Effects of Sleep Disturbance on the Inducibility of Atrial Fibrillation in Rats

Dae Wui Yoon, MS1*, Hong Euy Lim, MD, PhD2*, Seung Gwan Lee, PhD3, Se Joong Kim, MD, PhD4, Je Hyeong Kim, MD, PhD4, Seung Hoon Lee, MD, PhD6, Chang-Ho Yun, MD, PhD7, Chol Shin, MD, PhD, FCCP1,4,5

1Institute of Human Genomic Study, Korea University College of Medicine, Ansan, 2Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Korea University Ansan Hospital, 5Sleep Disorder Center, Department of Pulmonary and Critical Care Medicine, Ansan Hospital, Korea University College of Medicine, Ansan, 3Department of Otorhinolaryngology-Head and Neck Surgery, Ansan Hospital, Korea University College of Medicine, Ansan, 4Department of Neurology, Inha University College of Medicine, Incheon, Korea

Background and Objective The association of sleep disturbance (SD) with cardiovascular disease is through its induction of autonomic dysregulation and inflammation, which also could be important pathophysiological mechanisms responsible for atrial fibrillation (AF). However, no study has fully elucidated the causal relationship between SD and AF. We investigated whether SD influences AF vulnerability in an in vivo rat model.

Methods We divided 8-week-old Wister rats into 5 groups: 1) control, 2) 3-day SD, 3) 7-day SD, 4) 3-day sham SD, and 5) 7-day sham SD (n = 5 in each group), using the multiple platform method to create SD. We measured baseline heart rate, blood pressure (BP), and AF inducibility via burst atrial pacing (30 mA, 40 Hz, 30 seconds) by means of a transoesophageal electrode and used Sirius red staining to assess collagen deposition in the atriums.

Results Baseline heart rate, diastolic BP and AF inducibility were significantly higher in the SD groups than in the control and sham condition groups (p < 0.05). However, there were no differences between the 3- and 7-day SD groups. Systolic BP and induced AF duration did not statistically differ among any of the experimental groups. The Sirius red staining showed no differences in collagen deposition among any of the experimental groups.

Conclusions SD resulted in increase of AF inducibility in SD rats, suggesting that SD provides an atrial substrate for AF vulnerability, regardless of the disturbance period. However, induced AF duration showed no significant differences in SD rats.

Key Words Atrial Fibrillation, Transesophageal pacing, Sleep disturbance.

INTRODUCTION

Obstructive sleep apnea (OSA) is a common disorder, affecting more than 4% of males and 2% of females in the adult population1 and is a major cause of sleep disturbances (SD). It is defined as the occurrence of repetitive episodes of complete or partial pharyngeal obstruction during sleep.2 Physiologically, such episodes are often associated with increasing respiratory efforts, arousals, sleep fragmentation, intermittent hypoxemia, and hypercapnia.3

It is well known that OSA is strongly associated with a variety of cardiovascular diseases, including hypertension, coronary artery disease, stroke, heart failure, and arrhythmias.4,5 Atrial fibrillation (AF) is the cardiac arrhythmia that clinical practices encounter most frequently, and it derives from a variety of predisposing factors, such as old age, hypertension, diabetes mellitus, thyroid disease, and structural heart disease.6 A growing body of evidence has recently appeared indicating that OSA may be a new risk factor for AF. Previous epidemiologic or cross-sectional data demonstrated that AF associates with OSA, and, conversely, that OSA patients have
Sleep Disturbance and Atrial Fibrillation in Rats

