Brain Research 1644 (2016) 213–221

Contents lists available at ScienceDirect

Brain Research

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

Research report

Sleep restriction reduces the survival time and aggravates the neurological dysfunction and memory impairments in an animal model of cerebral hypoperfusion

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ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form
2 May 2016
Accepted 5 May 2016
Available online 9 May 2016

Keywords:
Sleep restriction
Cerebral hypoperfusion
Neurological function
Memory
Mortality rate

ABSTRACT

Cerebral blood flow is associated with the cerebrovascular prognosis. Sleep restriction (SR) may be a limiting factor of the prognosis after a cerebrovascular event, impairing the neurological recovery. We aimed to investigate the effects of SR on mortality rate and on behavioral and histological parameters of animals submitted to permanent cerebral hypoperfusion. Sixty male Wistar rats were distributed in 4 groups, according to the protocol of common carotid artery occlusion (CCAO) and SR: nSR+nCCAO, SR+nCCAO, nSR+CCAO, and SR+CCAO. The groups SR+nCCAO and SR+CCAO were submitted to SR during 10 days. The cerebral hypoperfusion was induced by the permanent CCAO. Neurological function and memory were assessed over 14 days of cerebral hypoperfusion. Analysis of neuropathological alterations were performed in the CA1 region of hippocampus. The mortality rate was 40% in the nSR+CCAO and SR+CCAO groups. SR significantly reduced the survival time of animals submitted to CCAO. After 7 and 14 days of cerebral hypoperfusion, 11% and 33% of the nSR+CCAO and SR+CCAO animals showed severe neurological dysfunction, respectively. A significant association between a high frequency of memory impairments with the group SR+CCAO was observed. The neuropathological alterations in CA1 region of hippocampus were similar among the groups. SR potentiates the negative effects of cerebral hypoperfusion conditions, suggesting that SR could be a factor associated with a worse prognosis after a cerebrovascular event.

1. Introduction

Sleep restriction (SR) is a condition frequently associated with several metabolic alterations. The reduction in total sleep time increases the arterial blood pressure, induces insulin resistance, and is related with weight gain and obesity (Grandner et al., 2014; Jackson et al., 2013). Consequently, SR is an important risk factor for cerebrovascular diseases, such as stroke (Eguchi et al., 2008).

Stroke is the third-leading cause of premature death and one of the most disabling disease in the general population (Murray et al., 2013), leading to several functional and cognitive sequelae (de Haan et al., 2006). A better prognosis for stroke patients is directly associated with the recovery of functional independence and cognitive function during the acute and chronic phases of disease. In animal models of stroke, it was also observed that histological alterations, comprising a higher infarct volume and brain swelling, were related with impairments in motor performance (Rogers et al., 1997). Different factors influence the short- and long-term prognosis of patients after a stroke, including the blood pressure during the acute phase of stroke (Castillo et al., 2004). Heiss et al. (1977) demonstrated that decreased cerebral blood flow after ischemic stroke is associated with poor neurologic deficits and, consequently, with a worst recovery of ischemic lesions. Acute SR can alter the cerebral blood flow. In the study of Poudel et al. (2012) sleep restricted patients showed a significant reduction in the right-lateralized fronto-parietal cerebral blood flow of attentional network. These findings could suggest that SR besides increasing the risk of cerebrovascular outcomes might also be a limiting factor of stroke prognosis, leading to impairments in the recovery of cognitive and neurological function of the patients. In this sense, we hypothesized that a sleep loss prior to an event of brain ischemia could promote changes in neuroprotective

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http://dx.doi.org/10.1016/j.brainres.2016.05.010
0006-8993/

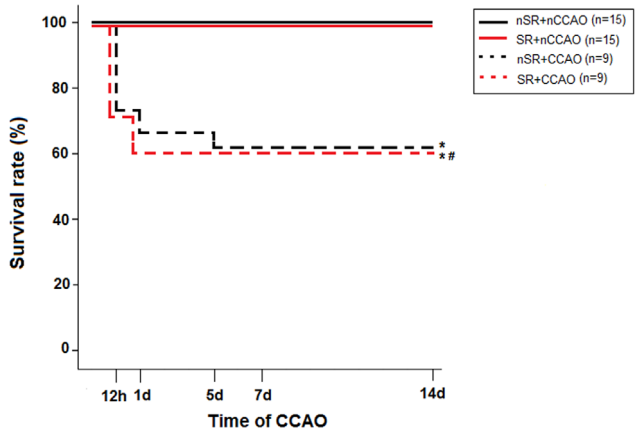

Fig. 1. Kaplan-Meier analysis with the survival rate of the animals after 12 h, 24 h, 5 days, 7 days, and 14 days of permanent CCAO. *nSR+CCAO and SR+CCAO groups showed an increased mortality rate compared to sham surgery animals ($P<0.01$). #SR+CCAO group had a lower survival time compared to nSR+CCAO ($P<0.01$). h: hours; d: days; nSR+nCCAO: non-sleep restricted+sham-surgery; SR+nCCAO: sleep restricted+sham-surgery; nSR+CCAO: non-sleep restricted+cerebral hypoperfusion; SR+CCAO: sleep restricted+cerebral hypoperfusion; CCAO: common carotid artery occlusion.

