Chapter 16
Oxidative Stress in the Central Nervous System Complications of Chronic Liver Failure

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16.1 Introduction

Chronic liver failure is the process of progressive destruction and regeneration of liver parenchyma leading to fibrosis and cirrhosis and would be responsible for an estimated 10.4, 7.3 and 5.3 deaths per 100,000 population in Europe, United States, and Canada, respectively [1–3]. The end-stage process of liver degeneration and failure, or cirrhosis, is the final common pathway of most forms of liver disease. Chronic liver failure may be caused by several conditions including, among others, alcohol, virus, non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), biliary disease as well as metabolic disorders. Many complications may arise from chronic liver failure, including portal hypertension, impaired metabolic capacity, synthesis dysfunction, malnutrition, ascites, hepatorenal syndrome, increased risk for the development of hepatocellular carcinoma as well as hepatic encephalopathy (Fig. 16.1).

Hepatic encephalopathy, which is observed in approximately 80 % of patients with chronic liver failure [4–6], is a debilitating neuropsychiatric complication of liver disease. Characterized by a constellation of symptoms, including cognitive, psychiatric, and motor disturbances, hepatic encephalopathy can progress to coma and death. Hepatic encephalopathy encompasses several clinical signs such as asterixis, stupor, seizures, and coma; its severity is usually graded with the West Haven Criteria (Table 16.1) [7]. However, in order to address the universal

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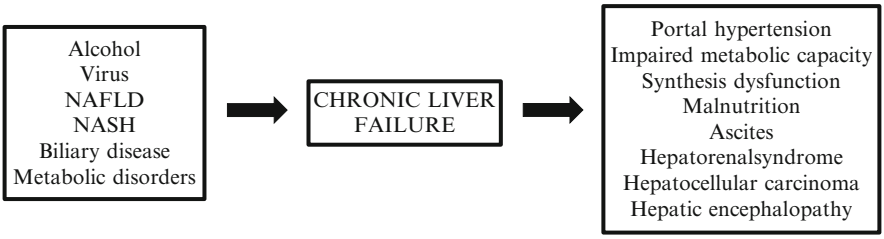

Fig. 16.1 Etiologic factors of chronic liver failure and resulting complications (*NAFLD* non-alcoholic fatty liver disease, *NASH* non-alcoholic steato-hepatitis)

Table 16.1 Grading of hepatic encephalopathy according to the West Haven criteria

Grade	Symptom
0	No signs or symptoms
I	Disturbed sleep-wake rhythm, restless, euphoria, anxiety, aimless, shortened attention span, trivial lack of awareness, impaired performance of addition
II	Lethargia or apathy, overt personality changes, disorientation for time/space, flapping, memory weakness, impaired performance for subtraction
III	Somnolence, stupor, confusion, disturbed articulation, responsive to verbal stimuli
IV	Coma

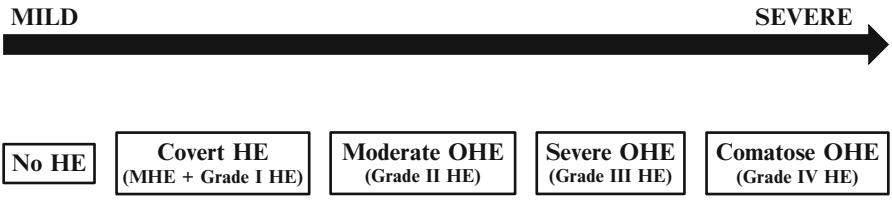

Fig. 16.2 Proposed ISHEN classification [8]. (*HE* hepatic encephalopathy, *MHE* minimal HE, *OHE* overt HE)

concern about the accuracy of the West Haven scale in diagnosing the severity of overt hepatic encephalopathy, the International Society for Hepatic Encephalopathy and Nitrogen metabolism (ISHEN), the official authority for issuing updates on terminology regarding hepatic encephalopathy, has recently proposed a revised classification (Fig. 16.2) [8]. Overall, hepatic encephalopathy has a significant impact on patients' quality of life and on their ability to function daily. Furthermore, hepatic encephalopathy leads to a poor prognosis and a greater risk of mortality [9]. This chapter will summarize the involvement of oxidative stress in the pathogenesis of hepatic encephalopathy, its consequences, and its potential role in therapeutic strategy.

16.2 Pathogenesis of Hepatic Encephalopathy

The pathophysiologic basis of hepatic encephalopathy is multifactorial and remains unclear. However, there is general agreement that ammonia plays a key role [10]. Ammonia accumulates in the brain in chronic liver failure leading to impaired bioenergetics [10, 11], altered neurotransmission [11–13], activation of peripheral benzodiazepine receptors [12, 14], leading to the synthesis of neurosteroids [15] as well as glutamate-mediated excitotoxicity [16, 17] and excessive production of glutamine [18, 19]. However, in the setting of chronic liver failure, the correlation between ammonia and severity of hepatic encephalopathy remains inconclusive [20, 21], suggesting that other pathogenic factors may be implicated. In recent years, oxidative stress has also been suggested to be part of the pathophysiologic cascade in hepatic encephalopathy. Among the factors responsible for oxidative stress development in the setting of hepatic encephalopathy are ammonia, manganese, intracellular calcium, mitochondrial permeability transition (MPT), electron transport chain, *N*-methyl D-aspartate (NMDA) receptors, peripheral benzodiazepine receptor, nuclear factor-Kappa B, inflammation, and glutamine. Data from the literature suggests that the relationship between these factors and oxidative stress in the pathogenesis of hepatic encephalopathy is complex. However, a detailed review of these factors in relation to hepatic encephalopathy is beyond the scope of this chapter. In the present chapter, these factors will be tackled emphasizing the implication of ammonia and manganese-induced oxidative stress in relation with hepatic encephalopathy.

