CHAPTER 8

Pharmacokinetics of prolonged-release melatonin mini-tablets in children with both autism spectrum disorder and a sleep disorder

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ABSTRACT

Sleep problems are highly prevalent among children with autism spectrum disorder (ASD), and these problems can significantly impact both the child and the child's family. Because conventional therapies are generally unsatisfactory, there is currently an urgent need for an effective intervention. Melatonin is effective for ameliorating sleep problems in this pediatric population, and its favorable short-term and long-term risk profile has led to the increasingly widespread clinical use of melatonin as an off-label medicine. Currently, no age-appropriate prolonged-release melatonin preparation is commercially available. Here, we performed a pharmacokinetics crossover study of Circadin 1 mg mini-tablets, a prolonged-release melatonin formulation, in 16 children and adolescents with both ASD and a sleep disorder. We tested 2 mg and 10-mg doses of Circadin based on the dose range we will use in an upcoming efficacy trial. Whole saliva samples were collected using passive drool collection, and melatonin concentration was measured in these samples. Adverse events were monitored throughout the study, and potential sedative effects were assessed using the Observer's Assessment of Alertness/Sedation (OAA/S) scale for 10 hours after administration. Pharmacokinetics parameters for melatonin were estimated using non-compartmental modeling. All 16 subjects (age range: 7-15 years) had a clinical diagnosis of autism spectrum disorder (based on DSM-IV-TR criteria). Mini-tablets were found to be both safe (i.e., none of the children choked) and acceptable to the children. The melatonin concentration peaked within two hours of administration and remained elevated for several hours thereafter. Circadin exposure was dose-linear, and clearance (1,000 L/hr) was similar between the dose groups. The median apparent terminal half-life was comparable between dosages. All reported side effects were consistent with known side effects. The highest levels of sedation (assessed using the OAA/S) were observed between 2 and 3 hours after administration of Circadin 2 mg and between 2 and 6 hours after administration of Circadin 10 mg. In conclusion, this study demonstrates the short-term safety, acceptability, and prolonged-release profile of Circadin mini-tablets in 16 school-age children and adolescents with ASD.

Introduction

Sleep problems are highly prevalent among children with autism spectrum disorder (ASD)[1,2], a very common spectrum of neurodevelopmental disorders that is characterized by impaired communication and social interaction together with restrictive and repetitive behaviors. Increased sleep latency, waking during the night, and difficulty awakening in the morning are among the most frequently reported problems in children with ASD[3–6]. These sleep problems can become prominent as early as two years of age, and they can persist for many years. Sleep disorders in these children can place a significant burden on the child's physical and mental health, thereby negatively affecting performance in school and creating stress for the child's family[7–12]. In addition, these problems are usually resistant to conventional sleep medications (e.g., benzodiazepines) or antipsychotic agents[12,13] – which in itself can cause adverse side effects or can lead to interactions with other drugs, and their use is contraindicated in some cases. Combining these drugs with behavioral approaches – which are generally difficult to apply, time-consuming, and usually require skilled expertise – is often unsatisfactory. Thus, there is an urgent need for an effective intervention[3,14].

Chronic sleep-wake disorders in children with ASD are associated with an inability to synchronize the circadian rhythm-generating biological system with environmental *zeitgebers*, thus resulting in abnormal melatonin secretion[15,16]. Moreover, a shift in peak melatonin secretion may underlie sleep-onset problems, whereas reduced rhythm amplitude may underlie sleep interruptions and early morning awakening[4]. Melatonin's efficacy in ASD was recently classified as grade A[17]. These findings – together with the observation that melatonin has a highly favorable short-term and long-term risk profile – have led to the increasingly widespread clinical use of melatonin as an off-label medicine. However, the quality of many melatonin preparations is questionable, as these preparations are available as a health supplement in some countries (for example, in the United States) and are therefore not necessarily manufactured in accordance with good manufacturing practice. In addition, no prolonged-release preparation for use in children is commercially available, even though prolonged-release preparations are preferred over fast-release preparations; prolonged-released formulations provide sustained blood levels[18] and are more useful for maintaining sleep[19]. Importantly, although young children may be able to swallow small tablets, they cannot swallow large tablets, and children with ASD can have difficulty swallowing tablets at any age. Therefore, there is a need for a reliable, commercially available, age-appropriate prolonged-release melatonin preparation that is licensed for use in children with ASD and sleep problems.

Circadin 2 mg is a prolonged-release melatonin formulation that is licensed in the EU for treating primary insomnia in patients 55 years of age and older. Previous studies demonstrated that Circadin 2 mg is both safe and efficacious in children and adolescents with neurodevelopmental and behavioral disorders[19–21]. In order to provide its intended release profile, Circadin tablets must be swallowed whole. An age-appropriate formulation was developed in the form of mini-tablets, which have the same *in vitro* dissolution profile as regular-size Circadin tablets and should therefore produce a concentration-time profile that mimics the physiological rhythm (depending on the amount and timing of the dose).

