Alterations in circadian rhythms of melatonin and cortisol in patients with bronchial asthma[1]

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ABSTRACT

AIM: To investigate the possible relationships between alterations in circadian rhythm of melatonin, cortisol and bronchial asthma. **METHODS:** Salivary melatonin and cortisol were measured simultaneously by radioimmunoassay in 10 mild intermittent or persistent patients, 11 moderate-to-severe persistent asthma patients, and 15 control subjects. Twelve salivary samples were collected in a series during a 24-h period in each subject. **RESULTS:** The results showed overall lower levels of salivary melatonin in asthma patients compared with control subject ($P<0.01$). The amplitude, peak-level, and baseline of salivary melatonin were significantly lower in mild intermittent or persistent ($P<0.01$, $P<0.05$) and moderate-to-severe persistent asthma patients ($P<0.01$) compared with control group. The 24-h mean level of salivary cortisol was greatly lower and the acrophase was markedly delayed in patients with mild intermittent or persistent asthma ($P<0.01$) and moderate-to-severe persistent asthma ($P<0.05$, $P<0.01$) compared with control subject. **CONCLUSION:** Disordered circadian rhythms of salivary melatonin and cortisol were found in asthma patients, which may be involved in the pathogenesis of bronchial asthma.

INTRODUCTION

Bronchial asthma is a characteristic circadian rhythm disease in many aspects. Patients with asthma often experience a nocturnal worsening of pulmonary function[1], asthmatic symptoms[2,3], and sleep quality[4]. Asthma patients also show a diurnal variation in death with the peak occurring during the early morning hours[5]. The mechanisms involved in circadian rhythms of asthma are multiple, interactive, and presently not fully understood[6].

Cortisol exhibits a circadian rhythm, reaching a peak level at approximately 07:00 (the time of awakening) and a trough around 23:00 in diurnally active subjects[7]. Since the corticosteroid can suppress key asthma relevant cell-inflammatory responses in the airways, it has been proposed that the alterations in airway function may be at least partly due to differences in serum cortisol in asthmatics and non-asthmatics.

Melatonin is a hormone that not only plays a major role in the regulation of circadian rhythms, but also may exert a function as part of the anti-oxidative defense system[8,9]. In addition to melatonin's immunostimulatory effects, some researches have identified its anti-inflammatory properties[10,11].

Many publications have demonstrated a significant positive correlation between the levels of salivary mela-

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tonin and cortisol with plasma or serum levels measured by radioimmunoassay[12,13]. The levels of salivary melatonin are valid indicators of the 24-h rhythmicity of the pineal secretory profile[14].

We hypothesize that melatonin and cortisol may be involved in alterations of circadian rhythm in patients with bronchial asthma. Therefore, present study aimed to investigate the possible connections between the alterations in circadian rhythms of melatonin, cortisol and bronchial asthma.

MATERIALS AND METHODS

Subject Thirty six subjects, including 10 mild intermittent or persistent asthma patients [5 men and 5 women, age range 24–33 years (27.7±4.2) years], 11 moderate-to-severe persistent asthma patients [5 men and 6 women, age range 20-35 years (26.5±4.5) years], and 15 healthy subjects [7 men and 8 women, age range 24-35 years (26.8±5.2) years] participated in the present study. The three groups were matched for age and sex. The asthmatic patients included inpatients and outpatients from the First Affiliated Hospital of Anhui Medical University. The diagnosis of bronchial asthma and the classification of asthma severity were made or defined according to the Expert Panel Report 2, Guidelines for the Diagnosis and Management of Asthma[15]. All subjects were not given any beta-blockers, barbiturates, or benzodiazepines and were free from anxiety or sleep disorder complaints. Patients who had fever (more than 37.5 ºC) and/or received steroids continuously or intermittently (including oral, intranasal, or parenteral corticosteroids) in the last one month before sampling were excluded from study. Some patients were on rescue treatment with beta-agonists and/or theophylline. Their physical health was assessed by a complete physical examination. All subjects had given written informed consent to participate and were told possible risks in the study; the protocol was approved by the Human Investigation Committee of Anhui Medical University. The experiment was performed from 2002 May 1 to 2002 July 26.

