METABOLIC EFFECTS

Inversion of Melatonin Circadian Rhythm in Chronic Alcoholic Patients during Withdrawal: Preliminary Study on Seven Patients

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Abstract — Aims: The inversion of melatonin circadian rhythm secretion in some alcoholics during both intake and acute withdrawal has been widely reported. In the same way, what happens to this inversion when these patients are in long-term withdrawal is not known. To document this abnormality in alcoholics after withdrawal we investigated melatonin secretion observed during chronic alcoholization and after withdrawal. Methods: We measured the urinary 6-sulfatoxymelatonin (6SM) (6SM/creatinine ratio), main metabolite of the hormone, in two fractions, one diurnal and the other nocturnal, in seven alcohol-dependent patients presenting with this abnormality during alcoholization at two times: in acute withdrawal phase (under benzodiazepines) and 15 days after beginning of withdrawal (free of any psychotropic treatment). Results: Our results show that this reversed rhythm of melatonin secretion as seen by the diurnal excretion of 6SM (6SM/creatinine ratio) persists during acute withdrawal in more than half of the patients and is still present 15 days after withdrawal in three patients. Conclusion: It is remarkable that the inversion of the melatonin rhythms gets corrected in four out of seven patients after withdrawal. But, the circadian disorganization of melatonin secretion in three patients could underline a desynchronization in some alcoholic patients and may indicate more widespread circadian temporal structure disturbances in these patients.

INTRODUCTION

While melatonin secretion is usually exclusively nocturnal, an inversion of the circadian rhythm in alcohol-dependent subjects was observed during alcoholization and the first 24 h of withdrawal (Majumdar and Miles, 1987; Murialdo et al., 1991; Fonzi et al., 1992, 1994; Mukai et al., 1998).

Thus, in alcohol-dependent patients, significant melatonin secretion was found in nearly 50% of the patients during the afternoon (a period during which the blood concentration are theoretically nil) prior to and after withdrawal (Majumdar and Miles, 1987). Two other studies have reported significantly higher diurnal urine melatonin rates in alcohol-dependent patients compared to controls (but not for the nocturnal fraction) (Murialdo et al., 1991; Fonzi et al., 1992). A third found that diurnal melatonin blood concentrations during alcoholization in alcohol-dependent patients were greater than those obtained after withdrawal and greater than those of the controls. The authors also found loss of the circadian melatonin secretion rhythm during acute withdrawal (Fonzi et al., 1994), an observation confirmed in two patients presenting delirium tremens (Mukai et al., 1998).

These observations suggest several hypotheses. The first is that alcohol stimulates melatonin secretion during the day as suggested by the observations showing diurnal melatonin secretion during alcoholization. We have previously tested this hypothesis (Danel and Touitou, 2006). By measuring the diurnal and nocturnal melatonin blood concentration in healthy volunteers subjected to alcoholization similar to that of the alcohol dependent (e.g. regular and significant alcoholization throughout nychtemeron: 256 g/24 h), we observed that the profile of melatonin secretion was not modified during the alcoholization session with respect to the placebo session (Danel and Touitou, 2006). Thus, high doses of alcohol do not induce detectable melatonin secretion in the healthy volunteer during the day. The hypothesis of alcohol stimulating diurnal melatonin secretion thus seems improbable. Two other hypotheses are possible:

- either chronic alcoholization induces desynchronization of circadian rhythms leading to an inversion of the circadian profile of the hormone, of which the elevation of melatonin during the day is merely a consequence, since this desynchronization has been observed with another hormonal system (Danel et al., 2006)
- or some alcoholics have a constitutional circadian melatonin disorder.

To test these hypotheses, we investigated anomalies observed during chronic alcoholization to see if they are corrected with prolonged withdrawal. If this were the case, one would expect chronic alcoholization itself to be responsible for the observed anomalies. If they are not corrected with abstinence, the hypothesis of a systemic circadian melatonin secretion disorder could be argued.

As it is known that the melatonin rhythm is evidenced by urinary 6-sulfatoxymelatonin (Arendt, 2006), we measured the diurnal and nocturnal urinary 6SM/creatinine ratio in alcohol-dependent patients during chronic alcoholization and included patients with a reversed night/day ratio. We then measured the diurnal and nocturnal urinary 6SM/creatinine ratio during the critical withdrawal period and after controlled withdrawal for at least 2 weeks.