Here, we performed a pharmacokinetics (PK) crossover study in children with both ASD and a sleep disorder. This initial study will be followed by a randomized, double-blind, placebo-controlled study to investigate the efficacy and long-term safety of Circadin mini-tablets in patients with a neurodevelopmental disorder. To minimize study-related burden and risk, melatonin concentration was measured non-invasively from saliva samples. Prior to the study, PK modeling and simulations were performed in order to determine the minimum number of sampling time points that would be needed and to adapt the sampling time points to accommodate the subjects as much as possible.

Methods

Subjects

Sixteen children with a DSM-IV-TR diagnosis of autistic spectrum disorder (ASD) with current sleep disorder were recruited from specialized child mental health clinics in the Netherlands. Current sleep disorder was defined as difficulty initiating or maintaining sleep, or non restorative sleep, for at least 1 month (DSM-IV 307.42). Subjects with an untreated medical or psychological condition that could be the etiology of sleep disturbances (e.g., restless leg syndrome) were not allowed to participate. The subject had to be able to comply with taking the study drug, collaborate freely with the study procedures and understand instructions in Dutch. Written informed consent was obtained prior to study initiation from parents having parental responsibility or from the legal guardian(s). In children aged 12 years or older, the written informed consent of the child was needed in addition to that of parents having responsibility/legal guardian. We excluded subjects with a history of difficulty with swallowing or easy choking, current symptoms suggestive of obstructive sleep apnea syndrome or any breathing related sleep disorders or periodic limb movements, or non-stable epileptic attacks within 3 months prior to screening (in case of a history of epilepsy). Other exclusion criteria included known clinically significant disturbance(s) in hepatic and/or renal function, current asthmatic symptoms, clinically relevant periodontal disease and/or oral injuries, pregnancy (at time of screening), known allergy to melatonin, and unstable use of allowed medication within 2 months prior to the screening. The subjects were not allowed to use any form of melatonin within one week prior to dosing. In addition, other concomitant medication was also not allowed within one week prior to study days, except for paracetamol, oral contraceptives or some topical medication (i.e., creams, ointments, gels or lotions used to induce a local effect without systemic exposure). Drugs or dietary supplements that can cause pharmacokinetic interactions with melatonin (by inhibition or induction of involved CYP450 isoenzymes), can alter melatonin secretion or release (like (in)direct beta-sympathicomimetic or -sympathicolytic drugs) or affect melatonin's mode of action (like $GABA_A$ receptor modulators and antidepressants) were not allowed from one week to the first occasion, throughout the study. The subjects were allowed to continue the use of the antipsychotics aripiprazole and risperidone, and of non-enzyme-inducing antiepileptic drugs. Stimulant drugs (methylphenidate, dexamphetamine) had to be discontinued at least 5 times the half-life of the specific drug to ensure complete wash-out during study days. On study days, the subjects had to refrain from aspirin use or drugs that contain ibuprofen. The subjects had to refrain from consumption of caffeine-containing products for 24 hours before each occasion until the end of the study day. On study days, the subjects had to refrain from eating bananas and chocolate and from drinks containing articifical colorants, caffeine or alcohol during the entire day. In addition, administration of medication and caffeine was questioned and a urine drug screen and pregnancy test were performed before any study-related procedures were started. The Central Committee on Research involving Human Subjects approved the study protocol.

Study design

This was an open label, single ascending dose, crossover study of 2 and 10 mg Circadin mini-tablets. The first occasion included a 24-hour baseline measurement day. This study followed a staggered approach: the study was completed in at least four children aged 11-17 years before commencing in the group of children 10 years of age or younger. Pubertal stage was assessed at screening. Female subjects were not studied during the stop week of their oral contraceptives. The subjects were confined to the clinical research unit for approximately 24 hours after melatonin administration. Subjects were given a 'paskaart' (stopping card) which he or she could show to indicate a study related activity should be stopped. The use of this card was explained during the screening and start of each occasion. The 'Gedragscode verzet minderjarigen' (Code of conduct in case of resistance in minors) and 'Gedragscode verzet

bij mensen met een verstandelijke handicap' (Code of conduct for physicians involved in the assessment of expressions of objection by people with mental disabilities) were to be followed in case a subject displayed resistance against any study related activity.
As this was an exploratory pharmacokinetic study in children and adolescents with ASD, no formal power calculation was performed. However, based on previous experience, it was expected that a sample size of 16 subjects would suffice for determination of PK parameters in saliva.

