Measurement of Urinary Melatonin: A Useful Tool for Monitoring Serum Melatonin after Its Oral Administration

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ABSTRACT

The relevance of measuring urinary melatonin (MLT) for human pineal research is sometimes questioned, and the relationship among serum levels of MLT, urinary excretion of the unmetabolized hormone, and excretion of MLT’s main metabolite, 6-hydroxymelatonin sulfate (aMT6s), is still uncertain.

We applied a well established RIA for measuring MLT in serum to urine samples, characterized its criteria of performance in this body fluid, and used it for human studies. In 16 adolescents, the endogenous overnight MLT secretion, expressed as the area under the concentration time curve, correlated significantly with the amounts of urinary aMT6s (r = 0.86; P < 0.0001) and urinary MLT (r = 0.70; P = 0.0027) excreted during a 16-h observation period. Oral administration of 3 mg exogenous MLT in 17 healthy volunteers resulted in peak MLT serum levels differing 28-fold among subjects (940–27,240 pg/mL; range). In this study urinary MLT, but not aMT6s, excretion was associated with blood MLT concentrations (r = 0.76; P = 0.0004 vs. r = 0.02; P = 0.93, respectively). Thus, endogenous MLT production can be assessed accurately by measuring either aMT6s or MLT excretion. After oral application of MLT, however, only measurement of MLT excretion is a reliable marker of serum concentrations. Determination of MLT in urine may prove to be a useful tool for drug monitoring after oral administration of the pineal hormone. (J Clin Endocrinol Metab 85: 666–670, 2000)

During the last decades, the physiological significance of the pineal gland and its hormone melatonin (MLT) has been unraveled in certain mammals for seasonal breeding (1, 2). In some species there is also evidence for aMT6s, is still uncertain.

We applied a well established RIA for measuring MLT in serum to urine samples, characterized its criteria of performance in this body fluid, and used it for human studies. In 16 adolescents, the endogenous overnight MLT secretion, expressed as the area under the concentration time curve, correlated significantly with the amounts of urinary aMT6s (r = 0.86; P < 0.0001) and urinary MLT (r = 0.70; P = 0.0027) excreted during a 16-h observation period. Oral administration of 3 mg exogenous MLT in 17 healthy volunteers resulted in peak MLT serum levels differing 28-fold among subjects (940–27,240 pg/mL; range). In this study urinary MLT, but not aMT6s, excretion was associated with blood MLT concentrations (r = 0.76; P = 0.0004 vs. r = 0.02; P = 0.93, respectively). Thus, endogenous MLT production can be assessed accurately by measuring either aMT6s or MLT excretion. After oral application of MLT, however, only measurement of MLT excretion is a reliable marker of serum concentrations. Determination of MLT in urine may prove to be a useful tool for drug monitoring after oral administration of the pineal hormone. (J Clin Endocrinol Metab 85: 666–670, 2000)
Material and Methods

Study 1

Sixteen adolescents, aged 14.7 ± 1.1 yr, of either sex (5 males and 11 females), were involved in this experiment. They were either healthy or scoliotic patients of the Orthopedic Department of the University of Vienna [details of this study were reported previously (14)]. They were not acutely ill, and drug medication was not given to any of the adolescents for at least 2 weeks before the experimental day. Consumption of caffeine and alcohol was not allowed 1 day before the test session.

All subjects were admitted into the hospital for 1 day, and 5 mL blood were obtained from each individual through an antecubital indwelling venous cannula at 1800, 2000, 2300, 0200, 0600, 0800 and 1000 h, respectively. Serum samples were stored at −20 C until melatonin was measured. In addition, urine was collected from 1800–1000 h, the volume was recorded, and a 10-mL aliquot was stored at −20 C until estimation of melatonin and aMT6s concentrations. The lights in the examination room were turned off at night (between 2100–0700 h), and if light was needed, it was restricted to less than 200 lux to avoid light-induced suppression of MLT production.

