Title

Aquaporin-4 deletion in mice reduces encephalopathy and brain edema in experimental acute liver failure

Authors

Rao, Kakulavarapu V Rama
Verkman, AS
Curtis, Kevin M
et al.

Publication Date

2014-03-01

DOI

10.1016/j.nbd.2013.11.018

Peer reviewed

Published in final edited form as:
Neurobiol Dis. 2014 March ; 63: 222–228. doi:10.1016/j.nbd.2013.11.018.

Aquaporin-4 Deletion in Mice Reduces Encephalopathy and Brain Edema in Experimental Acute Liver Failure

Kakulavarapu V. Rama Rao[1], **A. S. Verkman**[3], **Kevin M. Curtis**[4], and **Michael D. Norenberg**[1,2,4]

[1]Department of Pathology, University of Miami Miller School of Medicine, Miami, FL 33101

[2]Department of Biochemistry & Molecular Biology, University of Miami Miller School of Medicine, Miami, FL 33101

[3]Departments of Medicine and Physiology, University of California, San Francisco, CA 94143

[4]Veterans Affairs Medical Center, Miami, Fl 33125

Abstract

Brain edema and associated astrocyte swelling leading to increased intracranial pressure are hallmarks of acute liver failure (ALF). Elevated blood and brain levels of ammonia have been implicated in the development of brain edema in ALF. Cultured astrocytes treated with ammonia have been shown to undergo cell swelling and such swelling was associated with an increase in the plasma membrane expression of aquaporin-4 (AQP4) protein. Further, silencing the AQP4 gene in cultured astrocytes was shown to prevent the ammonia-induced cell swelling. Here, we examined the evolution of brain edema in AQP4-null mice and their wild type counterparts (WT-mice) in different models of ALF induced by thioacetamide (TAA) or acetaminophen (APAP). Induction of ALF with TAA or APAP significantly increased brain water content in WT mice (by 1.6 ± 0.3 and 2.3 ± 0.4 %, respectively). AQP4 protein was significantly increased in brain plasma membranes of WT mice with ALF induced by either TAA or APAP. In contrast to WT-mice, brain water content did not increase in AQP4-null mice. Additionally, AQP4-null mice treated with either TAA or APAP showed a remarkably lesser degree of neurological deficits as compared to WT mice; the latter displayed an inability to maintain proper gait, and demonstrated a markedly reduced exploratory behavior, with the mice remaining in one corner of the cage with its head tilted downwards. These results support a central role of AQP4 in the brain edema associated with ALF.

Keywords

Acetaminophen; acute liver failure; aquaporin-4; astrocyte swelling; brain edema; thioacetamide; transgenic mice

Address for correspondence: Michael D. Norenberg, M.D., Department of Pathology, University of Miami, Miller School of Medicine, PO Box 016960, Miami, Fl 33101, Voice: 305-585-7049, Fax: 305-585-5311, mnorenbe@med.miami.edu.

Introduction

Brain edema is a potentially lethal complication of acute liver failure (ALF), with an approximately 55–60% mortality (Escorsell et al., 2007; Lee et al., 2008). There is currently no effective treatment for the brain edema other than emergency liver transplantation (Farges et al., 1996; Hoofnagle et al., 1995; Vaquero et al., 2003). Cytotoxic brain edema, principally due to astrocyte swelling, is the major neuropathological finding in ALF (Jover et al., 2006; Kato et al., 1989; Martinez, 1968; Norenberg, 1977; Traber et al., 1989). Employing neuroimaging techniques, several reports have documented a significant intracellular accumulation of water in brain parenchyma in humans and experimental ALF of various etiologies, indicating the presence of cytotoxic brain edema. For review, see (Chavarria et al., 2011).

Several lines of evidence indicate that elevated blood and brain ammonia play major roles in the development of brain edema in ALF. For reviews, see (Blei, 1997). Blood and brain ammonia levels have been shown to correlate with the degree of encephalopathy and brain edema (Clemmesen et al., 1999; Kato et al., 1989; Ong et al., 2003; Traber et al., 1987), and ammonia is known to induce cell swelling in cultured astrocytes (Norenberg et al., 1991; Olson et al., 1992; Zwingmann et al., 2000) and in brain slices (Ganz et al., 1989).

The mechanisms by which ALF causes astrocyte swelling are not completely clear. It has recently been shown that altered ionic homeostasis, largely mediated by activation of the Na^+,K^+,$2Cl^-$ cotransporter (NKCC1) and associated cytoplasmic osmolar imbalance are involved (Jayakumar et al., 2008; Jayakumar et al., 2011). Such osmotic imbalance must be accompanied by the entry of water into cells so as to achieve osmotic equilibrium.

Water entry in some cell types is known to be mediated largely by aquaporin (AQP) water channels (King and Agre, 1996). AQP4 is the principal water channel in astrocytes (Nielsen et al., 1997), where it is involved in the development of brain edema in various neurological conditions, including ischemic stroke, traumatic brain injury, brain tumors and hyponatremia (Bloch et al., 2005; Manley et al., 2004; Papadopoulos and Verkman, 2007; Verkman et al., 2006). Knock-out mice lacking AQP4 are resistant to the development of the cytotoxic brain edema in hyponatremia and ischemic stroke (Manley et al., 2000).

Previous studies have documented that treatment of cultured astrocytes with ammonia resulted in increased AQP4 levels, which correlated well with the degree of cell swelling (Rama Rao et al., 2003). Additionally, silencing AQP4 in cultured astrocytes prevented the cell swelling following exposure to ammonia (Rama Rao et al., 2010). An increase in brain plasma membrane AQP4 was also documented in experimental ALF (Eefsen et al., 2010; Rama Rao et al., 2010). Together, these studies invoke a major role for AQP4 in the astrocyte swelling/brain edema associated with ALF. Despite these findings, one report found unaltered levels of brain AQP4 in experimental ALF (Wright et al., 2010).

