Ultrastructural changes in murine liver following HSV infection and stroke

Natalia Motorna[1], Svetlana Rybalko[2], Tatyana Kvitnitskaya-Ryzhova[3],
Daria Starosyla[2], Iryna Strokina[1], Rostyslav Kaminsky[1],
Sergey Savosko[1], Liudmyla Sokurenko[1], Yuri Chaikovsky[1]

[1] O.O. Bogomolets National Medical University, Department of Histology and Embryology, T. Shevchenko Blvd 13, 01601 Kyiv, Ukraine
[2] Gromashevsky L.V. Institute of Epidemiology and Infection Diseases of NAMS of Ukraine, Kyiv, Ukraine
[3] State Institution "D.F. Chebotarev Institute of Gerontology" of NAMS of Ukraine, Kyiv, Ukraine

ARTICLE INFO	ABSTRACT
	The study of herpetic infection is a topical problem. Although the peculiarities and consequences of acute HSV-I infection in the brain are quite well-studied, little is known about the damage to other organs which are not a source of latent HSV-I infection, the liver in particular. The current study is aimed at determining the ultrastructural changes in murine liver following HSV infection and stroke. Liver samples obtained from four groups of animals were studied: 1) intact mice; 2) mice with stroke; 3) mice infected with HSV-I; 4) mice aflicted with HSV-I and subsequently simulated stroke. The study showed the reproduction of the virus in hepatic endotheliocytes, although no virions were detected in the hepatocytes. Therefore, the described changes were considered the consequences of the infectious process. Pathological changes of hepatocytes consisted of deformation and fragmentation of the nuclei, as well as accumulation of osmiophilic granules, lysosomes and lamellary bodies. Latent HSV-I infection may reactivate in liver after the stroke, potentially causing the complications of the underlying disease.

INTRODUCTION

The study of herpetic infection is a topical problem. HSV infection has a latent course, and reactivates in situations of impaired immunity or pathologies of other bodily systems, the nervous system in particular [1,2]. Although the peculiarities and consequences of acute HSV-I infection in the brain are quite well-studied [3,4], little is known about the damage to other organs which are not a source of latent HSV-I infection, the liver in particular. What is more, the literature does not provide any reliable evidence on the post-stroke development of herpetic infection. This infection is detected late, and not infrequently, the diagnosis is made based on the autopsy results since the course of the disease is asymptomatic [5]. Elevated levels of HSV-I in the blood plasma and brain of infected mice after stroke were found in experiments [6], indicating the reactivation of HSV-I infection in the presence of brain damage. Thus, the current study is aimed at determining the ultrastructural changes in murine liver following HSV infection and stroke.

* **Corresponding author**
e-mail: l-sokurenko@i.ua

MATERIALS AND METHODS

The experiments were conducted on 36 BALB/c line mice weighing 20-24 g. Four groups of animals were studied: 1) intact mice; 2) mice with stroke; 3) mice infected with HSV-I; 4) mice aflicted with HSV-I and subsequently simulated stroke. The laboratory animals were infected with attenuated HSV-I (the museum strain of the virus specially adapted for studies using laboratory mice). In doing so, 0.03 ml of viral material which equals to LD_{50} was inoculated into the brains of mice. To simulate a stroke, 0.01 ml of autoblood was injected into the right hemisphere of the brain 30 days after infection. Subsequently, 10 days later, samples of liver were obtained for electron microscopy .

The liver fragments were fixed in a 2.5% solution of glutaraldehyde, in a phosphate buffer, with additional fixation in 1% buffered solution of osmium tetroxide. Dehydration was carried out in alcohols of ascending concentration (70%, 80%, 90%, 100%), as well as acetone. The biological specimens were embedded into a epon-araldite mixture. For targeted orientation, the semi-thin sections were stained with toluidine blue, followed by ultra-thin sectioning using a Reihert (Austria) ultratome. Contrasting was performed

with 2% solution of uranyl acetate and lead citrate. The ultra-thin sections were then studied under and photographed by electron microscope Tescan Mira 3 LMU (Czech Republic) with magnifications of ×10000-80000. All procedures with the laboratory animals were conducted in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986).

