Bioavailability of Oral Melatonin in Humans

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Abstract. We administered crystalline melatonin (80 mg) in gelatin capsules to 5 young male volunteers and measured serum and urinary melatonin levels at intervals. Changes in serum melatonin levels were best described by a biexponential equation with an absorption constant ($k_a$) of $1.72 \text{ h}^{-1}$ (half-life = 0.40 h) and an elimination constant ($k_e$) of 0.87 $\text{ h}^{-1}$ (half-life = 0.80 h). Peak serum melatonin levels, ranging from 350 to 10,000 times those occurring physiologically at nighttime, were observed 60–150 min after its administration, remaining stable for approximately 1.5 h. The fraction of ingested melatonin that was absorbed, estimated from the area under the curve describing serum melatonin concentrations as a function of time after melatonin administration (the concentration-time curve), varied by 25-fold among subjects. 3 additional volunteers received three melatonin-containing capsules (80 mg each) at 60-min intervals. This regimen extended the duration of elevated serum melatonin levels to 4–6 h. Melatonin excretion closely paralleled serum melatonin levels until 9 h after the hormone’s administration, after which urinary levels tended to be higher than those predicted from serum levels. However, the area under the concentration-time curve for serum melatonin correlated well ($r = 0.96$) with the cumulative melatonin excretion during the initial 15 h after melatonin’s administration, indicating that either approach can be used to estimate the absorption of orally administered melatonin.

Although melatonin, a hormone secreted by the mammalian pineal gland, has been administered to numerous human subjects in efforts to assess its possible physiological or psychological effects [9], available data on its physiological disposition in humans are fragmentary and sometimes inconsistent [22]. Most commonly the subjects received the melatonin orally. In view of melatonin’s limited solubility in water [8, 16], variations in its bioavailability among these subjects might have contributed to inconsistencies in its observed effects. Recently Iguchi et al. [4] described the rates at which melatonin, given as an intravenous bolus, is distributed ($t_{1/2} = 5.6 \text{ min}$) and eliminated ($t_{1/2} = 43.6 \text{ min}$) in human subjects. Data regarding melatonin’s bioavailability after its oral administration are scant [12, 23]; hence the present study was designed to monitor serum and urinary melatonin levels after oral administration of the crystalline hormone in capsules to normal human subjects. Although we observed considerable variability among subjects in actual serum melatonin levels, the kinetic parameters describing the hormone’s distribution and elimination were similar among subjects.

Subjects and Methods

8 healthy male volunteers, aged 20.5–29.5 years (tables 1, 11), were admitted to the Massachusetts Institute of Technology Clinical Research Center as inpatients for 36 h; they were in darkness between 10.00 p.m. and 7.00 a.m. and in light at other times. Before admission, the test protocol was fully explained to each volunteer, written informed consent was obtained, and a physical examination was performed. The test protocol had been sanctioned by the MIT Committee on Human Use as Experimental Subjects, and approval of the US Food and Drug Administration had been obtained for administration of melatonin (IND No. 19545). At the time of admission (7.30 a.m.) an indwelling catheter (Abbocath T-18) was inserted into an antecubital vein and kept open with a saline infusion (1,500 ml/24 h) for 36 h. On the following morning at 11.00 a.m. a gelatin capsule containing 80 mg crystalline melatonin (Nestle Co., Vevey, Switzerland) was administered to 5 of the volunteers. 3 additional volunteers received three such capsules at hourly intervals (11.00 a.m., 12.00 a.m., 1.00 p.m.). During the test
period each subject provided 46 blood samples (10 ml each) at various intervals (fig. 1, 3). Sera were separated by centrifugation and stored at −20 °C until assayed. Urine was collected during either 3-hour or 6-hour intervals (fig. 1, 3). The volume excreted during each time period was recorded and an aliquot was frozen at −20 °C until assay.

