

Bioavailability of a New Oral Spray Melatonin Emulsion Compared with a Standard Oral Formulation in Healthy Volunteers

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Abstract

Background and Objective: Melatonin, a pineal gland hormone secreted at night, is involved in the sleep-wake cycle and has been extensively used in the treatment of primary sleeping disorders. Up to 90% of blood melatonin is cleared by the liver in a single passage, thus its half-life is very short (30-60 minutes). To avoid the first pass effect responsible for up to 90% loss of the melatonin oral dosage and to ensure rapid onset of activity, a new food grade liquid emulsion has been formulated in a spray form, which assures an additional increase of the contact surface and the possibility of efficient mucosal absorption.

The purpose of the present study was to investigate the bioavailability profile of this new food grade liquid emulsion compared with a standard melatonin formulation in commercially available oral tablets.

Methods: In a single-dose, open-label, crossover study, eight subjects were randomly assigned to receive 5 mg of oral spray or oral melatonin (tablet). After a washout of one week, plasma was collected at six hours post dosing and assayed by a validated liquid chromatography tandem mass spectrometry method to determine the main pharmacokinetic parameters.

Results and Discussion: The student t Test for paired data highlighted a significant difference between C_{max} values ($p=0.021$) and the AUC ($p=0.045$). The absorption rate (T_{max}) of the two melatonin formulations did not show statistically significant differences (Friedman test). The amount of melatonin reaching the systemic circulation after oral spray administration was significantly higher than after oral tablet administration.

Keywords: Melatonin; Bioavailability; Pharmacokinetics; Spray; Oral

Introduction

In recent years, sleep disturbances have been documented to affect up to thirty percent of the Western hemisphere's population. Depression, anxiety and stress are the most common causes of sleep disturbances related to sleep-onset and sleep-maintenance difficulties. Sleep disturbances are common in the elderly, apart from psychological causes; they may be caused by side effects of medication or disease [1].

Melatonin (N-acetyl-5-methoxytryptamine) is a circulating hormone produced by the pineal gland during the dark phase of the day-night cycle. It is involved in the sleep-wake cycle and synthetic melatonin preparations have been extensively used in the treatment of primary sleeping problems [2]. The secretion of melatonin markedly decreases [3], as sleep fragmentation increases with age [4].

Up to 90% of blood melatonin is cleared by the liver in a single passage, making its half-life very short (30-60 minutes). It is metabolized in the liver by hydroxylation to 6-hydroxymelatonin and excreted in the urine following conjugation with sulfuric or glucuronic acid. Melatonin, but not its sulphate metabolites, readily crosses the blood-brain barrier [5]. Only blue light, of around 480 nm, suppresses the production of melatonin. Unfortunately, this type of light is present in many artificially lit environments [6]. The European Food Safety Authority (EFSA) has published guidelines on safety and efficacy in the use of melatonin, as a food supplement, at a dosage of 0.5-5 mg for the treatment of jet lag [7]. Melatonin has also been proposed as the standard agent for circadian rhythm sleep disorders, such as Delayed Sleep Phase Syndrome (DSPS) and non 24-hour sleep wake syndrome [8-10]. It can reduce sleep latency largely in patients with DSPS than in

patients with insomnia and it is especially effective in the elderly, who present physiologically low blood levels of the hormone [11].

To avoid the first pass effect responsible for up to 90% loss of the oral melatonin dose and to ensure rapid onset of activity, a new food grade liquid emulsion (Melatonin in emulsion spray by Medestea SpA) has been formulated in a spray form with an additional increase of the contact surface and the possibility of efficient mucosal absorption. These affirmations have been confirmed in an *in vitro* permeability study with porcine cheek epithelium [12].

Therefore, a pharmacokinetic (PK) study was necessary to verify whether the delivery of a melatonin emulsion at the buccal mucosa truly improved the amount and the rate of melatonin absorption compared to a standard oral tablet formulation. Parameters of primary interest were pharmacokinetic variables determined in plasma: Area Under the Curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}), Mean Residence Time (MRT), Time relative to the peak concentration (T_{max}), elimination half-life ($T_{1/2}$).