Saliva collection During the sampling period, the control subjects and outpatients were community living, inpatients were in the ward and all subjects were instructed to avoid heavy exercise. All samples were taken under indoor light conditions, and the intensity of the ambient light was restricted to 250 to 300 lux in full light (either daylight or artificial light), except at 01:00 and 04:00, when the light had to be turned off or the light intensity had to be less than 50 lux; in semidarkness a flashlight was used to illuminate only the sampling area (Gossen, Mavolux, digital-8C44276, Germany). Saliva was collected by placing wool swab of plain Salivettes (Sarstedt, Disposable Products, Regency Park, Adelaide) into the mouths of the subjects with chewing for 5 min. Saliva samples were taken at a total of 12 time points over a 24-h period for each subject. The samples was taken during the day (13:00 and 16:00) and at night (01:00 and 04:00), at the on-set (07:00, 08:00, 09:00, 10:00) light and the off-set (19:00, 20:00, 21:00, and 22:00) light. The samples were kept in Salivette tubes in the refrigerator at ±4 ºC until the end of each 24-h period. The Salivette tubes were centrifuged for 15 min at 3000 ×*g* and the samples were frozen at -20 ºC until assayed.

Salivary melatonin and cortisol assay Melatonin in saliva was measured by a direct radioimmunoassay (RIA). The kit is manufactured by LDN GmbH & Co, KG, Nordhorn Germany (Cat-No: DSL-BA-1200) and is specially made for the quantitative determination of melatonin in saliva. The cross-reactivity of the assay for *N*-acetylserotonin, 5-methoxytryptophol, and 5-methoxytryptamine is 0.80 %, 0.70 %, and 0.08 %. Serotonin, 5-methoxyindol-3-acetic acid is <0.01 %, respectively. The sensitivity of the assay is 1 ng/L. The intra- and inter-variations are 11.3 % and 12 %, respectively. Salivary cortisol concentrations were measured with a competitive solid-stage radioimmunoassay [Coat-A-Count, Diagnostic Products Corp (DPC)]. The protocol followed the instructions of Coat-A-Count Free Cortisol in Saliva kit from DPC.

Statistics To characterize the pattern of salivary melatonin in each subject, the periodic functions were fitted to each individual set of data points using the baseline cosine function (BCF)[16]:

$$Y(x)=b+H/2/(1-c)\cdot(\cos(x-Ø)-c+|(\cos(x-Ø)-c)|) \quad (1)$$

Y is the nth data point, x is the time of day (in radians), b is the baseline, H is the peak height (amplitude), c is cut-off constant, and $Ø$ is the acrophase. Peak duration [D; in(h)], *ie*, the time interval during which the model curve deviates from the baseline, was calculated from: $D=\arccos(c)/\pi\cdot 24$. These parameters were subjected to further calculations or common statistical analysis.

To characterize the pattern of salivary cortisol in each subject, the periodic function was fitted to each individual set of data points by another function that

has been suggested by Batschelet[17]:

$$Y(x)=M+A\cdot\cos(x-\varnothing+v\cdot\sin(x-\varnothing)) \quad (2)$$

A is the amplitude, $\varnothing$ is the acrophase, v determines the 'peakedness' of the curve and M is equal to the 24-h mean level.

One-way ANOVA procedure was used to compare the three groups of cases on one variable. A level of $P<0.05$ was considered to be significant.

RESULTS

The circadian rhythm profiles of salivary melatonin showed clear periodic patterns in the three groups, but exhibited more flattened profiles in mild intermittent or persistent asthma and the most flattened profiles in moderate-to-severe persistent asthma patients (Fig 1). Tab 1 shows the circadian rhythm parameters of salivary melatonin in the three groups. The amplitude and peak-level (night time) of salivary melatonin levels were significantly lower in mild group ($P<0.05$, $P<0.01$, respectively) and moderate-to-severe group ($P<0.01$, $P<0.01$, respectively) compared with control group, but there were no significant differences in the amplitude and the peak-level between mild and moderate-to-severe group ($P>0.05$, $P>0.05$, respectively). Also, the baseline levels (day time) of salivary melatonin were significantly lower in mild and moderate-to-severe group compared with control subjects ($P<0.05$, $P<0.01$, respectively), whereas there was no significant difference between mild group and moderate-to-severe group ($P>0.05$). There were no differences in the acrophase and the peak duration of salivary melatonin levels among the three groups ($P>0.05$, $P>0.05$, respectively).

Tab 1. Circadian parameters of salivary melatonin in control and asthmatic groups. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* control group. [d]*P*>0.05 *vs* mild group.