16.3 Oxidative Stress in Hepatic Encephalopathy

Oxidative stress, a condition in which the production of free radicals is far in excess of their rate of detoxification by endogenous mechanisms [22], refers to a state in which tissue and cellular redox balance is altered towards a more oxidizing environment [23, 24]. Precisely, oxidative stress results from an imbalance between the generation of reactive oxygen species and the cellular antioxidant defense capacity, potentially able to affect molecular structure and function. Reactive oxygen species include, among other, hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), superoxide anion ($O_2^{\bullet-}$), and peroxynitrite ($ONOO^-$). These reactive oxygen intermediates, which play important roles in cell signaling [25], are highly reactive due to the presence of unpaired valence shell electrons and are constantly produced during oxygen metabolism. However, in excess, reactive oxygen species are very harmful to the cell, for their reaction with cellular structures and macromolecules, and lead to cellular dysfunction. Indeed, oxidative stress affects major cellular components, including lipids, proteins, and DNA. In addition, the brain is absolutely dependent upon oxidative metabolism for cell survival and, being a highly aerobic tissue accounting for 20 % of total oxygen consumed by the body, is prone to dysfunction due to oxidative stress.

The neuropathology of hepatic encephalopathy in chronic liver failure reveals primarily changes in astrocytes (glial cells of the central nervous system) including cell swelling which consequently leads to brain edema [26–28]. Specifically, hepatic encephalopathy resulting from chronic liver failure reflects the clinical manifestation of a low-grade cerebral edema that develops after exhaustion of the volume-regulatory capacity of the astrocytes in response to ammonia and other hepatic encephalopathy-precipitating factors (e.g., inflammation, infection, hyponatremia) [29, 30]. As a consequence, astrocyte swelling triggers a complex signaling cascade which relies on NMDA receptor activation and elevation of intracellular calcium concentration, which result in increased formation of reactive oxygen species through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nitric oxide synthase (NOS) [29, 31]. Since oxidative stress in turn promotes astrocyte swelling, a self-amplifying signaling loop between osmotic- and oxidative stress is proposed [31, 32]. Among the consequences of this oxidative stress response are protein-tyrosine nitration, oxidation of RNA, and activation of Zn^{2+}-dependent transcription [31–35].

16.3.1 Ammonia-Induced Oxidative Stress in Hepatic Encephalopathy

Evidence for the involvement of oxidative stress in hepatic encephalopathy initially arose from the observation that Alzheimer type II astrocytes, a distinctive neuropathologic finding in brains of patient with hepatic encephalopathy, contain large amounts of lipofuscin pigments [36], consisting of peroxidized lipids [37]. Excessive amounts of lipofuscin pigments were also detected in ammonia-treated astrocyte cultures [37, 38]. Subsequently, more than 20 years ago, O'Connor and Costell [39] postulated that oxidative stress is implicated in the pathophysiologic mechanisms responsible for hepatic encephalopathy. They reported that hyperammonemic mice displayed evidence of lipid peroxidation in the brain [39]. Since these findings have been reported, substantial evidence from cell cultures and animal studies for an important role of hyperammonemia and oxidative stress in the pathogenesis of hepatic encephalopathy has been reported.

16.3.1.1 In Vitro Evidence of Ammonia-Induced Oxidative Stress in Hepatic Encephalopathy

Much of the evidence for a role of oxidative stress in ammonia neurotoxicity has been derived from cell culture studies [40]. Evidence suggests a close interrelation between astrocyte swelling, a characteristic feature of hepatic encephalopathy, and the production of reactive oxygen species leading to oxidative stress [41]. Specifically, astrocytes have been suggested to be a major source of reactive oxygen species under simulated hepatic encephalopathy conditions [41, 42]. Indeed, astrocytes exposed to

a pathophysiological concentration of ammonia were found to stimulate the production of free radicals and reactive oxygen species. In hypoosmotically treated cultured rat astrocytes, a NMDA receptor-dependent elevation of the intracellular calcium concentration was identified to be essential to swelling-dependent reactive oxygen species generation [43]. Ammonia-induced free radical generation via the activation of NADPH oxidase was observed in cultured astrocytes [42–44], while astrocyte swelling triggers a p47(phox)-dependent NADPH oxidase-catalyzed reactive oxygen species production [31]. In addition, in astrocyte cultures exposed to ammonia, increased heme oxygenase-1 (HO-1) [44] and inducible NOS (iNOS) expression as well as nitric oxide production were identified [34, 45]. Ammonia has also been shown to significantly increase soluble guanylyl cyclase [46], a source of nitric oxide, and decrease cellular glutathione (GSH) level [47], a major endogenous antioxidant, in cultured astrocytes. Furthermore, natriuretic peptides, which are known to attenuate the production of reactive oxygen species in other systems [47, 48], were shown to reduce the accumulation of reactive oxygen species in ammonia-treated cultured astrocytes [49]. Altogether, these studies suggest that oxidative stress is induced by astrocytes in conditions associated with increased levels of ammonia.