PCR and dot-ELISA were used to confirm the HSV-I in liver samples by detecting viral antigens in the Vero cell culture. The Vero cell culture was grown in sterile trays ("Nunc"). The infected trays were then incubated in culture medium (88% RPMI 1640 medium "Sigma") with the addition of 12% of heat inactivated fetal calf serum (FCS) and antibiotics at 37°C with 5% of CO_2. Cytopathic activity (CPA) – syncytia formation – served as a marker of viral reproduction. CPA was registered for 6-7 days, and HSV-I DNA in biological specimens was found by PCR via hybridization-fluorescent detection, using the set of reagents "AmplySensR HSV- I, II-FL". The "DNA-sorb-AM" set of reagents was used for DNA extraction. DNA was extracted from each studied specimen in the presence of an internal control specimen (BKO-FL). Dot-ELISA was performed on nitrocellulose filters. The filters with the applied specimens were dried and immersed in the solution containing 30 mg/ml BSA in 0.01 M Tris pH 7.5 – 0.15 M NaCl buffer, and incubated for two hours at 37°C. After the incubation in PAP (peroxidase-antiperoxidase complex), the filters were rinsed six times and, for the enzyme to develop, immersed into the substrate consisting of: 3.8 ml of DAB (diaminobenzidine tetrachloride) + 0.2 ml of 0.1% H_2O_2. The reaction was registered when the centers of the wells turned yellow. More detailed results of this study are presented in the earlier articles [6,7].

All laboratory (virology and experimental) procedures on animals were carried out in the Gromashevsky L.V. Institute of Epidemiology and Infection Diseases of NAMS of Ukraine in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was approved by the Bioethical committee for human subjects or animal research in Bogomolets National Medical University, Minutes №100, February 21, 2017.

RESULTS

The liver samples obtained from nine animals infected with HSV-I were studied by electron microscopy method. All samples revealed ultrastructural changes of liver, however the virions were found only in the endotheliocytes of one sample, although in vitro HSV-I were found in all nine samples of the infected animals. The diameter of electron-dense "core" of the virion was 280 nm, and with the outer contour of the capsid – 450 nm (Fig.1). In the liver of the infected mice, with stroke simulated on day 30, no virions were detected in endotheliocytes and hepatocytes, however the nature of ultrastructural changes was similar to those found in animals without stroke. PCR and dot-ELISA

in the selected liver samples of the group 4 animals were positive as well. Changes in the liver were focal, with deformation and fragmentation of hepatocytes nuclei, a significant number of osmiophilic granules, lysosomes and lamellar bodies in the cytoplasm of hepatocytes, edema of hepatocyte cytoplasm, fibroblasts and endothelial cells recorded. In separate cells, inclusions of glycogen and lipids were found.

Lipid inclusions, organelles reduction and hepatocytes cytoplasm edema were also found in non-infected mice with stroke, however the morphology of the hepatocytes nuclei bore no signs of fragmentation, inclusions or vacuolization.

The general morphology of the liver lobules was clearly visible in the investigated samples of all the animal groups. An RBC (red blood cell) stasis, impaired endothelial structure, and destruction of the basement membranes were found in the central veins and blood capillaries. These findings indicate the loss of the barrier function of the endothelium in the hepatic micro-vessels, which may explain the penetration of the infection into the liver via the circulatory system from the primary source of infection. Damage of liver endothelium in case of cerebral stroke, similar to our model of posttraumatic intracerebral hemorrhage, is an important factor in the systemic damage of vascular endothelium or in a structural manifestation of the development of endothelial dysfunction. It allows a better understanding of the delayed outcomes of stroke in other organs and infectious complications, in particular.
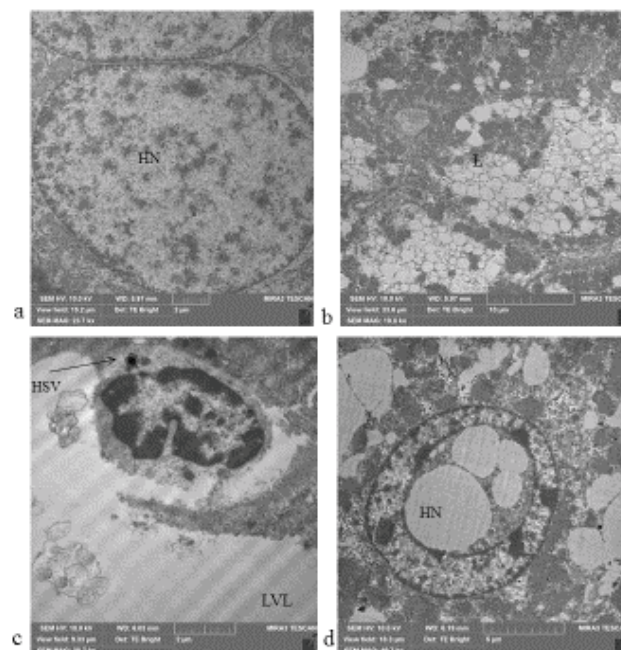
HN - hepatocyte nucleus; EN – endotheliocyte nucleus; E - erythrocyte; L - lipid inclusion; LVL - lumen of the lobular vein; HSV - HSV-I virion
Fig. 1. Transmission electron microscopy image of mice liver. Note: a - control group; b - group with stroke; c - HSV group; d - HSV group and stroke

