Journal of Autism and Developmental Disorders (2022) 52:2784–2793
https://doi.org/10.1007/s10803-021-05139-w

ORIGINAL PAPER

Melatonin Treatment and Adequate Sleep Hygiene Interventions in Children with Autism Spectrum Disorder: A Randomized Controlled Trial

Masaharu Hayashi[1] · Kazuo Mishima[2] · Michio Fukumizu[3] · Hiroyuki Takahashi[4] · Yuko Ishikawa[5] · Izumi Hamada[5] · Hideyuki Sugioka[5] · Osamu Yotsuya[5] · Yushiro Yamashita[6]

Accepted: 7 June 2021 / Published online: 28 June 2021

Abstract

Robust clinical evidence has not been available for melatonin, a drug commonly administered for treating sleep problems of children with autism spectrum disorder (ASD). In a phase 3 randomized, placebo-controlled clinical trial, we administered 1-mg melatonin (n = 65), 4-mg melatonin (n = 65), or placebo (n = 66) to196 children with ASD once daily before bedtime under adequate sleep hygiene interventions. The primary outcome was sleep onset latency (SOL) assessed with the electronic sleep diary. SOL shortened significantly in the 1- and 4-mg melatonin groups compared to the placebo group (− 22.0, − 28.0, and − 5.0 min, respectively; $p < 0.0001$ each). This therapeutic regimen of melatonin is a reasonable clinical approach to cope with ASD-emergent difficulties in children with ASD.

Keywords Autism spectrum disorder · Melatonin · Sleep hygiene interventions · Sleep problems · Randomized controlled trial

Introduction

Autism spectrum disorder (ASD), a neurodevelopmental condition with lifelong impacts (Lyall et al., 2017), is featured by persistent deficits in social communication and interaction and by restricted, repetitive patterns of behaviors (American Psychiatric Association, 2013). In 2016, ASD prevalence was 18.5 per 1,000 (one in 54) children aged 8 years, and ASD was 4.3 times as prevalent among boys as among girls (Maenner et al., 2020). Sleep problems (SPs)—common symptoms in children (Deliens et al., 2019; Rigney et al., 2018) and adults (Baker et al., 2019) with ASD—can impair social behaviors (Buck et al., 2014), cognitive daytime performance (Limoges et al., 2013), and quality of life (May et al., 2015). Therefore, increasing knowledge and understanding of SPs that bother children/patients with ASD will serve to improve their and their families' quality of life (Ballester et al., 2019).

The involvement of melatonin in regulating the sleep–wake cycle is well known, and children with ASD show decreased melatonin production (Rossignol et al., 2011), SPs (Deliens et al., 2019), and altered circadian rhythms (Veatch et al., 2015a). The abnormal synthesis (Melke et al., 2008), release patterns (Gagnon et al., 2018), concentrations (Rossignol et al., 2014), and metabolism of melatonin (Jonsson et al., 2010), disturbed signaling of intracellular melatonin receptor type 1A (Hong et al., 2016), immune signaling dysregulation (Meltzer et al., 2017), as well as the inflammation of the central and peripheral immune systems (Eissa et al., 2020) are found in ASD. A substantial reduction in serum melatonin levels

Masaharu Hayashi and Kazuo Mishima are contributed equally to this study.

✉ Masaharu Hayashi
Masaharu.hayashi@soc.shukutoku.ac.jp

[1] School of Nursing, College of Nursing and Nutrition, Shukutoku University, 673, Nitona-cho, Chuo-ku, Chiba 260-8703, Japan

[2] Department of Neuropsychiatry, Akita University Graduate School of Medicine, Akita, Japan

[3] Segawa Memorial Neurological Clinic for Children, Chiyoda-ku, Tokyo, Japan

[4] CMIC Co., Ltd., Minato-ku, Tokyo, Japan

[5] Nobelpharma Co., Ltd, Chuo-ku, Tokyo, Japan

[6] Department of Pediatrics and Child Health, Kurume University School of Medicine, Kurume, Fukuoka, Japan

is frequently associated with two polymorphisms of the acetylserotonin *O*-methyltransferase gene located in the promoter (rs4446909 and rs5989681) in ASD patients (Wu et al., 2020). Supplemental melatonin has been used to treat difficulties falling asleep in children with ASD, although the mechanism of action is uncertain (Goldman et al., 2014). A meta-analysis (Rossignol et al. 2011) indicated the association of melatonin treatment of ASD children with improved sleep parameters and better daytime behaviors; additional studies of melatonin treatment will help the confirmation and expansion of these findings. Psychiatrists, pediatricians, psychologists, educators, and parents recognize the importance of providing sleep hygiene interventions that are individually optimized to children's life rhythms and of conducting pharmacotherapy for their SPs under the interventions.

We hypothesized that 1- or 4-mg melatonin granules under adequate sleep hygiene interventions would be superior to placebo in efficacy for these children and would have an acceptable safety profile in Japanese children with ASD. We hereby provide clinicians, professionals in related fields, educators, and parents with clinical evidence that was obtained based on not the usual titration but parallel-group controlled design to examine the efficacy and safety of the 1- and 4-mg formulations of oral melatonin in combination with adequate sleep hygiene—first-line therapy for sleep disturbances experienced by children with neurodevelopmental disorders (NDDs) (Blackmer et al., 2016).

Methods

Study Population

Children were eligible when meeting all of the following inclusion criteria: (1) male or female, (2) 6 to 15 years of age at the onset of the pre-monitoring phase—children under the age of 6 years were not included because of considerable variability in nocturnal serum melatonin levels (Waldhauser et al., 1988) and of not reaching schoolable age in Japan, (3) ASD confirmed based on the diagnostic criteria for ASD in Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (American Psychiatric Association, 2013), (4) sleep onset latency (SOL; time between bedtime and sleep onset) of ≥ 30 min on average that persisted for 3 or more months, (5) capable (as with their caregivers) of being cooperative for sleep hygiene interventions, and (6) outpatients. Furthermore, their caregivers were cooperative with the entry in the electronic sleep diary, children and their caregivers could visit the hospital as scheduled, and their surrogate consenters provided written informed consent or competent children afforded written informed consent or assent prior to enrollment.

