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Original Article

Comparative Study between the use of Melatonin and A Solution with Melatonin, Tryptophan, and Vitamin B6 as an Inducer of Spontaneous Sleep in Children During an Auditory Response Test: An Alternative to Commonly Used Sedative Drugs

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OBJECTIVE: An elective investigation into the early diagnosis of deafness in children under the age of 4–5 years is performed using auditory evoked potentials of auditory brainstem responses (ABRs). In case of pediatric patients, the major difficulty includes being examined during spontaneous sleep, which is complicated to obtain, especially in the age range of 12 to 72 months. Recently, melatonin has been used as a "sleep inducer" in diagnostic tests with positive results. Our aim was to evaluate the use of melatonin and of a solution containing melatonin, tryptophan, and vitamin B6 as an inducer of spontaneous sleep on repeated ABR analyses as well as to evaluate the reduction in analyses with sedative drugs in case of uncooperative patients.

MATERIALS and METHODS: In total, 748 children aged between 12 and 48 months were included in the study and divided into three groups: A: placebo (n=235), B: melatonin (n=246), and C: melatonin, tryptophan, and vitamin B6 (n=267).

RESULTS: In groups B and C, in addition to physiological awakening, we observed a significant reduction in the number of repeated analyses as well as drug regimen usage.

CONCLUSION: This study confirms the strategic role of melatonin as an inducer of spontaneous sleep. However, above all, it suggests that the administration of a solution containing melatonin, tryptophan, and vitamin B6 significantly reduces the number of repeated ABR examinations as well as the percentage of repeated analysis performed using sedative drugs compared to both the control group and the melatonin-only group.

KEYWORDS: Melatonin, tryptophan, vitamin B6, sleep induction, auditory brainstem response

INTRODUCTION

A. Background and Aims

To evaluate the hearing of a child from the very first months of life is very important for the early detection of a possible hearing impairment, and in the case of hearing impairment, early treatment to prevent deafness may prevent the delay or failure in language acquisition [1]. Several programs of universal newborn hearing screenings have been started in different geographical regions, which included hearing tests in the first days of life and before the discharge of the baby from the neonatal unit. In total, 1 in 1,000 [2] newborns at birth has a hearing loss severe enough to affect the normal language development and 1 in 4000 suffers from total deafness (profound bilateral sensorineural hearing loss) [3, 4]. In younger children, it is difficult to conduct the traditional audiometric examination as a successful investigation requires that the patient should lay motionless [5]. An elective investigation in the early detection of deafness in children under the age of 4–5 years is performed using auditory evoked potentials of auditory brainstem responses (ABRs), which is an objective survey to evaluate a possible hearing impairment in young patients [6]. In the past few years, in uncooperative patients due to age and/or associated disabilities, the examination is performed under sedation and with long waiting lists as ours is the only pediatric facility able to meet this need [7]. During ABR testing, sedation is required in restless children who create some myogenic potential, which may be able to interfere and invalidate the examination [8–10].

B. Classical Methods for Sleep Induction used for ABR with Children

Non-pharmacological methods: Sleep deprivation. Sleep deprivation is a widely used technique in non-drug ABR examinations in case of uncooperative children, especially when aged between 12 and 72 months. It is commonly considered to be a useful tech-

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nique to facilitate children's sleep during an ABR examination and contributes to lower the failure of pharmacological techniques. However, sleep deprivation appears to be an uncomfortable technique for the whole family, and often, parents notice a significant irritability in the child with lack of sleep [11].

Pharmacological methods: Use of sedative/anesthetic drugs. Depending on the type and purpose of analysis, a non-cooperating patient may be treated with a pharmacological therapy based on sedatives, anesthetics, or a combination of the two [12]. The sedative agent generally used in the ABR survey is chloral hydrate, probably because it was the first molecule to be used [13]. The use of chloral hydrate requires the presence of an anesthetist and a medical team that monitors a patient's vital parameters [5].

