Effect of fluoxetine on circadian rhythm of melatonin in patients with major depressive disorder

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Abstract

OBJECTIVES: The purpose of this study is to explore the response of melatonin circadian rhythm to fluoxetine treatment and its relationship with clinical therapeutic effect.

METHODS: This study investigated salivary melatonin in 13 outpatients with major depressive disorder and age- and sex-matched healthy controls. Depressed patients received six weeks fluoxetine (20 mg/day) treatment, and saliva was collected before and four weeks after treatment. In sampling days, a total of 12 time-point salivary melatonin was measured over 24-hours. Multioscillator cosinor model was used to fit the rhythms.

RESULTS: There was no difference of circadian melatonin rhythms in depressed patients, and melatonin was not significantly lower after fluoxetine treatment. To our surprise, the Δ melatonin amplitude (Before minus After) was positively correlated with the improvement in Hamilton Depression Rating Scale (HDRS) scores at day 42 whereas there was no such correlation at day 28.

CONCLUSIONS: Melatonin rhythms were similar between depressed patients and matched healthy controls. The interesting finding that the difference of salivary melatonin amplitude was correlated with the clinical improvement after six weeks fluoxetine treatment deserve further study.

Introduction

Disruption of circadian rhythms has been hypothesized to be one causal factor in depression episode [13]. Melatonin with the highest concentration during the night and lowest levels during the day [19,24], is an accurate indicator of circadian timing [29]. Previous studies on circadian changes of melatonin in patients with major depression are equivocal [4,11,21,22,27,30]. While the lower amplitude, mesor and phase advanced melatonin have been reported by early studies [3,4,11,21],

recent studies found that patients with major depression showed a delayed peak secretion time [12] or no changes in melatonin amplitude and mesor in depression [22,30].

After antidepressants treatment, the changes of melatonin rhythm are varied. Three weeks Desipramine treatment increased melatonin secretion [28] whereas chronic imipramine, mianserin decreased daytime plasma melatonin [26]. In major depressive disorder, nocturnal melatonin was reduced by fluoxetine [10] while clomipramine did not influence melatonin diurnal secretion [3,4,27]. Furthermore, the relationship between melatonin circadian rhythm and treatment effect have been reported. Successful treatment with imipramine was correlated with higher peak levels and more secretion of melatonin in depressed patients [17]. In pregnant women with major depression, effective treatment with bright light was associated with phase advance of melatonin rhythm [14]. To our knowledge, there is no study concerning the treatment effect of serotonin selective re-uptake inhibitor – the most described medicine in the treatment of depression, on the changes of circadian rhythm of melatonin in depression. The aim of this study is to explore the circadian rhythms of salivary melatonin in major depression patients participating in a controlled trial of a serotonin selective reuptake inhibitor – fluoxetine, as well as their relationship with clinical effects.

Methods

Subjects

We included 13 depressed outpatients, age 19 to 35 years (26.85±4.71 Mean±SD) and 13 individually age- and sex-matched healthy control subjects (26.92±4.13 years old). All patients (seven males, six females) fulfilling the criteria for major depressive disorder of DSM-IV[1] were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) [15]. All patients scored at least 16 points on the Hamilton Depression Rating Scale (17 items) [16]. Each patient was given a physical and psychiatric examination, routine serum chemistry and hematology studies, electrocardiogram and electroencephalography. Each control subject was screened for physical and psychological health by physical examination, clinical interview and laboratory test if necessary. All subjects gave written informed consent after the procedure had been fully explained. The Institutional Review Board of the University of Science and Technology of China approved the study.

Exclusion criteria were as follows: manic or hypomanic episode before, prominent suicide tendency, alcohol or substance abuse, having taken electro-convulsive treatment (ECT) or long-acting antipsychotics within the last six months, having taken any antipsychotic or antidepressant within four weeks before the study, lactation or pregnancy. Sleep abnormalities, any personal or family history of psychiatric disorder were additional exclusion criteria for the healthy control subjects. The study was performed from August to December (summer and autumn) 2004 in Hangzhou (30 degree North), China. During the research period, control subjects had no medication, including oral contraceptives. All patients began to take fluoxetine, 20 mg a day at the next day when they finished principal assessment and collecting saliva. Five patients took estazolam or lorazepam during the first two weeks when necessary. The doses ranged from 0.5 mg to 2 mg per night before bedtime.

