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State of Astrocytes in the Mice Brain under Conditions of *Herpes* Viral Infection and Modeled Stroke

A. V. Gumenyuk,[1] A. A. Tykhomyrov,[2] S. I. Savosko,[1] M. M. Guzyk,[2] S. L. Rybalko,[3] A. O. Ryzha,[4] and Yu. B. Chaikovsky[1]

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Changes in astrocytes of the mice brain induced by infection with *herpes simplex* virus type 1 (HSV-1) and modeling of hemorrhagic stroke were examined by recording immunohistochemical labeling of glial fibrillary acidic protein (GFAP) and measuring the perimeters of astrocyte profiles. Five groups of BALB/c mice were examined: 1, intact animals (control); 2, animals infected with HSV-1 (museum strain, group HSV); 3, animals with modeled hemorrhagic stroke (HS); 4, animals with HSV-1 infection and subsequently developed HS (HSV+HS), and 5, animals infected with HSV-1 and with HS, which were treated by acyclovir (50 mg/kg, i.p., for 10 days; HSV+HS+ACV). Intracerebral hematomas in the HS groups were created by injection of autologous blood into the right hemisphere. Immunohistochemical assay revealed that herpetic infection induced hyperactivation of brain astroglial cells; somewhat more moderate activation of the astroglial cells was observed in the case of experimental stroke. Cortical and hippocampal astrocytes in the groups with HS and viral infection were characterized by significantly greater average values of visible perimeters of the sections of these cells. Administration of acyclovir to the infected mice provided significant reduction of the density and perimeter of the GFAP-positive astrocytes in the cortex and area CA1 of the hippocampus compared to groups 2, 3, and 4 ($P < 0.05$). Morphological and immunohistochemical changes in astrocytes indicate that acyclovir has a potential for modulation of the level of brain astroglia reactivation during herpetic infection. The specific protein of the astrocytes (GFAP) may serve as a marker of the efficacy of neurotropic action of antiviral drugs.

Keywords: astrocytes, reactive astrogliosis, glial fibrillary acidic protein (GFAP), intracerebral hemorrhage, *herpes simplex* virus, acyclovir.

INTRODUCTION

In the intact brain, astrocytes are responsible for a number of functions, including trophic, barrier, and protective ones. Under conditions of neurodegenerative diseases, this type of gliocytes also plays important roles [1]. Astrocytes respond to brain damage induced by different reasons (inflammation, ischemia, and injuries of other geneses) by an enlargement of their somata and elongation and arborization of the processes [2, 3]. In addition to hypertrophy, astrociytes may also proliferate and migrate, mostly toward the perivascular areas. Such a reaction is qualified as reactive gliosis. When brain tissues are damaged, increased expression of glial fibrillary acidic protein (GFAP) is observed in astrocytes under the respective conditions [4]; this allows one to use this protein as a marker of adverse effects on the CNS [5]. A decreased density of astrocytes surrounding the areas of ischemia in brain tissues may be indicative of degeneration of astroglia and subsequent loss of the functions of the latter. Conversely, hyperactivation of astroglial cells and formation of a glial scar can be related to neuroinflammation and inhibition of the reparative processes in neural tissues [7, 8].

Infection by *herpes simplex* virus type I (HCV-1) may result in penetration of this virus in brain tissues, and it is related to the intensification of GFAP expression [5]. Latent HCV-1 can be present in neurons for a long time, which makes

[1] Bogomolets National Medical University, Kyiv, Ukraine
[2] Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv, Ukraine
[3] Gromashevsky Institute of Epidemiology and Infectious Diseases, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
[4] Educational and Scientific Center "Institute of Biology and Medicine", Taras Shevchenko Kyiv National University, Kyiv, Ukraine
Correspondence should be addressed to S.I. Savosko
(e-mail: sergey.savosko@nmu.ua).