To more comprehensively assess the role of AQP4, the present study employed AQP4-null mice to examine the role of AQP4 in the evolution of brain edema and associated neurological deficits in ALF using two models produced by the hepatotoxins thioacetamide (TAA) and acetaminophen (APAP). We found a robust development of brain edema in wild-type (WT) mice, which was associated with an increase in AQP4 content in the plasma membrane, although total cortical levels remained unchanged. Brain edema was remarkably reduced in AQP4-null mice following ALF, and these mice showed delayed onset of coma. These findings suggest that AQP4 as an important determinant in the brain edema associated with ALF.

Materials and Methods

Transgenic AQP4-null mice

AQP4-null mice with CD1 background originally developed by Ma et al. (1997) were used in the present study. WT mice were generated by crossing CD1 males (Charles River Laboratories) with B6D2F1 females (Jackson Laboratories), and littermates of 10–14 weeks old (35–45 g) containing 50% of CD-1 and B6D2F1 background were used.

Thioacetamide (TAA) model of ALF

TAA (100 mg/kg, i.p.) was used to induce ALF as described previously by Jayakumar et al. (2011). These mice have been previously characterized with regard to blood levels of glucose, lactate, creatinine, hemoglobin (Sarhan et al., 1993; Caballero et al., 2001; Avraham et al., 2010; Lim JH et al., 2011; Chiang et al., 2011) In brief, TAA was injected i.p. daily for 3 days. To prevent hypoglycemia and dehydration, mice were given 0.5 ml/kg of fluid therapy every 12 h, s.c. (5% dextrose and 0.45% saline containing 20 mEq/l of potassium chloride). Control groups for both WT-type and AQP-4-null mice received normal saline (vehicle used for TAA). Mice were euthanized by decapitation at the onset of coma (Grade V HE) (Gammal and Jones, 1989).

Acetaminophen (APAP) model of ALF

The APAP model of ALF was induced in mice following the same protocol described for TAA, except that mice were fasted for 12 h before the injection of APAP (Kon et al., 2004; Shah et al., 2013). APAP (500 mg/kg) was freshly dissolved in pre-heated saline and a single dose was injected i.p. to WT and AQP4-null mice. Similar to the TAA model of ALF, these mice have been previously characterized with regard to blood levels of glucose, lactate, creatinine, hemoglobin, (Lim et al., 2010; Das et al., 2010; Grace-Lynn et al., 2012; Shah et al., 2013). To prevent hypoglycemia and dehydration, 3–4 h following APAP injection, mice were given 0.5 ml/kg of fluid therapy, s.c. (5% dextrose and 0.45% saline containing 20 mEq/l of potassium chloride). Control groups for both WT-type and AQP-4-null mice received normal saline (vehicle used for APAP). Following injection, mice had free access to food and water. All APAP-treated animals developed coma at 8±1.5 h, at which time they were euthanized by decapitation. Cerebral cortices were rapidly removed and then frozen at −80°C for subsequent Western blot studies. Both AQP4-null mice and WT-mice treated with hepatotoxins were euthanized at identical time points, i.e., at the time when WT-mice developed coma, so as to make a comparative analysis on brain water content in these mice.

All experimental procedures followed guidelines established by National Institute of Health Guide for the Care and Use of Laboratory animals and were approved by our Institutional Animal Care and Use Committee (IACUC).

Preparation of plasma membranes from cerebral cortex

Plasma membrane enriched fractions were isolated following the method of Marples et al. (Marples et al., 1995). In brief, cortical tissue was homogenized in 0.32 M sucrose-EDTA buffer containing a protease inhibitor cocktail (PIC, Roche Diagnostics), and centrifuged at 3,000 *g* for 5 min. The pellet was frozen at −80°C for 1 h to fracture the cells; then thawed and homogenized in 50 mM Tris-HCl (pH 8) containing PIC. The homogenates were centrifuged at 35,000 *g* for 30 min and the pellets were rehomogenized 2 times in 50 mM Tris-HCl buffer. The final pellet containing the plasma membrane enriched fraction was dissolved in 0.25 ml of lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 10% SDS, 1% NP-40, 5% sodium-deoxycholate and PIC.

Immunoblotting

The protein concentration in plasma membranes was determined by the bicinchoninic acid method (BioRad). Equal quantities of plasma membrane and tissue lysates were subjected to SDS-PAGE using 12% gels (Tris-HCl, pH 7.4) and then electrophoretically transferred to PVDF membranes. Blots were blocked with 5% nonfat dry milk in tris-buffered saline (TBS) containing Tween 20 (20 mM Tris-HCl, 150 mM NaCl, pH 7.4, and 0.05% Tween 20; TBS-T) for 2 h at room temperature and then incubated with rabbit anti-AQP4 (1:3000, Millipore), over-night at 4°C. PVDF membranes were then washed with TBS-T and incubated with HRP-conjugated secondary antibodies for 2 h at RT. After washing, membranes were visualized using enhanced chemiluminescence (ECL-plus; Amersham Biosciences, Piscataway, NJ). Optical densities of the bands were measured with the Chemi-Imager digital imaging system (Alpha Innotech, San Leandro, CA), and the results were quantified with the Sigma Scan Pro program (St. Louis, MO) as a proportion of the signal of a plasma membrane marker protein (Na^+-K^+-ATPase).

Immunohistochemistry

Mice were anesthetized and transcardially perfused with heparinized saline for 1 min, followed by fixation in 4% paraformaldehyde for 15 min. The heads were left in the same fixative for an additional 24 h at 5°C and cryoprotected with 30% sucrose in PBS. Coronal sections of brain were obtained and 20 μm thick sections were prepared with a cryostat. Frozen sections were blocked with 10% goat serum and incubated with specific antibodies to AQP4 (1:100) (Chemicon, CA), GLT-1 (1:100) overnight at 4°C. Sections were washed with tris-buffered saline (TBS) containing 0.1% Triton X 100 (TBS-T); incubated with fluorescent AlexaFlour-FITC and AlexaFlour-Rhodamine conjugated secondary antibodies (1:500) for 2 h; covered with commercial mounting media (Vector Laboratories), and examined with a laser scanning confocal microscope (Olympus, Japan). Fluorescent images were captured by randomly moving the microscope stage 5 mm^2 in all 4 directions.