Study 2

Seventeen healthy adults, aged 29.7 ± 7.9 yr, of either sex (12 males and 5 females), were included in the study. Subjects were required to have average body proportions (body weight within 10% of ideal, body mass index in the normal range for age). Heavy smoking and drug use (including any medication for at least 1 month before the study) were exclusion criteria, and volunteers were asked to refrain from consuming caffeine and alcohol for 24 h before the test session.

On the day of the study, after an overnight fast, the subjects consumed a light breakfast between 0700–0800 h. Starting at 0900 h, 14 blood samples were collected until 1700 h at either 15- or 60-min intervals (Fig. 4) through an antecubital indwelling venous cannula. Blood samples were centrifuged, and serum was stored at −20 C until assayed. At 1000 h, a tablet containing 3 mg melatonin (provided by Novartis Consumer Health, Nyon, Switzerland) was given orally to each volunteer for immediate swallowing with mineral water. In addition, urine was collected over 1- or 2-h intervals during the test period (Fig. 4). The urine volume was recorded, and a 10-mL aliquot was stored at −20 C until estimation of melatonin and aMT6s concentrations. During the entire test participants were not allowed to eat, but were asked to drink at least 1.5–2 L mineral water to produce enough urine at each collecting period.

Before entering a study, the experimental protocol was fully explained to the participants and, where appropriate, to the parents, and written informed consent was obtained. Approval by the local ethics committee of the Medical Faculty of the University of Vienna was obtained.

Assay of MLT in serum and urine

Serum melatonin concentrations were measured with a commercially available RIA kit (Bühlmann Laboratories AG, Allschwil, Switzerland), which uses 18 reverse phase columns for MLT extraction and the highly specific antimelanin serum G-280 (15) for MLT quantification. Our performance criteria were similar to those provided by the manufacturer. For quantification of physiological melatonin levels in urine we extracted 0.125–0.5 mL undiluted urine and applied the same assay procedure as that for serum.

Water blanks and MLT concentrations in MLT stripped afternoon urine were below the detection limit (1.0 pg/mL). The recovery of authentic MLT added at concentrations covering the entire standard curve to pooled afternoon urine samples was 103.9 ± 10.7% (mean ± sd). At melatonin concentrations of 3.6 and 35.4 pg/mL urine, the intraassay coefficients of variation were 5.3% (n = 15) and 8.3% (n = 15); at concentrations of 2.0 and 17.7 pg/mL, the interassay coefficients of variation were 18.1% (n = 42) and 10.2% (n = 45). A melatonin concentration curve produced by serial dilution of pooled morning urine samples paralleled the melatonin standard curve (Fig. 1). The 24-h melatonin excretion we observed in young adolescents was within the ranges reported by others (Table 1).

Assays of aMT6s in urine

aMT6s concentrations in urine were assayed using a commercially available RIA kit (Stockgrand Ltd., Guildford, UK), which is based on a method described by Arendt and co-workers (6, 16).

Statistical analysis

All data are given as the mean ± sd. Intra- and interassay coefficients of variation were calculated by a method described previously (17). The calibration curve was linearized by logit-log transformation of the bound/free ratio (18).

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TABLE 1. Melatonin excretion in humans according to selected publications

<table>
<thead>
<tr>
<th>Method</th>
<th>Age (yr)</th>
<th>Subjects</th>
<th>MLT (mean ± sd; ng)</th>
<th>Collection period (h)</th>
<th>Publication year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioassay</td>
<td>18–45</td>
<td>6</td>
<td>0.9–30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24</td>
<td>1975</td>
<td>Lynch et al. (25)</td>
</tr>
<tr>
<td>RIA</td>
<td>22–30</td>
<td>6</td>
<td>68.9 ± 27.4</td>
<td>Overnight</td>
<td>1979</td>
<td>Wetterberg et al. (26)</td>
</tr>
<tr>
<td>RIA</td>
<td>21–30</td>
<td>12</td>
<td>67 ± 29</td>
<td>24</td>
<td>1981</td>
<td>Lemaitre et al. (27)</td>
</tr>
<tr>
<td>RIA</td>
<td>26–51</td>
<td>14</td>
<td>38.2–179.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24</td>
<td>1981</td>
<td>Lang et al. (28)</td>
</tr>
<tr>
<td>RIA</td>
<td>Young adults</td>
<td>6</td>
<td>66.0</td>
<td>24</td>
<td>1990</td>
<td>Kivela et al. (29)</td>
</tr>
<tr>
<td>RIA</td>
<td>&gt;14</td>
<td>13</td>
<td>28.3</td>
<td>24</td>
<td>1997</td>
<td>Commentz et al. (30)</td>
</tr>
<tr>
<td>RIA</td>
<td>42 ± 3</td>
<td>12</td>
<td>18.8 ± 3.0</td>
<td>9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1998</td>
<td>Röjdmark et al. (31)</td>
</tr>
<tr>
<td>RIA</td>
<td>13–18</td>
<td>16</td>
<td>29.7 ± 13.2</td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1999</td>
<td>Present study</td>
</tr>
</tbody>
</table>

