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Original Article

Sleep interruption associated with house staff work schedules alters circadian gene expression

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ABSTRACT

Background: Epidemiological studies indicate that disruption of circadian rhythm by shift work increases the risk of breast and prostate cancer. Our studies demonstrated that carcinogens disrupt the circadian expression of circadian genes (CGs) and circadian-controlled genes (CCGs) during the early stages of rat mammary carcinogenesis. A chemopreventive regimen of methylselenocysteine (MSC) restored the circadian expression of CGs and CCGs, including *PERIOD 2* (*PER2*) and estrogen receptor β (*ERS2*), to normal. The present study evaluated whether changes in CG and CCG expression in whole blood can serve as indicators of circadian disruption in shift workers.

Methods: Fifteen shift workers were recruited to a crossover study. Blood samples were drawn before (6 PM) and after (8 AM) completing a night shift after at least seven days on floating night-shift rotation, and before (8 AM), during (1 PM), and after (6 PM) completing seven days on day shift. The plasma melatonin level and messenger RNA (mRNA) expression of *PER2*, nuclear receptor subfamily 1, group d, member 1 (*NR1D1*), and *ERS2* were measured, and the changes in levels of melatonin and gene expression were evaluated with statistical analyses.

Results: The mRNA expression of *PER2* was affected by shift ($p = 0.0079$); the levels were higher in the evening for the night shift, but higher in the morning for the day shift. Increased *PER2* expression ($p = 0.034$) was observed in the evening on the night versus day shifts. The melatonin level was higher in the morning for both day shifts ($p = 0.013$) and night shifts ($p < 0.0001$).

Conclusion: Changes in the level of *PER2* gene expression can serve as a biomarker of disrupted circadian rhythm in blood cells. Therefore, they can be a useful intermediate indicator of efficacy in future MSC-mediated chemoprevention studies.

1. Introduction

Epidemiological studies indicate that shift workers, including nurses, pilots, and flight attendants, are at an increased risk of breast, prostate, and colon cancers. By contrast, blind people, whose biological rhythms are not regulated by light and dark cycles and whose melatonin levels are diminished, show reduced risk of these cancers [1–3]. Recent meta-analysis results further provided evidence for the positive dose–response relationship between breast cancer risk and increasing years of employment and cumulative shift work [4]. These findings are corroborated by animal studies indicating that disruption of the biological clock by exposure to constant light, light at night, pinealectomy, or jet-lag protocols (repeated disruption of circadian rhythm) increase the incidence of spontaneous and carcinogen-induced tumors and accelerates tumor growth in rat mammary tumor models [5,6]. Based on these combined human and animal data, the International Agency for Research on Cancer classified disruption of circadian rhythm by shift work as a probable human carcinogen (Type 2A) [7].

The circadian rhythm regulates biological processes ranging from gene expression to behavior in a precise and sustained rhythm controlled by a molecular oscillator that functions with a periodicity of ~24 h. The biological clock functions not only in the central pacemaker,

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the suprachiasmatic nucleus (SCN), but also in most peripheral organs and cells, including mammary glands [8] and white blood cells [9]. The SCN synchronizes peripheral clocks through humoral (eg, melatonin and glucocorticoids) and neuronal pathways upon exposure to external cues, particularly light and nutrients. In mammalian cells, the molecular oscillator is regulated by the coordinated function of interlocked transcriptional/translational feedback loops. Heterodimers of Bmal1 and Clock activate transcription by binding to E-box elements in the promoters of circadian genes (CGs), including *Per*, *Cry*, and *Rev-ErbAα* (nuclear receptor subfamily 1, group d, member 1 (*Nr1d1*)), and in numerous circadian-controlled genes (CCGs) that include growth regulatory genes, DNA damage response and repair genes, and tumor suppressor genes [2]. Previous clinical studies indicated that shift work influences the regulation of peripheral CG and CCG expression in human blood cells; however, these findings remain controversial and inconclusive [10,11].

