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Interstitial ion homeostasis and acid-base balance are maintained in oedematous brain of mice with acute toxic liver failure

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Lactate*Abbreviation:*
ADC
Apparent diffusion coefficient
ALF
Acute liver failure
AOM
Azoxymethane
ATLF
Acute toxic liver failure
BBB
Blood brain barrier
CSF
Cerebrospinal fluid
ISF
Interstitial fluid
EB
Evans blue
MRI
Magnetic resonance imaging
$[K^+]_e$
Extracellular potassium concentration
$[Na^+]_e$
Extracellular sodium concentration
$[Lac]_e$
Extracellular lactate concentration

ABSTRACT

Acute toxic liver failure (ATLF) rapidly leads to brain oedema and neurological decline. We evaluated the ability of ATLF-affected brain to control the ionic composition and acid-base balance of the interstitial fluid. ATLF was induced in 10–12 weeks old male C57Bl mice by single intraperitoneal (i.p.) injection of 100 μg/g azoxymethane (AOM). Analyses were carried out in cerebral cortex of precomatous mice 20–24 h after AOM administration. Brain fluid status was evaluated by measuring apparent diffusion coefficient [ADC] using NMR spectroscopy, Evans Blue extravasation, and accumulation of an intracisternally-injected fluorescent tracer. Extracellular pH ($[pH]_e$) and ($[K^+]_e$) were measured *in situ* with ion-sensitive microelectrodes. Cerebral cortical microdialysates were subjected to photometric analysis of extracellular potassium ($[K^+]_e$), sodium ($[Na^+]_e$) and luminometric assay of extracellular lactate ($[Lac]_e$). Potassium transport in cerebral cortical slices was measured *ex vivo* as ^{86}Rb uptake. Cerebral cortex of AOM-treated mice presented decreased ADC supporting the view that ATLF-induced brain oedema is primarily cytotoxic in nature. In addition, increased Evans blue extravasation indicated blood brain barrier leakage, and increased fluorescent tracer accumulation suggested impaired interstitial fluid passage. However, $[K^+]_e$, $[Na^+]_e$, $[Lac]_e$, $[pH]_e$ and potassium transport in brain of AOM-treated mice was not different from control mice. We conclude that in spite of cytotoxic oedema and deregulated interstitial fluid passage, brain of mice with ATLF retains the ability to maintain interstitial ion homeostasis and acid-base balance. Tentatively, uncompromised brain ion homeostasis and acid-base balance may contribute to the relatively frequent brain function recovery and spontaneous survival rate in human patients with ATLF.

1. Introduction

Acute liver failure (ALF) develops in humans or experimental animals in consequence of viral infection or after ingestion of hepatotoxic drugs. While ALF in most instances presents multi-organ failure (Bernal et al., 2013; Lee, 2012, 2013), it is usually associated with neurological decline defined as hepatic encephalopathy (HE), with brain oedema being its major, often fatal complication (Blei, 2008; Gupta et al., 2017;

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McCormick et al., 2003). It is generally acknowledged that ALF-induced oedema is mainly cytotoxic in nature, resulting from water movement to the intracellular (predominantly intra-astrocytic) compartment, with moderate contribution of interstitial or vasogenic oedema (Chavarria et al., 2010; Scott et al., 2013). Acute toxic liver failure (ATLF) evoked by exposure of mice to azoxymethane (AOM) relatively faithfully models drug-induced ALF and symptoms of acute human HE. These include (i) reproducible development of four stages of neurological decline, (ii) increased levels of ammonia in the blood and in the brain, (iii) altered brain amino acids profile, (iv) severe neurological dysfunctions (Belanger et al., 2006; Matkowskyj et al., 1999; McMillin et al., 2014; Popek et al., 2017) and (v) brain oedema (Belanger et al., 2006; Chastre et al., 2014; Nguyen et al., 2006; Rangroo et al., 2012). The principal question of the present study was whether and in what degree brain oedema accompanying ATLF is associated with altered ion composition and pH of the interstitial space, parameters reflecting the ability of the brain to cope with pathophysiological consequences of cerebral fluid imbalance. To our knowledge, this problem has so far escaped analysis in the context of ATLF; only two studies known to us have dealt with potassium homeostasis in severe hyperammonemia, a condition not equivalent to ATLF (Rangroo et al., 2013; Sugimoto et al., 1997).

In this study we first analysed the nature of brain oedema in AOM -treated mice in which neurological HE symptoms progressed to the pre-comatose stage III (Matkowskyj et al., 1999; Popek et al., 2017). The MRI analysis revealed a decrease of the apparent diffusion coefficient (ADC), which is interpreted to define oedema as primarily cytotoxic (Chavarria and Cordoba, 2015). Subsequently, we confirmed leaky blood-brain barrier indicative of a vasogenic component, and found increased retention of an intracisternally administered fluorescent marker, indicating impairment of a paravascular cerebrospinal fluid (CSF)/interstitial fluid (ISF) flow, termed glymphatic system (Iliff et al., 2012). Having established the parameters of brain fluid balance, we then measured, *in vivo* in awake animals or in brain microdialysates, the extracellular concentrations of sodium ($[Na^+]_e$, potassium ($[K^+]_e$), lactate ($[Lac]_e$) and extracellular pH ($[pH]_e$). To account for the effect of ATLF on cerebral potassium transport we compared the uptake of a radiolabelled potassium surrogate, ^{86}Rb, in cerebral cortical slices derived from control and ATLF-affected brains.