The most common side effects of chloral hydrate are vomiting, excessive lethargy, dizziness, and disorientation [14]. The literature also reports of poisoning and cardiac arrest [15, 16]. Recently, other sedative molecules, such as propofol, were taken into account, which has shown good sedative power, especially if intravenously administered. However, as with chloral hydrate, propofol also requires the presence of an anesthetist and has major side effects such as respiratory depression, dyspnea, cardiac arrest, hallucinations, and nausea [15]. Because of the side effects due to the anesthetic/sedative agents, the use of this pharmacological class should not represent the routine treatment for the ABR survey. The use of pharmacological methods in an ABR examination involves a number of issues related to the safety of use in the pediatric field, the costs arising from the necessity of administration under the supervision of an anesthetist, and the interference of halogenated anesthetic agents on the detection of ABR parameters. In addition, not all centers that perform an ABR examination in pediatric subjects can rely on the presence of an anesthesiologist, and this increases the waiting time related to having this instrumental survey.

C. Using a Solution of Melatonin, Tryptophan and Vitamin B6 for Inducing Sleep During Auditory Evoked Potential Testing in Infants and Children

The above-discussed problems related to the classical methods of sleep induction during the ABR analysis emphasize the absolute need to conduct a test with the aid of a "sleep-inducing" molecule such as melatonin. This molecule is able to result in the correct performance of the examination without representing a risk factor for the child and a major expense for the National Health System [5]. Melatonin is a hormone (N-acetyl-5-methoxytryptamine) synthesized by the pineal gland whose secretion is regulated by the suprachiasmatic nucleus of the hypothalamus and has a peak between 2 and 3 a.m. [17, 18]. It is metabolized in the liver, and 80–90% of it is converted to 6-sulfatoxymelatonin [19], an inactive component excreted in the urine. It has proven to be effective in regulating the sleep/wake rhythm in children with neurological or psychiatric disorders (e.g. autism, ADHD) [20] at a dose of 3–6 mg every night.

D. Biosynthesis and Therapeutic Safety of Melatonin

The first stage of melatonin biosynthesis includes the hydroxylation of tryptophan (position 5 of the indole ring) in hydroxytryptophan by the enzyme "tryptophan hydroxylase"; the subsequent removal of the carboxyl group from the side chain by the "decarboxylase of the aromatic amino acids" leads to the synthesis of 5-hydroxytryptamine or serotonin. At this stage, vitamin B6 plays an important role as a coenzyme; the latter is acetylated by "N-acetyl transferase" into N-acetylserotonin, which is subsequently converted to melatonin by the enzyme "idrossindol-O-methyl-transferase", an enzyme found in the pineal gland and retina [21].

Scheme of the Biosynthesis of Melatonin *in vivo*

In the literature, toxicological data due to the use of melatonin in children cannot be found, which may be worrisome. Meta-analysis studies have shown that when using melatonin to reset the circadian rhythm, no adverse effects are induced, neither in short-term nor long-term use [22, 23]. Rossignol and Frye found that hormone use did not lead to adverse events in 7 of 12 studies in which children with ASD were treated with melatonin [24]. Since melatonin is metabolized by CYP1A2 and CYP2C19, the use of drugs that inhibit CYP1A2 can raise the plasma levels of melatonin [25]. Since melatonin can lead to temporary pressure fluctuations or a decrease in blood sugar level ranges, attention should be given to the simultaneous use with drugs that regulate blood pressure and glucose metabolism [26].

