Modification of sleep architecture in an animal model of experimental cirrhosis

Anabel Jiménez-Anguiano, Vanessa Díaz-Medina, Blanca Eugenia Farfán-Labonne, Gloria Giono-Chiang, David Kersenobich, Mario García-Lorenzana, Maria Concepción Gutiérrez-Ruiz, Javier Velázquez-Moctezuma

Anabel Jiménez-Anguiano, Vanessa Díaz-Medina, Mario García-Lorenzana, Javier Velázquez-Moctezuma, Area of Neuroscience, Department of Biology of Reproduction, Universidad Autónoma Metropolitana-Iztapalapa, CP 09340, Mexico City, Mexico
Blanca Eugenia Farfán-Labonne, Gloria Giono-Chiang, Maria Concepción Gutiérrez-Ruiz, Cell Physiology Laboratory, Department of Health Sciences, Universidad Autónoma Metropolitana-Iztapalapa, CP 09340, Mexico City, Mexico
David Kersenobich, Unit of Liver, Pancreas and Motility, Faculty of Medicine, Universidad Nacional Autónoma de México, Mexico City, D.F CP 09340, Mexico

Author contributions: Jiménez-Anguiano A, Gutiérrez-Ruiz MC and Velázquez-Moctezuma J contributed equally to this work; Jiménez-Anguiano A, Gutiérrez-Ruiz MC, Velázquez-Moctezuma J, Giono-Chiang G, Kersenobich D and García-Lorenzana M designed the research and analyzed the data; Jiménez-Anguiano A, Díaz-Medina V, Farfán-Labonne BE and Giono-Chiang G performed the research.

Supported by Grant 50633 from CONACyT to Jiménez-Anguiano A

Correspondence to: Dr. Javier Velázquez-Moctezuma, Area of Neuroscience, Department of Biology of Reproduction, Universidad Autónoma Metropolitana-Iztapalapa, CP 09340, Mexico City, Mexico, jvm@xanum.uam.mx

Abstract

AIM: To analyze the polygraphic sleep patterns during cirrhosis progression in a rat model by repeated CCl4 administration.

METHODS: Male Wistar rats received three weekly injections of CCl4 for 11 wk, and were analyzed before and during the induction of cirrhosis. Rats were implanted with electrodes to record their sleep patterns. Polygraph recordings were made weekly over 11 wk for 8 h, during the light period. After a basal recording, rats received three weekly injections of CCl4. Histological confirmation of cirrhosis was performed after 11 wk.

RESULTS: The results showed a progressive decrease in total wake time that reached statistical significance from the second week of treatment. In addition, there was an increase in total time of slow wave sleep (SWS) II and rapid eye movement sleep (REM sleep) in most of the 11 wk. SWS I showed no significant variations. During the final weeks, a significant increase in REM sleep frequency was also observed. Histological analyses of the livers showed unequivocal signs of cirrhosis.

CONCLUSION: These data suggest that hepatic failure produced by CCl4 administration is capable of modifying the sleep pattern even after only a few doses.

Key words: Experimental cirrhosis; Sleep; Rapid eye movement sleep; CCl4; Wakefulness

Peer reviewers: Vasily I Reshetnyak, MD, PhD, Professor, Scientist Secretary of the Scientific Research Institute of General Reanimatology, 25-2, Petrovka str., 107031, Moscow, Russia; Radha K Dhiman, Associate Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Jiménez-Anguiano A, Díaz-Medina V, Farfán-Labonne BE, Giono-Chiang G, Kersenobich D, García-Lorenzana M, Gutiérrez-Ruiz MC, Velázquez-Moctezuma J. Modification of sleep architecture in an animal model of experimental cirrhosis. World J Gastroenterol 2009; 15(41): 5176-5180 Available from: URL: http://www.wjgnet.com/1007-9327/15/5176.asp DOI: http://dx.doi.org/10.3748/wjg.15.5176

INTRODUCTION

The sleep-wakefulness cycle is an important circadian rhythm in mammals and is characterized by a reduction in the level of consciousness and by specific metabolic activities. Sleep is controlled by multiple areas of the brain and by several chemical factors, and can be readily modified by different activities, drugs, and pathological process, such as exercise, stress, alcoholism, depression, and metabolic diseases[1].

