20

Influence of the duration of circadian rhythm dis-turbance by light exposure on the morphology of the liver of laboratory rats

© S.S. Pakhomy, O.V. Zlobina, I.O. Bugaeva, G.N. Maslyakova, A.N. Ivanov, A.O. Moskvina

Saratov State Medical University,
410012 Saratov, Russia

e-mail: spakhomy03@gmail.com

Received December 20, 2021
Revised January 17, 2022
Accepted March 23, 2022

We studied the effect of long-term constant light exposure on the severity and reversibility of morphological changes in the liver of laboratory rats. Modeling of light exposure was carried out by means of 24-hour exposure to artificial light (in the daytime — 300 lk, at night — 500 lk). During the study we found that morphological changes in the liver were represented by damage to the parenchyma and circulatory disorders, the degree of which increased with increasing duration of exposure. After providing natural light in the laboratory, gradual recovery of the morphological structure of the organ was observed, which indicates the reversibility of the changes detected.

Keywords: light exposure, circadian rhythms, morphology, liver.

DOI: 10.21883/EOS.2022.06.54703.34-22

Introduction

Under present-day conditions a human is often exposed to long-term artificial light [1.2]. Light regime disturbances, appearing in case of daylight elongation, for instance, during work in the late or night hours or as a results of the jet lag, are one of the causes, resulting in biorhythms desynchronization [3–5]. These changes induce the development of functional and structural changes in organism, increasing a risk of cardiovascular and nervous systems diseases, endocrinopathy and oncology disease [6,7].

Circadian rhythms (diurnal and near-diurnal) disturbance, appearing as a response to photoperiod changes, is considered as one of the powerful stress factors, impacting the diurnal variations of cortisol and melatonin hormones levels in blood [5,8]. Increase of stress hormones concentration results in vasospasm and, consequently, development of blood circulation centralization phenomenon. At the same time melatonin level reduction increases the trombocytes functional activity. As a result, increase of blood platelets aggregation with microcirculation and clotting disorders, reduction of intravascular component functioning and substances transport efficiency occur as a response to the spasm in microcirculatory bloodstream vessels. These vascular abnormalities result the ischemia development, that is the cause of reduction of cells and tissues trophicity and appears as organs parenchyma damage [9,10].

Light exposure duration, lighting regime type and external oscillator power directly influence on severity of functional and structural disturbances. In the experiments [11–13] it was established, that at long-term exposure of constant light to gnawing animals the obesity and type II diabetes mellitus are developed. Relatively short-term presence (4−6 weeks) under conditions of 20-hour light-dark cycle, consisting of 10 h of light and 10 h of dark (Light-Dark 10:10), resulted in metabolic disturbance, body mass increase and increase of leptin, insulin and triglycerides concentration in blood plasma of the laboratory mice [14]. In the work [15] it was demonstrated, that these changes development in biochemical blood analysis of the laboratory animals is related to sleep duration and quality disturbance. In the studies we performed earlier [9,11], it was established, that severity of morphological changes, transformation resistance and level of microcirculatory disturbances reversibility depend on the light exposure intensity and duration.

Adverse effects of stress situations, appearing as a result of influence of various irritants, abnormal in terms of power and/or duration of exposure, on organism, are characterized with development of pathological changes [16]. Possibility of these changes reversibility is perceived as criterion of adaptation or deadaptation to the changed existence conditions. Until recent time the issues of influence of extended photoperiod with continuous lighting on severity and reversibility of morphological changes in visceral organs remain understudied. Therefore the purpose of this work is to study the influence of the long-term constant light exposure on severity and reversibility of the morphological changes in liver.

Materials and methods

The experimental study was carried out on the base of scientific laboratories of histology and pathological anatomy faculties of the Saratov SMU named after V.I. Razumovsky. The experiment was carried out using 60 white mongrel male rates with body weight of 250 ± 20 g in accordance with the international ethical norms of the European Convention for the protection of vertebrate animals used

for experimental and other scientific purposes (Strasbourg, 1986) and „International Guiding principles for Biomedical Research Involving Animals“ (2012), and based on the recommendations of the Ethics Committee of the Federal State-Owned Publicly-Funded Institution of Higher Education Saratov SMU named after V.I. Razumovsky of the Ministry of Health of the Russian Federation (protocol № 4 dated 06.12.2016). The experiment was carried out during fall period. The animals of all experimental groups had free access to water and food.