It has been proposed that characteristics shown in OSA patients, such as hypoxemia and frequent sleep interruption, are implicated in the cardiovascular diseases associated with OSA, including AF. However, determining whether a single factor on an interaction of multiple factors causes OSA’s effects on AF is impossible, in humans. Although a series of epidemiological and experimental studies reported a strong association between non-apneic SDs and cardiovascular diseases, to our knowledge only one study has reported that an acute sleep restriction, of less than the half the daily regular sleep time, significantly increased simple electrocardiographic markers (maximum P-wave duration (Pmax) and P-wave dispersion (Pd)), compared to baseline values. This suggests that non-apneic SD might contribute to AF’s development and/or recurrences. Thus, we primarily focused on one of OSA’s characteristics, sleep fragmentation, to determine OSAs effects on AF. Our animal model of sleep fragmentation, which employs platforms, was originally used to induce selective REM sleep deprivation. However, it also predominantly affects the REM stage by generating numerous awakenings, and could mimic the SDs shown in OSA patients. Such a model would be an effective for examining SDs’ effects on AF. To avoid confusing the terms, we refer to sleep fragmentation or deprivation as SD.

The study aimed, therefore, to investigate SDs’ effects on inducibility and duration of AF in this in vivo model.

METHODS

Animals

Eight-week old male Wistar rats (200-300 g) were housed in a colony maintained at 22 ± 2°C with a 12:12-hour light-dark cycle (lights on at 7 AM). The animals had free access to water and food inside standard polypropylene cages. The rats were randomly divided into the 5 groups: 1) control, 2) 3-day SD, 3) 7-day SD, 4) sham 3-day SD, and 5) sham 7-day SD (n = 5 in each group). We maintained both sham groups in a cage containing large platforms (14 cm in diameter) and sacrificed them on the same day as the 3- and 7-day SD rats. All rats were handled in accordance with the guidelines of the Animal Research Committee of Korea University and with the approval of the Ethics Committee of Korea University Medical Center.

Blood Pressure Measurement

After housing the rats for 2 weeks under consistent conditions, we non-invasively measured the baseline heart rate and blood pressure (BP), using a tail-cuff system warmed to 37°C (BP-2000 series II, BP Analysis System, Visitech Systems, Apex, N.C., USA). Rats were habituated to the BP measurement device for 5 days before BP recordings were begun. For each rat, we obtained at least 1 set of 10 measurements, with at least 9 or more successful readings.

Sleep Disturbance

To produce SDs, we employed a modified multiple-platform method as previously described. Briefly, experimental rats were placed in custom-made acryl cages (123 × 44 × 44 cm), each containing 14 platforms. These were either 6.5 cm (experimental) or 14 cm (sham) diameter platforms. The tanks were filled with water to about 1 cm below the platforms’ surfaces. When awake, the rats could move around by leaping from one platform to another, but when the rats entered REM sleep, the loss of muscle activity caused them to contact the water, prohibiting further sleep. This technique not only eliminates REM sleep, but also fragments slow wave sleep. To accustom the rats to the novel environment, we placed them on the platforms for 1 hr/day, for 3 consecutive days, before proceeding with the SD conditions.

AF Inducibility Test

For the transesophageal atrial burst pacing, anesthesia was induced with 5% isoflurane and maintained with 1.5-2% isoflurane during the entire AF inducibility test. A polygraph system (MacLab, GE, Horton, USA) continuously recorded the surface limb-leads electrocardiogram (ECG), with filtering between 0.1-100 Hz, and a real-time, fully automatic data analysis system (Pruka, GE, Horton, USA) analyzed the data. A clinically available 6-French decapolar electrodes catheter (St. Jude Medical, USA), whose diameter is similar to that of the gastric tubes for rats, with an interelectrode distance of 2 mm, was inserted into the esophagus under the monitoring of a fluoroscopy and an esophageal ECG. Rectangular pulses (pulse width 2 ms, ten times of the diastolic threshold, range from 30 mA to 40 mA) were delivered via 2 poles with the lowest pacing threshold. Burst atrial pacing at rates of 40 Hz for 30 seconds was performed to induce AF using an electrical stimulator (Grass S-88, Quincy, MA, USA). AF was induced three times with at least a 10-minute interval, and its duration was measured. Fig. 1A and 1B show the schematic study protocol and typical ECG tracings upon spontaneous restoration of the sinus rhythm after AF, respectively.