DISCUSSION

Light microscopy can detect only the consequences of an infectious process in the liver, although the immunohistochemical method has shown the intracellular localization of HSV in liver hepatocytes [8,9,10]. As shown in literature [6],

HSV-I is capable of reactivating in the brain. The purpose of the current study was to find signs of post-stroke HSV-I reactivation in the liver.

The results of this study have shown the virus to reproduce in the endothelial cells of the liver, although no virions were found in the hepatocytes. Therefore, the described changes were considered the consequences of the infectious process. Unfortunately, electron microscopy did not allow differentiating the detected changes in the liver between the groups of HSV-I infected mice with post-infection simulation of stroke. It was only the assessment of the hepatocyte nuclei morphology that revealed the domination of nuclei deformation and fragmentation in the setting of HSV-I infection as compared to the stroke group. As a rule, ultrastructural changes in the cells are non-specific for many forms of organ damage. Still, nuclear fragmentation does not look like necrosis, since from the pathogenetic point of view, cell necrosis is induced by multifactorial and versatile pathobiochemical impairments both within the cell, and in its environment. Vacuolization of the membrane organelles, impaired membrane integrity, reduced number of organelles, nuclear edema and chromatin dispersion (lysis of the nucleorprotein complexes) are characteristic evidence of cell necrosis. In our own study, a number of organelles, mitochondria in particular, as well as certain elements of the endoplasmic reticulum were present and often preserved in the samples with confirmed HSV-I. This undermines the fact that the revealed fragmentation of the hepatocyte nuclei is the initial manifestation of necrosis. We associate these changes with the consequences of the viral existence. At the same time, reduction of the cytosol organelles is a non-specific feature of cell damage or death. Detection of the mature virions would allow a better study of the virus-induced cytopathology. However, as shown by the results described in the article, this is quite a complex problem. It may be assumed that the appearance of virions, i.e. viral reproduction, occurs focally, i.e. in single cells.

In literature the stroke episode itself is not considered a factor of morphological liver damage. Moreover, liver changes have been described only in a few articles. Thus, in article [11] changes in bilirubin and liver enzymes following ischemic stroke reflect two phenomena which are simultaneously associated with the extent of brain infarction: 1) inflammation followed by an increase of C-reactive protein, WBC and gamma-glutamyl-transferase (γ-GT) and reduction of hemoglobin and non-conjugated bilirubin; and 2) unknown signal, independent from the inflammation, which leads to elevated levels of enzymes, indicating liver damage, amely glutamate-oxaloacetate-transaminase (GOT) and glutamate-piruvate-transaminase (GPT). However these articles are scarce and describe functional changes which are not confirmed by morphological data. Another quite a large category of articles describe cases of stroke development in the presence of chronic liver damage (liver cirrhosis, alcohol use) [12]. The authors describe the worsening of the body status and neurological disorders in the presence of a chronic disease, however, they do not refute the likelihood of emergence of such disorders [13]. There are no studies assessing the relation between liver damage and reactivation of latent infection in the case of acute hemorrhagic stroke. Thus, a latent HSV-I infection can be reactivated in the liver following a stroke, potentially causing complications of the underlying disease. However the small number of samples in our study and the lack of other similar studies looking at the association between the acute stroke and systemic complications in the body, require further and larger studies.

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