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Editorial

Infection, inflammation and hepatic encephalopathy, synergism redefined

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Every housestaff officer in charge of overnight admissions to a Hepatology service is aware of the need to rule out infection in patients with cirrhosis who present with hepatic encephalopathy (HE). During the day, he/she had been repeatedly taught that spontaneous bacterial peritonitis could exhibit changes in mental state as its sole clinical manifestation. In fact, the instructor should also highlight the entire spectrum of hepatic encephalopathy being influenced by the presence of infection and the concomitant systemic inflammatory response syndrome (SIRS), defined as a temperature greater than 38 °C or less than 36 °C; heart rate greater than 90 beats per minute; tachypnea greater than 20 breaths per minute or $PaCO_2$ less than 4.3 kPa; white cell count greater than 12×10^9/l or less than 4×10^9/l or the presence of greater than 10% immature neutrophils [1]. In acute liver failure, large clinical series have documented the higher prevalence of infection and SIRS among individuals with severe HE [2]. In a recent study of the US Acute Liver Failure group, progression of HE from mild to deeper stages was temporarily associated with the development of infection, especially in those with acetaminophen-induced ALF [3]. The development of acute-on-chronic liver failure, where HE and renal failure dominate the clinical picture [4], is often triggered by the presence of infection and its serious systemic consequences.

It is important to note that other inflammatory conditions associated with the development of SIRS result in changes in mental state. Some are not related to liver disease, such as pancreatitis and burns. In the case of liver disease, a subset of patients with severe alcoholic hepatitis may present with 'spontaneous' HE without an identified precipitant [5], with patients exhibiting features of the SIRS. Infection and concomitant inflammation are most noticeable in patients with cirrhosis and overt HE. In this issue of the Journal, Jalan and co-workers present data [6] to suggest that the subtlest manifestation of HE, minimal encephalopathy in subjects with cirrhosis, can also be added to this list.

The investigators studied patients with cirrhosis who presented with a diverse group of infections and who did not exhibit overt signs of HE. At baseline, within 24–36 h of starting antibiotics, neuropsychological testing was performed after the administration of an oral amino acid solution that results in a transient increase in blood ammonia levels without causing overt changes in mental state. Results of neuropsychological testing were abnormal but improved 1 week later, once the infection had been contained. Plasma levels of inflammatory cytokines were decreased. After resolution of the infection, the same amino acid challenge resulted in a similar rise in ammonia but without affecting neuropsychological reactions. The authors conclude that the SIRS to infection modulates the effects of ammonia on the brain.

Studies of the pathogenesis of hepatic encephalopathy have focused on the deleterious role of toxins on the brain. Zieve had postulated several decades ago a synergistic role of ammonia with products of bacterial metabolism in the gut, such as short chain fatty acids, mercaptans and phenols [7]. The clinical evidence reviewed above supports a different paradigm, where the question is reformulated: how does infection and subsequent inflammation affect mental state in liver disease? A role for ammonia need not be challenged, as the importance of ammonia is based on substantial experimental and clinical evidence [8]. Rather, the challenge is to determine how inflammation and infection exert synergistic effects with toxins such as ammonia.

There is surprisingly scant information in the hepatological literature to answer this question and it is useful to explore the experience of other groups. Patients with all forms of sepsis may develop changes in mental state. Up to 70% of patients with bacteremia exhibit neurological symptoms ranging from lethargy to coma, encompassing a range of symptoms and findings that has been termed sepsis-associated encephalopathy [9]. As often the case in

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metabolic encephalopathies, there may be multiple pathogenic factors and several postulated mechanisms.

1. Role of cytokines

SIRS is a response to the activity of pro-inflammatory cytokines, such as interleukin-1, IL-6, TNFα and interferons, generated in selected cells, such as macrophages, during infection. Higher levels of TNFα and IL-6 are already detectable in the serum of patients with fulminant hepatitis [10] and cirrhosis [11]. Inflammatory cytokines in patients with infections and liver disease could influence brain function at different levels.

(a) *The periphery*. Vagal afferents can be activated at sites where the inflammatory reaction occurs. Via this route, an intraperitoneal injection of lipopolysaccharide (LPS), a constituent of the wall of Gram-negative bacteria, will activate the nucleus tractus solitarius, the primary projection of the vagus nerve [12]. The induction of IL-1β mRNA in rat brain by peripheral IL-1β can be abrogated by a subdiaphragmatic vagotomy [13]. Activation of afferent nerve fibers may be a rapid form of transmission of signals from the inflamed tissue to brain and may underlie the genesis of 'sickness behavior' [14], a constellation of non-specific symptoms present in response to infection and inflammation.