Interventions

Circadin mini-tablets contain 1 mg of the active substance N-acetyl-5 methoxy-tryptamine, which is identical to endogenous melatonin. The mini-tablets were produced via traditional tableting methods, including dry-mixing and direct compression. A coating was applied to enhance palatability and reduce susceptibility to light (as melatonin is light sensitive). Most of the published studies as well as off-label use in clinical practice have shown that in the pediatric population melatonin efficacy is achieved in a certain range of doses, mainly 2-12 mg. Therefore, a unit dose of 1 mg was chosen to allow maximal flexibility in dose modification. Based on the anticipated dose range in the subsequent efficacy trial, dosages of 2 and 10 mg were included. In clinical practice, Circadin is taken in the evening, 1-2 hours before the child's usual bedtime. However, in order to avoid sleep disturbance due to repeated nighttime saliva sampling after melatonin administration, it was decided to administer melatonin during daytime (in the morning) instead of in the evening to minimize burden related to study participation. PK simulation indicated that the PK profile could be accurately evaluated after daytime administration (see Figure 1).

Circadin was administered in the morning immediately after a standardized breakfast. Administration was done in sitting position and in bright light (≥2,500 lux). To ensure that the mini-tablets were ingested in whole, mini-tablets could be taken together with standardized amounts of strawberry jam (5 ml), strawberry yoghurt (5 ml), orange juice (10 ml), semi-skimmed milk (10 ml) or water (10 ml). Melatonin has been shown to be stable in these common liquids and foods tested for up to 6 hours at room temperature (no degradation peak)[22]; hence it was unlikely that mixing the mini-tablets with these vehicles would affect melatonin dose. When mini-tablets were mixed in such a vehicle, it was recommended that it should be administered to the subject immediately. If immediate administration was not possible, the mixture was thrown away and a fresh dose was prepared using reserve mini-tablets. If the mini-tablets were administered mixed with a vehicle on the first occasion, mini-tablets were also administered in the same vehicle on the second occasion.

Swallowing was carefully observed. After deglutition the subject's mouth was inspected by the investigator using a flashlight and outcome of drug administration was scored as following[23]: (1) swallowed (no chewing during deglutition and no solid residuals found during oral inspection); (2) chewed (swallowed most of the tablet pieces, but small residuals found during oral inspection); (3) spat out (no observed deglutition and no solid found during oral inspection); (4) choked on (the mini-tablet was inhaled or a cough was caused during swallowing), or (5) refused to take (all actions preventing the physician/(co-)investigator/caretaker placing the mini-tablet in the mouth). In case of unsuccessful administration (e.g., spitting or visible chewing without other clear signs of obstruction), an attempt was made to administer a new dose using reserve mini-tablets available for the occasion. In case of visible chewing, the occasion was terminated and the new dose was given at least 7 days later. In addition, a saliva sample was obtained two minutes after ingestion, for determination of elevated melatonin concentrations (compared to pre-dose levels) due to contamination.
Standardized meals were provided following drug administration. Water (200 ml) was given every 2 hours post-dose to maintain all subjects on a uniform hydration schedule.

The wash-out period in-between occasions was at least 7 days and melatonin was administered at the same time of day in all subjects to avoid confounds from circadian variability.

Pharmacokinetic methods

SALIVARY MELATONIN COLLECTION AND BIOANALYSIS

For children who normally fall asleep before 12 hours prior to melatonin administration, saliva samples for baseline melatonin concentration determination were collected on the first occasion at t= -24, -20, -16 hrs, just before normal time of falling asleep, at a time point after falling asleep but before t=-5 hrs when the child wakes up on its own, -5 hrs, immediately after waking up, and 0 hrs pre-dose (not more than 10 min before dosing). For children who normally fall asleep after 12 hours prior to melatonin administration, baseline samples were collected at t= -24, -20, -16, -12 hrs, just before normal time of falling asleep, -5 hrs, immediately after waking up and 0 hrs pre-dose (not more than 10 min before dosing). Time points for PK sampling after administration were determined based on PK simulations (Figure 1). Whole saliva samples were collected by passive drool using the Saliva Collection Aid (Salimetrics Europe), a collection aid designed to simplify collection of whole saliva, hereby increasing subject compliance. The vented design helps avoid sample foaming and the device is constructed of polypropylene to avoid sample retention or contamination. Absorption loss and analyte degradation were further minimized by the use of low-protein binding storage cryovials (Salimetrics, UK), which are suitable for use with this collection aid. A minimum sampling volume of 1 ml was needed. Subjects and caregivers were trained prior to the first occasion on how to collect saliva using this collection aid. As bright light can suppress endogenous melatonin production, collection of saliva samples was done in dim light (< 8 lux). Plasma and salivary melatonin concentrations have been reported to increase when moving from a supine to a standing position and decrease when these positions are reversed, due to changes in plasma volume[24]. Therefore, saliva samples were collected after spending at least 15 minutes in a sitting position. Deviations were allowed during the night in order to minimize the duration that subjects need to be awake for saliva sampling; in these cases, duration of time spent in sitting position was recorded as a note.

