stabilizing the endothelial permeability. Recently, it has also been confirmed that melatonin can remove damaged mitochondria by activating the mitochondrial autophagocytes,[24—26] thereby inhibiting inflammation induced by brain injury. The Results of this study indicated that the more severe the degree of brain injury in preterm infants, the higher was the level of melatonin, which was consistent with previous studies. Excessive oxygen free radicals in preterm infants with brain injury may cause apoptosis and mitochondrial dysfunction by inflammatory cascade, which may ultimately lead to cell death. Melatonin has antioxidative effects and can activate the mitochondrial autophagocytes to remove damaged mitochondria, thereby inhibiting inflammation induced by brain injury, also, it may reduce the death of neurons and protect the nerves from injury by stabilizing endothelium permeability. We hypothesized that increased level of melatonin may be a mechanism of self-protection of the body. Melatonin is negatively correlated with the NBNA score at 40 weeks of corrected gestational age, indicating that the more severe the brain injury, the higher the melanin level would be. The AUC of the ROC of melatonin in diagnosing brain injury in preterm infants was 0.76, with 95% CI of (0.635, 0.886), with cutoff value of 69.5 pg/ml, sensitivity of 86.7%, and specificity of 68.4%, therefore the serum level of melatonin can be used for early assessment of brain injury in preterm infants.

SBDPs are the cleavage products of α-II spectrin.[6] α-II spectrin is abundantly present in axons and presynaptic terminals and originally found in the neurons as an important protein component of neurons. α-II spectrin is the substrate of calpain and caspase-3. Previous studies[16—20] have shown that in premature infants with brain injury, excessive oxygen free radicals can induce the of inflammatory reactions, including brain inflammation reactions,

and release pro-inflammatory cytokines, thus causing apoptosis and mitochondrial dysfunction as well as cell death. Cascade can activate Ca^{2+}-dependent enzymes including calpain and caspase-3.[27] α-II spectrin can be degraded by alpain and caspase-3, and the cleavage products are named SBDP145, SBDP150 and SBDP120 respectively according to their quality characteristics after electrophoresis. Previous studies showed that SBDP145, SBDP150, and SBDP120 increased in varying degrees in the cerebrospinal fluid of patients with severe brain trauma and in the serum of animals with brain injury or cerebral hypoxia-ischemia. Furthermore, the more severe the brain injury, the more obvious was the rise of SBDPs, and the worse the prognosis. The Results of this study indicated that the greater degrees of brain injury in preterm infants, showed higher levels of SBDP120 and SBDP145, which was consistent with previous studies, which were also negatively correlated with the NBNA score at 40 weeks of corrected gestational age. The AUCs of SBDP120 and SBDP145 in diagnosing brain injury in preterm infants were 0.97 and 0.77, with 95% CI of (0.635, 0.886) and (0.664, 0.891), cutoff values of 1.55 ng/ml and 1.35 ng/ml, sensitivity of 86.1% and 956.7%, and specificity of 96.7% and 85.3%, respectively. In conclusion, the serum levels of SBDP120 and SBDP145 are elevated in brain injury in preterm infants, they have diagnostic value for brain injury in preterm infants, and they can be beneficial biomarkers for diagnosing brain injury in preterm infants.

Due to limited conditions, this study did not detect the levels of melatonin, SBDP120, and SBDP145 at different time points during the disease course of preterm infants, so the dynamic changes of melatonin, SBDP120, and SBDP145 in the acute and convalescent stages of the disease, as well as related specific mechanisms are not known, future work will address these issues.

