The expression of Bcl-2 family proteins in liver cells of *C57Bl/6* mice under conditions of functional pinealectomy

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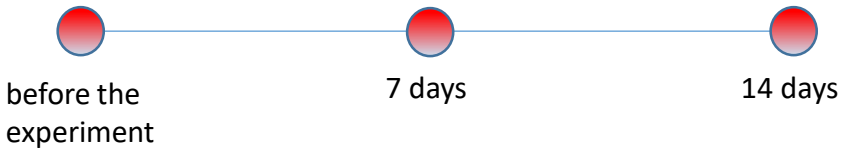
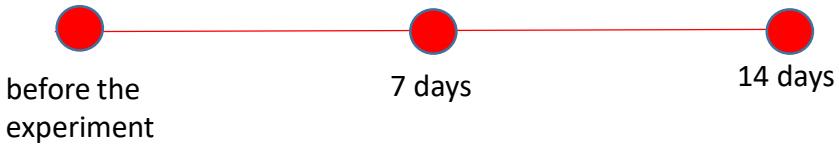
Motivation and Aim. It was found that blood circulatory and lymphatic flow disturbances develop in the liver lymphatic region under the influence of continuous lighting, which is an integral factor of modern life. These processes lead to the development of hypoxia, which adversely affects the structure and functions of the cell mitochondrial apparatus [Michurina S V.et al., 2008; Russart K.L.G. and Nelson R.J., 2018]. Apoptosis helps the body to get rid of unnecessary and defective cells. Most forms of apoptosis are realized by the mitochondrial pathway with the participation of Bcl-2 family proteins. It is believed that the ratio of the antiapoptotic Bcl-2 protein and proapoptotic Bad, Bax proteins is a "molecular switch", which determines whether tissue growth or atrophy will occur [Jeong S.Y. and Seol D.W., 2008]. The purpose of this study was to evaluate the influence of 24-hour lighting (24hL) on the expression of the proapoptotic Bad protein and the antiapoptotic Bcl-2 protein in liver cells of C57Bl/6 mice male.

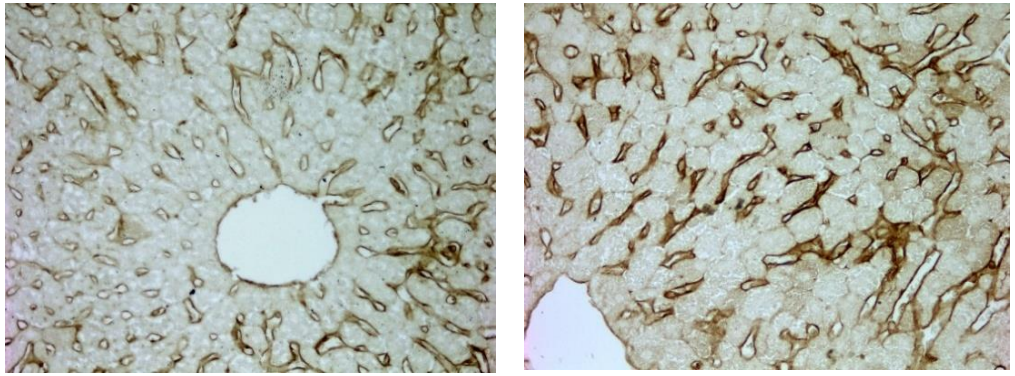
gr. "Control" (14/10 h)



gr. "24hL" (24-hour lighting) (24/0 h)

