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ORIGINAL ARTICLE

The circadian rhythm of tryptophan in breast milk affects the rhythms of 6-sulfatoxymelatonin and sleep in newborn

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Abstract

INTRODUCTION: The hormone melatonin regulates the sleep and this pineal hormone is synthesized in the organism from the amino acid tryptophan. It is known that breast-fed babies have better sleep patterns and a better entrained sleep/wake cycle than bottle-fed babies (adapted formula).
OBJECTIVE: To compare the circadian rhythm of 6-sulfatoxymelatonin (aMT6s) – the metabolite of melatonin excreted in the urine – in urine of bottle-fed and breast-fed children, and relate it to the circadian rhythm of tryptophan in breast milk, also evaluating the possible effects on the baby's night-time rest.
METHODS: 16 infants of 12 weeks of age were studied, divided into two groups depending on their exclusively natural or artificial feeding. The circadian rhythm of 6-sulfatoxymelatonin in urine was measured for the two groups of infants and for the breast-feeding mothers. In the breast milk, the circadian rhythm of the amino acid tryptophan was measured. The rest of the infants was tested by wrist actimeters for a week and the sleep parameters of the infants were measured and evaluated.
RESULTS: The tryptophan in the breast milk presented a circadian rhythm with acrophase at around 03:00. This affected the 6-sulfatoxymelatonin circadian rhythm with acrophase at 06:00 in the breast-fed infants, and also promoted nocturnal sleep. Assumed sleep, actual sleep, and sleep efficiency were significantly increased in the breast fed infants with respect the forrmula fed infants.
CONCLUSION: A temporal relationship was observed between the circadian rhythm of 6-sulfatoxymelatonin of the exclusively breast-fed babies and that of tryptophan in the mother's milk. Acting this aminoacide as a zeitgeber entrainment of the biological rhythms in the breast-fed infant.

Introduction

The hormone melatonin is synthesized in the organism from the amino acid tryptophan, mainly in the pineal gland. It presents high levels during the night which fall during the day [21]. The hormone has numerous endocrine and exocrine functions — antioxidant [15], oncostatic [8] and immunostimulatory [3,9,18]. But the best-known function is that of regulating biological rhythms together with the external cues (zeitgebers; light / dark alternation) [13]. It is known that these biological rhythms are still unentrained in the first stages of life. Studies on infants have shown that, until week 12 of life, the circadian rhythm of melatonin has yet to be established, and that the subsequent circadian consolidation of the pineal hormone is clearly reflected in coordination of the sleep/wake cycle [2]. Also, the nutritional contribution from milk is determinant in a baby's motor and psychic development, and it is known that breast-fed children present better sleep patterns than formula-fed children [10].

It has been demonstrated in both children [11] and adults [5] that oral administration of the amino acid tryptophan modifies the circulating levels of melatonin. It is therefore probable that, apart from genetic inheritance, environmental stimuli such as light, and the influence of the mother, breast milk has a strong influence on the melatonin levels of infants. This influence would be determined by the essential amino acid tryptophan, which is the precursor in melatonin's anabolism. Consequently, the purpose of the present study was to relate, over 24 hours, the levels of the amino acid tryptophan in breast milk [1,4] with the levels of 6-sulfatoxymelatonin (aMT6s)—the metabolite of melatonin excreted in the urine—in infants [17] who had been exclusively breast fed for three months, and to study the effects on the sleep of these infants compared with formula-fed infants.

Methods

Subjects

The study population, with the consent of the parents and under the supervision of the pædiatrician, consisted of two groups:

A. Eight healthy infants and their mothers. Their characteristics were as follows; males 5, females 3, ratio 5:3; age 12 weeks. The food was only breast milk provided regularly every 4 hours by each mother. None received formula.

B. Eight healthy infants. Their characteristics were as follows; males 3, females 5, ratio 3:5; age 12 weeks. The food was only formula (Blemil 1 Plus, Ordesa S.L., Spain) provided regularly every 4 hours in a feeding bottle.