Assay of Serum Melatonin

Melatonin in serum was determined by means of a radioimmunoassay procedure using antimelatonin serum [10] provided by Dr. L. Levine of Brandeis University, Waltham, Mass. Sodium hydroxide (0.5 ml, 1 M) was added to 1 ml serum or an appropriate dilution of serum, and the mixture was extracted with 5 ml CHCl₃. The aqueous phase was discarded and the organic phase was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.55 ml Tris buffer (0.01 M Tris, pH 7.5, 0.14 M NaCl containing 0.05% gelatin). The buffer extract was then washed with 1 ml petroleum ether. 500 µl of this extract or of a standard melatonin solution was mixed with 100 µl of antiserum solution (diluted 1:5000 with Tris buffer containing 0.15% gelatin) and 100 µl 3H-melatonin (New England Nuclear Co., Boston, Mass.), also diluted with Tris buffer containing 0.15% gelatin, to yield 1,750 cpm/100 µl. The mixture was then shaken using a vortex mixer and incubated at 35 °C for 60 min. 1 ml of saturated ammonium sulfate was then added, and the mixture was incubated overnight at 4 °C. After centrifugation the precipitate was dissolved in 200 µl 0.1 N NaOH, scintillation fluid ('Biofluor', New England Nuclear Co.) was added, and its radioactivity was counted. Melatonin concentrations were estimated by means of the logit log plot [18]. The sensitivity of the assay (B/BO = 85%) varied between 5 and 10 pg/ml serum. The recovery of added melatonin was 86.9 ± 2.1%(mean ± SEM); all present values are corrected for recovery. In control samples containing 38.5 or 188 pg melatonin/ml serum, the intrasubject coefficients of variation were 13.8 and 7.9%, respectively. The corresponding interassay coefficients of variation were 21.2 and 12.5%.

Assay of Urinary Melatonin

Urinary melatonin levels were determined by adjusting 5-ml aliquots of each urine sample to pH 5 and extracting the hormone into 8 ml of chloroform. The organic extract was washed successively with 3 ml 0.1 N sodium hydroxide and 3 ml of water, and then evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.6 ml of chloroform: 1.2 ml of 0.01 M phosphate buffer (pH 6.5) containing 0.1% gelatin and 6 ml of heptane were added. The tubes were then capped, mechanically shaken, and centrifuged. Duplicate 0.5-ml aliquots of the buffer extract were submitted to radioimmunoassay.

To obviate a cumbersome chromatographic purification step in the preparation of urine extracts for assay [11], we used a highly specific rabbit antimelatonin serum (provided by Dr. G. Brown, McMaster University, Hamilton, Ontario) [15]. The antiserum and 3H-melatonin are incubated for 1 h at 37 °C in the presence of graded concentrations of unlabeled melatonin or aliquots of urine extract. After addition of saturated ammonium sulfate solution, the reaction mixture is incubated overnight at 4 °C. The antibody-bound 3H-melatonin is collected as a precipitate by centrifugation, dissolved in 0.1 N NaOH, and its radioactivity counted in scintillation fluid.

Melatonin concentrations were estimated from a plot of logit (B/BO) versus a log transformation of the dose axis [20]. In seven successive assays, samples of pooled urine to which 100 or 200 pg of authentic melatonin were added yielded mean recoveries of 74 and 78%, respectively. The intraassay and interassay coefficients of variation were 6 and 17%, respectively, at both melatonin levels. Estimates of urinary melatonin reported here were corrected for recovery. The limit of sensitivity of this assay procedure (B/BO = 85%) ranged from 10 to 16 pg per tube.

Mathematical Analysis

The exponential equations which best described serum melatonin values in individual subjects and in the means of the 5 subjects receiving a single dose were characterized using the 'feathering method' [13] (fig. 2). For this purpose we utilized serum melatonin values obtained during the interval starting 15 min after its ingestion (these values did not differ from normal daytime values) and terminating when serum levels were 3 times greater than those observed the previous night. The exponents of this equation were used to estimate melatonin's absorption constants (kₐ) and elimination constants (kₑ). The biological half-life (τₑ) for absorption was calculated according to the formula τₑ = 0.693/kₐ [13]. Variations in the efficiencies with which orally administered melatonin was absorbed were assessed by comparing the areas under each subject's curve (AUC), relating its serum melatonin concentration to the time after melatonin administration. For this calculation we utilized serum melatonin concentrations obtained during the interval starting with melatonin's administration and ending when serum melatonin levels had fallen to 3 times those observed during the previous night. Each area was estimated by means of the 'trapezoidal method' [13].

Results

Serum Melatonin Levels and Melatonin Excretion in Subjects Receiving a Single Melatonin Dose

All subjects showed a normal nocturnal increase in the serum melatonin concentration on the day before receiving the melatonin capsule. Peak values were observed throughout the dark period. 1 subject (No. 6) exhibited elevated serum melatonin values through the night and an additional high value was observed at 8 a.m. in the light (fig. 1). Highest values were observed 60-150 min after the hormone was ingested. Even though at that time serum melatonin levels were 350-10,000 times higher than the peak levels observed during the previous night, serum melatonin returned to its physiological nocturnal range within 8-19 h (fig. 1).