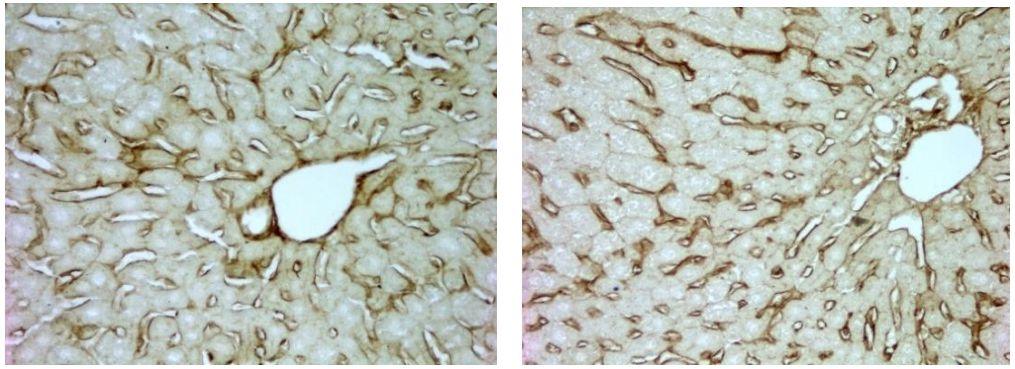


Material and Methods. The males of C57Bl/6 mice aged of 10-12 weeks were kept in the SPF-vivarium center of ICG SB RAS. The intact animals (the group "Control", n=5) were kept at the standard lighting mode – **light 14 h/dark 10 h.** The mice of the experimental group (the gr. "24hL", n=6) were kept for 14 days under conditions of 24-hour lighting – **light 24 h/dark 0 h.** Immunohistochemical detection of the antiapoptotic Bcl-2 protein expression and the proapoptotic Bad protein expression was performed in liver paraffin sections using the indirect immunohistochemical ABC method. Computer morphometric analysis of digital photos at magnification x400 was carried out using "Image J" program. The relative areas of zones staining and the ratio of the Bcl-2 expression area to the Bad expression area were calculated.



“Control” mice *“24hL” mice*

The Bad protein in pericentral zones

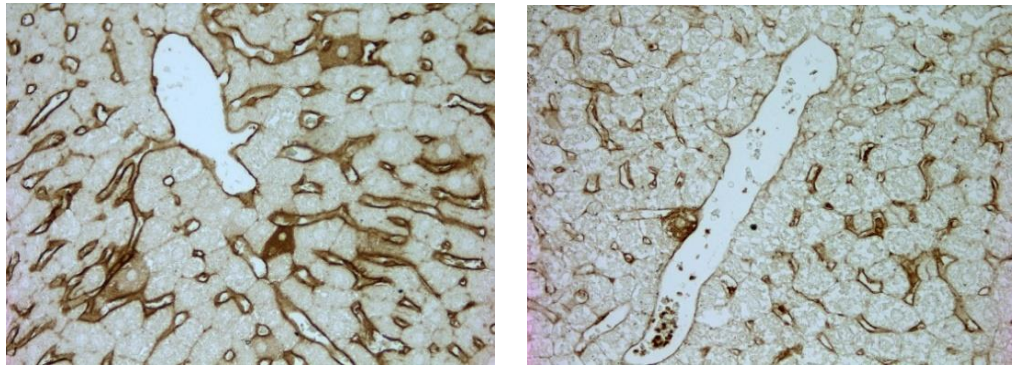


“Control” mice *“24hL” mice*

The Bad protein in periportal zones

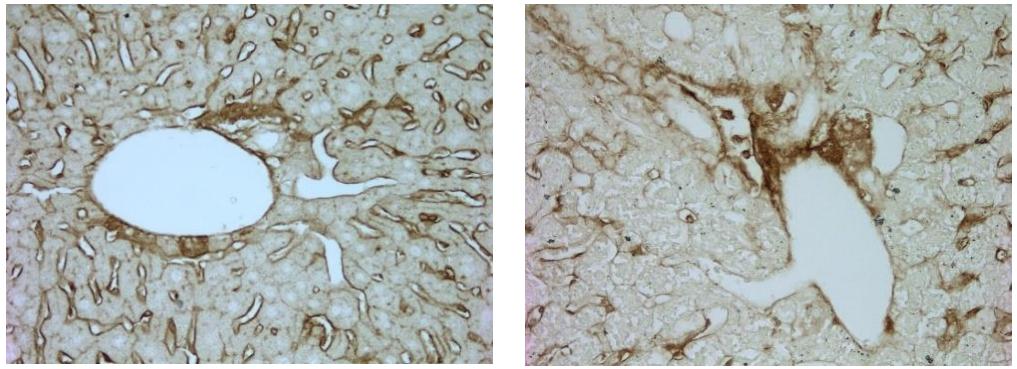
Fig. 1. (x400). The pronounced **immunohistochemical staining** of **the proapoptotic Bad protein** of sinusoidal cells of blood capillaries was revealed in the liver of C57Bl/6 mice males kept under 24-hour lighting. The Bad-positive signal was detected in the endothelium of interlobular veins and in the ductal epithelium of triad bile ducts, and it was also sometimes found in hepatocytes located mainly in periportal (a,b) and pericentral zones (c,d) of hepatic lobes.

Fig. 2. (x400). At the same time, weak **immunohistochemical staining** of **the antiapoptotic Bcl-2 protein** was revealed in sinusoidal liver cells and in single hepatocytes. Staining for Bcl-2 was not determined in the ductal epithelium of triad bile ducts.