Measurement of brain edema

Brain water content was determined by the wet/dry weight method. Approximately 10 mg tissue (3–4 pieces from each animal) of cerebral cortex were dissected; wet weights of tissue determined; tissue dried overnight in an oven at 120°C; and dry weights determined. The difference in wet/dry weights were converted to percent water content (tissue wet weight − tissue dry weight)/wet weight ×100).

Statistical Analysis

Data are presented as mean ± SEM of control and experimental groups consisting of 5–8 WT and AQP4-null mice. The data were subjected to analysis of variance (ANOVA) followed by Neuman-Keuls post-hoc analysis. A $p<0.05$ was considered significant.

Results

Thioacetamide (TAA) model

All mice were clinically monitored and the extent of encephalopathy was graded as previously described (Gammal and Jones, 1989). TAA-treated mice appeared normal during the first 24 h after the administration of TAA. Between 24 and 36 h, WT mice gradually developed Grade I encephalopathy (Fig. 1A). Symptoms progressively worsened over time, and by approximately 60–72 h, the animals evolved to Grade II–III encephalopathy. This rapidly progressed to Grade V encephalopathy at approximately 90 h, at which time mice displayed loss of righting reflexes, and unresponsiveness to pain, followed by reduced

corneal reflexes. By contrast, AQP4-null mice treated with TAA were less symptomatic as compared to WT mice and only reached Grade III encephalopathy at 90 h (Fig. 1A).

Serum ammonia levels in both WT and APQ4-null mice increased by approximately 5-fold after TAA treatment at the time WT mice were euthanized (385 ± 39 in WT-TAA; 421 ± 36 in AQP4-TAA vs. 69 ± 10 μM in WT-control). The extent of liver injury was assessed by measuring serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), both of which showed an approximately 10-fold increase in AST activity and a 13-fold increase ALT activity in TAA-treated animals as compared to controls in both WT and AQP-null mice (Table 1). There was also a significant increase plasma levels of total bilirubin and creatinine in both WT and AQP4 mice given TAA (Table 2).

Acetaminophen (APAP) model

In contrast to TAA, the APAP model of ALF showed an extremely rapid onset of liver damage and encepahalopathy resulting in neurological deterioration (8–10 h) as compared to TAA (90 h) (Table 2 and Fig. 1B). Mice were slightly lethargic 2–3 h after the administration of APAP, which progressed to stage II–III encephalopathy as shown by difficulty in retaining a normal gait and remaining confined to one corner of the cage with the head tilted downward. This condition further worsened to coma, which was associated with the loss of righting and corneal reflexes. This stage was achieved in WT mice at 8 ± 1.5 h, whereas AQP4-null mice similarly treated with APAP displayed a comparable level of encephalopathy after 12–14 h after (data not shown).

Serum ammonia levels were increased by 7-fold in both WT and AQP4-null mice treated with APAP (WT-control 71 ± 18 vs. 589 ± 11 μM in WT-mice-APAP; 613 ± 88 in AQP4-null-APAP). Extensive liver damage in APAP-induced ALF was found where serum levels of ALT increased 60-fold and AST levels increased 10-fold (Table 3). AQP4-null mice treated with APAP also showed comparable AST and ALT levels (Table 2). There was also a significant increase in plasma levels of total bilirubin and creatinine in both WT and AQP4 mice given APAP (Table 4).

Brain water content

WT mice treated with TAA showed a significant increase in brain water content (1.64 ± 0.5%, $p<0.01$) by 80–90 h as compared to saline-treated controls (Fig. 2). By contrast, identically treated AQP4-null mice showed no, or only a minimal, increase in brain water content as compared to WT mice treated with TAA. Similar results were also found after APAP treatment to mice, but the extent of increase in brain water content was higher (2.36 ± 0.7%, $p<0.01$) than in TAA-treated mice (Fig. 3).

AQP4 protein content

AQP4 protein content was determined in plasma membrane fractions in WT mice treated with TAA or APAP. Treatment of WT mice with TAA showed an 85% increase in AQP4 protein at the coma stage (Fig. 4). APAP treatment of WT mice showed approximately 150% increase in AQP4 protein content in the plasma membrane fraction.

Immunohistochemistry of AQP4

Immunofluorescence of AQP4 in cortical sections in TAA- and APAP-treated WT mice displayed an increase in AQP4 expression in close relationship to capillaries and small blood vessels (Fig. 5), consistent with the perivascular localization of AQP4 in astrocytic end-feet plasma membranes. We also found a significant colocalization of AQP4 with the capillary plasma membrane marker glucose transporter 1 (GLUT 1).

Discussion

In this study, transgenic mice deficient in AQP4 (AQP4-null mice) showed a remarkably reduced level of brain edema as compared to WT mice following ALF produced by two different hepatotoxins, TAA or APAP. AQP4-null mice also showed a lesser degree of encephalopathy at the time WT mice with ALF were comatose. Levels of AQP4 were found to be significantly increased in the plasma membrane fraction (PM) in WT mice in two different models of ALF. Such increment in AQP4 levels correlated with the degree of brain edema and encephalopathy in WT-mice. Together, these events strongly suggest AQP4 in the mechanism of brain edema in ALF.

We previously documented that treatment of cultured astrocytes with a clinically relevant concentration of ammonia caused a significant increase in AQP4 protein content in the plasma membrane (Rama Rao et al., 2003). A similar finding was also reported by Bodega et al. (Bodega et al., 2012). More recently, two reports have documented increased brain plasma membrane levels of AQP4 in rats with ALF without any change in total tissue AQP4 protein (Eefsen et al., 2010; Rama Rao et al., 2010).