Clinical assessment

Clinical efficiency of fluoxetine treatment was assessed by using Hamilton Depression Rating Scale (HDRS) and Zung Self-Rating Depression Scale (SDS) [32]. The severity of illness item (CGI-S) and the improvement item (CGI-I) of Clinical Global Impression Scale (CGI) were also used before each test. The Apathy Evaluation Scale by a clinician (AES-C) [20] was used for measuring apathy. Beck Depression Inventory (BDI) [6] and Beck anxiety Inventory (BAI) [7] were used for the assessment of global depression and anxiety. State-Trait Anxiety Inventory (STAI) [25] was also used for measuring state and trait anxiety. Each healthy subject was also assessed by these scales(SDS, BDI, BAI, and STAI), their scores were in normal area.

Saliva collection

The depressed patients and healthy controls were community living. They were instructed to avoid heavy exercise, sexual intercourse or eating cheese, and to avoid drinking alcohol or caffeine on the sampling day. The subjects were instructed to brush their teeth without toothpaste and to rinse their mouths with water 10 minutes before sampling during daytime. All samples were taken under normal light except at 0100 h and 0400 h when they were in darkness or the light intensity was <50 lux. Saliva was collected by placing Salivettes (Sarstedt, Numbrecht, Germany) in the mouths without chewing for five min, then salivettes were stored at 4 °C immediately during the day time and placed in a cold box during the night. Saliva was collected from 1900 h to 1600 h the next afternoon. Between 2230 h and 0630 h the next morning, the subjects went to bed/got up with the light turned off/on respectively. This schedule is not significantly different from their habitual rest-activity routine. The time points was every three hour during the day (1300 h, 1600 h) and the night (0100 h, 0400 h), and every one hour during the on-set (1900 h, 2000 h, 2100 h, 2200 h) and off-set (0700 h, 0800 h, 0900 h, 1000 h). A total of 12 time points were taken. The subjects were in a sitting position on sampling time except in a leaning backwards position during the night (0100 h, 0400 h). All of the depressed patients got help for sampling from their relatives. At the end of each sampling day the samples were centrifuged for 15 min at 3000 G , and the supernants were kept at –20 °C until assayed. In depressed patients, saliva was collected before and after four weeks fluoxetine treatment respectively.

Salivary melatonin assay

Salivary melatonin was measured by competitive radioimmunoassay (RIA) using commercial kits (Cat No: BA-1200 Labor Diagnostika Nord Gmbh & Co. KG, Nordhom, Germany). The average intra-assay coefficient of variation (CV) was 7.5% and average inter-assay coefficient of variation was 12%. Cross reactivity for both N-Acetylserotonin and 5-methorytryptophol were <1%. The protocol used was offered by Labor Diagnostika Nord Gmbh & Co. KG. The range of standards was 0–256 pg/ml and sensitivity was 0.33 pg/ml. Personnel who were unaware of the clinical variables in the same environment analyzed all samples in duplicate.

Statistics

One-way analysis of variance (ANOVA) was used for the clinical data to evaluate the therapeutic effect of fluoxetine. Circadian rhythm of salivary melatonin was studied with baseline cosinus function (BCF) [23]. Curve fitting was performed in each set of data using constrained non-linear regression analysis (SPSS11.5). Salivary melatonin profiles were fitted with the following equation [23]:

$$Y(x)=b+H/2/(1-c) * [\cos(x-\phi) - c + |\cos(x-\phi) - c|]$$

Where x represents the time of day (in radians). Y is the predicted value of melatonin at time x. b is the baseline and H, the peak height (amplitude). ϕ, the acrophase and c, the cut-off constant. Peak duration (D, in hours) refers to the time interval during which the model curve is above the base level, which is calculated from $D=\arc \cos(c)*24/\pi$.

