Original research

Immunohistochemical analysis of GFAP expression in the experimental sepsis-associated encephalopathy

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article;
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Pathophysiology of sepsis-associated encephalopathy (SAE) is linked to blood-brain barrier breakdown, neuroinflammation and neurotransmitter imbalance in the brain. Astroglia, the most abundant cell population within the brain, plays the critical role in control of all kinds of homeostatic processes, thereby regulating the adaptive reactions of the brain to various challenges. Astroglia are highly heterogenous across the brain regions, therefore, damaging factors stimulate heterogenous astroglial reactivity and response in different brain regions.

The aim of this study was determining immunohistochemical features of GFAP expression in various brain regions in the model of rodent experimental sepsis.

Materials and methods. The experiment was performed in Wistar rats: control group of 5 sham-operated rats and the main group of 20 rats subjected to cecum ligation and puncture (CLP) procedure. The immunohistochemical study of GFAP expression in the sensorimotor cortex, subcortical white matter, hippocampal, thalamic and caudate nucleus/putamen regions was performed from 20 to 48 hours of the postoperative period.

Results. Starting from the 12th hour after CLP, animals began display progressive increase in signs of periorbital exudation, piloerection, fever-/hypothermia, diarrhea, social isolation, lethargy, and respiratory impairment. In the period of 20–38 hours, 9 animals showed expressed previously listed symptoms and were euthanized (CLP-B – lethal group), 11 rats survived until 48 hours of the experiment (CLP-A – survived group). In the lethal group, starting from 20 to 38 hours after the CLP procedure, a significant (relative to control) regionally-specific dynamic increase in the level of GFAP expression was observed in the brain: in the cortex – by 465 %, in the subcortical white matter – by 198 %, in the hippocampus – by 250 %, from the 23rd hour – in the caudate nucleus/putamen by 18 %. In the thalamus, no significant changes in the level of GFAP expression were observed. In the cortex and hippocampus of survived animals, 48 h after CLP, higher values of GFAP expression were observed comparing to the group of non-survived animals.

Conclusions. Under conditions of the experimental SAE, an early dynamic increase in the astroglial reactivity was observed in the cortex, hippocampus, white matter, and caudate nucleus/putamen of the brain with the most significant increase of indicators in the cortex and hippocampus, which potentially indicates relatively more vulnerable areas of the brain to damaging factors, as well as places of the most active intercellular interaction in the condition of systemic inflammation. Higher values of GFAP expression in the cortex and hippocampus of survived animals at 48 hours of the experiment, compared with indicators of non-survived group, indicate increased astroglial reactivity in these brain regions at the noted time period, accompanied by relatively more favorable clinical course of the disease.

Імуногістохімічний аналіз експресії GFAP при експериментальній сепсис-асоційованій енцефалопатії

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Патогенез сепсис-асоційованої енцефалопатії (САЕ) пов'язують із пошкодженням гематоенцефалічного бар'єра, нейрозапаленням і дисбалансом нейромедіаторів у мозковій тканині. Астроглія, найчисленніша популяція клітин у головному мозку, відіграє критичну роль у контролі всіх видів гомеостатичних процесів, регулюючи адаптивні реакції мозку у відповідь на різні фактори його пошкодження. Астроглія дуже неоднорідна в різних відділах мозку, тому фактори, що викликають пошкодження тканини, ініціюють гетерогенні клітинні реакції.

Мета роботи – визначення імуногістохімічних особливостей експресії GFAP у різних відділах мозку за умов експериментальної моделі сепсису в щурів.

Матеріали та методи. Дослідження здійснили на щурах лінії Вістари: контрольна група включала 5 хибнооперованих щурів; 20 тваринам виконали перев'язку та пункцію сліпої кишки (CLP). Здійснили імуногістохімічне дослідження експресії маркера GFAP у сенсомоторній корі, підкірковій білій речовині, гіпокампі, таламусі, хвостатому ядрі/лушпині в період з 20 до 48 години після CLP-процедури.

Результати. Починаючи з 12 години після CLP-процедури, у тварин почали прогресувати ознаки періорбітальної ексудації, пілоерекція, гіпер-/гіпотермія, діарея, соціальна ізоляція, летаргія та респіраторні порушення. У період з 20 до 38 години 9 тварин на тлі суттєвого погіршення стану були евтаназовані (підгрупа CLP-B – загиблі щури), 11 особин вижили до кінця експерименту, 48 годин (підгрупа CLP-A – тварини, які вижили). У підгрупі CLP-B у строк із 20 до 38 години після процедури CLP у головному мозку спостерігали вірогідне (щодо контролю) регіонально-специфічне динамічне

збільшення рівня експресії GFAP: у корі – на 465 %, підкірковій білій речовині – на 198 %, гіпокампі – на 250 %; з 23 години – на 18 % у хвостатому ядрі/лушпині. У таламусі не визначили вірогідну зміну рівня експресії GFAP. У корі та гіпокампі тварин, які вижили, через 48 годин після CLP встановили вищі значення експресії GFAP порівняно з групою загиблих щурів.

