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Zaleplon increases nocturnal melatonin secretion in humans

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ABSTRACT

Insomnia is the most common sleep condition. Many hypnotics decrease nocturnal melatonin secretion. The aim of this research consists of studying the effect of the hypnotic drug zaleplon on melatonin secretion. Twelve non-smoker drug-free healthy male subjects participated in the study. All participants were normal sleepers and aged 33.2 ± 11.7 years. They orally took 10 mg of zaleplon at 22:00 h in a double-blind, randomized, cross-over design. The study was carried out during two consecutive days in a week-end. Blood samples were extracted at 22:00, 23:00, 24:00, 01:00, 02:00 and 12:00 h. Melatonin was measured by an ELISA assay. All the subjects had a circadian rhythm of melatonin secretion. Zaleplon compared to placebo increased significantly the melatonin levels at 23:00, 24:00 and 01:00 h. No differences in melatonin levels between placebo and zaleplon were found at 12:00, 22:00 and 02:00 h. Zaleplon compared to placebo increased by 46% the Area Under the Curve of melatonin secretion. The present study indicates that zaleplon increases nocturnal melatonin secretion without increasing daytime melatonin levels. We suggest that when clinicians prescribe a hypnotic, the effect on melatonin levels should be another parameter to be taken into account.

1. Introduction

Insomnia is the most common sleep condition. About 40% of patients attending general practitioner clinics suffer from this problem (Blais et al., 2001) and approximately 2/3 of those who see a psychiatrist complain that they are not satisfied with the restorative quality of their sleep (Berrios and Shapiro, 1993). Benzodiazepines (BZD) and non-BZD hypnotics are the most frequently prescribed drugs for sleep disorders (Paulose-Ram et al., 2004). Melatonin has been used as a hypnotic (Lemoine et al., 2007), at the same time, melatonin is the main hormone secreted by the pineal gland and is involved in the light–dark/wake–sleep cycle regulation (Reiter, 2003). Thus, the interest of studying the effect of the different hypnotics on this hormone is paramount. Most BZD have been reported to decrease nocturnal melatonin levels (Kabuto et al., 1986; McIntyre et al., 1988; Monteleone et al., 1989; McIntyre et al., 1993; Hajak et al., 1996; McNulty et al., 1996). No effect of BZD on nocturnal melatonin secretion has also been reported (McIntyre et al., 1988; Copinschi et al., 1990; Allen et al., 1994; Norman et al., 2001). Non-BDZ hypnotics seem not to affect nocturnal melatonin levels (Copinschi et al., 1995; Mann et al., 1996; Norman et al., 2001). Zaleplon, a non-BZD hypnotic drug, has been reported to increase nocturnal melatonin secretion in rabbits (Noguchi et al., 2003). The purpose of this research is to study the effect of zaleplon on melatonin secretion of healthy human beings.

2. Methods

2.1. Subjects

Twelve non-smoker drug-free healthy male volunteers, aged 33.2 ± 11.7 years (mean ± standard deviation), participated in the study. Subjects were recruited among the researcher's acquaintances. Women were excluded to avoid the effect of the menstrual cycle and contraceptives on melatonin secretion (Brun et al., 1987). None of them had a history of medical, neurological or psychiatric disease. Routine laboratory parameters were normal and they did not fulfill criteria for any type of sleep disorder according to the Fourth Revision of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2000). The study was carried out in accordance with the Helsinki Declaration and all subjects gave written informed consent before inclusion. The protocol was approved by the Ethics Committee of the University of La Laguna.

Exclusion criteria were: having made a transoceanic flight in the last month before the study, sleep problems, abnormal results in the metabolic or urine tests and having a work on night shifts. They were asked to refrain from using sun glasses, drinking coffee, tea or alcohol

Abbreviation list: AUC, Area Under the Curve; BZD, Benzodiazepines; cAMP, cyclic Adenosine MonoPhosphate; CNS, Central Nervous System; CYP, Human Cytochrome P450; DSIP, Delta Sleep-Inducing Peptide; ELISA, Enzyme-Linked ImmunoSorbent Assay; GABA, Gamma-AminoButyric Acid; HIOMT, HydroxyIndole-O-MethylTransferase; NAAG, N-AcetylAspartylGlutamate; NAT, N-AcetylTransferase; VIP, Vasoactive Intestinal Polypeptide.

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containing beverages 12 h before and for the duration of the experiment. Naps were not allowed during the experiment.

2.2. Study design

All subjects orally took one capsule containing placebo or 10 mg of zaleplon at 22:00 h in a randomized, double-blind and cross-over design. At 21:00 h a butterfly needle was inserted into the antecubital vein and kept patent with diluted heparinised saline, which was discarded before sampling. The first blood extraction was taken just before the ingestion of the capsule. Blood was drawn at 22:00, 23:00, 24:00, 01:00, 02:00 and 12:00 h. One hour before the first extraction subjects were to bed and stayed there until the end of the last night sampling. Next day they were to bed at 11:00 h. They stayed in bed with their eyes open receiving the normal day-light intensity until 12:00 h when blood was drawn. This was done to avoid the body postural effect on melatonin levels (Deacon and Arendt, 1994). Lights were turned off at 21:00 h and the eyes of each subject were covered with a mask to avoid unexpected light contamination. Blood was collected in the dark by using a lantern with low intensity red light. To avoid the seasonal effect on melatonin secretion (Morera and Abreu, 2006), the study was carried out during two consecutive days on the second week-end of August. The same routine was followed during the two sessions.

