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Diurnal neurobiological alterations after exposure to clozapine in first-episode schizophrenia patients

Hong-Qiang Sun[a,1], Su-Xia Li[b,1], Fang-Bin Chen[c,1], Yan Zhang[c], Peng Li[a,c], Mei Jin[c], Yan Sun[b], Fan Wang[d], Wei-Feng Mi[a], Le Shi[a,b], Jing-li Yue[a], Fu-De Yang[d], Lin Lu[a,e,*]

[a] *Peking University Sixth Hospital/Institute of Mental Health and Key Laboratory of Mental Health, Ministry of Health, Peking University, Beijing 100191, China*
[b] *National Institute on Drug Dependence, Peking University, Beijing 100191, China*
[c] *102 Military Hospital of China, Changzhou 213000, China*
[d] *Beijing Hui-Long-Guan Hospital, Peking University, Beijing 100096, China*
[e] *National Clinical Research Center for Mental Disorder, Peking University, Beijing 100191, China*

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ABSTRACT

Background: Irregular circadian rhythm and some of its most characteristic symptoms are frequently observed in patients with schizophrenia. However, changes in the expression of clock genes or neuropeptides that are related to the regulation of circadian rhythm may influence the susceptibility to recurrence after antipsychotic treatment in schizophrenia, but this possibility has not been investigated.
Methods: Blood samples were collected from 15 healthy male controls and 13 male schizophrenia patients at 4 h intervals for 24 h before and after treatment with clozapine for 8 weeks. The outcome measures included the relative expression of clock gene mRNA PERIOD1 (PER1), PERIOD2 (PER2), PERIOD3 (PER3) and the levels of plasma cortisol, orexin, and insulin.
Results: Compared with healthy controls, schizophrenia patients presented disruptions in diurnal rhythms of the expression of *PER1*, *PER3*, and *NPAS2* and the release of orexin, accompanied by a delayed phase in the expression of *PER2*, decreases in *PER3* and *NPAS2* expression, and an increase in cortisol levels at baseline. Several of these disruptions (*i.e.*, in *PER1* and *PER3* expression) persisted after 8 weeks of clozapine treatment, similar to the decreases in the 24-h expression of *PER3* and *NPAS2*. Clozapine treatment for 8 weeks significantly decreased the 24-h levels of *PER2* and increased the 24-h levels of insulin.
Conclusion: These persistent neurobiological changes that occur after 8 weeks of clozapine treatment may contribute to the vulnerability to recurrence and efficacy of long-term maintenance treatment in schizophrenia.

1. Introduction

Schizophrenia is a neurodevelopmental psychotic disorder that is characterized by hallucinations, disorganized thought, and cognitive impairment, resulting in chronic and severe social and occupational dysfunction. However, the etiology and pathogenesis of schizophrenia remain a mystery. Previous studies have shown that schizophrenia patients are characterized by disruptions in the circadian rhythm of hormones and neuropeptides, such as significant phase advances of serum tryptophan, prolactin, and melatonin secretion (Rao et al., 1994, 1995).

Mammalian circadian timing is partially controlled by "clock genes" that are expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus, other brain regions, and peripheral tissues. Circadian rhythms are driven by self-regulatory interactions between a set of clock genes and their protein products (Ko and Takahashi, 2006; Mazzoccoli et al., 2012). The expression of proteins from one positive loop and one negative loop oscillates, forming a circadian rhythm. The positive drive of the daily clock consists of helix-loop-helix, PAS domain-containing transcription factor genes (Clock and Bmal1). The negative loop mainly consists of Per and Cry proteins that provide negative feedback signaling for the Clock/Bmal1 drive to complete the 24-h cycle (Mazzoccoli et al., 2012).

* Corresponding author at: Peking University Sixth Hospital/Institute of Mental Health/National Clinical Research Center for Mental Disorder, Peking University, 51 Huayuan Bei Road, Beijing 100191, China. Fax: +86 10 62032624.
E-mail address: linlu@bjmu.edu.cn (L. Lu).
[1] Equally contributed to this work.

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Evidence that links circadian clock gene polymorphisms with schizophrenia is limited. A previous study indicated that the T3111C polymorphism of the *CLOCK* gene presented a transmission bias in a sample of 145 Japanese schizophrenia subjects compared with healthy controls (Takao et al., 2007). Another study suggested that *PER3* but not *PER2* abnormalities were associated with schizophrenia (Mansour et al., 2006). The expression of *PER1* mRNA in the temporal lobe in schizophrenia subjects significantly decreased compared with age-matched normal controls (Aston et al., 2004). However, the association between clock genes and schizophrenia is far from clear.

The first atypical antipsychotic that was developed, clozapine, was shown to dramatically improved the outcome of treatment in schizophrenia patients (Advokat, 2005). It markedly reduces psychotic symptoms without eliciting significant neurological side effects and was the most effective drug for treatment-resistant schizophrenia (Naheed and Green, 2001; Tuunainen et al., 2000), yielding symptom improvement in 30% of patients within 6 weeks (Kane et al., 1988) and 61% within 1 year (Meltzer, 1992; Meltzer et al., 1989). Approximately 50% of schizophrenia patients who did not respond to other antipsychotics benefitted from clozapine (Reynolds, 2012). Clozapine effectively improved both psychiatric symptoms and circadian rhythm disturbances (Lewis et al., 2006; Meltzer et al., 2010; Wirz-Justice et al., 2000, 2001). After treatment with olanzapine for 4 weeks in schizophrenia patients, hypothalamic–pituitary–adrenal (HPA) axis activity was reduced, with a decrease in plasma cortisol levels compared with baseline conditions, and the characteristic growth hormone peak around sleep onset was markedly reduced (Mann et al., 2006). Because of poor methodological feasibility, few studies had investigated circadian rhythms in schizophrenia patients (Vanelle, 2009).