SUBJECTS AND METHODS

Seven patients (Table 1) meeting the DSM IV TR criteria of alcohol dependence syndrome (American Psychiatric
Inversion of Melatonin Rhythm during Alcohol Withdrawal

Table 1. Patients’ characteristics of the seven patients meeting the DSM IV-TR alcohol dependence criteria

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Age at start of consumption (years)</th>
<th>Age at start of dependence (years)</th>
<th>Duration of the dependence (years)</th>
<th>Alcohol (g/day)</th>
<th>Cushman (withdrawal)</th>
<th>MCV (fl)</th>
<th>GGT (IU/l)</th>
<th>ALAT (IU/l)</th>
<th>ASAT (IU/l)</th>
<th>PT (%)</th>
<th>BMI</th>
<th>Associated troubles</th>
<th>Duration of abstinence (in months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>49</td>
<td>20</td>
<td>1</td>
<td>160</td>
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<td>120</td>
<td>41</td>
<td>26</td>
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<tr>
<td>2</td>
<td>M</td>
<td>39</td>
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<td>5</td>
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<td>99</td>
<td>112</td>
<td>35</td>
<td>42</td>
<td>100</td>
<td>23</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>51</td>
<td>18</td>
<td>31</td>
<td>150</td>
<td>10</td>
<td>100</td>
<td>450</td>
<td>68</td>
<td>30</td>
<td>88</td>
<td>25</td>
<td>arteritis</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>52</td>
<td>12</td>
<td>22</td>
<td>30</td>
<td>6</td>
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<td>18</td>
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<td>5</td>
<td>M</td>
<td>47</td>
<td>20</td>
<td>45</td>
<td>2</td>
<td>200</td>
<td>9</td>
<td>239</td>
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<td>93</td>
<td>22</td>
<td></td>
<td>18</td>
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<tr>
<td>6</td>
<td>M</td>
<td>46</td>
<td>18</td>
<td>31</td>
<td>15</td>
<td>300</td>
<td>6</td>
<td>102</td>
<td>56</td>
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<td>100</td>
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<td></td>
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<tr>
<td>7</td>
<td>M</td>
<td>40</td>
<td>20</td>
<td>24</td>
<td>16</td>
<td>350</td>
<td>5</td>
<td>95</td>
<td>909</td>
<td>314</td>
<td>248</td>
<td>99</td>
<td>pancreatitis</td>
<td>22.5</td>
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<tr>
<td>Mean</td>
<td></td>
<td>46.29</td>
<td>17.32</td>
<td>14.29</td>
<td>222.86</td>
<td>7.00</td>
<td>100.71</td>
<td>267.57</td>
<td>99.00</td>
<td>69.71</td>
<td>97.14</td>
<td>23.50</td>
<td></td>
<td>9.14</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>5.09</td>
<td>2.93</td>
<td>11.09</td>
<td>12.54</td>
<td>74.10</td>
<td>1.83</td>
<td>9.05</td>
<td>317.92</td>
<td>113.59</td>
<td>81.94</td>
<td>4.78</td>
<td></td>
<td>8.49</td>
</tr>
</tbody>
</table>

MCV, mean corpuscular volume; GGT, gamma glutamyl transpeptidase; ALAT, alanine-amino transferase; ASAT, aspartate-amino transferase; PT, prothrombin time; BMI, body mass index.

Association, 2000), presenting with a reversed night/day ratio of 6SM/creatinine, were included after obtaining their informed written consent. The study was consistent with the standards and ethical principles for research on biological rhythms on humans (Toutou et al., 2006). Lifestyle, physical health and clinical status were assessed by clinical and laboratory examinations to determine eligibility for the study. All subjects were synchronized with diurnal activity (8 am to 11 pm) and nocturnal rest (11 pm to 8 am). No subject had a current or past diagnosis of substance abuse or dependence other than alcohol and tobacco. They worked no rotating shifts, took no transmeridian flights and had had no infection or disease for at least 1 month before the session. No subject had a current or past psychosis disorder. The entire study took place in the months of February and March to eliminate the effects of seasons and duration of the photoperiod (patients were not exposed to light during bedtime).