	Control group (*n*=15)	Mild group (*n*=10)	Moderate-to-severe group (*n*=11)
Baseline	8.5±2.0	5.3±1.8[b]	3.7±1.1[cd]
Amplitude	79±29	43±12[c]	18±5[cd]
Peak level	88±29	49±15[c]	22±4[cd]
Acrophase	1:52±00:38	1:37±00:44[a]	3:33±1:53[a]
Peak duration	10.3±0.9	9.4±1.1[a]	9.6±1.1[a]

Notes: baseline:daytime melatonin level (ng/L); acrophase: clock time at which the nocturnal melatonin reaches peak level (h:min); amplitude: distance from baseline to peak level; peak level: highest nocturnal melatonin level (ng/L); peak duration: time interval during which the periodic curve deviates from the baseline level (h:min). *P* value: One-way ANOVA.

The circadian rhythm profiles of salivary cortisol exhibited clear circadian patterns (Fig 2). Tab 2 shows the circadian rhythm parameters of salivary cortisol levels. The 24-h mean level was significantly different

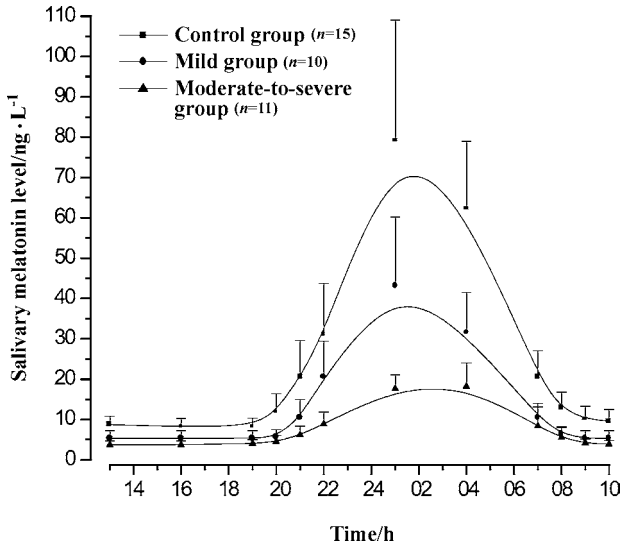

Fig 1. Mean circadian profile of salivary melatonin (solid dots) and fitted model curve for baseline cosine function (BCF). Mean±SD.

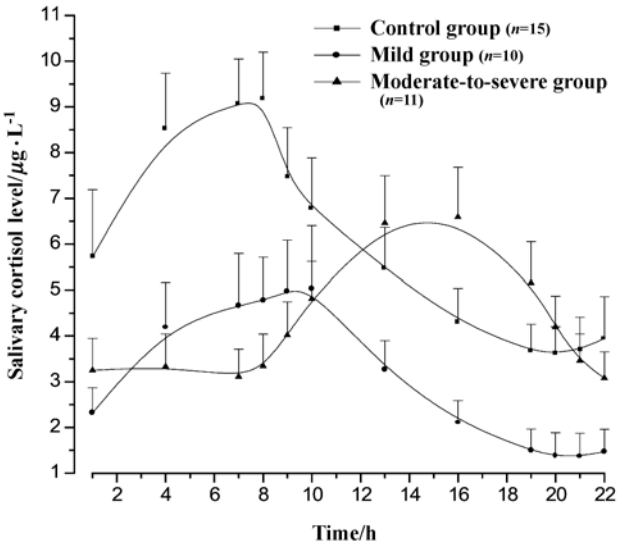

Fig 2. Circadian profile of salivary cortisol (solid dots) and fitted model curve for equation 2. Mean±SD.

Tab 2. Circadian parameters of salivary cortisol in control and asthmatic groups. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control group. $^{f}P<0.01$ *vs* mild group.

	Control group (n=15)	Mild group (n=10)	Moderate-to-severe group (n=11)
Acrophase	7:23±1:26	9:33±0:53^{c}	15:21±1:54^{c,f}
Amplitude	4.4±0.9	2.7±0.6	3.9±0.6
24-h mean level	6.1±0.7	3.0±0.4^{c}	4.9±1.2^{b}

Notes: acrophase: clock time at which the cortisol levels reaches its peak (h:min);amplitude: distance from mean to peak levels (μg/L); the 24-h mean level (μg/L); *P* values: One-way ANOVA.

among the three groups ($P<0.01$) as analyzed by ANOVA. Post hoc analysis showed that the 24-h mean level was significantly lower in mild and moderate-to-severe groups ($P<0.01$, $P<0.05$, respectively) as compared with control group. The amplitude of salivary cortisol was not significantly different among the three groups ($P>0.05$). The acrophase of salivary cortisol was significantly different among three groups ($P<0.01$). *Post hoc* analysis showed that the acrophase was delayed about 2 h in mild group ($P<0.01$) and delayed about 8 h in moderate-to-severe group ($P<0.01$).