A study by Wright et al. (Wright et al., 2010), found no change in total tissue levels of AQP4 in brain in a rat model of ALF induced by galactosamine, which is in agreement with observations by other investigators (Eefsen et al., 2010; Rama Rao et al., 2010). Based on this finding, these investigators postulated that AQP4 plays no role in the brain edema in ALF. However, this study, did not examine levels of AQP4 in the plasma membrane, the site where AQP4 facilitates water movement. Such plasma membrane increase in AQP4 was found in brains of rats with ALF (Eefsen et al., 2010; Rama Rao et al., 2010). Complementary to our observations, a recent study also reported increased plasma membrane levels of AQP4 in human postmortem cerebral cortex from patients with ALF (Thumburu et al., 2013).

Both *in vitro* and *in vivo* studies strongly suggest a close association between the development of brain edema and increased brain AQP4 expression in the plasma membrane in ALF. However, whether AQP4 critically contributes to the development of brain edema in ALF has not been fully established. The present study therefore employed two different models of ALF (TAA and APAP hepatotoxicity) in AQP4-null mice and their WT counterparts and established the extent of brain edema in both models. The results of these studies showed a complete absence or a marked reduction in brain edema in AQP4-null mice following ALF as compared to WT-mice, supporting a crucial role of AQP4 in the development of brain edema in ALF.

Despite the absence of brain edema in AQP4-null mice, these mice still developed encephalopathic symptoms, although with a significantly delayed time course as compared to WT mice. The explanation for the encephalopathy in these mice in the absence of brain edema is not completely clear. However, studies have previously documented that increased cerebral blood flow (hyperemia) may contribute to an increase in intracranial pressure (ICP) in patients, as well as in experimental animals with ALF (Jalan, 2005; Larsen et al., 2001). It is therefore possible that hyperemia and associated increase in ICP, without overt brain edema, may have contributed to the encephalopathy in AQP4-null mice. It is also possible that elevated levels of brain ammonia due to increased cerebral blood flow may have contributed to encephalopathy.

While mechanisms of astrocyte swelling/cytotoxic edema in ALF are not completely clear, the majority of reports indicate that the edema is cytotoxic in origin i.e., an intracellular accumulation of water principally in astrocytes (Kato et al., 1989; Martinez, 1968;

Norenberg, 1977; Traber et al., 1989). T2-weighted MRI scans in patients with ALF displayed a significant reduction in ADC values, corresponding to an increase in intracellular water in brain, indicating that the brain edema in ALF is indeed cytotoxic. For review, see (Chavarria et al., 2011) Moreover, in a model of ALF induced by thioacetamide, a complete absence of blood brain-barrier disruption was observed (Szumanska and Albrecht, 1997), further supporting the prevalence of cytotoxic brain edema in ALF. Nevertheless, there may also be an element of vasogenic edema in the end-stage liver failure, likely due to secondary complications (sepsis, hypotension and associated hypoxia).

AQP4 has been shown to facilitate the formation of cytotoxic edema by allowing rapid water movement from blood into astrocytes across the astrocyte plasma membrane. For review, see (Papadopoulos and Verkman, 2013). Conversely, AQP4 has also been shown facilitate the resolution of vasogenic edema by enhancing the clearance of extracellular water into the perivenous space (Papadopoulos and Verkman, 2013). Thus, while AQP4 gene deletion has been shown to exert protection against cytotoxic brain edema, AQP4 gene deletion was shown to be detrimental in disorders associated with vasogenic edema, as may occur with neoplasms, abscess and subarachnoid hemorrhage. For review, see (Papadopoulos and Verkman, 2013). The present study showing a marked reduction in the brain edema of AQP4-null mice follows the general pattern observed in cytotoxic edema.

The means by which an increase in plasma membrane levels of AQP4 occurs in ALF is not known. It has been proposed that α–syntrophin, a member of the dystrophin family of proteins, tethers AQP4 to its C-terminal PDZ domain, thereby promoting a greater anchoring (i.e., stability) of AQP4 in the plasma membrane of astrocytes (Neely et al., 2001). It is therefore possible that increased stability of AQP4, facilitated by α–syntrophin, might occur in ALF. In partial support of this view, we recently found that α-syntrophin protein expression was increased in brains of rats with ALF induced by thioacetamide (Rama Rao et al., 2010). It is thus possible that an increase in α-syntrophin might also have occurred in the present study in WT mice with ALF. Another possible mechanism for increased plasma membrane level of AQP4 could be a reduction and/or a delay in the degradation. of AQP4, a process mediated by ubiquitination and subsequent proteasomal degradation, as such a process has been found following injury to retinal Müller cells (Dibas et al., 2008). Whether or not such an event occurs in ALF in not known.

Conclusions

In conclusion, this study demonstrates that ALF induced by thioacetamide or acetaminophen results in the development of brain edema in WT mice, which was associated with a concomitant increase in brain AQP4 in the plasma membrane of astrocytes. AQP4-null mice were resistant to the development of brain edema following ALF. Together, these results indicate that AQP4 represents an important determinant in the brain edema associated with ALF and thus a potential therapeutic target for patients with ALF.

Acknowledgments

This work was supported by NIH Grant DK06331 and by a Department of Veterans Affairs Merit Review Award (MDN) and by NIH grant DK35124 (ASV).