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the respective treatment quite challenging [9]. Effects of viral infection are opposed by activation of endogenous protective mechanisms in the brain, in particular due to increased synthesis of interferon-λ (IFN-λ). Infection-related events in astrocytes induced by HCV-1 are accompanied by intensification of the production of this interferon, which is indicative of the antiviral activity of the latter [10]. Often, activation of endogenous mechanisms of the brain resistivity with respect to viral infections is insufficient, which requires administration of exogenous specific medications. Among such agents, there is acyclovir, which was shown to provide antiherpetic effects via inhibition of herpetic DNA-polymerase. Reactions of astrocytes to attempts for treatment of HSV-1 infection have not been studied earlier.

In our experiments, we have studied reactions of astrocytes in the murine brain to HSV-1 infection, to modeled hemorrhagic stroke (HS), to a combined action of the above factors, and to the treatment of the latter experimental pathology by acyclovir. We examined changes in the GFAP expression and those in dimensions and form of the astrocytes in different experimental groups.

METHODS

The experiments were carried out on albino BALB/c mice with a mean body mass of 18–20 g. The following groups of animals were formed: 1, control (intact animals, n = 10); 2, mice infected with HSV-1 (n = 15); 3, animals with modeled hemorrhagic stroke (HS, n = 15); 4, mice infected with HSV-1 with subsequent modeling of stroke (group HSV+HS, n = 40), and 5, animals infected with HSV-1 and with modeled stroke, which received acyclovir (group HSV+HS+ACV, n = 10).

The mice of groups 2, 4, and 5 were infected with HSV-1 by injection of a museum strain of this virus adapted for research on mice; injections were performed into junctions of the zygomatic and squamose bones of the skull. The dose used (0.03 ml of the standard solution corresponded to 4.0 lg LD_{50}). Regression of the symptoms of viral infection was observed on days 15–16 after the injection. From this time, HSV-1 was considered to transfer into its latent form. For modeling of HS in groups 3 and 4, we used injections of 0.15–0.2 ml of autologous blood into tissues of the right hemisphere (L = 1.5; H = 3.0; B = –1.0 from the bregma) [12, 13]. This procedure was made in groups 4 and 5 on day 30 after infection with HSV-1, thus reproducing a combined stroke + viral infection pathology. In group 5 of the animals, acyclovir was administered for 10 days (i.p., 50 mg/kg). Animals of group 1 were withdrawn from the experiment on day 40, while those with modeled stroke were withdrawn on day 10 after induction of the latter.

The brains of experimental animals were taken off after decapitation, fixed in 4% paraformaldehyde solution, dehydrated in ethanol, and embedded in paraplast (Leica Surgipath Paraplast Regular, Germany). Six-μm-thick frontal brain sections were prepared on a Thermo Microm HM 360 microtome (Thermo Fisher Scientific Microm, Germany). The sections were deparaffinized in xylene and descending ethanol concentrations, air-dried, and washed with buffered normal saline (PBS). Then, deparaffinized sections were blocked in 3% solution of bovine serum albumin (BSA) in PBS, which contained 0.05 % Triton X-100 (PBST), for 60 min at room temperature. Then, the sections were incubated with anti-GFAP antibodies (rabbit anti-GFAP antibodies, sc-9065, Santa Cruz Biotechnology, USA; dilution in PBST 1:200 for 18 h at +4°C). The sections were washed three times in PBST and incubated for 60 min at room temperature with the appropriate secondary antibodies, conjugated with fluorescein isothiocyanate (FITC, dissolved 1:500 in PBST). The washed sections were then stained with a fluorescent dye, Hoechst-33342 (1.0 μg/ml), which allowed us to visualize the cell nuclei of living cells. Results of immunofluorescent staining were observed using an LSM 510 Meta laser scanning microscope (Carl Zeiss, Germany) and subsequently processed with Zeiss ZEN software. CarlZeiss (AxioVision SE64 Rel.4.9.1) software was used to measure changes in the dimensions of GFAP-positive cells by measuring the perimeters of their pericaryonal sections (μm). These perimeters were determined by contours of the visible GFAP-positive profiles, which included the soma and proximal parts of the astrocyte processes. The number of measured perimeters of cortical and hippocampal astrocytes in the above-mentioned experimental groups varied from 22 to 40.