Sleep hygiene interventions—defined as a set of standardized and positive bedtime routines that promote effective sleep (e.g., activities related to sleep environment, sleep schedule, sleep practices, and physiologic factors) (Abel et al., 2017; Blackmer et al. 2016; Jan et al., 2008)—constitute the first-line therapy for sleep disturbances experienced by children with NDDs (Blackmer et al., 2016; Grigg-Damberger et al., 2013; Jan et al., 2008). We endeavored to ensure the implementation of adequate sleep hygiene interventions by the following approaches: (1) instructing children and their caregivers to acquire favorable sleep–wake cycles and desirable sleep habits through the sponsor-delivered leaflet and the investigators' face-to-face guidance for children and their caregivers; (2) precisely assessing sleep-related variables (e.g., time for medication, bedtime, and time of sleep onset) based on information obtained by means of the electronic sleep diary for which the rigorous rules for data entry were established for caregivers; and (3) monitoring proper adherence of studied children to the abovementioned instructions and guidance.

The key exclusion criteria were as follows: (1) children who had intellectual disabilities that were adjudicated to "severe" or greater in severity concerning one or more conceptual, social, or practical domains; (2) children who had a history of melatonin treatment; (3) children who received a melatonin analog with an indication for the improvement of difficulty falling asleep in insomnia within 4 weeks prior to the onset of the pre-monitoring phase; and (4) children who were complicated by breathing-related sleep disorders, hepatic function disorder, schizophrenia, or bipolar disorder.

Eligible children, who met all of the following criteria that were examined in the last 7 days of the 14-day screening phase, were subsequently included in the 14-day, double-blind, randomization phase: (1) SOL lasting ≥ 30 min over 3 or more days; (2) successful adherence (≥ 5 days) to time for medication and bedtime that were specified for individual children, with allowance ranges of 15 min and 30 min, respectively; and (3) successful adherence (≥ 5 days) to the entry in the electronic sleep diary and/or to medication. All children underwent sleep hygiene interventions at the time of informed consent acquisition.

Study Design

A multicenter, collaborative, three parallel-group, randomized, double-blind, placebo-controlled clinical trial was conducted to examine the efficacy and safety of oral melatonin as compared to placebo in preventing difficulties falling asleep among numerous SPs that children with ASD presented. The study sponsor, Nobelpharma Co., Ltd., provided investigational drugs for this study—melatonin granules and placebo. Nobelpharma Co., Ltd. manufactured placebo, and an independent third party organization

ensured indistinguishability between active drug—1- and 4-mg melatonin granules—and placebo. The trial consisted of the following 5 phases: (1) the 7-day pre-monitoring phase, in which sleep status was checked; (2) the 14-day screening phase, during which SOL was assessed; (3) the 14-day, parallel-group, double-blind randomization phase, in which eligible children were assigned 1:1:1 to receive 1 mg of melatonin (1-mg melatonin group), 4 mg of melatonin (4-mg melatonin group), or placebo (placebo group) once daily (before bedtime), and the drug's effects on SOL and aberrant behaviors were assessed; (4) the 42-day open-label phase, in which all children were ensured to receive the active drug—1-, 2-, or 4-mg melatonin—according to the titration procedure (initial dose: 1 mg) for assessing its clinical benefits and a greater number of children were to be assessed for evaluating the efficacy and safety of the drug in this first randomized clinical trial in Japan; and (5) the 14-day post-monitoring phase, in which withdrawal symptoms or rebound phenomenon was examined. The use of other sleep medications was exclusionary during the study period. The dose levels of melatonin, time for medication, and duration of each phase were determined in consideration of the study designs of previous clinical studies.

Children were recruited at 34 medical institutions in Japan, and the trial protocol was approved by the institutional review boards thereof. Between June 2016 and September 2018, the trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Written informed consent was obtained from the surrogate consenters of children prior to enrollment.

Outcomes

The primary outcome was the change from the median of SOL recorded during the last 7 days of the screening phase (baseline) to the median of SOL recorded during the last 7 days of the randomization phase or before medication discontinuation. Furthermore, caregivers were instructed to enter all of information on the sleep status of their children (i.e., time of drug intake, bedtime, time of sleep onset, and wakening time) in the sponsor-provided electronic sleep diary after awakening; however, the entry exceeding the next night (23:59) was not permitted. The diary, which was installed with the robust, especially developed program to facilitate data entry by caregivers, allowed for the timely collection of information on the sleep variables of their children and ensures, together with objective measures (actigraphy), the more precise assessment of SOL that was established as the primary outcome because previous clinical studies (Gringras et al., 2012; Malow et al., 2012; Wright et al., 2011) had provided the most reliable clinical evidence among a number of sleep-related variables. The key secondary outcomes were as follows: (1) sleep variables assessed with the actigraph (FS-760; ACOS Co., Ltd., Handa, Nagano, Japan)—[SOL, number of wakenings after sleep onset (number of wakenings between time of falling asleep and wakening time), wakening time after sleep onset (total time of staying awake between time of falling asleep and wakening time), total sleep time (TST), and sleep efficiency [(SE) defined as TST divided by time in bed]; (2) changes in aberrant behaviors after melatonin treatment assessed with the aberrant behavior checklist (Aman MG, 1994) Japanese version (ABC-J); and (3) safety of melatonin treatment.

Randomization, Medication, and Sleep Hygiene Interventions

Information on the sleep- and medication-related criteria among the inclusion criteria, which was obtained through the electronic sleep diary, was systematically checked through a program especially developed to the present clinical trial. Subsequently, Medidata Balance® (Medidata Solution, Inc.; New York, NY, USA) was used to allocate the random numbers to eligible children that had been generated by an independent manager. The minimization method for randomization used a history of ramelteon treatment and the median SOL during the last 7 days of the screening phase in an attempt to avoid the skew randomization to either of the 3 study groups of children with the history or a biased median SOL, if any.