<sup>a</sup> Range.
<sup>b</sup> 2200–0700 h.
<sup>c</sup> 1800–1000 h.
Results

Study 1

Serum MLT concentrations displayed a circadian variation with low daytime (1.1 ± 1.1 pg/mL at 1800 h) and high nighttime values (44.3 ± 21.5 pg/mL at 2300 h; Table 2 and Fig. 2). The urinary aMT6s excretion of the subjects well represented the endogenous MLT secretion, as the area under the concentration time curve of MLT (AUCMLT) correlated significantly with the amount of aMT6s excreted during the observation period (r = 0.86; P < 0.0001; Fig. 3a). The urinary MLT excretion of the subjects also correlated significantly with the AUCMLT (r = 0.70; P = 0.0027; Fig. 3b), indicating a good representation of endogenous MLT secretion by urinary MLT excretion as well. Remarkably, there was also a good correlation between urinary aMT6s excretion and urinary MLT excretion (r = 0.85; P < 0.0001). AUCMLT or its log(10) did not correlate with the MLT concentration or its log(10) in urine (data not shown).

Study 2

Oral administration of 3 mg MLT to healthy volunteers resulted in peak serum MLT concentrations 60 min after its application (Table 2 and Fig. 4), although peak MLT concentrations differed 28-fold, and the AUCMLT differed 16-fold among subjects (Table 2).

Although both urinary aMT6s and MLT excretion rose after oral MLT application, urinary aMT6s excretion did not represent serum MLT concentrations, as the AUCMLT did not correlate with the amount of aMT6s excreted during the observation period (r = 0.02; P = 0.93; Fig. 5a). By contrast, urinary MLT excretion correlated significantly with the AUCMLT (r = 0.76; P = 0.0004; Fig. 5b), indicating a good representation of serum MLT levels by MLT excretion in urine after its oral administration.

Discussion

We applied to urine a RIA kit well established for quantification of MLT in serum and saliva and examined its criteria of performance and specificity in this body fluid. Results were within usual limits for RIA and allowed the utilization of this assay for urinary measurements in clinical studies.

In an examination of endogenous nocturnal MLT secretion in adolescents over 16 h, we found an average amount of some 30 ng unmetabolized MLT excreted with the urine. This roughly amounts 1/1000 of the 30 μg MLT produced per night according to pharmacokinetic calculations (13) and experimental evidence (19). It further complies with the observation that less than 1% of endogenous MLT is excreted in an unmetabolized form (20, 21) and also with previous reports concerning the excretion of MLT in humans.

Endogenous melatonin secretion during the night, as estimated by the area under the concentration time curve, correlated as expected with the excretion of aMT6s during the same period (6, 7) and also correlated with the excretion of MLT. Although the latter correlation coefficient was somewhat lower (r = 0.70) than that with aMT6s (r = 0.86), the total MLT excretion, nevertheless, accurately reflected serum MLT. Thus, excretion of MLT may be used for projection of serum MLT levels and assessment of endogenous MLT secretion. In contrast to a previous study (22) MLT concentrations in urine collected overnight did not correlate with serum MLT in our cohort of adolescents. After oral application of MLT in volunteers, their serum MLT, judged according to peak levels and the area under the concentration time curve, varied roughly up to a factor of 25, confirming previous reports (10, 11). The urinary MLT excretion displayed a similar variation and correlated well with the area under the concentration time curve.

