Pathogenesis of Hepatic Encephalopathy in Acute Liver Failure

Javier Vaquero, M.D.,[1,2] Chuhan Chung, M.D.,[2] Michael E. Cahill, B.A.,[2] and Andres T. Blei, M.D.[2]

ABSTRACT

Hepatic encephalopathy (HE) in acute liver injury signifies a serious prognosis. Brain edema and intracranial hypertension are major causes of death in this syndrome. Comparison of HE in acute liver failure (ALF) with that of cirrhosis allows recognition of important differences and similarities. A key role for ammonia in the pathogenesis of both HE and brain edema is now firmly supported by clinical and experimental data. Additional factors, such as infection, products of the necrotic liver, and synergistic toxins, may contribute to an altered mental state. A low plasma osmolarity, high temperature, and both high and low arterial pressure may affect brain water content. A combined derangement of cellular osmolarity coupled with cerebral hyperemia can explain the development of brain edema in ALF. Increasingly, study of the mechanisms responsible for brain swelling provides critical information for understanding the pathogenesis of HE.

KEYWORDS: Hepatic encephalopathy, brain edema, acute liver failure, ammonia, cerebral blood flow

Objectives: Upon completion of this article, the reader should be able to (1) understand the key role played by ammonia in the pathogenesis of hepatic encephalopathy and brain edema in acute liver failure and (2) recognize the influence of other important additional factors in the development of these complications.

Accreditation: Tufts University School of Medicine (TUSM) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. TUSM takes responsibility for the content, quality, and scientific integrity of this CME activity.

Credit: TUSM designates this educational activity for a maximum of 1 Category 1 credit toward the AMA Physicians Recognition Award. Each physician should claim only those credits that he/she actually spent in the educational activity.

The development of HE in patients with ALF signals a critical phase of the illness (also defined as fulminant hepatic failure[1]) and is associated with a reduced survival. In epidemiological studies performed in the pretransplant era, spontaneous recovery of liver function was 70% in stages I and II encephalopathy and was reduced to < 20% in stages III and IV encephalopathy.[2] Death *in* hepatic coma is common in patients with cirrhosis and advanced liver failure, but a unique feature of ALF is death *from* cerebral edema and intracranial hypertension.

Fulminant Hepatic Failure; Editor in Chief, Paul D. Berk, M.D.; Guest Editor, Roger Williams, C.B.E., M.D., FRCP, FRCS, FRCPE, FRACP, F.Med.Sc., FRCPI (Hon), FACP (Hon). *Seminars in Liver Disease*, volume 23, number 3, 2003. Address for correspondence and reprint requests: Dr. Andres T. Blei, Searle 10–573, 303 East Chicago Ave., Chicago, IL, 60611. E-mail: a-blei@northwestern.edu. [1]Post-doctoral Research Fellow, [2]Section of Hepatology, Department of Medicine, Lakeside Veterans Administration Medical Center and Northwestern University Feinberg School of Medicine, Chicago, Illinois. Tel: +1(212) 584-4662. 0272–8087,p;2003,23,03,259,270,ftx,en;sld00229x.

A CLINICAL OVERVIEW: COMPARISON OF HE IN ALF AND CIRRHOSIS

Encephalopathy in ALF shares features and exhibits differences with the encephalopathy of cirrhosis. Five aspects deserve specific consideration.

Grading of HE

The West Haven criteria, designed for clinical studies in cirrhosis,[3] have also been used in patients with ALF. However, the precise characteristics of each stage often overlap, and differences between stages I and II or between II and III can be blurred. Certain clinical features of ALF are not well-represented in this classification, especially severe agitation, which can be an initial neurological symptom in ALF and pose serious problems in management (including the need to sedate the patient with loss of neurological end points for follow-up). An excitatory behavioral phase is consistent with robust experimental findings of an increased extracellular brain glutamate in this condition.[4]

Once stage IV encephalopathy is reached, the Glasgow coma scale, initially developed for patients with neurotrauma,[5] provides a numerical continuous score from 3 (worst) to 15 (best). Although it has not been formally evaluated in metabolic encephalopathies, it is better suited for examining patients in stages III and IV encephalopathy than the West Haven criteria, as was recently shown.[6]

Precipitating Factors

The pathogenic role of precipitating factors, well-recognized in the encephalopathy of cirrhosis, is often overlooked in ALF. Patients with acute liver failure may develop encephalopathy from the use of sedatives, as disturbances of sleep or agitation may be an early prodrome and are often medicated prior to arrival at a specialized center. Gastrointestinal hemorrhage, uremia, and electrolyte disturbances need to be ruled out. Infection, however, is the key precipitant to consider; the role of infection is discussed in the next section.

Seizures

Seizures have traditionally been viewed as a rare event in hepatic encephalopathy. A retrospective review of electroencephalogram tracings in 94 patients with cirrhosis described epileptiform abnormalities in 14% of subjects with deep encephalopathy who did not receive a liver transplant.[7]

Seizure activity has been reported in previous clinical series of ALF[8] and is a well-recognized complication of acute hyperammonemia in urea-cycle disorders.[9] In a recent controlled trial, subclinical seizure activity was detected in 10 of 22 patients enrolled as controls in a trial of prophylactic phenytoin in ALF.[10] Measurements of low oxygen saturation in the jugular vein led to the conclusion that poor cerebral perfusion and tissue anoxia were potential determinants of seizure development. At autopsy, patients in the nontreated group had greater evidence of cerebral edema. The high frequency of subclinical seizures reported in this series awaits confirmation from other centers.

Brain Edema

Death from intracranial hypertension has now been reported in patients with cirrhosis and deep hepatic encephalopathy in the setting of acute-on-chronic liver failure.[11,12] The magnetization transfer ratio, an indirect reflection of brain water content on spectroscopy, was clearly abnormal in patients with cirrhosis,[13] suggesting low-grade brain edema. The paradigm has shifted, with an increasing realization that a disturbance in brain water regulation is central to the process responsible for hepatic encephalopathy.[14,15] Nonetheless, a neurological death is a rare event in patients with cirrhosis.

Cerebral Perfusion

In cirrhosis, a reduction in cerebral blood flow has been described in patients with overt[16] and minimal[17] encephalopathy. A hyperdynamic circulatory state is a characteristic finding in liver failure, and the response of the cerebral circulation needs to be considered in this context. Recently, Guevara and colleagues[18] postulated a direct relation between the reduction in cerebral and renal blood flow in patients with cirrhosis and ascites. The decrease in perfusion of both territories was viewed as a response to systemic arterial vasodilatation, a sequence well-accepted for the renal vasoconstriction of cirrhosis.[19] The absence of signs of encephalopathy in these patients adds further credence to the view that the cerebral circulation also reacts to the generalized hemodynamic disturbance of liver failure.[20] In ALF, an initial reduction of cerebral blood flow (CBF) may reflect similar mechanisms.[21] However, a rise in CBF is prominently seen in patients with overt brain edema.[22]

PATHOGENESIS OF HE IN ALF—SYSTEMIC FACTORS

Conceptually, hepatic encephalopathy arises from exposure of the brain to circulating neurotoxins. In an early stage of research in this area, the absence of a critical trophic factor for brain function was postulated.[23] Recently, this idea has been revived in experiments performed in isolated liver-brain preparations.[24] However, multiple elements point at the role of circulating toxins, most conclusively the development of HE in the presence of a normal liver.[25]

Ammonia

A pathogenic role for ammonia has been the focus of experimental and clinical studies. Death from cerebral edema and intracranial hypertension is well-recognized in children with urea cycle enzyme deficiencies and severe hyperammonemia.[9] In human ALF, arterial ammonia levels > 200 μg/dL were associated with cerebral herniation within 24 hours of reaching stage III–IV encephalopathy.[26] These data have been subsequently confirmed[27] and point at levels of < 150 μg/dL as a cutoff below which the risk of neurological death may be substantially decreased.