Because distributions of the measured numerical values differed significantly from the normal law, the nonparametric Kruskal–Wallis test was used in intergroup comparisons. The numerical values are presented below as medians and 25 to 75% quartile intervals (Q1–Q3). Data samplings were analyzed using Origin Lab, version 8.0.

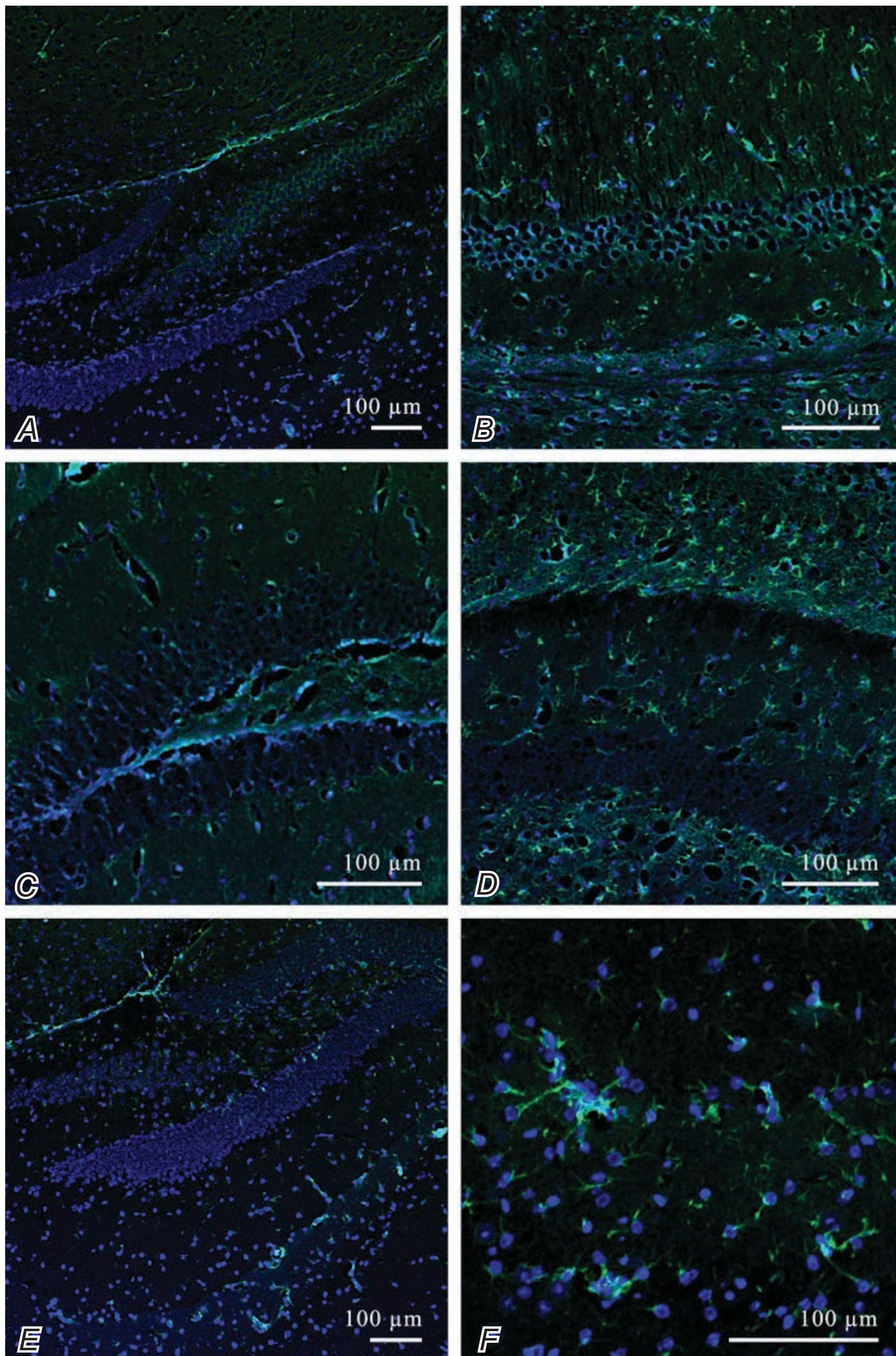
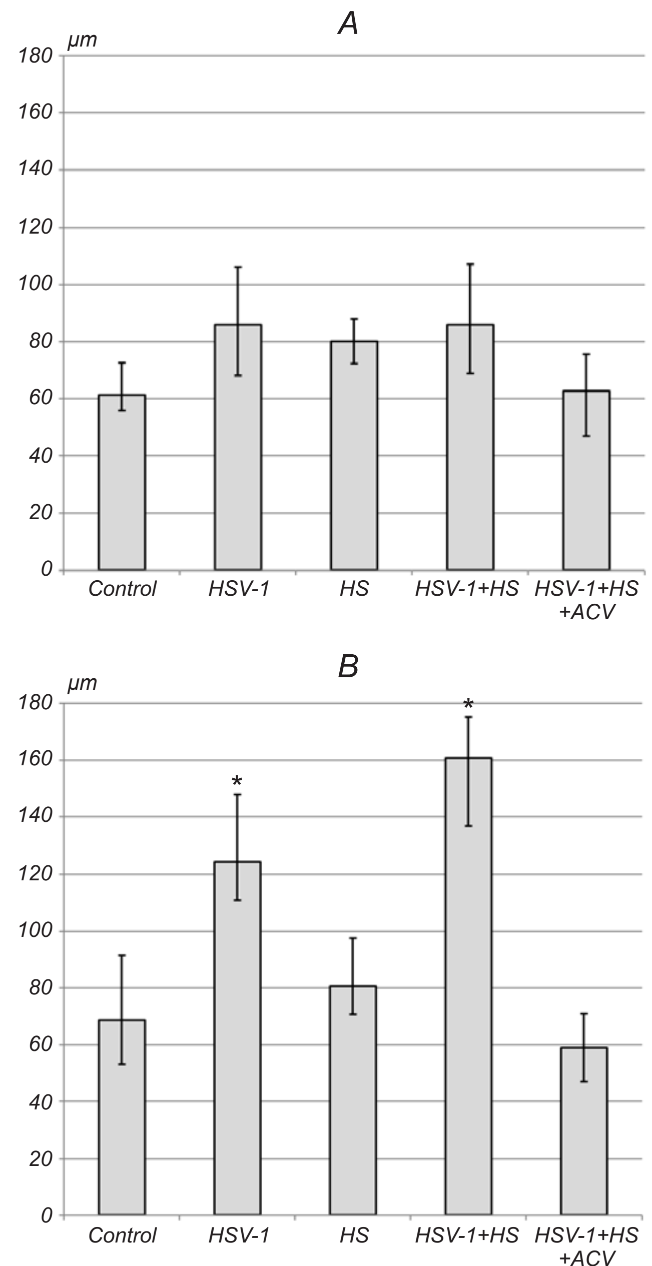