E. Use of Tryptophan and Vitamin B6 as Elements for Increasing the Action of Melatonin

Tryptophan is an essential amino acid that must be taken through the diet because the human body cannot synthesize it [27-29]. Therefore, the biosynthesis of melatonin depends on the availability of tryptophan. It is well documented that during cases of acute tryptophan depletion, there is a significant decrease in plasma levels of melatonin [30] and those of urinary 6-hydroxy melatonin sulfate [31, 32]. Thus, tryptophan deficiency is due to sleep disorders associated with a lack of melatonin [33, 34]. Vitamin B6 is an interesting molecule involved in a wide range of physiological processes. Due to the water solubility and high reactivity of the phosphorylated form, it is an excellent co-factor in various metabolic processes, most of which are related to the synthesis and degradation of amino acids [35]. Vitamin B6 consists of three related compounds, pyridoxine, pyridoxal, and pyridoxamine, all of which differ in the position 4 substituent. All forms, however, are phosphorylated by a kinase, which represents the basic requirement related to the co-factor activity of vitamin B6 [36, 37]. Vitamin B6 is required in the biosynthesis of serotonin [38] as a co-factor in the transformation of L-5-hydroxy tryptophan into serotonin by the enzyme carboxylase of aromatic amino acids [39]. Thus, vitamin B6 is essential in the biosynthesis of melatonin, and its molecular deficiency causes a sleep disorder.

Purpose of the Study

For some years, audiological diagnostic examinations of infant hearing loss have been performed under sedation for uncooperative patients (because of age and/or associated disabilities); there is a waiting list of several months for this test because only our pediatric facility is able to meet this need. Our aim was to evaluate an alternative method to commonly used drugs that provides for the introduction of melatonin or a solution containing melatonin, tryptophan, and vitamin B6 as sedating agents and sleep inducers. This compared to a control group without any treatment, referred to the number of repeated ABR analysis as well as the evaluation of the reduced analysis carried out with sedative drugs in case of uncooperative patients. The survey will allow us to extrapolate additional data of clinical interest related to clinical safety, to reduce health costs, and to possibly reduce waiting lists.

MATERIALS and METHODS

A comparative study for the ABR investigation, was performed on three groups. The control group (Group A) received no integration. Groups B and C received melatonin (Melamil, Humana Italia S.p.A./Milte Italia S.p.A., Milano, Italia), 4 drops/ die (1 mg) and a solution containing melatonin, tryptophan, and vitamin B6 (Melamil Tripto or Melamil Plus, Humana Italia S.p.A./ Milte Italia S.p.A., Milano, Italia) 0.5 mL/ die (1 mg of melatonin, 20 mg of tryptophan, and 1.4 mg of vitamin B6), respectively, each evening beginning from seven days before starting the analysis (30 minutes before bedtime) and 30 minutes before starting the ABR investigation. The success of the treatment was evaluated at the end of the study period. The number of awakenings and repeated analyses were recorded in a structured diary, while the information questionnaires were filled in by health care professionals to monitor any adverse effects and other parameters that emerged during ABR investigation. Both products used in this study are in the category of nutritional supplements. This type of comparative study did not require approval by the Ethics Committee nor the compilation of the informed consent from parents of enrolled children.

Statistical Analysis

The Chi^2 test (Chi-square test) and the Monte Carlo method were used. The Monte Carlo method was created to obtain a more precise evaluation of the critical value obtained from the theoretical distribution of the Chi^2 test. At the end of the probabilistic analysis, the calculation continues with the Marascuilo procedure, only in case the Ho hypothesis should be rejected, to identify any differences between the proportions in the programmed groups. The comparisons were conducted at a probability level of $p<0.05$.

Statistical Methods

The nominal or ordinal variables were evaluated using the Chi^2 technique and the Marascuilo procedure.

Data Processing

All statistical analyses were performed on Macintosh Imac computers using the Software program XLSTAT ver. 5-6-2014 (Addinsoft, New York, USA) [40].

RESULTS

Children (748 cases) aged between 12 and 48 months were included in the study. The first group received no integration (Group A; n=235), the second group received only melatonin (Group B, n=246), and the third group received a solution containing melatonin, tryptophan, and vitamin B6 (Group C, n=267). To classify the study, we noted the absence of drop-outs, side-effects, and other relevant parameters. The two statistical tests applied to show a high significance among the examined groups ($Chi^2 = 6$, $p<0.0001$). More specifically:

A. Repeated ABR Analyses

The detected percentages of the examined phenomenon were 71.1% (A), 34.6% (B), and 16.5% (C), with percentage changes referred to reduction: B vs. A (−74%), C vs. A (−49.1%), and B vs. C (−48.2%). These results were highly significant after comparison with the Marascuilo procedures (Table 1).