Children took melatonin or placebo at 45 min before bedtime as follows: placebo during the screening phase; 1 mg of melatonin, 4 mg of melatonin, or placebo during the randomization phase; and 1 mg of melatonin, increasing to 2 or 4 mg accordingly during the open-label phase—dose escalation was admitted after a minimum of 7-day administration at the previous dose, while dose tapering was permitted without restriction. Patients and personnel who were involved in the present study were blinded to treatment assignments. The doses, time for medication, and study phases were established in consideration of previous clinical studies of melatonin in which its doses ranged between 0.5 mg and 12 mg, time for medication before bedtime between 30 and 45 min, and study duration between 12 and 14 weeks (Gringras et al., 2012; Malow et al., 2012; Wright et al., 2011).

Safety

Adverse events (AEs), laboratory assessments, vital signs, height, weight, withdrawal symptoms, and rebound phenomenon were included in the safety analysis. The preferred terms listed on the Japanese version of the Medical Dictionary for Regulatory Activities version 19.0 (MedDRA, 2018) were used to express AEs and drug-related adverse events.

Statistical Analyses

Efficacy outcomes during the double-blind phase were analyzed in the full analysis (randomized children who received at least 1 dose of a double-blind study drug). Required sample size was calculated to be 180 according to the bootstrap method stimulation under the conditions of ≥ 90% power at 2.5% 2-sided level to conduct 2 between-group comparisons for the 1- and 4-mg melatonin groups and the placebo group. The primary outcome was analyzed according to Steel's test. The safety analysis dataset consisted of children who were randomly assigned to a treatment group and received at least 1 dose of an investigational drug.

Continuous and categorical variables are expressed as medians with the interquartile range, and between-group comparisons were made using Wilcoxon signed rank test. Steel test was conducted to adjust the multiplicity of nonparametric analyses on the primary efficacy outcome. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using SAS 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Subject Disposition and Their Characteristics

A total of 229 children were assessed for eligibility at 34 institutions in Japan (Fig. 1); in 31 excluded children who did not meet the inclusion criteria among them, 4.8% (11/229) were excluded because of the insufficient cooperation of children and/or their caregivers, while SOL failed to meet the inclusion criterion in most of other excluded children. A total of 196 children (male, 61.7%; mean age, 11.2 years) were enrolled. Subsequently, enrolled children were randomly assigned 1:1:1 to the 1-mg melatonin group (n = 65), the 4-mg melatonin group (n = 65), or the placebo group (n = 66); they were followed up for 14 days (final visit, September 14, 2018). The study groups did not show great differences in the demographic and baseline characteristics of randomized children (Table 1). The full analysis set and the safety analysis set included 196 children each.

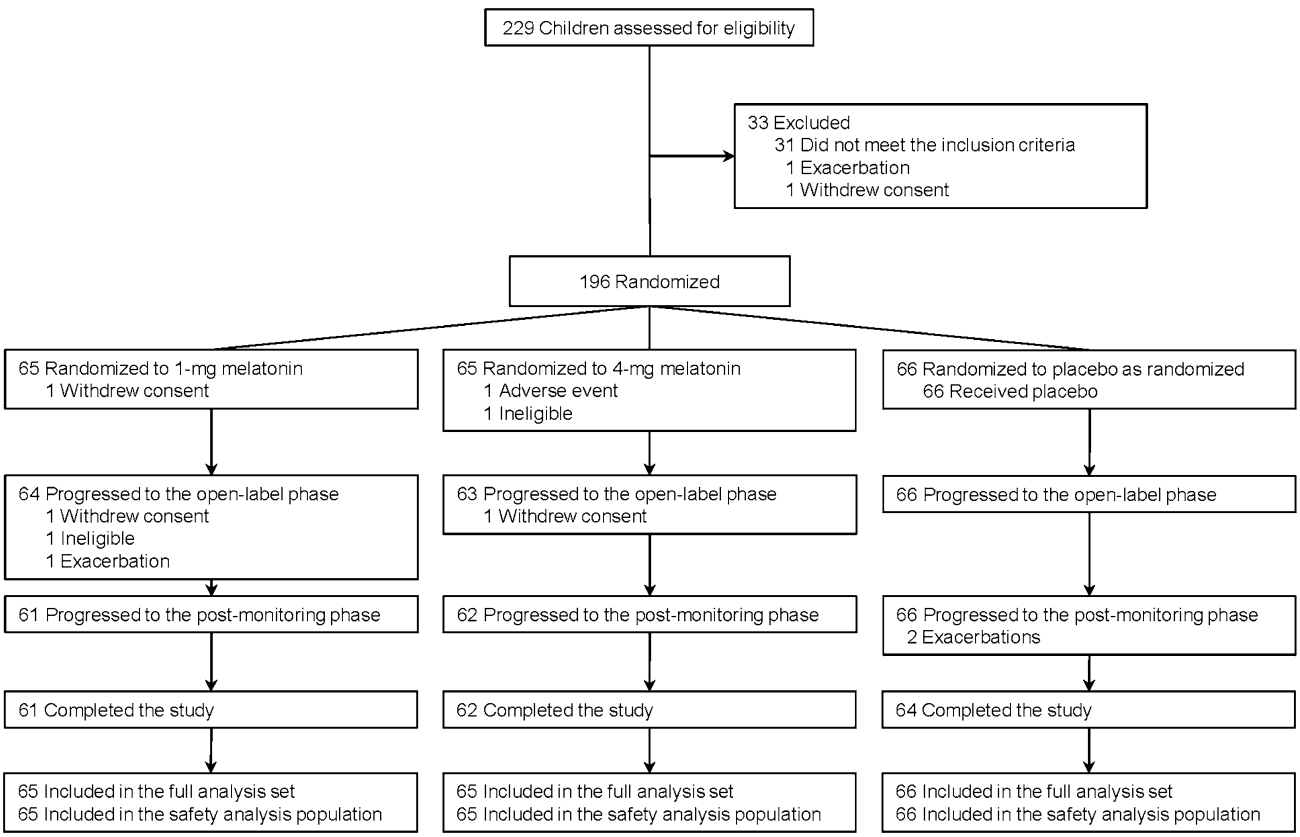

Fig. 1 A diagram depicting child disposition

Table 1 Demographic and baseline characteristics of randomized children, full analysis set