Another factor by which ammonia and oxidative stress have been related to hepatic encephalopathy is the MPT, a calcium-dependent process characterized by the opening of the permeability transition pore (PTP) in the inner mitochondrial membrane. Oxidative stress triggers the induction of the MPT and, as a consequence, there is an increased permeability to protons, ions, and other solutes <1,500 Da [50], leading to a collapse of the mitochondrial inner membrane potential. Loss of the mitochondrial membrane potential results in osmotic swelling of the mitochondrial matrix, movement of metabolites across the inner membrane, defective oxidative phosphorylation, cessation of ATP synthesis, and the generation of reactive oxygen species. It was reported that, in cultured astrocytes, oxidative stress would be involved in the induction of the MPT by ammonia, suggesting that oxidative stress and the subsequent induction of the MPT contribute to the pathogenesis of hepatic encephalopathy [51, 52]. Also, treatment of cultured astrocytes with ammonia caused a significant dissipation of the mitochondrial membrane potential as well as an increase in the mitochondrial permeability to 2-deoxyglucose. Both of these changes were blocked by cyclosporin A, a MPT inhibitor. Similarly, ammonia caused a decrease in the mitochondrial calcein fluorescence (an index of the MPT), which was also blocked by cyclosporin A [53].

16.3.1.2 In Vivo Evidence of Ammonia-Induced Oxidative Stress in Hepatic Encephalopathy

Evidence for the implication of oxidative stress in the pathogenesis of hepatic encephalopathy has also been reported in studies in experimental animals. For example, NOS activity has been shown to be increased in the brains of portacaval-shunted rats [54]. iNOS and neuronal NOS (nNOS) protein expression is also increased in the brains of these animals [22, 55, 56], whereas brain endothelial NOS

(eNOS) protein expression is increased in thioacetamide (TAA)-induced cirrhosis in the rat [57]. It was also reported that astrocyte swelling stimulated the production of cerebral nitric oxide in ammonia-treated rats [58]. An increase in HO-1 mRNA expression as well as a decrease in copper/zinc-superoxide dismutase (Cu/Zn SOD) gene expression have been reported in the brains of portacaval-shunted rats [59]. Protein tyrosine nitration, a consequence of oxidative stress, was also demonstrated in the cerebral cortex of these animals [34, 56].

In a rat model of chronic liver failure (portacaval anastomosis), neurons were immunoreactive to nNOS, whereas iNOS was expressed in pyramidal-like cortical neurons and perivascular astrocytes [56]. In the same animals, nitrotyrosine immunoreactivity was found in pyramidal-like cortical neurons and in perivascular astrocytes. It was also demonstrated that nNOS and iNOS are produced in the Purkinje (neuronal) cells and Bergmann glial cells in rats following portacaval anastomosis [55]. TAA-cirrhotic rats showed nNOS immunoreactivity in stellate and basket neurons and eNOS immunoreactivity in perivascular glial cells of the white matter [57]. In the same experimental model of chronic liver failure, eNOS was located in Purkinje cell bodies and vessels endothelial cells [57]. Taken together, these data suggest that neuronal, glial, and endothelial cells are all sources of free radicals and nitric oxide in hepatic encephalopathy indicating that oxidative stress in hepatic encephalopathy is a multicellular phenomenon.

Systemically, the relationship between hyperammonemia and oxidative stress differs from that depicted in the brain. Indeed, in another model of chronic liver failure and hyperammonemia/hepatic encephalopathy, the bile-duct ligated rat, Bosoi et al. [60] observed the presence of systemic oxidative stress and cerebral edema. The authors suggested that systemic oxidative stress might be an important "first hit," which, followed by increases in ammonia, leads to the onset of brain edema [60]. In a similar model of cirrhosis, an increase in lipid peroxidation and reduction in antioxidant enzymes in the cerebral cortex and cerebellum were reported [61]. Interestingly, the administration of *N*-acetylcysteine exerted a protective effect through the attenuation of oxidative stress [61].

16.3.1.3 Human Studies

While considerable evidence indicates the presence of oxidative stress markers in experimental models of hepatic encephalopathy, documentation of oxidative stress in humans is limited. Increased amount of lipofuscin pigments was found in brains of patients with hepatic encephalopathy [36, 40]. Elevated blood levels of reactive oxygen species were also identified in patients with hepatic encephalopathy resulting from chronic alcohol consumption, which was associated with decrease antioxidant capacity [62]. Increased SOD activity, thiobarbituric acid reactive substances, and decreased catalase activities were observed in cirrhotic children [63]. Increased nitric oxide after transjugular intrahepatic portosystemic shunt insertion in patients with cirrhosis was also reported [64]. Furthermore, postmortem cortical brain tissue samples from patients with cirrhosis dying with or without hepatic encephalopathy

were analyzed and compared with brains from patients without liver disease [35]. The results indicate that hepatic encephalopathy in patients with cirrhosis is associated with oxidative stress, protein tyrosine nitration, and RNA oxidation [35].

In postmortem human brain tissue obtained from autopsies of patients with cirrhosis and hepatic encephalopathy, a whole human genome microarray approach revealed altered expression of genes related to oxidative stress [65]. Specifically, expression levels of genes involved in oxidative stress defense, such as HO-1, selenoprotein-V, peroxiredoxin-4, and peroxisome proliferator-activated receptor α (PPARα), were elevated in patients with cirrhosis with hepatic encephalopathy but not in patients with cirrhosis without hepatic encephalopathy, when compared with controls [65]. Taken together, these data strongly suggest a role for oxidative stress in the pathogenesis of hepatic encephalopathy in patients with chronic liver failure and indicate that cerebral oxidative stress is a hallmark of hepatic encephalopathy in patients with liver failure.

16.3.2 Manganese-Induced Oxidative Stress in Hepatic Encephalopathy

Manganese is an essential trace element found in a variety of biological tissues and is necessary for normal functioning of several physiological processes including amino acid, lipid, protein, and carbohydrate metabolism [66]. Manganese is also an important component of a number of cerebral enzymes, in particular, glutamine synthetase, an ammonia detoxifying enzyme. At low levels, manganese binds with superoxide dismutase to form MnSOD, an important mitochondrial antioxidant enzyme [67]. However, when excessive, manganese contributes to neurological abnormalities such as parkinsonism and dystonia [68]. Occupational exposure to excessive manganese levels leads to neurotoxicity, referred to as manganism, which resembles Parkinson's disease [69]. Chronic exposure of various cell types to manganese was shown to induce oxidative stress [70–72].