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Methods

Subjects

The study was carried out in accordance with the principles of the current revision of the Declaration of Helsinki and its amendments; the International Conference Guidelines for Good Clinical Practices and local legal and regulatory requirements. The Ethics Committee of the Fondazione IRCCS Policlinico San Matteo Hospital approved the study protocol.

Before enrollment in the study, subjects were fully informed of the aim, methods, expected study duration, anticipated risks and possible discomfort and afterwards, written informed consent was obtained.

Eight healthy volunteers (five males, three females) aged between 21 and 51 years were included based on their medical history, clinical and physical examinations, vital signs, biological tests and electrocardiogram (ECG). In particular, renal and hepatic functions were normal in all volunteers, and no clinically relevant abnormal laboratory values or relevant clinical abnormalities were observed during the screening phase. Exclusion criteria included the presence or history of any significant disease, alcohol and/or drug abuse, smoke abuse, positive HIV and/or Hepatitis B and C test. No concomitant therapy was permitted. Female patients of childbearing age were excluded if not using or if not willing to continue using a medically reliable method of contraception for the entire study duration. Any concomitant assumption of products containing melatonin or derivate inhibitors and inductors of CYP450 were not allowed during the study period. Patients treated with any investigational study medication or who underwent any medical procedure in the previous 90 days were excluded.

Study design and clinical procedures

This open-label, randomized crossover study was designed to investigate 5 mg doses of the oral melatonin formulation (Melatonin in emulsion spray by Medestea SpA, 1 mg of melatonin for each spray and Melatonin tablet containing 5 mg of melatonin manufactured by SIRC). During scheduled visits, the investigator dispensed 5 mg (5 sprays of a food grade liquid emulsion directly under the tongue) of melatonin (Medestea SpA) (product A) or a 5 mg tablet (1 *per os*) of Melatonin (SIRC) (product B), according to the randomization schedule, including a washout period of one week.

At each visit, concomitant medication, physical examination, dispensing of study medication, collection of PK blood samples, and adverse events were registered. The two products were administered in the morning under fasting conditions (for at least 12 hours). For the evaluation of melatonin plasma concentrations, 6-8 mL of blood were collected by venipuncture at time 0, predose, and at 5, 10, 20, 30, 40, 60, 90, 120, 180, 240, and 360 minutes (min) after administration of each preparation. Blood samples were collected in heparinized disposable tubes. After centrifugation, resulting plasma samples were stored at -80°C until analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS) [13].

Determination of plasma melatonin concentrations

Chemicals: A Melatonin reference standard was obtained from Sigma Aldrich (Milan, Italy), N-Acetyltryptamine an Internal Standard (IS) reference was obtained from Space (Milan, Italy).

All solvents were of HPLC grade or higher. Acetonitrile, Propan-

2-ol, Methanol, Formic Acid were obtained from Carlo Erba (Milan, Italy); Ammonium Formiate from Fluka (Milan, Italy); purified water from Direct-Q, Millipore system (Malsheim, France). A pool of human plasma was obtained from the Department of Transfusion Medicine, Fondazione IRCCS Policlinico San Matteo, Pavia (Italy).

Chromatographic conditions: Melatonin plasma concentrations were detected by reverse phase high performance liquid chromatography, coupled with a tandem mass spectrometer (HPLC-MS/MS).

A TSQ Quantum Access spectrometer (Thermo Scientific, San Jose, CA, USA), equipped with an Electrospray Ion Source (ESI), operating in positive mode, was employed for all analyses, in the selected reaction monitoring assay (SRM). The observed transitions (precursor \rightarrow product ions) were: m/z 233 \rightarrow 173.99 for Melatonin and m/z 203 \rightarrow 144.03 for the Internal Standard. Signals were resolved chromatographically (t_R 2.78 min and 3.06 min respectively) using a Gemini - NX 3 μ C18 110A, 100 \times 4.6 mm column (Phenomenex) and a gradient elution with two mobile phases (A: purified water - Ammonium Formiate 2 mM - HCOOH 0.1%; B: CH₃CN) at a flow-rate of 650 μ L/min. The column oven was maintained at 30°C and the autosampler tray at 15°C.