References

Avraham Y, et al. Cannabidiol improves brain and liver function in a fulminant hepatic failure-induced model of hepatic encephalopathy in mice. Br J Pharmacol. 2011; 162:1650–1658. [PubMed: 21182490]

Blei AT. Brain edema and intracranial hypertension: a focus for the use of liver support systems. Artif Organs. 1997; 21:1182–1184. [PubMed: 9384323]

Bloch O, et al. Aquaporin-4 gene deletion in mice increases focal edema associated with staphylococcal brain abscess. J Neurochem. 2005; 95:254–262. [PubMed: 16181429]

Bodega G, et al. Ammonia induces aquaporin-4 rearrangement in the plasma membrane of cultured astrocytes. Neurochem Int. 2012; 61:1314–1324. [PubMed: 23022607]

Caballero ME, et al. Epidermal growth factor reduces multiorgan failure induced by thioacetamide. Gut. 2001; 48:34–40. [PubMed: 11115820]

Chiang CH, et al. Investigation of hepatoprotective activity of induced pluripotent stem cells in the mouse model of liver injury. J Biomed Biotechnol. 2011:219060. 2011. [PubMed: 21808596]

Chavarria L, et al. Neuroimaging in acute liver failure. Neurochem Int. 2011; 59:1175–1180. [PubMed: 21945201]

Clemmesen JO, et al. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology. 1999; 29:648–653. [PubMed: 10051463]

Dibas A, et al. Changes in ocular aquaporin-4 (AQP4) expression following retinal injury. Mol Vis. 2008; 14:1770–1783. [PubMed: 18836575]

Eefsen M, et al. Brain expression of the water channels aquaporin-1 and -4 in mice with acute liver injury, hyperammonemia and brain edema. Metab Brain Dis. 2010; 25:315–323. [PubMed: 20938728]

Escorsell A, et al. Acute liver failure in Spain: analysis of 267 cases. Liver Transpl. 2007; 13:1389–1395. [PubMed: 17370334]

Farges O, et al. Incidence of rejection and infection after liver transplantation as a function of the primary disease: possible influence of alcohol and polyclonal immunoglobulins. Hepatology. 1996; 23:240–248. [PubMed: 8591847]

Gammal SH, Jones EA. Hepatic encephalopathy. Med Clin North Am. 1989; 73:793–813. [PubMed: 2657263]

Ganz R, et al. Ammonia-induced swelling of rat cerebral cortical slices: implications for the pathogenesis of brain edema in acute hepatic failure. Metab Brain Dis. 1989; 4:213–223. [PubMed: 2796874]

Ghosh J, et al. Taurine prevents arsenic-induced cardiac oxidative stress and apoptotic damage: role of NF-kappa B, p38 and JNK MAPK pathway. Toxicol Appl Pharmacol. 2009; 240:73–87. [PubMed: 19616567]

Grace-Lynn C, et al. Evaluation of the hepatoprotective Effects of Lantadene A, a pentacyclic triterpenoid of Lantana plants against acetaminophen-induced liver damage. Molecules. 2012; 17:13937–13947. [PubMed: 23178309]

Hoofnagle JH, et al. Fulminant hepatic failure: summary of a workshop. Hepatology. 1995; 21:240–252. [PubMed: 7806160]

Jalan R. Pathophysiological basis of therapy of raised intracranial pressure in acute liver failure. Neurochem Int. 2005; 47:78–83. [PubMed: 15927306]

Jayakumar AR, et al. Na-K-Cl cotransporter-1 in the mechanism of ammonia-induced astrocyte swelling. J Biol Chem. 2008

Jayakumar AR, et al. The Na-K-Cl cotransporter in the brain edema of acute liver failure. J Hepatol. 2011; 54:272–278. [PubMed: 21056502]

Jover R, et al. Brain edema and inflammatory activation in bile duct ligated rats with diet-induced hyperammonemia: A model of hepatic encephalopathy in cirrhosis. Hepatology. 2006; 43:1257–1266. [PubMed: 16729306]

Kato M, et al. Electron microscopic study of the blood-brain barrier in rats with brain edema and encephalopathy due to acute hepatic failure. Gastroenterol Jpn. 1989; 24:135–142. [PubMed: 2744330]

King LS, Agre P. Pathophysiology of the aquaporin water channels. Annu Rev Physiol. 1996; 58:619–648. [PubMed: 8815812]

Kon K, et al. Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. Hepatology. 2004; 40:1170–1179. [PubMed: 15486922]

Larsen FS, et al. Cerebral hyperemia and nitric oxide synthase in rats with ammonia-induced brain edema. J Hepatol. 2001; 34:548–554. [PubMed: 11394654]

Lee WM, et al. Acute liver failure: Summary of a workshop. Hepatology. 2008; 47:1401–1415. [PubMed: 18318440]

Lim AY, et al. Histopathology and biochemistry analysis of the interaction between sunitinib and paracetamol in mice. BMC Pharmacol. 2010; 10:14. [PubMed: 20950441]

Lim JH, et al. Protective Effects of Platycodon grandiflorum Aqueous Extract on Thioacetamide-induced Fulminant Hepatic Failure in Mice. J Toxicol Pathol. 2011; 24:223–228. [PubMed: 22319234]

Ma T, et al. Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. J Clin Invest. 1997; 100:957–962. [PubMed: 9276712]

Manley GT, et al. New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice. Neuroscience. 2004; 129:983–991. [PubMed: 15561413]

Manley GT, et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med. 2000; 6:159–163. [PubMed: 10655103]

Marples D, et al. Redistribution of aquaporin-2 water channels induced by vasopressin in rat kidney inner medullary collecting duct. Am J Physiol. 1995; 269:C655–664. [PubMed: 7573395]

Martinez A. Electron microscopy in human hepatic encephalopathy. Acta Neuropathol (Berl). 1968; 11:82–86. [PubMed: 5699273]

Neely JD, et al. Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. Proc Natl Acad Sci U S A. 2001; 98:14108–14113. [PubMed: 11717465]

Nielsen S, et al. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci. 1997; 17:171–180. [PubMed: 8987746]

Norenberg MD. A light and electron microscopic study of experimental portal-systemic (ammonia) encephalopathy. Progression and reversal of the disorder. Lab Invest. 1977; 36:618–627. [PubMed: 559221]

Norenberg MD, et al. Ammonia-induced astrocyte swelling in primary culture. Neurochem Res. 1991; 16:833–836. [PubMed: 1944774]

Olson JE, et al. Astrocyte volume regulation and ATP and phosphocreatine concentrations after exposure to salicylate, ammonium, and fatty acids. Metab Brain Dis. 1992; 7:183–196. [PubMed: 1294868]

Ong JP, et al. Correlation between ammonia levels and the severity of hepatic encephalopathy. Am J Med. 2003; 114:188–193. [PubMed: 12637132]

