Immunohistochemical study of the brain glutamine synthetase expression in the rat septic model

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Severe sepsis is accompanied by multiple organ dysfunction where acute liver failure (ALF) plays one of the most critical roles. The principal sign of acute hepatic encephalopathy accompanying ALF is ammonia induced edema of astrocytes. In case of ALF in sepsis, one can suppose increase in systemic and brain ammonia. Astroglia are target cells for ammonia metabolism as they are principal source of glutamine-synthetase (GS). Previous studies have reported that ALF stimulates increase in astroglial GS which correlates with deterioration of the animal state.

The aim of the study was to determine the level of glutamine synthetase expression in different brain regions of rats in the conditions of experimental sepsis.

Materials and methods. The study was conducted in Wistar rats: 5 sham-operated (control) animals and 20 rats with cecum ligation and puncture (CLP) septic model. Immunohistochemical study of GS expression was performed in the cortex, white matter, hippocampus, thalamus, caudate nucleus/putamen in the period of 20–48 h after CLP.

Results. Starting at 12th h after CLP, operated animals displayed progressive impairment finished by profound lethargy and respiratory failure. Between 20–38 h, 9 animals expressed final mentioned symptoms and were euthanized (CLP-B, non-survived), 11 rats displayed less expressed suffering up to 48 h (CLP-A, survived). At 23 h in CLP-B and 48 h in CLP-A rats, liver tissue displayed morphological signs of the focal irreversible damage which was aggravated with time after CLP which could be observed dynamically in non-survived group. Both CLP-A and CLP-B rats showed gradual elevation of GS in all studied brain regions. From 24 to 38 h after CLP, non-survived animals showed significant region-specific dynamic increase in GS expression: in the cortex – by 69.35 %, hippocampus – by 53.6 %, thalamus – by 50.0 %; with the most substantive elevation in the cortex – 1.69-fold increase compared to control.

Conclusions. In CLP model, 24 h after operation there is significant dynamic increase in GS level in the cortical, hippocampal and thalamic regions of the rat brain with the most prominent in the cortex. Heterogeneous increase in GS indicates regions more or less vulnerable for incoming systemic agents as well as region-specific reactiveness of the brain tissue, including local astroglia, to these factors in septic conditions. Morphological signs of sepsis-associated liver damage preceding significant increase in the brain GS by 1 h, might suppose addition of the liver failure to the course of sepsis which is accompanied by increased level of hepatogenic toxins (presumably including ammonia) in the blood and in the brain parenchyma by 23 h after CLP. The latter might propose an active implication of hepatogenic detrimental agents in sepsis pathophysiology and involvement of reactively increased brain GS levels in the complex mechanisms of sepsis-associated encephalopathy.

Імуногістохімічне дослідження експресії глутамінсинтетази в головному мозку щурів в умовах моделі сепсису

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Тяжкий перебіг сепсису супроводжується синдромом поліорганної недостатності, в якому гостра печінкова недостатність (ГПН) відіграє одну з найважливіших ролей. Головна ознака гострої печінкової енцефалопатії, що супроводжує ГПН, – набряк астроцитів, індукований аміаком. У разі розвитку ГПН під час сепсису можна також припустити підвищення рівнів системного та мозкового аміаку. Астроцити – ключові клітини для метаболізму аміаку в мозку, оскільки є основним джерелом глутамінсинтетази (GS). Попередні дослідження показали: ГПН призводить до підвищення рівнів астрогліальної GS, що корелює з погіршенням стану експериментальних тварин.

Мета роботи – визначення рівня експресії глутамінсинтетази в різних відділах мозку щурів в умовах експериментального сепсису.

Матеріали та методи. Дослідження здійснили на щурах лінії Вістар: 5 хибнооперованих (контрольних) і 20 тварин із септичною моделлю перев'язки та пункції сліпої кишки (CLP). Імуногістохімічне дослідження експресії GS виконали в корі, білій речовині, гіпокампі, таламусі, хвостатому ядрі/лушпині в період між 20 і 48 год після CLP.

