LYVE-1 expression in liver cells of mice with functional pinealectomy

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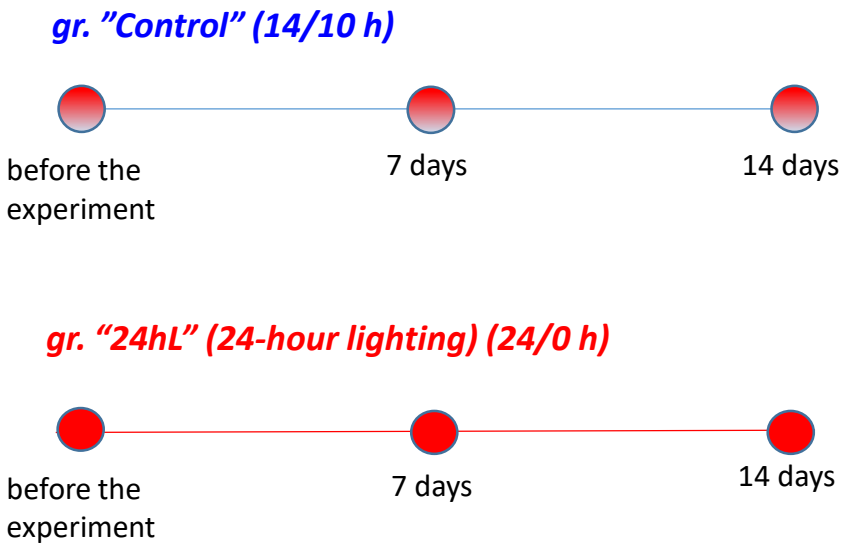
Motivation and Aim. An integral factor of modern life is the use of continuous artificial lighting and constant changes in light mode. As a result of the long existence of a person under such conditions, the production of the pineal gland hormone melatonin significantly decreases/stops in his body (functional pinealectomy), there is an imbalance of cyclicity in the work of body systems, desynchronosis develops. It is known that the liver is not only a unique metabolic and immunological organ, it is the largest source of lymph production in the body, and it accounts for up to 50% of the lymph entering the thoracic duct [Tanaka M. and Iwakiri Y., 2016]. It was found that under the influence of constant lighting in the liver and its lymphatic region, circulatory and lymphatic flow disorders develop. These processes lead to the development of hypoxia, which negatively affects the structure and function of the organ parenchyma cells [Michurina S.V. et al., 2008, 2018; Tanaka M. and Iwakiri Y., 2018]. The aim of this study was to evaluate the effect of 24-hour illumination (24hL) on the expression of the LIVE-1 marker (lymphatic vessel endothelial receptor-1) in liver cells of male C57Bl / 6 mice.



Material and Methods. The males of C57Bl/6 mice aged 10-12 weeks were kept in the SPF-vivarium center of ICG SB RAS. Food and water were provided to animals *ad libitum*. The intact animals (the group "Control", n=6) were kept at the standard lighting mode – photoperiod light/dark: 14/10 hour (h). The mice of the experimental group (the gr. "24hL", n=6) were kept for 14 days under conditions of 24-hour lighting - photoperiod light/dark: 24/0 h. Immunohistochemical determination of the LIVE-1 receptor was performed in paraffin sections of the liver. Computer morphometric analysis of digital photos at magnification x400 was carried out using "Image J" program. The relative areas and brightnesses of zones staining for LIVE-1 were calculated. In this case, the brightness of the stained area is a parameter inversely proportional to the marker concentration in this zone.

