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## BRAIN AQP4 DURING EXPERIMENTAL ACUTE LIVER FAILURE

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Hepatic encephalopathy (HE) was defined as a complex neuropsychiatric syndrome triggered by severe liver pathology and manifesting by covert and overt alterations up to hepatic coma and death [1]. Acute liver failure (ALF) results in acute hepatic encephalopathy (AHE) characterized by brain edema caused by complex mechanisms closely linked to ammonia toxicity [2]. Astrocytes are central brain cells the most sensitive to ammonia as being primarily source of glutamine synthetase (GS), therefore astrocyte swelling is a principal feature of AHE brain [1-3]. Aquaporin-4 (AQP4) is one of the central astrocyte molecules responsible for water homeostasis and cell volume in health and disease and presents the most abundant water channel in the CNS. According to current HE pathophysiology, alteration of AQP4 regulation can play a central role in the brain edema progression [1]. Considering high heterogeneity of astroglia populations in the CNS, AQP4 involvement to the links of HE can also sustain mentioned conventional diversity. The purpose of the study was determining the level of AQP4 in different rat brain regions in the conditions of ALF.

**Materials and methods.** The study was conducted in Wistar rats: 5 sham (control) animals and 10 rats with acetaminophen induced liver failure model (AILF) [4]. Starting from the 6<sup>th</sup> h after acetaminophen treatment all AILF-animals showed progressive impairment of ALF, evidenced histologically by spread liver centrilobular necrosis that finished in 6 rats by comatose state up to 24 h (constituted subgroup AILF-B, "non-survived"). 4 animals survived until the 24 h - subgroup AILF-A, "survived". In control "AILF-C" group, all animals survived up to 24 h. The IHC study of AQP4 was carried out in the sensorimotor cortex, white matter, hippocampus, thalamus and caudate nucleus/putamen regions between 12 and 24 h after treatment and included detection of immunopositive labels using rabbit polyclonal anti-AQP4 primary Ab (Thermo Scientific, USA) and Ultra Vision Quanto Detection imaging system with diaminobenzidine (Thermo Scientific Inc., USA). The results were

assessed at 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). AQP4 expression was assessed as a percentage of the relative area (S rel., %) of labels to the total area in the SFV. Five SFVs of each region were examined for 1 animal.

**Results.** Control brains demonstrated heterogeneous staining on AQP4 among different 5 regions with the highest level in the sensorimotor cortex – 2.32 (2.12; 3.45) % and the lowest in the subcortical white matter – 0.45 (0.25; 1.06) % (Table 1). AQP4+ labelling in all brain regions of controls was related to astroglial endfeet processes both vascular and, in lesser extent, parenchymal ones. In the AILF-B group, starting from 16 to 24 h after acetaminophen treatment, a significant (relative to control) regionally-specific dynamic increase AQP4 levels was observed in the brain: in the cortex – by 405.17 %, in the hippocampus – by 387.38%, in the caudate nucleus/putamen – by 314.11%, from 12<sup>th</sup> h: in the thalamus – by 342.66% and in the subcortical white matter – by 297.77%; with the highest elevation of AQP4 expression in the cortex among other studied regions: by 5.05 times. Mentioned results in comparison to control values are summarized in (Table 1). After AILF-procedure, non-survived animals displayed the growing dynamics in AQP4 level in all 5 studied brain regions with the highest values 24 h after the injection. As early as 12 h, sacrificed animals of AILF-B group displayed reliable gain in AQP4 staining compared to control in the cortex, hippocampus and caudate/putamen, whereas white matter and thalamic elevation reached statistical validity only by 16 h after acetaminophen administration.

Table 1

**Brain AQP4 level in different experimental groups expressed in the percent of immune positive labels in the SFV. Data are presented as median (Me) and lower, upper quartiles (Q1; Q3)**

Brain region	AILF-A	AILF-B	AILF-C
Cortex	9.46 (7.68; 9.72) *†	11.72 (10.11; 12.54) *†	2.32 (2.12; 3.45)
Subcortical white matter	1.53 (1.10; 1.85) *	1.79 (1.27; 1.92) *	0.45 (0.25; 1.06)
Hippocampus	10.21 (8.93; 10.54) *	10.43 (9.15; 10.87) *	2.14 (2.07; 3.23)
Thalamus	3.20 (2.89; 4.43) *	3.32 (3.10; 4.56) *	0.75 (0.43; 1.68)
Caudate/putamen	3.43 (2.91; 4,59) *	3.52 (3.25; 4.77) *	0.85 (0.36; 1.72)

Notes: Reliable differences in indicators compared to the control animals ( $p < 0.05$ ) are marked with an asterisk (\*). Reliable differences between AILF-A and AILF-B groups in the same brain region ( $p < 0.05$ ) are marked with the dagger (†); "AILF-A" – survived; "AILF-B" – non-survived; "AILF-C" – control.

After AILF-procedure, non-survived AILF-B-animals displayed the growing dynamics in AQP4 expression in all 5 studied brain regions with the highest values 24 h after the injection. As early as 12 h, sacrificed animals of AILF-B group displayed reliable gain in AQP4 staining compared to control in the cortex, hippocampus and caudate/putamen, whereas white matter and thalamic elevation reached statistical validity only by 16 h after treatment. The present study evidenced that AILF-model provokes increased AQP4 in the cortex, hippocampus and caudate

nucleus/putamen by 12th h after treatment and by 16th h – in the white matter and thalamus. This is comparable with our previous study [5], where AILF model caused upregulation of GS in the same brain regions, however, in the cortex and hippocampus in later terms. The latter suggests that AQP4 alterations in mentioned regions could precede changes in the astrocytic glutamate-glutamine shuttle and means that AQP4 elevation can be reasoned by other factors than the glutamine hyperosmolarity. Nevertheless, increase in AQP4 and GS levels in the white matter and thalamus was found to be simultaneous indicating the potential overlapping regulatory mechanisms for these two proteins in the conditions of AHE.

**Conclusion.** AILF in rats induces dynamic increase in AQP4 levels in the cortex, hippocampus and caudate nucleus/putamen by 12<sup>th</sup> h and in the white matter and thalamus – by 16<sup>th</sup> h after the acetaminophen overdosing with the highest elevation in the cortex. The heterogeneity in the degree of AQP4 elevation among different brain regions may indicate brain territories more susceptible for systemic toxic exposure and damage in ALF. Furthermore, the earliest reliable increase of AQP4 in the cortex, hippocampus and caudate/putamen might propose the faster reactivity of the local astroglial populations in response to the hyperammonemia among other regions. Consequently, the later and lower rates of AQP4 elevation in the white matter might indicate local astroglia as less reactive and/or more protected from the harmful exposure at a certain time period of the experiment. The higher cortical levels of AQP4 in the non-survived animals compared to survived ones reflect the significance of AQP4-involving mechanisms in the aggravation of AHE, as well as the role of AQP4 alterations in thanatogenesis in the conditions of acute liver failure.

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