Manganese has also been implicated in the pathogenesis of hepatic encephalopathy [73]. Manganese highly accumulates in astrocytes [74, 75], which renders these cells more vulnerable to its toxicity. Consistent with this vulnerability, manganese has been shown to decrease antioxidant capacity [72] and generate oxidative stress [72, 76, 77], which are prevented by pre-treatment with *N*-acetylcysteine [78]. Manganese also brings about mitochondrial dysfunction [79, 80], including decreased energy production [72] and the induction of the MPT [81], and causes histopathological changes in astrocytes (Alzheimer type II change) [82]. Interestingly, morphologic and functional changes after exposure of astrocytes to manganese are similar to those observed after ammonia treatment. Cultured astrocytes exposed to ammonia (5 mM) or manganese acetate (100 mM) were shown to increase both free radicals production and L-arginine uptake (a precursor of nitric oxide), and such effects were synergized when manganese was co-treated with ammonia [77, 83]. Similarly, exposure of primary cortical astrocytes to a low

concentration of manganese (10 μM) was shown to potentiate interferon-gamma and tumor necrosis factor-alpha-induced expression of iNOS mRNA and protein along with an increased production of nitric oxide [84]. The potentiating effect was a consequence of the activation of soluble guanylate cyclase and mitogen-activated protein kinase (MAPK) signaling pathways [84]. Cultured astrocytes exposed to manganese were also shown to inhibit glutamate uptake by a process involving oxidative stress [85, 86]. Additionally, it was demonstrated that treatment of rats with manganese chloride led to an increase in manganese level in brain that was accompanied by the development of pathological changes similar to those seen in hepatic encephalopathy (Alzheimer type II astrocytosis), and such changes were significantly reduced when rats were treated with antioxidant *N*-actetylcysteine. In primary rat cortical neurons exposed to manganese, an increase in biomarkers of oxidative damage (F(2)-isoprostanes), which was prevented by pretreatment with the antioxidant Trolox (hydrophilic analog of vitamin E), was reported [87]. These results were confirmed in mice exposed to manganese [87]. Finally, it was recently demonstrated that manganese leads to an increase in markers of oxidative stress in rat brain chronically exposed to manganese [88]. These studies suggest that manganese contributes to oxidative stress in hepatic encephalopathy and that such effect is exacerbated in the presence of ammonia.

Patients with chronic liver failure and those who had surgically created portal-systemic shunts have elevated plasma and brain manganese levels, most likely reflecting the combined effects of hepatocellular failure, impaired biliary excretion, and the presence of portal-systemic shunting of blood [89]. This may lead to selective manganese accumulation in the globus pallidus, caudate nucleus, and putamen, and the adjacent areas of the basal ganglia manifest as hyperintensity of these brains areas on T1-Magnetic Resonance Imaging [89, 90]. Indeed, elevated manganese levels were found in the globus pallidus obtained at autopsy from patients with chronic liver failure [90, 91]. Disturbances of manganese homeostasis may partly account for the cognitive impairment associated with chronic liver failure [92].

16.4 Antioxidant Strategies for the Treatment of Hepatic Encephalopathy

Treatment of experimental animals suffering from hyperammonemia and hepatic encephalopathy with antioxidants (e.g., ascorbate, alpha-tocopherol, dimethylsulfoxide) was shown to have beneficial effects by improving antioxidant status as well as their clinical condition [93, 94]. The antioxidant melatonin was shown to reduce blood and brain ammonia level as well as attenuate brain lipid peroxidation in rats after TAA injection [95]. Additionally, increased malondialdehyde levels and decreased glutathione peroxidase, catalase, and SOD activities were found in the hippocampal tissue of rats with portal hypertension (a model of low-grade hepatic encephalopathy), and such effects were reversed when rats were

treated with curcumin, a known antioxidant [96]. Rats treated with morin (3,4,7,2′, 4′-pentahydroxyflavone), a flavonol, were shown to be protected against oxidative stress in brains of chronic hyperammonemic rats [97]. It was recently demonstrated that guanosine, a nucleoside exhibiting antioxidant properties [98, 99], was neuroprotective in a rat model of chronic hepatic encephalopathy by reducing oxidative stress markers in the brain [100].

The antioxidant *N*-acetylcysteine has proven useful in reducing brain edema in acute liver failure [101] and in the management of patients with acute liver failure [102–105]. In addition, *N*-acetylcysteine was shown to delay the progression of encephalopathy in azoxymethane-induced acute liver failure in mice, as well as to reduce brain water content [101]. Finally, hypothermia, which has been shown to improve brain edema in animals and humans with acute liver failure, is also known to reduce free radical production [106]. Interestingly, *N*-acetylcysteine was able to ameliorate spatial memory and motor coordination deficits observed experimental chronic liver failure (bile-duct ligated rats) [61]. *N*-Acetylcysteine supplementation decreased lipid peroxidation and was also able to restore the activity of antioxidant enzymes as well as structural deficits observed in the cortex and cerebellum of cirrhotic animals with hepatic encephalopathy. Together, these data clearly demonstrate that the protective effect of *N*-acetylcysteine in experimental hepatic encephalopathy is mediated through attenuation of oxidative stress, suggesting a therapeutic role for *N*-acetylcysteine in patients afflicted with hepatic encephalopathy.