Characteristics	Melatonin groups		Placebo group	Overall
	1-mg (n = 65)	4 mg (n = 65)	n = 66	n = 196
	n (%)	n (%)	n (%)	n (%)
Age, mean (SD), y	10.8 (2.3)	12.0 (2.4)	10.8 (2.6)	11.2 (2.5)
Sex				
Male	39 (60.0)	45 (69.2)	37 (56.1)	121 (61.7)
Female	26 (40.0)	20 (30.8)	29 (43.9)	75 (38.3)
Weight, mean, kg	40.30 (14.72)	45.58 (13.56)	40.00 (15.90)	41.95 (14.91)
< 40	36 (55.4)	23 (35.4)	39 (59.1)	98 (50.0)
≥ 40	29 (44.6)	42 (64.6)	27 (40.9)	98 (50.0)
Height, mean, cm	143.22 (14.71)	150.83 (15.41)	144.71 (16.24)	146.25 (15.74)
< 145	36 (55.4)	21 (32.3)	32 (48.5)	89 (45.4)
≥ 145	29 (44.6)	44 (67.7)	34 (51.5)	107 (54.6)
Neurodevelopmental disorders				
Intellectual disabilities				
Absent	54 (83.1)	57 (87.7)	60 (90.9)	171 (87.2)
Present	11 (16.9)	8 (12.3)	6 (9.1)	25 (12.8)
Communication disorders				
Absent	61 (93.8)	64 (98.5)	64 (97.0)	189 (96.4)
Present	4 (6.2)	1 (1.5)	2 (3.0)	7 (3.6)
Attention-deficit/hyperactivity disorder				
Absent	27 (41.5)	32 (49.2)	29 (43.9)	88 (44.9)
Present	38 (58.5)	33 (50.8)	37 (56.1)	108 (55.1)
Specific learning disorder				
Absent	58 (89.2)	59 (90.8)	62 (93.9)	179 (91.3)
Present	7 (10.8)	6 (9.2)	4 (6.1)	17 (8.7)
Motor disorders				
Absent	59 (90.8)	60 (92.3)	60 (90.9)	179 (91.3)
Present	6 (9.2)	5 (7.7)	6 (9.1)	17 (8.7)
Anamnesis				
Absent	22 (33.8)	26 (40.0)	29 (43.9)	77 (39.3)
Present	43 (66.2)	39 (60.0)	37 (56.1)	119 (60.7)
Concurrent diseases				
Absent	3 (4.6)	5 (7.7)	8 (12.1)	16 (8.2)
Present	62 (95.4)	60 (92.3)	58 (87.9)	180 (91.8)
Sleep onset latency[a], min				
< 50	29 (44.6)	27 (41.5)	29 (43.9)	85 (43.4)
≥ 50	36 (55.4)	38 (58.5)	37 (56.1)	111 (56.6)
History of ramelteon treatment				
Absent	47 (72.3)	48 (73.8)	48 (72.7)	143 (73.0)
Present	18 (27.7)	17 (26.2)	18 (27.3)	53 (27.0)

SD standard deviation

[a]As assessed with the electronic sleep diary

Primary Outcome

During the randomization phase, the changes in the "medians of SOL recorded during the last 7 days of the randomization phase or before medication discontinuation" from the "median of SOL recorded during the last 7 days of the screening phase (baseline)" were as follows: − 5.0 min, − 22.0 min, and − 28.0 min for the placebo group, the 1-mg melatonin group, and the 4-mg melatonin group, respectively. SOL shortened significantly ($p < 0.0001$) more in the melatonin groups than in the placebo group (Table 2). Furthermore, the subgroup analyses

Table 2 Sleep onset latency as assessed with the electronic sleep diary during the clinical trial, full analysis set

Groups	Phase	Visit No.	N	Summary statistics Change[a] from baseline[c], median, min	Steel's test[c] *p* value
Placebo	Pre-monitoring	2	66	–	–
	Screening	3	66	52.5	–
	Randomization	4	66	− 5.0	–
	Open-label	5	66	− 27.3	–
	Open-label	6	66	− 30.0	–
	Post-monitoring	7	65	− 10.0	–
1-mg melatonin	Pre-monitoring	2	64	–	–
	Screening	3	65	51.0	–
	Randomization	4	65	− 22.0	< 0.0001
	Open-label	5	64	− 24.0	–
	Open-label	6	63	− 24.5	–
	Post-monitoring	7	61	− 10.0	–
4-mg melatonin	Pre-monitoring	2	65	–	–
	Screening	3	65	54.0	–
	Randomization	4	65	− 28.0	< 0.0001
	Open-label	5	63	− 25.0	–
	Open-label	6	61	− 24.5	–
	Post-monitoring	7	62	− 11.3	–
Melatonin	Screening	3	193	52.0	–
	Open-label	5	193	− 25.0	–
	Open-label	6	190	− 27.0	–
	Post-monitoring	7	188	− 10.0	–

[a]The median of sleep onset latency recorded during 7 days immediately before each visit—the median of sleep onset latency recorded during 7 days immediately before visit 3

[b]The median of sleep onset latency recorded during 7 days immediately before visit 3

[c]Against the placebo group

of the primary outcome were conducted with respect to sex, age, weight (< 40 kg, ≥ 40 kg), SOL at baseline, history of ramelteon treatment, intellectual disabilities, and attention-deficit/ hyperactivity disorder (ADHD), as well as height (< 145 cm, ≥ 145 cm) (Supplementary Tables S1–8). Consequently, SOL shortened significantly ($p < 0.05$) more in the 1-mg melatonin group than in the placebo group with respect to all subgroups except "female," "previous history of ramelteon treatment," and "≥ 145 cm in height." SOL shortened significantly ($p < 0.05$) in the 4-mg melatonin group than in the placebo group with respect to all subgroups. In the 42-day open-label phase, SOL shortened more in children who had received placebo or 1-mg melatonin in the 14-day double-bind, randomization phase. In the 14-day post-monitoring phase, SOL shortening was sustained although its magnitude reduced. Data missing did not occur during the screening and randomization phases. Dose increment was required for some of studied children. In summary, melatonin treatment provided clinical outcomes that met its primary endpoint and that were better at a greater dose.

