Sleep Science 9 (2016) 232–235

HOSTED BY

Contents lists available at ScienceDirect

Sleep Science

journal homepage: www.elsevier.com/locate/ssci

Full length article

Sleep pattern and locomotor activity are impaired by doxorubicin in non-tumor-bearing rats☆

Fabio Santos Lira[a], Andrea Maculano Esteves[b,*], Gustavo Duarte Pimentel[c], José Cesar Rosa[d], Miriam Kannebley Frank[b], Melise Oliveira Mariano[e], Josiane Budni[f], João Quevedo[f], Ronaldo Vagner dos Santos[g], Marco Túlio de Mello[h]

[a] *Exercise and Immunometabolism Research Group, Department of Physical Education, Universidade Estadual Paulista (UNESP), Presidente Prudente, SP, Brazil*
[b] *Faculdade de Ciências Aplicadas, Universidade Estadual de Campinas, Limeira, Brazil*
[c] *Laboratory of Research in Clinical Nutrition and Sports (Labince), Nutrition Faculty (FANUT), Federal University of Goias (UFG), Goiânia, GO, Brazil*
[d] *Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil*
[e] *Departamento de Psicobiologia, Universidade Federal de São Paulo, Brazil*
[f] *Laboratory of Neurosciences, National Institute for Translational Medicine, and Center of Excellence in Applied Neurosciences of Santa Catarina, Postgraduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina, 88806-000 Criciúma, SC, Brazil*
[g] *Departamento de Biociências, Universidade Federal de São Paulo, Santos, Brazil*
[h] *Departamento de Esportes, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

ARTICLE INFO

Keywords:
Sleep
Locomotor activity
Doxorubicin
Rats

ABSTRACT

Purpose: We sought explore the effects of doxorubicin on sleep patterns and locomotor activity. To investigate these effects, two groups were formed: a control group and a Doxorubicin (DOXO) group.
Methods: Sixteen rats were randomly assigned to either the control or DOXO groups. The sleep patterns were examined by polysomnographic recording and locomotor activity was evaluated in an open-field test.
Results: In the light period, the total sleep time and slow wave sleep were decreased, while the wake after sleep onset and arousal were increased in the DOXO group compared with the control group ($p < 0.05$). In the dark period, the total sleep time, arousal, and slow wave sleep were increased, while the wake after sleep onset was decreased in the DOXO group compared with the control group ($p < 0.05$). Moreover, DOXO induced a decrease of crossing and rearing numbers when compared control group ($p < 0.05$).
Conclusions: Therefore, our results suggest that doxorubicin induces sleep pattern impairments and reduction of locomotor activity.

1. Introduction

Although sleep is essential for good health and quality of life, according to Bonnet and Arand [1], one-third or more of normal adults suffer from significant sleep loss. In addition, several studies have shown that cancer chemotherapy treatment with doxorubicin alters sleep patterns and health status, leading to distressing symptoms and fatigue [2–4].

Doxorubicin (DOXO), a member of the antineoplastic antibiotic family of anthracyclines, is a chemotherapeutic agent developed in the 1960s [5], that is still widely used in the treatment of a variety of malignancies, such as acute leukemia, non-Hodgkin lymphomas, breast cancer, Hodgkin's disease, and sarcomas [6,7].

Savard et al. [4] showed that breast cancer patients treated with doxorubicin have impaired sleep-wake activity rhythms. Moreover, the first administration of chemotherapy is associated with a disruption of the sleep-wake rhythm, and the repeated administration of this chemotherapy results in more enduring impairments of the sleep-wake rhythms. Moreover, the DOXO treatment in breast cancer women is associated with disturbance sleep, sleep efficiency and poor sleep quality [8].

Neural systems implicated in the control of sleep also impact the functioning of host defenses. The challenge for future research is to determine the ultimate implication of the sleep loss effects in molecular terms to clarify the mechanistic processes involved in the impairment of cellular functional activity, and the impact of sleep deprivation on

Peer review under responsibility of Brazilian Association of Sleep.
* Correspondence to: Faculdade de Ciências Aplicadas, Universidade de Campinas, Rua Pedro Zaccaria, 1300 - Jardim Santa Luiza, Limeira, São Paulo CEP 13484350, Brazil.
E-mail address: andrea.esteves@fca.unicamp.br (A.M. Esteves).

http://dx.doi.org/10.1016/j.slsci.2016.10.006
Received 16 May 2016; Received in revised form 20 October 2016; Accepted 27 October 2016
Available online 05 November 2016

other essential inflammatory markers for immune function [9]. On the other hand, other studies have reported that chemotherapy may promote and/or aggravate inflammation status which impairs sleep quality [10,11].

Therefore, in this study, we sought to determine effects of DOXO on the sleep pattern and locomotor activity in the rats.

2. Methods

2.1. Animals

The Experimental Research Committee of the Universidade Federal de São Paulo approved all procedures for the care of the animals used in this study (0619/09). A total of 16 male Wistar rats approximately 14 weeks of age (weighing 350–380 g) were used. They were housed four per cage in an animal room under a 12-h light-dark cycle at 22 ± 1 °C and 60 ± 5% humidity and received a chow diet and water ad libitum. The experiments were carried out after a one-week acclimation period. The rats were randomly divided into two groups: (i) a saline Control group (n=8) and (ii) a doxorubicin (DOXO) group (n=8).

