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 amount of melatonin reaching the systemic circulation after oral spray administration was significantly higher than after oral tablet administration. 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 (AUCinf), peak plasma concentration (Cmax), Mean Residence Time (MRT), Time relative to the peak concentration (Tmax), elimination half-life (T1/2).

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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 inducers 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 Medesta 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 and Melatonin tablet containing 5 mg of melatonin manufactured in emulsion spray by Medesta SpA, 1 mg of melatonin for each spray (5 sprays of a food grade liquid emulsion directly under the tongue) by SIRC. During scheduled visits, the investigator dispensed 5 mg (5 sprays of a food grade liquid emulsion directly under the tongue) of melatonin (Medesta 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 conical glass tubes and evaporated to dryness under a gentle stream of nitrogen at room temperature. Residues were reconstituted in 100 µL of acidified water (HCOOH 0.1%) and transferred into a micro-vial. 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 50 μL aliquots of working solutions of melatonin were added to 450 μL of human plasma/water (blank) in 1.5 mL polypropylene tubes, to yield the final concentrations reported in standard samples and quality controls (QC).

In brief, 50 µL ofIS (20 ng/mL), 50 µL of 10% Ammonium Hydroxide solution were added to each standard/unknown sample (500 µL), 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 µL of acidified water (HCOOH 0.1%) and transferred into a micro-vial. Aliquots of 10 µL 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:

\[ C_{\text{max}} = \text{peak plasma concentration (ng/ml);} \]
\[ T_{\text{max}} = \text{Time relative to the peak concentration (min);} \]
\[ AUC_{0-\infty} = \text{Area Under the Curve plasma concentration - time curve from 0 to } \infty \text{ (ng*min/ml), calculated by using the linear trapezoidal rule (Kinetica TM Software version 4, InnaPhase Corporation Philadelphia PA);} \]
\[ F(A/B) = \text{Relative bioavailability of product A versus product B;} \]
\[ MRT = \text{Mean Residence Time (min);} \]
We considered \( AUC_{0-\infty} \) as the true estimate of the relative bioavailability \( F \) of melatonin, obtained by comparing \( AUC \) from time 0 to \( \infty \). 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 in quantitative variables. Parametric or non parametric tests were used.

Table 1 summarizes demographic and baseline characteristics of the healthy enrolled volunteers. Eight subjects were enrolled and completed the study. Table 1 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 quantification (LLOQ) for melatonin was 0.156 ng/mL. In all curves, the non-zero intercept was used to obtain the best fit of data for the calibration curves.

The mean AUC \(0-\infty \) [SD] were 1719.93 [918.47] (ng* min/mL) and 1719.93 [918.47] (ng* min/mL) for product A and B, respectively. Cmax and AUC \(0-\infty \) were analyzed by the Student t Test for Paired Data. Tmax difference was tested by the Wilcoxon signed-rank test.

**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).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean [SD] Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37 [13] 21-51</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 [14] 54-94</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171 [10] 163-190</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/3</td>
</tr>
</tbody>
</table>

Table 1: Baseline demographics of the 8 healthy volunteers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean [SD] Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (0-\infty) (ng*min/mL)</td>
<td>1719.93 [918.47] 761.49-3167.70</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>17.2 [9.3] 7.4-37.4</td>
</tr>
<tr>
<td>Tmax (min)</td>
<td>42.5 [24.9] 20.0-90.0</td>
</tr>
<tr>
<td>T1/2(min)</td>
<td>54.0 [9.6] 45.3-75.6</td>
</tr>
<tr>
<td>K (min(^{-1}))</td>
<td>0.0311 [0.0188] 0.0127-0.0656</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>57.2 [18.2] 32.0-86.3</td>
</tr>
</tbody>
</table>

Product A

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mean [SD] Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.38 [0.47] 0.27</td>
</tr>
<tr>
<td>5</td>
<td>1.09 [0.65] 0.75</td>
</tr>
<tr>
<td>10</td>
<td>4.79 [3.67] 3.11</td>
</tr>
<tr>
<td>20</td>
<td>10.84 [7.20] 7.74</td>
</tr>
<tr>
<td>30</td>
<td>13.06 [6.29] 11.23</td>
</tr>
<tr>
<td>40</td>
<td>14.13 [5.97] 11.10</td>
</tr>
<tr>
<td>60</td>
<td>11.43 [4.73] 11.78</td>
</tr>
<tr>
<td>90</td>
<td>8.52 [5.61] 6.85</td>
</tr>
<tr>
<td>120</td>
<td>6.58 [4.70] 4.71</td>
</tr>
<tr>
<td>180</td>
<td>3.31 [1.37] 1.92</td>
</tr>
<tr>
<td>240</td>
<td>1.66 [2.08] 0.55</td>
</tr>
<tr>
<td>360</td>
<td>0.39 [0.48] 0.21</td>
</tr>
</tbody>
</table>

Table 2: Mean melatonin plasma concentrations.

Pharmacokinetic and statistical results

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 in quantitative variables. Parametric or non parametric tests were used.

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

The mean $C_{\text{max}}$ 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 $C_{\text{max}}$ 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<sub>max</sub>) 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 $T_{\text{max}}$ and $K_{\text{a}}$ 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 superimposable 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.

### References