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## New insight into the mechanism of cytotoxic brain edema in acute liver failure: focus on the functionality of mitochondria.

Cerebral edema is the most fatal complication of hepatic encephalopathy (HE) resulting from acute liver failure. HE is a set of neuropsychiatric syndrome manifested by loss of consciousness, intellectual and motor functions disorders, and in the worst cases lead to coma and death of patients. Cerebral edema occurs in up to 70% of patients with acute liver failure. Currently, no specific treatment of acute HE-associated brain edema and coma other than whole-body cooling is available. Brain edema progresses quickly and with intracranial hypertension remain a leading cause of death in acute HE. HE is a multifactorial disease and exact mechanism remains unknown. It is assumed that the two main pathological factors causing the development of the disease are high concentrations of ammonia, which results from the lack of metabolism in the damaged liver, and inflammation, which is manifested by elevated levels of cytokines.

Cerebral edema has two components: vascular, related to damage to the blood-brain barrier, and cytotoxic, caused by swelling of brain cells - astrocytes. The dominant role in the development of edema in acute HE is ascribed to cytotoxic. The mechanism of brain edema in acute HE is more complex and involvement of glutamine (Gln) was postulated. This amino acid is produced in the brain specifically in astrocytes, in the reaction of ammonia and glutamate (Glu). Contribution of Gln in cytotoxic edema assumes interplay in i) intracellular osmotic imbalance followed by water penetration into cells and ii) mitochondria dysfunction related to elevated levels of Gln, described by the “Trojan horse” hypothesis from 2006. According to this hypothesis, Gln accumulating in the cytoplasm enters mitochondria and is metabolized by enzyme PAG to ammonia and Glu and, ammonia is the trigger of subsequent deleterious events. In consequence it elicits mitochondrial membrane potential loss, reactive oxygen species production and further megachannel opening. This causes osmotic swelling of the mitochondrial matrix and contributes to the formation of cytotoxic edema. Since 2006, no astrocyte mitochondria glutamine transporter was detected, and the hypothesis was confirmed by in vitro and ex vivo studies using histidine (His) as an inhibitor of glutamine transport.

Our unique observations in the mouse model of acute HE and the local silencing of the astrocytic Gln transporter - SN1 model suggest that despite astrocyte edema and accumulation of Gln in the brain cells of both models, morphological changes in the mitochondria only in the acute HE was noticed. Moreover, recent work indicates that the ASCT2 glutamine transporter variant has been localized at the inner membrane of mitochondria in pancreatic cancer cells. Thus, we hypothesize that the astrocytic accumulation of glutamine itself does not cause damage to the mitochondria, and presence of additional factors (hyperammonemia and/or cytokines) that influence glutamine transport to the mitochondria and/or other transporter, namely ASCT2, may contribute to the mechanisms of cytotoxic cerebral edema.

Morphological analysis of mitochondria 3D in astrocytic cells using confocal microscopy, measurement of mitochondrial functionality parameters and measurement of the level and activity of the ASCT2 transporter in isolated astrocytic mitochondria in both animal models will help achieve the assumed goal. Additionally, it is planned to study the activity and expression of ASCT2 in conditions reproducing the main pathogenic factors in acute HE in in vitro studies. The use of new molecular biology techniques, which have not been used so far in research on cytotoxic edema, will allow to capture the role of elevated glutamine levels in mitochondrial damage in an innovative way. The model of local silencing of the SN1 transporter in mice, to our knowledge, uniquely reproduces the increase in glutamine levels in astrocytes as the only factor studied. Therefore, it is possible to highlight the role of glutamine in the study of the mechanism of cerebral edema.

We are expecting that the results will renew the 'Trojan horse' hypothesis and provide a better understanding of mitochondria-related cytotoxic brain edema mechanism considering ASCT2 role and high glutamine, ammonia and cytokines level. In a longer perspective, unraveling the mitochondria-engaged edema formation mechanism may help fine-tune HE therapy. However mitochondrial dysfunction is becoming one of the most emerging pathological process in the etiology of many neurological disorders as Huntington's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Epilepsy, Schizophrenia, Multiple sclerosis, Neuropathic pain and Alzheimer's disease. A better understanding of the general mechanism of their damage and the factors influencing their dynamics will provide a major contribution to general knowledge and may have an impact on research on all these diseases.