Two circadian infant formulas produce differential cerebellum gene expression in lactating rat neonates

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Introduction

Sleep is important for all humans, especially babies. It is essential for growth and development of the central nervous system as well as maturation of many physiological processes. Sleep develops a circadian rhythm over time when the infant attains the typical mature phases of sleep and adjusts to the day–night cycle. In this maturation process the production of serotonin and melatonin by the pineal gland is essential for the consolidation of the wake/sleep cycle. Previous studies have shown that concentrations of some nutrients in breast milk vary throughout the day. This seems to indicate that different nutrient intake over the 24-h period may help babies to establish appropriate sleep/wake rhythms.

Studies with infants have shown the effect of tryptophan on the different parameters of infant sleep. Furthermore, other nutrients have also been found to affect the sleep/wake cycle.

With the final objective to facilitate the circadian wake–sleep rhythm of formula fed infants we designed and developed two different infant formulas with different nutrient composition.

Materials and methods

A special method for feeding rat neonates was developed combining maternal and artificial milk. Animals were kept artificially on 12-h cycles of light and dark. Artificial feeding was done during the dark phase under a red light environment which has been described it is not detected by rats. Three different groups of lactating neonate rats were fed with a combination of each of the new formulas or a standard formula. Control group was fed with standard formula ($n = 18$), the other two groups were fed with day ($n = 8$) and night formula ($n = 18$), respectively. They were sacrificed 5 days after birth; the cerebellum was extracted and processed for RNA extraction. Labelled cyanidine fluorophors c-RNA were synthesized from total RNA and hybridised in a DNA chip containing probes for 1,265 different genes. Genes were included based on the possible relationship with circadian rhythms or of toxicity or pharmacological interest.

Two different infant formulas were developed with different nutrients to facilitate the circadian wake–sleep rhythm. The milk designed for the day/wake cycle was higher in protein, cytidine, guanosine and inosine as well as vitamins A, B₁₂ and C, whilst the sleep promoting formula contained more L-tryptophan, carbohydrates, medium chain triglycerides, the nucleotides, uridine and adenosine (see Table 1). Clinical efficacy of the new combination of formulas was tested with a control formula in 21 healthy babies in a randomised, double blind, crossover study in which babies received both formulas over a week each.

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Table 1 Compositional differences between day and night experimental formulas (Puigjaner et al.)

Ingredient	Day	Night
Tryptophan	+	+++
α -Lactalbumin	–	+
LC-PUFA	+	–
MCT	–	+
Adenosine, uridine	–	++
Cytidine, guanosine, inosine	++	–
Vitamins A, C, E and B ₁₂	++	+
Carbohydrates	++	+++

LC-PUFA long chain polyunsaturated fatty acids, MCT medium chain triglycerides

Results

Small gene expression changes were observed in lactating neonate rats cerebellum as a consequence of different artificial nutrition. Since nutrition intervention was partial, only small changes were expected. Figures 1 and 2 summarize the genes that have a differential expression (up or down-regulated).

Animals fed the sleep facilitating formula showed a decreased expression of pro-apoptotic genes Bax and Bcl-2 and oxidative stress related genes cytochrome p450 and cytochrome-C oxidase, whilst nuclear factor-kappaB (NF- κ B) gene showed an increased expression compared animals fed the standard formula. NF- κ B is a transcription factor that when activated promotes production of several sleep-promoting substances such as interleukin-1beta (IL-1 β), tumor necrosis factor (TNF)-alpha, and nerve growth factor [1–3]. Animals fed with the wake facilitating milk showed a decreased expression of the α 3-subunit of GABAA, GABAB-R1 receptors and TNF compared with those fed the standard formula. GABA receptors are common targets for sleep inductor drugs [4]. Decreases in TNF have been

Fig. 1 Up or down-regulated genes in the cerebellum of lactating rat neonates of day formula fed animals compared to standard formula **Fig. 2** Up or down-regulated genes in the cerebellum of lactating rat neonates of night formula fed animals compared to standard formula

associated with suppression of spontaneous sleep [5]. Randomised, double blind, crossover clinical trial ($n = 21$) results are summarized in Figs. 3, 4 and 5. Babies fed with day and night formula combination showed better sleep parameters. They significantly slept for a longer time and had more immobility minutes and their sleep latency was shorter [6].

The gene expression experiments results in rats had a concordance with the clinical macroscopic observed effects.

Fig. 3 Comparison of slept hours between standard and day/night formula fed babies (Puigjaner et al.) **Fig. 4** Comparison of immobility minutes between standard and day/night formula fed babies

Fig. 5 Comparison of sleep latency (min) between standard and day/night formula fed babies

Conclusions

Our results seem to indicate that the specifically designed nutrient composition of the formula may influence expression of genes related to sleep or waking.

Combination of nutrient dissociation formula produces an increase in the number of hours slept and immobility minutes and a decrease of sleep latency in healthy babies compared with a standard formula.

Animal experiment and clinical trials seem to indicate that nutrition may help to establish circadian rhythms

References

1. Kubota T, Fang J, Kushikata T, Krueger JM (2000) Interleukin-13 and transforming growth factor-beta1 inhibit spontaneous sleep in rabbits. *Am J Physiol Regul Integr Comp Physiol* 279(3):R786–R792
2. Kubota T, Kushikata T, Fang J, Krueger JM (2000) Nuclear factor-kappaB inhibitor peptide inhibits spontaneous and interleukin-1beta-induced sleep. *Am J Physiol Regul Integr Comp Physiol* 279(2):R404–R413
3. Kubota T, Fang J, Brown RA, Krueger JM (2001) Interleukin-18 promotes sleep in rabbits and rats. *Am J Physiol Regul Integr Comp Physiol* 281:R828–R838
4. Staley KJ, Soldo BL, Proctor WR (1995) Ionic mechanisms of neuronal excitation by inhibitory GABAA receptors. *Science* 269:977–981
5. Takahashi S, Tooley DD, Kapas L, Fang J, Seyer JM, Krueger JM (1995) Inhibition of tumor necrosis factor in the brain suppresses rabbit sleep. *Pflugers Arch* 431:155–160
6. Rivero M, Barriga C, Rodriguez AB, Cubero J, Narciso D, Aparicio S, Garau C, Chifré R, Martín M, Rial RV (2005) Circadian dissociated baby formula consolidates the wake/sleep rhythm in lactating neonates. *SAJCN* 49(S1):393