Observed serum melatonin levels after its oral administration were best described by a biexponential equation of the form

\[ C = A e^{-k_a t} + B e^{-k_e t}, \]

where C is concentration. The parameters for this equation (derived from mean serum melatonin values by the 'feathering method') are shown in figure 2. Individual subject's val-
Fig. 1. Serum melatonin levels (a) and urinary melatonin excretion (b) in 5 male volunteers (subjects 4, 5, 6, 9, 10) before and after oral administration of 80 mg melatonin in a gelatin capsule.

ues for the exponents found for this equation are given in table 1. From these exponents a mean (± SEM) absorption constant ($k_s$) = 1.72 ± 0.12 h⁻¹, $t_{1/2}$ = 0.40 h and a mean elimination constant ($k_d$) = 0.86 ± 0.03 h⁻¹ ($t_{1/2}$ = 0.80 h) were calculated for orally administered melatonin. The fact that a biexponential equation best fit the data indicates that melatonin's distribution after its oral administration obeys either a one-compartment open model or a two-compartment open model with very rapid exchange between the compartments [13].

Peak serum melatonin levels remained relatively stable during the interval occurring 60–150 min after its ingestion (fig. 1, 2).

Marked differences were noted in the serum and urinary melatonin levels of the 5 subjects receiving a single dose of the hormone (fig. 1): the area under the concentration-time curve of individual subjects varied by as much as 25-fold (table 1).

Urinary melatonin levels paralleled serum values until 9 h after melatonin administration. Thereafter, at a time
when serum melatonin values had already dropped to their normal nighttime range, melatonin excretion was still approximately 100 times greater than that normally observed at nighttime (fig. 1).

**Serum Melatonin Levels and Melatonin Excretion in Subjects Receiving Three Doses of Melatonin**

The 3 subjects that received three 80-mg doses at hourly intervals maintained peak serum melatonin levels for a longer period (4–6 h) than those receiving a single dose (fig. 3); however, peak levels were no higher. The areas under the serum concentration-time curves showed variations similar to those seen in subjects receiving a single dose of melatonin (table II); the pattern of melatonin excretion was also similar (fig. 3).

**Relationship between Serum and Urinary Melatonin after Its Administration**

The excretion of melatonin in the initial 9 h after its administration correlated well (r = 0.96) with the area under the concentration-time curve (fig. 4). Thus, melatonin's absorption after its oral administration can be assessed from either its excretion or the area under its serum concentration-time curve.

**Discussion**

These data show that orally administered melatonin causes very rapid elevations in serum melatonin levels, with plateaus persisting for several hours. Soon after the discovery of melatonin, Kopin et al. [6] described the biphasic

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**Table I. Characteristics and absorption parameters of subjects receiving a single dose of melatonin**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>ket (h⁻¹)</th>
<th>t₁/₂(ket) (h)</th>
<th>Kₙ (h⁻¹)</th>
<th>t₁/₂(kₙ) (h)</th>
<th>Area (ng h/ml)</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>24.50</td>
<td>175.9</td>
<td>97.2</td>
<td>0.90</td>
<td>0.77</td>
<td>2.14</td>
<td>0.32</td>
<td>61.89</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>24.50</td>
<td>172.5</td>
<td>72.8</td>
<td>0.79</td>
<td>0.88</td>
<td>1.78</td>
<td>0.39</td>
<td>1,605.84</td>
<td>25.95</td>
</tr>
<tr>
<td>6</td>
<td>21.25</td>
<td>170.1</td>
<td>67.8</td>
<td>0.90</td>
<td>0.77</td>
<td>1.57</td>
<td>0.44</td>
<td>493.75</td>
<td>7.98</td>
</tr>
<tr>
<td>9</td>
<td>20.50</td>
<td>174.5</td>
<td>69.5</td>
<td>0.95</td>
<td>0.73</td>
<td>1.68</td>
<td>0.41</td>
<td>97.69</td>
<td>1.58</td>
</tr>
<tr>
<td>10</td>
<td>27.50</td>
<td>190.5</td>
<td>85.4</td>
<td>0.78</td>
<td>0.89</td>
<td>1.44</td>
<td>0.48</td>
<td>63.23</td>
<td>1.02</td>
</tr>
</tbody>
</table>