Результати. Починаючи з 12 години після CLP, у прооперованих тварин спостерігали погіршення стану, що прогресувало. У 9 тварин між 20 і 38 год клінічний перебіг завершився розвитком летаргії та важких респіраторних порушень; вони були евтаназовані – група «тварин,які загинули», CLP-B. В 11 щурів спостерігали менш виражені порушення стану до 48 год – група «тих, які вижили», CLP-A. Через 23 год у щурів групи CLP-B, а також через 48 год у тварин групи CLP-A у тканині печінки виявили морфологічні ознаки вогнищевого незворотного пошкодження, що мало тенденцію до прогресування з часом після CLP-процедури. У CLP-A і CLP-B щурів спостерігали поступове підвищення рівня GS

у всіх ділянках мозку, що дослідили. Від 24 до 38 год після CLP у групі CLP-B визначили вірогідне регіон-специфічне динамічне збільшення експресії GS: у корі – на 69,35 %, гіпокампі – на 53,6 %, таламусі – на 50,0 %; найістотніше підвищення – в корі, збільшення в 1,69 раза порівняно з контролем.

Висновки. У моделі CLP через 24 години після операції спостерігали вірогідне динамічне підвищення рівня GS у корковій, гіпокампальній і таламічній ділянках мозку щура, найсуттєвіше підвищення – в корі. Гетерогенне підвищення рівнів GS вказує на регіони мозку, що більше чи менше вразливі до системних нейротоксичних чинників, а також підтверджує регіональну специфічність реактивності мозкової тканини, в тому числі місцевої астроглії, до системних впливів в умовах сепсису. Морфологічні ознаки сепсис-асоційованого пошкодження печінки, що на 1 годину передують вірогідному збільшенню GS в мозку, можуть вказувати на приєднання печінкової недостатності до перебігу сепсису з підвищенням рівня гепатогенних токсинів (імовірно, включаючи аміак) у крові та мозковій тканині через 23 години після CLP. Отже, можна припустити активну участь гепатогенних шкідливих молекул у патофізіології сепсису, а також це свідчить про залучення реактивно підвищених рівнів мозкової GS у комплексних механізмах сепсис-асоційованої енцефалопатії.

In 2016, sepsis was defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Sepsis-associated encephalopathy (SAE) – is an acute brain dysfunction ranging from delirium to coma and accompanies sepsis in up to 70 % of ICU patients [2].

Mechanisms of SAE are complex and still need to be clarified. Current knowledge about SAE is focused on neurotransmitter imbalance, blood-brain barrier breakdown, neuroinflammation, ischemic focal lesions, excessive/deficient glial reactivity, selective neuronal death, alteration of water homeostasis and brain edema [3]. Severe course of sepsis is often accompanied by multiple organ dysfunction syndrome where acute liver failure (ALF) plays one of the most critical roles [4]. It was evidenced decades ago, that ALF also determines high mortality rates in ICU departments, including patients with sepsis [5]. The principal sign of acute hepatic encephalopathy (AHE) accompanying ALF is ammonia induced cytotoxic edema of astrocytes leading to generalized brain edema-swelling [6]. Astrocytes, the main homeostatic cellular population in the brain, are responsible for constant supporting of all mentioned fundamental processes on physiological level. Representing the most numerous cell population in the brain, in pathological state they might cause deep failure of the tissue homeostasis in diverse spectrum and lead to progressive brain dysfunction [7,8].