Papadopoulos MC, Verkman AS. Aquaporin-4 and brain edema. Pediatr Nephrol. 2007

Papadopoulos MC, Verkman AS. Aquaporin water channels in the nervous system. Nat Rev Neurosci. 2013; 14:265–277. [PubMed: 23481483]

Rama Rao KV, et al. Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. Neuroreport. 2003; 14:2379–2382. [PubMed: 14663195]

Rama Rao KV, et al. Brain aquaporin-4 in experimental acute liver failure. J Neuropathol Exp Neurol. 2010; 69:869–879. [PubMed: 20720509]

Sarhan S, et al. Effects of inhibition of ornithine aminotransferase on thioacetamide-induced hepatogenic encephalopathy. Neurochem Res. 1993; 18:539–549. [PubMed: 8474573]

Shah N, et al. Role of toll-like receptor-4 in mediating multi-organ dysfunction in acetaminophen induced acute liver failure in mice. Liver Transpl. 2013

Szumanska G, Albrecht J. Lectin histochemistry of the rat brain following thioacetamide-induced hepatic failure. Mol Chem Neuropathol. 1997; 32:163–177. [PubMed: 9437665]

Thumburu KK, et al. Expression of astrocytic genes coding for proteins implicated in neural excitation and brain edema is altered after acute liver failure. J Neurochem. 2013 Accepted Article. 10.1111/jnc.12511

Traber P, et al. Effect of body temperature on brain edema and encephalopathy in the rat after hepatic devascularization. Gastroenterology. 1989; 96:885–891. [PubMed: 2914649]

Traber PG, et al. Electron microscopic evaluation of brain edema in rabbits with galactosamine-induced fulminant hepatic failure: ultrastructure and integrity of the blood-brain barrier. Hepatology. 1987; 7:1272–1277. [PubMed: 3679092]

Vaquero J, et al. Pathogenesis of hepatic encephalopathy in acute liver failure. Semin Liver Dis. 2003; 23:259–269. [PubMed: 14523679]

Verkman AS, et al. Three distinct roles of aquaporin-4 in brain function revealed by knockout mice. Biochim Biophys Acta. 2006

Wright G, et al. Role of aquaporin-4 in the development of brain eodema in liver failure. J Hepatol. 2010; 53:91–97. [PubMed: 20451280]

Zwingmann C, et al. Effects of ammonia exposition on glioma cells: changes in cell volume and organic osmolytes studied by diffusion-weighted and high-resolution NMR spectroscopy. Dev Neurosci. 2000; 22:463–471. [PubMed: 11111163]

HIGHLIGHTS

- Acute liver failure (ALF) in mice resulted in a marked brain edema (swelling).
- Aquaporin-4-null mice (AQP-4-null) with ALF show a marked reduction in brain edema.
- AQP-4-null mice with ALF also show markedly reduced neurological deficits.

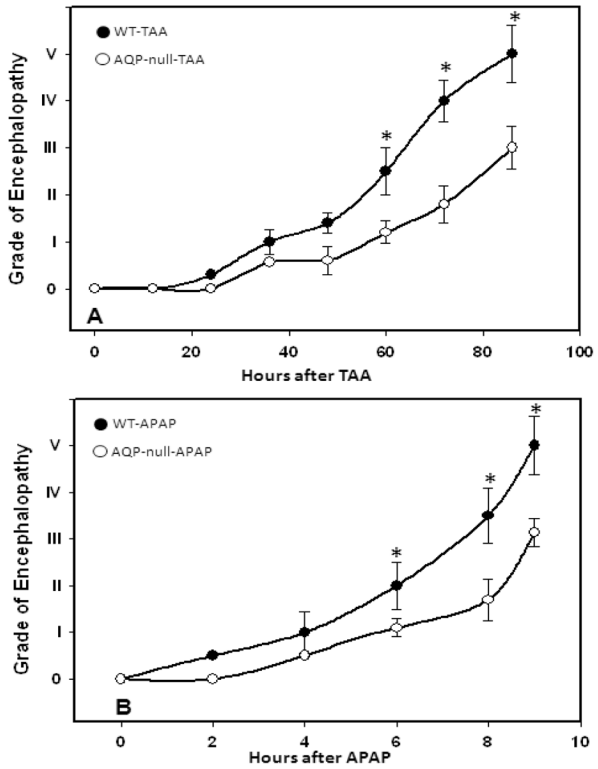

Figure 1.

A. Grades of encephalopathy in WT and AQP4-null mice with ALF induced by thioacetamide (TAA). AQP4 gene deletion significantly delays the onset of encephalopathy in mice. Values are mean ± S.E.M. of 6–7 animals in each experimental group. * vs. AQP4-null mice, $p<0.05$. **B.** Grades of encephalopathy in WT and AQP4-null mice with ALF induced by acetaminophen (APAP). AQP4 gene deletion significantly delayed the development of encephalopathy. Values are mean ± S.E.M. of 6–7 animals in each experimental group. * vs. AQP4-null mice, $p<0.05$.

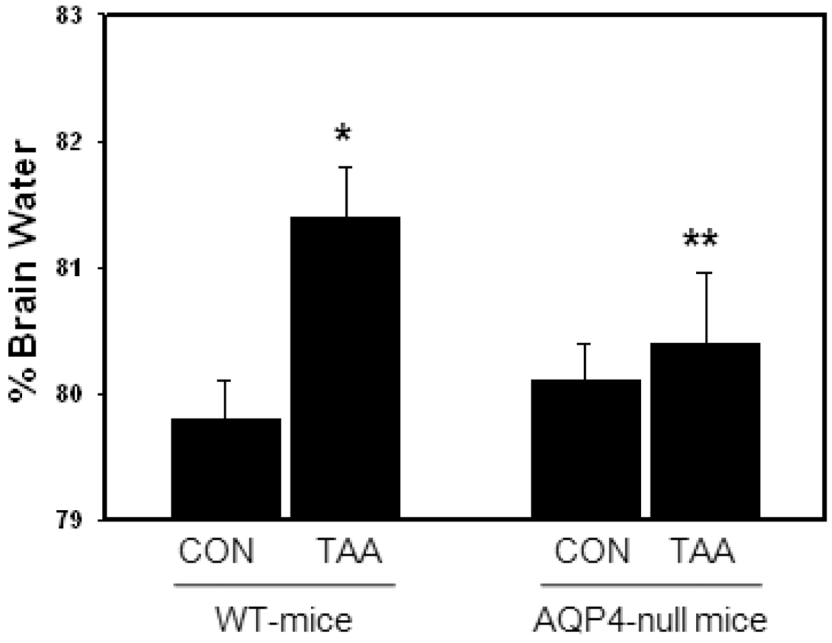

Figure 2.