Sample preparation: 50 μ L aliquots of working solutions of melatonin were added to 450 μ L of human plasma/water (blank) in 1.5 mL polypropylene tubes, to yield the final concentrations reported in standard samples and quality controls (QC).

Seven different standard concentrations were used for the calibration curve ranging from 0.156 ng/mL to 10 ng/mL. To calculate sample concentrations above 10 ng/mL, the calibration curve was extended to 50 ng/mL. A calibration curve was analyzed with each batch. Quality control samples were prepared at the following concentrations: 0.5, 4, 8 and 25 ng/mL. Three sets of QC samples were assayed with each run [14].

In brief, 50 μ L of IS (20 ng/mL), 50 μ L of 10% Ammonium Hydroxide solution were added to each standard/unknown sample (500 μ L), followed by a liquid-liquid extraction with 2 mL of dichloromethane.

Samples were vortex mixed. Tubes were then centrifuged for 10 min at 3000 g at 4°C and the lower organic phase was transferred into conical glass tubes and evaporated to dryness under a gentle stream of nitrogen at room temperature. Residues were reconstituted in 100 μ L of acidified water (HCOOH 0.1%) and transferred into a micro-vial. Aliquots of 10 μ L were injected into the HPLC-MS/MS apparatus and then analyzed.

Pharmacokinetic and statistical analysis: No pharmacokinetic model has been hypothesized, and the following parameters were determined from the measured data:

C_{max} = peak plasma concentration (ng/ml);

T_{max} = Time relative to the peak concentration (min);

$AUC_{0-\infty}$ = Area Under the Curve plasma concentration - time curve from 0 to ∞ (ng*min/ml), calculated by using the linear trapezoidal rule (Kinetica TM Software version 4, InnaPhase Corporation Philadelphia PA);

F (A/B) = Relative bioavailability of product A versus product B;

MRT = Mean Residence Time (min);

$T_{1/2}$ = elimination half-life (min).

We considered $AUC_{0-\infty}$ as the true estimate of the relative bioavailability F of melatonin, obtained by comparing AUC from time 0 to ∞ ($AUC_{0-\infty}$).

For the estimation of the absorption rate constant (K_a) an one compartment open model with oral absorption was hypothesized.

Power consideration: the primary endpoint was to compare the AUC of two formulations of oral melatonin. We hypothesized that after sublingual administration, the amount of melatonin delivered to the general circulation might be much higher, about 50% or more, than after oral administration. A two sided t-test achieves 84% power to infer the mean ratio is not 1.000 when the total sample size of a 2x2 cross-over design is 8, the actual mean ratio is 1.550, the coefficient of variation is 0.250 and significant level is 0.0500.

The mean and standard deviation [SD] were used to summarize quantitative variables. Parametric or non parametric tests were used according data distribution (Shapiro's Test). To evaluate the differences between the two products, $AUC_{0-\infty}$ and C_{max} , (after log transformation) were analyzed by the Student t Test for Paired Data. T_{max} difference was tested by the Wilcoxon signed-rank test.

$P < 0.05$ was considered statistically significant. All tests were two-sided. Data analysis was performed with the STATA statistical package (release 9.0, 2006, Stata Corporation, College Station, Texas, USA).

Results

Subjects

Eight subjects were enrolled and completed the study.

Table 1 summarizes demographic and baseline characteristics of the healthy enrolled volunteers.

Table 2 describes mean plasma concentrations after administration of Melatonin in emulsion spray (product A) and Melatonin tablet (product B).