Висновки. В умовах експериментальної САЕ спостерігали раннє динамічне підвищення реактивності астроглії в корі, гіпокампі, білій речовині і хвостатому ядрі/лушпині головного мозку з найбільшим підвищенням показників у корі та гіпокампі. Це потенційно вказує на відносно вразливіші для факторів пошкодження ділянки мозку, а також на місця найактивнішої міжклітинної взаємодії під час системного запалення. Вищі значення експресії GFAP у корі та гіпокампі тварин, які вижили до 48 години експерименту, щодо показників групи загиблих щурів указують на посилення астрогліальної реактивності в названих ділянках мозку в цей період на тлі відносно сприятливішого клінічного перебігу захворювання.

Иммуногистохимический анализ экспрессии GFAP при экспериментальной сепсис-ассоциированной энцефалопатии

Т. В. Шулятникова, В. А. Туманский

Патогенез сепсис-ассоциированной энцефалопатии (САЭ) связывают с повреждением гематоэнцефалического барьера, нейровоспалением и дисбалансом нейромедиаторов в мозговой ткани. Астроглия, наиболее многочисленная популяция клеток в головном мозге, играет главенствующую роль в контроле всех видов гомеостатических процессов, регулируя адаптивные реакции ткани при воздействии различных повреждающих факторов. Астроглия очень неоднородна в разных отделах мозга, поэтому повреждающие факторы активируют гетерогенные клеточные реакции.

Цель работы – определение иммуногистохимических особенностей экспрессии GFAP в разных отделах мозга в условиях экспериментальной модели сепсиса у крыс.

Материалы и методы. Эксперимент проведён на крысах линии Вистар: контрольная группа включала 5 ложно-оперированных крыс; 20 животным выполнена перевязка и пункция слепой кишки (CLP). Иммуногистохимическое исследование экспрессии белка GFAP проведено в материале коры, подкоркового белого вещества, гиппокампа, таламуса, хвостатого ядра/скорлупы в срок от 20 до 48 часов после CLP-процедуры.

Результаты. Начиная с 12 часов после CLP-процедуры, у животных начали прогрессировать признаки периорбитальной экссудации, пилоэрекция, гипер-/гипотермия, диарея, социальная изоляция, летаргия и респираторные нарушения. В период с 20 до 38 часа 9 животным на фоне значительного ухудшения состояния проведена эвтаназия (подгруппа CLP-B – погибшие крысы), 11 особей выжили до конца эксперимента, 48 часов (подгруппа CLP-A – выжившие животные). В подгруппе CLP-B в сроке с 20 до 38 часа после процедуры CLP в головном мозге наблюдали достоверное (относительно контроля) регионально-специфическое динамическое увеличение уровня экспрессии GFAP: в коре – на 465 %, подкорковом белом веществе – на 198 %, гиппокампе – на 250 %; с 23 часа – на 18 % в хвостатом ядре/скорлупе. В таламусе достоверное изменение уровня экспрессии GFAP не выявлено. В коре и гиппокампе выживших животных через 48 ч после CLP установлены более высокие значения экспрессии GFAP по сравнению с группой погибших крыс.

Выводы. В условиях экспериментальной САЭ отмечено раннее динамическое повышение реактивности астроглии в коре, гиппокампе, белом веществе и хвостатом ядре/скорлупе головного мозга с наиболее существенным повышением показателей в коре и гиппокампе. Это потенциально указывает на относительно более уязвимые для повреждающих факторов области мозга, а также на места наиболее активного межклеточного взаимодействия при системном воспалении. Более высокие значения экспрессии GFAP в коре и гиппокампе выживших животных к 48 часам эксперимента относительно показателей группы погибших крыс указывают на усиление астроглиальной реактивности в данных областях мозга в этот период на фоне относительно более благоприятного клинического течения заболевания.

Sepsis is one of the most common life-threatening states leading to intensive care units (ICU) admission and patient's death [1]. Sepsis-associated encephalopathy (SAE) – is a complex brain dysfunction on the background of sepsis with the incidence up to 70% of all septic ICU patients [2]. Clinical course of SAE ranges widely from sickness behavior to coma and associated with higher risks of lethal outcome and frequent development of long-term cognitive impairment [3,4].

Pathophysiology of SAE is linked to blood-brain barrier (BBB) breakdown, neuroinflammation, water and neurotransmitter disbalance, glial and neuronal dysfunction and death, meanwhile, these mechanistic components seem to be not universal but rather context-dependent in each case and region-specific for the brain.