Cirrhosis, on the other hand, is an irreversible dysfunction of the liver characterized by damage of the parenchyma, alteration of the reticular structure and the connective tissue that sustains the lobules and sinusoids[2]. Cirrhosis can progress to hepatic encephalopathy
and to coma[8], but even before these advanced stages, functional alterations of several brain nuclei have been detected during early stages by magnetic transfer ratio, a magnetic resonance imaging technique[9]. Cirrhosis could also impact pulmonary function and might be involved in the development of obstructive sleep apnea syndrome (OSAS) in patients with ascites; however, the early stages have not been associated with OSAS[8]. In the advanced stages, cirrhotic patients show an increased frequency of moderate obstructive sleep apnea[8]. In addition, cirrhotic patients with metabolic alterations frequently show abnormalities in electroencephalographic recordings[7]. Furthermore, patients with severe cirrhosis show a significant increase in power potency associated with the theta frequency and a decrease associated with the alpha frequency in the electroencephalogram (EEG)[8,9]. Recently, Mostacci et al[10] reported significant sleep alterations in 178 patients with cirrhosis when compared to normal control subjects using questionnaires: the basic Nordic sleep and the Epworth Sleepiness Scale. They reported that patients with cirrhosis complained of more daytime sleepiness, because they had fragmented nocturnal sleep caused by frequent nocturnal wakening and had difficulties for falling asleep.

Through questionnaires of quality of life, sleep disturbances have been recognized as one of the early signs in patients with cirrhosis and hepatic encephalopathy[11]. Sleep disturbances in cirrhosis has not been correlated with clinical parameters or with cognitive impairment. Cirrhotic subjects with unsatisfactory sleep show higher scores for depression and anxiety, raising the possibility that the effects of these chronic emotional alterations might underlie the pathogenesis of sleep disturbances. Moreover, cirrhotic patients show reduced sleep time, increased latencies to sleep and frequent wakening. These alterations are not due to previously prescribed medications, but are related to abnormalities of the circadian system[12,13].

From this evidence we can conclude that hepatic cirrhosis causes a series of cerebral changes, which could modify several behaviors, such as sleep. Alterations of the sleep pattern as hepatic disease develops have not been reported. In addition, chronic administration of CCl4 in rats induces reactive free radicals that attack membrane components, culminating in cell death[13] and promoting fibrosis[14]. This is a reliable procedure to induce experimental hepatic cirrhosis. The aim of this study was to analyze the sleep pattern in rats chronically treated with CCl4 as hepatic damage progresses.

**MATERIALS AND METHODS**

**Animals**

Ten adult male Wistar rats (250-280 g) were used in this study. The animals were housed in a temperature-controlled room (22°C) and under a 12:12 normal light-dark cycle (light ON at 08:00 am). They were kept in individual clear polycarbonate cages with food and water available ad libitum. The experiments were performed following the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

**Sleep recording**

Under deep general anesthesia induced with a cocktail (ketamine: 3.75 mg/100 g, xylazine: 0.19 mg/100 g, and acepromazine: 0.038 mg/100 g ip), rats were chronically implanted with a standard set of electrodes for sleep recording. Two stainless steel screw electrodes were implanted in the frontal and parietal cortex for the EEG and flexible wires were inserted in the neck muscles to record an electromyogram.

**CCl4 treatment**

One week after surgery, and after 3 d of habituation to the recording conditions, rats (n = 10) polygraph recordings were made to obtain their basal sleep parameters. Thereafter, animals received an intraperitoneal (ip) injection, three times a week, containing different dilutions of CCl4 and mineral oil, always in a total volume of 0.25 mL. Treatment lasted for 11 wk under the following pattern of administration: in the first week the animals received a solution with one part of CCl4 and six parts of mineral oil (1:6). In the second week, the proportion of the solution changed to 1:5. In the third week the proportion of the solution was 1:4. From week four to week eleven the proportion of the solution was 1:3.