For evaluation of the extended photoperiod influence on liver morphology the animals from the experimental groups were subject to continuous artificial light exposure using Light/Light (L/L) model. L/L model assumes the continuous artificial lighting in a laboratory during day time with power of 300 lk, during night time — 500 lk.

The study was divided into two series, each one included two experimental groups of animals ($n = 12$). The first one was dedicated to the study of light exposure duration influence on liver morphology: animals from the experimental groups were subject to continuous lighting for 10 and 21-st days respectively. Sampling was performed on the next day after the experiment completion. The second one was focused on the observed changes reversibility study: animals from the experimental groups were under continuous lighting for 10 and 21-st days respectively, and then the natural lighting was recovered in the laboratory. Sampling was performed on 14 day after lighting recovery. Time periods of the experimental model are defined based on data on staging of the stress reactions forming in organism, characterized with development of the general adaptation syndrome on the 10-th day and adaptation mechanisms failure on 21-st day [17]. The animals from the control group were under standard day-night lighting conditions for 21-st days.

Animals from all experimental groups were removed from the experiment by preparation overdosing: intramuscular combination of Telazol at a dose of 0.2 ml/kg and Xylanit at a dose of 0.2 mg/kg.

During morphological study the liver samples were processed using standard histological equipment, were colored with hematoxylin and eosin. Perls reaction was used for hemosiderin pigment granules detection.

Morphometric analysis of histological preparations was performed using the system of analysis of the digital images of medical microvisor μ Vizo-101 LOMO in the lens field of 63x. During the morphometric study the following indices were used: parenchyma normalization coefficient (PNC), number of nonparenchymal liver elements (NLE) and bicyclic hepatocytes. PNC allows to evaluate the intensity of changes, developing in connection with the long-term light exposure, and is calculated as a ratio of a number of hepatocytes with cytoplasm dystrophic changes to a number of hepatocytes under necrosis [11].

Statistical processing of the study results was performed using Statistica 10.0 software (Stat Soft Inc, USA). In case of difference of values distribution in a sample from the normal, median and quartiles were calculated. Significance of differences (p) was calculated using a non-parametric test of Mann–Whitney. Changes at $p < 0.05$ were considered significant.

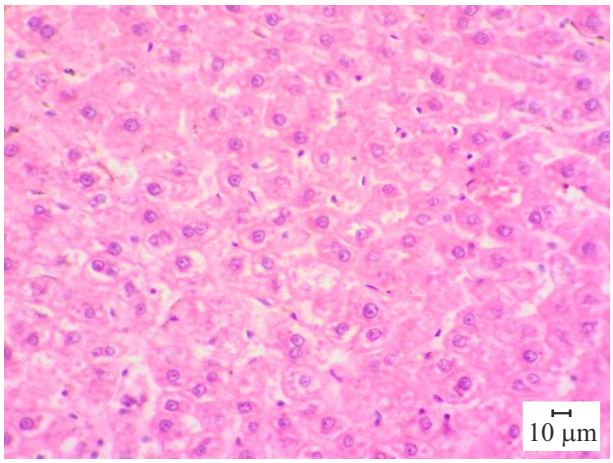

Figure 1. Moderate dystrophic changes in hepatocytes. 10-th day of light exposure. Col. H.-E. Magn. Magn. 246.4.

Study results

It is established, that severity of the morphological changes in liver depended on duration of the circadian rhythms disturbance, caused by constant exposure of the light irritant. In the first series of the experiment on the 10-th day of light exposure the damage of the organ parenchyma, presented with moderate dystrophy and necrosis of hepatocytes, development of moderate blood filling in stroma, erythrocytes intravascular hemolysis and small hemosiderin pigment granules accumulation in Kupffer cells, was observed in the liver (fig. 1, 2).

Experiment duration extension to 21-st days was accompanied with appearance of more pronounced signs of parenchyma damage up to formation of the hepatocytes focal necrosis nidus (fig. 3). Moderate congestion and edema were observed in the organ stroma, erythrocytes ghosts and fibrin were located in vessels lumen. Moderate accumulation of hemosiderin pigment granules was observed in Kupffer cells.

The results of the morphometric study of light stimulation duration influence on liver morphology are presented in the table.