The subjects' health status was determined from a physical examination, a follow-up, and considering these characteristics. The subjects took no drugs during the study that would disturb the MT6's and tryptophan levels. The study was approved by the Ethical Research Committee of Extremadura University, Badajoz (Spain).

Study protocol

The samples were taken over a period of 24 h. Urine was collected to measure the main urinary aMT6s. Each baby's urine was collected in adhesive bags, and each mother's urine was collected in a urine bottle. An aliquot of 2 ml was transferred to vials (Eppendorf, USA) and frozen at –30°C pending assay. An two ml of breast milk was collected to assay the amino acid tryptophan. The samples were transferred to polypropylene vials and frozen at –30°C pending assay. The samples were divided into A.M. and a P.M. uniform groups of 12h each: (00:00–12:00) and (12:00–00:00).

Biochemical methods

Tryptophan in the breast milk after alkaline hidrolisys, were measured by HPLC (Beckman's System 6300/7300). The results were expressed as μmol/l.

Urinary aMT6s levels were measure by an ELISA method, using the direct urinary aMT6s ELISA kit from IBL Hamburg (Germany). The results were expressed as nanograms/millilitre. The correlation between aMT6s and other variables (delivery, sex, feeding schedule, sleep/wake) was assessed.

Sleep analysis

Sleep patterns in dark cycles were recorded by actigraphy (Actiwatch) for a 7-day period, with the device put on the baby's leg. The sleep was automatically scored, based on the activity data, and analyzed with the software package Sleep Analysis (Cambridge Neurotechnology, U.K.).

After logging the activity for a week by means of Actiwatch, we proceeded to the study of the following sleep parameters:

- Assumed sleep: The period of time between the onset of sleep with the first radical decline in activity, until a new increase of activity was detected coinciding with awakening.
- Actual sleep: The time of the sleep period in which activity is below a threshold denominated sensitivity, described for each individual.
- Sleep efficiency: Percentage of time asleep relative to the time in the crib.
- Sleep latency: The time lapse from laying the baby in the crib until sleep.

Mathematical analysis

Statistical analysis. We evaluated the normality of variables using the Kolmogorov-Smirnov statistical procedure. All the data are presented as mean and standard deviation. The statistical analysis was performed using a non-parametric test (Kruskal-Wallis), and matched pairs signed rank tests were performed to detect differences between 2 study samples, $p<0.05$ was considered statistically significant.

Chronobiological analysis. The tryptophan and aMT6s 24 hour time series data were fitted by a sinusoidal function (1).

$$y(t) = y0 + A \sin(\omega t + \varphi) \quad (1)$$

[y (0) = mesor; average of the variable over one cycle; A = amplitude; ω = angular frequency; φ= phase] using the software package IGOR PRO 4.0 1988–2000 (WaveMetrics, Inc. Late Oswego, Oregon, USA).

Results

In the circadian rhythm study, the 24-h period sinusoidal function (1) was fitted to the breast milk tryptophan data and to the urinary 6-sulfatoxymelatonin excretion data. Equation (2) is the fit corresponding to tryptophan levels in the breast milk:

$$y(t) = 67.95 + 13.12 \sin(0.26 t + 0.76) \quad (2)$$

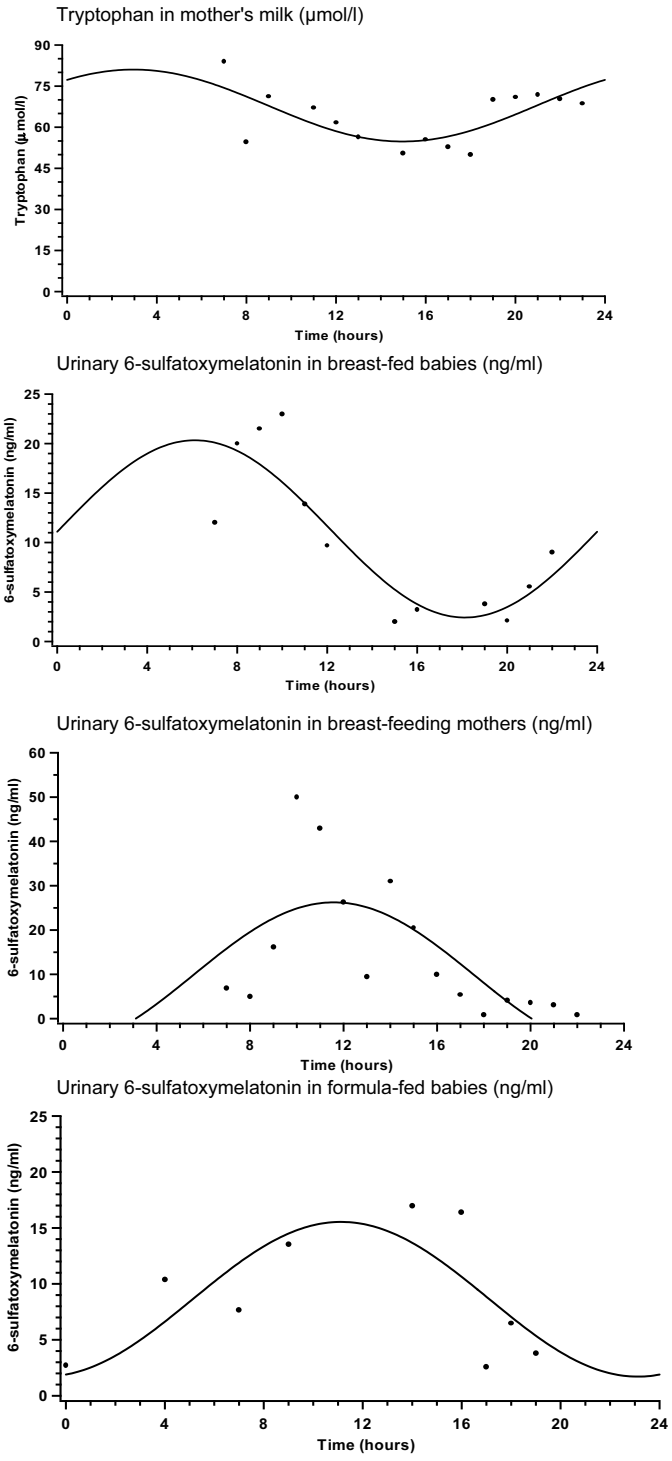

Figure 1. Plots of the sinusoidal fits to the 24-h data of tryptophan in breast milk and of 6-sulfatoxymelatonin in the urine of breast-fed babies (n=8), their mothers (n=8), and formula-fed babies (n=8).