Brain water content in wild-type (WT) and AQP4-null mice treated with thioacetamide (TAA). WT-mice treated with TAA showed a 1.64 ± 0.3% increase in brain water content as compared to WT-control mice. Values are mean ± S.E.M. of 6–8 animals in each experimental group. * vs. control, $p<0.01$; ** vs. WT-TAA, $p<0.05$. CON, control.

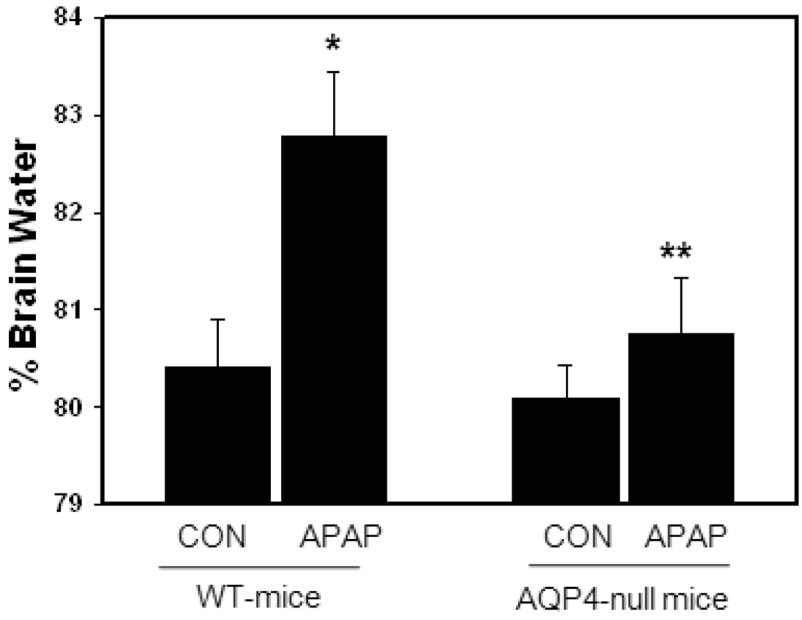

Figure 3.

Brain water content in WT and AQP4-null mice treated with acetaminophen (APAP). WT-mice treated with APAP displayed a 2.36 ± 0.39% rise in brain water content as compared to WT-control mice. Values are mean ± S.E.M. of 5–7 animals in each experimental group. * vs. control, $p<0.01$; ** vs. WT-APAP, $p<0.05$. CON, control.