DISCUSSION

In the present study, we observed overall lower levels of salivary melatonin in mild intermittent or persistent and moderate-to-severe persistent asthma patients, while clear circadian patterns of salivary melatonin were present in the three groups. The amplitude and peak-level (nighttime) and baseline (daytime) of salivary melatonin levels were significantly lower in asthma patients. Consistent with our observations, Kos-Kudla *et al*[18] demonstrated that considerable decrease in the 24-h mean level and amplitude in serum melatonin in asthma patients as compared to healthy controls.

The amplitude of circadian rhythms is considered to be one of the most important parameters in analyzing circadian rhythms. It stems primarily from opposite differences in the low daytime melatonin and the higher levels of melatonin at night and represents the endogenous fluctuations of hormones under physiological and pathological conditions. In the present study, the highest amplitude of salivary melatonin was found in control group, the lower and the lowest in mild intermittent or persistent and moderate-to-severe asthma patients, respectively. These findings showed that the lower amplitude of salivary melatonin levels might be related to bronchial asthma itself, or at least to the state of the disease. However, this did not necessarily imply that there was a causal association between the alteration of melatonin level and bronchial asthma.

The baseline level (daytime melatonin) is also an interesting question in analyzing circadian rhythm and is regulated by the environmental light cues. It has been reported that hospitalized patients have significantly higher daytime plasma melatonin levels (baseline) than those from the community group[19,20]. Lewy *et al*[21] also demonstrated that room light of less intensity, which is sufficient to suppress melatonin secretion in other mammals, failed to do so in humans. In the present study, a puzzle of the low baseline level was found in asthmatic patients. Since the hospitalized asthma patients received less intensive light, if there were a light effect, they should have had higher melatonin baseline level. Therefore, the low melatonin baseline level in the present study may also be involved in the pathological state of asthma.

The underlying mechanism causing the decline of melatonin in patients with bronchial asthma is not known. Barriga *et al*[22] reported that the decline in melatonin levels in rats might be either due to the direct inhibitory effect of corticosterone on pinealocytes or because the melatonin is more rapidly metabolized during the stress of a pathological condition.

Our results demonstrated that there was still a diurnal rhythm of salivary cortisol in patients with bronchial asthma. The 24-h mean level of salivary cortisol was significantly lower in asthma patients. Consistent with our results, some investigators observed that the 24-h mean level decreased significantly[18,23]. Most interestingly, we found that the acrophase was significantly delayed in mild intermittent or persistent asthma patients, particularly in moderate-to-severe persistent ones. Haen *et al*[24] demonstrated that the time of serum cortisol peak level occured (acrophase) later in asthmatics than in non-asthmatic controls. Nevertheless, other authors observed the decreased cortisol concentration with the acrophase within the normal range[18,23]. Additionally, in the present study, we can observe clearly from Fig 2 that the cortisol level decreased at about 08:00 in control group when cortisol level began to increase and reached its peak level at about 15:30 in mod-

erate-to-severe persistent asthma patients. Consistent with our observations, Fujitaka *et al*[25] reported that the plasma cortisol concentrations in patients with asthmatic attacks exceeded those in the mild persistent asthma phase in the afternoon and presented an opposite diurnal pattern with control group. This phenomenon was explained as a consequence of the different phase shift of cortisol level caused by different severity of the disease. Whereas, the shortcomings of the present study were the fact that some of small waveform and phase shift in salivary cortisol (ultradian rhythms) might not be sensitive enough to be detected.

According to the results discussed above, it has been suggested that the cortisol level is disturbed not only in the quantitative evaluation but also in the rhythmic pattern of the hormone in asthmatic patients. Since cortisol is anti-inflammatory, and is very effective in reducing the intensified airway inflammation and decrement in lung function associated with asthma[7]. Therefore, the lower cortisol level along with disordered rhythmic release in asthmatic patients may contribute to the worsening of asthma and the falling of lung function. It has been suggested that chronotherapy should be applied to optimize the desired effects of medications and to minimize undesired ones in asthmatic patients.

In conclusion, our experimental results confirm that disordered circadian rhythms of salivary melatonin and cortisol are found in patients with bronchial asthma, which may be involved in the pathogenesis of asthma.

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