The subjects (6 men and 1 woman) averaged 46.29 years of age (SD 5.09). Their average daily alcohol consumption was 222.86 g/day (SD 74.10). The study of biological indicators of alcohol misuse showed on average a mean corpuscular volume of 100.71 fl (SD 9.05) and gamma glutamyl transpeptidase at 267.57 IU/l (SD 317.92). No patient presented hepatocellular deficiency (prothrombin time >95% in all patients). The BMI was 23.50 (SD 1.64). The intensity of the withdrawal syndrome, measured on the Cushman scale (minimum 0; maximum 21; 7–14 = moderate withdrawal) (Castaneda and Cushman, 1989) was 7.00 (SD 1.83) and justified medically assisted withdrawal at the patients’ home under the supervision of a nurse.

A nurse who was specifically trained in alcoholic withdrawal performed the medical control and supervision of the withdrawal twice daily. Urine was collected by the nurse at the alcohol-dependent patients’ homes in three circumstances: the first during alcoholization, the second during the first day of withdrawal while the patients undertook medical withdrawal treatment during 8 days (60 mg of diazepam: 20 mg at 8.00 am, 20 mg at 12.00 am and 20 mg at 6.00 pm; and vitamins of group B), and the third 15 days after the beginning of alcohol withdrawal with medical withdrawal treatment having ceased for at least 8 days. With each sampling, the 24-h urine collection was divided into two. The patients were asked to empty their bladders at 8 pm and to collect their urine for the first sample from 8 pm to 8 am the next day. A second sampling was made from 8 am to 8 pm. After noting the diuresis, a fraction of the urine samples was frozen. The analysis of the rate of urinary 6-sulfatoxymelatonin was performed blind of clinical data in the laboratory and was measured with a commercial enzyme-linked immunosorbent assay (ELISA) (Boeringer Mannheim, France) in a fully automated analyser (ES 700). The progression of the patients when abstaining from alcoholic beverages was regularly documented during 18 months of observation. In the follow-up to the study, of the seven patients entered in the study, one recommenced consumption of alcohol immediately after withdrawal (patient no. 1), three following a longer period of abstinence (patients 2, 6 and 7, respectively, 6, 3 and 1 months) and three were still abstaining 18 months after withdrawal (patients 3, 4, 5). The non-parametric Wilcoxon test for paired series was used to test the significance of changes in day and night secretion of the 6SM/creatinine ratio in the three protocol times. For the correlation analyses, we calculated the coefficient for Spearman’s correlation.

RESULTS (TABLE 2)

The results are expressed in the form of the 6SM/creatinine ratio over 12-h temporal sequences, a diurnal period from 8 am to 8 pm and a nocturnal period from 8 pm to 8 am.

During alcoholization, we observed a night/day ratio of excretion of abnormal urinary 6SM/creatinine ratio (i.e. ratio < 1) in all subjects. All patients had inversion of secretion during alcoholization. The anomaly was corrected in two of the patients on withdrawal. And finally, 15 days after beginning of withdrawal, three patients showed an anomaly of persistent secretion (inversion or equalization of night/day ratio) but four had a normalization of their secretion (night/day ratio > 1). Nonetheless, treatment of the data using the Wilcoxon test does not show a significant change in the distribution of melatonin. We did not find significant correlation between reversed ratios (during and after withdrawal) and duration and degree of alcoholism and recidivism. We only found significant positive correlations between reversed ratios during alcoholization and the age at start of consumption (rho = 0.874, P = 0.010) and the age at start of dependence (rho = 0.758; P = 0.048).
DANIEL