16.5 Conclusion

Oxidative stress has evolved in recent years as a major pathogenetic factor in hepatic encephalopathy and experimental evidence for oxidative stress in brain in experimental models of hepatic encephalopathy due to chronic liver failure is increasing. Indeed, several reports suggest that oxidative stress participates in the pathophysiologic cascade responsible for hepatic encephalopathy. While the factors responsible for oxidative stress formation in hepatic encephalopathy remain incompletely understood, it appears that ammonia and manganese would be partly responsible for the production of reactive oxygen species. Although increased oxidative stress in hepatic encephalopathy resulting from chronic liver failure has been demonstrated by some groups, its consequences are not fully established. Additional studies on the role of oxidative stress in chronic hepatic encephalopathy are warranted. Increased oxidative stress has been documented in several studies and antioxidants were shown to be protective against ammonia-induced astrocyte swelling and cerebral edema in liver failure. Antioxidant therapy such as *N*-acetylcysteine is already being used in the management of acute liver failure and its complications. Other antioxidants could prove to be valuable adjuncts to traditional hepatic encephalopathy therapies, such as ammonia-lowering strategies, in the context of chronic liver failure. Further studies are needed in order to assess these possibilities.

References

1. Zatoński WA, Sulkowska U, Mańczuk M, Rehm J, Boffetta P, Lowenfels AB et al (2010) Liver cirrhosis mortality in Europe, with special attention to Central and Eastern Europe. Eur Addict Res 16:193–201
2. FASTSTATS—chronic liver disease or cirrhosis [Internet]. [cited 2014 May 28]. Available from http://www.cdc.gov/nchs/fastats/liver-disease.htm
3. Canadian Liver Foundation (2013) Liver disease in Canada—a crisis in the making. Canadian Liver Foundation
4. Selberg O, Böttcher J, Tusch G, Pichlmayr R, Henkel E, Müller MJ (1997) Identification of high- and low-risk patients before liver transplantation: a prospective cohort study of nutritional and metabolic parameters in 150 patients. Hepatology 25:652–657
5. Hartmann IJ, Groeneweg M, Quero JC, Beijeman SJ, de Man RA, Hop WC et al (2000) The prognostic significance of subclinical hepatic encephalopathy. Am J Gastroenterol 95: 2029–2034
6. Bismuth M, Funakoshi N, Cadranel J-F, Blanc P (2011) Hepatic encephalopathy: from pathophysiology to therapeutic management. Eur J Gastroenterol Hepatol 23:8–22
7. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT (2002) Hepatic encephalopathy—definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 35:716–721
8. Mullen KD, Prakash RK (2012) Hepatic encephalopathy. Humana Press, Totowa
9. Stewart CA, Smith GE (2007) Minimal hepatic encephalopathy. Nat Clin Pract Gastroenterol Hepatol 4:677–685
10. Butterworth RF (2002) Pathophysiology of hepatic encephalopathy: a new look at ammonia. Metab Brain Dis 17:221–227
11. Mousseau DD, Butterworth RF (1994) Current theories on the pathogenesis of hepatic encephalopathy. Proc Soc Exp Biol Med 206:329–344
12. Norenberg MD, Huo Z, Neary JT, Roig-Cantesano A (1997) The glial glutamate transporter in hyperammonemia and hepatic encephalopathy: relation to energy metabolism and glutamatergic neurotransmission. Glia 21:124–133
13. Szerb JC, Butterworth RF (1992) Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. Prog Neurobiol 39:135–153
14. Desjardins P, Butterworth RF (2002) The "peripheral-type" benzodiazepine (omega 3) receptor in hyperammonemic disorders. Neurochem Int 41:109–114
15. Ahboucha S, Pomier-Layrargues G, Mamer O, Butterworth RF (2006) Increased levels of pregnenolone and its neuroactive metabolite allopregnanolone in autopsied brain tissue from cirrhotic patients who died in hepatic coma. Neurochem Int 49:372–378
16. Hermenegildo C, Marcaida G, Montoliu C, Grisolía S, Miñana MD, Felipo V (1996) NMDA receptor antagonists prevent acute ammonia toxicity in mice. Neurochem Res 21:1237–1244
17. Marcaida G, Felipo V, Hermenegildo C, Miñana MD, Grisolía S (1992) Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. FEBS Lett 296:67–68
18. Hawkins RA, Jessy J, Mans AM, De Joseph MR (1993) Effect of reducing brain glutamine synthesis on metabolic symptoms of hepatic encephalopathy. J Neurochem 60:1000–1006
19. Takahashi H, Koehler RC, Brusilow SW, Traystman RJ (1991) Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. Am J Physiol 261: H825–H829
20. Ong JP, Aggarwal A, Krieger D, Easley KA, Karafa MT, Van Lente F et al (2003) Correlation between ammonia levels and the severity of hepatic encephalopathy. Am J Med 114: 188–193
21. Kundra A, Jain A, Banga A, Bajaj G, Kar P (2005) Evaluation of plasma ammonia levels in patients with acute liver failure and chronic liver disease and its correlation with the severity of hepatic encephalopathy and clinical features of raised intracranial tension. Clin Biochem 38:696–699

