Chapter 9

Postoperative radiotherapy for acromegaly delays the circadian phase of melatonin secretion

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ABSTRACT

Objective
Postoperative radiotherapy for acromegaly is associated with a considerable increase in pituitary insufficiencies. In general, the notion is that this is due to side effects of radiotherapy on the pituitary, but the hypothalamus may also be involved. Circadian variations in melatonin secretion are under the control of endogenous clock signals arising from the suprachiasmatic nucleus of the hypothalamus. Therefore, the aim of this study was to assess the effects of postoperative radiotherapy on characteristics of diurnal melatonin secretion in patients cured from acromegaly.

Design
cross-sectional study in 3 groups of 8 subjects (4 men in each group) matched for age, gender and BMI.

Patients and methods
The groups consisted of: 1) patients treated with postoperative radiotherapy 14 ± 2 years prior to this study, 2) patients treated with transsphenoidal surgery, and 3) healthy controls. Melatonin concentrations were measured each hour during 24h and circadian rhythmicity was appraised by a skewed baseline cosine curve fit procedure.

Results
Mean serum melatonin concentrations were highest during nighttime and lowest during the afternoon compared to the morning. Mean morning, afternoon, nighttime or total melatonin concentrations did not differ between the groups. The peak level and the onset and offset of melatonin did not differ between the groups. The acrophase, however, was delayed in patients treated with postoperative radiotherapy compared to healthy controls.

Conclusion
There is a delayed acrophase in melatonin circadian rhythmicity in patients treated by postoperative radiotherapy for acromegaly, potentially reflecting altered timing of the suprachiasmatic nucleus.
INTRODUCTION

Postoperative radiotherapy for acromegaly is effective in reducing persistent growth hormone (GH) excess. A decline of ~50% in serum GH levels is observed within the first two years after radiotherapy and of ~75% within 5 years (1-3). On the other hand, radiotherapy for pituitary tumors can lead to deficiencies in anterior pituitary hormones either due to direct effects on healthy pituitary tissue and/or alterations in hypothalamic functioning. Despite advances in modern radiation treatment there is still uncertainty with respect to the dosimetric accuracy of potential radiation damage to the hypothalamic nuclei. Moreover, the hypothalamus is considered more vulnerable to radiation damage than the pituitary (4). In addition, radiotherapy negatively influences quality of life in these patients, especially with respect to general and physical fatigue (5).

Within the hypothalamus, the suprachiasmatic nucleus (SCN) is considered to be the central circadian pacemaker of the body. Altered regulation of endogenous rhythms could contribute to the increased fatigue observed during long-term follow-up in patients previously treated by postoperative radiotherapy (5;6). For instance, cranial radiation therapy in childhood is associated with objective and subjective changes in the sleep–wake rhythm in adulthood (7). Unfortunately, diurnal variations of pituitary hormones can not be used for assessment of the diurnal regulation by the SCN in patients previously treated for pituitary adenomas, because anterior pituitary deficiencies are frequently seen after radiotherapy due to the combined effects on healthy pituitary and hypothalamic tissue (8). Another circadian output regulated by the SCN is the diurnal variation of melatonin secretion by the pineal gland (9). We, therefore, hypothesized that radiation damage to the hypothalamus, especially to the SCN, could be reflected in altered diurnal variation of melatonin secretion. Therefore, the aim of the present study was to compare the 24h circadian variation of melatonin secretion in patients, who had been cured from GH excess by combined surgery and radiotherapy, to patients cured by surgery only and healthy controls.

PATIENTS AND METHODS

Patients

Patients were recruited from a cohort of patients with biochemical remission after acromegaly, who have been described extensively (2;10). We selected 3 groups that were carefully matched for age, gender and body mass index.

The first group consisted of acromegalic patients who had been treated and cured by postoperative radiotherapy (n=8), because of persisting postoperative disease activity. The second group consisted of patients who had been treated by transsphenoidal surgery only (n=8) and who were in long-term remission. The third group consisted of healthy controls (n=8).
Exclusion criteria for patients and healthy controls were hypertension, diabetes mellitus, pregnancy and recent transatlantic flights. Healthy controls did not use any medication. All patients had normal glucose-suppressed GH concentrations (<0.38 μg/L) and normal IGF-1 levels corrected for sex and age at the time of inclusion. Radiation had been given with a conventional linear accelerator (8 MeV) in a rotating field; total tumor dose was 40-45 Gy, fractionated in 20 sessions over a period of 4-5 weeks.