LC/MS/MS

Regression analysis with the weighted linear regression method (1/X) was used to obtain the best fit of data for the calibration curves. The curve for all accepted runs had an $r^2 \geq 0.9896$. The lower limit of quantification (LLOQ) for melatonin was 0.156 ng/mL and the upper limit of quantification was 50 ng/mL. In all curves, the non-zero standards were within $\pm 15\%$ of their nominal value in accordance with the acceptance criteria. The LLOQ samples were within $\pm 20\%$ of their nominal value, inter-assay precision was 9.25% and inter-assay accuracy was 102.81%, in accordance with the acceptance criteria. For the QC standards, the inter assay-accuracy ranged from 97.58% to 103.84% and the inter-assay precision was $\leq 6.72\%$ [14].

	Mean	Standard Deviation	Median	Min-Max
Age (years)	37	13	44	21-51
Weight (kg)	71	14	70	54-94
Height (cm)	171	10	165	163-190
Sex (M/F)	5/3			

Table 1: Baseline demographics of the 8 healthy volunteers.

Time min	Product A			Product B		
	Mean ng/mL	SD	Median	Mean ng/mL	SD	Median
0	0.38	0.47	0.27	0.00	0.00	0.00
5	1.09	0.85	0.75	0.29	0.26	0.22
10	4.79	3.87	3.11	3.21	3.84	1.64
20	10.44	7.20	7.74	7.01	5.24	6.56
30	13.08	6.29	11.23	9.09	4.36	9.43
40	13.14	5.97	11.10	10.62	5.65	10.66
60	11.43	4.73	11.78	9.41	7.04	7.97
90	8.52	5.61	6.85	6.55	5.09	5.57
120	6.58	4.70	4.71	4.77	3.65	3.99
180	3.31	3.17	1.92	1.76	1.61	1.44
240	1.66	2.09	0.55	0.72	0.58	0.67
360	0.39	0.48	0.21	0.18	0.15	0.21

Table 2: Mean melatonin plasma concentrations.

Product A		
Parameters	Arithmetic Mean mean [SD]	Range
$AUC_{0-\infty}$ (ng*min/mL)	1719.93 [918.47]	761.49-3167.70
C_{max} (ng/mL)	17.2 [9.3]	7.4-37.4
T_{max} (min)	42.5 [24.9]	20.0-90.0
$T_{1/2}$ (min)	54.0 [9.6]	45.3-75.6
K_a (min ⁻¹)	0.0311 [0.0188]	0.0127-0.0656
MRT (min)	57.2 [18.2]	32.0-86.3
Product B		
$AUC_{0-\infty}$ (ng*min/mL)	1179.23 [776.80]	232.84-2579.90
C_{max} (ng/mL)	12.4 [6.6]	3.6-22.9
T_{max} (min)	37.5 [11.7]	20.0-60.0
$T_{1/2}$ (min)	51.9 [13.4]	34.0-69.1
K_a (min ⁻¹)	0.0233 [0.0049]	0.0176-0.0293
MRT (min)	51.6 [13.8]	33.4-72.9
Ratio A/B		
Variable	mean [SD]	Range
$AUC_{0-\infty}$ (ng*min/mL)	1.86 [1.17]	0.87-4.07
C_{max} (ng/mL)	1.5 [0.5]	0.9-2.5

Table 3: Pharmacokinetic parameters of Melatonin in emulsion spray Product A and Melatonin tablet Product B (8 subjects; dose 5 mg)

Pharmacokinetic and statistical results

The mean and standard deviation values of $AUC_{0-\infty}$, C_{max} , T_{max} , $T_{1/2}$, K_a and MRT are shown in table 3.

Figure 1 shows the disposition of melatonin after administration of product A (Test Preparation), or product B (Reference Preparation) and the average curves of plasma concentration-time as a result of product A or product B.

The mean $AUC_{0-\infty}$ [SD] were 1719.93 [918.47] (ng* min/mL) and 1179.23 [776.80] ng* min/mL for product A and B, respectively.

Significant differences were observed in $AUC_{0-\infty}$ values obtained after treatment with A and B ($P=0.0446$).