According to morphometric analysis data the signs of parenchyma damage of various severity were observed in both experimental groups of the first series of the experiment. The most pronounced changes were observed in the experimental group with light exposure duration of 21-st days: increase of hepatocytes number under necrosis to 19 [16,22] and PNC reduction to 1.7 [1.5,2] were observed. The observed results indicate the more

Results of liver morphometric study

Examined indices in the lens field of 63x	Observation groups				
	Control group	10 days of light exposure		21-st days of light exposure	
		LL	Reversibility	LL	Reversibility
Number of hepatocytes with dystrophic changes	36 [30; 43]	40 [35; 43]	36 [31; 37]	33 [29; 39]	35 [30; 43]
Number of hepatocytes under necrosis	16 [13; 19]	18 [15; 21]*	16 [14; 20]	19 [16; 22]*	17 [14; 20]
PNC	2.3 [2; 2.7]	2.2 [2; 2,5]	2.2 [2; 2,5]	1.7 [1,5; 2]*	2.0 [1,8; 2,2]*
Number of liver NLE	7 [5; 8]	7 [5; 8]	13 [11; 16]*	5 [4; 6]*	16 [11; 19]*
Number of bicyclic hepatocytes	5 [3; 6]	6 [4; 8]*	4 [2; 6]	4 [2; 5]	5 [3; 7]

Note. * — significance of differences with control group ($p < 0.05$).

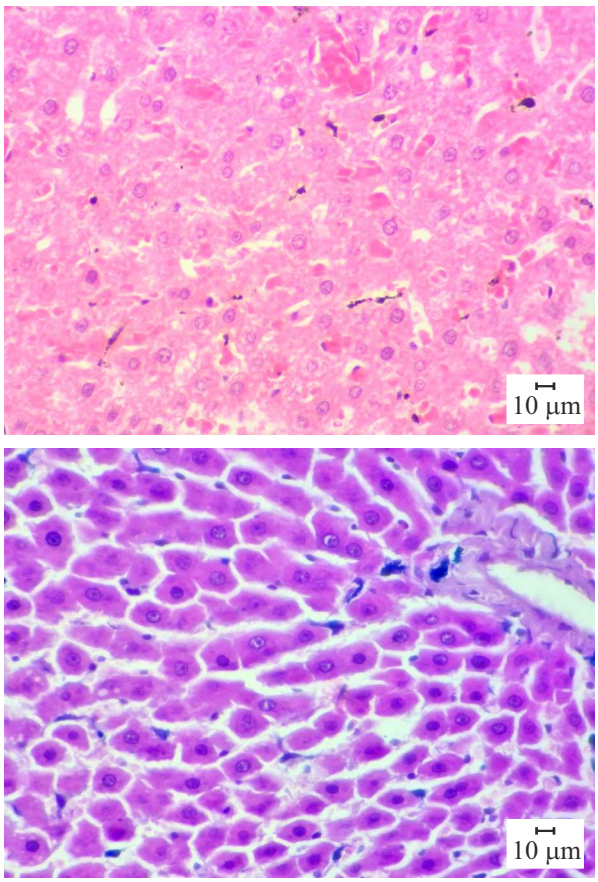

Figure 2. Accumulation of hemosiderin pigment in liver. 10-th day of light exposure. *a* — col. H.-E. Magn. Magn. 246.4. *b* – col. Perls reaction. Magn. 246.4.

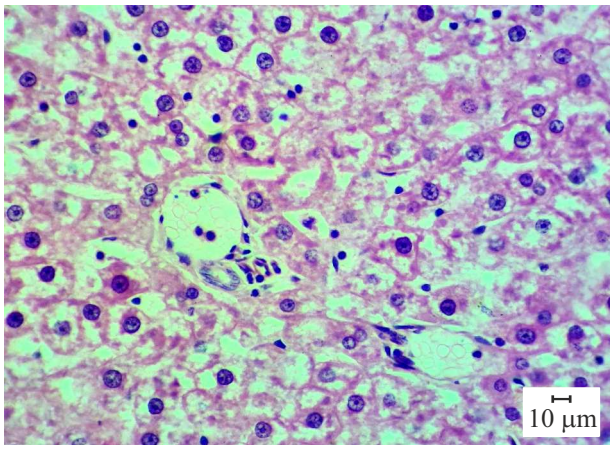

Figure 3. Expressed dystrophic and necrotic changes in hepatocytes. 21-st day of light stimulation. Col. H.-E. Magn. 246.4.

pronounced damage of the organ parenchyma in connection with the light exposure duration increase.

Influence on macrophagal and lymphocytic systems of the organ was evaluated based on results of liver NLE number count (lymphocytes, Kupffer cells, Ito cells). Number of NLE of liver on the 10-th day of the experiment did not differ from the control group. However, with increase of experiment duration to 21-st days the reduction of this index from 7 [5,8] to 5 [4,6] was observed.