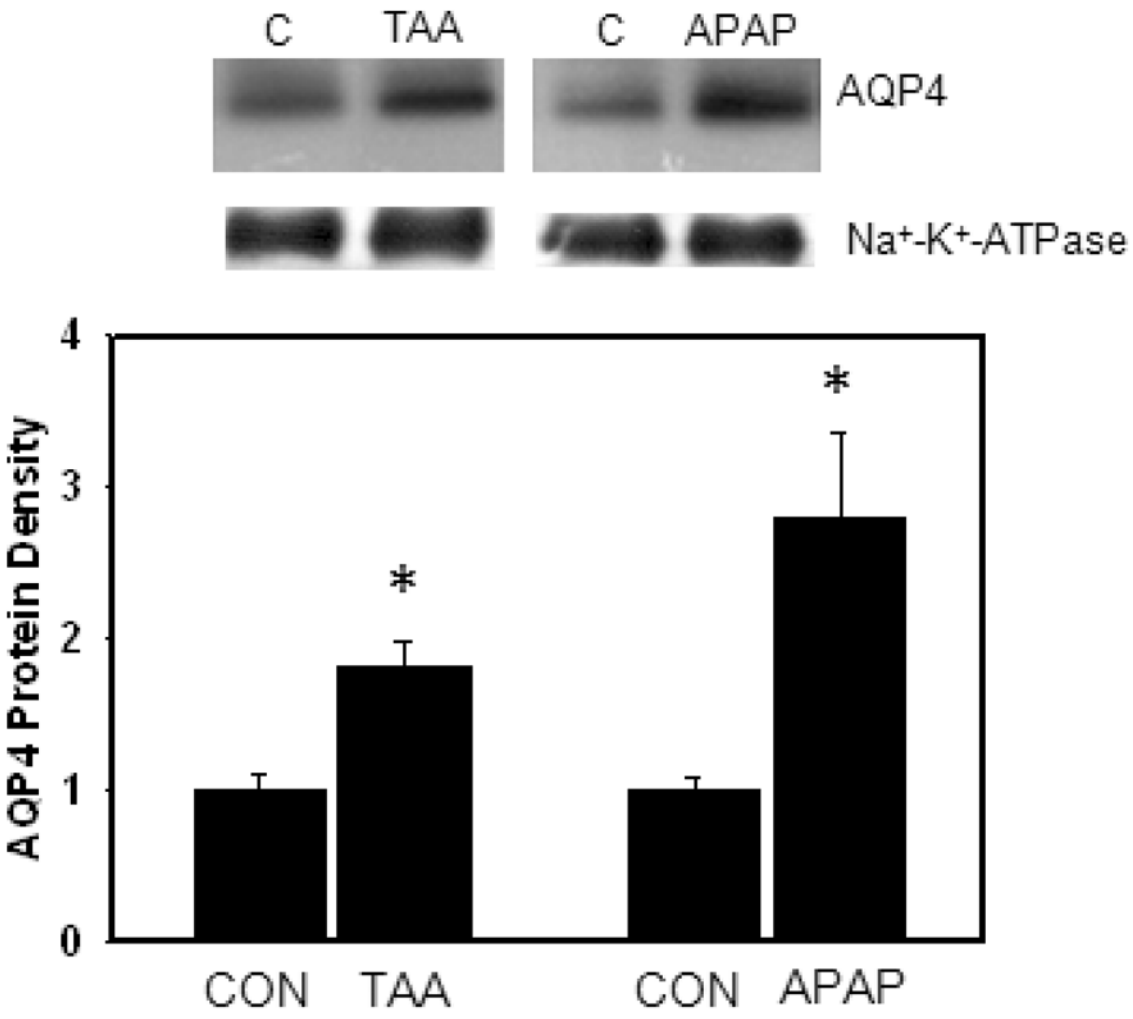

Figure 4.

A. AQP4 protein expression in brain plasma membranes of WT mice treated with TAA or APAP show a significant increase in AQP4 content. B. Quantitation of AQP4 protein content in plasma membrane fraction. Plasma membrane AQP4 protein content was normalized to protein content of Na^+,K^+ ATPase Values are mean ± S.E.M. of 5 animals in each experimental group. * vs. control, $p<0.01$. CON, control.

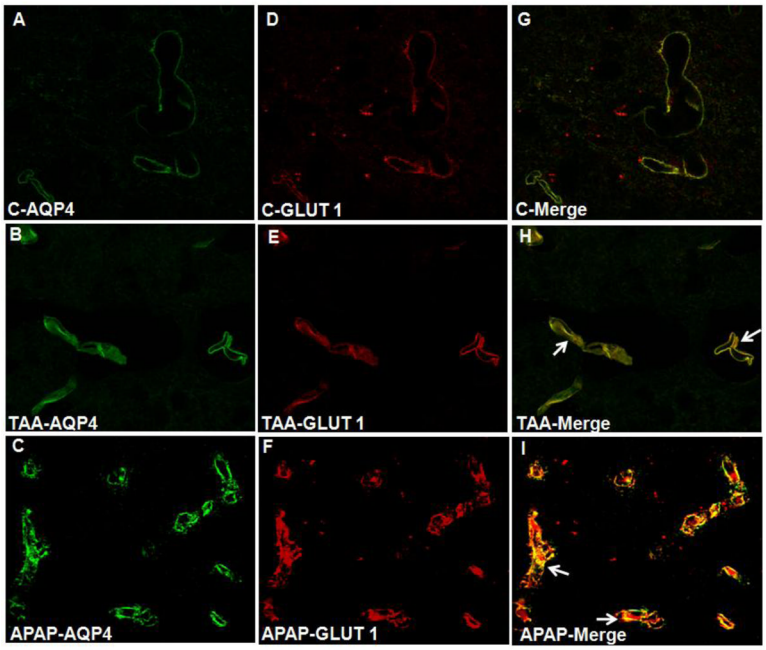

Figure 5.

Immunohistochemistry of AQP4 (A, B, C), and the capillary endothelial marker GLUT 1 (D, E, F) in cerebral cortex of control (C), thioacetamide (TAA)-and acetaminophen (APAP)-treated WT-mice. Merged immunofluorescent images (G, H, I) display a co-localization of AQP4 with GLUT 1, consistent with the perivascular localization of AQP4 on astrocytic plasma membranes. Immunofluorescence shows an increase in AQP4 (arrows) in WT-mice treated with TAA or APAP. Scale bar = 50 μm.

Table 1

Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In control, wild-type (WT) and AQP4-null mice treated with TAA (AQP4-null-TAA).

Enzyme	WT-control	WT-TAA	AQP-null-TAA
AST	292 ± 87	2646 ± 973*	2377 ± 898*
ALT	66.16 ± 22	1084 ± 168*	963 ± 198*

Values are mean ± S.E.M (n=6–7 in each group).

*p<0.01 vs. WT control. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 2

Biochemical parameters in the blood plasma of control, wild-type (WT) and AQP4-null mice treated with TAA (AQP4-null-TAA).

Metabolite	WT-control (mM)	WT-TAA (mM)	AQP4-null-TAA (mM)
Glucose	10.6 ± 1	11.1 ± 1.2	11.4 ± .96
Lactate	5.1 ± .6	4.7 ± .56	4.5 ± 0.6
Creatinine	0.022 ± 0.003	0.051 ± 0.004*	0.058 ± 0.006*
Total bilirubin	0.00102 ± 0.0002	0.0047 ± 0.0004*	0.0052 ± 0.0003*

Values are mean ± S.E.M (n=5 in each group).

* $p<0.01$ vs. control (CON).

Table 3

Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in control, wild-type (WT) and AQP4-null mice treated with APAP (10 h) (AQP4-null-APAP).

Enzyme	WT-control	WT-APAP	AQP-null-APAP
AST	342 ± 95	3578 ± 873*	3298 ± 498*
ALT	72.16 ± 14	4569 ± 569*	4286 ± 476*

Values are mean ± S.E.M (n=6–7 in each group).

* $p<0.01$ vs. WT-control.

Table 4

Biochemical parameters in the blood plasma of control, wild-type (WT) and AQP4-null mice treated with APAP (AQP4-null-APAP).

Metabolite	WT-control (mM)	WT-APAP (mM)	AQP4-null-APAP (mM)
Glucose	11.2 ± 1.3	10.56 ± 1.3	9.9 ± .96
Lactate	6.1 ± .72	4.6 ± .71	6.0 ± 0.56
Creatinine	0.019 ± 0.002	0.039 ± 0.0014*	0.046 ± 0.002*
Total bilirubin	0.0011 ± 0.0003	0.006 ± 0.0003*	0.0067± 0.0006*

Values are mean ± S.E.M (n=5 in each group).

* $p<0.01$ vs. WT-control.