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Melatonin for Chronic Insomnia in Angelman Syndrome: A Randomized Placebo-Controlled Trial

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Previous studies suggested that melatonin improves sleep in insomniac patients with Angelman syndrome. To assess the efficacy of melatonin, a randomized placebo-controlled study was conducted in 8 children with Angelman syndrome with idiopathic chronic insomnia. After a 1-week baseline period, patients received, depending on age, either melatonin 5 or 2.5 mg, or placebo, followed by 4 weeks of open treatment. Parents recorded lights off time, sleep onset time, wake-up time, and epileptic seizures in a diary. Salivary melatonin levels were measured at baseline and the last evening of the fourth treatment week. Melatonin significantly advanced sleep onset by 28 minutes, decreased sleep latency by 32 minutes, increased total sleep time by 56 minutes, reduced the number of nights with wakes from 3.1 to 1.6 nights a week, and increased endogenous salivary melatonin levels. Parents were satisfied with these results. Indications that melatonin dose in Angelman syndrome patients should be low, are discussed.

Keywords: Angelman syndrome; melatonin; sleep problems

Angelman syndrome is characterized by severe intellectual disability, motor impairment, seizures and subtle dysmorphic facial features and is associated with 4 types of chromosomal abnormalities involving the chromosome 15q11-q13 region (deletions, paternal uniparental disomy, methylation imprinting mutations, and UBE3A and other presumed single gene mutations). Most individuals with Angelman syndrome exhibit behavioral features such as excessive laughter, hyperactivity, noncompliance, speech impairment, and sleep problems.[1-3] Sleep problems include settling problems and frequent night waking.[4,5] They appear resistant to behavior therapy and conventional sleep medication.[5]

Melatonin, a hormone synthesized and released by the pineal gland during darkness, is a chronobiotic drug with hypnotic properties.[6] There is increasing evidence that chronic sleep problems in persons with intellectual disabilities are the result of circadian rhythm disorders and that melatonin therapy could be effective. However, only a few randomized trials with melatonin in persons with intellectual disabilities are performed.[7] In insomniac patients with intellectual disabilities, melatonin decreases sleep latency, but does not influence sleep maintenance.[8-10] An open-label trial of 13 children with Angelman syndrome found that melatonin decreased sleep latency and increased total sleep time as well.[11]

Method

Participants

Parents of patients with Angelman syndrome and chronic idiopathic insomnia that were referred to our sleep center by local general practitioners, were asked to participate in a placebo-controlled trial with melatonin. Criteria for participation were sleep latency more than 30 minutes, or 2 or more wakes, lasting more than 15 minutes a night, at least 5 nights a week, during more than 1 year preceding inclusion. Exclusion criteria were prior use of melatonin, liver disease, renal failure, chronic pain, and age less than 24 months. Patients were examined by a physician (first author) for patients with intellectual disabilities who is specialized in sleep disorders, assessing that behavioral and social sleep hygiene measures had been attempted unsuccessfully and no signs for a physical cause for the insomnia were present. The trial was performed in the home setting of each individual. It followed the 1983 revised provisions of the 1975 Declaration of Helsinki.

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The local Medical Ethical Committee approved the study. The patients' parents gave written informed consent.

The trial consisted of 2 consecutive periods: a 1-week qualification period and 4 weeks of treatment during which participants were randomly allocated to melatonin or placebo therapy. Saliva specimens for measuring melatonin concentrations were collected in the qualification period (baseline) to examine whether there was a delayed sleep phase syndrome or another disturbance in melatonin circadian rhythm and at the end of the last week of the treatment period to examine whether 4 weeks of treatment had changed endogenous melatonin circadian rhythm. After the 4-week placebo controlled phase of the study, all patients received 4 weeks open treatment with melatonin. At follow-up parents were asked to tell if they were satisfied with the result of the treatment. Patients were not allowed to change their co-medication. All investigators involved in the study were unaware of the treatment allocation. The code was broken when the data of all patients were recorded in the database.