In case of SAE astrocytes rapidly became reactive performing heterogeneous morpho-functional phenotypes in region- and time-dependent manner as was shown in our recent study [9]. In case of ALF development in sepsis, one can suppose increase of systemic and brain ammonia levels. Astroglia are the target cells responsible for ammonia metabolism inside the brain as they are the principal cellular pool expressing glutamine-synthetase (GS) – enzyme essential to convert ammonia and glutamate to glutamine [10]. As was shown in our previous yet not published study, in rat acetaminophen-induced acute liver failure (AILF) model there was substantive region- and time-dependent increase in astroglial GS expression which correlated with deterioration of the animal state up to coma in non-survived animals during first 24 hours after acetaminophen overdosing. Considering high heterogeneity of astroglial population through the brain regions in healthy and diseased brain, it seems rational to study characteristic features of GS expression alteration in the same brain regions to estimate astroglial reactiveness in the conditions of severe sepsis model reflecting a similar state in human sepsis.

Aim

The aim was to determine the immunohistochemical level of glutamine synthetase expression in different brain regions of rats in the conditions of experimental sepsis.

Materials and methods

The study was performed in Wistar rats, 200–300 g ("Biomodelservice", Kyiv, Ukraine). 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. Animals were subjected to the cecal ligation and puncture (CLP) model of sepsis. Rats were divided into 2 groups: CLP group (n = 20) and sham-operated (control) group (n = 5). All further experimental stages were conducted according to previously displayed manner [11].

After CLP-procedure, rats were routinely observed up to 48 h after operation. Beginning from the 12th hour the following clinical signs as periorbital exudation, piloerection, diarrhea, fever/hypothermia, social isolation, deep lethargy and severe respiratory disorders dynamically increased in animals. During the period of 20-38 h after CLP-procedure, 9 rats showed highly expressed mentioned clinical symptoms and were euthanized ("CLP-B" – non-survived/lethal), 11 animals with less expressed symptoms survived until 48 h – end-point of the experiment ("CLP-A" – survived). Sham-operated rats ("CLP-C") showed no lethal outcomes. All animals from CLP-A and CLP-C groups were sacrificed at 48 h after CLP-procedure by intraperitoneal overdosing of sodium thiopental.

Samples of the brain and liver tissue were processed according to standard procedures with formation of paraffin blocks. For general histopathological analysis hematoxylin-eosin stained sections were used. Immunohistochemical (IHC) study involved detection of immunopositive labels using rabbit polyclonal anti-GS primary antibody (Thermo Scientific, USA) and Ultra Vision Quanto Detection imaging system with diaminobenzidine (Thermo Scientific Inc., USA). The results of IHC reaction were assessed at magnification x200 in a standardized field of view (SFV) of the microscope Scope. A1 "Carl Zeiss" (Germany) using Jenoptik Progres Gryphax 60N-C1"1,0x426114 (Germany) camera and the program Videotest-Morphology 5.2.0.158 (Video Test LLC, RF). The expression of GS was assessed as a percentage of the relative area (S rel., %) of immunopositive labels

Table 1. The indicators of GS expression in different brain regions in animals of different experimental groups expressed in the percent of immune-positive labels in the SFV, Me (Q1; Q3)

Brain region	CLP-A	CLP-B	CLP-C
Cortex	4.25 (4.12; 5.25)*	5.25 (4.23; 5.72)*	3.10 (2.56; 3.85)
Subcortical white matter	0.38 (0.29; 1.20)	0.42 (0.32; 1.39)	0.34 (0.27; 0.41)
Hippocampus	1.72 (1.63; 2.03)*	1.92 (1.74; 2.11)*	1.25 (0.72; 1.52)
Thalamus	1.68 (1.53; 2.11)*	1.71 (1.65; 2.25)*	1.14 (0.38; 1.34)
Caudate/putamen	0.97 (0.48; 1,67)	1.04 (0.52; 1.76)	0.92 (0.47; 1.52)

*: significant differences in indicators compared to control group (P < 0.05) are marked with an asterisk; **CLP-A:** survived group; **CLP-B:** non-survived group; **CLP-C:** control.

to the total area of the tissue section in the SFV. For the comparative analysis of the GS expression sensorimotor cortex, subcortical white matter, hippocampus, thalamus and caudate nucleus/putamen regions were selected. Five SFV of each noted region were analyzed for each animal.