(b) *The cerebral endothelial cell*. Cytokines are large molecules whose entry into brain is limited by the tight blood–brain barrier. Receptors for IL-1β and TNFα are present in brain endothelial cells, whose ligation can result in the transduction of signals culminating in the intracerebral synthesis of nitric oxide and prostanoids [15]. Recent studies have identified perivascular cells of macrophage origin as a target of cytokine effects [16].

(c) *Astrocytes*. HE is characterized by anatomical and functional changes of astrocytes [17]. Cytokines may directly affect astrocyte function, entering the central nervous system through sites lacking a blood–brain barrier (such as the circumventricular organs), diffusing into brain parenchyma and activating transcription factors that in astrocytes increase the production of cytokines, such as IL-6 (reviewed in [18]). In a recent study in patients with uncontrolled intracranial hypertension in acute liver failure, increased brain efflux of IL-1β, TNFα and IL-6 was noted [19], supporting the concept that intracerebral synthesis of cytokines may be an important consequence of the characteristic astrocyte swelling seen in this condition.

2. Oxidative stress

Addition of ammonia to isolated astrocytes generates reactive oxygen species (ROS), a process related to the synthesis of glutamine, as addition of methionine-sulfoximine, an inhibitor of glutamine synthetase, prevents the appearance of ROS [20]. The toxicity of superoxide is enhanced when combined with nitric oxide to form peroxynitrite, a free radical than in the presence of carbon dioxide can modify tissue proteins to form a stable product, nitrotyrosine. In vivo, an infusion of ammonia that causes astrocyte swelling and brain edema results in immunohistochemical evidence of nitrotyrosine accumulation in astrocytes [21].

Nitrosative stress has been proposed as an additional mechanism of injury in HE [22]. Generation of nitric oxide in the brain has been demonstrated in vivo in models of hyperammonemia [23] and sepsis [24]. In primary astrocyte cultures, LPS and interferon γ induce the formation of nitric oxide via activation of inducible nitric oxide synthase [25], an activation also seen when ammonia is added to such cultures [22]. The role of brain iNOS mediating such effects in liver disease in vivo is less clear, as several groups [26,27] note the lack of an increased expression of iNOS mRNA, protein and/or activity in the brain of rats with acute and chronic liver failure.

Cells develop lines of defense against oxidative/nitrosative stress. Microarray studies in brains of rats after portacaval anastomosis point at a decreased mRNA expression of Cu–Zn superoxide dismutase, a key antioxidant enzyme [27]. In sepsis, decreased activities of antioxidant enzymes may be of pathogenic importance [28], including effects on astrocytes [25]. Oxidative stress is a pathogenic mechanism underlying many neurodegenerative disorders, from Parkinson's to Alzheimer dementia to amyotrophic lateral sclerosis [29]. In liver disease, elucidation of the mechanisms by which oxidative stress in astrocytes may influence brain function has emerged as a major focus of research in HE [30].

3. Hemodynamic aspects

Peripheral vasodilatation is a characteristic hemodynamic alteration seen in both liver failure and sepsis. Cerebral blood flow is generally decreased in patients with cirrhosis, a reduction that may reflect a decrease in brain metabolic activity; this concept has been examined in rats with portacaval anastomosis, where an early reduction of cerebral glucose metabolism [31] and blood flow [32] has been demonstrated. An alternative explanation for the reduction of cerebral blood flow in cirrhosis suggests a vasoconstrictor response to the presence of systemic vasodilatation, similar to that seen in the renal circulation [33].

Cerebral blood flow is reduced in patients with the sepsis syndrome [34], with a reduction in brain oxidative

metabolism. Administration of endotoxin to human volunteers resulted in a reduction of cerebral blood flow, explained by the development of hypocapnia [35]. However, in spite of a rise in circulating cytokines (TNFα, IL1-β and IL-6), cerebral oxidative metabolism was preserved, suggesting additional mediators of brain injury in sepsis.

In acute liver failure, an increase in cerebral blood flow has been shown to worsen the neurological picture by increasing brain water and intracranial pressure (reviewed in [36]). Could the reduction in cerebral perfusion seen in patients with cirrhosis and infection be harmful to brain function? Are the effects of liver failure and infection on cerebral perfusion additive? Answers to such questions are important to define new therapeutic strategies in patients with HE.

In conclusion, Jalan and co-workers should be commended for highlighting the importance of infection and inflammation even in minimal alterations of cognitive function in liver disease. In the meantime, our housestaff officer should be reassured he/she is in the right track by doggedly pursuing a possible infection in the patient admitted with HE. While his/her search for other precipitants should not be overlooked, a broader view notes the impact of infection and inflammation on the mental state of all forms of hyperammonemic liver failure. Over the years, several theories have been proposed to explain the mechanisms responsible for HE. The search has been reinvigorated by a new perspective on synergism.

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