Subjects had to rinse their mouths out with water 10 minutes before each saliva collection and were instructed not to express mucus or sputum from the back of the throat into the collection tube. Saliva was collected in tubes wrapped in aluminum foil to protect the samples from light. After saliva collection, saliva was transferred to duplicate transport tubes (also wrapped in aluminum foil; approximately 1 ml per tube) and stored within 2 hours after collection at a maximum temperature of -20°C. Saliva melatonin concentrations were determined by ABL (Analytisch Biochemisch Laboratorium BV, Assen, The Netherlands) using a LC-MS/MS method (precision and accuracy, CV ≤ 15%; validated range, 2 – 20,000 pg/ml).

URINARY 6-SULPHATOXYMELATONIN CONCENTRATIONS

Urine samples were collected for determination of 6-sulphatoxymelatonin (6-SMT). Urine was collected after spontaneous voiding in potty-trained children or using a urine collection bag in prepubertal non-potty-trained children. All urine passed over 12 hour periods was collected into a standard urine bottle. The total volume was measured and recorded and approximately 5 ml was kept at -20°C. No preservative was required, as 6-SMT is stable in urine for one day at room temperature, 2 days at 4°C and for at least 2 years at -20°C. 6-SMT concentrations were determined by ABL (Analytisch Biochemisch Laboratorium BV, Assen, The Netherlands) using a qualified radioimmunoassay (^{125}I label) (Stockgrand Ltd.; validated upper limit of quantification, ULOQ 2,500 pg/ml).

Safety

At screening, pregnancy testing was done in females who had a menstrual cycle using a qualitative, color immuno-chromatografic detection of HCG in urine using CARDS O.S. H.C.G.-urine test kits (Pacific Biotech, Inc., San Diego, CA 92121, USA). Adverse events were monitored throughout the study.

Assessment of sedation

As Circadin was given in the morning, it was anticipated that daytime sleepiness and slight lowering of body temperature could occur. As children with ASD may be unable to verbally describe level of sedation, the Observer's Assessment of Alertness/Sedation (OAA/S) Scale was used pre-dose and 1, 2, 3, 6, and 10 hours after melatonin administration. This scale was developed to more objectively measure the level of alertness in subjects who are sedated and has been shown to be reliable and valid and to be sensitive to the effects of for example midazolam[25] and is comparable to the Ramsey scale which is commonly used in the pediatric intensive care unit to assess level of consciousness[26].

Statistical analysis

PHARMACOKINETIC ANALYSIS

Exploratory individual and summary concentration-time profiles of melatonin and 6-SMT were generated to identify potential outliers. Observations below the lower limit of quantification were excluded from the analysis. Saliva samples taken 2 minutes after dosing ('contamination samples') were excluded from analysis, as these were unlikely to reflect actual (excreted) melatonin saliva concentrations. PK parameters were estimated using R (v2.12.0, R Foundation for Statistical Computing, Vienna, Austria, 2010).

Results

Subjects

A total of 16 subjects (12 male, 4 female) with ASD were enrolled in 2013-2014. The average age was 10 years (range: 7-15 years), and the average weight was 40.6 kg (range: 26-67 kg). Most subjects were prepubescent (Tanner Stage 1, 50%), with fewer subjects having an intermediate pubescent stage (Stage 2, 19%; Stage 3 and 4, 6% each) or having adult features (Stage 5, 19%). Comorbidities included 22q11 deletion syndrome, migraine, asthma, eczema, ADHD, cystitis, and constipation. The medications that were continued during the study included propranolol (10 mg twice daily, by one subject), flixotide (250 µg twice daily, by two subjects), ventolin (200 µg as needed, by one subject), desloratine (1 mg twice daily, by one subject), macrogol (6.9 g once daily, by one subject), and an antibiotic treatment (unspecified, for cystitis, by one subject). Antipsychotic agents (aripiprazole, 1-3 mg once daily, by two subjects; and risperidone, 0.5 mg once daily, by three subjects) were also continued during the study. Any disallowed concomitant medication was discontinued prior to the study in accordance with the protocol. Seven subjects had used melatonin prior to the study. During the study, all administrations were successful, and none of the subjects choked or visibly chewed on the study medication. All of the subjects completed the study. Subject 9 (a 45-kg 13-year-old boy with Tanner Stage 5) and subject 10 (a 58-kg 15-year-old boy with Tanner Stage 5) received an incorrect melatonin formulation that did not comply with the intended *in vitro* release profile. Thus, the data obtained from these two subjects were excluded from the PK analysis and evaluation of the OAA/S scores; however, these two subjects were included in the safety analysis. Both of these subjects used melatonin prior to the study. In addition, it is suspected that three subjects (subjects 5, 6, an 11) held the tablets in their mouth for a long time after administration of 2 mg Circadin, resulting in measured melatonin levels at 1 hour which exceeded the levels of other subjects (up to 10-fold) probably because melatonin was released in their mouth before swallowing. These children were excluded from the sub-analysis PK population.