Secondary Outcomes

Sleep variables assessed with the actigraph are summarized (Supplementary Table 9). SOL assessed with the actigraph shortened significantly ($p < 0.0001$) more in the 1- and 4-mg melatonin groups (− 21.0 min and − 20.0 min, respectively) than in the placebo group (1.0 min). TST and the number of wakenings after sleep onset did not change in any melatonin groups. SE improved in both the 1- and 4-mg melatonin groups compared to the placebo group, with a statistically significant difference in the 4-mg melatonin group (2.35%, $p = 0.0408$), but not in the 1-mg melatonin group (2.07%, $p = 0.1365$). In the 1-mg melatonin group, wakening time after sleep onset extended significantly ($p = 0.0072$) despite TST and SE tended to improve as compared to the baseline values.

Of special note for physicians, professionals in related fields, and caregivers who are dedicated to the treatment of children with ASD is the fact that during the 14-day, double-blind, randomization phase, there was no significant difference between the melatonin groups and the placebo

group with respect to all of 5 aberrant behaviors assessed with the ABC-J (Supplementary Tables S10–14), although they improved significantly ($p < 0.0001$; except stereotypic behavior, $p = 0.0014$) at the completion of the subsequent 42-day open-label phase.

Safety

During the randomization phase, AEs occurred in 9 (13.8%), 19 (29.2%), and 12 (18.2%) of children in the 1-mg melatonin group, the 4-mg melatonin group, and the placebo group, respectively (Supplementary Table S15). Drug-related AEs occurred in 0 (0.0%), 5 (7.7%), and 3 (4.5%) in the 1-mg melatonin group, the 4-mg melatonin group, and the placebo group, respectively. Neither serious AEs nor death occurred during medication. Somnolence provoked temporary interruption and medication discontinuation in 1 child each in the 1- and 4-mg melatonin groups; the causality with melatonin was denied by the investigator in the former because of having assessed that insomnia was one of the original SPs of the child, but not in the latter. The most predominant AEs were infections and infestations in 3 (4.6%), 7 (10.8%), and 5 (7.6%) in the 1-mg melatonin group, the 4-mg melatonin group, and the placebo group, respectively, followed by nervous system disorders in 2 (3.1%), 4 (6.2%), and 2 (3.0%), respectively. During the open-label phase, infections and infestations occurred most predominantly in 39 (20.2%); the second most predominant AEs were nervous system disorders in 13 (6.7%). Pharyngitis occurred most predominantly: during the randomization phase, 2 (3.1%), 2 (3.1%), and 4 (6.1%) in the 1-mg melatonin group, the 4-mg melatonin group, and the placebo group, respectively; and during the open-label phase, 25 (13.0%) in the melatonin group.

Neither serious AEs nor death occurred during the randomization and open-label phases. Withdrawal symptoms or rebound phenomenon did not occur not only immediately after the completion of the 42-day open-label phase but also during the 14-day post-monitoring phase. During the randomization and open-label phases, melatonin treatment was safe in the vast majority of children examined. Nevertheless, 2 children were admitted to the hospital due to the abrupt deterioration of irritability—one of the aberrant behaviors of ASD—on the next day of the last medication. According to clinical trial regulations in Japan, hospitalization led to categorize the AE serious. We speculate that they were incapable of accommodating themselves to medication discontinuation and developed excessive excitement—an ASD-specific feature. Hence, these episodes advert clinicians to be cautious when modifying the therapeutic intervention for children with ASD.

Discussion

Untreated SPs of children with ASD are extremely diverse and characteristic. Clinicians who treat children with ASD are fully aware that the implementation of adequate sleep hygiene interventions is indispensably required to obtain desirable sleep patterns and eventless daytime activities in an attempt to empower them to sustain good social activities and behaviors during the day (e.g., intercommunication, interaction, and school attendance). The present randomized placebo-controlled trial allowed the quantified assessment of SPs, which clearly demonstrated the efficacy and safety of pharmacotherapy with 1- and 4-mg melatonin when the interventions were appropriately implemented as a therapeutic platform.

Children (< 18 years) with neurodevelopmental disorders have a higher prevalence of SPs (Abdelgadir et al., 2018; Deliens et al., 2019; Limoges et al., 2013; May et al., 2015; Rigney et al., 2018; Rossignol et al., 2011; Veatch et al., 2015a), and the efficacy and safety of melatonin treatment for them were demonstrated in a number of randomized controlled trials that had compared melatonin with placebo or other interventions (Abdelgadir et al., 2018); however, the heterogeneity and inconsistency among these trials limited the overall quality of the evidence obtained (Abdelgadir et al., 2018). Children with ASD showed a greater number of SP episodes and shorter sleep time than controls, and SPs were related to inadequate sleep hygiene (e.g., unhealthy bedtime routines, bad eating habits, excessive light in the bedroom, and uncomfortable room temperature)—a finding that warrants a strong focus on sleep hygiene improvements because many of these children are concurrently affected by both ASD and ADHD (van der Heijden et al., 2018). Since insufficient sleep in children is associated with deficits in higher-order and complex cognitive functions and an increase in behavioral problems (Astill et al., 2012), the development of healthy bedtime routines should always be the first step in the management of sleep disturbances (van der Heijden et al., 2018); further replication studies are required to corroborate this therapeutic strategical approach. SPs may exacerbate core symptoms of ASD [e.g., decreased social communication skills (Veatch et al., 2015b) and increased restricted and/or interests and/or behaviors (Goldman et al., 2011), and poor sleep has a negative impact on children's well-being (Giallo et al., 2013). Additional studies should assess direct relations between sleep/behaviors and sleep hygiene intervention outcomes (Abel et al., 2017). We consider that the present study is salient in the following: (1) study design consisting of 3 parallel-group, double-blind, parallel-group, randomized, placebo-controlled clinical trial—a design