Arterial sampling is important, because AV differences of ammonia can be considerable in ALF. In a recent human study, arterial concentration was 160 ± 53 versus 110 ± 35 μg/dL in the femoral vein, a significant difference.[28] Under normal circumstances (Fig. 1), the splanchnic release of ammonia, derived from the breakdown of glutamine in the intestine and the increased activity of colonic bacteria, results in 10-fold higher levels of ammonia in the portal vein. An efficient hepatic uptake mechanism, related to both urea (high capacity, low affinity) and glutamine (low capacity, high affinity) synthesis, results in tight control of ammonia levels reaching the periphery, with a hepatic extraction rate of 0.8 to 0.9.[29] In ALF, hepatic vein measurements showed higher levels of ammonia (242 ± 118) than those seen in arterial blood (182 ± 80 μg/dL, n = 22).[28] In the setting of an acutely failing liver, ammonia levels in the hepatic vein are similar to those seen in the portal vein.

Muscle uptake of this increased ammonia load results in the formation of glutamine. Whereas release and recirculation of glutamine will result in the regeneration of ammonia, splanchnic generation of ammonia

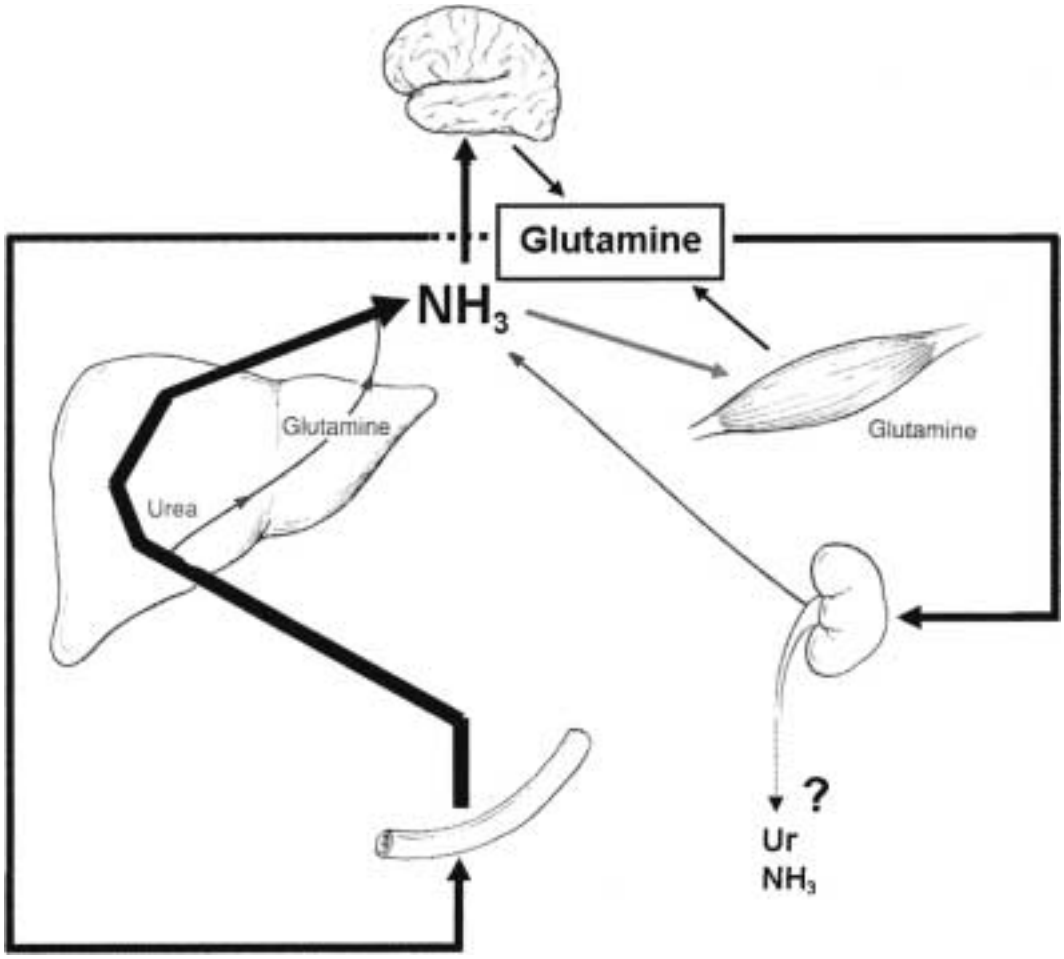

Figure 1 Interorgan trafficking of ammonia and glutamine. In normal conditions, gut release of ammonia results in high portal vein ammonia levels. Ammonia is efficiently removed by the liver via the urea cycle and glutamine synthesis, resulting in lower levels of ammonia in hepatic venous blood compared with arterial levels. Under normal conditions, arterial ammonia values are tightly controlled. In ALF, the liver extracts portal venous ammonia poorly. The subsequent increase of arterial ammonia levels leads to increased disposition of ammonia in other tissues. Both the brain and muscle lack a complete urea cycle and rely on the formation of glutamine. Thus, the brain and muscle become ammonia-uptake and glutamine-releasing organs. Because the regeneration of ammonia from glutamine that will occur in the intestines and kidney appears to have a saturation point, the capacity of the muscle to detoxify ammonia represents a potential therapeutic target. Finally, the capacity of the kidney to excrete ammonia in ALF is under investigation.

appears saturable.[28] Thus, the capacity of muscle to detoxify ammonia may be of importance. Ornithine-aspartate stimulates muscle glutamine synthetase in experimental ALF and prevents the development of brain edema.[30] The role of amino acids in ammonia disposal in ALF deserves further attention.

A net uptake of ammonia also occurs in the brain,[28] where it amidates both alpha-ketoglutarate and glutamate.[31] Glutamine is formed and cycles from astrocytes to presynaptic neurons, where glutamate is formed. After release into the synaptic cleft, reuptake of glutamate occurs in astrocytes. A profound alteration of this cycle has been demonstrated in experimental studies[31] and underlies the development of brain edema.

Recent studies have raised the possibility that the kidney may be an important route for ammonia elimination in cirrhosis.[32] Such findings await additional confirmation. In any case, the extent of renal ammonia elimination in ALF may be affected by the development of renal failure, a common finding in this syndrome.

