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Pharmacokinetics of orally administered melatonin in critically ill patients

Abstract: Critically ill patients exhibit reduced melatonin secretion, both in nocturnal peaks and basal daytime levels. Oral melatonin supplementation may be useful for known sedative and antioxidant properties. Its early enteral absorption and daily pharmacokinetics were determined in two cohorts of six high-risk patients in this prospective trial. During their third and fourth Intensive Care Unit (ICU) day, they underwent two different sets of repeated blood samples to detect serum melatonin levels through radio-immuno-assay. Cohort 1: samples taken at 20:00, 20:45, 21:30, 24:00, 03:00, 06:00, 14:00, 20:00 to describe the daily pharmacokinetics. Cohort 2: 20:00, 20:05, 20:10, 20:20, 20:30, 20:45 to study the early absorption. On ICU day 3, endogenous levels were measured, while the absorption of exogenous melatonin was determined on ICU day 4 after administration, at 20:00, of 3 mg melatonin. All basal levels were below the expected values. Following enteral administration, pharmacological levels were already reached in 5 min, with a serum peak after 16 min (half-absorption time: 3 min 17 s). The maximum serum level observed was 11040 pg/mL and the disappearance rate indicated a half-elimination time of 1 hr 34 min. Serum melatonin levels decreased significantly after midnight; pharmacological levels were maintained up to 10 hr following administration. No excessive sleepiness was reported in this patient group. Critically ill patients exhibited reduced melatonin secretion, as reported in the literature. Despite the critical illness, the oral bioavailability was satisfactory: serum levels after oral administration showed basically unchanged intestinal absorption, while disappearance rate was slower than reported elsewhere in healthy volunteers.

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Introduction

The clinical relevance of melatonin is gaining ground [1]. The role of melatonin in the treatment of sleep disturbances [2], to prevent jet-lag [3], as an antioxidant [4, 5] and oncostatic agent [6, 7], or as part of the sepsis treatment [8], is widely discussed; yet the role of melatonin in critically ill patients still calls for extensive investigation [9].

Critically ill patients suffer from severe sleep disturbances during their stay in an Intensive Care Unit (ICU) [10, 11]. Several studies found melatonin levels in critically ill patients to be severely depressed [12–15], either in terms of nocturnal peaks or basal daytime serum levels [16]. Moreover, these patients require high levels of antioxidants due to their critical illness [17, 18].

Whether low serum levels of melatonin are due to reduced production or higher consumption [19] as an antioxidant is unknown; however, such evidence could have a role in the significant prevalence of sleep disorders among ICU patients.

The causes of sleep disturbances during critical illnesses have been largely investigated and discussed and could be reasonably related to pain, noise, interventions as well as

nocturnal check-ups, daytime sleep, biological clock impairment, drug interactions [12, 20] (mainly opioids, benzodiazepines, vasopressors and corticosteroids) and emotional stress. Finally, it is unknown whether sleep disturbances merely result from the harsh ICU environment, or are primarily due to low melatonin levels [21].

Oral melatonin supplementation could be a useful therapeutic approach [22–24] but there is a lack of data on the pharmacological interactions and first-passage effects of oral melatonin in high-risk patients. Organ failure and gut function might affect absorption and explain differences compared with healthy subjects. This study investigated the enteral absorption and pharmacokinetics of orally administered melatonin in high-risk critically ill patients during their first 4 days of ICU stay.

Materials and methods

Study population

We planned a prospective, interventional trial enrolling high-risk patients consecutively admitted to general ICU with an estimated need for mechanical ventilation of more than 4 days [25]. Exclusion criteria were: underage, hepatic failure (Child-Pugh class C), dialytic treatment and gut impracticability [26]. As soon as their clinical and neurological conditions improved, patients were duly informed of the study and their consent was obtained. Tablets of 125 mg were produced (Procemsa, Torino, Italy) containing 3 mg of pure melatonin (Helsinn, Biasca, Switzerland), with microcrystalline cellulose (70 mg), calcium phosphate (47 mg), magnesium stearate (2.5 mg) and sodium carboxymethyl cellulose (2.5 mg) as excipients.