22. Rao VLR (2002) Nitric oxide in hepatic encephalopathy and hyperammonemia. Neurochem Int 41:161–170
23. Medina J, Moreno-Otero R (2005) Pathophysiological basis for antioxidant therapy in chronic liver disease. Drugs 65:2445–2461
24. Albano E (2006) Alcohol, oxidative stress and free radical damage. Proc Nutr Soc 65: 278–290
25. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
26. Bosoi CR, Parent-Robitaille C, Anderson K, Tremblay M, Rose CF (2011) AST-120 (spherical carbon adsorbent) lowers ammonia levels and attenuates brain edema in bile duct-ligated rats. Hepatology 53:1995–2002
27. Wright G, Soper R, Brooks HF, Stadlbauer V, Vairappan B, Davies NA et al (2010) Role of aquaporin-4 in the development of brain oedema in liver failure. J Hepatol 53:91–97
28. Davies NA, Wright G, Ytrebø LM, Stadlbauer V, Fuskevåg O-M, Zwingmann C et al (2009) L-ornithine and phenylacetate synergistically produce sustained reduction in ammonia and brain water in cirrhotic rats. Hepatology 50:155–164
29. Häussinger D, Görg B (2010) Interaction of oxidative stress, astrocyte swelling and cerebral ammonia toxicity. Curr Opin Clin Nutr Metab Care 13:87–92
30. Shah NJ, Neeb H, Kircheis G, Engels P, Häussinger D, Zilles K (2008) Quantitative cerebral water content mapping in hepatic encephalopathy. Neuroimage 41:706–717
31. Reinehr R, Görg B, Becker S, Qvartskhava N, Bidmon HJ, Selbach O et al (2007) Hypoosmotic swelling and ammonia increase oxidative stress by NADPH oxidase in cultured astrocytes and vital brain slices. Glia 55:758–771
32. Häussinger D, Schliess F (2008) Pathogenetic mechanisms of hepatic encephalopathy. Gut 57:1156–1165
33. Kruczek C, Görg B, Keitel V, Pirev E, Kröncke KD, Schliess F et al (2009) Hypoosmotic swelling affects zinc homeostasis in cultured rat astrocytes. Glia 57:79–92
34. Schliess F, Görg B, Fischer R, Desjardins P, Bidmon HJ, Herrmann A et al (2002) Ammonia induces MK-801-sensitive nitration and phosphorylation of protein tyrosine residues in rat astrocytes. FASEB J 16:739–741
35. Görg B, Qvartskhava N, Bidmon H-J, Palomero-Gallagher N, Kircheis G, Zilles K et al (2010) Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy. Hepatology 52:256–265
36. Brunk UT (1989) On the origin of lipofuscin; the iron content of residual bodies, and the relation of these organelles to the lysosomal vacuome. A study on cultured human glial cells. Adv Exp Med Biol 266:313–320; discussion 321–322
37. Gregorios JB, Mozes LW, Norenberg MD (1985) Morphologic effects of ammonia on primary astrocyte cultures. II. Electron microscopic studies. J Neuropathol Exp Neurol 44:404–414
38. Gregorios JB, Mozes LW, Norenberg LO, Norenberg MD (1985) Morphologic effects of ammonia on primary astrocyte cultures. I. Light microscopic studies. J Neuropathol Exp Neurol 44:397–403
39. O'Connor JE, Costell M (1990) New roles of carnitine metabolism in ammonia cytotoxicity. Adv Exp Med Biol 272:183–195
40. Norenberg MD (1987) The role of astrocytes in hepatic encephalopathy. Neurochem Pathol 6:13–33
41. Schliess F, Görg B, Häussinger D (2006) Pathogenetic interplay between osmotic and oxidative stress: the hepatic encephalopathy paradigm. Biol Chem 387:1363–1370
42. Norenberg MD, Rao KVR, Jayakumar AR (2005) Mechanisms of ammonia-induced astrocyte swelling. Metab Brain Dis 20:303–318
43. Schliess F, Foster N, Görg B, Reinehr R, Häussinger D (2004) Hypoosmotic swelling increases protein tyrosine nitration in cultured rat astrocytes. Glia 47:21–29
44. Warskulat U, Görg B, Bidmon H-J, Müller HW, Schliess F, Häussinger D (2002) Ammonia-induced heme oxygenase-1 expression in cultured rat astrocytes and rat brain in vivo. Glia 40:324–336