Infection

A classic precipitant factor of the encephalopathy in chronic liver disease is the development of infection. Recent clinical observations indicate a strong association between parameters of infection and the course of encephalopathy in ALF[33] (see Bernal[34] in this issue). A recent report from the U.S. Acute Liver Failure Study group supports and extends these observations.[35] Only patients with early encephalopathy were analyzed. In a prospective evaluation of acetaminophen-induced ALF (n = 96), a positive diagnosis of infection preceded or coincided with the progression of stage I–II to deeper stages of encephalopathy in 79% of individuals. In subjects without demonstrable infection, a group that included both acetaminophen and nonacetaminophen etiologies (n = 168), a greater number of components of the systemic inflammatory response syndrome (SIRS) was associated with a stepwise progression of encephalopathy from 25% (0 components), 34.7% (1 component), and 50% (2 to 3 components).[35] An explanation of the components of SIRS can be found in Table 1.

Table 1 Definition of SIRS

The systemic inflammatory response to a variety of severe clinical insults.[103]
The response is manifested by two or more of the following conditions:
Temperature > 38 °C or < 36 °C
Heart rate > 90 beats/min
Respiratory rate > 20 breaths/min or $PaCO_2$ < 32 torr (< 4.3 kPa)
WBC > 12,000 cells/mm^3, < 4000 cellls/mm^3, or > 10% immature (band) forms

From: Muckart DJ, Bhagwanjee S. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions of the systemic inflammatory response syndrome and allied disorders in relation to critically injured patients. Crit Care Med 1997;25:1789–1795

How infection triggers encephalopathy in liver failure is poorly understood. The encephalopathy of sepsis is not similar to that of ALF.[36] Binding of cytokines to receptors in cerebral endothelial cells with subsequent signal transduction into the brain is a likely scenario.[37] Interactions of this process with other toxins, such as ammonia, have not been examined and may yield important clues to the pathogenesis of HE.

The Necrotic Liver

Scattered reports indicate improvement of the clinical condition in ALF after total hepatectomy.[38] In two well-studied cases, intracranial pressure was reduced and liver transplantation successfully performed when a donor organ became available.[39,40] A reduction in liver-derived cytokines was suggested as a reason for this beneficial effect.[40] However, critical examination of this experience notes the development of mild to moderate hypothermia after removal of the liver. Reductions of temperature to 32 to 35°C have been associated with reductions in brain edema and intracranial pressure in both experimental models[41] and human ALF.[42] In a controlled trial of hypothermia in patients with head trauma, reduced levels of interleukin (IL)-1β accompanied body temperatures of 34°C.[43] At this time, the role of the necrotic liver in the development or progression of encephalopathy is uncertain.

Synergism

In the mid-1970s, Zieve and Nicoloff[44] coined the concept of "synergistic toxins," in which a wide array of gut-derived substances potentiated ammonia's deleterious effects on the brain. These studies focused on mortality associated with ammonia administration to rats, noting a reduction of the LD50 of ammonia with the addition of short-chain fatty acids, mercaptans, and phenols. Octanoic acid had previously received attention as a putative cause of brain edema in Reye's syndrome.[45] Its role in ALF is uncertain.

The impact of compounds that cross the blood-brain barrier and activate gamma-aminobutyric acid (GABA)–ergic pathways has undergone a vast change since originally proposed more than 20 years ago. Although the existence of endogenous benzodiazepine ligands in the brain of patients with ALF has been reported previously,[46] current evidence supports a potentiating effect of ammonia on GABA-induced neurotransmission.[47] These aspects are discussed in greater detail elsewhere in this issue.[48]

Tryptophan is an amino acid whose levels are increased in the plasma of patients with ALF.[49] Its entry

into the brain is favored by activation of the neutral amino acid carrier at the level of the blood-brain barrier in exchange for glutamine, the brain levels of which are increased as a result of ammonia detoxification in astrocytes. Tryptophan is a precursor of serotonin, but the role of serotoninergic abnormalities in the encephalopathy of ALF is uncertain. A report of increased brain quinolinic acid, a peripheral derivative of tryptophan, in human brain does not suggest a major role for this pathway in the encephalopathy of ALF.[50]

BRAIN EDEMA: PART OF THE SPECTRUM OF HE

For many years, the presence of brain edema was viewed as a unique complication of ALF, a distinct entity from the classic picture of HE. Our views on this separation have undergone major changes in recent years. The results of in vitro studies, animal experimentation, and human data point at a common disturbance of water accumulation, present in the entire spectrum of clinical manifestations (Fig. 2). According to this view, the clinical expression of brain edema, a rise in intracranial pressure, is prominent in ALF but can also be detected in subjects with cirrhosis and deep hepatic coma.[11,12] New technical developments have allowed the estimation of an increased water content in the brain of patients with cirrhosis,[13] supporting the notion of low-grade brain edema.[14]

In 1999, we proposed a mechanism responsible for the development of brain edema based on a combination of experimental and clinical observations.[51] An initial osmotic disturbance of the brain, when combined with an increase in cerebral blood flow, results in this unique complication of liver failure. Many of our views of the pathogenesis of brain edema and intracranial hypertension have originated from work in the rat after portacaval anastomosis receiving an ammonia infusion. Although this model is not one of ALF, the reliable development of brain edema within a few hours of infusion allows the study of factors responsible for swelling in the absence of confounding variables seen in the setting of ALF. Such variables may also be critically important and will be reviewed after we espouse our basic concepts.

An Osmotic Disturbance

SELECTIVE CELLULAR SWELLING

Brain edema represents a net increase in total brain water content. Multiple studies point at cortical astrocytes as the cellular element initially swollen in ALF. An anatomic breakdown of the blood-brain barrier is not a feature of ALF, as noted in experimental models[52,53] and after examination of cerebral capillaries in human brain.[54] Neuroanatomic studies are difficult to perform in autopsy material,[54] so animal models have been very useful for supporting a primary event in astrocytes.[52,53] Furthermore, isolated astrocytes can be induced to swell when exposed to some of the circulating toxins of liver failure.[55] The demonstration of astrocyte swelling in animals with portacaval anastomosis alone[56] supports a spectrum of changes, in which glial swelling can occur without brain edema. The term low-grade brain edema has been coined for this earlier disturbance of water homeostasis.[14]

CHANGES IN ORGANIC OSMOLYTES

Direct measurements in experimental animals[57] and nuclear magnetic resonance (NMR) spectroscopic findings in humans[58] have repeatedly shown a marked increase in brain glutamine, the product of ammonia detoxification in astrocytes. Inhibition of glutamine synthetase prevents