Treatment

During the 4 treatment weeks, patients 6 years of age and older received a daily dose of 5 mg of melatonin (Duchefa Farma BV, Haarlem, The Netherlands) orally at 7 PM, mixed with carboxymethylcellulose in a fast-release tablet, or an identically looking placebo. A 5-mg dose was chosen because most placebo-controlled trials with melatonin used this dose.[12-14] Patients under 6 years of age received a daily dose of 2.5 mg of melatonin orally at 6 PM. Compliance was tested by comparison of the number of tablets returned with the number prescribed.

Outcome Measures

Primary outcome measures were the between-group differences of sleep latency, sleep onset, wake up time, and number and length of wakes. Secondary outcome measures were the between group differences of salivary melatonin concentrations, number of epileptic seizures, as well as adverse reactions of melatonin. Sleep variables were assessed by parents and recorded in a sleep diary. Parents were asked to listen or watch their child every 10 minutes from bedtime until the time they found their child asleep. Furthermore, they reported the times at night when they heard or saw that their child was awake. The parents were encouraged to describe any suspected adverse effects in the diary. Results were discussed with parents at the end of the double-blind phase and after 4 weeks of open treatment. At both times, parents were asked to judge the result as small, moderate, or strong improvement or unchanged. Because of contradictory reports of proconvulsant and anticonvulsant effects of exogenous melatonin,[15,24] seizure control was asked for at any visit.

Endogenous Melatonin

On the last night of the baseline week and the last night of the fourth treatment week (at which night no melatonin was given) salivary samples were collected hourly during 5 consecutive hours. In children 2–4 years of age from 5 PM, in children 5 and 6 years of age from 6 PM, and in older children from 7 PM. To prevent suppression of melatonin secretion by bright light[16] the children remained indoors with curtains closed during that period. Dim light (<50 lux) was allowed. Saliva was collected in a cotton plug either chewed on by the patient or by sweeping the cavity of the patients' mouth by 1 of the parents. The limit of detection of the assay, which had to contain at least 0.5 mL of saliva, was 0.39 pg/mL with an intra-assay variation of 14.5%/mL.[17] The maximum detection level was 50 pg/mL.

Statistical Analyses

Results of a 1-sample Kolmogorov-Smirnov analysis showed that data of each variable were normally distributed. Unpaired *t*-tests were conducted to test differences in change between melatonin versus placebo treatment on the sleep variables. Statistical significance was accepted at $P < .05$.

Results

A total of 13 children and adults with Angelman syndrome and chronic sleep problems were referred to our sleep center. Four of them were not included because their sleep problems did not meet criteria for inclusion. After receiving information on the design of the trial, the parents of 1 patient decided not to participate because they thought that the trial was too burdensome for their child. The remaining 8 patients were randomly assigned to melatonin or placebo conditions. Thus, the melatonin and the placebo group each consisted of 4 patients.

Seven patients showed sleep onset as well as sleep maintenance problems, and 1 patient suffered from sleep onset problems only (Table 1). There were no statistically significant differences between patients who were allocated to melatonin and placebo group on all parameters during baseline week.

During melatonin treatment, sleep latency decreased by 32 minutes, sleep onset advanced by 28 minutes, and total sleep time increased by 56 minutes on average. The mean number of nights with wakes decreased from 3.1 to 1.6 a week on average. These changes significantly differed from those during placebo treatment (Table 2). There was no significant change in lights out time and sleep offset time.

The parents of 2 children (#4 and 8) who received melatonin considered the treatment outcome as a moderate improvement, and 2 (#2 and 5) as a strong improvement. After 4 weeks of open treatment with melatonin, the parents of 2 children (#2 and 8) decided upon continuation of

Table 1. Participant Characteristics and Medication Use During the Study

	Patient							
	1	2	3	4	5	6	7	8
Type of Angelman syndrome	Deletion	Deletion	Disomy	Deletion	Deletion	Disomy	Deletion	Deletion
Age, y:mo	4:10	12:10	6:7	5:7	8:10	9:2	20:11	13:4
Gender	Male	Female	Female	Male	Female	Female	Male	Female
Weight, kg	20	48	26	22.5	31	37	56	40
Sleep onset problem	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nighttime awakening	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Antiepileptic drug use	cbz,clb,vpa	–	vpa	–	esm	–	vpa	clb,vpa
Present sleep medication	mid	pip	–	–	–	–	–	–

NOTE: cbz = carbamazepin; clb = clobazam; esm = ethosuximide; vpa = valproate; mid = midazolam; pip = pipamperon.