Concentration-time profiles of melatonin in saliva

For the endogenous melatonin concentrations measured prior to administration of the Circadin mini-tablets, each individual had several observations that exceeded the assay's lower limit of quantification (LLOQ; i.e., >2 pg/ml). For 14 out of 16 subjects, no saliva samples were taken from 23:00 (11:00 PM) through 7:00 AM in order to minimize patient burden.

In one subject (subject 4), the melatonin concentration measured in the saliva sample taken at t=3 hours exceeded the assay's upper limit of quantification (ULOQ; i.e., >20,000 pg/ml). Because the number of freeze/thaw cycles for this sample exceeded the number of validated cycles, this observation was excluded from further analysis. None of the other PK samples had a concentration that exceeded ULOQ. Three and 8 samples that were taken two minutes after administration of 2 and 10 mg ('contamination samples') had a concentration that exceeded the ULOQ.

The apparent terminal elimination rate constant and its derived parameters – including the apparent terminal half-life, the area-under-the-curve (AUC) from dosing to infinity (including extrapolated percentages), the apparent drug clearance rate, and the apparent volume of distribution – were excluded for subject 6 (on the 2 mg dosing occasion) and subject 14 (on both occasions), as this parameter could not be accurately estimated for these occasions based on the regression plots.

The individual concentration-time profiles are presented in Figure 2. The melatonin concentration peaked within two hours of administration and remained elevated for several hours thereafter (Figure 3). Circadin exposure (derived from the AUC data) was dose-linear, and apparent clearance (approximately 1,000 L/hr) was similar between the dose groups (Table 1). The median apparent terminal half-life was similar between the 2-mg and 10-mg dose groups. The apparent volume of distribution in the saliva decreased (with corresponding clearance) with increasing dose.

Urine 6-sulphatoxymelatonin concentrations

Urine 6-sulphatoxymelatonin concentrations in samples collected during the first 12 hours of administration exceeded ULOQ (i.e., >2,500 pg/ml) for 1/14 subjects following a 2-mg dose and for all 14 subjects following a 10-mg dose. The mean total 6-SMT recovered from urine during the baseline period was 4.2 µg/12 daytime hours and 13.5 µg/12 nighttime hours. Following administration with Circadin 2 mg, the mean amount of total 6-SMT recovered from urine was 989.5 µg/12 daytime hours and 95.3 µg/12 nighttime hours. Following administration with Circadin 10 mg, many of the 6-SMT values were above the ULOQ during the collection period 0-12 hours following dosing (further dilution of the samples was not validated).

Sedation level

Shortly after waking up in the morning (at the time point of the first assessment of the level of sedation), several subjects assessed by the Observer's Assessment of Alertness/Sedation Scale transitory scored 'drowsy/normal speech'; one subject was scored 'drowsy' just prior to the administration of Circadin 10 mg (Figure 4). Following morning administration of Circadin (2 mg or 10 mg), most subjects assessed by the Observer's Assessment of Alertness/Sedation Scale scored to have a mild increase in sedation. The highest levels of sedation were observed between 2 and 3 hours after administration of Circadin 2 mg and between 2 and 6 hours after administration of Circadin 10 mg, with up to 6/14 (2 mg) and 7/14 (10 mg) of children scoring 'drowsy to normal speech' or a higher sedation score.

Adverse effects

In the 16 subjects, the following adverse events were potentially or likely associated with the administration of 2 and/or 10 mg Circadin: fatigue (in seven and eight subjects after 2 and 10-mg doses, respectively), a sensation of heaviness (in one subject after a 2-mg dose), somnolence (in three and two subjects after 2 and 10-mg doses, respectively), falling asleep (in two subjects after both the 2 mg and 10-mg dose), headache (in one and three subjects after 2 and 10-mg doses, respectively), and nausea (in one subject after a 10-mg dose). All of the adverse events were transient and mild, and no serious adverse events were reported. Nausea, fatigue and headache were more frequently reported following Circadin 10 mg compared with Circadin 2 mg.

Questionnaire regarding participation

Overall, the subjects and their caregivers were positive about the burden and duration of the study. Nearly all subjects (69%) and caregivers (88%) stated that they would consider (consent for) participating in a similar study again. Most of the children did not find the saliva sampling to be bothersome. The four subjects who indicated that saliva sampling was somewhat bothersome were 10, 11, 13, and 15 years old. The one child who would not participate again found participating in the study rather nice, but found the study day duration long and the urine sampling a bit bothersome. The one parent who would not consent for participation in similar research found the child's participation very nice, but thought saliva and urine sampling were a bit bothersome, as did the child. The child itself found participating not so nice and did not know if it would participate again.