45. Sinke AP, Jayakumar AR, Panickar KS, Moriyama M, Reddy PVB, Norenberg MD (2008) NFkappaB in the mechanism of ammonia-induced astrocyte swelling in culture. J Neurochem 106:2302–2311
46. Konopacka A, Zielińska M, Albrecht J (2008) Ammonia inhibits the C-type natriuretic peptide-dependent cyclic GMP synthesis and calcium accumulation in a rat brain endothelial cell line. Neurochem Int 52:1160–1166
47. Woodard GE, Rosado JA (2008) Natriuretic peptides in vascular physiology and pathology. Int Rev Cell Mol Biol 268:59–93
48. De Vito P, Incerpi S, Pedersen JZ, Luly P (2010) Atrial natriuretic peptide and oxidative stress. Peptides 31:1412–1419
49. Skowrońska M, Zielińska M, Albrecht J (2010) Stimulation of natriuretic peptide receptor C attenuates accumulation of reactive oxygen species and nitric oxide synthesis in ammonia-treated astrocytes. J Neurochem 115:1068–1076
50. Zoratti M, Szabò I (1995) The mitochondrial permeability transition. Biochim Biophys Acta 1241:139–176
51. Bai M, Qi X, Yang Z, Yin Z, Nie Y, Yuan S et al (2011) Predictors of hepatic encephalopathy after transjugular intrahepatic portosystemic shunt in cirrhotic patients: a systematic review. J Gastroenterol Hepatol 26:943–951
52. Rao KV, Panickar KS, Jayakumar AR, Norenberg MD (2005) Astrocytes protect neurons from ammonia toxicity. Neurochem Res 30:1311–1318
53. Bai G, Rao KVR, Murthy CR, Panickar KS, Jayakumar AR, Norenberg MD (2001) Ammonia induces the mitochondrial permeability transition in primary cultures of rat astrocytes. J Neurosci Res 66:981–991
54. Rao VL, Audet RM, Butterworth RF (1995) Selective alterations of extracellular brain amino acids in relation to function in experimental portal-systemic encephalopathy: results of an in vivo microdialysis study. J Neurochem 65:1221–1228
55. Suárez I, Bodega G, Rubio M, Fernández B (2005) Down-regulation of astroglial proteins in the rat cerebellum after portacaval anastomosis. Neuropathol Appl Neurobiol 31:163–169
56. Suárez I, Bodega G, Arilla E, Felipo V, Fernández B (2006) The expression of nNOS, iNOS and nitrotyrosine is increased in the rat cerebral cortex in experimental hepatic encephalopathy. Neuropathol Appl Neurobiol 32:594–604
57. Hernández R, Martínez-Lara E, Moral MLD, Blanco S, Cañuelo A, Siles E et al (2004) Upregulation of endothelial nitric oxide synthase maintains nitric oxide production in the cerebellum of thioacetamide cirrhotic rats. Neuroscience 126:879–887
58. Master S, Gottstein J, Blei AT (1999) Cerebral blood flow and the development of ammonia-induced brain edema in rats after portacaval anastomosis. Hepatology 30:876–880
59. Song G, Dhodda VK, Blei AT, Dempsey RJ, Rao VLR (2002) 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 68:730–737
60. Bosoi CR, Yang X, Huynh J, Parent-Robitaille C, Jiang W, Tremblay M et al (2012) Systemic oxidative stress is implicated in the pathogenesis of brain edema in rats with chronic liver failure. Free Radic Biol Med 52:1228–1235
61. Dhanda S, Kaur S, Sandhir R (2013) Preventive effect of N-acetyl-L-cysteine on oxidative stress and cognitive impairment in hepatic encephalopathy following bile duct ligation. Free Radic Biol Med 56:204–215
62. Negru T, Ghiea V, Păsărică D (1999) Oxidative injury and other metabolic disorders in hepatic encephalopathy. Rom J Physiol 36:29–36
63. Ribeiro L, Andreazza AC, Salvador M, da Silveira TR, Vieira S, Nora DB et al (2007) Oxidative stress and S100B protein in cirrhotic children. Neurochem Res 32:1600–1603
64. Jalan R, Olde Damink SWM, Ter Steege JC, Redhead DN, Lee A, Hayes PC et al (2011) Acute endotoxemia following transjugular intrahepatic stent-shunt insertion is associated with systemic and cerebral vasodilatation with increased whole body nitric oxide production in critically ill cirrhotic patients. J Hepatol 54:265–271

65. Görg B, Bidmon H-J, Häussinger D (2013) Gene expression profiling in the cerebral cortex of patients with cirrhosis with and without hepatic encephalopathy. Hepatology 57:2436–2447
66. Aschner M, Erikson KM, Dorman DC (2005) Manganese dosimetry: species differences and implications for neurotoxicity. Crit Rev Toxicol 35:1–32
67. Holley AK, Dhar SK, St Clair DK (2010) Manganese superoxide dismutase versus p53: the mitochondrial center. Ann N Y Acad Sci 1201:72–78
68. Pal PK, Samii A, Calne DB (1999) Manganese neurotoxicity: a review of clinical features, imaging and pathology. Neurotoxicology 20:227–238
69. Olanow CW (2004) Manganese-induced parkinsonism and Parkinson's disease. Ann N Y Acad Sci 1012:209–223
70. Sun AY, Yang WL, Kim HD (1993) Free radical and lipid peroxidation in manganese-induced neuronal cell injury. Ann N Y Acad Sci 679:358–363
71. Desole MS, Esposito G, Migheli R, Fresu L, Sircana S, Zangani D et al (1995) Cellular defence mechanisms in the striatum of young and aged rats subchronically exposed to manganese. Neuropharmacology 34:289–295
72. Chen C-J, Liao S-L (2002) Oxidative stress involves in astrocytic alterations induced by manganese. Exp Neurol 175:216–225
73. Butterworth RF (2010) Metal toxicity, liver disease and neurodegeneration. Neurotox Res 18:100–105
74. Aschner M, Vrana KE, Zheng W (1999) Manganese uptake and distribution in the central nervous system (CNS). Neurotoxicology 20:173–180
75. Aschner M, Gannon M, Kimelberg HK (1992) Manganese uptake and efflux in cultured rat astrocytes. J Neurochem 58:730–735
76. Erikson KM, Dorman DC, Lash LH, Aschner M (2005) Persistent alterations in biomarkers of oxidative stress resulting from combined in utero and neonatal manganese inhalation. Biol Trace Elem Res 104:151–163
77. Jayakumar AR, Rao KVR, Kalaiselvi P, Norenberg MD (2004) Combined effects of ammonia and manganese on astrocytes in culture. Neurochem Res 29:2051–2056
78. Zhang F, Xu Z, Gao J, Xu B, Deng Y (2008) In vitro effect of manganese chloride exposure on energy metabolism and oxidative damage of mitochondria isolated from rat brain. Environ Toxicol Pharmacol 26:232–236
79. Gavin CE, Gunter KK, Gunter TE (1990) Manganese and calcium efflux kinetics in brain mitochondria. Relevance to manganese toxicity. Biochem J 266:329–334
80. Heron P, Cousins K, Boyd C, Daya S (2001) Paradoxical effects of copper and manganese on brain mitochondrial function. Life Sci 68:1575–1583
81. Rao KVR, Norenberg MD (2004) Manganese induces the mitochondrial permeability transition in cultured astrocytes. J Biol Chem 279:32333–32338
82. Olanow CW, Good PF, Shinotoh H, Hewitt KA, Vingerhoets F, Snow BJ et al (1996) Manganese intoxication in the rhesus monkey: a clinical, imaging, pathologic, and biochemical study. Neurology 46:492–498
83. Hazell AS, Norenberg MD (1998) Ammonia and manganese increase arginine uptake in cultured astrocytes. Neurochem Res 23:869–873
84. Moreno JA, Sullivan KA, Carbone DL, Hanneman WH, Tjalkens RB (2008) Manganese potentiates nuclear factor-kappaB-dependent expression of nitric oxide synthase 2 in astrocytes by activating soluble guanylate cyclase and extracellular responsive kinase signaling pathways. J Neurosci Res 86:2028–2038
85. Volterra A, Trotti D, Tromba C, Floridi S, Racagni G (1994) Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. J Neurosci 14:2924–2932
86. Hazell AS, Norenberg MD (1997) Manganese decreases glutamate uptake in cultured astrocytes. Neurochem Res 22:1443–1447
87. Milatovic D, Gupta RC, Yu Y, Zaja-Milatovic S, Aschner M (2011) Protective effects of antioxidants and anti-inflammatory agents against manganese-induced oxidative damage and neuronal injury. Toxicol Appl Pharmacol 256:219–226