The parameters of those individual that showed a significant fit (p<0.1) to the BCF were used for further calculation of means. To analysis the differences for melatonin between depressed patients (before and after four weeks treatment) and healthy controls, Students' t-test was used. Circular statistics were applied for the acrophase data, Jupp's Phi and S for mean angle and angular standard deviation. Mardian-Watson-Wheller x^2 test for evaluation of acrophase difference between depressed patients and healthy controls. Circular correlation was used for assessing the correlation of melatonin acrophase and clinical variables [2]. Multiple regressions were performed to determine the correlation between parameters (except acrophase) of melatonin rhythm and clinical variables. A level of P≤0.05 was considered to be significant.

Results

Clinical response with fluoxetine treatment

Thirteen patients were included in the study and treated with fluoxetine, nine completed the whole study for six weeks. Two changed to taking other antidepressants and withdrew their consents. One preferred psychotherapy rather than continued antidepressants. One lost connection. These four patients all quit this program in the first week. Patients' clinical scores at different evaluation time are shown in Table 1. There was a 74% mean decrease in HDRS scores after six weeks of treatment. The mean decrease was 36% in SDS scores. Eight out of nine patients responded well to fluoxetine (lowering of HDRS scores >50%). After four weeks treatment, BDI score and BAI score were significant lower than the first day (BDI 26.89±12.21 vs. 13.00±7.42 p=0.025; BAI 41.00±12.47 vs. 29.29±3.15 p=0.041). The reduction of State-anxiety scale score and Trait-anxiety scale score were also significant (State-anxiety 58.6±12.58 vs. 43.43±8.50 p=0.037; Trait-anxiety 58.30±12.21 vs. 46.86±8.97 p=0.047, respectively). AES total score also reduced significantly (30.10±7.03 vs. 11.86±3.02 p=0.001).

Comparison of circadian variation of free melatonin in depressed patients before and after fluoxetine treatment

In thirteen patients, the saliva of one patient who quit in the first week was not enough for melatonin assay, so the corresponding control's saliva was not assayed too. Of the 33 data sets, 31 exhibited significant periodic pattern over when using the BCF to fit the data (Figure 1, Table 2). In depressed patients before treatment, the amplitude of salivary melatonin was similar to that in healthy controls. After 4 weeks fluoxetine treatment the amplitude and baseline were lower, however the difference did not reach significant level. The acrophase were not significantly advanced in depressed patients (including before and after treatment) compared with healthy controls. The distribution of acrophase is shown

Table 1. Clinical Effects after 6 weeks of Fluoxetine Treatment(mean±S.D.).

	Day0 (n=13)	Day7 (n=9)	Day14 (n=9)	Day28 (n=9)	Day42 (n=9)	p-value
HDRS	20.08±2.91	13.50±2.51	10.75±3.37	8.13±4.16	5.25±3.33	0.000
SDS	53.00±7.21	46.75±5.92	42.12±4.94	37.50±5.58	33.75±5.52	0.000
CGI-S	3.78±0.83	3.43±0.54	3.14±0.69	2.29±0.95	1.86±0.9	0.000
CGI-I		3.14±0.69	2.86±0.38	2.00±1.00	1.86±1.07	0.008