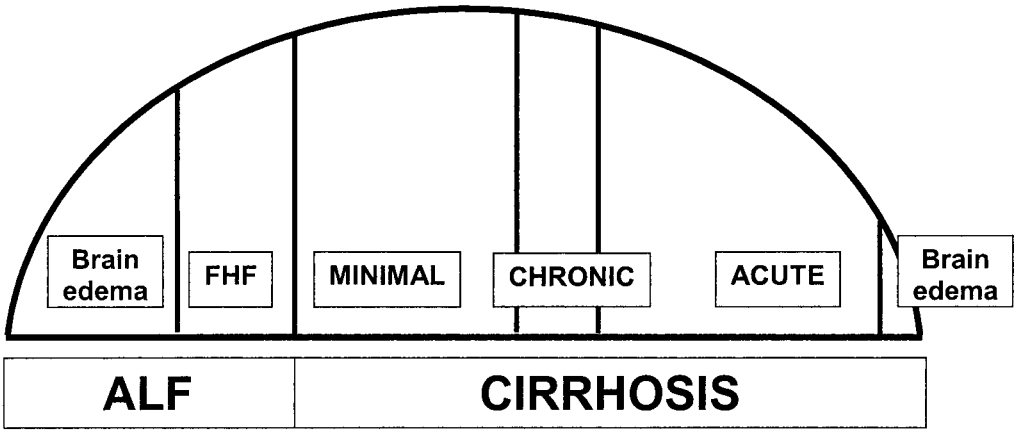

Figure 2 The spectrum of hepatic encephalopathy. An increasing amount of data points to a common disturbance of brain water accumulation underlying the entire spectrum of neurological manifestations of both acute and chronic liver disease. The intensity and acuteness of the insult, together with the influence of other concurrent systemic factors, will determine which part of this spectrum will be clinically apparent.

ammonia-induced swelling of isolated astrocytes[59] as well as brain edema in vivo.[60,61] The increase in brain glutamine can be fourfold to sixfold, although the limited capacity of astrocytic glutamine synthetase results in a steady high level of glutamine throughout the course of the neurological disturbance.

Cells exhibit short- and long-term adaptive mechanisms to adjust for changes in osmolarity.[62] A high extracellular brain potassium has been shown to occur acutely in an experimental model of acute hyperammonemia,[63] consistent with the effects of regulatory volume decrease in isolated cells.[62] Potassium levels are increased in the jugular vein of patients with ALF,[64] suggesting an increased exit from brain tissue. In the case of chronic adaptation, reduction of the levels of myoinositol, a key intracellular organic osmolyte, is accomplished slowly over several days.[62] Such osmotic adaptation may be a factor that explains the lower frequency of brain edema in subacute or subfulminant hepatic failure.[65] Consistent with these temporal changes in osmotic adaptation is the finding of an elevated glutamine and a low brain myoinositol in patients with cirrhosis, as seen with brain NMR spectroscopy.[58]

An Increase in Cerebral Blood Flow

In 1986, Ede and Williams[66] observed an increase of CBF in a subset of patients with ALF and deep HE. They proposed this increased CBF reflected the systemic vasodilatation seen in ALF. Subsequent clinical studies showed a more complex picture. In a series of 30 patients with ALF, Wendon et al[67] noted a wide range of values of CBF, with most having reduced CBF. In an American series, 24% of patients had an elevated CBF, which was associated with brain edema and a higher mortality.[22]

CEREBRAL ANOXIA IN ALF?

The cerebral metabolic rate for oxygen ($CMRO_2$) can be estimated in humans by the product of CBF and the arteriovenous oxygen difference. A small cerebral arteriovenous oxygen difference (arterial-jugular vein content) was seen in many of the patients with low or normal CBF,[67] which is suggestive of tissue anoxia. In ALF, values of $CMRO_2$ are low, in some cases less than those thought necessary to maintain cerebral viability.[68] However, these patients can achieve a full neurological recovery after transplantation.[22,67]

Alternatively, the finding of a low $CMRO_2$ in patients with normal CBF may be indicative of relative hyperemia, with a dissociation of CBF and the brain's metabolic needs.[69] In order to study the response of $CMRO_2$ to alterations in CBF, Larsen and colleagues[70] measured blood flow and oxygen extraction after infusion of noradrenaline. Their findings indicate a preservation of cerebral oxidative metabolism, arguing against the concept of tissue anoxia.

FAILURE OF CEREBROVASCULAR AUTOREGULATION

Under normal conditions, cerebral autoregulation maintains a stable CBF in the face of fluctuations in systemic pressure. The limits of autoregulation, between 60 and 160 mmHg, can be shifted in chronic disease states such as arterial hypertension. In resistance vessels, a myogenic component of autoregulation is normally based on the rapid response of vascular smooth muscle to changes in transmural pressure. In ALF, Larsen assessed the cerebrovascular autoregulation after a noradrenaline challenge. When mean arterial pressure rose by 30 mmHg, transcranial Doppler measurements in the middle cerebral artery showed an increase of velocity of 41%.[71] This loss of autoregulation was restored within 1 day after liver transplantation or within 4 days in subjects with spontaneous recovery. Of note, the therapeutic use of hypothermia also restored cerebrovascular autoregulation in a series of 14 patients with ALF.[72]

RESPONSE TO CHANGES IN PCO_2

Under normal conditions, the cerebral circulation responds exquisitely to changes in hypercapnia and hypocapnia with concomitant vasodilatation and vasoconstriction, respectively. CBF changes linearly from 2 to 4% for every millimeter of mercury change in pCO_2, also termed the CO_2 reactivity coefficient. Hypoxia must be profound, with a pO_2 less than 60 mmHg triggering cerebral vasodilatation.[73]

In patients with ALF, evidence supports the existence of a dilated cerebral vasculature. Hyperventilation leading to hypocapnia results in an appropriate reduction in CBF.[74] Furthermore, it can restore cerebrovascular autoregulation.[75] Hypercapnia, however, does not result in further increases in CBF,[74] an indicator of a markedly reduced CO_2-reactivity coefficient. In an already dilated cerebral vasculature, further vasodilatory stimuli are unlikely to result in additional effects.

MECHANISMS

In our experimental model, a predictable and selective rise in CBF occurs prior to the development of brain edema and intracranial hypertension in the setting of stable systemic hemodynamics.[61,76] Two important observations have been made in this model that shed light on the pathogenesis of this complex phenomenon.

1. *Brain edema can be prevented with measures that impede the rise in CBF.* Both indomethacin[77] and mild hypothermia[41] have been shown to reduce CBF and prevent the development of brain swelling and intracranial pressure (ICP) elevation. The case of indomethacin is especially significant as the drug has limited entry into the brain. Whereas hypothermia exhibits multiple effects on brain metabolism, vasoconstriction induced by indomethacin can be effective in human disease.[78] An increase in blood flow may underlie the movement of water into brain following

the principles of Starling's law, as recently postulated by Larsen and Wendon.[70]

2. *The signal that triggers the increase in CBF occurs after the generation of glutamine in astrocytes*. Inhibition of glutamine synthesis with methionine-sulfoximine ameliorates the rise in CBF seen in our model.[76] This compound also restores the cerebrovascular response to CO_2 in normal rats,[60] suggesting that an impaired cerebral autoregulation develops once glutamine is generated.

The link between the synthesis of glutamine and the subsequent onset of cerebral hyperemia is a critical question in this model and has not been elucidated. The finding of a high nitric oxide (NO) efflux from the sagittal sinus[76] raised a possible role for NO generated from an increased activity of neuronal NO synthase.[80] However, selective and nonselective inhibitors of this enzyme failed to prevent the rise in CBF.[81] The high CBF values seen in human ALF in deep coma highlight the importance of the search for a brain-derived signal that results in cerebral hyperemia. An increase in blood flow is associated with an increase in ammonia uptake,[82] a factor that has recently been shown to increase the likelihood of cerebral herniation in patients with ALF.[83]

OXIDATIVE AND NITROSATIVE STRESS: PATHOGENIC MECHANISMS IN BRAIN EDEMA AND HE?