Table 2. Difference in Sleep Parameters From Baseline to Week 4

	Baseline					Intervention (4 weeks)				
	Melatonin Mean (SD)	Placebo Mean (SD)	*t*	*df*	*P*	Melatonin Mean (SD)	Placebo Mean (SD)	*t*	*df*	*P*
Lights out time[a]	20:10 (0:55)	20:09 (1:12)	.011	6	.99	20:14 (0:52)	20:14 (1:12)	0	6	1
Sleep onset time[a]	21:00 (1:10)	21:05 (1:30)	–.096	6	.93	20:32 (0:56)	21:10 (1:31)	–.715	6	.02[c]
Sleep latency[b]	50.00 (17.49)	56.50 (21:17)	–.473	6	.65	17.5 (5.74)	55.75 (20.64)	–3.570	6	.01[c]
Sleep offset time[a]	07:49 (0:36)	07:02 (0:33)	1.715	5	.15	07:42 (0:38)	07:02 (0:02)	1.768	5	.14
No. of nights/week with wakes	3.1 (2.95)	5.9 (1.41)	–1.724	6	.14	1.6 (2.01)	5.6 (1.24)	–3.357	6	.01[d]
No. of wakes/night	1.5 (1.41)	1.7 (0.69)	–.127	6	.9	0.9 (0.61)	1.8 (0.79)	–1.926	6	.1
Duration of wakes[b]	39.50 (35.25)	55.00 (37.53)	–.602	6	.57	11.75 (9.87)	60.75 (45.02)	–2.126	6	.08
Total sleep time[a]	10:08 (1:22)	9:40 (0:37)	.545	5	.61	11:04 (0:46)	9:31 (0:28)	2.996	5	.03[c]

a. Hours:minutes.
b. Minutes.
c. $P < .05$.
d. $P < .01$.

the treatment. The parents of 1 child (#5), however, reported an increase of the number of night wakes and stopped treatment. The parents of the fourth child (# 4) did not want to continue treatment, because they were disappointed with the treatment result.

The parents of the 4 children who received placebo reported that the sleep problems of their children did not change. After 4 weeks of open melatonin treatment, the parents of 3 children (#1, 3, and 6) wanted to continue melatonin treatment because of the strong improvement of the sleep of their child, whereas the parents of 1 child (#7) stopped treatment because the sleep problems in their child remained unchanged.

In 35 of 80 salivary samples, the amount of saliva was too low for a melatonin analysis. Visual inspection of the data on the remaining 45 samples (Table 3) shows that salivary melatonin concentrations, on a day no study medication was given, increased substantially after melatonin treatment (ie, child 2, 4, 5, and 8), whereas melatonin concentrations after placebo treatment (ie, child 1, 3, 6, and 7) did not change. In patients 4 and 8, salivary melatonin concentrations were above 50 pg/mL at the first evening after discontinuation of 4 weeks of melatonin treatment. In patient 5, the first sample (collected at 5 PM) contained more than 50 pg/mL.

Of 4 patients receiving melatonin in the melatonin group, 2 used antiepileptic drugs (ie, child 5 and 8; see Table 1). Last reported seizure was, respectively, 3 months and 2 years before entering the study. No seizures were reported by their parents during the present study, or by parents of the other 6 patients.