After each extraction, blood was centrifuged at 3000 rpm for 10 min, and serum was separated and frozen at −30 °C until assayed for melatonin. Serum melatonin was determined by an Enzyme-Linked ImmunoSorbent Assay (ELISA), using commercial kits purchased from Immuno Biological Laboratories (IBL, Hamburg, Germany). The detection limit of this assay was 1.6 pg/ml; intra and interassay coefficients of variation were 3–11.4% and 6.4–19.3% respectively.

2.3. Statistical analysis

Data were analyzed using the 15th version of the Statistical Package for the Social Sciences (Chicago, Illinois, USA). The trapezoidal rule (Whittaker and Robinson, 1967) was used to calculate the melatonin Area Under the Curve (AUC). Serum melatonin AUC for the placebo and zaleplon condition was calculated for a period of 4 h (23:00–02:00 h). Change in melatonin AUC was measured by subtracting the melatonin AUC following zaleplon challenge from the respective melatonin AUC following placebo ingestion (Δ Melatonin = zaleplon melatonin AUC − placebo melatonin AUC). Wilcoxon non-parametric *t*-paired test was used to analyze differences between placebo and zaleplon melatonin values, as well as to compare midday placebo melatonin level with all night melatonin placebo levels. Data are presented as mean and standard error of mean for clarity.

3. Results

Comparison of melatonin levels for each time and drug condition is presented in Table 1. Melatonin levels increased significantly at 23:00, 24:00 and 01:00 h after zaleplon ingestion. Melatonin levels at 22:00, 02:00 and 12:00 h were not significantly different between placebo and zaleplon conditions. Comparison of placebo nocturnal melatonin values with midday placebo melatonin value showed that

Table 1
Melatonin levels (pg/ml) in placebo and zaleplon condition.

Time	Placebo	Zaleplon	*p*
22:00	12.5 ± 2.1	13.6 ± 2.9	0.387
23:00	18.3 ± 2.5	26.2 ± 5.3	0.012
24:00	16.6 ± 6.5	24.6 ± 3.1	0.002
01:00	24.4 ± 7.5	60.5 ± 17.0	0.015
02:00	28.3 ± 7.7	28.7 ± 2.9	0.529
12:00	7.2 ± 1.1	11.7 ± 2.6	0.135

Data are given as mean ± sem.

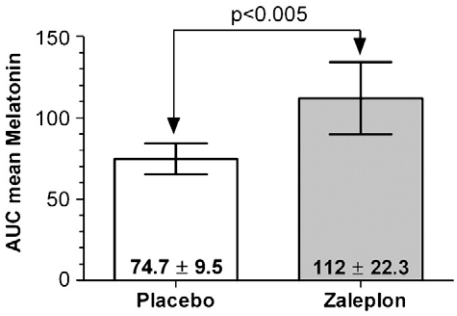

Fig. 1. Comparison of mean melatonin Area Under the Curve (AUC) between placebo and zaleplon challenge.

all subjects had a clear circadian rhythm of melatonin secretion, with significantly higher nocturnal levels compared to the diurnal levels ($p<0.05$, comparisons not shown). Applying the trapezoidal rule to the melatonin levels at 23:00, 24:00, 01:00 and 02:00 h, placebo administration produced an AUC of 767 (100%) while zaleplon produced an AUC of 1120.2 (146%). Zaleplon compared to placebo increased the total melatonin AUC by 46%. Fig. 1 shows the comparison of mean melatonin AUC for the time period from 23:00 to 02:00 h. Mean melatonin AUC was significantly higher in the zaleplon than in the placebo condition.

4. Discussion

To the best of our knowledge, this is the first time that the effect of zaleplon on melatonin secretion is investigated in healthy human subjects. The principal finding of this research is that 10 mg of zaleplon, a normal adult therapeutic dose, increases nocturnal melatonin secretion without producing an increase of melatonin levels the following day. Our results confirm the existence of a circadian rhythm of melatonin secretion with higher nocturnal than diurnal levels.

Among the BZD that have been reported to decrease nocturnal melatonin levels are flunitrazepam (Kabuto et al., 1986; Hajak et al., 1996), alprazolam (McIntyre et al., 1988, 1993), diazepam (Monteleone et al., 1989), triazolam and temazepam (McNulty et al., 1996). No significant effects on nocturnal melatonin secretion have also been reported for alprazolam (McIntyre et al., 1988; Copinschi et al., 1990) and temazepam (Allen et al., 1994; Norman et al., 2001). The apparent contradictory effect for alprazolam on melatonin secretion may be explained by the fact that the alprazolam effect on melatonin may be dose-dependent. McIntyre et al. (1988) found that 0.5 mg of alprazolam had no effect on nocturnal melatonin secretion but 2 mg of alprazolam significantly suppressed nocturnal melatonin secretion. Those results were later confirmed by Copinschi et al. (1990) and McIntyre et al. (1993).

The controversial results of temazepam on melatonin secretion are more difficult to explain. McNulty et al. (1996) reported that 10 mg of temazepam significantly reduced the amount of secreted melatonin. Allen et al. (1994) gave 20 mg of temazepam to seven nurses who at times had worked night shifts. They did not find any effect of temazepam on nocturnal melatonin secretion. This result may be explained by the fact that the sample was mostly (5 out of 7) comprised by nurses who had worked night shifts. Another source of bias may stem from the fact that compliance with medication cannot be assured, because medication was taken at home. Norman et al. (2001) gave 20 mg of temazepam to a group of eight healthy subjects. They found that temazepam did not significantly affect nocturnal melatonin secretion but they reported a decrease of nocturnal melatonin levels. The authors point out that the effect of temazepam may be dose-dependent, with higher doses causing a statistically significant suppression of nocturnal melatonin secretion. However, in a previous report by McNulty et al. (1996), half of the dose of