“Control” mice *“24hL” mice*

The Bad protein in pericentral zones



“Control” mice *“24hL” mice*

The Bad protein in periportal zones

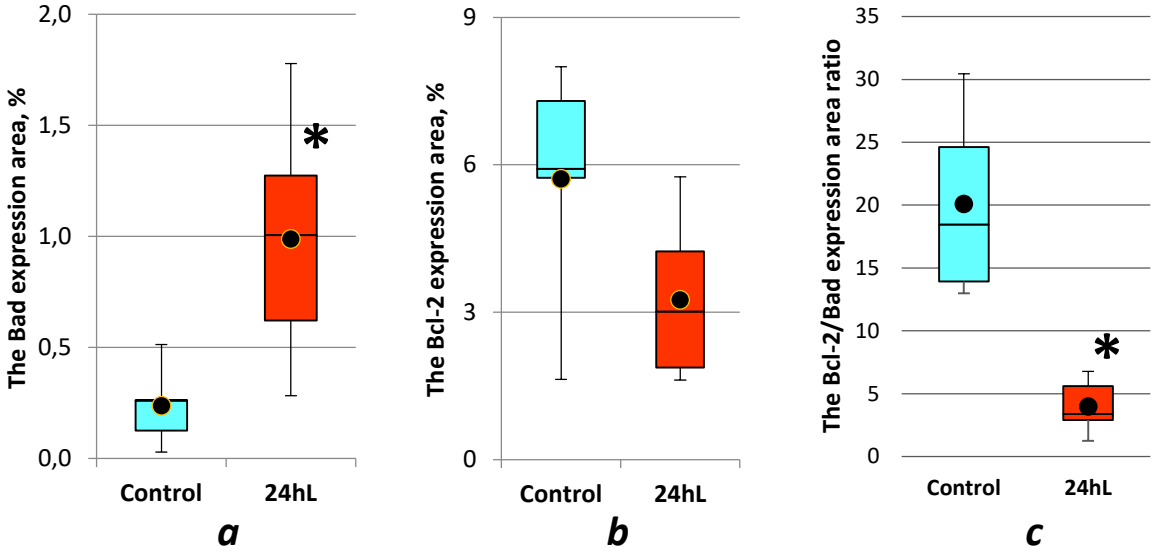


Fig. 3. <u>Morphometric analysis</u> of liver preparations in the group "24hL" showed an increase in the expression area of **the proapoptotic Bad** protein (***a***) compared to animals exposed to standard light (* the Mann-Whitney U-test; p<0,05). At the same time, the expression area of **the antiapoptotic Bcl-2 protein** (***b***) in this group did not change significantly compared to control animals, but tended to decrease. **The Bcl-2/Bad expression area ratio** (***c***) decreased significantly in the "24hL" mice compared with the "Control" animals due to an increase in the Bad expression area (* the Mann-Whitney U-test; p<0,05).

It is known that a violation of the light regime, and in particular long-term round-the-clock lighting, leads to a decrease in melatonin production resulted to the development of desynchronosis followed by the development of organic pathology [Michurina S V.et al., 2008, 2018]. Due to apoptosis, the body gets rid of defective cells without starting the process of inflammation. Moreover, most of its forms are realized not through cell death receptors, but by the mitochondrial pathway with the participation of BCL-2 family proteins. Numerous data indicate that mitochondria are the site of melatonin synthesis itself. These organelles contain the enzymes N-acetyltransferase and hydroxyindole-O-methyltransferase. Melatonin is a mitochondrial antioxidant, protecting these organelles by binding reactive oxygen species. Studies have shown that melatonin reduces the rate of apoptosis, prevents the opening of mitochondrial pores, has a stabilizing effect and supports the mitochondrial membrane potential, prevents the release of cytochrome C, prevents electron leakage and preserves mitochondrial functions. In addition, mitochondrial biogenesis and dynamics are also regulated by melatonin [Reiter R.J. et al, 2018]. Since apoptosis is triggered by the inactivation of Bcl-2 when it binds to the Bad protein, the increase in the staining area of the proapoptotic protein Bad set by us indicates a decrease in antiapoptotic protection and in the apoptosis development along the mitochondrial pathway in liver cells. This is also confirmed by a decrease in the ratio of Bcl-2/Bad expression areas in the liver of mice kept under 24-hour lighting (*light 24 h/dark 0 h*) compared to control animals (*light 14h/dark 10h*).

Conclusion. So, our results indicate a weakening of the anti-apoptotic protection of organ cells, which creates the conditions for the activation of the "mitochondrial branch" of apoptosis in animal liver cells with light-induced functional pinealectomy.

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