Data were statistically processed using Statistica® for Windows 13.0 (StatSoft Inc., license No. JPZ804I382130ARCN10-J) with evaluating median (Me), lower and upper quartiles (Q1; Q3). For data comparison between two and more than two groups, Mann–Whitney and Kruskal–Wallis tests were used. The results were considered significant at 95 % (P < 0.05).

Results

Starting from the 12th hour after CLP-procedure, all operated animals displayed progressive impairment in periorbital exudation, piloerection, fever-/hypothermia, diarrhea, social isolation, lethargy, and respiratory impairment. Between 20 and 38 h, 9 animals expressed pronounced mentioned symptoms and were euthanized ("CLP-B"), while 11 rats survived until 48 h of the experiment ("CLP-A"). From 23 h in non-survived, as well as 48 h in survived animals, pathohistological study of the liver tissue revealed signs of spread ballooning degeneration of hepatocytes of presumably centrilobular localization with selective coagulative necrosis of individual hepatocytes, as well as foci of centrilobular necrosis of lobules, neutrophilic infiltration and signs of moderate cholestasis. Mentioned changes were aggravated with time after CLP-procedure in case of non-survived group.

At 24 h control brains showed heterogeneous GS expression among different regions with the highest level in the sensorimotor cortex – 3.10 (2.56; 3.85) % and the lowest in the subcortical white matter region – 0.34 (0.27; 0.41) % (Table 1). GS labeling in all brain regions of control animals was predominantly associated with the vascular astroglial endfeet and in the lesser degree was related to parenchymal astrocytic processes (Fig. 1).

IHC analyses of all studied brain regions of rats in CLP-A and CLP-B groups revealed enhanced GS staining. In the sensorimotor cortex, GS+ staining predominantly was related to the superficial first two layers and was associated with perikaryons of hypertrophically changed astrocytic bodies as well as equally distributed over cytoplasm of diverse types of cellular processes. Such distribution led to neuropil diffuse-focal GS+ staining (Fig. 2).

In the CLP animals, the alteration of GS expression was characterized by heterogenous mode among different brain regions. The highest elevation of the GS expression was detected in the cortex and the lowest – in the caudate
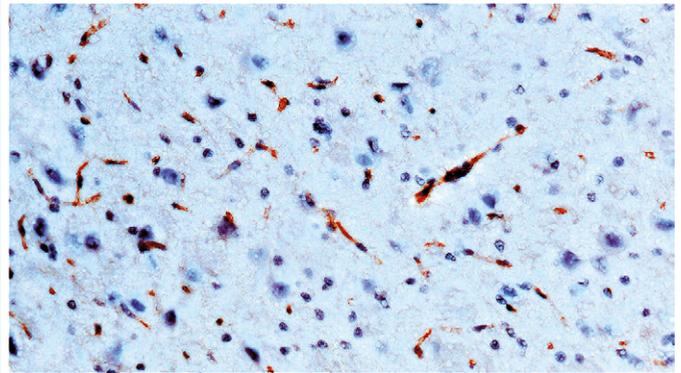
Fig. 1. GS expression in the cortex of the control rat (CLP-C group) 48 h after the sham operation (anti-GS, Thermo Scientific, USA). ×200.
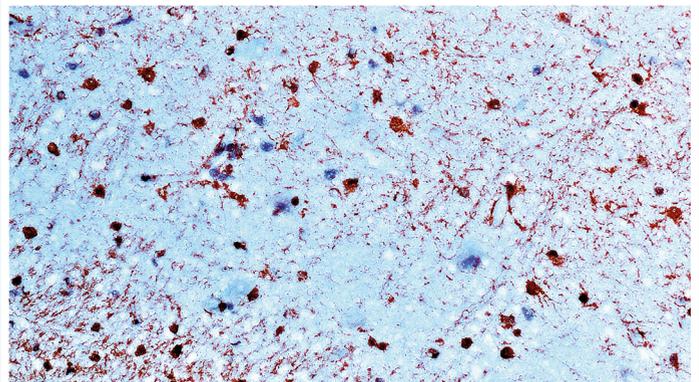
Fig. 2. GS expression in the cortex of the non-survived rat (CLP-B group) 38 h after the CLP procedure (anti-GS, Thermo Scientific, USA). ×200.