Intensity of proliferative processes in liver was studied using bicyclic hepatocytes number count: on the 10-th day of the experiment the increase of bicyclic hepatocytes number to 6 [4,8], and on the 21-st day — decrease of this index to 4 [2,5] was observed.

When studying the morphological structure of liver on the 14-th day after completion of the continuous lighting exposure the organ structure recovery was observed in both experimental groups: reduction of stroma edema and vascular congestion, reduction of severity of dystrophy and necrosis of hepatocytes (fig. 4).

According to morphometric study data on the 14-th day after completion of light exposure for 10 days the number of hepatocytes with dystrophic changes in cytoplasm and under necrosis, PNC did not differ from values in the control group. At the same time during study of the reversibility in the group with light exposure for 21-st days, PNC remained below control values and was equal to

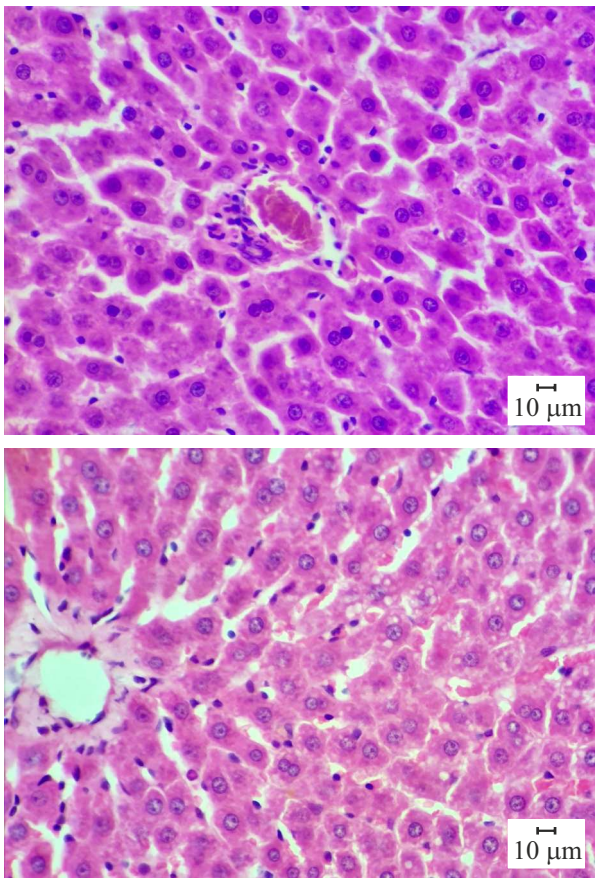

Figure 4. Dystrophic change in hepatocytes on the 14-th day afyer completion of light stimulation for 10 days (*a*) and 21-st days (*b*). Col. H.-E. Magn. Magn. 246.4.

2.0 [1.8;2.2]. The observed data indicates the dependence of recovery processes rate for liver parenchyma on light exposure duration.

Morphometric changes from the side of monocytic-macrophagal system on the 14-th day after completion of the continuous light exposure in both experimental groups were characterized with sharp increase of liver NLE number. The highest number of NLE was observed in the experimental group with light exposure duration of 21-st days — 16 [11;19], that exceeded the indices in the control group by a factor of almost 2.5 — 7 [5;8]. The observed results indicate the activation of the processes of proliferation and differentiation of monocytic-macrophagal system cells. Number of bicyclic hepatocytes in both experimental groups was equal to the values in the control group.

As a result of this study it was established, that the long-term constant light exposure results in development of liver parenchyma damage and circulation failure in the organ, severity of which was increased with exposure duration increase. On the 10-th day of the experiment the moderate dystrophy of hepatocytes, signs of failure of circulation and proliferation and differentiation of monocytic-macrophagal system cells were developed in liver. This morphological pattern is probably related to activation of stress-implementing systems in organism and indicates the development of stress stability stage in connection with the constant light exposure. Experiment duration increase to 21-st days was accompanied with the further damage of the organ parenchyma and circulation failure, that indicated the stress transition to exhaustion stage. The observed results correspond to the data, published earlier, and indicate the development of stress reaction in organism in connection with the long-term light exposure [9,11,18].

Recovery of the natural light regime in the laboratory results in gradual normalization of the organ blood filling and activation of liver parenchyma reparation mechanisms. Increase of number of functional cells is perceived by us as a demonstration of regeneration processes, focused on liver tissue recovery.