Discussion

Four weeks of melatonin treatment in Angelman syndrome children with chronic insomnia was more effective in decreasing sleep latency, advancing sleep onset, and increasing total sleep time than placebo. Furthermore, the number of nights per week with wakes decreased more during melatonin treatment. The reduction in sleep latency and the

Table 3. Melatonin in Saliva at Baseline and the Last Day of the Fourth Treatment Week[a]

		Patient							
		1	2	3	4	5	6	7	8
Melatonin or placebo[b]	Time (PM)	P	M	P	M	M	P	P	M
Baseline week	5				<0.5	<0.5			
	6	4.9		3.7	<0.5	1.4	<0.5		
	7	4.0	0.6	3.1	0.6	#	#	1.4	#
	8	4.7	0.7	4.2	#	3.3	#	<0.5	<0.5
	9	#	1.6	#	#	#	#	<0.5	#
	10	#	#	#			#	#	9.1
	11		#					#	
Week 4	5				>50	>50			
	6	2.5		2.7	>50	6.1	<0.5		
	7	1.1	22.7	#	>50	#	<0.5	<0.5	#
	8	#	#	#	>50	9.5	2.2	1.3	34.0
	9	#	#	4.6	>50	19.2	#	4.2	>50
	10	#	#	#			#	#	>50
	11		#					#	>50

a. Pound signs indicate that not enough saliva was collected.
b. Study medication during weeks 1–4 double-blind phase: P = placebo; M = melatonin.

advance of sleep-onset is consistent with earlier findings in studies with melatonin in children with chronic sleep onset.[14,18] The beneficial effect on night waking and total sleep time differed from findings in earlier studies showing that melatonin does not influence sleep maintenance.[8-10,19]

An average of half an hour earlier getting to sleep and sleeping 1 hour longer is a statistically significant difference. However, one might ask whether this is of a meaningful benefit to the patients and their parents. When asked whether they were satisfied with the results after 4 weeks of melatonin (or in the case of placebo after 4 weeks of open melatonin use), parents responded positively. Half an hour earlier, getting to sleep meant that parents needed less time-consuming efforts to try to let their child stay in bed. A mean increase in total sleep time by approximately 1 hour, meant less or not waking up anymore in the middle of the night and waking up by parents and siblings. But next to this, parents reported that their children's behavior was easier to manage and that they were less sleepy and more attentive at daytime. This latter finding is consistent with another placebo-controlled study showing that melatonin improved health status.[18]

Diary measures have measurement error, but are frequently used in nonclinical settings. Sleep diaries based on observations by others, however, yield more satisfactory data compared with actigraphy than sleep diaries based on self-report.[20] Besides, parents of sleep-disturbed infants were accurate reporters of actigraphically assessed sleep onset and sleep duration.[21] Sadeh et al[22] showed an 85.3% agreement rate between actigraphic sleep–wake scorings compared with those of polysomnography. Measures were only used to compare between the results of the 2 consecutive periods and not used to determine sleep parameters per se.

Melatonin levels in the baseline week were low. It is possible that in our patients melatonin levels increased after the last sampling time. Late melatonin onset is a key characteristic of the delayed sleep phase syndrome.[18] Consequently, sleep onset insomnia in Angelman syndrome could be the result of a delayed sleep phase syndrome. Delayed sleep phase syndrome is defined as an abnormally delayed sleep–wake rhythm. The major symptoms of this syndrome are extreme difficulty to initiate sleep at a conventional hour of the night and great difficulty to wake up on time in the morning. Delayed sleep phase syndrome is associated with late dim light melatonin onset.[12] This circadian rhythm disorder responds very well to melatonin treatment. Zhdanova et al[11] also found low melatonin levels in children with Angelman syndrome in the baseline week. They attributed these low levels, in part, to the use of valproate. Sodium valproate is known to suppress plasma melatonin levels.[23] In our study, 4 children used valproate, but their melatonin levels in the baseline week were not lower than those of children who did not use valproate.

The question whether melatonin influences seizure frequency remains a matter of discussion. Melatonin may increase seizure frequency.[15] However, melatonin also has anticonvulsant effects.[24] The melatonin treatment period in our patients was perhaps too short to draw conclusions about the influence of melatonin on seizure frequency.