Several recent observations support the presence of oxidative and nitrosative stress in the brain of models of HE. The formation of free radicals can be indirectly surmised from a series of clinical and experimental observations. In humans, lipofuscin pigment, reflecting the peroxidation of lipids, can be detected in Alzheimer type II astrocytes.[84] We have shown an increase in gene expression of brain heme oxygenase-1 and the reduction of Cu/Zn superoxide dismutase in rats after portacaval anastomosis, findings that support the presence of oxidative stress.[85] Activities of neuronal NO synthase are increased in this model,[86] and we have reported an increase in brain NO efflux, another free radical, in rats after portacaval anastomosis receiving an ammonia infusion.[76]

The strongest evidence for this concept arises from cellular studies. The formation of free radicals can be detected in astrocytes exposed to ammonia.[87] Astrocytes exposed to ammonia also develop the mitochondrial permeability transition (MPT).[88] This effect, in which the opening of a large nonselective pore in mitochondria results in morphological and functional abnormalities, leads to defective oxidative phosphorylation and to the generation of even more free radicals. Cultured neurons did not develop the MPT when exposed to ammonia.[88] In support of a pathogenic role of glutamine, inhibition of glutamine synthetase prevented the development of the MPT in isolated astrocytes exposed to ammonia.[88]

Free radicals can nitrosylate proteins, and nitrotyrosine, a stable product of this reaction, can be demonstrated in isolated astrocytes exposed to ammonia.[89] This finding can also be seen in vivo.[89] We have recently completed preliminary studies in rats after portacaval anastomosis receiving an ammonia infusion. Clear evidence of nitrotyrosine accumulation in astrocytes was noted (Fig. 3). The functional implications of these changes and their relation to the pathogenesis of HE is an evolving concept but one likely to be important in the manifestations of HE in both ALF and cirrhosis.

CLINICAL PATHOPHYSIOLOGY

In the previous section, we analyzed paradigms that have evolved from experimental models. Clinical observations support the role of such pathophysiological mechanisms.

Plasma Osmolarity

Earlier series of patients with ALF had noted hyponatremia in patients with encephalopathy.[90] In the experimental animal, hyponatremia (mean serum sodium of 117) aggravates ammonia-induced brain edema.[91] The osmotic disturbance in the brain of patients with ALF is anisosmotic, reflecting the generation of osmoles within the brain,[62] and will be potentiated by a decrease in plasma osmolarity. Patients with cirrhosis who developed intracranial hypertension after placement of a transjugular intrahepatic portal-systemic stent-shunt (TIPS) were all hyponatremic.[11] Further support of this concept can be seen with the deterioration of mental state associated with rapid fluid shifts in hemodialysis.[92]

Temperature

Fever aggravates the clinical picture in ALF.[93] Fever (> 38°C) is a component of SIRS, as described earlier, and in experimental animals, an increase in cerebral blood flow and metabolic rate accompanies the hyperthermic state.[94] Preliminary evidence supports an association between temperatures above 38°C and the development of intracranial hypertension in patients with ALF.[95] Whereas fever can accompany systemic infection, septic encephalopathy is associated with an intact cerebrovascular CO_2 reactivity and pressure autoregulation,[36] a conspicuous difference with the changes seen in human ALF.

Arterial Pressure

As a consequence of the loss of cerebrovascular autoregulation, patients with ALF are susceptible to the effects of both a reduction and an increase in arterial pressure. Cerebral ischemia can ensue as a result of the former. Cerebral hyperemia, the result of the latter, can aggravate the development of brain edema. Maintenance of an adequate level of blood pressure is critical to prevent either possibility.

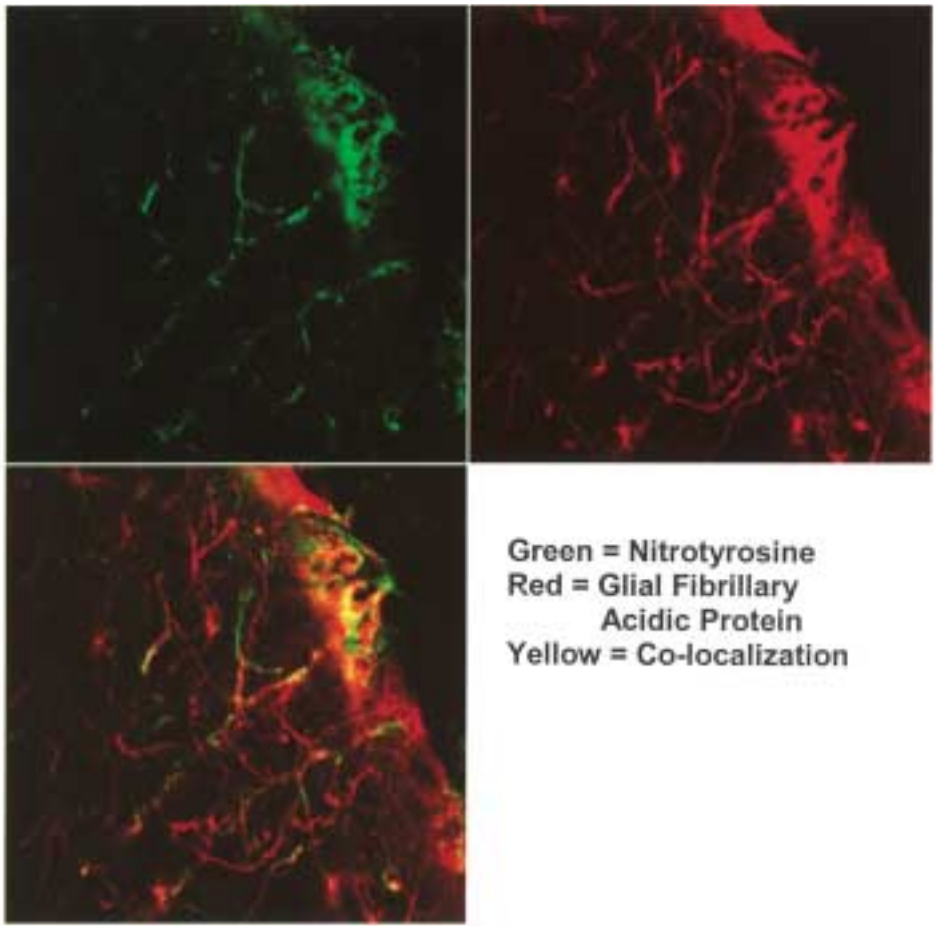

Figure 3 Staining for nitrotyrosine in the cerebral cortex of a portacaval-shunted rat receiving an ammonia infusion. The upper right-hand picture shows glial fibrillary acidic protein (GFAP) positive astrocytes in the cerebral cortex. The upper left-hand picture shows nitrotyrosine staining in astrocytes; the lower right-hand picture reveals the overlap of GFAP and nitrotyrosine staining in cortical astrocytes. Methods: Ammonia (55 μmol/kg/min) was infused through the femoral vein for 3 hours. At the end of the infusion, the brain was fixed by the perfusion-fixation method. After blocking with 1% bovine serum albumin (BSA) and 5% goat serum, cerebral cortex sections were incubated overnight in rabbit antinitrotyrosine antibody (Upstate Group [Charlottesville, VA], 1:75) at 4°C followed by incubation in Alexa Fluor 488 antirabbit secondary antibody (Molecular Probes [Eugene, OR], 1:400) for 1 hour at 25°C. Following nitrotyrosine staining, astrocytes labeling was revealed with an overnight incubation in mouse anti-GFAP antibody (Chemicon International [Temecula, CA], 1:1000) at 4°C followed by incubation in Alexa Fluor 568 anti–mouse secondary antibody (Molecular Probes, 1:400) for 1 hour at 25°C. The fluorescent-stained slides were scanned using a LSM 510 confocal microscope.