Discussion

This is the first study to investigate the pharmacokinetics (PK), acceptability, and short-term safety of a new prolonged-release melatonin formulation in children with ASD. The ability to deliver pediatric medicines accurately and safely is essential in order to ensure that the correct dose is received and that the formulation is safe, easy to use, and acceptable from both the child's and the caregiver's perspective. Due to age-related differences in the anatomy of the buccal cavity[27], young children may not be able to swallow a large tablet, and children with ASD or other neurodevelopmental disorder might experience difficulty swallowing a tablet at any age. Therefore, Circadin mini-tablets were developed as an age-appropriate formulation for this patient population. These 3-mm diameter mini-tablets were produced using traditional tableting methods (i.e., dry-mixing and direct compression) that can be reproduced easily and do not require sophisticated manufacturing equipment. A coating was applied to the tablets to enhance their palatability and to reduce the active ingredient's exposure to light. Previous studies showed that this type of formulation is safe for use in healthy pre-school and school-age children up to six years of age[28 23]. In our study, this formulation was ingested safely (i.e., none of the children choked) and was acceptable to the children. However, children who had difficulty swallowing or had a history of choking easily were excluded from the study; therefore, the results may not be directly applicable to this population.

Because Circadin mini-tablets have the same *in vitro* dissolution profile as regular-size Circadin tablets, the melatonin concentration-time profile of the mini-tablets was expected to mirror the profile of regular-size Circadin tablets. The PK properties of Circadin tablets have been investigated in both adults and children, as well as in children and adolescents with neurodevelopmental and behavioral disorders[19–21]. For our study, we chose 2-mg and 10-mg doses based on the anticipated dose range in an upcoming efficacy trial in which the children will initially receive 2 mg Circadin, after which they will have the option to modify the dose, first increasing to 5 mg, and then increasing to 10 mg if the lower doses are not efficacious. Based on the results of a recent randomized clinical trial in which the long-term effects and safety of melatonin were investigated in children[29], we anticipate that some of the children in our upcoming study will need a dose of 10 mg. Other studies[18,30] suggest that per kg body weight, children require a higher amount of melatonin than adults[19]; this difference may be due to the higher endogenous melatonin levels in healthy children, as prepubescent children metabolize melatonin faster than adults[31 32]. Ideally, the *in vivo* release of Circadin mini-tablets should peak within 2-4 hours of administration, and the levels should remain elevated for 4-5 hours thereafter. In our study, salivary drug concentrations were measured starting one hour after administration (and thereafter), and the oral cavity was rinsed after the mini-tablets were swallowed in order to minimize residual oral contamination. The melatonin concentration peaked within two hours following treatment with both 2 and 10 mg Circadin mini-tablets, and the concentration remained elevated for several hours thereafter. Circadin exposure in the saliva was dose-linear, and clearance in the saliva was similar

between the two dose groups. Determination of the melatonin metabolite, 6-SMT, in urine indicated extensive metabolism and excretion in the first 12 hours following dose administration. Consistent with previous research[33 34], melatonin PK in saliva varied widely. Given the fairly consistent shape of the individual saliva curves, and given the known variability of melatonin in plasma[33 34], this variability is likely due to variability in absorption and clearance, rather than variability in the saliva-to-plasma ratio. For example, considerable variability in bioavailability has been reported in healthy adults, with values ranging from 0.01 to 0.3[35].

Following melatonin ingestion, melatonin saliva and plasma concentrations are closely correlated[34], and orally administered melatonin emerges in the serum and saliva with nearly parallel time courses[33]. Assuming that the average saliva-to-plasma ratio is 0.37 after the administration of melatonin (based on previous research[33]), the mean estimated maximum plasma concentration after administration of Circadin 2 mg mini-tablets in children with ASD may actually be higher than the reported maximum plasma concentration in healthy adult volunteers with similar exposure (based on the estimated AUC in plasma). After administration of Circadin 2 mg tablets, the average C_{max} plasma value in adults was 483-1000 pg/ml. However, these results are difficult to interpret, as the groups varied in both age and gender, factors that can affect melatonin kinetics. For example, women generally have a C_{max} value that is four-fold higher than men. If exogenous melatonin appears in the plasma with the same time course as in saliva, the maximum concentration in the plasma after taking Circadin 2 mg mini-tablets will be reached earlier in children with ASD than in healthy adults after taking Circadin 2 mg tablets. This difference could be due to a faster absorption rate for mini-tablets compared to conventional sustained-release tablets (due to the larger surface area of the mini-tablets). In healthy adults, a half-life of 6 hours has been reported after taking Circadin 2 mg tablets. In our study, the median apparent terminal half-life was approximately 4 to 5 hours, which may suggest that children – or children with ASD – metabolize melatonin more rapidly than adults, or the volume of distribution may be smaller in children than in adults. To date, relatively little research has been performed regarding melatonin metabolism in children with ASD. A study in which serum melatonin and urine 6-SMT were measured following an intravenous infusion of melatonin in prepubescent, pubescent, and adult subjects[32] found that prepubescent children metabolize melatonin more rapidly than adults, and a second study found that melatonin metabolism may be delayed in children with Asperger syndrome[36]. In our study, because the group size was relatively small, it was not possible to perform subgroup analyses in order to compare PK between pubescent and prepubescent children. Following morning administration of Circadin, most subjects were assessed by the Observer's Assessment of Alertness/Sedation Scale to have a mild increase in sedation, with most scores indicating 'drowsy/normal speech' or 'slow reaction to verbal'. Sedation after the 2 mg Circadin dose peaked at (and after) 2 hours, which is around the T_{max} time of the PK profile, demonstrating PK/PD correlation.