nucleus/putamen region. Interestingly, both CLP-A and CLP-B animals were noted by significant (compared to control) elevation in GS expression only in the cortex, hippocampus and thalamus, whereas subcortical white matter and caudate/putamen regions showed insignificant rise (Table 1). Increased values of GS expression in all studied regions had no significant difference between CLP-A and CLP-B groups (P > 0.05), although CLP-B values were higher compared to CLP-A indicators (Table 1).

In the CLP-B group, the relative area of GS expression displayed the most prominent increase in the cortex compared to control: 5.25 (4.23; 5.72) % and 3.10 (2.56; 3.85) %, respectively, P < 0.05, which was equal to 69.35 % or 1.69-fold increase if compare medians of indicators. At 48 h of the experiment, CLP-A group both showed significant increase in cortical GS expression – 4.25 (4.12; 5.25) % (Table 1).
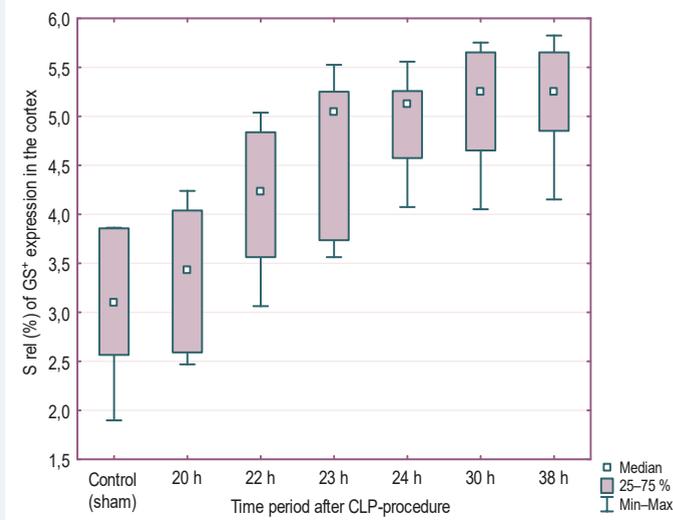
Fig. 3. Dynamics of the GS expression in the cortex of CLP-B rats after CLP-procedure.
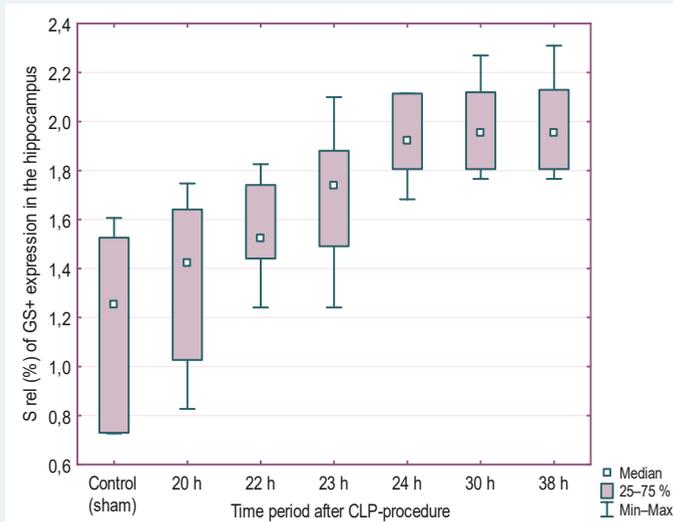
Fig. 4. Dynamics of the GS expression in the hippocampus of CPL-B rats after CLP-procedure.
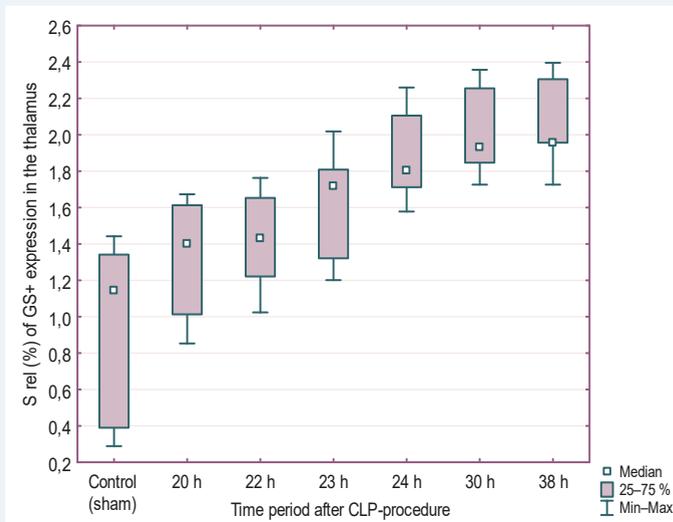
Fig. 5. Dynamics of the GS expression in the thalamus of CLP-B rats after CLP-procedure.