2. Materials and methods

2.1. Azoxymethane (AOM) model of ALF

Experiments were performed on 10–12 weeks old male C57Bl mice, with approval and under surveillance of the IVth Local Ethical Committee, National Drug Institute, Warsaw or of the University Committee on Animal Resources, University of Rochester Medical Center. ATLF was induced according to the protocol described in Popek et al. (2017). Mice received a single intraperitoneal (i.p.) injection of 100 μg/g azoxymethane (AOM) (Sigma-Aldrich). Hypothermia in AOM-injected animals was prevented/corrected by using the heating pads exactly as described by Belanger et al. (2006). Two i.p. injections (at 8 and 16 h post-AOM) of 5% glucose in saline (500μl volume) were given to prevent both dehydration and hypoglycaemia. All brain tissue analyses were performed 18–22 h after AOM administration, i.e. in the period in which by neurological criteria the animals were in the pre-comatous stage. Biochemical analyses performed at 20 h post-AOM showed increased serum concentrations of inflammatory cytokines (IL-6 and TNFα increased by 21 and 4.7 fold, respectively), blood ammonia (a 2.7-fold rise), and activity of liver damage marker enzymes: ALT (a 12-fold rise) and AST (a 4.7-fold rise) (Popek et al., 2017).

2.2. Determination of apparent diffusion coefficient (ADC)

Brains of control and AOM-treated animals were scanned with Bruker BioSpec 70/30 Advance III system working at 7T, with a transmit cylindrical radiofrequency coil (8.6 cm inner diameter) and a mouse brain dedicated receive-only array coil (2 × 2 elements) positioned over the animal's head. The animals were positioned prone with the head placed in the stereotactic apparatus and anaesthesia mask, and were anesthetized at around 1.5–2% isoflurane in a mixture of oxygen and air. Respiration was monitored throughout the experiment.

Structural transverse MR images covering the whole brain were acquired with T2-weighted TurboRARE (TR/TE = 6000/30 ms, RARE factor = 4, spatial resolution = 78 μm × 78 μm x 500 μm, 35 slices, no gaps, number of averages (NA) = 4, scan time = 25min). Spin echo diffusion-weighted images covering the whole brain were acquired (TR/TE = 7000/27 ms, b-values = 0, 600, 1110 s/mm^2, number of directions = 1, spatial resolution = 156 μm × 156 μm x 700 μm, 25 slices, no gaps, scan time = 33 min). ADC maps were calculated using Bruker ParaVision 5.1 software. Atlas of each individual brain structure was obtained by automatically labelling whole brain using MRM NeAt atlas and transformation matrix (obtained in the normalization step). That automatic labelling algorithm was implemented as custom-made MATLAB script (http://www.mathworks.com/products/matlab/) exploiting modified functions provided by IBASPM software (http://www.thomaskoenig.ch/Lester/ibaspm.htm). At the top of the analysis, ADC maps were overlaid with the corresponding individual brain structure atlas and mean ADC values from each structure voxels were calculated using custom-made MATLAB scripts.

2.3. Evans blue extravasation measurement

The Evans Blue (EB) (4 ml/kg in saline) was injected intravenously and allowed to circulate for 2 h. Mice were then perfused transcardially under anaesthesia with heparinised saline until perfusion fluid in effluent became colourless. Tissue samples (cortex and hippocampus) were extracted and weighed, homogenized in 50% trichloracetic acid and centrifuged at 4 °C. Supernatant absorbance was measured spectrophotometrically at 620 nm (FLUOstar Omega microplate reader). Results were quantified using a standard curve.

2.4. Extracellular potassium ($[K^+]_e$) and pH measurements with ion-sensitive microelectrodes in awake animals

The animals were anesthetised using isoflurane (2.5% in air), a 3 mm cranial window was prepared over somatosensory cortex, and dura mater was removed. The exposed scull was submerged in ACSF with 3 mM KCl. During the experiment lasting ~2 h, awake but restrained mice were resting on heated pads to maintain body temperature at 37 °C.

In vivo recordings were obtained from somatosensory cortex (200 μm below the exposed pial surface). Ion-sensitive microelectrodes (ISE) for K^+, and H^+ were pulled from single-barreled pipette glass, salinised and loaded with a ~300 μm column of valinomycin-based K^+ ion exchange resin (Potassium Ionophore I-Cocktail B, Sigma-Aldrich), or H^+ ion exchange resin (Hydrogen Ionophore I-Cocktail A, Sigma-Aldrich) and backfilled with 150 mM KCl or PBS with a pH of 7.4, respectively. A reference electrode was backfilled with 150 mM NaCl. The ISEs were calibrated before and following the K^+/H^+ recording using aCSF solutions with a stepwise K^+ or H^+ gradient. The extracellular ion concentrations data were sampled at 10 kHz and filtered from 0.1 to 2 kHz using an MultiClamp 700A/700B, digitized using Digidata 1322A, recorded using Clampex 9.2 and analysed using Clampfit 10.2 (Ding et al., 2016). The surgery for insertion of the microelectrode used for K^+ and pH recording was carried out within the 20th hour post AOM. First 15 min of recording served to equilibrate the system ("equilibration recording", Fig. 2A, inset) and the results from this period were not included. The results obtained during the next 1 h of recording ("genuine recording", Fig. 2A, inset) were averaged.