Table 1. Marascuilo procedure. The comparison between the proportions of group B vs group A (34.6% vs 71.1%) has statistical significance. The reduction in percentage terms of ABR is 49% in favor of the first group (85-167/167). Comparing 16.5% vs 71.1% is also significant with a variation of 74%. There is a difference between the two treatments, 16.5% vs. 34.6%, with a variation of 48%

Contrast	Value	Critical value	Significant
|p (group A=control) – p (group B=melatonin)|	0.365	0.104	Yes
|p (group A=control) – p (group C=melatonin, tryptophan, and vitamin B6)|	0.546	0.091	Yes
|p (group B=melatonin) – p (group C=melatonin, tryptophan, and vitamin B6)|	0.181	0.093	Yes

ABR: Auditory Brainstem Response

Table 2. Marascuilo procedure. The comparison between the proportions of group B vs group A (17.1% vs 35.3%) has statistical significance. The reduction in percentage terms of ABR is 49.4% in favor of the first group (42-83/83). Comparing 6.4% vs 35.3% is also significant with a variation of 79.5%. There is a difference between the two treatments, 17.1% vs 6.4%, with a variation of 59.5%

Contrast	Value	Critical value	Significant
|p (group A=control) – p (group B=melatonin)|	0.182	0.096	Yes
|p (group A=control) – p (group C=melatonin, tryptophan, and vitamin B6)|	0.290	0.085	Yes
|p (group B=melatonin) – p (group C=melatonin, tryptophan, and vitamin B6)|	0.107	0.069	Yes

ABR: Auditory Brainstem Response

B. Repeated ABR Analysis with Patients under Sedation

The detected percentages of the examined phenomenon were 35.3% (A), 17.1% (B), and 6.4% (C), with percentage changes referred to reduction: B vs. A (−49.4%), C vs. A (−79.5%), and C vs. B (−59.5%). These results were highly significant after comparison with the Marascuilo procedure (Table 2).

The reduction in repeated analyses and the number of analyses that required the use of sedative drugs for Group B and Group C were statistically more significant vs Group A. The percentage of repeated analyses for Group A and the number of repeated analyses with sedative drugs were, 71.1% and 35.3%, respectively, for Group B were 34.6% and 17.1%, respectively, and those for Group C were 16.5% and 6.4%, respectively. Equally significant was the comparison between Group C and A (16.5% vs 71.1%) in terms of repeated analyses with a variation of 74%. A marked difference was also shown between Group B and C treatment (16.5% vs 34.6%), with a variation of 48%, and also significant in terms of analyses that required the use of drugs (6.4% vs. 17.1%) with a variation of 59.5% (Figures 2, 3).

We also registered a spontaneous awakening in all patients of Groups B and C, similar to a physiological one, without the typical side effects of drug therapies from use of sedative agents.