88. Fernsebner K, Zorn J, Kanawati B, Walker A, Michalke B (2014) Manganese leads to an increase in markers of oxidative stress as well as to a shift in the ratio of Fe(II)/(III) in rat brain tissue. Metallomics 6:921–931
89. Inoue E, Hori S, Narumi Y, Fujita M, Kuriyama K, Kadota T et al (1991) Portal-systemic encephalopathy: presence of basal ganglia lesions with high signal intensity on MR images. Radiology 179:551–555
90. Layrargues GP, Shapcott D, Spahr L, Butterworth RF (1995) Accumulation of manganese and copper in pallidum of cirrhotic patients: role in the pathogenesis of hepatic encephalopathy? Metab Brain Dis 10:353–356
91. Krieger D, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H (1995) Manganese and chronic hepatic encephalopathy. Lancet 346:270–274
92. Tarter RE, Hegedus AM, Van Thiel DH, Schade RR, Gavaler JS, Starzl TE (1984) Nonalcoholic cirrhosis associated with neuropsychological dysfunction in the absence of overt evidence of hepatic encephalopathy. Gastroenterology 86:1421–1427
93. Bruck R, Aeed H, Shirin H, Matas Z, Zaidel L, Avni Y et al (1999) The hydroxyl radical scavengers dimethylsulfoxide and dimethylthiourea protect rats against thioacetamide-induced fulminant hepatic failure. J Hepatol 31:27–38
94. Guerrini VH (1994) Effect of antioxidants on ammonia induced CNS-renal pathobiology in sheep. Free Radic Res 21:35–43
95. Túnez I, Muñoz MC, Medina FJ, Salcedo M, Feijóo M, Montilla P (2007) Comparison of melatonin, vitamin E and L-carnitine in the treatment of neuro- and hepatotoxicity induced by thioacetamide. Cell Biochem Funct 25:119–127
96. Roselló DM, Balestrasse K, Coll C, Coll S, Tallis S, Gurni A et al (2008) Oxidative stress and hippocampus in a low-grade hepatic encephalopathy model: protective effects of curcumin. Hepatol Res 38:1148–1153
97. Subash S, Subramanian P (2009) Morin a flavonoid exerts antioxidant potential in chronic hyperammonemic rats: a biochemical and histopathological study. Mol Cell Biochem 327: 153–161
98. Petronilho F, Périco SR, Vuolo F, Mina F, Constantino L, Comim CM et al (2012) Protective effects of guanosine against sepsis-induced damage in rat brain and cognitive impairment. Brain Behav Immun 26:904–910
99. Schmidt AP, Lara DR, Souza DO (2007) Proposal of a guanine-based purinergic system in the mammalian central nervous system. Pharmacol Ther 116:401–416
100. Paniz LG, Calcagnotto ME, Pandolfo P, Machado DG, Santos GF, Hansel G et al (2014) Neuroprotective effects of guanosine administration on behavioral, brain activity, neurochemical and redox parameters in a rat model of chronic hepatic encephalopathy. Metab Brain Dis 29(3):645–654
101. Bémeur C, Vaquero J, Desjardins P, Butterworth RF (2010) N-acetylcysteine attenuates cerebral complications of non-acetaminophen-induced acute liver failure in mice: antioxidant and anti-inflammatory mechanisms. Metab Brain Dis 25:241–249
102. Harrison PM, Wendon JA, Gimson AE, Alexander GJ, Williams R (1991) Improvement by acetylcysteine of hemodynamics and oxygen transport in fulminant hepatic failure. N Engl J Med 324:1852–1857
103. Jones AL (1998) Mechanism of action and value of N-acetylcysteine in the treatment of early and late acetaminophen poisoning: a critical review. J Toxicol Clin Toxicol 36:277–285
104. Walsh TS, Hopton P, Philips BJ, Mackenzie SJ, Lee A (1998) The effect of N-acetylcysteine on oxygen transport and uptake in patients with fulminant hepatic failure. Hepatology 27: 1332–1340
105. Wendon JA, Harrison PM, Keays R, Williams R (1994) Cerebral blood flow and metabolism in fulminant liver failure. Hepatology 19:1407–1413
106. Globus MY, Alonso O, Dietrich WD, Busto R, Ginsberg MD (1995) Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J Neurochem 65:1704–1711