The patients who had been by postoperative radiotherapy underwent an insulin tolerance test (ITT) for establishing GH reserve. To be included for this study a subnormal GH-peak response of less than 3 μg/L was required in the presence of glucose nadir <2.2 mmol/L (11), because that would mean that due to postoperative radiotherapy at least 1 hypothalamic-pituitary axis was affected. We have previously shown that an insufficient GH response to the ITT after postoperative radiotherapy is diagnostic for GHD after acromegaly (12). All patients in the surgery only group had normal GH reserve. All subjects underwent identical 24h blood sampling studies (see below).

Premenopausal women were defined as LH/FSH deficient when secondary amenorrhoea was present for more than 1 year. In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 μmol/l) after an insulin tolerance test. If results were below the lower limit of the respective reference ranges, substitution with thyroxine, hydrocortisone or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided. All patients with pituitary deficiencies were on stable conventional substitution therapy, for at least 12 months prior to the study. None of the postmenopausal women used estrogens.

Written informed consent was obtained from all the patients and control subjects. The study was approved by the ethical committee of the Leiden University Medical Center.

Sampling protocol

Patients and controls were admitted to the hospital on the day of study. An indwelling i.v. cannula was inserted into a vein of the forearm 60 min before hourly sampling began starting at 09.30h A.M. for the next 24h. A slow infusion of 0.9% NaCl and heparin (1 U/ml) was used to keep the line patent. Meals were served at 0800h, 1230h and 1730h. The subjects were free to ambulate, but not to sleep during daytime. Lights were turned off between 2200h and 2400h, when patients indicated they wanted to go sleep to ensure similarity with normal out-of-hospital sleeping patterns. Plasma samples were collected on ice in heparinized tubes. The samples were centrifuged at 4°C for 7 minutes; the plasma was then separated, frozen and stored at -20°C until the assays were performed.
Assay
Plasma melatonin concentrations were determined by RIA (LDN Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany). Sensitivity of the assay is 2 pg/ml. The intra-assay coefficient of variation is 12.1-12.3 % in the range of 15.1-157 pg/ml. All serial samples in this study were run in the same assay.

Data analysis
First, the mean concentrations of melatonin in the morning, afternoon and night were obtained from the average values in samples obtained during the morning (samples of 9.30h, 10.30h, 11.30h, 12.30h), during the afternoon (samples of 14.30h, 15.30h, 16.30h, 17.30h), and during the night (1.30h, 2.30h, 3.30h, 4.30h). The area under the curve (AUC) was estimated using trapezoidal integration.

Subsequently, diurnal variations in melatonin concentrations were appraised with a skewed baseline cosine function proposed by van Someren and Nagtegaal (13). This method has been validated to best describe the typical range of melatonin profiles and combines a fixed baseline with skewness of the cosine function, allowing differences in the steepness of the rising and falling limb of the melatonin peak. Outcome measures for statistical analyses were the peak level of melatonin and the corresponding acrophase (clock-time of the maximal melatonin concentration), a melatonin onset marker (up-cross time: the time after which the curve exceeds one-quarter of its amplitude) and a melatonin offset marker (down-cross time: the time after which the curve drops below one quarter of its amplitude).

Statistics
Results are presented as the mean ± standard error of the mean (SEM), unless stated otherwise. Statistical contrasts between groups were evaluated with the non-parametric Mann-Whitney U-test. SPSS for windows version 14.0 (SPSS Inc., Chicago, IL) was used for data analysis. P<0.05 was considered significant.