Glucose

A recently proposed candidate for a synergistic role in the development of intracranial hypertension in ALF is hyperglycemia. In a preliminary report, values > 12 mmol/L (> 200 μg/dL) were associated with higher values of ICP.[96] In other neurological conditions, hyperglycemia is known to aggravate the effects of brain trauma[97] and ischemia[98] in relation to the increased generation of brain lactate as a result of anaerobic glycolysis. Lactate levels are increased in the brain of experimental models[99] and human[100] ALF, although the mechanism responsible for the rise may be different.[100]

The Rise in Intracranial Pressure

Water in the brain exists in three forms: intracellular water, blood, and cerebrospinal fluid. The latter is decreased in ALF, as seen in imaging of the brain in which shrinking of ventricular size is a common finding.[101] Swelling of the gray matter, where astrocytes constitute 30% of the cellular elements, has been recently demonstrated using NMR techniques.[102] Although cerebral blood volume is difficult to measure, the presence of cerebral vasodilatation and hyperemia suggests an increase in this compartment. With the enlargement of the cellular compartment and in the setting of a limited compliance imposed by the rigid skull, small increases in blood volume will cause an inordinate rise in ICP. The article by Jalan et al[103] in this issue provides further insight into the monitoring and management of intracranial hypertension.

CONCLUSION

Our article has highlighted the close relationship between the process that results in brain edema and the pathogenesis of hepatic encephalopathy. ALF is a good

example of how factors traditionally thought to account for brain edema are also implicated in the pathogenesis of hepatic encephalopathy. Elucidation of the mechanisms responsible for brain water accumulation is likely to provide new insights into rational therapeutic approaches to hepatic encephalopathy.

ACKNOWLEDGMENTS

Supported by a Merit Review from the VA Research Administration and the Stephen B. Tips Memorial Fund at Northwestern Memorial Hospital. Dr. Vaquero is supported by Fondo de Investigacion Sanitaria (BEFI/FIS), Madrid, Spain.

ABBREVIATIONS

ALF	acute liver failure
CBF	cerebral blood flow
$CMRO_2$	cerebral metabolic rate of oxygen
HE	hepatic encephalopathy
ICP	intracranial pressure
NMR	nuclear magnetic resonance
NO	nitric oxide
SIRS	systemic inflammatory response syndrome

REFERENCES

1. Trey C, Davidson CS. The management of fulminant hepatic failure. Prog Liver Dis 1970;3:282–298
2. Trey C. The fulminant hepatic failure surveillance study. Brief review of the effects of presumed etiology and age of survival. Can Med Assoc J 1972;106(Suppl):525–527
3. Atterbury CE, Maddrey WC, Conn HO. Neomycin-sorbitol and lactulose in the treatment of acute portal-systemic encephalopathy. A controlled, double-blind clinical trial. Am J Dig Dis 1978;23:398–406
4. Butterworth RF. Hepatic encephalopathy and brain edema in acute hepatic failure: does glutamate play a role? Hepatology 1997;25:1032–1034
5. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet 1974;2:81–84
6. Vaquero J, Oliver D, Hassanein T, et al. Evaluation of mental state in a clinical trial of MARS for patients with acute hepatic encephalopathy: comparison of two scales. In: Chamuleau R, Jones A, Meijers A, eds. Encephalopathy and Nitrogen Metabolism in Liver Failure. Amsterdam: Kluwer Academic Publishers; 2003:55–69
7. Steg RE, Wszolek ZK. Electroencephalographic abnormalities in liver transplant recipients: practical considerations and review. J Clin Neurophysiol 1996;13:60–68
8. Rakela J, Lange SM, Ludwig J, Baldus WP. Fulminant hepatitis: Mayo Clinic experience with 34 cases. Mayo Clin Proc 1985;60:289–292
9. Michalak A, Butterworth RF. Ornithine transcarbamylase deficiency: pathogenesis of the cerebral disorder and new prospects for therapy. Metab Brain Dis 1997;12:171–182
10. Ellis AJ, Wendon JA, Williams R. Subclinical seizure activity and prophylactic phenytoin infusion in acute liver failure: a controlled clinical trial. Hepatology 2000;32:536–541
11. Jalan R, Dabos K, Redhead DN, et al. Elevation of intracranial pressure following transjugular intrahepatic portosystemic stent-shunt for variceal haemorrhage. J Hepatol 1997; 27:928–933
12. Donovan JP, Schafer DF, Shaw BW Jr, Sorrell MF. Cerebral oedema and increased intracranial pressure in chronic liver disease. Lancet 1998;351:719–721
13. Cordoba J, Alonso J, Rovira A, et al. The development of low-grade cerebral edema in cirrhosis is supported by the evolution of (1)H-magnetic resonance abnormalities after liver transplantation. J Hepatol 2001;35:598–604
14. Haussinger D, Kircheis G, Fischer R, et al. Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral edema? J Hepatol 2000;32:1035–1038
15. Blei AT. Brain edema and portal-systemic encephalopathy. Liver Transpl 2000;6:S14–S20
16. Almdal T, Schroeder T, Ranek L. Cerebral blood flow and liver function in patients with encephalopathy due to acute and chronic liver diseases. Scand J Gastroenterol 1989;24:299–303
17. Rodriguez G, Testa R, Celle G, et al. Reduction of cerebral blood flow in subclinical hepatic encephalopathy and its correlation with plasma-free tryptophan. J Cereb Blood Flow Metab 1987;7:768–772
18. Guevara M, Bru C, Gines P, et al. Increased cerebrovascular resistance in cirrhotic patients with ascites. Hepatology 1998;28:39–44
19. Arroyo V, Jimenez W. Complications of cirrhosis. II. Renal and circulatory dysfunction. Lights and shadows in an important clinical problem. J Hepatol 2000;32:157–170
20. Groszmann RJ. Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. Hepatology 1994;20:1359–1363
21. Bihari DJ, Gimson AE, Williams R. Cardiovascular, pulmonary and renal complications of fulminant hepatic failure. Semin Liver Dis 1986;6:119–128
22. Aggarwal S, Kramer D, Yonas H, et al. Cerebral hemodynamic and metabolic changes in fulminant hepatic failure: a retrospective study. Hepatology 1994;19:80–87
23. Geiger A, Magnes J, Taylor RM, Veralli M. Effect of blood constituents on uptake of glucose and on metabolic rate of the brain in perfusion experiments. Am J Physiol 1954;177: 138–149
24. Alexander B, Aslam M, Benjamin IS. An investigation of the relationship between the liver and brain using an isolated perfused rat brain preparation. J Pharmacol Toxicol Methods 1999;42:31–37
25. Crespin J, Nemcek A, Rehkemper G, Blei AT. Intrahepatic portal-hepatic venous anastomosis: a portal-systemic shunt with neurological repercussions. Am J Gastroenterol 2000; 95:1568–1571
26. Clemmesen JO, Larsen FS, Kondrup J, et al. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology 1999;29:648–653
27. Madl C, Kramer L, Gendo A, et al. Prognostic accuracy of sensory evoked potentials (SEP) and arterial ammonia in predicting development of cerebral edema and death by cerebral herniation in patients with fulminant hepatic failure (FHF). Gastroenterology 2000;118:1007A
28. Clemmesen JO, Kondrup J, Ott P. Splanchnic and leg exchange of amino acids and ammonia in acute liver failure. Gastroenterology 2000;118:1131–1139
29. Nomura F, Ohnishi K, Terabayashi H, et al. Effect of intrahepatic portal-systemic shunting on hepatic ammonia extraction in patients with cirrhosis. Hepatology 1994;20:1478–1481

