Circadian rhythms of melatonin, cortisol, and clock gene expression in the hyperacute phase of wake-up stroke: study design and measurement

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To the Editor: Wake-up stroke (WUS) is the name given to an ischemic stroke that occurs primarily during night-time sleep and accounts for approximately 20% of all cases of acute ischemic stroke (AIS). Epidemiological studies have demonstrated a circadian distribution of AIS, with a higher frequency in the morning and a lower frequency during sleep.[1] It remains unclear whether WUS is a stochastic event or if a distinct pathophysiologic process, such as circadian rhythm disorder, differentiates WUS from daytime stroke (non-WUS).

The circadian distribution of AIS may be attributed to the circadian rhythmicity of some cardiovascular parameters, such as blood pressure (BP), fibrinolytic activity, platelet aggregation, and others.[2] The rhythmicity of these parameters is generated by complex networks of endogenous clocks, referred to collectively as the biological clock, which regulates circadian rhythms. Therefore, researchers have proposed the possibility of decoupling the circadian clock protective mechanisms of the brain in WUS patients. Some clinical findings support this proposed mechanism. Lundholm et al.[3] compared BP variability in WUS vs. non-WUS patients, and considered that nocturnal autonomic nerve instability may warrant further study as a potential mechanism of WUS. The stability of the autonomic nervous system is controlled by the endogenous clocks. Obstructive sleep apnea (OSA) is a recognized risk factor for WUS; some scholars have suggested that a key factor in the causal link between OSA and vascular disease risk factors may be an abnormal circadian rhythm.[4] Taking these previous findings together, we propose a novel hypothesis that, in some WUS patients, the night-time rhythmicity may be attenuated or abolished. Circadian rhythm refers to endogenous 24-h cycles driven by endogenous clocks, which are located in the anterior hypothalamus as a pair of nuclei known as suprachiasmatic nuclei (SCN). The activity of the SCN is dependent on the expression of auto-regulatory positive and negative feedback loops of the core clock genes, including CLOCK, BMAL1, two cryptochrome genes (CRY1, CRY2), three period genes (PER1, PER2, PER3), and others. These internal molecular clocks are very accurate, even when external cues are missing. Notably, it is now accepted that peripheral blood cells contain a circadian clock similar to that in the SCN.[5] Melatonin and cortisol are two traditional markers of circadian rhythms that have often been measured as circadian biomarkers. Melatonin can be considered as a reliable and accurate, even when external cues are missing. Notably, it is now accepted that peripheral blood cells contain a circadian clock similar to that in the SCN.[5] Melatonin and cortisol are two traditional markers of circadian rhythms that have often been measured as circadian biomarkers. Melatonin can be considered as a reliable biomarker.

Therefore, we plan to evaluate the circadian phase variations in WUS patients (via the plasma concentrations of cortisol and melatonin as well as clock gene expression in peripheral blood cells) compared with non-WUS patients and healthy volunteers. The proposed study was approved by the Ethics Committee of the First Affiliated Hospital of Suzhou University (No. 2019053) and was registered in the Chinese Clinical Trial Registry (No. ChiCTR1900024381).

This single-center, cross-sectional, observational study has three arms: the WUS group, the non-WUS group, and the healthy control group. The WUS group is defined as those who go to night-time sleep healthy and wake up with...
stroke symptoms. The non-WUS group is defined as those who experience stroke with a definite onset time during the daytime. The healthy control group is composed of healthy age- and sex-matched volunteers. All AIS patients who arrive at our Emergency Department are reviewed to determine eligibility. Strict inclusion and exclusion criteria are set up to minimize the confusing factors which may affect circadian rhythm. The detailed inclusion criteria for this study are: aged 40 to 80 years; lesions located in the anterior circulation (for patients); clear consciousness; time of hospital arrival between 6 AM and 6 PM. And the exclusion criteria are based on one of the following: lesions involving the thalamus (for patients); treatment with mechanical thrombectomy (for patients); drinking of caffeinated beverages for 24 h before the study; confirmed depression or other psychiatric disorders; history of stroke, brain tumor or other neurological disease; history of sleep disorder, blindness, severe liver or renal dysfunction or malignant disease; shift work or travel across two or more time zones within 6 weeks; medication history including β-receptor blockers that inhibit melatonin secretion, steroids, benzodiazepines, opioids, and immunsuppressant agents [Figure 1].

All enrolled patients are required to complete the basic examination within 24 h of admission, including physical and nervous system examination, laboratory tests, electrocardiogram, cerebral computed tomography (CT), CT angiography, and perfusion scanning. Participants are asked to maintain their normal nocturnal sleep patterns for the duration of the study. Daytime naps are not allowed. Patients, as well as volunteers, are exposed to the natural and conventional fluorescent light during the awake time, and the lights are turned off between 20:00 and 22:00 depending upon individual sleeping habits. All participants are instructed to wear activity bracelet monitors (HUAWEI honor 4) and eye masks while sleeping. During the overnight sampling period, a pen-sized dim flashlight is used to avoid light-induced suppression of melatonin secretion. Blood samples are taken at 6-h intervals beginning at a fixed time point of design after each participant is enrolled in the study. Each blood sample is tested for the concentrations of melatonin and cortisol and the expression levels of the following seven genes: CLOCK, BMAL1, three period genes (PER1, PER2, PER3), and two cryptochrome genes (CRY1, CRY2). During the course of the 24-h study, the heart rate, body temperature, and BP of all patients are recorded each hour. Sleep parameters, including the total sleep time, number of awakenings, rapid eye movement sleep percentage, light sleep percentage, and deep sleep percentage, are obtained after participants wake up. The recruitment target for each group is 15 subjects with an anticipated dropout rate of 25%, resulting in approximately 11 subjects in each group. The reasons for dropout will be recorded and are expected to include reduced sleep quality during the research period or the observation of lesions located in the thalamus or posterior circulation on magnetic resonance imaging at a later stage.

The primary end-points of this study are the circadian rhythmicity of the plasma melatonin concentration, the
plasma cortisol concentration, the expression of seven clock genes, heart rate, body temperature, and BP in the WUS group, and the differences in the circadian rhythms among the three groups. The secondary endpoints are the possible impacts of the biological clock on the occurrence of WUS.

We will compare the study endpoints among the three arms using MetaCycle R package and corrected for multiple comparison testing using the Fisher method. Continuous variables will be presented as mean ± standard deviation or median (interquartile range) depending on the normality of distribution. Analysis of variance (ANOVA) or Welch ANOVA will be used to compare variables among the three groups according to variance homogeneity evaluated by F tests. Categorical variables will be compared by the Chi-square test. The P value for significance will be set to 0.05.

To our knowledge, this study is among the first to investigate the circadian rhythm in the hyperacute phase of WUS. However, this study has the well-known limitations of a cross-sectional study, and only association can be evaluated, not causation. If positive results are found, this will only imply the association between circadian rhythm and WUS. We will not be able to infer temporality or causality between them. Nonetheless, if our hypothesis is supported, the association may provide novel insights for further exploration of the pathogenesis of WUS.

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Conflicts of interest

None.

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