RESULTS
Patients
Age, gender, BMI and IGF-I concentrations did not differ between the three groups (Table 1). Radiotherapy was applied 13.9 ± 2.1 years prior to this study in the patients treated by postoperative radiotherapy. Two patients in the postoperative radiotherapy group had TSH deficiency, 1 patient had ACTH deficiency and 2 premenopausal female patients were treated with estrogens in combination with progesterone or and 2 male patients with testosterone. In contrast, none of the patients who were treated by surgery only suffered from anterior pituitary deficiencies.
Mean melatonin concentrations during the morning, afternoon, and night and total melatonin secretion

Mean serum melatonin concentrations were highest during nighttime (85.2 ± 10.1 pg/ml, p<0.001 compared to morning and afternoon) and lowest during the afternoon (27.0 ± 2.2 pg/ml, p=0.021 compared to morning and p<0.001 compared to nighttime) and in between these values during the morning (35.3 ± 3.0 pg/ml). Mean morning, afternoon, and nighttime concentrations did not differ between the three groups (Figure 1). Mean AUC did not differ between patients treated with postoperative radiotherapy (68653 ± 10391 pg/ml/24h), patients treated by surgery only (60524 ± 10550 pg/ml/24h), and healthy controls (68019 ± 9286 pg/ml/24h, p=NS).

Diurnal analysis

Representative examples of 24h melatonin secretion profiles in patients treated by postoperative radiotherapy and surgery only are shown in Figure 2. The peak level was not different between the three groups. Onset (up-cross time) and offset (down-cross time) were both later in patients treated with postoperative radiotherapy compared to surgery only and controls although these differences did not reach statistical significance. However, the acrophase was

Table 9/1: Clinical characteristics of patients with acromegaly treated by postoperative radiotherapy, patients treated by surgery only, and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Radiotherapy (n=8)</th>
<th>Surgery (n=8)</th>
<th>Controls (n=8)</th>
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</thead>
<tbody>
<tr>
<td>Age (years, mean (range))</td>
<td>52 (37-62)</td>
<td>52 (43-69)</td>
<td>53 (37-77)</td>
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<tr>
<td>Gender (n)</td>
<td>4/4</td>
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<td>4/4</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>29.2 ± 1.8</td>
<td>30.2 ± 1.2*</td>
<td>25.6 ± 1.2</td>
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<tr>
<td>IGF-I (μg/l)</td>
<td>90 ± 14</td>
<td>125 ± 16</td>
<td>122 ± 9</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SEM unless stated otherwise.
*P=0.038 vs. controls in the Mann-Whitney U-test.

Figure 9/1: Mean morning, afternoon, and night melatonin concentrations in patients treated with postoperative radiotherapy (white bars), patients treated with surgery alone (black bars), and healthy controls (grey bars).
Figure 9/2: Serum melatonin time series in three representative subjects. A 62-yr old male patient cured by surgery and postoperative radiotherapy; B a 47-yr old male patient cured by surgery; C a 43-yr old healthy control male subject.
significantly delayed in patients treated with postoperative radiotherapy compared to healthy controls\( (p=0.038, \) Table 2\). In addition, the acrophase was also later in patients treated with postoperative radiotherapy compared to patients treated with surgery only, but this difference did not reach statistical significance. The point-estimation of the acrophase in patients treated with surgery only was in between patients treated with postoperative radiotherapy and healthy controls.

**DISCUSSION**

Radiotherapy for acromegaly is effective in lowering GH and IGF-I concentrations in acromegalic patients\( (1-3)\), whereas on the other hand it is associated with hypopituitarism and impairment in quality of life in these patients\( (5)\). The hypothalamus is considered to be even more vulnerable to radiation damage than the pituitary gland\( (4)\). Within the hypothalamus, the suprachiasmatic nucleus (SCN) regulates various circadian rhythms, including those of pituitary hormones and of melatonin by the pineal gland\( (9)\). Therefore, damage to the hypothalamus could translate into alterations in the diurnal profiles of behavior and physiology. Indeed, cranial radiation therapy in childhood is associated with objective and subjective changes in the sleep–wake rhythm in adulthood\( (7)\). The data presented here give additional support to the contention of SCN alterations by radiotherapy by showing that previous radiotherapy is associated with a shift in acrophase timing in diurnal melatonin secretion in acromegalic patients.