Astrocytes, the most numerous cell population inside the central nervous system (CNS), constantly provide wide range of fundamental homeostatic functions including neurotransmitter and water balance, microcirculation, BBB permeability, synaptic function and plasticity etc. [5].

In conditions of SAE, as well as in the whole spectrum of neuropathologies, astrocytes become reactive but in case of loose homeostatic capacity (astrodegeneration with astroglial atrophy and pathological remodeling of astrocytes) might account to the failure of tissue homeostasis leading to diverse severe functional CNS disorders [6]. Astrogliosis combines remodelling of genetic, morphological and functional phenotype of cells leading to their hypertrophy, upregulation of glial fibrillary acidic protein (GFAP), changing in receptors function and secretome modification [7]. GFAP is one of the intermediate filament proteins of mostly matured astrocytes and its increased expression is considered as a key marker of astroglial reactivity and is the most commonly used as a hallmark of reactive astrocytes in both human and animal brain [8]. Animal models and tissue cultures studies provided

evidence of highly beneficial effects of increased GFAP expression during tissue damage in context of various pathologies suggesting protective potential of astroglial reactivity [9], although for many decades and still this mention is highly speculative [10].

The role of reactive astrogliosis and its specificity throughout the brain during SAE is still ambiguous and needs further clarification determining the relevance of presented study. Considering close interaction between neuroglial populations in healthy and diseased brain as well as the results of our previous study showing region-specific diversity of microglial reactivity during experimental SAE, it seems critical to reveal the level of astroglial reactivity in the same brain regions to assess compatibility of glial reactiveness.

Aim

To determine immunohistochemical features of GFAP expression in the various regions of the brain in the model of rodent experimental sepsis.

Materials and methods

The study was performed in Wistar rats, 200–300 g ("Biomodelservice", Kyiv, Ukraine). The animals were kept under standard conditions, with free access to food (standard food for rats, "Biomodelservice", Kyiv, Ukraine) and water. All experimental procedures were ruled in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, 18 March 1986; ETS No. 123) and the Directive 2010/63/EU on the protection of animals used for scientific purposes.

Experimental animals were subjected to the cecal ligation and puncture (CLP) septic model in rodents. Rats were divided into 2 groups: CLP group (n = 20) and sham-operated control group (n = 5). All further experimental stages were conducted by the way displayed in our previous paper [11]. Within 2 hours after CLP-procedure, rats were routinely observed every 30 min, further every 6 hours up to 48 h-time-point after operation. Starting from the 12th hour the following clinical signs gradually increased in animals as periorbital exudation, piloerection, diarrhea, fever/hypothermia, social isolation, deep lethargy and severe respiratory disorders. During the period of 20–38 h after CLP-procedure, in the main group of animals, 9 rats showed expressed mentioned clinical symptoms and were euthanized (CLP-B – non-survived/lethal), 11 animals survived until 48 hours – end-point of the experiment (CLP-A – survived). Sham-operated control rats (CLP-C) showed no lethal outcomes. Animals from CLP-A and CLP-C groups were sacrificed at 48 hours after CLP-procedure by intraperitoneal overdosing of sodium thiopental.

Transversal sections of the rat brain were fixed in 10 % buffered formalin, conventionally processed and embedded in paraffin. Histopathological analysis of samples was performed using hematoxylin-eosin stained sections. Immunohistochemical (IHC) study was run in accordance with the protocol of the antibody manufacturer using mouse monoclonal anti-GFAP primary antibody

Table 1. The level of GFAP expression in the various regions of the rat brain in different studied groups expressed in the percent of positive labels in standardized field of view of the microscope (S rel. (%)). Data are displayed as median (Me) and lower, upper quartiles (Q1; Q3)

Brain region	CLP-A	CLP-B	CLP-C
Cortex	16.32 (16.06; 17.51)*†	14.75 (12.97; 15.36)*†	2.61 (2.58; 3.20)
Subcortical white matter	30.47 (26.90; 32.92)*	30.01 (25.32; 30.60)*	10.04 (6.98; 11.27)
Hippocampus	19.44 (18.78; 20.60)*†	15.49 (16.94; 18.21)*†	4.43 (2.92; 5.04)
Thalamus	4.04 (3.78; 4.58)	3.92 (3.20; 4.46)	3.83 (3.20; 4.42)
Caudate/putamen	8.18 (7.56; 11,17)*	8.09 (6.27; 11.26)*	6.82 (5.66; 7.48)

*: statistically significant differences compared to control values (P < 0.05) are marked with an asterisk; **†:** significant differences between CLP-A and CLP-B groups (P < 0.05); **CLP-A:** survived; **CLP-B:** non-survived; **CLP-C:** control.