The next highest growth in GS values was revealed in hippocampus and thalamus of CLP-B group: 1.92 (1.74; 2.11) % (1.53-fold or 53.6 % compared medians to control) and 1.71 (1.65; 2.25) % (1.5-fold or 50 % compared to control), respectively. In CLP-A group, hippocampal and thalamic expression also demonstrated significant increase compared to control, however values did not differ from CLP-B group (P > 0.05), (*Table 1*).

Less pronounced GS level alteration in CLP-B animals was related to subcortical white matter and caudate/putamen region: 0.42 (0.32; 1.39) % (1.23-fold/23 % compared medians to control) and 1.04 (0.52; 1.76) % (1.13-fold/13.04 % compared medians to control), respectively. Nevertheless, the revealed excess in indicators had no significance compared to control (P > 0.05).

From the 20th h after operation, non-survived animals performed dynamic elevation in GS expression in all studied brain regions. Interestingly, the significant increase in indicators was found only in animals died starting from 24 h after CLP-procedure with the maximal values in all studied brain regions at 38 h – the time-point when the last CLP-B animal was euthanized due to decompensated state (*Figs. 3–5*).

In sum, in the postoperative period of CLP-procedure, the highest GS expression was typical for the CLP-B animals in all studied brain regions with the most substantial and significant compared to control elevation in the cortical, hippocampal and thalamic regions. Furthermore, in the latter regions, significant and dynamic rising of GS was revealed only starting from 24 h after CLP-procedure and reached maximum at 38 h.

Discussion

In sepsis, besides the brain damage conditioned by the action of systemic neurotoxins and products of dysregulated immune response, in case of sepsis-associated liver dysfunction development, one can suppose the influence of the additional detrimental factors caused by the failure of detoxifying and other functions of the liver.

Current concepts on pathogenesis of the brain damage in SAE and AHE indicate the presence of similar links in their mechanisms, including neuroinflammation, abnormal glial reactivity, neurotransmitter imbalance, disturbed water metabolism, brain edema, etc. [2,12].