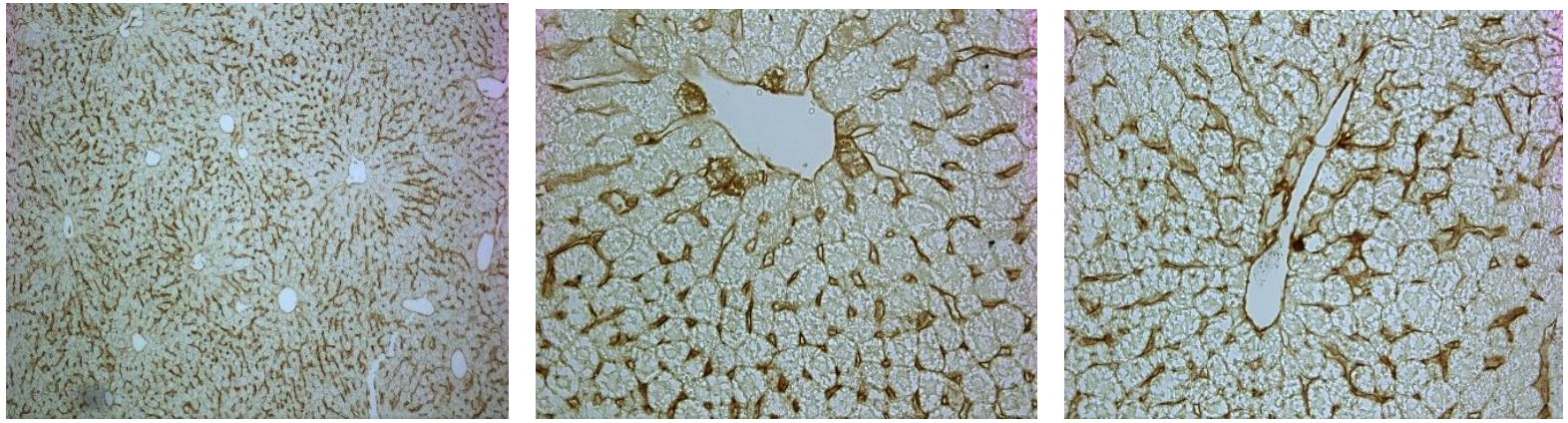


Fig. 1. "Control" mice: a - general view of the liver preparation (magnification x100), b – the pericentral zone (x400), c - the periportal zone (x400).

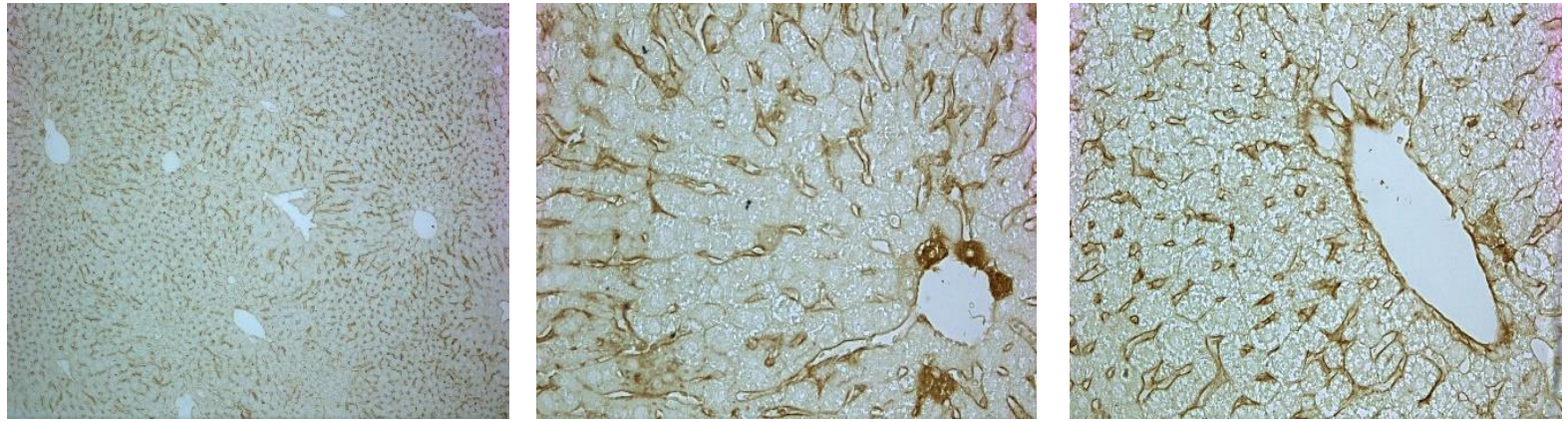
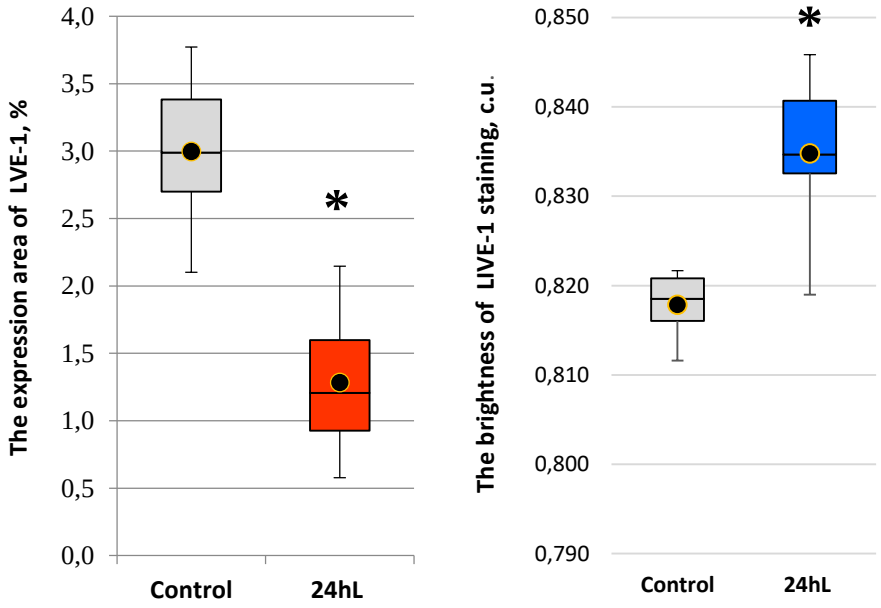


Fig. 2. "24hL" mice: a - general view of the liver preparation (x100), b – the pericentral zone (x400), c - the periportal zone (x400).