The ratio F (A/B), between the $AUC_{0-\infty}$ values obtained after

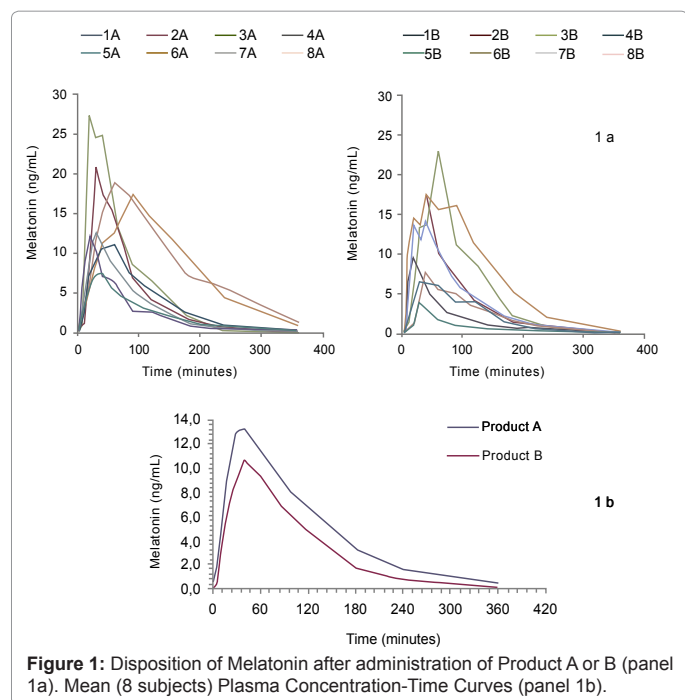


Figure 1: Disposition of Melatonin after administration of Product A or B (panel 1a). Mean (8 subjects) Plasma Concentration-Time Curves (panel 1b).

administration of product A or product B was 1.86 [1.17], when considering the arithmetic mean.

The mean C_{max} obtained after administration of product A or product B was 17.2 [9.3] ng/mL and 12.4 [6.6] ng/mL, respectively. Significant differences were observed in C_{max} values obtained after treatment with A or B ($P=0.0210$). The concentration peak for melatonin was obtained at 42.5 [24.9] and 37.5 [11.7] min (T_{max}) for product A and product B, respectively. The difference was not statistically significant ($P=0.7227$).

Safety

The preparations and the study treatments were well tolerated without any adverse reactions.

Discussion

Melatonin is a good candidate for sublingual administration, considering its variable oral absorption, short biological half-life and extensive first pass metabolism. With oral melatonin doses of 2-4 mg, only 15% of the dose reaches the systemic circulation [15].

Alternatively, oral mucosa absorption avoids the first-pass effect, associated with liver metabolism of melatonin. In general compounds that come in contact with the mucous membrane quickly diffuse through it, since the connective tissue beneath the epithelium is highly vascularized.

This special spray emulsion, showed improvement in most of the issues that have so far hampered the clinical efficacy of melatonin.

The current study characterized the bioavailability of a new oral spray formulation of melatonin in comparison with a standard oral formulation.

The AUC values obtained following a single dose of the two preparations showed significant differences. The amount of melatonin reaching the systemic circulation after administration of oral spray melatonin was significantly higher than that of the oral tablet formulation.

Data obtained in this study showed that the extent of melatonin absorption after oral spray delivery was 1.8 times that observed after administration of the standard oral tablet; the peak concentration was also significantly higher, 1.5 times the corresponding oral tablet value. The absorption rate expressed as T_{max} and K_a was comparable between the two products. After administration of product A (Melatonin in emulsion spray) concentrations of melatonin at 5, 10, and 20 min respectively were 4.6, 3.5, 3.0 times higher than the corresponding concentrations of product B (Melatonin tablet). Thereafter, the concentrations of melatonin after administration of product A were 1.5-2.4 times the corresponding concentrations of product B.

Product A (Melatonin spray) in emulsion and the reference preparation, Product B, (Melatonin tablet) were not super impossible in terms of extent of absorption.

The present study indicates that the new formulation Melatonin food supplement in emulsion spray improves melatonin absorption, ensuring higher concentrations after administration, compared to the standard oral tablet formulation of melatonin.

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