Sirius Red Stain

After completing the AF inducibility test on each rat, heart was rapidly removed and the atriums were isolated from the heart. To assess collagen deposition in the atriums, we performed Sirius red staining, as previously described. After photographing the stained slides with a digital camera against 5 fields at ×100 magnification (Micropublisher 3.3 RTV, QIMAGING, Surrey, British Columbia, Canada), we measured the degree of fibrosis via a digital image analysis system (Imaga-Pro Plus, MediaCybenetics, Bethesda, Maryland, USA). Briefly, we calibrated the baseline optical balances of all photographs and determined the red and yellow values via a positive control slide. We expressed the result by multiplying the stained area (pixel) by the pixel intensity, but in units of gigapixels (G, ×109) × pixel intensity. An investigator blinded to the slides’ group allocations performed all image anal-
Analysis processes.

**Statistical Analysis**

All the data are expressed as means ± SD. Nonparametric Kruskal-Wallis method was used to assess the inter-group differences. Post hoc analysis was performed with the Mann-Whitney’s U-test. All statistical analyses were conducted using SPSS statistical software, version 13.0 (SPSS, Inc., Chicago, IL, USA). A p value of < 0.05 was taken to be statistically significant.

**RESULTS**

**Heart Rate and Blood Pressure**

Table 1 shows the baseline and follow-up heart rate (HR) and systolic and diastolic BP. Baseline measurements did not differ among the groups. At the end of experimental period, heart rate and diastolic BP were significantly higher in both the 3- and 7-day SD groups than in the control and sham groups (p < 0.05), but sys-

---

**Table 1. Heart rate and blood pressure in control, sham, and sleep disturbance groups**

<table>
<thead>
<tr>
<th>Hemodynamic variables</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>331.0 ± 29.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>141.0 ± 7.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>105.0 ± 18.3</td>
</tr>
</tbody>
</table>

* † p < 0.05 vs. control or sham, * p < 0.01 vs. control or sham. SD: sleep disturbance.
tolic BP did not differ statistically. The control and sham groups showed no significant changes in HR and systolic and diastolic BP as compared to their baseline measurements. HR and BP measurements showed no significant differences between 3-day SD and 7-day SD groups.

**AF Inducibility Test**

AF was induced in 40%, 53.4%, and 60% of the control, 3-day sham and 7-day sham groups, respectively. There were no statistical differences among these groups. However, AF was induced in 86.7% of the 3-day SD and 80% of the 7-day SD groups. These were significantly higher than rates in the control and sham groups were (p < 0.05)(Fig. 2A). Mean duration of induced AF episodes was 32.3 ± 28.1, 33.5 ± 31.7, 48.6 ± 43.4, and 31.5 ± 32.3 seconds in the control, 3-day sham and 3-day SD, 7-day sham and 7-day SD groups, respectively. However, these showed no significant differences among them (Fig. 2B).

**Sirius Red Stain**

Values for the stained fibrotic areas were 11.6 ± 3.6, 10.2 ± 3.5, 11.1 ± 6.4, 9.8 ± 4.4, and 10.6 ± 5.1 G pixels × pixel intensity in the control, 3-day sham, 3-day SD, 7-day sham, and 7-day SD groups, respectively. Collagen deposition showed no significant differences between 3- and 7-day SD groups as compared to the control and sham groups.

**DISCUSSION**

Our study is the first in vivo animal study to provide evidence that SD may be participate in the pathogenesis of AF. In the present study, we observed increased HR, diastolic BP, and AF inducibility in SD rats as compared to the control and sham groups. However, induced AF duration showed no significant differences among the groups.