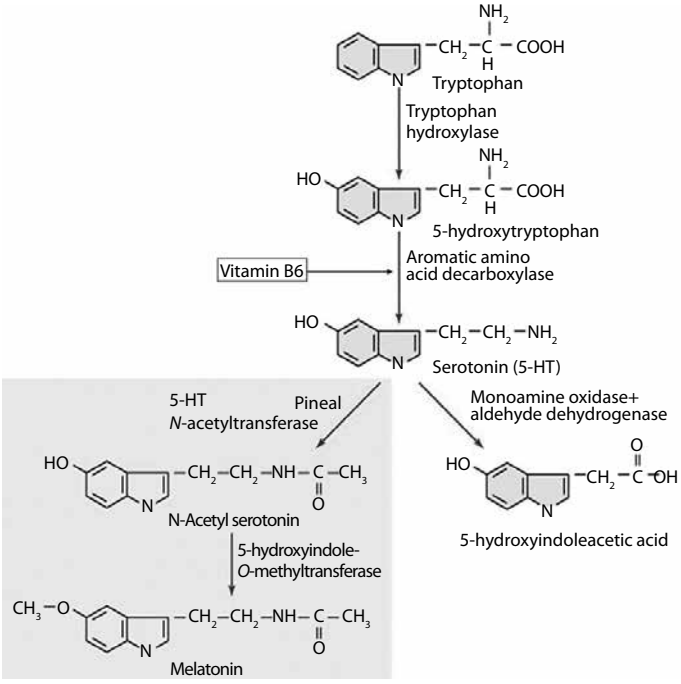

Figure 1. Biosynthesis of melatonin

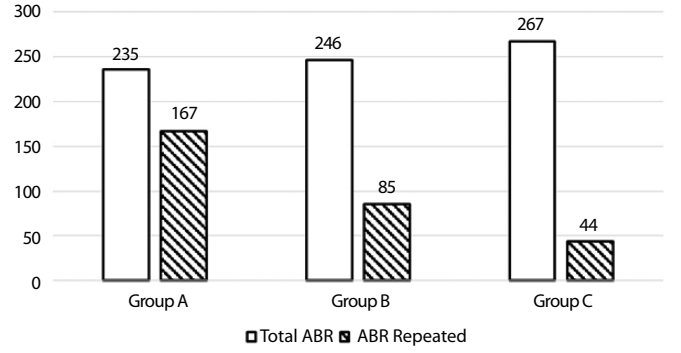

Figure 2. Total number of ABR examinations performed versus the number of repeated ABR examinations. Group A (control), Group B (melatonin), and Group C (melatonin, tryptophan, and vitamin B6).

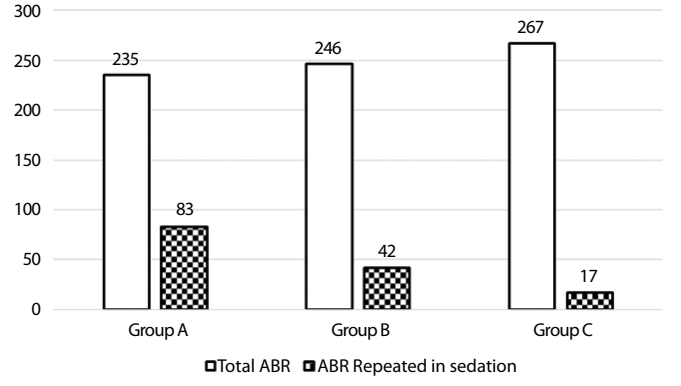

Figure 3. Total number of ABR examinations performed versus the number of ABR examinations repeated under sedation. Group A (control), Group B (melatonin), and Group C (melatonin, tryptophan, and vitamin B6).

DISCUSSION

This study confirmed the strategic role of melatonin as a spontaneous sleep inducer, as shown in other clinical studies, but it especially suggests that the administration of a solution containing melatonin, tryptophan, and vitamin B6 significantly reduces the number of repeated ABR examinations as well as the percentage of repeated analyses through the use of sedating drugs compared to the melatonin-only group and control group. Considering the same quantity of administered melatonin, it can be deduced that the addition of tryptophan and vitamin B6 had a decisive role in the reduction in the observed parameters. The use of an integration based on melatonin, tryptophan, and vitamin B6 might be an alternative solution to the use of traditional methods during ABR investigations or similar analyses that require spontaneous sleep, especially in children aged between 12 and 48 months. This melatonin, tryptophan, and vitamin B6 formulation may lead to a better management of parental anxiety and cost savings for the National Health System.

Ethics Committee Approval: This type of comparative study did not require approval by the Ethics Committee.

Informed Consent: N/A.

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