Conclusion

As a result of this study it was established, that at long-term light exposure the circulation failure, dystrophy and necrosis of hepatocytes, increase of Kupffer cells number are developed in liver. Severity of morphological changes in liver increases with the experiment duration increase and reaches the maximum values on the 21-st day. After provision of natural lighting in the laboratory on the 14-th day the gradual recovery of liver morphological pattern is observed, thus indicating the reversibility of the observed changes. Intensity of the recovery processes in liver depends on light exposure duration.

Funding

The work has been performed under the state assignment of the Federal State-Owned Publicly-Funded Institution of Higher Education „Saratov SMU named after V.I. Razumovsky“ of the Ministry of Health of the Russian Federation on the subject of „Development of mathematical model for evaluation of transformation rate of functional changes in the whole organism under the light desynchronosis into irreversible morphological changes of target organs during experiment“.

0.1. Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] D.J. Stenvers, R. van Dorp, E. Foppen, J. Mendoza, A.-L. Opperhuizen, E. Fliers, P.H. Bisschop, J.H. Meijer, A. Kalsbeek, T. Deboer. Sci. Rep., **6**, (35662) (2016). DOI: 10.1038/srep35662.

[2] Y. Cho, S.H. Ryu, B.R. Lee, K.H. Kim, E. Lee, J. Choi. Chronobiol. Internat., **32** (9), 1294–1310 (2015). DOI: 10.3109/07420528.2015.1073158

[3] J. Cedernaes, N. Waldeck, J. Bass. Genes Dev., **33** (17–18), 1136–1158 (2019). DOI: 10.1101/gad.328633.119.
[4] V.A. Snezhinskij, N.F. Pobivanceva. Zhurnal Grodnenskogo gosudarstvennogo medicinskogo universiteta, **1**, 9–13 (2013). (in Russian)
[5] C.E. Koch, B. Leinweber, B.C. Drengberg et al. Neurobiol. Stress., **6**, 57–67 (2017). DOI: 10.1016/j.ynstr.2016.09.001
[6] J.O. Early, A.M. Curtis. Seminars in Immunology, Immunometabolism., **28** (5), 478–490 (2016). DOI: 10.1016/j.smim.2016.10.006.
[7] V.N. Anisimov, I.A. Vinogradova, A.V. Bukalev i dr. Vopr. onkol., **60** (2), 15–27 (2014). (in Russian)
[8] T.A. LeGates, D.C. Fernandez, S. Hattar. Nat Rev Neurosci., **15** (7), 443–454 (2014). DOI: 10.1038/nrn3743
[9] K.I. Zhurkin, O.V. Zlobina, A.N. Ivanov i dr. Tromboz, gemostaz i reologiya, **3** (67), 164–166 (2016). (in Russian) DOI: 10.15372/SSMJ20200303
[10] P. Poredos, M.K. Jezovnik. Angiology, **7** (69), 564–567 (2017).
[11] O.V. Zlobina, I.O. Bugaeva, S.S. Pahomij, A.N. Ivanov, Yu.A. Slyusarenko, E.D. Usol'ceva. Vestnik novyh medicinskih tekhnologij. Elektronnoe izdanie, **5**, 250–254 (2018). (in Russian) DOI: 10.31857/S0869813921030109
[12] L.K. Fonken et al. PNAS., **107**, 18664–18669 (2010).
[13] C.P. Coomans et al. FASEB journal, **27** (4), 1721–1732 (2013). DOI: 10.1096/fj.12-210898
[14] L.P. Casiraghi, A. Alzamendi, A. Giovambattista, J. Chiesa1, A.D. Golombek. Physiological Reports, **4** (8), 12743 (2016). DOI: 10.14814/phy2.12743
[15] D.J. Phillips, M.I. Savenkova, I.N. Karatsoreos. Brain, Behavior, and Immunity, **47**, 14–23 (2015). DOI: 10.1016/j.bbi.2014.12.008.
[16] S.N. Ezhov, S.G. Krivoshchekov. Byull. Sibir. otd-ya RAMN, **4**, 77–83 (2004). (in Russian)
[17] V.N. Morozov, A.A. Hadarcev. Vestnik novyh medicinskih tekhnologij, **1**, 15–17 (2010). (in Russian)
[18] O.V. Zlobina, S.S. Pahomij, I.O. Bugaeva, G.N. Maslyakova, A.N. Ivanov. Vestnik novyh medicinskih tekhnologij. Elektronnoe izdanie, **5**, 245–249 (2018). (in Russian)