Timing of blood samples

After a period of clinical stabilization, the 2-day pharmacokinetic study began on ICU day 3. All study days started at 20:00 and lasted 24 hr. In the first six patients (Cohort 1), we evaluated the endogenous profile of melatonin with blood samples collected at 20:00, 20:45, 21:30, 24:00, 03:00, 06:00, 14:00 and 20:00 hr, just before melatonin administration. Three milligrams of melatonin were then administered by nasogastric (or naso-jejunal) tube after crushing the tablet and mixing it with 20 mL of water, followed by another 20 mL to flush out the remnants inside the tube. Blood samples in study day 2 were then collected at the same times as on study day 1.

The analysis of data from Cohort 1 highlighted rapid melatonin enteral absorption, with the highest serum concentration reached in the first blood sample (45 min after melatonin administration). Hence, to investigate the early kinetics of melatonin, six more critical patients were enrolled (Cohort 2). They underwent blood sampling on study day 1 at 20:00, 20:05, 20:10, 20:20, 20:30 and 20:45. Three milligrams of melatonin were then given at the beginning of study day 2, and blood samples were collected at the same times as on study day 1.

Extreme attention was paid to maintaining darkness at night when the blood samples were taken via central venous catheters. Patients' sleepiness was monitored by nurses, assuming that observing a calm patient with closed eyes meant that the patient was sleeping. All patients received standard intensive treatment including ventilatory, cardiovascular, nutritional support (continuous enteral nutrition or a combination of enteral and parenteral nutrition) and sedatives based on their clinical needs.

To evaluate the pharmacokinetic values of exogenous melatonin, differences between serum melatonin levels on study day 1 and 2 were calculated (corrected concentration-time curves), assuming basal secretion to be substantially unchanged according to unmodified clinical conditions.

Serum melatonin measurements

To measure melatonin levels, blood samples were collected, as previously described, from central venous catheters placed in the internal jugular vein before the beginning of the study. Samples were collected in plastic tubes without anticoagulant agents. Serum samples were immediately separated by centrifugation and stored at −20°C until assayed. Melatonin concentrations were measured using a commercial radio-immuno-assay (RIA) kit for human melatonin (BioSource Europe S.A., Belgium). Serum samples (200 μL) were incubated overnight with [^{125}I]-melatonin and melatonin antibody. The next day, the bound fraction was counted after precipitation. In this assay, sensitivity was 2 pg/mL. The reproducibility of the RIA tests was investigated by determining the intra- and inter-assay coefficient of variation (CV) by repeated measurements of three serum samples with different melatonin concentrations. The intra-assay CV from 15 different runs was 12.1% (15.1 pg/mL), 9.8% (50.1 pg/mL) and 12.3% (157 pg/mL); the inter-assay CV from 15 different runs was 12.3%, 9.6% and 16.2% at concentrations of 21.4, 39.4 and 205.4 pg/mL, respectively.

Statistical analyses

Data were statistically analysed using GraphPad Version 4 (GraphPad Software Inc., La Jolla, CA, USA). Serum melatonin concentration data were analysed by one-way ANOVA, non-linear regression and fitted with this function: $Y = \text{Span}*\exp(-K_1*X) - \text{Span}*\exp(-K_2*X) + \text{Plateau}$. Pharmacokinetic parameters were also determined.

Results

Twelve patients were enrolled from March to June 2007: 4 had pneumonia, 2 cardiac arrest, 2 cardiac failure after myocardial infarction, 1 septic shock, 1 pancreatitis, 1 trauma and 1 hypovolemic shock in enterocolitis. Demographical and clinical data are reported in Table 1.

On study day 1, melatonin levels after 48–60 hr from ICU admission were significantly depressed (Table 2) compared with the values reported in the literature for healthy volunteers [27]. In five out of six patients no circadian rhythm was found (Fig. 1).