(clone ASTRO6, Thermo Scientific, USA) and Ultra Vision Quanto Detection system with DAB (Thermo Scientific Inc., USA). The products of the immunohistochemical reaction were evaluated at x200 in the standardized field of view (SFV) of the Scope. A1 "Carl Zeiss" (Germany), Jenoptik Progres Gryphax 60N-C1"1,0x426114 (Germany) photo-camera and Videotest-Morphology 5.2.0.158 (Video Test LLC, RF) program. The level of GFAP protein expression in the tissue section was analyzed as the relative area (S rel., %) of GFAP$^+$-stained labels to the total area of an SFV. For the comparative analysis of the noted criterion, such brain regions as sensorimotor cortex, subcortical white matter, thalamic, hippocampal, and caudate nucleus/putamen regions were studied. We analyzed five SFVs of each listed brain region for each rat.

Statistical procedures were performed using Statistica® for Windows 13.0 (StatSoft Inc., license No. JPZ804I382130ARCN10-J) with evaluation of the median (Me) and lower and upper quartiles (Q1; Q3). The comparison of the differences between studied groups was carried out using the Mann–Whitney U-test and Kruskal–Wallis test. The results were considered statistically significant at 95 % (P < 0.05).

Results

During conventional light optical histopathological analysis of the samples from different brain regions of all experimental groups, the shape and size of astrocytic bodies and nuclei did not show prominent changes. While immunohistochemical study revealed substantial morphological shifting of astroglia. Comparing different brain regions in control CLP-C sham-operated rats at 48 h of the experiment we noted significant nonequivalence of the GFAP expression with the highest level in the subcortical white matter – 10.04 (6.98; 11.27) % and the lowest one in the cortex – 2.61 (2.58; 3.20) % (*Table 1*). In control animals the morphology of conditionally intact astrocytes presented region-dependent diversity. Cortical GFAP$^+$ astroglia were mostly represented by typical protoplasmic forms with relatively small nucleus and cell body with moderate branching of fine medium length processes forming small extensions in the pericapillary endfeet. GFAP-positive astrocytes of the white matter were characterized by morphology of fibrous forms – had slightly smaller cell body with lesser branching but more thick processes, which as well as cortical forms did not overlap processes of the nearest astrocytes. GFAP$^+$
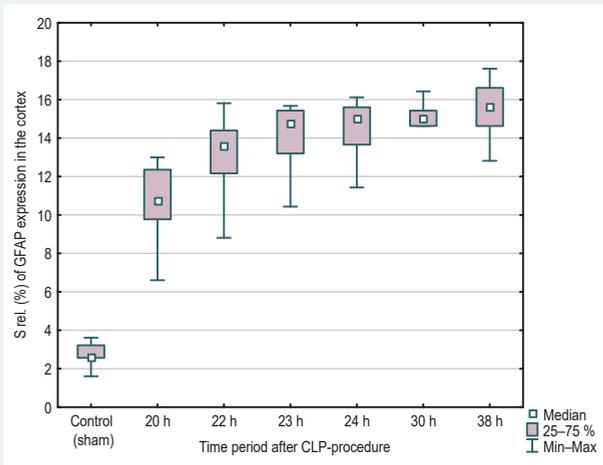
Fig. 1. The dynamics of GFAP⁺ expression after CLP-procedure in the cerebral cortex of animals in the lethal group.
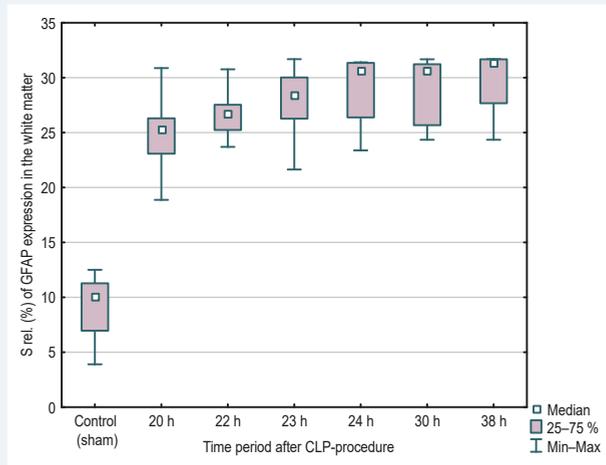