that avoids the ambiguous interpretation of data originating from dose escalation or tapering; (2) adequate sleep hygiene interventions; (3) strictness of data collection (i.e., digitally monitored entry of the sleep data provided by caregivers) and of data interpretation for randomization (i.e., systematic checkup of the satisfied sleep-related criteria among the inclusion criteria); (4) randomized assignment of eligible children with ASD who had SPs even after having undergone adequate sleep hygiene interventions; (5) provision of clinical evidence, which is helpful to the confirmation and expansion of known clinical findings, through the high-quality data that are less limited in overall quality than the case of meta-analysis data derived from multiple clinical studies and corroborates the therapeutic strategic adequacy of melatonin treatment under adequate sleep hygiene interventions because results from the preset study clearly indicate the therapeutic efficacy and safety of our approach for studied children including those with intellectual disabilities and those with ADHD, and (6) suggestion of a need for a longer duration of assessment on the relationship of melatonin treatment under adequate sleep hygiene interventions with aberrant behaviors of children with ASD.

Statistical analyses on the primary outcome data obtained with the electronic sleep diary and the actigraph revealed the significant efficacy of the 1- and 4-mg melatonin groups as compared to the placebo group and the inefficacy of the placebo group in which children had undergone sleep hygiene interventions alone. Furthermore, the subgroup analyses thereof indicated that the 1-mg group melatonin group was not sufficient for children with ASD in the subgroups "$\geq$145 cm in height," "female," and "previous history of ramelteon treatment."; however, the 4-mg melatonin group was significantly effective than the placebo group with respect to all these subgroups, although medication was discontinued due to somnolence in 1 child with ASD during the randomization phase. Hence, caution should be taken to the potential development of somnolence. These data clinically imply that melatonin treatment can be initiated at 1 mg for children with ASD and suggest that melatonin dose incrementation may be required for some of them. The data from the 42-day open-label phase indicate SOL shortening changes in response to the regimen provided in the 14-day, parallel-group, double-blind randomization phase.

TST did not improve significantly in the melatonin groups as compared to the placebo group, presumably due to the facts that many of studied children had the delayed sleep phase disorder. Wakening time after sleep onset was significantly longer in the 1-mg melatonin group than in the placebo group. However, we consider that this finding causes less clinical concerns because TST and SE improved in the 1-mg melatonin group as compared to the baseline values. Melatonin treatment, when compared before and after administration, significantly improved all of 5 aberrant behaviors examined throughout the study period, with the exception of the 14-day double-blind randomization phase in which the melatonin groups were compared with the placebo group. We surmise that these differing results were owing to a small sample size and the short duration of the randomization phase.

Limitations

Our study has several limitations. First, the present clinical study afforded limited findings on the melatonin safety in and aberrant behaviors of studied children with ASD: however, this shortcoming was recently covered by a long-term open-label clinical study of melatonin treatment in children with neurodevelopmental disorders that provided the long-term safety profile of melatonin treatment and its favorable effects on their aberrant behaviors (Yuge et al., 2020). Second, children under the age of 6 years and over the age of 15 years were not included. Therefore, findings from the present study may not be applicable to the relevant children; further clinical evidence originating from repeated melatonin treatment needs to be accumulated in the clinical settings. Third, the threshold for the actigraph used in the present study was not validated for children with ASD. However, the actigraph is a medical device that is very useful to obtain the objective, precise data on their sleep status without influences by caregivers. Therefore, we consider that actigraphic measurement provided a given level of clinical evidence to the monitor body activity of studied children. Fourth, we could not obtain any clinical evidence for precisely determining the time for medication termination nor concretely examining the pathophysiology of 2 children who had deteriorated ASD immediately after medication termination. Fifth, findings from the present melatonin treatment are not appliable to younger children incapable of adhering to the instructions on adequate sleep hygiene interventions. Nevertheless, several applications to record sleep-related variables are currently downloadable to the cell phone of caregivers who are expected to follow adequate sleep hygiene interventions by using them to the extent that their children can address.

Conclusions

Melatonin treatment, when provided under adequate sleep hygiene interventions and with adequate dose adjustments, was shown to be a reasonable therapeutic approach for children with NDDs, especially ASD.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10803-021-05139-w.

Acknowledgments The authors are grateful to all investigators and subinvestigators, and clinical research coordinators at respective medical institutions for child recruitment, data acquisition, and study conduct, to children along with their families for their participation in the present study, and to Satoshi Sakima, MD, for valuable discussions about the manuscript; his effort was compensated by Nobelpharma Co., Ltd.

Author Contributions MH and KM conceived the study. MH, KM, MF, HT, YI, IH, HS, OY, and YY contributed to the study design and planning. IH made the analyses. MH prepared the first draft of the manuscript. MH and KM finalized the manuscript. All authors edited and contributed to the final version of the manuscript and gave final approval to the submitted version.

Funding The present study was funded by Nobelpharma Co., Ltd.

Data Availability The dataset used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. MH reported receiving consulting fees from Nobelpharma during the conduct of the study. KM reported receiving grants from the Japanese Ministry of Health, Labour and Welfare (H29-Seishin-Ippan-001, 19GC1012), the Japanese Ministry of Education, Science, and Technology, and the National Center of Neurology and Psychiatry Intramural Research Grant for Neurological and Psychiatric Disorders, consulting fees from Nobelpharma and Taisho Pharmaceutical, and lecture fees from Eisai, MSD, Takeda Pharmaceutical, Astellas Pharma, and Janssen Pharmaceutical. MF reported receiving grant support from Nobelpharma. YY reported receiving grant support from Nobelpharma. Nobelpharma Co., Ltd. is a company that provided the investigational drugs. HT is an employee of CMIC. YI, IH, HS, and OY are employees of Nobelpharma Co., Ltd.

Ethical Approval This study was performed in accordance with the Declaration of Helsinki. Ethical approval was obtained from the institutional review board at each participating institution.