Morphometric analysis of mouse liver preparations after two weeks of continuous illumination revealed a two-fold **decrease in the area** of expression of the LIVE-1 marker against the background of **an increase in brightness** of LIVE-1 area staining by 2% (inversely proportional to the marker concentration in this zone; (*) the Mann-Whitney U-test; $p<0,01$).

The study of the expression of the LIVE-1 marker in the liver of **intact mice under standard lighting** mode revealed pronounced immunohistochemical staining of sinusoid cells of blood capillaries in the periportal regions and especially in the perineal zones of the hepatic lobes. In the parenchyma, individually colored hepatocytes were sometimes found (Fig. 1).

In **animals kept under round-the-clock lighting**, a weaker expression of LIVE-1 was detected in the endothelium of sinusoid capillaries, especially in the intermediate zones (Fig.2).



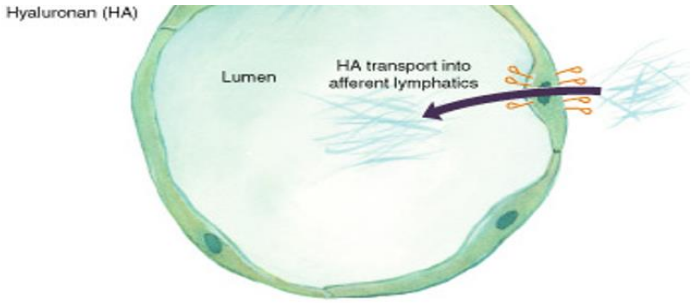
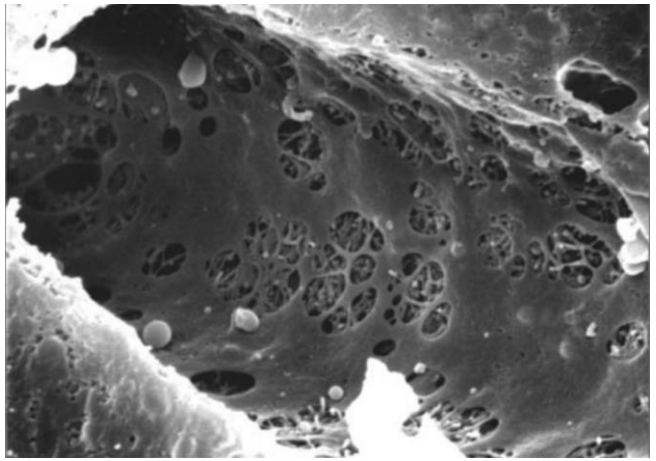


Figure adapted from Jackson D.G. *et al.* (2001) Trends Immunol. **22**:317.

LYVE-1 is known to be a transmembrane protein receptor in endothelial cells of lymphatic vessels for hyaluronic acid (hyaluronan = HA), an extracellular matrix mucopolysaccharide [Breslin J.W. et al., 2018]. Hyaluronan is the most powerful water-binding molecule in our body; it can absorb water 1000 times its mass [Day A.J and Sheehan J.K., 2001].

The pronounced expression of LIVE-1 in the endothelium of liver sinusoids in intact mice is confirmed by the results of other researchers. It was found that LYVE-1 is expressed in the liver not only by endothelial cells of lymphatic vessels but also by endothelial cells of blood sinusoid capillaries, as well as in some macrophages. In our study, the staining on LIVE-1 of portal and especially middle zones of liver acini in intact animals was revealed.

In liver diseases, the expression of LIVE-1 may be reduced. Thus, in cirrhosis and hepatocellular carcinoma, LYVE-1 expression is suppressed [Carreira Mouta C.C. et al., 2001]. In chronic hepatitis and cirrhosis of the liver, a decrease in the expression of LYVE-1 in the endothelium of sinusoids was detected, especially in areas adjacent to active inflammatory or fibrotic lesions. It is known that parenchyma cells in the intermediate zones are most sensitive to oxidative stress. In this case, the sinusoidal endothelium in the affected areas, demonstrating the weakening of LYVE-1, loses fenestration, which the authors attribute to the progression of liver disease [ArimotoJ. et al., 2010]. Disorders in the lymphatic vasculature that cause inadequate drainage can lead to a condition characterized by tissue swelling, which is the result of the accumulation of protein and fluid in the interstitium [Saharinen P. et al., 2004].



Sinusoid of a rat liver with fenestrated endothelial cells. Scanning electron micrograph from Vollmar B. and Menger M.D. (2009) Physiol Rev **89**: 1269–1339.

Conclusion. In this regard, the weak expression of LYVE-1 detected by us on the membranes of endothelial cells of sinusoidal capillaries in mice kept for a long time under continuous illumination may indicate a violation of the functioning of the fields of fenestration of these cells. This can lead to a decrease in the endocytotic activity of the latter, difficulty in blood-tissue exchange, deterioration of lymphatic drainage in the liver, and development of tissue hypoxia.

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