Among sequences of ALF, systemic and brain hyperammonemia have been considered to be principal factors in the mechanisms of acute hepatic encephalopathy. The target cell population in the brain for ammonia exposure are astroglia as they are primarily ones containing glutamine synthetase and metabolize ammonia [10]. Our recent animal studies used CLP model of sepsis and acetaminophen-induced liver failure (AILF) model of ALF, have revealed the early astroglial reactive changes in the cortical, white matter, hippocampal, thalamic and caudate nucleus/putamen regions of the rat brain. In sepsis model, dynamic significant increase in GFAP protein expression in the cortex, white matter and hippocampus was found as early as 20 h after CLP-procedure [9], while in AILF-model, the trend of GFAP alterations was represented by the form of a dynamic downward curve from 16 to 24 h after acetaminophen treatment (yet unpublished data). These

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results suggest high and fast responsiveness of astroglia both on systemic inflammatory challenge and on hepatogenic toxemia which demonstrates contrary effects on protein expression.

Studies on GS, another principal player of astroglial metabolism and functions including reactive astrogliosis, have demonstrated in AILF-model pronounced but heterogeneous increase in GS expression in the cortex, white matter, hippocampus, thalamus and caudate/putamen 16 h after acetaminophen treatment and maximal values at 24 h [13]. The latter fact potentially could indicate brain regions more or less permeable for ammonia and other toxic agents as well as heterogeneous sensitivity of different brain regions to damage factors in conditions of ALF. Coincidence of the high GS expression with the period of animal's decompensation might indicate close involvement of high GS levels in the development of AHE.

GS is known to be a central enzyme in the regulation of neurotransmitters glutamate and GABA homeostasis and therefore, supporting functionality of synapses and neuronal circuicity [14]. SAE is characterized by the violation of the neurotransmitter tone in the nervous tissue, leading to pronounced brain dysfunction up to coma state [3]. The present study has demonstrated the dynamic increase in GS expression in the CLP-rat brains with slightly higher rates in the non-survived animals (likewise it was observed in AILF-model of the acute liver failure) [13], although the rise of expression in CLP-study was less significant than in AILF and was significant only 24 h after the procedure. The appearance of morphological signs of focal irreversible liver damage, which preceded significant increase in GS expression by 1 hour, might suppose addition of the liver failure of a certain degree to a course of sepsis with increased levels of hepatogenic toxins (presumably including ammonia) in the blood and directly in the brain parenchyma by 23 h after CLP-procedure.

The highest increase in GS expression in the cortex potentially might indicate the brain region most exposed to detrimental systemic incoming agents, as well as specificity of the local astroglial reactivity in given complex condition. The significant rise in GS expression from 24 to 38 h, demonstrate implication of high levels of GS protein expression in the mechanisms of SAE progression and suppose involvement of hepatogenic detrimental influence among mechanisms of sepsis decompensation. Although, the absence of a significant difference between GS expression values in survived and non-survived animals, as well as positive correlation between GS expression level and animal survival, may indicate that the severity of the putative liver failure is insufficient to significantly influence the mechanisms of thanatogenesis in the CLP-model of sepsis.

Conclusions

1. In the conditions of CLP sepsis, as soon as 24 h after operation, there is significant dynamic increase in GS level in the cortical, hippocampal and thalamic regions of the rat brain with the most prominent in the cortex. Heterogeneous alteration in GS expression potentially indicates zones more or less acceptable for incoming systemic agents as well as region-specific reactiveness

of the brain tissue, including local astroglial populations, to these factors in the conditions of sepsis.

2. Revealed morphological signs of sepsis-associated liver damage preceding significant increase in the brain GS by 1 h, might suppose addition of the liver failure of a certain degree to the course of sepsis, which is accompanied by increased level of hepatogenic toxins (presumably including ammonia) in the blood and in the brain tissue by 23 h after CLP. The latter might propose active implication of hepatogenic detrimental agents in sepsis pathophysiology and involvement of reactively increased brain GS levels in the complex mechanisms of SAE.

Prospects for further research. Pathophysiology of SAE still needs further clarifying. Considering growing body of evidence for central role of glial responsibility and/ or functional disability in suspected mechanisms, it is highly expected to continue further research in the field of glial reactivity to systemic endotoxic challenge of different origin.