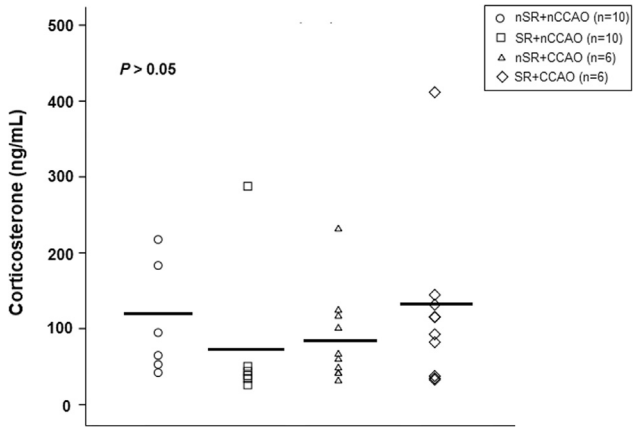

Fig. 2. Plasmatic levels of corticosterone among the groups (ng/mL). nSR+nCCAO: non-sleep restricted+sham-surgery; SR+nCCAO: sleep restricted+sham-surgery; nSR+CCAO: non-sleep restricted+cerebral hypoperfusion; SR+CCAO: sleep restricted+cerebral hypoperfusion; Black line: mean value of corticosterone.

mechanisms in central nervous system, leading to a higher long-term susceptibility to the chronic ischemic lesions.

Animal models of cerebral hypoperfusion have been increasingly used to mimic the clinical effects of reduced cerebral blood flow or permanent brain ischemia, as observed in conditions of vascular dementia (Farkas and Luiten, 2001). The use of animal models could be an important approach to elucidate the consequences of SR during the early phase of decreased cerebral blood flow. Thus, the present study aimed to investigate the effect of a prior exposition to SR on the mortality rate, neurological function, and short- and long-term memory of animals submitted to a model of permanent cerebral hypoperfusion. We also analyzed the neuropathological alterations in the CA1 region of the hippocampus.

2. Results

2.1. Mortality rate

In the 2 sham-surgery groups, the survival rate of the animals was 100%. In the comparison between the 2 groups subjected to the cerebral hypoperfusion model, no significant effect of SR was observed on the mortality rate. Both nSR+CCAO and SR+CCAO groups showed a mortality rate of 40% during the 14 days of permanent CCAO (Fig. 1), differing significantly from the sham-surgery animals ($X^2=14.9$; gl=3; $P<0.01$). The survival time of the animals was significantly lower in the SR+CCAO group compared with nSR+CCAO ($P<0.01$). The SR+CCAO group showed a mortality of 26.7% during the first 12 h post-surgery and 40% after 24 h. On the other hand, in the nSR+CCAO group, the survival time was up to 5 days. (Fig. 1).

2.2. Corticosterone levels

No significant effect of SR and CCAO were observed in the plasmatic levels of corticosterone. The levels of corticosterone were similar among the 4 experimental groups (Fig. 2).

2.3. Neurological function

All animals showed a normal neurological function during baseline and after 10 days of SR and 24 h of CCAO (Fig. 3(A)–(C)), indicating no isolated and acute effect of SR and cerebral hypoperfusion, respectively, on the neurological function of the animals. After 7 days of cerebral hypoperfusion, 33% of the SR+CCAO and 11% of the nSR+CCAO animals presented a lack of resistance to the dorsal push, indicating severe neurological dysfunction (Fig. 3(D)). The same proportion of animals with neurological function impairments was observed after 14 days of brain ischemia (Fig. 3(E)). In the comparison among the groups, it was observed a significant association between higher frequencies of neurological dysfunction and the group SR+CCAO ($X^2=10.2$; gl=3; $P<0.05$).

2.4. Short- and long-term memory

Five rats from nSR+nCCAO group, 6 from SR+nCCAO, 1 from nSR+CCAO, and 2 from SR+CCAO were excluded from the analysis for remaining >20 sec with locomotor inactivity or without objects exploration. These frequencies were not statistically significant ($X^2=2.6$; gl=3; $P>0.05$). No significant effect of locomotor activity was observed on the performance of the animals in the novel object recognition test (Fig. 4). The number of rearing behavior, total and central locomotion, and also the ratio central/total locomotion were similar among the groups in the 2 tests.

During both Test 1 and Test 2, a significant effect of the cerebral hypoperfusion induction was observed on the discrimination (Test 1 – $F_{3,30}=41.0$; $P<0.001$; Test 2 – $F_{3,30}=25.2$; $P<0.001$) and recognition indexes (Test 1 – $F_{3,30}=41.0$; $P<0.001$; Test 2 – $F_{3,30}=25.2$; $P<0.001$) (Fig. 5(A) and (B)). nSR+CCAO and SR+CCAO groups spent more time exploring the familiar object compared with the sham-surgery groups. No significant effect of SR was observed on the discrimination and recognition indexes.

We stratified the sample according to the normality cut-offs of discrimination and recognition indexes, categorizing as discrimination index ≥ 0 or <0 and recognition index $\geq 50\%$ or $<50\%$. It was observed a significant association of higher frequencies of memory impairments in the group SR+CCAO (Fig. 5(C) and (D)). In the sham-surgery groups, nSR+nCCAO and SR+nCCAO, 100% of the animals showed normal discrimination and recognition indexes values in Test 1 and Test 2. In the nSR+CCAO, 37.5% and 50% of the animals showed negative values of discrimination and recognition index $<50\%$ in Test 1 and Test 2, respectively. The higher proportion of memory impairments was observed in the SR+CCAO group. In Test 1 and Test 2, 86% SR+CCAO animals showed negative discrimination values (Test 1 – $X^2=19.9$; gl=3; $P<0.001$; Test 2 – $X^2=20.2$; gl=3; $P<0.001$) and