2.2. Design

In the first day the animal were subjected to electrode insertion surgery. Seven days after surgery (ninth day), the animal received doxorubicin cloridrato (Eurofarma Laboratory, Campinas, Brazil) (15 mg/kg, i.p.) [12] or saline (i.p.). The sleep recording was conducted for 24 h (12-h light-dark) after 48 h doxorubicin-treatment (a single administration) and the locomotor activity was evaluated 48 h after doxorubicin-treatment in the open-field test. The doxorubicin and saline were administered at 7:00 AM.

2.3. Experimental Protocols

2.3.1. Surgical preparation

The rats were anesthetized with diazepam and ketamine (5 and 100 mg/kg body weight, i.p., respectively). They were then placed in the stereotaxic apparatus, and two bipolar electrodes with 4 stainless-steel screws (Ø 0.9 mm) were placed into the skull through small holes bored into the right lateral frontoparietal region (1 pair of screws) and the left medial frontoparietal region (another pair) in order to monitor the bipolar electrocorticogram (ECoG) [13].

For the electromyography recording (EMG), one pair of electrodes was inserted in the cervical musculature. After the electrode insertion surgery, the rats were placed in individual compartments for 7 days of recovery and then given 2 days of adaptation while connected to the polysomnographic recording (PSG) device.

2.3.2. Electrocorticography recording

During the Electrocorticography (ECoG) recording, the rats remained in individual compartments with unrestricted access to food and water. The ECoG recording was made with a Nihon-Kohden model QP 223 polygraph (digital signal acquisition) using three pairs of channels: two ECoG and one EMG for the cervical musculature. The recording was analyzed for two 12 h periods (12-h light–dark). In the literature, rats were shown to have 62% sleep efficiency during the light period (7:00–19:00 h) and 33% during the dark period (19:00–7:00 h) [14]. Each 10 s period was classified in accordance with Timo-Iaria et al. [15]: wakefulness (W) was defined as low amplitude waves with fast ECoG and EMG activation; slow wave sleep (SWS) was defined as high amplitude waves and slow ECoG and EMG activation; and paradoxical sleep was defined (PS) as fast ECoG activity, the regular presence of a theta hippocampal rhythm and the absence of EMG activity. At the end of the analysis, the sleep parameters were quantified using the Polysmith Software program®.

The sleep parameters collected were the following: sleep efficiency (SE; percentage of total sleep time during the recording period), latency to sleep (time lag between the start of the recording and the first sleep period), slow wave sleep (SWS; percentage of all periods featuring high delta content during the recording period), paradoxical sleep (PS; percentage of all periods during the recording period), PS latency (time lag between the start of the recording and the first PS period), wake after sleep onset (WASO; percentage of all periods of wakefulness throughout the recording period number of awakenings) and number of arousals (number of awakenings).

2.3.3. Open-field test

The rats were treated with doxorubicin (15 mg/kg), and saline 48 h before the exposure to the open-field apparatus (light phase), in order to assess the possible effects of drug treatment on spontaneous locomotor activity. Analysis of the rat's spontaneous activity was carried out in an open field apparatus, which is a 45 cm×60 cm arena surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 rectangles (15 cm×20 cm each) by black lines [16]. Animals were gently placed on the left rear quadrant, and left to explore the arena for 5 min. The number of horizontal (crossings) and vertical (rearings) activities performed by each rat during the 5 min observation period was counted by an expert observer.

2.4. Statistical analysis

The statistical analysis was performed using the GraphPad Prism statistics software package version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). The data are expressed as the mean ± SEM. Implementation of the Kolmogorov-Smirnov test revealed that the results of the experiments were distributed normally. The data were analyzed using two-way ANOVA followed by the Tukey test and unpaired Student's *t*-test for comparison between the two groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Doxorubicin administration impairs the sleep pattern

A significant increase was detected on the wakefulness of the DOXO group in light period in relation to control group. Statistical differences were found in both the light/dark period in the DOXO and control groups for wakefulness. In addition, the Sleep Efficiency of the DOXO group demonstrated a significant decrease in relation to the control group in the light period group; no statistical differences were found in either the light/dark period in the DOXO group to Sleep Efficiency. Fig. 1.

The sleep parameter data are shown in Table 1. The two-way ANOVA revealed the main effects of the group (arousal), time (TST,

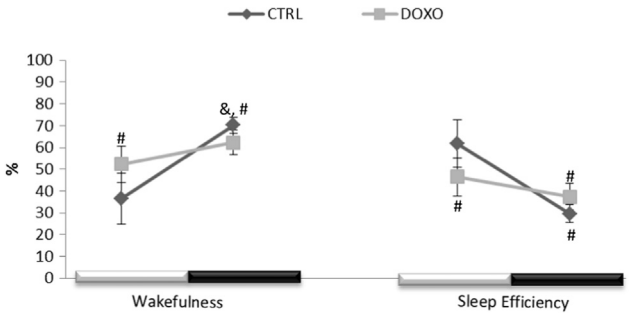

Fig. 1. Wakefulness (%) and Sleep Efficiency (%) during light and dark periods of sleep in both Control (CTRL) and Doxorubicin (DOXO) groups. Two-way ANOVA followed by the Tukey post hoc test ($p < 0.05$) comparison of groups for the time factor (# differ Light Control; & differ Light DOXO). The bars mark of periods light (left) and dark (right) of sleep. Animals: CTRL (n=8), DOXO (n=8). Dose: 15 mg/kg, (i.p.) – DOXO or saline.