HDRS: Hamilton Depression Rating Scale; SDS: Zung Self-Rating Depression Scale; CGI-S: the severity of illness item in Clinical Global Impression Scale; CGI-I: the improvement item in Clinical Global Impression Scale
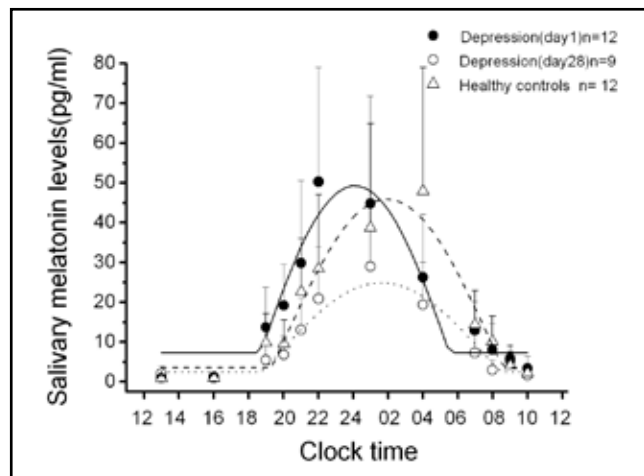
Figure 1. Mean circadian profile of salivary melatonin and fitted model curve for baseline consinus function (BCF). Solid line, depression (day0); Dot line, depression (day28); Dashed line, healthy control. Vertical lines represent S.D.
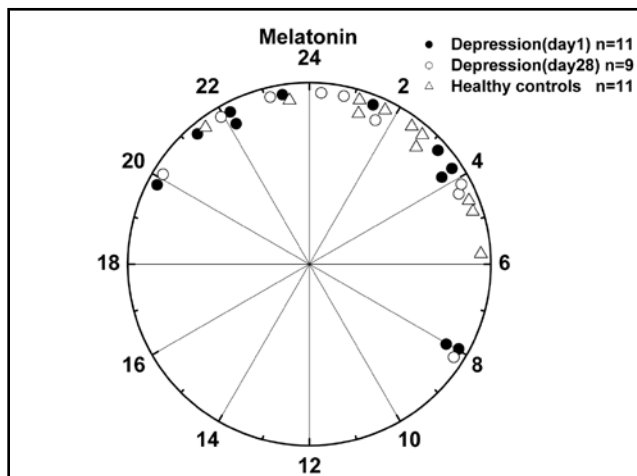

Figure 2. Distribution of acrophase of melatonin in healthy controls and depressed patients before and after fluoxetine treatment. n: number of data sets, which the significance level of fitted curve was less than 0.10.

in Figure 2. There was no any significant difference in other parameters (peak level, peak duration, area under curve of original concentration) between depressed patients before and after fluoxetine treatment.

Correlation between clinical responses and circadian rhythms of melatonin in depressed patients

There was no correlation between depression severity and melatonin rhythms both before and after treatment. Difference of melatonin amplitude (Difference is equal to the amplitude before treatment minus the amplitude after treatment, i.e., Difference(Y)=Before–After) was positively correlated with the improvement in HDRS scores at day 42 (Improvement is equal to HDRS score before treatment minus HDRS score after treatment, divided by HDRS score before treatment and times 100%) (Improvement(X)=(Before–After)/Before×100%) (Figure 3). The difference of melatonin amplitude and the improvement in HDRS scores at day 28 did not show significant correlation.

Discussion

In the present study, we confirmed a clear circadian rhythm of salivary melatonin in patients with major depression before and after fluoxetine treatment. There was no any difference in the circadian rhythms of salivary melatonin between depressed patients and healthy subjects. This result is consistent with most recent studies [10,22,30]. Rubin et al.[22] studied 38 endogenous depressed patients and individually matched controls. They found depressed patients showed a trend toward a significantly elevated average nocturnal melatonin. When 14 premenopausal women were excluded, the postmenopausal female and male depressive patients did not differ significantly from their matched controls. Childs et al.[10] studied ten patients with major depressive disorder and age- and sex-matched healthy controls, they found nocturnal melatonin levels were similar in depressed patients and healthy controls. Another study reported there was no any significant difference of

Table 2. Circadian Parameters of Salivary Melatonin in Healthy Controls and Depressed Patients before and after 4 Weeks Fluoxetine Treatment (mean±S.D.).

	Before treatment	After treatment	Control subjects
Baseline (pg/ml)	8.45±3.45	4.36±2.05	3.30±1.98
Amplitude (pg/ml)	70.97±38.76	42.84±23.16	67.03±27.95
Acrophase (h:m)	1:13±3:39	1:08±3:11	2:16±2:11
Peak level (pg/ml)	86.82±35.72	42.13±24.43	68.18±28.08
Peak duration (h)	6.76±2.93	6.03±2.37	9.59±2.76
Area under curve	430.93±85.12	241.25±58.71	396.93±89.57