**Polygraph recordings**

Polygraph recordings of the rats were obtained for 8 h within the light period, once every week, with the Nihon-Kohden model polygraph. Thus, sleep recordings were done before treatment and after every three injections. In addition to the polygraph recordings, rat behavior was observed during the recording period. The polygraph recordings were scored visually according to standard criteria[8]. The frequency and the duration of wakefulness, slow wave sleep (SWS) I, SWS II, and rapid eye movement (REM) sleep were quantified.

**Histological liver slices**

Half of the animals died during the treatment (Initial n = 10; final n = 5). After sleep recordings, animals were killed by an overdose of pentobarbital and their livers were removed for histological examination. Liver slices were stained with hematoxylin-eosin and their morphological characteristics were determined.

**Statistical analysis**

All data were expressed as mean ± SD and analyzed by SPSS 11.0 software. Data were analyzed using an ANOVA for repeated measurements, followed by Fisher post hoc comparisons to detect significant differences between groups. P < 0.05 was considered statistically significant.

**RESULTS**

**Sleep pattern**

Treatment with CCl4 induced behavioral changes in all
rats, characterized mainly by a progressive decrease in locomotion. During the final 2 wk, four out of the five surviving animals showed ascitis. Five of the animals died before the end of the 11-wk period, so the size of the group decreased as the treatment progressed. Concerning sleep, CCl administration elicited a decrease of wake time throughout the 11 wk of treatment. The decrease was observed from the first week of treatment but only reached statistical significance from the second week. This decrease in wake time grew larger as the treatment progressed, and during weeks 10 and 11, wake time was less than 50% of pretreatment values (Figure 1A). Concerning SWS I, no significant modifications were observed. However, SWS II time showed a significant increase from the second week of treatment. The increase remained constant with only small variations during the 11 wk. Only during weeks six and seven did the increase not reach statistical significance (Figure 1B). REM sleep showed a significant increase in the first week of treatment (Figure 1C). However, REM sleep duration returned to control levels during the second and third weeks of treatment and thereafter, there was a significant increase that lasted until week eleven. The increase of REM sleep time was due mainly to the duration of each period, because no significant increases in REM sleep frequency were observed during most of the weeks. Only during weeks eight, ten, and eleven were there significant increases in REM sleep frequency (Figure 2).

Table 1 summarizes the data obtained concerning all the parameters recorded during pretreatment and during the 11 wk of recording.

### Histological liver slices

Histological examination revealed the effects of chronic CCl treatment on liver parenchyma. Figure 3 shows a sample of a liver treated with CCl. A normal liver can be observed in Figure 3A. In Figure 3B, the effects of CCl treatment for 11 wk on liver histological features are shown. Clear indications of cirrhosis were observed (fibrosis, lipidic vacuoles, pycnotic nuclei, and necrosis).