Several studies have shown that atypical antipsychotics may modulate HPA axis activity, which is involved in the etiology of schizophrenia (Walker et al., 2008). Previous studies have found changes in molecules that are linked to insulin resistance and glucose handling in schizophrenia patients, including patients who have not received antipsychotic medications (Arranz et al., 2004; van Nimwegen et al., 2008). Sleep disorders are well known to be a common problem in schizophrenia patients. Orexin-containing neurons project from the lateral hypothalamic area to many central nervous system (CNS) areas, including the paraventricular nucleus of the hypothalamus, arcuate nucleus, locus coeruleus (Date et al., 1999; Horvath et al., 1999; Peyron et al., 1998), ascending arousal system in the brainstem (monoaminergic, cholinergic), and basal forebrain (cholinergic) (Baumann and Bassetti, 2005). These projections suggest that orexin plays a central role in promoting wakefulness and mediating stress.

The hypothesis in the present study was that clozapine can ameliorate disruptions in circadian rhythms in schizophrenia patients, with significantly higher response rates compared with other antipsychotics. We detected the rhythmic expression of mRNA of specific clock genes that are important components of the molecular clock mechanism and are related to schizophrenia and the circadian rhythms of blood levels of hormones and peptides (*i.e.*, cortisol, orexin, and insulin) to test our hypothesis.

2. Methods and materials

2.1. Subjects

The clinical sample consisted of 15 male first-episode patients with schizophrenia and 15 age- and gender-matched controls. The patients were consecutively recruited from the 102 Military Hospital of China, Changzhou, China. Recruitment occurred between August 2009 and August 2010. The patients' ages ranged from 18 to 45 years (mean ± SD: 22.54 ± 3.84 years). Thirteen patients who reported no history of any other psychiatric disorders and no history of taking any antipsychotic medications completed the study. The patients met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; (American Psychiatric Association, 1994), criteria for schizophrenia. The diagnosis was established using the Structured Clinical Interview for DSM-IV disorders (SCID), which was performed by two experienced psychiatrists. A minimum score of 60 points on the 30-item Positive and Negative Syndrome Scale (PANSS) was required to be enrolled in the study. The duration of the present schizophrenia episode ranged from 1 to 36 months (mean ± SD: 12.08 ± 13.49 months). The exclusion criteria included (i) age <18 years or >45 years, (ii) comorbid DSM-IV diagnosis, including alcohol or illicit drug abuse, and other Axis I psychiatric disorders, (iii) current or past serious physical illness (*e.g.*, active tuberculosis, acute hepatitis, cirrhosis, renal illness, cardiovascular illness, or unstable diabetes), and (iv) diastolic blood pressure <60 mm Hg or heart rate <60 beats per minute.

Control participants included 15 physically and mentally healthy male volunteers who were recruited from the local community and matched for age (age range, 18–45 years; mean ± SD: 22.20 ± 2.14 years). Screening for psychiatric disorders was performed according to SCID which was also performed by two experienced psychiatrists. The following exclusion criteria were used: (i) any of the exclusion criteria for the experimental group, (ii) past DSM-IV Axis I or Axis II disorders, (iii) sleep disturbances, (iv) shift work, (v) use of any medication within the past 30 days, (vi) any current or past physical illness that would aggravate or reappear if the individual participated in the study.

The results of the participants' physical examination, electrocardiogram, hepatorenal function, and routine urinalysis were within normal limits. All of the experimental procedures were performed in accordance with the Declaration of Helsinki and approved by the ethics committee of the Beijing Hui-Long-Guan Hospital, Beijing, China. All of the procedures were performed with adequate understanding and written consent by the subjects. Each patient was provided free clozapine (Jiangsu Nhwa Pharmaceutical Corporation Ltd., Jiangsu, China) for 8 weeks. The clozapine dosage began with 25 mg daily (taken after dinner) for the first day and gradually increased to 100–450 mg during the first 2 weeks (Matsuda et al., 1996). The dosage was then maintained until the end of 8 weeks. Before the first dose of clozapine and on weekends during the study period, we assessed hemograms for blood side effects. Electrocardiograms were assessed at the end of the 1st, 2nd, 4th, and 8th weekends for heart side effects. Each patient was paid 400 RMB (equivalent to USD$64.35) upon completion of the study, and control participants were paid 200 RMB (equivalent to USD$32.17). The schizophrenia patients were transitioned to other antipsychotic medications or continued to take clozapine at the end of the study.

2.2. Experimental procedure

All of the schizophrenia patients underwent two experimental sessions, as described previously (Li et al., 2013; 2009b) and as briefly detailed below. The first session began before the beginning of clozapine treatment, and the second session occurred on day 57 of clozapine treatment. Each healthy volunteer underwent one experimental session.

Each participant arrived at the experimental ward at least 5 h before the first blood sample was drawn and remained there until the last blood sample was drawn the next day. After the participant arrived, an intravenous catheter was inserted into a forearm vein. Before the first blood sample was drawn, the participant sat in a recliner to adapt to the experimental environment but was not allowed to sleep. A total of seven blood samples were collected. Collection occurred every 4 h at 0400, 0800, 1200, 1600, 2000, and