Fig. 2. The dynamics of GFAP⁺ expression after CLP-procedure in the subcortical white matter of animals in the lethal group.
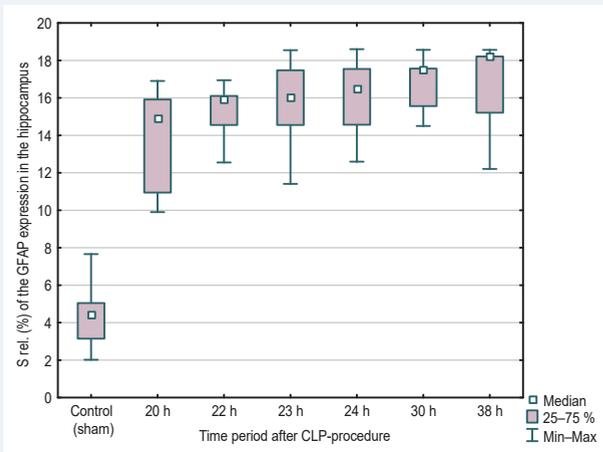

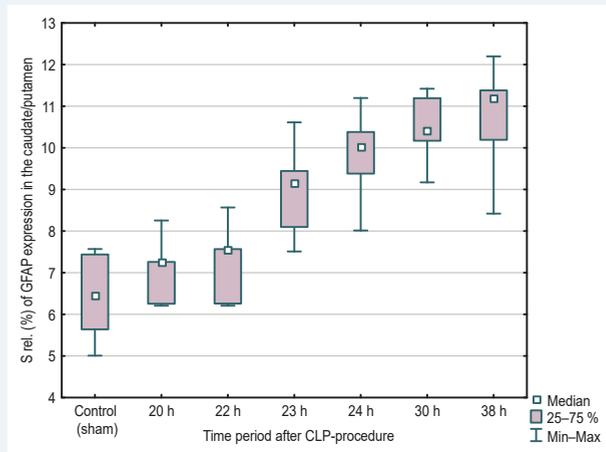**Fig. 3.** The dynamics of GFAP⁺ expression after CLP-procedure in the hippocampus of animals in the lethal group.

Fig. 4. The dynamics of GFAP⁺ expression after CLP-procedure in the caudate nucleus/putamen of animals in the lethal group.

astrocytic pool of the hippocampus, thalamus and caudate nucleus/putamen differed by relative morphological complexity and included the both mentioned morphological phenotypes with the prevalence of the fibrous one. In the vast majority of brain regions of sham-operated rats GFAP⁺ astroglia tended to localize in close contact with the vascular walls, forming a well-defined coupling (*Figs. 5, 7, 9, 11*).

Experimental CLP-A (survived) and CLP-B (non-survived) groups showed more serious difference both in the GFAP expression degree and in the time period of its increase during the experimental course.

As well as in control animals the highest numerical indicators of GFAP expression in rats after CLP were in subcortical white matter. Thus, in CLP-A group the relative area of GFAP⁺ labels had the highest data among all brain regions and experimental groups, and was equal to 30.47 (26.90; 32.92) %, P < 0.05 comparing to control, thereby exceeding control indicators by 3 times (by 203 %). In the CLP-B non-survived rats, elevation of GFAP S rel. (%) also displayed statistical validity and was equal to 30.01 (25.32; 30.60), exceeding control indicators by 198 %. Simultaneously, there was no statistically significant dif-

ference between indicators of CLP-A and CLP-B groups in this region (*Table 1*).

When comparing the reliable increasement of GFAP level in relation to control values, it was found that the lowest one was typical for caudate nucleus/putamen region and was equal to 8.18 (7.56; 11,17), (exceed control indicators by 20 %) and 8.09 (6.27; 11.26), (exceed control indicators by 18 %) for CLP-A and CLP-B groups respectively, with no statistical difference between noted groups (*Table 1*).

In the hippocampus the reliable increasement of the GFAP S rel. (%) comparing to control was even more expressed then in subcortical white matter and was equal to more than 4 times increasement in the CLP-A group of survivors. Thus, the values of GFAP S rel. (%) in CLP-A and CLP-B groups were respectively: 19.44 (18.78; 20.60), (exceed control indicators by 339 %) and 15.49 (16.94; 18.21), (exceed control indicators by 250 %); (significant at P < 0.05 comparing to control and between each other) (*Table 1*).

The most substantive increasement of the GFAP expression was noted in the cortex of CLP-A survival animals, where it was equal to more than 6 times compared
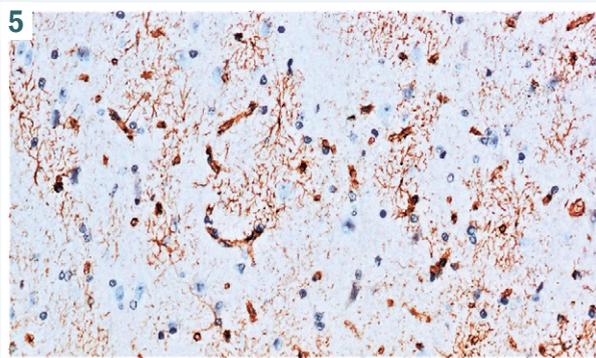
Fig. 5. Cortical expression of GFAP in the control rat 48 h after the sham procedure (anti-GFAP, Thermo Scientific, USA). ×200.
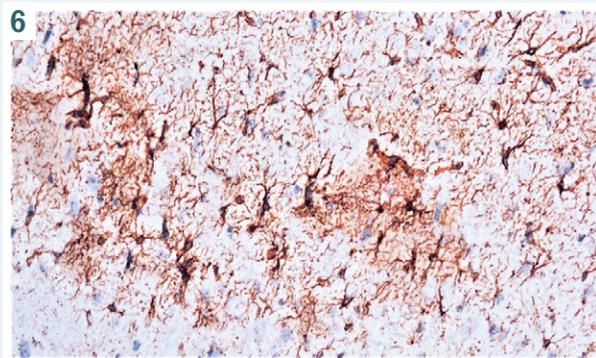