---

### Table 1 CCl effect on sleep architecture for 11 wk of treatment (min ± SE)

| Treatment     | Wake total time | SWS 1 total time | SWS 2 total time | REM sleep total time | Duration of REM sleep epochs | Frequency of REM sleep | Latency of REM sleep | Latency of SWS |
|---------------|-----------------|------------------|------------------|----------------------|-------------------------------|-----------------------|---------------------|------------------|
| Control (n = 10) | 169.03 ± 14.94  | 89.67 ± 9.21     | 174.82 ± 9.51    | 42.32 ± 5.47         | 2.16 ± 0.21                   | 17.3 ± 1.315          | 48.84 ± 11.54      | 37.06 ± 6.78     |
| 1 wk (n = 10)  | 142.93 ± 9.21   | 109.69 ± 12.89   | 158.40 ± 13.32   | 67.64 ± 4.89         | 2.95 ± 0.27                   | 23 ± 2.27             | 69.72 ± 26.61      | 25.60 ± 6.05     |
| 2 wk (n = 10)  | 128.85 ± 9.74   | 87.45 ± 8.45     | 213.62 ± 6.07    | 49.52 ± 7.39         | 2.56 ± 0.14                   | 17.7 ± 1.93           | 80.27 ± 19.98      | 26.57 ± 5.58     |
| 3 wk (n = 9)   | 133.40 ± 9.26   | 74.36 ± 14.90    | 214.38 ± 18.94   | 57.30 ± 5.44         | 2.44 ± 0.13                   | 22.2 ± 2.17           | 63.80 ± 17.81      | 17.84 ± 3.61     |
| 4 wk (n = 9)   | 107.77 ± 11.07  | 80.69 ± 10.27    | 225.89 ± 10.20   | 58.60 ± 7.94         | 2.44 ± 0.16                   | 22.2 ± 2.17           | 63.80 ± 17.81      | 17.84 ± 3.61     |
| 5 wk (n = 9)   | 107.52 ± 12.58  | 74.27 ± 13.55    | 232.54 ± 14.85   | 67.24 ± 5.45         | 2.70 ± 0.27                   | 23.55 ± 1.74          | 73.67 ± 19.52      | 25.04 ± 4.98     |
| 6 wk (n = 7)   | 121.19 ± 11.64  | 86.08 ± 8.86     | 208.17 ± 16.42   | 63.64 ± 3.37         | 2.72 ± 0.26                   | 22.42 ± 2.01          | 79.99 ± 18.22      | 17.58 ± 2.30     |
| 7 wk (n = 7)   | 126.87 ± 7.28   | 93.07 ± 10.69    | 191.5 ± 13.90    | 61.47 ± 8.03         | 2.31 ± 0.21                   | 24.85 ± 3.47          | 71.49 ± 23.91      | 19.81 ± 4.54     |
| 8 wk (n = 7)   | 99.64 ± 12.18   | 82.11 ± 8.89     | 230.48 ± 14.96   | 66.93 ± 9.00         | 1.96 ± 0.23                   | 31.42 ± 2.17          | 75.81 ± 11.97      | 18.08 ± 4.95     |
| 9 wk (n = 6)   | 120.06 ± 17.93  | 78.53 ± 7.85     | 215.29 ± 16.85   | 65.41 ± 3.90         | 2.56 ± 0.40                   | 24.33 ± 2.78          | 85.26 ± 32.18      | 20.03 ± 6.91     |
| 10 wk (n = 5)  | 82.03 ± 8.25    | 66.91 ± 13.22    | 256.29 ± 22.37   | 68.89 ± 3.72         | 2.60 ± 0.50                   | 26.6 ± 2.56           | 60.46 ± 36.58      | 17.15 ± 7.64     |
| 11 wk (n = 5)  | 72.79 ± 11.77   | 77.34 ± 17.61    | 257.84 ± 18.77   | 71.28 ± 7.67         | 2.63 ± 0.41                   | 26.4 ± 2.56           | 39.64 ± 10.70      | 17.24 ± 5.22     |

*a* P < 0.05, *b* P < 0.01 vs control. Repeated measure ANOVA.

---

[Figure 1](#) Percentage of Wakefulness (A), SWS II (B), and REM sleep (C) in weekly 8 h sleep recordings during treatment with CCl. Numbers at the top of the bars express the number of subjects. *a* P < 0.05, *b* P < 0.01 vs control. Repeated measurements were analyzed by ANOVA.

www.wjgnet.com
DISCUSSION

The present results indicate that sleep-wake patterns changed as experimental cirrhosis progressed. REM sleep showed an acute response to the first administrations of CCl₄. Wake and SWS II time were significantly modified after 2 wk of CCl₄ treatment, and these effects showed a steady and slight increase during the following weeks. These results are consistent with previous reports showing that cirrhotic patients display a reduction in α activity and an increase in theta and delta activity.[6]