Fig. 1. GFAP immunofluorescence in the CA1 area of the hippocampus; laser confocal scanning microscopy. A) Intact animal (group 1, control); B) mouse infected with HSV-1 (group 2, HSV); C) mouse with experimental hemorrhagic stroke (group 3, HS); D) animal with combined HSV infection and stroke (group 4, HSV+HS), and E and F) mice with a combination of viral infection and stroke treated with acyclovir (group 5, HSV+HS+ACV). Green color, GFAP immunofluorescence; blue color, DNA staining with Hoechst 33342.

RESULTS

Significant reactive changes of astrocytes in the brain cortex and area CA1 of the hippocampus were observed after both introduction of HSV-1 and modeling of HS. Results of the respective immunohistochemical assays are shown in Fig. 1. In the control group 1 (Fig. 1 A), GFAP-positive sites were found only around the nuclei and in single short processes of the astrocytes. In general, it should be concluded that astrocytes in the control group 1 practically showed no signs of reactive changes. In the brains of mice of group 2 (HSV), the intensity of GFAP-positive staining of astrocytes was significantly greater (Fig. 1 B). The respective cells were characterized by greater lengths and more intense arborization of the processes, which should be considered signs of clear reactive changes. In animals of group 3 (HS), immunostaining of the astrocytes was more moderate, as compared to that in group 2 (Fig. 1 C). Intense GFAP-positive staining was observed around a number of vessels and in astrocytes of deep layers of the brain cortex. Mice of group 4 (HSV+HS) also demonstrated strong manifestations of reactive astrogliosis, especially in the CA1 area of the hippocampus (Fig. 1 D). In group 5 (HSV+HS+ACV), the level of specific GFAP immunofluorescence was significantly lower in comparison with those in the cortex and hippocampus of mice of groups 2, 3, and 4 (Fig. 1 E, F). Assessment of the astrogliosis topography showed that reactive changes noticeably prevailed in astrocytes within the CA1 area of the hippocampus. This can be related to a closer location of this cerebral area to the site of the hemorrhage in mice of groups 3 and 4. It should, however, be mentioned that intense reactive gliosis was observed in this hippocampal region also in mice of group 2, i.e., in animals with no modeled HS. Thus, it can be concluded that neurons and astrocytes of the area CA1 were significantly damaged by viral infection. This conclusion is confirmed by a considerably smaller intensity of Hoechst-33342 fluorescence in mice of groups 2 and 4, which is indicative of noticeable nucleolysis (in other words, death of gliocytes and neurons) (Fig. 1 B, D). In group 5 (HSV+HS+ACV), the level of Hoechst-33342 fluorescence was significantly higher in comparison with that in groups 2, 3, and 4 (Fig. 1 E, F). This fact is indicative of a higher number of survived astrocytes and neurons in the brain areas of group-5 mice, i.e., of a significantly lower level of neurodegeneration.

The measurements of perimeters of the visible GFAP-positive astrocyte profiles (those of the soma and proximal parts of the processes) showed that both viral infection and modeling of HS (either separately or in combination) resulted in significant increases of these values of astrocytes in the brain cortex and CA1 area of the hippocampus ($P < 0.05$ in all cases of comparisons, Fig. 2 A, B).



F i g. 2. Averaged values of the perimeter (μm) of pericaryonal sections of astrocytes in different experimental groups. A and B) Cortical astrocytes and those of area CA1 of the hippocampus. Median values and limits of the second and third quartiles of distributions are shown. Asterisks show cases of significant differences ($P < 0.05$) in comparisons with the control.

After modeling of stroke, averaged (modal) values of the perimeters of sections of cortical and hippocampal astrocytes were greater than those in the control group by about 31 and 17%, respectively. Infection by HSV-1 exerted noticeably more intense effects on dimensions of cortical and hippocampal astrocytes; the increments of the analyzed parameter of cortical neurons in groups 2 (HSV) and 4 (HSV+HS) were about 40%. Increases in the dimensions of astrocytes of the hippocampal CA1 area in these two groups were much greater (Fig. 2 B). In group 2, the respective increment was more than 80%, while in group 4 (HSV+HS), the increase in the average perimeter was more than twofold (+134%).

The treatment of experimental animals subjected to combined influences of viral infection and modeled stroke by acyclovir (group 5) led to significant normalization of the measured dimensional characteristic of astrocytes (perimeter of their sections). With respect to cortical neurons, such normalization was nearly complete; in hippocampal neurons, the modal value of the perimeter in the above group was even smaller than in the control (Fig. 2 B).