Fig. 6. Cortical expression of GFAP in the survived rat 48 h after the operation (anti-GFAP, Thermo Scientific, USA). ×200.
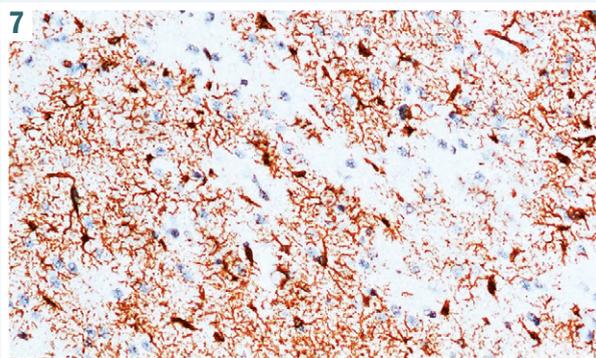

Fig. 7. White matter expression of GFAP in the control rat 48 h after the sham procedure (anti-GFAP, Thermo Scientific, USA). ×200.
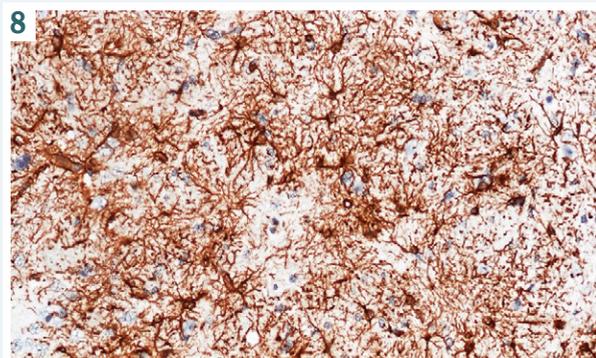

Fig. 8. White matter expression of GFAP in the survived rat 48 h after the operation (anti-GFAP, Thermo Scientific, USA). ×200.
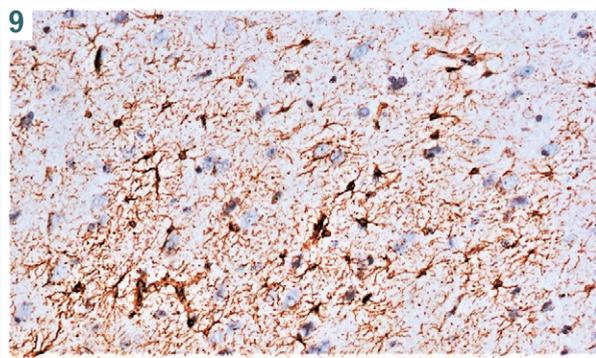

Fig. 9. Hippocampal expression of GFAP in the control rat 48 h after the sham procedure (anti-GFAP, Thermo Scientific, USA) ×200.
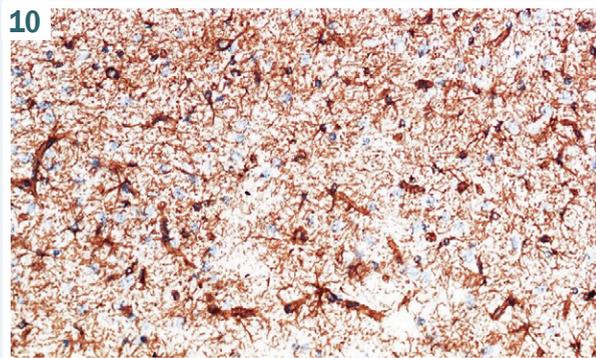