Table 2. Abnormal day time and night time ratio of 6SM/creatinine in 7 alcohol-dependent subjects during alcoholization, during withdrawal under benzodiazepines and after withdrawal in alcoholic beverages (without benzodiazepines)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>During alcoholization</th>
<th>During withdrawal under benzodiazepines</th>
<th>After withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.00–20.00</td>
<td>20.00–8.00</td>
<td>Ratio (night/day)</td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>0.48</td>
<td>0.96*</td>
</tr>
<tr>
<td>2</td>
<td>1.37</td>
<td>0.66</td>
<td>0.48*</td>
</tr>
<tr>
<td>3</td>
<td>1.35</td>
<td>0.87</td>
<td>0.64*</td>
</tr>
<tr>
<td>4</td>
<td>3.82</td>
<td>1.11</td>
<td>0.29*</td>
</tr>
<tr>
<td>5</td>
<td>1.17</td>
<td>1.07</td>
<td>0.91*</td>
</tr>
<tr>
<td>6</td>
<td>0.53</td>
<td>0.40</td>
<td>0.75*</td>
</tr>
<tr>
<td>7</td>
<td>2.68</td>
<td>1.67</td>
<td>0.62*</td>
</tr>
<tr>
<td>Mean</td>
<td>1.63</td>
<td>0.89</td>
<td>0.66</td>
</tr>
<tr>
<td>SD</td>
<td>1.21</td>
<td>0.44</td>
<td>0.24</td>
</tr>
</tbody>
</table>

ND = non-determined. The excretion is considered abnormal (*) when the ratio is <1.

DISCUSSION

Melatonin circadian rhythm shows a peak between 2 am and 4 am, in consequence with an elevated rate of urinary 6SM in urine excreted at night and collected in the morning, with a ratio of nocturnal emission to diurnal emission of 6SM approaching 5 (Tribli et al., 2003). As Nowak et al. (1987) demonstrated, urinary 6-SM excretion rates are reliable indices of serum melatonin concentrations and a good way to monitor human circadian rhythms. This is why we decided to collect the urine by 12-h periods (from 8 am to 8 pm and 8 pm to 8 am).

Our results show that our patients in a period of alcoholization have an abnormal 6SM excretion profile, urinary metabolite reflection of melatonin secretion by the pineal gland (Arendt, 2006). This disorganization demonstrates a loss of normal circadian organization in the alcoholic during a period of alcoholization (Rupp et al., 2007). It persists during acute withdrawal in certain cases. Rather more surprisingly, this abnormality persists in some patients: three patients in seven have a diurnal urinary 6SM/creatinine ratio that is greater than the nocturnal ratio. It is possible that the circadian rhythm is disturbed systemically. But the fact that some subjects remained abnormal after withdrawal could be explained by the presence of an abnormal ratio before they became alcoholic (which would be a counter to the conclusion that alcoholism causes the abnormal ratio). Another explanation could be that benzodiazepines can potentiate inhibition of melatonin synthesis and secretion (Garfinkel et al., 1995). In their study on rats, Djeridane and Touitou showed that diazepam affected pineal melatonin synthesis and plasma melatonin levels (~40%) in vivo but not in vitro (Djeridane and Touitou, 2003).

Significant positive correlations between reversed ratios during alcoholization and the age at start of consumption could mean that the earlier the onset, the more the inversion of the ratio. It is known that pubertal brains are more susceptible to alcohol damage than adult brains (Monti et al., 2005); therefore, the beginning of alcohol consumption is important and have to be considered for future studies.

Very few physiopathological conditions were reported as being at the origin of such an abnormality. One of them, Smith-Magenis syndrome (De Leersnyder, 2006; De Leersnyder et al., 2006), secondary to a chromosomal deletion (17p11.2), could constitute a model for future investigation. The patients regularly present dysmorphia, late development, psycho-behavioural disorders and significant sleep disorders attributed to circadian disturbance of melatonin secretion that, paradoxically, takes place during the day.

The interesting point of this observation may be the parallel with sleep disorders observed in alcoholics during alcoholization, withdrawal and sometimes abstinence. Further work is necessary to establish correlations between the anomalies in melatonin secretion and the sleep and attention disorders observed in alcoholics. With this in mind, the patient presenting an inversion of the melatonin secretion rhythm could be proposed therapy involving the prescription of beta-adrenergic blocking agents in the morning and melatonin at the end of the afternoon (Lewy et al., 2006), which is a strategy proposed for Smith-Magenis syndrome (Carpizo et al., 2006).

REFERENCES