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References

[1] Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J. D., Coopersmith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., van der Poll, T., Vincent, J. L., & Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*, *315*(8), 801-810. https://doi.org/10.1001/jama.2016.0287

[2] Mazeraud, A., Righy, C., Bouchereau, E., Benghanem, S., Bozza, F. A., & Sharshar, T. (2020). Septic-Associated Encephalopathy: a Comprehensive Review. *Neurotherapeutics: the journal of the American Society for Experimental NeuroTherapeutics*, *17*(2), 392-403. https://doi.org/10.1007/s13311-020-00862-1

[3]   Chung, H. Y., Wickel, J., Brunkhorst, F. M., & Geis, C. (2020). Sepsis-Associated Encephalopathy: From Delirium to Dementia? *Journal of clinical medicine*, *9*(3), 703. https://doi.org/10.3390/jcm9030703

[4]   Woźnica, E. A., Inglot, M., Woźnica, R. K., & Łysenko, L. (2018). Liver dysfunction in sepsis. *Advances in clinical and experimental medicine: official organ Wroclaw Medical University*, 27(4), 547-551. https://doi.org/10.17219/acem/68363

[5]   Warrillow, S., & Bellomo, R. (2015). Intensive Care Management of Severe Acute Liver Failure. *Annual Update in Intensive Care and Emergency Medicine 2015*, *2015*, 415-430. https://doi.org/10.1007/978-3-319-13761-2_30

[6]   Liotta, E. M., & Kimberly, W. T. (2020). Cerebral edema and liver disease: Classic perspectives and contemporary hypotheses on mechanism. *Neuroscience letters*, *721*, 134818. https://doi.org/10.1016/j.neulet.2020.134818

[7]   Verkhratsky, A., & Nedergaard, M. (2018). Physiology of astroglia. *Physiological Reviews, 98*, 239-389. https://doi.org/10.1152/physrev.00042.2016

[8]   Zorec, R., Županc, T. A., & Verkhratsky, A. (2019). Astrogliopathology in the infectious insults of the brain. *Neuroscience letters*, *689*, 56-62. https://doi.org/10.1016/j.neulet.2018.08.003

[9]   Shulyatnikova, T., & Tumanskiy, V. (2021). Immunohistochemical analysis of GFAP expression in the experimental sepsis-associated encephalopathy. *Pathologia, 18*(3), 295-302. https://doi.org/10.14739/2310-1237.2021.3.240033.

[10]   Zhou, Y., Eid, T., Hassel, B., & Danbolt, N. C. (2020). Novel aspects of glutamine synthetase in ammonia homeostasis. *Neurochemistry international*, *140*, 104809. https://doi.org/10.1016/j.neuint.2020.104809

[11]   Shulyatnikova, T. V., & Shavrin, V. A. (2021). Mobilisation and redistribution of multivesicular bodies to the endfeet of reactive astrocytes in acute endogenous toxic encephalopathies. *Brain research*, *1751*, 147174. https://doi.org/10.1016/j.brainres.2020.147174

[12]   Shulyatnikova, T. V., & Shavrin, V. A. (2017). Modern view on hepatic encephalopathy: basic terms and concepts of pathogenesis. *Pathologia*, *14*(3), 371-380. https://doi.org/10.14739/2310-1237.2017.3.118773

[13]   Shulyatnikova, T. & Tumanskiy, V. (2021). Glutamine synthetase expression in the brain during experimental acute liver failure (immunohistochemical study). *Journal of Education, Health and Sport*, *11*(10), 342-356. https://doi.org/10.12775/JEHS.2021.11.10.033

[14]   Walls, A. B., Waagepetersen, H. S., Bak, L. K., Schousboe, A., & Sonnewald, U. (2015). The glutamine-glutamate/GABA cycle: function, regional differences in glutamate and GABA production and effects of interference with GABA metabolism. *Neurochemical research*, *40*(2), 402-409. https://doi.org/10.1007/s11064-014-1473-1

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