Table 2. Serum and urinary MLT/aMT6s during endogenous overnight MLT secretion (Exp 1) and after ingestion of 3 mg MLT (Exp 2)

<table>
<thead>
<tr>
<th></th>
<th>Exp 1</th>
<th>Exp 2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Serum peak MLT (pg/mL)</td>
<td>44.3 ± 21.5g</td>
<td>22.2–103.2 g</td>
</tr>
<tr>
<td>AUCMLT (pg/h/mL)</td>
<td>557.9 ± 178.4</td>
<td>284.6–992.5</td>
</tr>
<tr>
<td>aMT6s (μg)</td>
<td>11.1 ± 5.0f</td>
<td>4.1–24.2f</td>
</tr>
<tr>
<td>MLT (ng)</td>
<td>29.7 ± 13.2e</td>
<td>9.0–59.1e</td>
</tr>
<tr>
<td></td>
<td>4,701 ± 6,415b</td>
<td>940–27,240b</td>
</tr>
<tr>
<td></td>
<td>9,514 ± 9,152</td>
<td>2,451–40,302</td>
</tr>
<tr>
<td></td>
<td>1,800 ± 404d</td>
<td>791–2,570d</td>
</tr>
<tr>
<td></td>
<td>2,280 ± 1,673d</td>
<td>742–7,067d</td>
</tr>
</tbody>
</table>

a At 2300 h.
b Sixty minutes after ingestion of 3 mg MLT.
c Excreted between 1800–1000 h.
d Excreted within 7 h after oral administration of 3 mg MLT.
curve, whereas the excretion of aMT6s did not. Thus, after oral administration, serum MLT levels can be assessed from MLT, but not from aMT6s, excretion.

In endogenous MLT production, the hormone is manufactured by the pineal gland and collected by the venous capillary system, emptying into the vena cerebri magna and further into the venous portion of the systemic circulation (23). Through the arterial portion of the systemic circulation it is distributed to all body tissues. A part of this MLT that is distributed to the liver is metabolized to aMT6s and, in this form, excreted with the urine. After oral application, all absorbed MLT is collected by the hepatic venous system and has to pass the liver, where 30–60% (13) is immediately metabolized to aMT6s and, in this form, excreted with the urine. After oral application, all absorbed MLT is collected by the hepatic venous system and has to pass the liver, where 30–60% (13) is immediately metabolized to aMT6s (first pass effect). Thus, a certain portion of absorbed MLT never appears in the systemic circulation or in the body tissues, but is excreted as aMT6s with the urine. Therefore, aMT6s excretion increases after oral MLT application, but it does not reflect serum MLT levels.

In many human studies MLT is orally administered for physiological, pharmacological, and therapeutic purposes. However, the huge differences in bioavailability after oral MLT application outlined above may confound the results if the differences in blood levels are not taken into account (12). In some experiments serum and saliva MLT levels may serve this purpose, although frequent sample collection and numerous MLT measurements may be painful, tedious, and costly. In sleep studies examining the sedative/hypnotic effect of MLT, saliva and serum collection is usually prohibited. Urinary MLT estimation may turn out to be the method of choice for drug monitoring in this area, which is currently a subject of intensive investigation (8, 24).

The data presented indicate that urinary MLT excretion can be accurately estimated with a commercially available RIA kit and that the data obtained by this procedure concur with our present understanding of pineal physiology and MLT metabolism. With a somewhat lesser degree of precision, urinary MLT excretion reflects endogenous MLT secretion, as does urinary aMT6s excretion. After oral MLT application, aMT6s excretion is not useful for the assessment of serum MLT concentrations, whereas MLT excretion correlates significantly with serum levels. Thus, measurement of MLT excretion is proposed as a useful tool for drug monitoring after ingestion of MLT.