30. Rose C, Michalak A, Pannunzio P, et al. L-ornithine-L-aspartate in experimental portal-systemic encephalopathy: therapeutic efficacy and mechanism of action. Metab Brain Dis 1998;13:147–157
31. Felipo V, Butterworth RF. Neurobiology of ammonia. Prog Neurobiol 2002;67:259–279
32. Olde Damink SW, Jalan R, Redhead DN, et al. Interorgan ammonia and amino acid metabolism in metabolically stable patients with cirrhosis and a TIPSS. Hepatology 2002;36: 1163–1171
33. Rolando N, Wade J, Davalos M, et al. The systemic inflammatory response syndrome in acute liver failure. Hepatology 2000;32:734–739
34. Bernal W. Changing patterns of causation and the use of transplantation in the United Kingdom. Semin Liver Dis 2003;23:227–238
35. Vaquero J, Polson J, Chung C, et al. Infection and the progression of encephalopathy in acute liver failure. Gaastroenterology 2003; (in press)
36. Matta BF, Stow PJ. Sepsis-induced vasoparalysis does not involve the cerebral vasculature: indirect evidence from autoregulation and carbon dioxide reactivity studies. Br J Anaesth 1996;76:790–794
37. Licinio J, Wong ML. Pathways and mechanisms for cytokine signaling of the central nervous system. J Clin Invest 1997;100:2941–2947
38. Ringe B, Lubbe N, Kuse E, et al. Total hepatectomy and liver transplantation as two-stage procedure. Ann Surg 1993;218:3–9
39. Rozga J, Podesta L, LePage E, et al. Control of cerebral oedema by total hepatectomy and extracorporeal liver support in fulminant hepatic failure. Lancet 1993;342:898–899
40. Jalan R, Pollok A, Shah S, et al. Liver derived pro-inflammatory cytokines may be important in producing intracranial hypertension in acute liver failure. J Hepatol 2002;37:536
41. Cordoba J, Crespin J, Gottstein J, Blei AT. Mild hypothermia modifies ammonia-induced brain edema in rats after portacaval anastomosis. Gastroenterology 1999;116:686–693
42. Jalan R, Damink SW, Deutz NE, et al. Moderate hypothermia for uncontrolled intracranial hypertension in acute liver failure. Lancet 1999;354:1164–1168
43. Marion DW, Penrod LE, Kelsey SF, et al. Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 1997;336:540–546
44. Zieve L, Nicoloff DM. Pathogenesis of hepatic coma. Annu Rev Med 1975;26:143–157
45. Trauner DA. Pathologic changes in a rabbit model of Reye's syndrome. Pediatr Res 1982;16:950–953
46. Basile AS, Hughes RD, Harrison PM, et al. Elevated brain concentrations of 1,4-benzodiazepines in fulminant hepatic failure. N Engl J Med 1991;325:473–478
47. Basile AS. Direct and indirect enhancement of GABAergic neurotransmission by ammonia: implications for the pathogenesis of hyperammonemic syndromes. Neurochem Int 2002;41:115–122
48. Butterworth R. Molecular neurobiology of acute liver failure. Semin Liver Dis 2003;23:251–258
49. Record CO, Buxton B, Chase RA, et al. Plasma and brain amino acids in fulminant hepatic failure and their relationship to hepatic encephalopathy. Eur J Clin Invest 1976;6:387–394
50. Basile AS, Saito K, al-Mardini H, et al. The relationship between plasma and brain quinolinic acid levels and the severity of hepatic encephalopathy. Gastroenterology 1995;108: 818–823
51. Blei AT, Larsen FS. Pathophysiology of cerebral edema in fulminant hepatic failure. J Hepatol 1999;31:771–776
52. Traber PG, Dal Canto M, Ganger DR, Blei AT. 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
53. Gove CD, Hughes RD, Ede RJ, Williams R. Regional cerebral edema and chloride space in galactosamine-induced liver failure in rats. Hepatology 1997;25:295–301
54. Kato M, Hughes RD, Keays RT, Williams R. Electron microscopic study of brain capillaries in cerebral edema from fulminant hepatic failure. Hepatology 1992;15:1060–1066
55. Norenberg MD. Astroglial dysfunction in hepatic encephalopathy. Metab Brain Dis 1998;13:319–335
56. Swain MS, Blei AT, Butterworth RF, Kraig RP. Intracellular pH rises and astrocytes swell after portacaval anastomosis in rats. Am J Physiol 1991;261:R1491–1496
57. Cordoba J, Gottstein J, Blei AT. Glutamine, myo-inositol, and organic brain osmolytes after portocaval anastomosis in the rat: implications for ammonia-induced brain edema. Hepatology 1996;24:919–923
58. Laubenberger J, Haussinger D, Bayer S, et al. Proton magnetic resonance spectroscopy of the brain in symptomatic and asymptomatic patients with liver cirrhosis. Gastroenterology 1997;112:1610–1616
59. Norenberg MD, Bender AS. Astrocyte swelling in liver failure: role of glutamine and benzodiazepines. Acta Neurochir Suppl (Wien) 1994;60:24–27
60. Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. Am J Physiol 1991;261: H825–H829
61. Blei AT, Olafsson S, Therrien G, Butterworth RF. Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. Hepatology 1994;19: 1437–1444
62. McManus ML, Churchwell KB, Strange K. Regulation of cell volume in health and disease. N Engl J Med 1995;333: 1260–1266
63. Sugimoto H, Koehler RC, Wilson DA, et al. Methionine sulfoximine, a glutamine synthetase inhibitor, attenuates increased extracellular potassium activity during acute hyperammonemia. J Cereb Blood Flow Metab 1997;17:44–49
64. Larsen FS, Moller K, Strauss G. Specific cerebral efflux of potassium in patients with fulminant hepatic failure. Liver Transpl 2001;7:C22
65. O'Grady JG, Schalm SW, Williams R. Acute liver failure: redefining the syndromes. Lancet 1993;342:273–275
66. Ede RJ, Williams RW. Hepatic encephalopathy and cerebral edema. Semin Liver Dis 1986;6:107–118
67. Wendon JA, Harrison PM, Keays R, Williams R. Cerebral blood flow and metabolism in fulminant liver failure. Hepatology 1994;19:1407–1413
68. Powers WJ, Grubb RL Jr, Darriet D, Raichle ME. Cerebral blood flow and cerebral metabolic rate of oxygen requirements for cerebral function and viability in humans. J Cereb Blood Flow Metab 1985;5:600–608
69. Larsen FS. Cerebral circulation in liver failure: Ohm's law in force. Semin Liver Dis 1996;16:281–292
70. Larsen FS, Ejlersen E, Clemmesen JO, et al. Preservation of cerebral oxidative metabolism in fulminant hepatic failure: an autoregulation study. Liver Transpl Surg 1996;2:348–353