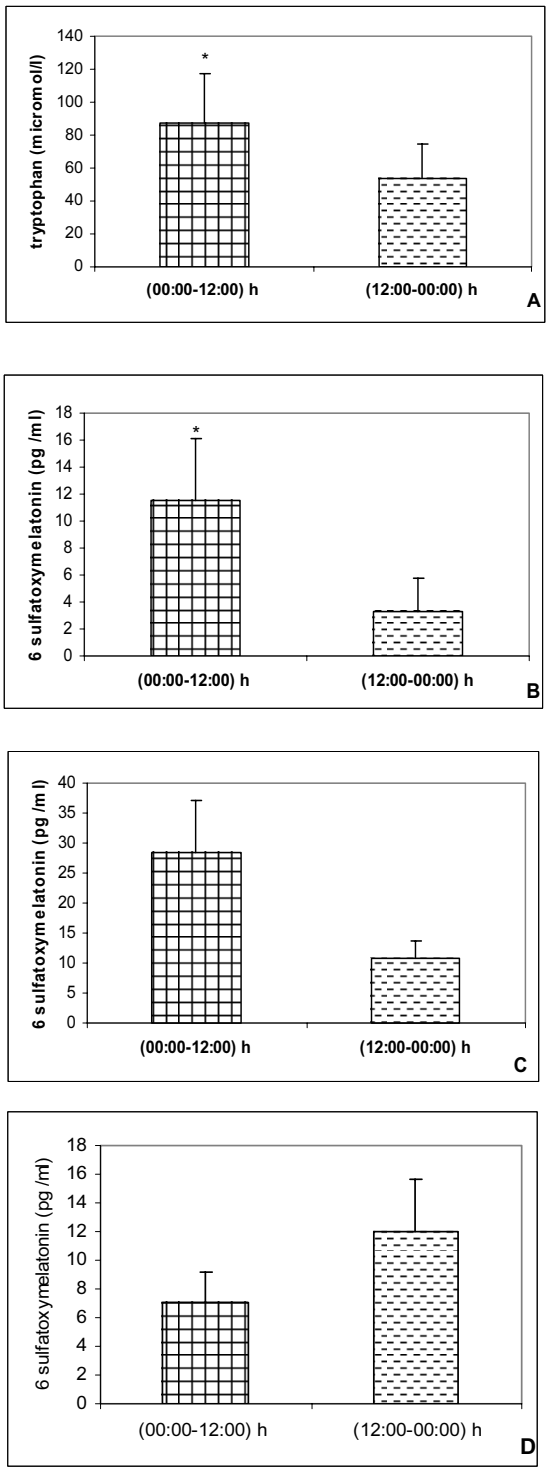

Figure 2. Bar charts of tryptophan in breast milk (A), and of 6-sulfatoxymelatonin in the urine of breast-fed infants (B), of their mothers (C), and of formula-fed infants (D), (n=8). *(p<0.05) with respect to the time interval (12:00–00:00).

Table 1. Chronobiological parameters of urinary 6-sulfatoxymelatonin in breast-fed babies (n=8), their breast-feeding mothers (n=8), and formula-fed babies (n=8), and of tryptophan in breast milk (n=8).

Parameters	Mesor	Acrophase	Nadir
tryptophan (breast milk) µmol/l	67.95 µmol/l	03:00 h	15:00 h
6-sulfatoxymelatonin (natural) ng/ml	11.45 ng/ml	06:00 h	18:00 h
6-sulfatoxymelatonin (mathers) ng/ml	9.72 ng/ml	12:00 h	20:00 h
6-sulfatoxymelatonin (formula) µg/ml	9.82 ng/ml	11:00 h	00:00 h

Table 2. Comparison of the sleep parameters of breast-fed (n=8) and formula-fed (n=8) babies.

	Formula	Breast milk
Time in the crib	10h 2min ± 1h 10min	9h 54min ± 2h 36min
Assumed sleep	8h 53min ± 52min	9h 35min ± 41min [a]
Actual sleep	7h 7min ± 43min	8h 30min ± 49min [a]
Sleep efficiency	70.512 ± 5.78 %	81.45 ± 5.48 % [a]
Sleep latency	1h ± 45min	30min ± 17min

[a] $p<0.05$ with respect to the formula milk.

Equations (3–5) are the fits corresponding to the 6-sulfatoxymelatonin levels in the urine of, respectively, the breast-fed babies, their mothers, and the formula-fed babies:

$$y(t) = 11.44 + 8.97 \sin (0.26\, t - 0.05) \quad (3)$$

$$y(t) = 9.72 + 16.44 \sin (0.26\, t - 1.44) \quad (4)$$

$$y(t) = 9.82 + 8.28 \sin (0.26\, t - 1.52) \quad (5)$$

These four sets of data and the corresponding fits are plotted in Figure 1. One observes that the acrophases of tryptophan in the breast milk and the breast-fed infants' urinary 6-sulfatoxymelatonin levels are both the closer. The infant's 6-sulfatoxymelatonin rhythm lags ($\Phi 3 - \Phi 2$) by 2h 44min behind the rhythm of tryptophan in the breast milk. The mothers' 6-sulfatoxymelatonin rhythm is not in phase with these values — its acrophase is at around 12:00, the rhythm lags ($\Phi 4 - \Phi 3$) by 5h 21min behind the rhythm of infant's 6-sulfatoxymelatonin. The 6-sulfatoxymelatonin circadian rhythm of the formula-fed babies had an acrophase at 11:00 and a nadir at 00:00, rhythm lags ($\Phi 5 - \Phi 3$) by 5h 39min behind the breast milk infant' 6-sulfatoxymelatonin rhythm. The chronobiological parameters corresponding to Equations (2–5) are listed in Table 1.