On study day 2, the corrected concentration-time curve after melatonin administration in Cohort 1 is reported in Table 2 and Fig. 2. Mean maximal melatonin serum concentration was reached in the first sample (45 min from

Table 1. Epidemiological and clinical data

	Cohort 1 (6 patients)	Cohort 2 (6 patients)	*P*-value
Age (yr)	62 [58–71]	74 [56–81]	0.518
Sex (M:F)	5:1	5:1	1
Weight (kg)	80 [70–85]	79 [75–90]	0.872
Height (cm)	170 [160–170]	173 [170–175]	0.351
SAPS II (points)[a]	56 [31–69]	59 [53–64]	0.575
ICU-LOS (days)	9 [6–23]	29 [5–70]	0.378
MV length (days)	7 [5–10]	7 [5–33]	0.809
SOFA (points)[b]	7 [3–9]	7 [4–9]	0.872
Urea (mg/dL)[b]	124 [102–135]	155 [37–218]	0.423
Creatinine (mg/dL)[b]	2.5 [1.6–3.2]	1.9 [1.2–2.5]	0.575
Total Bilirubin (mg/dL)[b]	0.5 [0.2–0.5]	2.2 [1.2–3.1]	0.044
Hospital mortality (%)	66	33	0.248

SAPS, Simplified Acute Physiology Score (range: 0–163); ICU-LOS, intensive care unit length of stay; MV, mechanical ventilation; SOFA, sequential organ failure assessment (range: 0–14).
Values are median [interquartile range] or absolute numbers. Variables were compared with Wilcoxon rank-sum test or Fischer Exact test.
[a]Values referred to the first 24 hr in intensive care unit.
[b]Values referred to the 3rd day in intensive care unit.

Table 2. Serum melatonin levels

	Study day 1 (ICU day 3)		Study day 2 (Treatment)	
Time	Cohort 1	Cohort 2	Cohort 1	Cohort 2
20:00	3 [3–10]	3 [3–5]	–	–
20:05	–	6 [3–10]	–	4691 [4026–4997]
20:10	–	4 [3–9]	–	6168 [3627–12074]
20:20	–	4 [3–11]	–	5483 [3148–11138]
20:30	–	4 [3–11]	–	2641 [1498–5310]
20:45	4 [3–15]	3 [3–11]	9588 [5874–9695]	1281 [889–6475]
21:30	6 [3–15]	–	6531 [4397–6641]	–
24:00	3 [3–10]	–	1616 [1248–1735]	–
03:00	11 [6–17]	–	439 [274–1311]	–
06:00	10 [8–20]	–	138 [50–251]	–
14:00	11 [9–12]	–	48 [16–118]	–
20:00	5 [3–12]	7 [4–9]	21 [9–82]	–

Values are median [interquartile range], expressed as pg/mL serum melatonin.

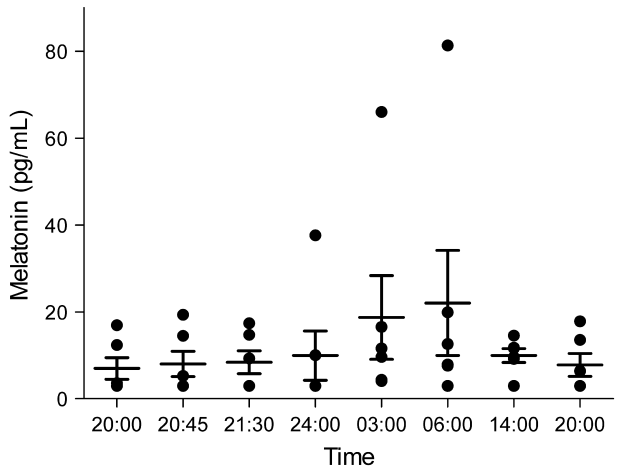

Fig. 1. Daily endogenous melatonin levels in critically ill. Serum melatonin values measured in critically ill patients of Cohort 1 during their ICU day 3. Points are the absolute values; error bars represent mean and standard deviation.

administration) in all patients (Table 2). Serum melatonin pharmacological levels [28, 29] were observed up to 10 hr following oral administration. In Cohort 1, the K_2 value (absorption constant) could not be determined.