Fig. 10. Hippocampal expression of GFAP in the survived rat 48 h after the operation (anti-GFAP, Thermo Scientific, USA). ×200.
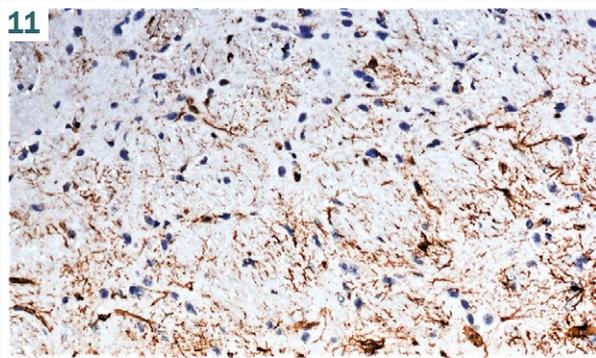

Fig. 11. Caudate nucleus/putamen expression of GFAP in the control rat 48 h after the sham procedure (anti-GFAP, Thermo Scientific, USA). ×200.
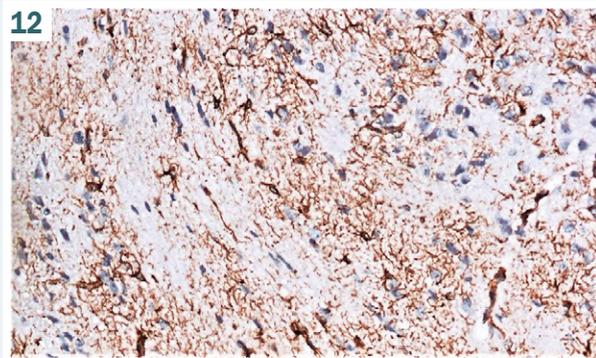

Fig. 12. Caudate nucleus/putamen expression of GFAP in the survived rat 48 h after the operation (anti-GFAP, Thermo Scientific, USA). ×200.

to control. In CLP-A the expression of the marker was substantially higher than in CLP-B group, respectively: 16.32 (16.06; 17.51), (exceeding control indicators by 525 %) and 14.75 (12.97; 15.36), (exceeding control indicators by 465 %); (significant at P < 0.05 comparing to control and between CLP-A and CLP-B) (*Table 1*).

Although the values of GFAP expression were increased comparing to sham-operated rats in the thalamus, the difference between all three groups for this region was not statistically reliable (*Table 1*).

Summarizing the above, the highest values of GFAP+ S rel. (%) in all studied regions were typical for the survived CLP-A animals with the most substantial increase in the cortex and hippocampus (comparing to control rates). The significant difference in values of GFAP expression between operated groups was revealed for the cortical and hippocampal regions with higher medians in survived animals 48 h after procedure compared to the medians of CLP-B indicators across all time-points.

In CLP-B group, during the postoperative period the elevation of GFAP indicators reflected the smooth dynamic curve (*Figs. 1–4*). Depending on the time period after CLP-procedure when animals of CLP-B group began displaying profound clinical signs of systemic decompensation and was sacrificed, the levels of GFAP expression both displayed specificities. The maximal significant values of the GFAP S rel. (%) was found 38 h after the operation in all the studied regions except thalamic, where increased indicators were not statistically reliable (*Figs. 1–4*). Unlike the cortical, white matter and hippocampal regions, where significant elevation of the GFAP expression was found at 20 h after CLP-procedure, in caudate nucleus/putamen region it was noted only at 23 h after CLP-procedure.

During histopathological study of ICH sections, the increased numbers of GFAP+ cells per SFV were immediately noted. The morphology of GFAP-positive astrocytes differed by appearance of territorial domain overlapping between neighboring cells when their cell bodies lay in close proximity to each other, and their numerous thickened processes crossed with each other, forming a kind of rough fibrous network. Capillary endfeet of such astrocytes thickened, clearly delineating the vascular walls over a large extent. The most expressed mentioned morphological shifting was typical for subcortical white matter and hippocampal regions (*Figs. 6, 8, 10, 12*).

Discussion

In the healthy mammal brain, a wide array subtypes of astrocytes were recognized according to their developmental lineage, genomics, proteomics, receptor expression, morphology and physiology [8,12,13]. Besides protoplasmic and fibrous glia there are other morpho-functional phenotypes as radial glia known as neuronal and glial progenitors during brain development, ependymal glia, perivascular glia, tanycytes, Bergmann cerebellar glia, Müller glia of retina and velate glia [12]. Within the human CNS astroglial population also includes special unique forms as interlaminar astrocytes and the varicose projection astrocytes [12,14]. Molecular heterogeneity of astroglia seems much complex. Studies have revealed

that the same morphological phenotype can differ by expression of molecules involved in cell function either across different brain regions as well as within the same area. It is supposed that noted proteomic and functional heterogeneity of astroglia may determine the special vulnerability of distinct brain regions to specific inner and outer insults [14]. Regarding special astroglial markers, they were not exclusion. Among the most broadly used molecular markers for astroglial identification including GLT-1 (EAAT2), GLAST (EAAT1), aquaporin-4, S100β, Aldh1L1, glutamine synthetase, connexins 30 and 43, GFAP takes a leading position. Even physiologically all mentioned markers are not equally expressed by all subpopulations of astrocytes across the brain and display substantial diversity, allowing to assume that mentioned heterogenous phenotypes do not shape the whole extension of suggested diversity and the latter might be greatly specified by the brain region, age, physiological state of the brain and the kind of pathological stimuli.