*Paired t-test. Before treatment group vs. Control subjects, n=11; Before treatment vs. After treatment, n=9; After treatment vs. Control subjects, n=9.
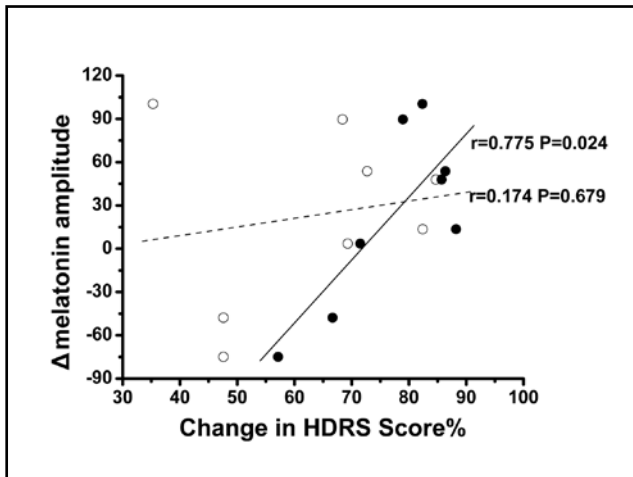
Figure 3. Δ melatonin amplitude show positive relationship with change in Hamilton Depression Rating Scale (HDRS) score following fluoxetine treatment. Δ melatonin amplitude=Before–After. Change in HDRS score=(Before–After)/Before * 100%. Dot and solid line means day 42. Circus and dash line refers to day 28. Note clinical improvement at day 42 after treatment was significantly associated with decrease of melatonin amplitude.

melatonin profile across 24 hour in nine melancholic depressed patients and age- and sex-matched healthy controls [30].

This result did not support early finding that low nocturnal melatonin was correlated with depression [3–5,8]. These early studies mainly concentrated to nocturnal melatonin secretion, not collected samples across day and night. The discrepancy may also be related to methodological differences such as whether have individually matched healthy controls, as well as disparities in medication status, age and season among patients [18,22]. Moreover, in the above mentioned studies the total amount of melatonin was measured in plasma samples. Levels of plasma binding of hormones may affect the biological activity of the hormones [9]. Salivary melatonin is a measure of free melatonin, it avoids the assay noise by binding of the radioimmunoligand to plasma proteins other than melatonin [31].

In the present study, amplitude, peak level and baseline of melatonin were not significantly lower in depressed patients after treatment. To our surprise, the difference of melatonin amplitude before and after treatment was positively correlated with clinical improvement. This correlation may not simply just attributed to direct pharmacological effects on lowering nocturnal melatonin [10]. Since two patients showed higher melatonin amplitude after fluoxetine treatment; And one patient who improved the least, the average melatonin level did not change after treatment.

To our knowledge, this is the first study to examine the relationship between clinical response to fluoxetine treatment and change in salivary melatonin rhythm. Our findings are consistent with a previous study, which measured nocturnal salivary melatonin in 10 pregnant women with major depression before and after bright

light therapy [14]. They observed successful treatment was associated with phase advance of melatonin rhythm. Because the samples were only collected before bedtime, it is not clear how the clinical response related to the change of melatonin amplitude or nocturnal levels. Hariharasubramanian et al.[17] reported that peak levels and 24-hour plasma secretion of melatonin significantly increased in 10 patients with major depression after four weeks imipramine treatment. As a group, these patients achieved clinical remission. In four subjects, melatonin rhythm was also measured after two weeks treatment, no difference was found compared with before treatment, accompanied by a partial or no clinical improvement. Because only one patient responded poor to fluoxetine (lowering of HDRS <50%), we were unable to perform discriminant analysis between good and poor treatment responders with respect to melatonin rhythm parameters. The clinical implications of the present study are also limited due to the relative small sample size. The bigger sample sizes with longer follow-up deserved for the future studies.

In conclusion, salivary melatonin showed clear circadian rhythm in depressed patients before and after fluoxetine treatment. Melatonin rhythms were similar between depressed patients and matched healthy controls. The interesting finding that the difference of salivary melatonin amplitude was correlated with the clinical improvement after six weeks fluoxetine treatment deserve further research.

Acknowledgements

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