The relationship between SD and increased AF inducibility may involve several mechanisms. First, alterations of autonomic nervous system have been implicated in initiation. It has been known that sympathetic stimulation acts as a trigger which is responsible for onset of AF. Several human studies have been demonstrated to show that SD leads to increased sympathetic and decreased parasympathetic modulation, as assessed by spectral analysis of heart rate variability and BP variability, and increased level of circulating epinephrine and norepinephrine with attendant increases of BP and HR, and resulted in a shift of sympathovagal balance toward sympathetic dominance. Consequently, these findings reflect increased sympathetic nervous system activity in SD subjects. In another study, which examined the effect of 24-hour total sleep deprivation on muscle sympathetic nerve activity (MSNA) in healthy men by measuring burst rate and burst incidence, total SD elevated BP through arterial baroreflex resetting. Moreover, insufficient sleep significantly increased BP and HR, as monitored by a portable multi-biomedical recorder, in male technical workers. Another study found similar results with 24-h BP monitoring of hypertensive subjects. Chronic SD also decreased heart rate variability indices and erythrocyte magnesium levels, while it increased norepinephrine levels, in healthy males. Additionally, previous animal studies demonstrated that heterogeneous changes in atrial sympathetic innervations (neural remodeling), which is characterized by increased sympathetic nerve sprouting and hyperinnervation in the atrium, provide a substrate for AF. Taken together, these results indicate that either short-term or long-term SD may cause autonomic imbalances and contribute to AF development.

Second, SD may increase AF inducibility by increasing the inflammatory response, a well-known mediating factor in AF initiation and/or perpetuation. In one study of healthy human adults, SD led to increased levels of peripherally-circulating leukocytes, interleukin 6, and high-sensitivity C-reactive protein, suggesting that SD may contribute to inflammation. An animal experiment also revealed that paradoxical SD in rats induced an inflammatory response with increases in serum pro-inflammatory cytokines. The exact pathophysiological mechanism(s) whereby inflammation generates AF is unknown, but researchers have suggested that inflammation leads to "atrial myocarditis," with subsequent electrical and structural changes (i.e., "remodeling processes"), which consequently leads to AF initiation and maintenance.
The stress response elicited by sleep disruption has long been considered a confounding factor in studies attempting to isolate the specific role of sleep loss itself. Indeed, SD by the platform method has been known as a stressful procedure to experimental animals and, thus, elevates corticosterone level and sympathetic nervous system activity. Therefore, it is possible to think that increased inducibility of AF in SD rats is not due to the effect of SD per se but merely stress effect from SD procedure, since the stress responses has a negative impact on the cardiovascular system. However, even other, less stressful SD methods, such as gentle handling and disk-over-water methods also induce some kind of stress responses as well. Thus, perfectly determining the effect of SD itself is not possible. Considering such a stress response as a critical component of SD's physiological state, rather than regarding it as an unwanted experimental confound, (as McEwen proposed to explain sleep loss's deleterious effects via the biological model of allostatic and allostatic load33) might be more reasonable.

In the present study, we found no significant differences in the induced AF duration, not only in the 3- and 7-day SD groups, but also in the control and sham groups. Such results could be explained by a critical mass theory, in which the rats' atrial masses might not have been enough to sustain AF. Maintenance of AF is also influenced by heterogeneous conduction properties of tissue, which is usually affected by structural remodeling.6,34 To determine whether structural remodeling has occurred in the atrium of SD rats, we conducted Sirius red staining and examined the fibrosis extent in the atriums. However, fibrotic content did not differ significantly among any of the experimental groups. This result might also explain why induced AF duration did not differ among the groups in our study.

In conclusion, we found that SD increases AF inducibility in rats, suggesting that SD provides an atrial substrate for AF vulnerability in rats, regardless of the disturbance period. However, induced AF duration did not differ significantly among the SD rats. As previously mentioned, these results may be due to the experimental rats' low atrial masses. Further studies are required to clarify the exact underlying pathophysiologic mechanisms of SD that leads to AF genesis.

Acknowledgments
Grateful thanks to Ms. Hyeon Kim for her linguistic revision of the paper. This study was performed at the Korea University Hospital, Ansan, South Korea.

Conflicts of Interest
The authors have no financial conflicts of interest.

REFERENCES
24. Ogawa Y, Kanbayashi T, Saito Y, Takahashi Y, Kitajima T, Takahashi K, et al. Total sleep deprivation elevates blood pressure through arterial baro-