Figure 2 shows the urinary 6-sulfatoxymelatonin levels of the three study groups and the breast milk tryptophan levels split into two equal time intervals — (00:00–12:00) and (12:00–00:00). The levels of tryptophan in the breast milk (Figure 2A) and of urinary 6-sulfatoxymelatonin for the breast-fed infants (Figure 2B) were significantly higher in the A.M. interval (00:00–12:00) than in the P.M. interval (12.00–00:00) ($p<0.05$).

Table 2 presents a summary of the results of the night-time sleep study. The assumed sleep, actual sleep, and sleep efficiency were greater ($p<0.05$) in the breast-fed than in the formula-fed babies.

Discussion

The chronobiological variables for the tryptophan content of the breast milk over a 24-h period showed significant variation reflecting a circadian rhythm in this parameter. The acrophase (maximum) occurred at around 03:00, and the mesor (mean) was 67.95 µmol/l.

The levels of 6-sulfatoxymelatonin, melatonin's catabolic metabolite excreted in the urine, were not constant over the 24-h period in either group of infants (breast-fed and formula-fed) or in the breast-feeding mothers. All three groups presented a circadian rhythm, but with different chronobiological parameters. The acrophase of the rhythm in the mothers was at 12:00 with a mesor of 9.72 ng/ml, while in the exclusively breast-fed three-month-old infants the acrophase was at 06:00 with a mesor of 11.45 ng/ml. This corroborates the findings of Ardura et al. [2] which indicated the appearance of the circadian rhythm of melatonin at the end of the neonate period. These exclusively breast-fed children already presented a fully established activity/rest rhythm, with actual sleep during the night (8h 30min ± 49min) that was significantly longer than that of the formula-fed infants (7h 7min ± 43min). [6, 10]

Thus, in the breast-fed infants, the rhythm of 6-sulfatoxymelatonin excretion seems to be entrained and influenced by the rhythm of tryptophan in the mother's milk. The infant's 6-sulfatoxymelatonin rhythm lags by 2h 44min behind the rhythm of tryptophan in the breast milk. Other authors have suggested that this lag may be the result of the sum of two metabolic processes: (i) the formation of melatonin from the ingested tryptophan, with an estimated time of 60 minutes [12, 5]; and (ii) the catabolism of melatonin to 6-sulfatoxymelatonin, which is then excreted with an elimination half-life of between 30 and 50 minutes [12, 19].

Hence, in the initial stages of life, the rhythm of the amino acid tryptophan has a marked repercussion on the anabolism of the hormone melatonin, and on the consolidation of rest during the nocturnal cycle.

There are major circadian variations in the composition of human milk, and it would be reasonable to suppose that these changes would have a non-negligible functional importance. There has, however, not as yet been any development of infant-food formulas that take these aspects into account, even though information on the circadian variability of breast milk has been available for some time. For example, there are numerous sources in the literature giving different values of the acrophase and nadir according to the component in question. Thus, the maximum of cytoplasm organelles dispersed in the milk occurs after dusk, while there are early-morning maxima of copper, zinc, iron [7, 14] and lactose concentrations, this last being inversely correlated with other oligosaccharides [20]. The amino acids alanine, glutamine, threonine, glutamate, and aspartic acid reach maximum levels at the beginning of the afternoon [16].

In the initial stages of life, therefore, the rhythm of the amino acid tryptophan in the mother's milk, through its anabolism to the hormone melatonin, could be like a zietgeber and closely affect the consolidation of the breast-feeding infant's night-time rest. This thus supports the idea of the importance of the composition of infant foods in consolidating the infant's sleep/wake cycle, and that the amino acid tryptophan provided through breast-feeding intervenes in that consolidation.

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