Saliva melatonin concentrations at baseline were characteristic of daytime sampling, being low in the morning and increased over 12 hours towards the evening yet showed overall low endogenous levels. These results were confirmed by baseline 6-SMT measured in urine. This confirms the low levels found in earlier studies in children with ASD. Children with ASD can have an abnormal circadian melatonin profile, including lower nighttime melatonin levels[15,37-39]. Because nearly half of our subjects had used melatonin prior to the study, the study population may have been biased towards subject with lower baseline melatonin levels. However, because exogenous melatonin does not appear to affect the production of endogenous melatonin in terms of amplitude[40], and because endogenous melatonin was not suppressed by the administration of Circadin tablets in previous studies (our unpublished data), it is unlikely that these subjects had low baseline endogenous levels due to negative feedback from taking exogenous melatonin prior to the study. On the other hand, endogenous melatonin profiles may be age-specific or disorder-specific. For example, healthy children show a progressive decline from pre-school age in both nocturnal serum melatonin[31,41-43] and urinary metabolite excretion rate[44], suggesting that circadian rhythm decreases with

sexual maturation[31], causing a small increase in melatonin levels in pubescent children[45]. The putative effect of age on endogenous melatonin levels has been investigated only rarely in subjects with ASD. One recent study found no correlation between age and melatonin levels in children with Asperger syndrome[38], suggesting that melatonin levels are suppressed in early childhood in this population[38]; however, this apparent lack of relationship could also be attributed to other factors, including small sample size.

Although the administration of Circadin led to supraphysiologic melatonin levels, treatment was generally were tolerated, with no severe, serious or significant adverse events. Reported adverse events were consistent with known side effects[46,47]. The most frequent adverse events such as fatigue, somnolence and onset of sleep were to be expected based on melatonin's mechanism of action.

In conclusion, this study demonstrates the short-term safety, acceptability, and prolonged-release profile of Circadin mini-tablets in school-age children and adolescents with ASD. Importantly, the potential applications for this new, flexible formulation are not limited to children with neurodevelopmental disorders, but could also include other populations with sleep problems, including children and adolescents with ADHD, adult patients with a neurological disorder leading to dysphagia, and geriatric patients; however, the feasibility of giving mini-tablets to patients with difficulty swallowing should be determined. This initial study will be followed by a randomized, double-blind, placebo-controlled study designed to investigate the efficacy and long-term safety of Circadin mini-tablets in children and adolescents with neurodevelopmental disorders.

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TABLE 1 **Summary PK parameters of melatonin per treatment**

Treatment	N	C_{max} (pg/ml)	t_{max} (h)	$^{a}AUC_{0\text{-last}}$ (pg.h/ml)	$^{b}AUC_{0\text{-}\infty}$ (pg.h/ml)	$t_{1/2}$ (h)
Circadin 2 mg	14	965 (1,170)	1.57 (0.762)	2,370 (1,240)	2,420 (1,100)	5.74 (3.31)
Circadin 2 mg*	11	410 (210)	1.73 (0.792)	1,960 (1,030)	2,150 (960)	4.87 (1.87)
Circadin 10 mg	14	3,970 (2,830)	1.37 (0.640)	12,300 (7,830)	13,00 (7,680)	4.44 (1.69)

Summary of melatonin pharmacokinetic parameters (mean and SD) in the PK population. Data from subject 9 and 10 were excluded from analysis as these subjects were dosed with a melatonin formulation that erroneously did not comply with the desired in vitro release profile. *sub-analysis PK population. **a.** Area under the saliva concentration-time curve from the first to the last observation; **b.** Area under the saliva concentration-time curve from the first observation to infinity.

FIGURE 1 **Graphs showing simulated concentration-time profiles in plasma (black line) after 2-mg morning dose (upper left panel), 10-mg morning dose (upper right panel), 2-mg evening dose (lower left panel) and 10-mg evening dose (lower right panel) administered to a child of 35 kg body weight in fed condition.** In the current study, a morning dose was proposed. An allometric scaling approach was used, assuming dose linearity from 2 to 10 mg, a smaller distribution volume (25 L instead of 50 L and higher metabolism rate (half-life of 0.42 hr) in a healthy child compared to an average adult. Concentration-time profile of endogenous melatonin concentrations are shown in black and was fitted based on data from healthy children. The baseline profile (represented by grey line) in the study population can be different from this profile. Sampling time points after Circadin administration are indicated by vertical black lines.