Informed Consent All children provided written informed ascent/consent and all their caregivers provided written informed consent before taking part.

References

Abdelgadir, I. S., Gordon, M. A., & Akobeng, A. K. (2018). Melatonin for the management of sleep problems in children with neurodevelopmental disorders: A systematic review and meta-analysis. *Archives of Disease in Childhood, 103*(12), 1155–1162. https://doi.org/10.1136/archdischild-2017-314181

Abel, E., Kim, S. Y., Kellerman, A. M., & Brodhead, M. T. (2017). Recommendations for identifying sleep problems and treatment resources for children with autism spectrum disorder. *Behavior Analysis in Practice, 10*(3), 261–269. https://doi.org/10.1007/s40617-016-0158-4

Aman, M. G., & Singh, N. (1994). *Aberrant behavior checklist—community*. Slosson Educational Publishing Inc.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). American Psychiatric Association.

Astill, R. G., Van der Heijden, K. B., Van Ijzendoorn, M. H., & Van Someren, E. J. (2012). Sleep, cognition, and behavioral problems in school-age children: A century of research meta-analyzed. *Psychological Bulletin, 138*(6), 1109–1138. https://doi.org/10.1037/a0028204

Baker, E. K., Richdale, A. L., & Hazi, A. (2019). Employment status is related to sleep problems in adults with autism spectrum disorder and no comorbid intellectual impairment. *Autism, 23*(2), 531–536. https://doi.org/10.1177/1362361317745857

Ballester, P., Martínez, M. J., Javaloyes, A., Inda, M. D., Fernández, N., Gázquez, P., Aguilar, V., Pérez, A., Hernández, L., Richdale, A. L., & Peiró, A. M. (2019). Sleep problems in adults with autism spectrum disorder and intellectual disability. *Autism Research, 12*(1), 66–79. https://doi.org/10.1002/aur.2000

Blackmer, A. B., & Feinstein, J. A. (2016). Management of sleep disorders in children with neurodevelopmental disorders: A review. *Pharmacotherapy, 36*(1), 84–98. https://doi.org/10.1002/phar.1686

Buck, T. R., Viskochil, J., Farley, M., Coon, H., McMahon, W. M., Morgan, J., & Bilder, A. (2014). Psychiatric comorbidity and medication use in adults with autism spectrum disorder. *Journal of Autism & Developmental Disorders, 44*(12), 3063–3071. https://doi.org/10.1007/s10803-014-2170-2

Deliens, G., & Peigneux, P. (2019). Sleep-behaviour relationship in children with autism spectrum disorder: Methodological pitfalls and insights from cognition and sensory processing. *Developmental Medicine & Child Neurology, 61*(12), 1368–1376. https://doi.org/10.1111/dmcn.14235

Eissa, N., Sadeq, A., Sasse, A., & Sadek, B. (2020). Role of neuroinflammation in autism spectrum disorder and the emergence of brain histaminergic system. Lessons also for BPSD? *Front Pharmacol*. https://doi.org/10.3389/fphar.2020.00886

Gagnon, K., & Godbout, R. (2018). Melatonin and comorbidities in children with autism spectrum disorder. *Current Developmental Disorders Reports, 5*(3), 197–206. https://doi.org/10.1007/s40474-018-0147-0

Giallo, R., Wood, C. E., Jellett, R., & Porter, R. (2013). Fatigue, well-being and parental self-efficacy in mothers of children with an autism spectrum disorder. *Autism, 17*(4), 465–480. https://doi.org/10.1177/1362361311416830

Goldman, S. E., Adkins, K. W., Calcutt, M. W., Carter, M. D., Goodpaster, R. L., Wang, L., Shi, Y., Burgess, H. J., Hachey, D. L., & Malow, B. A. (2014). Melatonin in children with autism spectrum disorders: Endogenous and pharmacokinetic profiles in relation to sleep. *J Autism Dev Disord, 44*(10), 2525–2535. https://doi.org/10.1007/s10803-014-2123-9

Goldman, S. E., McGrew, S., Johnson, K. P., Richdale, A. L., Clemons, T., & Malow, B. A. (2011). Sleep is associated with problem behaviors in children and adolescents with autism spectrum disorders. *Research in Autism Spectrum Disorders, 5*(3), 1223–1229. https://doi.org/10.1016/j.rasd.2011.01.010

Grigg-Damberger, M., & Ralls, F. (2013). Treatment strategies for complex behavioral insomnia in children with neurodevelopmental disorders. *Current Opinion in Pulmonary Medicine, 19*(6), 616–625. https://doi.org/10.1097/MCP.0b013e328365ab89

Gringras, P., Gamble, C., Jones, A. P., Wiggs, L., Williamson, P. R., Sutcliffe, A., Montgomery, P., Whitehouse, W. P., Choonara, I., Allport, T., & Edmond, A. (2012). Melatonin for sleep problems in children with neurodevelopmental disorders: Randomised

double masked placebo controlled trial. *BMJ*. https://doi.org/10.1136/bmj.e6664

Hong, L. J., Jiang, Q., Long, S., Wang, H., Tian, Y., Wang, C. K., Cao, J. J., Tao, R. R., Huang, J. Y., Liao, M. H., & Lu, Y. M. (2016). Valproic acid influences mtnr1a intracellular trafficking and signaling in a β-arrestin 2-dependent manner. *Mol Neurobiol, 53*(2), 1237–1246. https://doi.org/10.1007/s12035-014-9085-y

Jan, J. E., Owens, J. A., Weiss, M. D., Johnson, K. P., Wasdell, M. B., Freeman, R. D., & Ipsiroglu, O. S. (2008). Sleep hygiene for children with neurodevelopmental disabilities. *Pediatrics, 122*(6), 1343–1350. https://doi.org/10.1542/peds.2007-3308

Jonsson, L., Ljunggren, E., Bremer, A., Pedersen, C., Landén, M., Thuresson, K., Giacobini, M., & Melke, J. (2010). Mutation screening of melatonin-related genes in patients with autism spectrum disorders. *BMC Medical Genomics*. https://doi.org/10.1186/1755-8794-3-10