The corrected concentration-time curve after melatonin administration in Cohort 2 is reported in Table 2, Fig. 3 and Fig. 4: these observations made it possible to determine the K_2 (absorption constant, Table 3). Assuming this K_2 value as adequate for pharmacokinetic behaviour also in Cohort 1, it was then possible to determine the absorption half-life in this group, as well as other pharmacokinetic parameters (Table 3). Maximal serum melatonin concentration, as measured in Cohort 2, was reached 16 min 52 s (T_{max}) after melatonin administration. Melatonin did not increase sleepiness and no reduction of sedatives was necessary after melatonin administration.

Discussion

Patients enrolled in this prospective, interventional trial were extremely high-risk: their severity at admission and

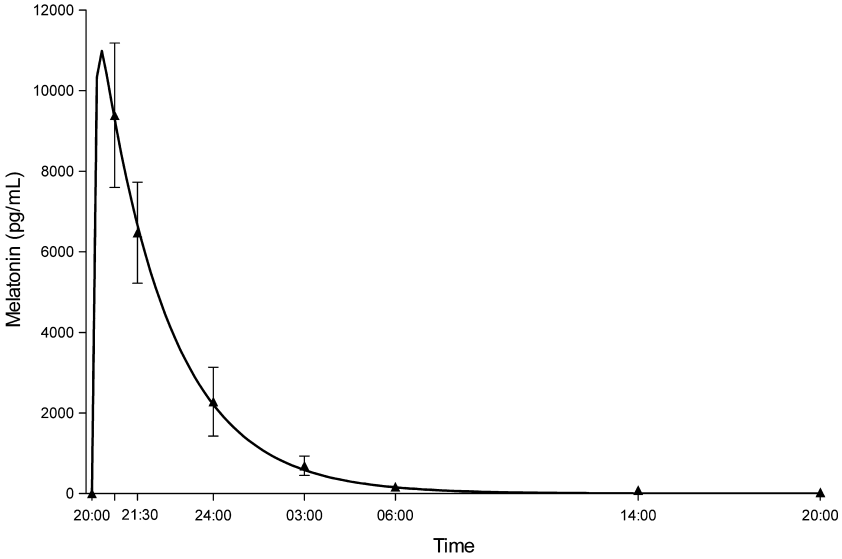

Fig. 2. Baseline-corrected daily serum melatonin levels. Non-linear fit of baseline-corrected serum melatonin values in the Cohort 1 of critically ill patients receiving orally 3 mg melatonin on ICU day 4. To complete the earlier part of this pharmacokinetic figure, the K_2 value calculated from the patients of Cohort 2 was used.

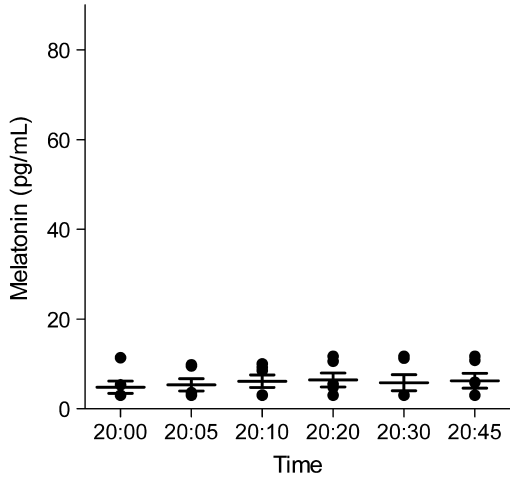

Fig. 3. Baseline-corrected early serum melatonin levels. Non-linear fit of baseline-corrected serum melatonin values in the Cohort 2 of critically ill patients receiving orally 3 mg melatonin on ICU day 4.