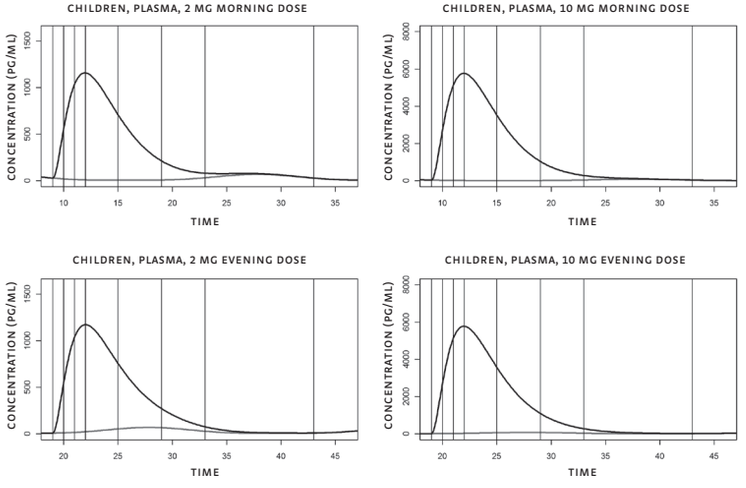

FIGURE 2 Individual concentration-time profile of melatonin measured in saliva on a semi-log scale. The continuous line represents the measured concentrations after 2 mg Circadin mini-tablets; the dashed line the concentrations after 10 mg Circadin mini-tablets. The assay's lower limit of quantification (2 pg/ml) is plotted as a horizontal dashed line.

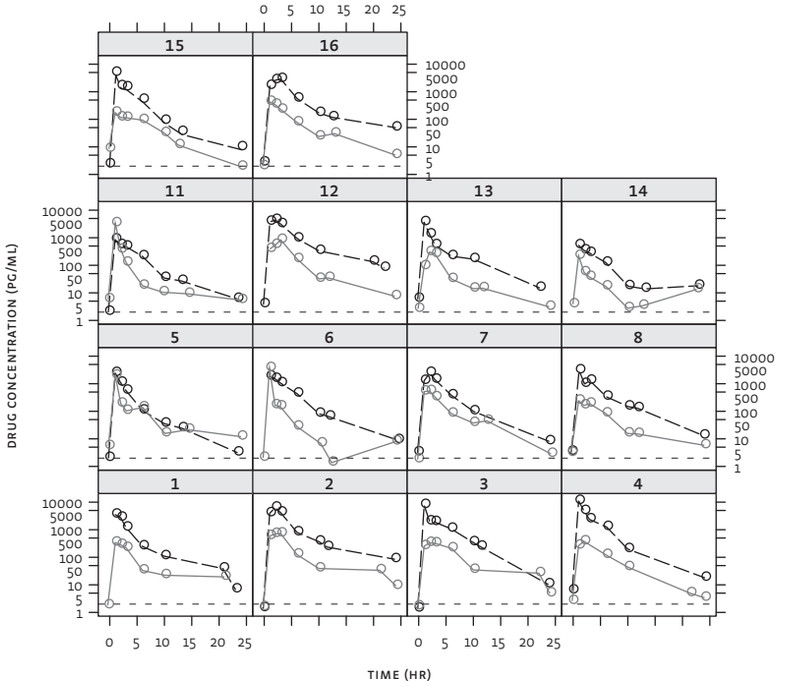

FIGURE 3 Concentration-time profile of melatonin measured in saliva.

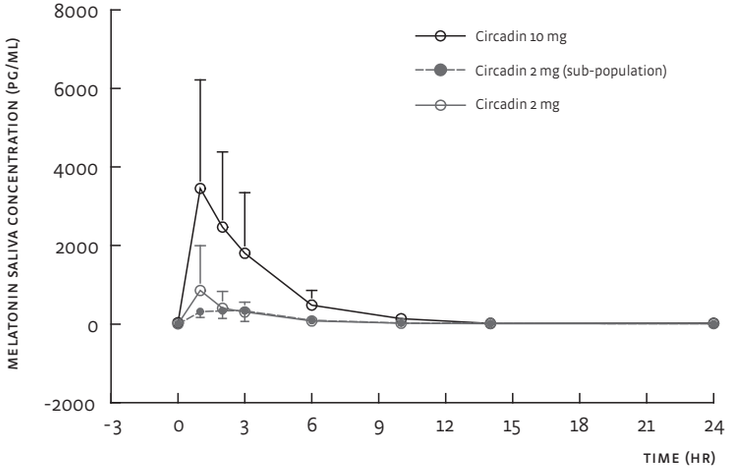

FIGURE 4 Observer's Assessment of Alertness/Sedation Scale scores before and after administration of 2 mg Circadin or 10 mg Circadin, PK population. Number of subjects in PK population with score 'awake/oriented', 'slow reaction to verbal, 'drowsy/normal speech' are presented per time point. None of the subjects scored 'reacts to soft touch'.