Due to GFAP molecule has eight different isoforms, there is increased possibility of its diverse expression by different subpopulations of astrocytes [15,16]. It has been estimated that astrocytic volume is much bigger than their GFAP+ profiles display, as their fine processes are mainly GFAP- and although currently GFAP considered as the optimal marker for astroglial detection, it can reveal only up to 15 % of total cell volume in rodent brain [12]. Upregulation of GFAP is a sign of the most reactive astrocytes, however, not all variety, therefore does not decode the function and heterogeneity of the population studied with this marker.

It was evidenced that during aging in rodents the expression of GFAP increased regionally including such areas as basal ganglia, hippocampus and corpus callosum [14] and may vary even across different areas of hippocampus [17]. Results of the recent study have demonstrated the higher GFAP expression in the adult spinal cord compared to the whole brain in health and after focal demyelinating injury [18].

Neuroinflammation is one of the principal processes involved in the pathophysiology of SAE as well as excitotoxicity due to neurotransmitter dysfunction, and ischemic lesions. Astrocytes are considered as critical players of neuroinflammatory CNS response. They involved in engaging and retaining leukocytes at damaged brain regions, produce both anti-inflammatory and proinflammatory cytokines, anti-oxidants, free radicals etc., thus controlling inflammatory response magnitude. In case of systemic inflammatory challenge, astrocytes tend to increase secretion of pro-inflammatory cytokines acquiring neurotoxic properties and persist longer in pro-inflammatory phenotype in relation to microglial counterparts [7].

In our recent study linked to microglial phagocytic activity in the experimental SAE, we have revealed the brain regions where glial reactiveness was the most intense in the white matter and caudate/putamen which was suggested as indication of the regions with the most profound tissue damage and active neuroinflammatory response to systemic inflammation [19]. The current study has evidenced that astroglial reactivity generally showed a similar trend in timing and localization of the most active events in comparison to microglial reactions in the same

pathologic conditions. In all studied cases of non-survived CLP-B group of animals there was early (starting from the 20 h) increase in GFAP expression in the sensorimotor cortex, subcortical white matter and hippocampus and in caudate nucleus/putamen region at the 23rd h after CLP-procedure. This results almost completely reflect the behavior of microglia within noted brain regions except only caudate/putamen region where astroglial reactiveness was belated compared to microglial one. Thalamic area in both our studies displayed absence of statistically significant increase in GFAP expression which likely may indicate this region or astroglial population of it as the least susceptible to the toxic influence of aggressive factors that arise in the brain medium under conditions of systemic inflammation. The most pronounced en-hancement of the GFAP expression compared to control showed cortical and hippocampal regions of survived animals (6-fold and 4-fold, respectively) which potentially indicate the most vulnerable brain regions for incoming systemic aggressive molecules and/or local damaging factors, as well as locations of the most active glia-glia/glia-neuron interactions in response local homeostatic failure. These regions should receive more attention in further studies on SAE pathophysiology as zones of highly up-regulated glial reactivity, which per se might serve as a marker of tissue processes of particular importance in pathogenesis of any kind of brain pathology. The higher values of GFAP expression in the cortex and hippocampus of survived CLP-A animals in respect to CLP-B levels in the same areas may indicate the principal role of reactive astrogliosis in the mechanisms of tissue adaptation in the noted regions to the arisen pathologic condition and may suggest the particular significance of mentioned regions in the mechanisms of SAE.

Conclusions

1. Starting from the 20th hour after CLP-operation (from the 23rd h in caudate nucleus/putamen), the reliable (relative to control) region-specific dynamic increase in GFAP expression was observed in the cortex, subcortical white matter, hippocampus, caudate nucleus/putamen of the rat brain (except the thalamus) and the most substan-tive increase of indicators in cortex and hippocampus. The latter potentially may indicate the most vulnerable brain regions affected by damaging factors, as well as locations of the most active intercellular communication in response to systemic inflammatory challenge.

2. Higher values of GFAP expression in the cortex and hippocampus of survived animals at 48 hours of the experiment, compared with indicators of non-survived group, indicate increased astroglial reactivity in these brain regions at the noted time period, accompanied by relatively more favorable clinical course of the disease.

Prospects for further research. Given the mecha-nisms of SAE are still to be elucidated and suggesting special role of glial reactivity in these processes, it would be reasonable to proceed further studies in the field of brain intercellular communication in response to systemic inflammation.

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