DISCUSSION

HSV-1 is a polytropic virus capable of reproducing in brain cortical neurons [14], astrocytes [15, 16], and endotheliocytes [17]. Immunohistochemical assay allowed us to conclude that astrocytes respond to infection with HSV-1 (when the latter is in the latent form) by the development of clearly expressed reactive gliosis. It was also found that astrogliosis in the CA1 area of the hippocampus under conditions of viral infection (group 2) is comparable or even more pronounced than that in the same hippocampal area in the group with modeled stroke (group 3). In this study, we cannot formulate a strictly unambiguous answer for the cause of dramatic astrogliosis under conditions of the combined pathology (HSV+HS), i.e., what plays a leading role, injury/ischemia or a direct effect of HSV-1 on astrocytes? Nevertheless, to the best of our knowledge, our paper is the first report describing the combine effect of stroke and HSV-1 infection on astrocytes (astroglial response). Based on our previous results [5, 18], we can hypothesize that HSV-1 may be reactivated after simulation of HS (formation of a local brain hematoma), although there is a lack of information related to the above problem in the existing literature. Some publications [19], however, described changes in the GFAP expression in response to HSV-1 in the murine BALB/c model; the increased expression of this glial protein was shown in astrocytes under *in vitro* conditions [20]. Altogether, these findings are indirect, while significant proof in favor of possible HSV-1 reactivation in astrocytes should be taken into account. Thus, changes in the GFAP level can be considered, from a certain aspect, a marker of the viral reproduction activity. As was found [19], the increased GFAP expression in the murine cornea was observed in the acute phase of inflammation, and this was followed by a decrease in the GFAP level. It was assumed that lowering of the GFAP content can be related to transition of the virus to a long-time latency.

It should be specially noted that, in our experiments, hippocampal neurons in the CA1 area were found to be specifically sensitive to HSV-1 infection. Changes in their dimensions under conditions of isolated action of HSV-1 and combined action of this virus and modeled HS were much more intense than those in cortical neurons ($P < 0.05$ in both cases).

As for changes in the astrocyte responses to HSV-1 under the influence of acyclovir, the existing data are scarce and contradictory. Ramakrishna et al. [21] analyzed changes in the GFAP levels in the cerebrospinal fluid (CSF) of patients suffering from multiple sclerosis and treated with acyclovir. The authors found no considerable changes in the above index within the period of treatment. An association of acyclovir effects with some alleviation of the neurological deficit and level of IL-6 in mice was shown [17]; the authors, however, did not examine in this case the state of astrocytes using immunohistochemical tests.

Thus, we found that infection with HSV-1 leads to the development of rather intense reactive astrogliosis in the mice brain; changes in the GFAP level are quite demonstrative markers of pathological changes in the brain induced by HSV-1 infection. The latter infection may probably noticeably complicate the consequences of stroke. The state of astroglial cells can be used as a marker of regression of viral infection-related inflammation under the action of antiviral agents, acyclovir in particular.

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All procedures for laboratory animals were conducted in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986), and Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The authors, A. V. Gumenyuk, A. A. Tykhomyrov, S. I. Savosko, M. M. Guzyk, S. L. Rybalko, A. O. Ryzha, and Yu. B. Chaikovsky, declare the absence of any conflict in commercial or financial relations, relationships with organizations or persons that in any way could be related to the study, and also in interrelations of the co-authors.