In 3 of the 4 children who received melatonin, salivary melatonin levels were extremely high after 4 weeks of treatment. The parents of these children reported the return or increase of night wakes when visiting the clinic after week

4 of the open melatonin treatment phase, or at the next evaluation 1 month later.

The postmelatonin high levels of salivary melatonin could be due to a supraphysiological dose of exogenous melatonin. However, the elimination half-life of melatonin has been reported as 0.8 hours with an absorption half-life of 0.4 hours, while melatonin levels ranged from 350 to 10 000 times those occurring physiologically.[25] Furthermore, in children 6–12 years of age who participated in a study using 5 mg of melatonin in a similar study design as in the present study, salivary melatonin levels measured 24 hours after the last exogenous melatonin intake, were normal and only showed a phase advance.[14,18] If there were a large advance of the endogenous melatonin rhythm, so that endogenous melatonin started to increase largely before 5 PM, patients should have been sleepy in the afternoon. Parents did not report this. Another explanation for the high salivary melatonin levels approximately 24 hours after the last intake of melatonin is that the melatonin metabolism is disturbed in Angelman syndrome. Melatonin is metabolized in the liver by cytochrome P450 1A2 to its main primary metabolite 6-hydroxymelatonin. Exogenous melatonin has a half-life between 30 and 50 minutes. Genetic variations influence the expression of specific cytochrome P450 isoenzymes in individuals and this finding influences their capacity to metabolize certain drugs.[26] Assuming that exogenous melatonin does not stimulate endogenous melatonin secretion, it is likely that the high melatonin levels after 4 weeks of treatment with melatonin in 3 of 4 children in this study, found 24 hours after the last oral melatonin dose, were caused by a low activity of cytochrome P450 1A2 or another disturbance in the metabolism of melatonin. However, the possibility that the elevated levels were the result of down-regulation of endogenous melatonin or enzyme inhibition by antiseizure medication can in some cases not be ruled out. Only 3–4% of the white population is a poor metabolizer of cytochrome P450 1A2. That 3 of 4 children with Angelman syndrome in the melatonin group had elevated melatonin levels after 4 weeks of treatment raises the question whether a low activity of cytochrome P450 1A2 is part of the Angelman syndrome phenotype.

We are studying the above-discussed explanations for a possible disturbed melatonin metabolism in Angelman syndrome patients. Until the results are published, we suggest testing melatonin metabolism in each individual Angelman syndrome patient before considering (long-term) melatonin treatment, for instance by measuring salivary melatonin hourly during 24 hours after melatonin administration. Consequently, the best pharmacotherapeutic dose can be determined, taking into account the individually measured elimination half-life of melatonin. If salivary melatonin levels do not reach basal values within 12 hours after melatonin administration, consider lowering the dose with at least 50%.

This first placebo-controlled study of melatonin in insomniac Angelman syndrome patients has several shortcomings. The number of patients (ie, n = 8) was low. Many parents referred to our sleep clinic, when asked for permission to let their child participate in this study, refused to do so because they only wanted immediate help for their child's problems instead of risking the possibility of another 4 weeks of disturbed sleep due to placebo treatment. In this study, sample size was very small and the power of the *t*-test was low. Despite low power, significant and meaningful differences were found between placebo and melatonin conditions. It should be noted that a randomized controlled trial with a larger sample size would have a larger statistical power. However, it is extremely difficult if not impossible to include a large number of participants with Angelman syndrome in any randomized controlled trial, because Angelman syndrome is a relatively rare genetic disorder.

We relied on parental sleep diaries rather than more objective measures, such as actigraphy. Melatonin levels would better have been measured more than 24 hours, for example, 48 hours, after the last intake of exogenous melatonin. The sample was biased by referrals to a highly specialized outpatient clinic for insomniac patients with intellectual disabilities. These shortcomings threaten the validity of our conclusions. Nevertheless, the results show that melatonin treatment may help insomniac Angelman syndrome patients. However, further studies need to be performed on melatonin metabolism in Angelman syndrome to establish the best dose. This study indicates that it is possible that melatonin dose in Angelman syndrome should be lower than generally prescribed.

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