In our study, total melatonin secretion and diurnal timing of melatonin secretion were unaffected in patients treated for acromegaly by surgery only. Only a few studies have addressed circadian melatonin rhythms in patients with acromegaly. Data on melatonin secretion in active acromegaly are conflicting. In one study, total melatonin secretion was decreased compared to healthy controls, whereas the acrophase occurred earlier\( (14)\). In contrast, another study reported an increased average 24h melatonin secretion, without any evidence of disturbed circadian timing\( (15)\). Additional studies showed increased melatonin levels during daytime.
Radiotherapy in acromegaly delays circadian melatonin phase

In patients with acromegaly (16;17) and patients with other intrasellar pituitary region tumors (18).

In our present study, radiotherapy did not affect total melatonin secretion, which contrasts with a previous study reporting diminished nocturnal melatonin secretion in 8 patients who had been treated with radiotherapy for acromegaly (19). However, in contrast to our study patients, those 8 patients still showed elevated growth hormone levels with failure of suppression during an oral glucose suppression test and two of these patients had elevated prolactin concentrations. To our knowledge, there are no other studies of the effects of radiotherapy on the pituitary gland on melatonin circadian secretion.

We found that radiotherapy was associated with a delayed acrophase of the circadian melatonin secretion profile, even though total melatonin secretion was unaffected. Melatonin secretion is under the control of endogenous cyclic signals arising from the SCN, the main circadian pacemaker (9). The close proximity of the pituitary gland to the ventromedial hypothalamic location of the SCN may make the latter vulnerable for scattered radiation aimed at the pituitary. Additional leads to support this hypothesis were found in the altered timing of sleep in patients during long term follow up after treatment for large non-functioning macroadenomas (20). In those patients, mid-sleep timing was clearly delayed. Interestingly, mid-sleep timing is highly correlated with the melatonin phase (21), in accordance with the delay in acrophase of melatonin concentrations observed in the current study. It is likely that with evaluation of radiotherapeutical precision techniques the scattering of radiation to the hypothalamus could be limited.

Alternatively, rather than direct radiation damage to the SCN per se, other hypothalamic nuclei that control SCN function could be damaged by radiotherapy resulting in altered excitatory/ inhibitory input to the SCN. GHRH, which is predominantly produced by the paraventricular nucleus, mediates specific feed back signals to the SCN (22). There are indications that acromegalic patients previously treated by postoperative radiotherapy have a diminished GHRH tone (12;23). In this respect, it is interesting, that intra-SCN injection of GHRH during daytime was found to advance circadian phase in hamsters (22).

Circadian melatonin rhythms are relatively stable over time under controlled conditions like in our experiment (24). Indeed, it takes several days for the external factors to shift the phase of the body clock (24), which makes it unlikely that possible changes in the time that the lights were turned off in our experiment compared to the home situation influenced our findings. Melatonin secretion are also influenced by other factors such as environmental factors (transatlantic flights, night shift work), aging, alcohol consumption, and depression (24). However, the patients were matched for age and patients or controls who had recently undertaken transatlantic flights were excluded.

During long-term follow-up of patients with strict biochemical control of acromegaly, persisting complaints of general and physical fatigue have been observed, especially in relation to radiotherapy (5;6). Indeed, adequate sleep quality and quantity is obtained only when aligned
with the most favorable circadian timing window for sleep, e.g. during the high nocturnal levels of melatonin (25). In addition, melatonin is suggested to be important for optimal functioning of other human circadian systems (25). From this perspective, a phase delay in melatonin acrophase could thus not only be a consequence of alterations in hypothalamic circadian timing, but could in itself contribute to the complex persisting morbidity seen in patients treated for acromegaly especially in case of applied postoperative radiotherapy.

In conclusion, this study indicates that radiotherapy for acromegaly induces a delay in the acrophase of melatonin circadian rhythmicity, which could be a reflection of altered hypothalamic circadian timing.
Radiotherapy in acromegaly delays circadian melatonin phase

REFERENCE LIST

1. Biermasz NR, Dulken HV, Roelfsema F 2000 Postoperative radiotherapy in acromegaly is effective in reducing GH concentration to safe levels. Clin Endocrinol (Oxf) 53:321-327
6. van der Klauuw AA, Biermasz N, Hoftijzer HC, Pereira AM, Romijn JA 2008 Previous radiotherapy negatively influences quality of life during four years of follow-up in patients cured from acromegaly. Clin Endocrinol (Oxf) In press