In conclusion, the serum levels of melatonin, SBDP120, and SBDP145 in the early stage of brain injury in preterm infants are significantly elevated and are associated with the degree of brain injury, suggesting that melatonin, SBDP120, and SBDP145 may be favorable biomarkers for diagnosing brain injury in preterm infants.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Galinsky R, Lear CA, Dean JM, Wassink G, Dhillon SK, Fraser M, et al. Complex interactions between hypoxia-ischemia and inflammation in preterm brain injury. *Dev Med Child Neurol* 2018;**60**:126–33.
2. Leviton A, Allred EN, Fichorova RN, O'Shea TM, Fordham LA, Kuban KKC, et al. Circulating biomarkers in extremely preterm infants associated with ultrasound indicators of brain damage. *Eur J Paediatr Neurol* 2018;**22**:440–50.
3. Hu Y, Wang Z, Pan S, Zhang H, Fang M, Jiang H, et al. Melatonin protects against blood-brain barrier damage by inhibiting the TLR4/NFκB signaling pathway after LPS treatment in neonatal rats. *Oncotarget* 2017;**8**:31638–54.
4. Moretti R, Zanin A, Pansiot J, Spiri D, Manganozzi L, Kratzer I, et al. Melatonin reduces excitotoxic blood-brain barrier breakdown in neonatal rats. *Neuroscience* 2015;**311**:382–97.
5. Marseglia L, D'Angelo G, Manti S, Rulli I, Salvo V, Buonocore G, et al. Melatonin secretion is increased in children with severe traumatic brain injury. *Int J Mol Sci* 2017;**18**. pii: E1053.
6. Witek MA, Fung LW. Quantitative studies of caspase-3 catalyzed αII-spectrin breakdown. *Brain Res* 2013;**1533**:1–15.
7. Chen S, Shi Q, Zheng S, Luo L, Yuan S, Wang X, et al. Role of α-II-spectrin breakdown products in the prediction of the severity and clinical outcome of acute traumatic brain injury. *Exp Ther Med* 2016;**11**:2049–53.
8. Schober ME, Requena DF, Davis LJ, Metzger RR, Bennett KS, Morita D, et al. Alpha II Spectrin breakdown products in immature Sprague Dawley rat hippocampus and cortex after traumatic brain injury. *Brain Res* 2014;**1574**:105–12.
9. Wu H, Li Z, Yang X, Liu J, Wang W, Liu G. SBDPs and Tau proteins for diagnosis and hypothermia therapy in neonatal hypoxic ischemic encephalopathy. *Exp Ther Med* 2017;**13**:225–9.
10. Madan A, Hamrick SEG, Ferriero DM. Central nervous system injury and neuroprotection. In: Taeusch HW, Ballard RA, Gleason CA, editors. *Avery's diseases of the newborn*. 8th ed. Philadelphia: Elsevier Saunders; 2005. p. 970–6.
11. Bonifacio SL, Gonzalez F, Ferriero DM. Central nervous system injury and neuroprotection. In: Taeusch HW, Ballard RA, Gleason CA, editors. *Avery' s diseases of the newborn*. 9th ed. Philadelphia: Elsevier Saunders; 2011. p. 869–91.
12. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978;**92**:529–34.
13. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. *Behav Brain Res* 1992;**49**:1–6.
14. Lin ZL, Yu HM, Lin J, Chen SQ, Liang ZQ, Zhang ZY. Mild hypothermia via selective head cooling as neuroprotective therapy in term neonates with perinatal asphyxia: an experience from a single neonatal intensive care unit. *J Perinatol* 2006;**26**:180–4.
15. Douglas-Escobar M, Weiss MD. Biomarkers of brain injury in the premature infant. *Front Neurol* 2013;**3**:185.
16. Kennaway DJ, Rowe SA. Effect of stimulation of endogenous melatonin secretion during constant light exposure on 6-sulphatoxymelatonin rhythmicity in rats. *J Pineal Res* 2000;**28**:16–25.
17. Mishima K, Okawa M, Shimizu T, Hishikawa Y. Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. *J Clin Endocrinol Metab* 2001;**86**:129–34.
18. Patra A, Huang H, Bauer JA, Giannone PJ. Neurological consequences of systemic inflammation in the premature neonate. *Neural Regen Res* 2017;**12**:890–6.
19. Paton MCB, McDonald CA, Allison BJ, Fahey MC, Jenkin G, Miller SL. Perinatal brain injury as a consequence of preterm birth and intrauterine inflammation: designing targeted stem cell therapies. *Front Neurosci* 2017;**11**:200.
20. Ginsberg Y, Khatib N, Weiss B, Arison S, Ross MG, Weiner Z, et al. Magnesium sulfate (MG) prevents maternal inflammation induced offspring cerebral injury evident on MRI but not via IL-1β. *Neuroscience* 2017;**353**:98–105.
21. Shimada A, Hasegawa-Ishii S. Histological architecture underlying brain-immune cell-cell interactions and the cerebral response to systemic inflammation. *Front Immunol* 2017;**8**:17.
22. Endesfelder S, Weichelt U, Strauß E, Schlör A, Sifringer M, Scheuer T, et al. Neuroprotection by caffeine in hyperoxia-

induced neonatal brain injury. *Int J Mol Sci* 2017;**18**. pii: E187.
23. Lorente L, Martín MM, Abreu-González P, Pérez-Cejas A, Ramos L, Argueso M, et al. Serum melatonin levels in survivor and non-survivor patients with traumatic brain injury. *BMC Neurol* 2017;**17**:138.
24. Yao L, Lu P, Ling EA. Melatonin suppresses toll like receptor 4-dependent caspase-3 signaling activation coupled with reduced production of proinflammatory mediators in hypoxic microglia. *PLoS One* 2016;**11**:e0166010.
25. Colella M, Biran V, Baud O. Melatonin and the newborn brain. *Early Hum Dev* 2016;**102**:1—3.
26. Lin C, Chao H, Li Z, Xu X, Liu Y, Hou L, et al. Melatonin attenuates traumatic brain injury-induced inflammation: a possible role for mitophagy. *J Pineal Res* 2016;**61**:177—86.
27. Figueira RL, Gonçalves FL, Simões AL, Bernardino CA, Lopes LS, Castro E Silva O, et al. Brain caspase-3 and intestinal FABP responses in preterm and term rats submitted to birth asphyxia. *Braz J Med Biol Res* 2016;**49**. pii: S0100-879X2016000700703.