Indirect tests, such as actigraphic recordings and questionnaires on sleep, have suggested that cirrhotic patients suffer from unsatisfactory sleep, mainly due to the reduction in the quality of sleep produced by several awakenings throughout the night.[12-15]. However, Steindl et al.[16] did not find differences in polysomnographic recordings of cirrhotic patients compared to matched healthy controls. However, sleep diaries of these patients indicated more frequent nocturnal awakenings and daytime naps. Moreover, these researchers measured the levels of melatonin and found a significant increase during daytime, when melatonin is normally absent.[6]. Recently, Velissaris et al.[17] corroborated these results; they showed that melatonin circadian patterns were altered in cirrhotic patients without clinical encephalopathy.[6-7]. In a higher magnification, the bold arrow indicates hepatocytes with lipidic vacuoles.
COMMENTS

Background
Sleep disturbances have been described in patients with advanced cirrhosis. However, the development and mechanisms of these alterations are not known. In this study, the sleep pattern of rats submitted to experimental cirrhosis was analyzed as liver damage progressed. The results showed an early decrease of wake time and sustained increases of slow wave sleep (SWS) and later, of REM sleep. This data suggest that sleep alterations could be the warning signs of liver disease.

Research frontiers
This study adds information on the relationship between liver function and brain function disturbances.

Innovations and breakthroughs
The animal model of liver cirrhosis induced by chronic administration of CCl4 is a suitable model to analyze the relationship between liver failure and brain function disturbances.

Applications
This study highlights the need for gastroenterologists to pay attention to sleep disturbances as early signs of liver failure.

Terminology
SWS and rapid eye movement sleep and the two major sleep stages in most animal species.

Peer review
The content of the article will be interesting not only for the gastroenterologists, but also for other specialists. Further investigations of mechanisms of sleep alterations might yield potentially efficacious approaches to its clinical management.