REFERENCES

1. A. A. Tykhomyrov, A. S. Pavlova, and V. S. Nedzvetsky, "Glial fibrillary acidic protein (GFAP): on the 45th anniversary of its discovery," *Neurophysiology*, **48**, No. 1, 54-71 (2016).
2. M. A. Anderson, J. E. Burda, Y. Ren, et al., "Astrocyte scar formation aids CNS axon regeneration," *Nature*, **532**, No. 7598, 195-200 (2016).
3. S. Sirko, M. Irmler, S. Gascón, et al., "Astrocyte reactivity after brain injury: the role of galectins 1 and 3," *Glia*, **63**, No. 12, 2340-2361 (2015).
4. M. Sofroniew, "Astrocyte barriers to neurotoxic inflammation," *Nat. Rev. Neurosci.*, **16**, No. 5, 249-263 (2015).
5. A. V. Gumenyuk, S. L. Rybalko, S. I. Savosko, et al., "GFAP as a marker of reactive astrocytes in the mice brain following hemorrhagic stroke and HSV-I," *Biopolym. Cell,* **33**, No. 6, 415-423 (2017).
6. A. Gumenyuk, N. Motorna, S. Rybalko, et al., "Development of herpetic infection associated with stroke and its correction with acyclovir," *Curr. Issues. Pharm. Med. Sci*., **30**, No. 1, 20-23 (2017).
7. J. E. Burda, A. M. Bernstein, and M. V. Sofroniew, "Astrocyte roles in traumatic brain injury," *Exp. Neurol.*, **275**, No. 3, 305-315 (2016).
8. S. L. McKenney, F. F. Mansouri, A. D. Everett, et al., "Glial fibrillary acidic protein as a biomarker for brain injury in neonatal CHD," *Cardiol. Young*, **26**, No. 7, 1282-1289 (2016).
9. J. P. Wang, G. N. Bowen, S. Zhou, et al., "Role of specific innate immune responses in Herpes Simplex Virus infection of the central nervous system," *J. Virol.*, **86**, No. 4, 2273-2281 (2012).
10. J. Li, L. Ye, X. Wang, et al., "Induction of IFN-lambda contributes to TLR3-mediated HSV-1 inhibition in astrocytes," *J. Neurosci. Res.*, **90**, No. 2, 399-406 (2012).
11. Y.-C. Jiang, H. Feng, Y.-C. Lin, and X.-R. Guo, "New strategies against drug resistance to herpes simplex virus," *Int. J. Oral Sci.,* **8**, No. 1, 1-6 (2016).
12. A. V. Gumenyuk, N. V. Motorna, S. L. Rybalko, et al., "Mutual influence of herpes virus infection activation and cerebral circulation impairment on the state of brain cells," *Biopolym. Cell,* **32**, No. 2, 126-130 (2016).
13. G. Paxinos and K. B. J. Franklin, *The mouse brain in stereotaxic coordinates*. Academic Press, San Diego (2001).
14. A. Majer, K. A. Caligiuri, K. K. Gale, et al., "Induction of multiple miR-200/182 members in the brains of mice are associated with acute Herpes Simplex Virus 1 encephalitis," *PLoS ONE*, **12**, No. 1, e0169081 (2017).
15. L. S. Reinert, L. Harder, C. K. Holm, et al., "TLR3 deficiency renders astrocytes permissive to herpes simplex virus infection and facilitates establishment of CNS infection in mice," *J. Clin. Invest.,* **122**, No. 4, 1368-1376 (2012).
16. L. Yue, S. Guo, Y. Zhang, et al., "The modulation of phosphatase expression impacts the proliferation efficiency of HSV-1 in infected astrocytes," *PLoS ONE*, **8**, No. 11, e79648 (2013).
17. N. V. Motorna, S. L. Rybalko, L. M. Sokurenko, et al., "Patterns of herpetic infection reactivation in the liver," *Mikrobiol. Zh.*, **79**, No. 5, 70-79 (2017).
18. G. Zhao, H. Chen, Z. Song, et al., "Glial fibrillary acidic protein expression during HSV-1 infection in mouse cornea," *APMIS,* **122**, No. 2, 128-135 (2014).
19. M. J. Carlucci, L. A. Scolaro, and E. B. Damonte, "Inhibitory action of natural carrageenans on Herpes simplex virus infection of mouse astrocytes," *Chemotherapy*, **45**, No. 6, 429-436 (1999).
20. M. Pekny and M. Pekna, "Astrocyte reactivity and reactive astrogliosis: costs and benefits," *Physiol. Rev.* **94**, No. 4, 1077-1098 (2014).
21. C. Ramakrishna, M. Golub, A. Chiang, et al., "Effects of acyclovir and IVIG on behavioral outcomes after HSV1 CNS infection," *Behav. Neurol.*, **2017**, 5238402 (2017).