ket = Elimination constant; kₙ = absorption constant; t₁/₂ ket = half-life of elimination; t₁/₂ kₙ = half-life of absorption. The factor was determined by assigning a value of 1 to the subject with the lowest area under the concentration-time curve (subject 4) and expressing other areas as a multiple of that area; the renal clearance of exogenous melatonin, calculated from these data, was 161.1 ± 33.0 ml/h (mean ± SEM).
disappearance of intravenously administered radioactive melatonin from the whole mouse. The biological half-life in the first 10 min after administration was estimated to be approximately 2 min; thereafter it was about 35 min. The hormone was subsequently found to be taken up within practically all tissues, but especially within the pineal and ovaries [24]. Subsequently, a similar biphasic pattern was observed for the disappearance of intravenous radioactive [6, 17] or nonradioactive [3, 4, 12] melatonin from the blood in several mammalian species, including humans. The biological half-life for the early distribution phase was found to be in the range of a few minutes, and that of the elimination phase ranged from 20 to 50 min. Similar biexponential patterns of disappearance have been described following placement of exogenous melatonin into the CSF of rhesus monkeys [18, 19] or sheep [21].

One group of investigators monitored serum melatonin levels in sheep given melatonin orally [5]. Peak levels were reached within 30 min and a stable plateau was sustained for at least 7 h. This prolonged elevation of serum melato-
Table II. Characteristics and absorption parameters of subjects receiving three doses of melatonin

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Area (ng h/ml)</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29.5</td>
<td>179.7</td>
<td>76.6</td>
<td>161.63</td>
<td>1.00</td>
</tr>
<tr>
<td>B</td>
<td>22.5</td>
<td>172.0</td>
<td>60.1</td>
<td>720.11</td>
<td>4.46</td>
</tr>
<tr>
<td>C</td>
<td>22.5</td>
<td>180.1</td>
<td>81.6</td>
<td>682.89</td>
<td>4.23</td>
</tr>
</tbody>
</table>

The factor was determined by assigning a value of 1 to the subject with the lowest area under the concentration-time curve (subject A) and expressing other areas as a multiple of that area.

Fig. 4. Correlation and regression between the area under the melatonin concentration-time curve (in arbitrary units) and the amount of melatonin excreted within 15 h of its administration. $r = \text{Correlation coefficient; } p = \text{probability.}$

...in after its oral administration was thought to be a peculiarity of this species and a result of the mixing of the administered melatonin with the contents of the animal's commodious rumen and its subsequent slow release.

In preliminary observations on human subjects, it was observed that 1 male volunteer exhibited highest melatonin values 30 min after oral administration of 100 mg melatonin [23], while in 2 other subjects peak melatonin values were found 30–90 min after ingestion of 2.5 mg melatonin [12]. Although neither of these publications provided sufficient data to permit estimation of pharmacokinetic parameters, the shapes of the serum concentration-time curves from all 3 subjects were similar to those exhibited by our volunteers.

Our data show that oral melatonin is rapidly absorbed in humans: its elimination is more rapid than in sheep, but slower than in rodents. The elimination constant after its oral administration is similar to that observed by others [12, 23] after its intravenous administration. Changes in serum melatonin values were best characterized by a biexponential equation, suggesting either a one-compartment open model with first-order absorption or a two-compartment open model with rapid flux between the compartments and first-order absorption [13]. Given melatonin's biphasic disappearance [4, 6, 14, 17] with a rapid distribution phase after its intravenous administration [6, 21], the latter model seems to be appropriate.

After a single oral dose, relatively stable serum melatonin concentrations are maintained for approximately 1.5 h (fig. 1). This time can be extended with little cumulative effect by giving the same dose repeatedly at hourly intervals (fig. 3).

Although there is little between-subject variation in the rate constants for melatonin's absorption and elimination, substantial interindividual differences were observed in the areas under their concentration-time curves. The basis of these major differences in melatonin's absorption is not clear. They may reflect the use of a crystalline preparation of melatonin; perhaps a melatonin solution would have achieved more uniform absorption.

Interestingly, melatonin's excretion closely paralleled its serum concentration; however, during the late hours after melatonin administration, its excretion was considerably higher than would have been predicted from concurrent serum values. Endogenous melatonin is mainly metabolized in the liver to 6-OH-melatonin and excreted as conjugates of sulfuric and glucuronic acids [7]. The pharmacological dosage used may have exceeded the metabolic capacity of the liver and thus increased the percentage of unchanged melatonin which was excreted.

Exogenous melatonin has been found by several investigators to facilitate sleep onset [1, 2]; the indole significantly decreased self-reported alertness and increased sleepiness as measured by the Profile of Mood States and the Stanford Sleepiness Scale self-report mood questionnaires [22]. A better understanding of the fate and distribution of the exogenous hormone may accelerate the growth of knowledge concerning its possible roles and uses.

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