Limoges, E., Bolduc, C., Berthiaume, C., Mottron, L., & Godbout, R. (2013). Relationship between poor sleep and daytime cognitive performance in young adults with autism. *Research in Developmental Disabilities, 34*(4), 1322–1335. https://doi.org/10.1016/j.ridd.2013.01.013

Lyall, K., Croen, L., Daniels, J., Fallin, M. D., Ladd-Acosta, C., Lee, B. K., Park, B. Y., Snyder, N. W., Schendel, D., Volk, H., Windham, G. C., & Newschaffer, C. (2017). The changing epidemiology of autism spectrum disorders. *Annual Review of Public Health, 38*, 81–102. https://doi.org/10.1146/annurev-publhealth-031816-044318

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., Christensen, D. L., Wiggins, L. D., Pettygrove, S., Andrews, J. G., Lopez, M., Hudson, A., Baroud, T., Schwenk, Y., White, T., Rosenberg, C. R., Lee, L., Harrington, R. A., Huston, M., … Dietz, P. M. (2020). Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network 11 sites, United States, 2016. *MMWR Surveill Summ, 69*(4), 1–12. https://doi.org/10.15585/mmwr.ss6904a1

Malow, B., Adkins, K. W., McGrew, S. G., Wang, L., Goldman, S. E., Fawkes, D., & Burnette, C. (2012). Melatonin for sleep in children with autism: A controlled trial examining dose, tolerability, and outcomes. *Journal of Autism & Developmental Disorders, 42*(8), 1729–1737. https://doi.org/10.1007/s10803-011-1418-3

May, T., Cornish, K., Conduit, R., Rajaratnam, S. M., & Rinehart, N. J. (2015). Sleep in high-functioning children with autism: Longitudinal developmental change and associations with behavior problems. *Behavioral Sleep Medicine, 13*(1), 2–18. https://doi.org/10.1080/15402002.2013.829064

MedDRA (2018). Medical dictionary for regulatory activities. Available at: https://meddra.org. Accessed 22 November 2018.

Melke, J., Botros, H. G., Chaste, P., Betancur, C., Nygren, G., Anckarsäter, H., Rastam, M., Ståhlberg, O., Gillberg, I. C., Delorme, R., & Chabane, N. (2008). Abnormal melatonin synthesis in autism spectrum disorders. *Molecular Psychiatry, 13*(1), 90–98. https://doi.org/10.1038/sj.mp.4002016

Meltzer, A., & Van de Water, J. (2017). The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology, 42*(1), 284–298. https://doi.org/10.1038/npp.2016.158

Rigney, G., Ali, N. S., Corkum, P. V., Brown, C. A., Constantin, E., Godbout, R., Hanlon-Dearman, A., Ipsiroglu, O., Reid, G. J., Shea, S., & Smith, I. M. (2018). A systematic review to explore the feasibility of a behavioural sleep intervention for insomnia in children with neurodevelopmental disorders: A transdiagnostic approach. *Sleep Medicine Reviews, 41*, 244–254. https://doi.org/10.1016/j.smrv.2018.03.008

Rossignol, D. A., & Frye, R. E. (2011). Melatonin in autism spectrum disorders: A systematic review and meta-analysis. *Developmental Medicine & Child Neurology, 53*(9), 783–792. https://doi.org/10.1111/j.1469-8749.2011.03980.x

Rossignol, D. A., & Frye, R. E. (2014). Melatonin in autism spectrum disorders. *Current Clinical Pharmacology, 9*(4), 326–334. https://doi.org/10.2174/1574884711308666007 2

van der Heijden, K. B., Stoffelsen, R. J., Popma, A., & Swaab, H. (2018). Sleep, chronotype, and sleep hygiene in children with attention-deficit/hyperactivity disorder, autism spectrum disorder, and controls. *European Child & Adolescent Psychiatry, 27*(1), 99–111. https://doi.org/10.1007/s00787-017-1025-8

Veatch, O. J., Goldman, S. E., Adkins, K. W., & Malow, B. A. (2015). Melatonin in children with autism spectrum disorders: How does the evidence fit together? *Journal of Natural Sciences, 1*(7), e125.

Veatch, O. J., Maxwell-Horn, A. C., & Malow, B. A. (2015). Sleep in autism spectrum disorders. *Current Sleep Medicine Reports, 1*(2), 131–140. https://doi.org/10.1007/s40675-015-0012-1

Waldhauser, F., Weiszenbacher, G., Tatzer, E., Gisinger, B., Waldhauser, M., Schemper, M., & Frisch, H. (1988). Alterations in nocturnal serum melatonin levels in humans with growth and aging. *Journal of Clinical Endocrinology & Metabolism, 66*(3), 648–652. https://doi.org/10.1210/jcem-66-3-648

Wright, B., Sims, D., Smart, S., Alwazeer, A., Alderson-Day, B., Allgar, V., Whitton, C., Tomlinson, H., Bennett, S., Jardine, J., McCaffrey, N., Leyland, C., Jakeman, C., & Miles, J. (2011). Melatonin versus placebo in children with autism spectrum conditions and severe sleep problems not amenable to behaviour management strategies: A randomised controlled crossover trial. *Journal of Autism & Developmental Disorders, 41*(2), 175–184. https://doi.org/10.1007/s10803-010-1036-5

Wu, Z. Y., Zou, J. J., Wang, Q. X., Naveed, M., Bao, H. N., Wang, W., Fukunaga, K., & Han, F. (2020). Autism spectrum disorder (ASD): Disturbance of the melatonin system and its implications. *Biomedicine & Pharmacotherapy*. https://doi.org/10.1016/j.biopha.2020.110496

Yuge, K., Nagamitsu, S., Ishikawa, Y., Hamada, I., Takahashi, H., Sugioka, H., Yotsuya, O., Mishima, K., Hayashi, M., & Yamashita, Y. (2020). Long-term melatonin treatment for the sleep problems and aberrant behaviors of children with neurodevelopmental disorders. *BMC Psychiatry, 20*(1), 445. https://doi.org/10.1186/s12888-020-02847-y