71. Larsen FS, Ejlersen E, Hansen BA, et al. Functional loss of cerebral blood flow autoregulation in patients with fulminant hepatic failure. J Hepatol 1995;23:212–217
72. Jalan R, Olde Damink SW, Deutz NE, et al. Restoration of cerebral blood flow autoregulation and reactivity to carbon dioxide in acute liver failure by moderate hypothermia. Hepatology 2001;34:50–54
73. Vavilala MS, Lee LA, Lam AM. Cerebral blood flow and vascular physiology. Anesthesiology Clinics of North America 2002;20: v, 247–264
74. Larsen FS, Adel Hansen B, Pott F, et al. Dissociated cerebral vasoparalysis in acute liver failure. A hypothesis of gradual cerebral hyperaemia. J Hepatol 1996;25:145–151
75. Strauss G, Hansen BA, Knudsen GM, Larsen FS. Hyperventilation restores cerebral blood flow autoregulation in patients with acute liver failure. J Hepatol 1998;28:199–203
76. Master S, Gottstein J, Blei AT. Cerebral blood flow and the development of ammonia-induced brain edema in rats after portacaval anastomosis. Hepatology 1999;30:876–880
77. Chung C, Gottstein J, Blei AT. Indomethacin prevents the development of experimental ammonia-induced brain edema in rats after portacaval anastomosis. Hepatology 2001;34: 249–254
78. Clemmesen JO, Hansen BA, Larsen FS. Indomethacin normalizes intracranial pressure in acute liver failure: a twenty-three-year-old woman treated with indomethacin. Hepatology 1997;26:1423–1425
79. Larsen FS, Wendon J. Brain edema in liver failure: basic physiologic principles and management. Liver Transpl 2002; 8:983–989
80. Rao VL, Audet RM, Butterworth RF. Increased neuronal nitric oxide synthase expression in brain following portacaval anastomosis. Brain Res 1997;765:169–172
81. Larsen FS, Gottstein J, Blei AT. Cerebral hyperemia and nitric oxide synthase in rats with ammonia-induced brain edema. J Hepatol 2001;34:548–554
82. Chung C, Gottstein J, Vaquero J, et al. Vasopressin infusion accelerates the development of brain edema in rats after portacaval anastomosis. J Hepatol 2003;39:193–199
83. Strauss GI, Knudsen GM, Kondrup J, et al. Cerebral metabolism of ammonia and amino acids in patients with fulminant hepatic failure. Gastroenterology 2001;121:1109–1119
84. Norenberg MD. The astrocyte in liver disease. In: Fedoroff S, Hertz L, eds. Advances in Cellular Neurobiology, Vol. 2. New York: Academic Press; 1981:303–352
85. Song G, Dhodda VK, Blei AT, et al. GeneChip analysis shows altered mRNA expression of transcripts of neurotransmitter and signal transduction pathways in the cerebral cortex of portacaval shunted rats. J Neurosci Res 2002;68: 730–737
86. Rao VL, Audet RM, Butterworth RF. Increased nitric oxide synthase activities and L-[3H]arginine uptake in brain following portacaval anastomosis. J Neurochem 1995;65:677–678
87. Murthy CR, Rama Rao KV, Bai G, Norenberg MD. Ammonia-induced production of free radicals in primary cultures of rat astrocytes. J Neurosci Res 2001;66:282–288
88. Bai G, Rama Rao KV, Murthy CR, et al. Ammonia induces the mitochondrial permeability transition in primary cultures of rat astrocytes. J Neurosci Res 2001;66:981–991
89. Schliess F, Gorg B, Fischer R, et al. Ammonia induces MK-801-sensitive nitration and phosphorylation of protein tyrosine residues in rat astrocytes. FASEB J 2002;16: 739–741
90. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology 1989;97:439–445
91. Cordoba J, Gottstein J, Blei AT. Chronic hyponatremia exacerbates ammonia-induced brain edema in rats after portacaval anastomosis. J Hepatol 1998;29:589–594
92. Davenport A, Will EJ, Davison AM. Continuous vs. intermittent forms of haemofiltration and/or dialysis in the management of acute renal failure in patients with defective cerebral autoregulation at risk of cerebral oedema. Contrib Nephrol 1991;93:225–233
93. Munoz SJ, Moritz MJ, Bell R, et al. Factors associated with severe intracranial hypertension in candidates for emergency liver transplantation. Transplantation 1993;55:1071–1074
94. Katsumura H, Kabuto M, Hosotani K, et al. The influence of total body hyperthermia on brain haemodynamics and blood-brain barrier in dogs. Acta Neurochir (Wien) 1995; 135:62–69
95. Kodakar SK, Gopal PB, Wendon JA. Intracranial pressure is related to body temperature in acute liver failure. Liver Transpl 2001;7:C87
96. Kodakar SK, Gopal PB, Wendon JA. Hyperglycemia is associated with intracranial hypertension in patients with acute liver failure. Liver Transpl 2001;7:C21
97. Lam AM, Winn HR, Cullen BF, Sundling N. Hyperglycemia and neurological outcome in patients with head injury. J Neurosurg 1991;75:545–551
98. Nakakimura K, Fleischer JE, Drummond JC, et al. Glucose administration before cardiac arrest worsens neurologic outcome in cats. Anesthesiology 1990;72:1005–1011
99. Therrien G, Giguere JF, Butterworth RF. Increased cerebrospinal fluid lactate reflects deterioration of neurological status in experimental portal-systemic encephalopathy. Metab Brain Dis 1991;6:225–231
100. Tofteng F, Jorgensen L, Hansen BA, et al. Cerebral microdialysis in patients with fulminant hepatic failure. Hepatology 2002;36:1333–1340
101. Wijdicks EF, Plevak DJ, Rakela J, Wiesner RH. Clinical and radiologic features of cerebral edema in fulminant hepatic failure. Mayo Clin Proc 1995;70:119–124
102. Venkatasubramanian PN, Tom B, Blei AT, Wyrwicz AM. Monitoring of brain water by chemical shift imaging during ammonia-induced brain swelling in rats after portacaval anastomosis. Artif Organs 2001;25:551–557
103. Jalan R. Intracranial hypertension in acute liver failure: pathophysiological basis of rational management. Semin Liver Dis 2003;23:271–282