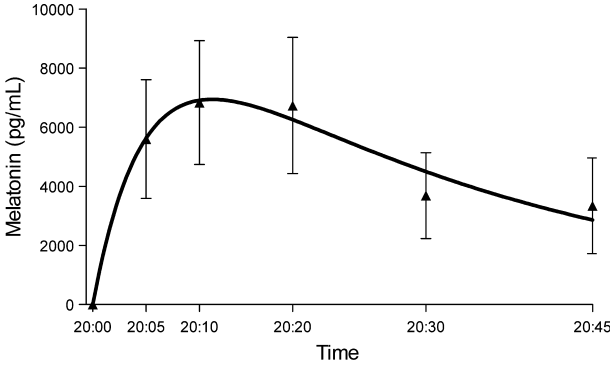

Fig. 4. Early endogenous melatonin levels in critically ill patients. Serum melatonin values measured in the critically ill patients of Cohort 2 during their ICU day 3. Points are the absolute values; error bars represent mean and standard deviation.

Table 3. Pharmacokinetic parameters calculated in the patients of Cohort 1. To determine T_2, the K_2 measured in the patients of Cohort 2 was used

K_1	0.44
K_2	12.62
T_1	1.57 hr
T_2	0.06 hr
AUC	28231.29 pg/mL*hr
T_{max}	0.27 hr
C_{max}	11039.84 pg/mL

K_1, elimination constant; K_2, absorption constant; T_1, elimination half life; T_2, absorption half life; AUC, area under concentration-time curve; T_{max}, time to reach maximal serum concentration; C_{max}, maximal serum concentration.

degree of organ impairment (at ICU day 3, median SOFA score of 7 on a 14 point-scale) resulted in a high complexity of care (Table 1). All patients required invasive ventilatory support for over 5 days, six received vasopressors, all had prolonged ICU stay, and the mortality rate was high.

Serum melatonin concentrations in these critically ill patients were dramatically reduced, both during daytime basal secretion and during the night when an endogenous melatonin peak was expected. This evidence substantially confirms the results highlighted in the literature [12, 13, 16].

When analysing the pharmacokinetic results, we noticed that melatonin peaks after exogenous administration of the indole were reached earlier in ICU patients (Fig. 4) than in healthy volunteers [29] (16 min versus 48 min) with levels almost ten times higher. The rate of melatonin disappearance was slightly slower in the former group (Fig. 2) than in the latter [29] (half-life 94 min versus 63 min).

Unexpectedly, intestinal absorption was substantially unchanged despite the critical illness. Crushing the tablets and administering them by nasogastric tube increased the area of intestinal mucosa exposed to melatonin [30], thus possibly explaining the halving time needed to reach the peak serum concentration compared with healthy volunteers. Continuous enteral nutrition might facilitate melato-

nin absorption as a consequence of its intrinsic prokinetic effect, while the lipid content might make the lipophylic melatonin molecule more absorbable. Finally, the use of prokinetic drugs and postpyloric access in three patients could have also accelerated melatonin absorption.

Although it seemed to exist a difference between the mean serum melatonin levels at 45 min after administration in the two cohorts, this difference was not statistically significant ($P = 0.0547$). The increase in serum melatonin half-life is difficult to explain, because these patients had no overt hepatic dysfunction, and most of them presented only moderate acute renal impairment (Table 1).

A limitation of this study lies in the need to enrol two different ICU patient cohorts to fully describe the pharmacokinetics of oral melatonin, with the intrinsic risk of clinical differences (though not statistically significant except for serum bilirubin) between the two groups. Moreover, to avoid the large number of blood samples required by this study design, pharmacokinetic parameters described in the literature were taken for healthy volunteers. No side effects (in particular, no excessive sleepiness) were reported in this small group of critically ill patients.

Our findings confirm that critically ill patients have reduced blood melatonin levels, with no physiological nocturnal peaks. Exogenous orally administered melatonin proved to be readily and adequately absorbed, even in the early phase of a critical illness. After oral administration of melatonin, the following changes were observed in this patient sample: (i) the serum melatonin peak was reached after approximately 15 min, and (ii) melatonin pharmacological levels were observed up to 10 hr after oral administration. Further studies on the clinical effects of exogenous melatonin administration in critically ill patients need to take into account its substantial satisfactory oral bioavailability.

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