REFERENCES

1. Siegel J. Sleep as a circadian rhythm. In: Siegel J, editor. The neural control of sleep & wakening. New York: Springer-Verlag; 2002: 93-101
2. Ham AW, Cormack DH. Histology. Mexico: Interamericana, 1984; 783-812
3. Butterworth RF. Complications of cirrhosis III. Hepatic encephalopathy. J Hepatol 2000; 32: 171-180
4. Iwasa M, Kinosada Y, Nakatsuka A, Watanabe S, Adachi Y. Magnesium transfer contrast of various regions of the brain in liver cirrhosis. AJNR Am J Neuroradiol 1999; 20: 652-654
5. Nikaina I, Pastaka C, Zachou K, Dalekos GN, Gourgoulianis K. Sleep apnoea syndrome and early stage cirrhosis: a pilot study. Eur J Gastroenterol Hepatol 2006; 18: 31-35
6. Tanne F, Gagnado F, Chazouilleres O, Fleury B, Wendum D, Lasnier E, Lebeau B, Poupon R, Serfaty L. Chronic liver injury during obstructive sleep apnea. Hepatology 2005; 41: 1290-1296
7. Papenberg J, Lanzinger G, Kommerer B, Hoyer S. Comparative studies of the electroencephalogram and the cerebral oxidative metabolism in patients with liver cirrhosis. Klin Wochenschr 1975; 53: 1107-1113
8. Amodio P, Del Piccolo F, Petteno E, Mapelli D, Angeli P, Iemmlor R, Muraca M, Musto C, Gerunda G, Rizzo C, Merkel C, Gatta A. Prevalence and prognostic value of quantified electroencephalogram (EEG) alterations in cirrhotic patients. J Hepatol 2001; 35: 37-45
9. Bahn E, Nolte W, Kurth C, Ramadori G, Rüther E, Wiltfang J. Quantification of the electroencephalographic theta/alpha ratio for the assessment of portal-systemic encephalopathy following implantation of transjugular intrahepatic portosystemic stent shunt (TIPS). Metab Brain Dis 2002; 17: 19-28
10. Mostacci B, Ferlisi M, Baldi Antognini A, Sama C, Morelli C, Mondini S, Cirignotta F. Sleep disturbance and daytime sleepiness in patients with cirrhosis: a case control study. Neurol Sci 2008; 29: 237-240
11. Sherlock S, Summerskill WH, White LP, Phear EA. Portal-systemic encephalopathy; neurological complications of liver disease. Lancet 1954; 267: 454-457
12. Córdoba J, Cabrera J, Lataix L, Penev P, Zee P, Blei AT. High prevalence of sleep disturbance in cirrhosis. Hepatology 1998; 27: 339-345
13. Martínez M, Mourtelle M, Muriel P. Protective effect of colchicine on acute liver damage induced by CCl4. Role of cytochrome P-450. J Appl Toxicol 1995; 15: 49-52
14. Desmoulère A, Xu G, Costa AM, Yousef IM, Gabbiani G, Tuchweber B. Effect of pentoxifylline on early proliferation and phenotypic modulation of fibrogenic cells in two rat models of liver fibrosis and on cultured hepatic stellate cells. J Hepatol 1999; 30: 621-631
15. Takeuchi E. [Polygraphical study on the wakefulness-sleep cycle of the rat] Shinrigaku Kenkyu 1970; 41: 248-256
16. Steindl PE, Finn B, Bendok B, Rothke S, Zee PC, Blei AT. [Changes in the 24-hour rhythm of plasma melatonin in patients with liver cirrhosis--relation to sleep architecture] Wien Klin Wochenschr 1997; 109: 741-746
17. Velissaris D, Karanikolas M, Kalogeropoulos A, Solomou E, Polychronopoulos P, Thomopoulos K, Labropoulou-Karatzia C. Pituitary hormone circadian rhythm alterations in cirrhosis patients with subclinical hepatic encephalopathy. World J Gastroenterol 2008; 14: 4190-4195
18. Ogata T, Nomura M, Nakaya Y, Ito S. Evaluation of episodes of sleep apnea in patients with liver cirrhosis. J Med Invest 2006; 53: 159-166
19. Diemer NH. Size and density of oligodendroglial nuclei in rats with CCl4-induced liver disease. Neurobiology 1975; 5: 197-206
20. Luse SA, Wood WG. The brain in fatal carbon tetrachloride poisoning. Arch Neurol 1967; 17: 304-312
21. Steindl PE, Finn B, Bendok B, Rothke S, Zee PC, Blei AT. Disruption of the diurnal rhythm of plasma melatonin in cirrhosis. Ann Intern Med 1995; 123: 274-277
22. Zee PC, Mehta R, Turek FW, Blei AT. Portacaval anastomosis disrupts circadian locomotor activity and pineal melatonin rhythms in rats. Brain Res 1991; 560: 17-22
23. Del Piccolo F, Sacerdotti D, Amodio P, Bombonato G, Bolognesi M, Mapelli D, Gatta A. Central nervous system alterations in liver cirrhosis: the role of portal-systemic shunt and portal hyperperfusion. Metab Brain Dis 2003; 18: 51-62
24. Hazell AS, Butterworth RF. Hepatic encephalopathy: An update of pathophysiologic mechanisms. Proc Soc Exp Biol Med 1999; 222: 99-112
25. Bergeron M, Reader TA, Layrargues GP, Butterworth RF. Monoamines and metabolites in autopsied brain tissue from cirrhotic patients with hepatic encephalopathy. Neurochem Res 1989; 14: 853-859
26. Rose C, Felipo V. Limited capacity for ammonia removal by brain in chronic liver failure: potential role of nitric oxide. Metab Brain Dis 2005; 20: 275-283

S-Editor Tian L  L-Editor Stewart GJ  E-Editor Zheng XM