Accumulating evidence indicates that the circadian clock prevents carcinogenesis and that frequent disruption of circadian rhythm is an important tumor-promoting factor [1,12,13]. Long-term shift work is associated with repeated phase shifts over the course of several days, leading to delayed resetting of the circadian clock. Chronic jet-lag protocols, which model long-term shift work, promote tumor development in mice by disrupting the circadian expression of major CGs (eg, period 2 [*Per2*]), activating oncogene expression, and inhibiting DNA repair signaling pathways [13]. Models of chronic jet lag also accelerate liver carcinogenesis by eliminating the rhythmic expression of *Per2* and other tumor suppressor genes [14,15]. Moreover, *in vivo* and *in vitro* studies have demonstrated that knocking out or mutating the *Per2* gene increases the incidence and accelerates the growth of spontaneous and chemical-induced tumors [1,2], suggesting that chronic disruption of normal circadian rhythm also promotes carcinogenesis. Our previous studies demonstrated that disruption of CG expression (eg, *Per2*) by a single carcinogenic dose of mammary tumor-specific carcinogen, *N*-nitroso-*N*-methylurea (NMU), ablated the rhythmic expression of several key DNA damage responsive and repair genes in rat mammary glands. The rhythmic expression of *Per2* and most DNA response and repair genes was also disrupted in the mammary glands of rats exposed to a weeklong, 12-h-advanced jet-lag protocol (unpublished data). A chemopreventive regimen of dietary *L*-methylselenocysteine (MSC), which reset the rhythms and restored the expression levels of CGs and CCGs, especially *Per2* and estrogen receptor β (*ERβ*, *Ers2*), inhibited rat mammary carcinogenesis [8,16]. These results suggested that the circadian expression of *Per2* plays a pivotal role in mammary carcinogenesis and its prevention.

Light exposure at night and sleep deprivation influence circulating levels of several hormones including melatonin (a hormone with direct oncostatic properties), cortisols, and reproductive hormones to increase the risk of breast cancer [2,17,18]. Among shift workers, melatonin secretion was strongly influenced by the number of night shifts, specific work schedule, and light intensity exposure during the study period [19–22]. Chronic reduction in melatonin levels among night-shift workers may be an important carcinogenic mechanism.

Given that up to 30% of the workforce is employed in occupations that require chronic rotation into work shifts resulting in exposure to light at night (hospital staff, flight crews, janitorial staff, firefighters, police, soldiers, factory workers, etc.), the health implications of circadian disruption may be significant [23,24]. In addition, exposure to increasing amount of light due to light pollution, television and computer monitors, smartphones, and other electronics continue to extend the daily period of light exposure [25]. Therefore, strategies that mitigate the effects of light at night on sleep patterns and disruption of circadian rhythm have the potential to significantly improve public health. Given the individual variation in phase and susceptibility to circadian disruption, finding molecular biomarkers for circadian disruption is essential to developing intervention strategies for preventing the adverse effect of chronic work schedule changes. The aim of the present study was to determine whether expression changes in CGs and CCGs in peripheral blood samples can serve as biomarkers for assessing the peripheral circadian rhythm in shift workers.

2. Materials and methods

2.1. Study design and subject recruitment

The study design was approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey (now Rutgers, the State University of New Jersey). Briefly, the goal was to recruit 15 hospital interns and residents to a crossover biomarker study. Blood samples were drawn before (6 PM) and after (8 AM) completing a night shift after seven days on a floating night-shift rotation, and before (8 AM), during (1 PM), and after (6 PM) completing seven days on the day shift. The plasma melatonin level and mRNA expression levels of *PER2*, *NR1D1*, and *ERS2* were measured in the peripheral whole blood samples.

Study volunteers were recruited among the residents and interns of the Departments of Family Medicine and Pediatrics at the Robert Wood Johnson University Hospital after consultation with the residency program director and the chief resident. Volunteers were recruited primarily by using flyers and communicating with the chief resident.

2.1.1. Subject selection criteria

When a potential subject contacted the recruiting office, the recruiter briefly screened the subject over the phone using a questionnaire to verify that they were healthy and eligible to participate in the research study. If no exclusions were present, an initial appointment was scheduled in the morning at the end of the night shift. All appointments in the Clinical Research Center (CRC) of Robert Wood Johnson Medical School were held in the Robert Wood Johnson University Hospital. Informed consent was obtained from each subject at this time. The first round of study blood samples was collected, with a fasting ChemScreen and complete blood count (CBC), to verify the absence of significant abnormalities.

2.1.2. Inclusion criteria

Healthy men and women, aged 21–34 years, who regularly work the night shift and who were not taking selenium-containing supplements were eligible for inclusion in the study.

2.1.3. Exclusion criteria

Exclusions beyond the current use of selenium supplements included pregnancy or breast-feeding, major cardiovascular conditions, major chronic lung disease, and current or past cancer therapy. Common medical conditions and their treatments such as diabetes, asthma, obesity, and use of medications, including hormonal contraceptives, were recorded but not noted for exclusion in this working population. Subjects with significant anemia or liver function test abnormalities were excluded. Subjects received the results of the laboratory tests to share with their personal physician.

2.2. Sleep logs

To enhance compliance with completing the daily sleep log, subjects received a weekly phone call or e-mail reminder, with their permission. Each sleep log documents the daily sleep and wake time, and nap and medications taken. The subjects were asked to sleep a total of at least 8 h in bed, that is, 10 PM to 6 AM for the day shift and 10 AM to 6 PM for the night shift. Fig. 1 is a representative example of a completed standard sleep log.