Treatment of Hyperammonemia in Liver Failure: A Tale of Two Enzymes

See "Efficacy of L-ornithine L-aspartate in acute liver failure: a double-blind, randomized, placebo-controlled study," by Acharya SK, Bhatia V, Sreenivas V, et al, on page 2159.

The occurrence of encephalopathy in patients with acute liver injury defines acute liver failure (ALF) and changes the natural history of the disease.[1] Cytotoxic brain edema and intracranial hypertension occurring in encephalopathic ALF patients account for a large number of deaths owing to cerebral herniation.[2] From a pathophysiological perspective, the cell type in the brain that is primarily affected is the astrocyte, which in both humans and in animal model systems can be shown to be swollen in the ALF brain.[3] Ammonia has been suggested to play a central role in the pathogenesis of brain edema in ALF.[4–7] Several studies have demonstrated that high arterial ammonia levels in ALF predict the occurrence of intracranial hypertension and death from cerebral edema.[4–7] Elevated arterial ammonia levels correlate closely with brain ammonia uptake; ammonia within the brain is then detoxified by astrocytes via glutamine synthetase (Figure 1), resulting in accumulation of glutamine.[8] Increased astrocytic glutamine concentrations are thought to cause the osmotic stress that results in cell swelling. Although the blood–brain barrier is thought to be intact at least initially in ALF, superimposed inflammatory stress commonly observed in these patients may augment the deleterious effects of hyperammonemia on brain swelling, "unlocking the blood–brain barrier" to the effect of this inflammation.[6,9,10] Lowering plasma ammonia levels is therefore an obvious strategy for the prevention and treatment of brain swelling in ALF.

In the present issue of GASTROENTEROLOGY, Acharya et al[11] describe the results of a large and quite definitive clinical trial of the drug L-ornithine L-aspartate (LOLA) in ALF. LOLA was intended to lower plasma ammonia levels. The rationale for LOLA's use is based on an enhanced understanding of ammonia metabolism. Studies focusing on inter-organ ammonia metabolism in patients with cirrhosis indicate that the liver, muscle, kidney, and small bowel are important in regulating circulating levels of ammonia. In variance with the widely held view that most of the ammonia is produced in the colon from the action of bacteria, recent studies have demonstrated that ammonia is produced primarily in the small bowel from uptake of glutamine, being metabolized to glutamate and ammonia by the enzyme glutaminase.[12,13] Ammonia would normally be converted to urea by the liver, but, in liver failure, ammonia increases to toxic levels. The protein expression and the activity of the enzyme glutamine synthetase has been shown to be up-regulated in muscle in liver failure, which enhances the detoxification of ammonia into glutamine, which is nontoxic and should be excreted effectively.[14] Studies in rats with liver failure have suggested that the administration of LOLA was associated with a lowering of plasma and cerebrospinal fluid ammonia, and a reduction in brain edema.[14] The proposed mechanism of action of LOLA then would be an enhancement of the conversion of L-ornithine to glutamate in muscle, suggesting that muscle could be targeted as an alternative site of ammonia detoxification.[14,15] This important clinical trial by Acharya et al[11] tested this hypothesis in an appropriately powered design in a large, tertiary teaching hospital in New Delhi, India. The authors concluded that treatment of ALF patients with LOLA was not associated with a reduction in ammonia, improvement in encephalopathy, or an improvement in survival.[11]

Controlled clinical trials are very difficult to perform in ALF and the authors are to be commended for successfully conducting a large trial, even if it was a negative one. A major problem with clinical trials in ALF is the confounding effect of liver transplantation that does not allow determination of the true value of intervention as patients are (rightly) rescued by transplantation. In India, unfortunately, liver transplantation is not readily available. That lack of transplantation as rescue allows a clearer determination of the role of other interventions. The authors used appropriate definitions of ALF and randomized 201 of 278 patients who were screened for inclusion (the largest published clinical trial in ALF) in their study to receive either LOLA or placebo in a double-blind manner. They excluded patients who had a >90% chance of mortality defined by their previous published studies.[11] The primary end point for the study was survival and the secondary end points, among others, were effect of the intervention on arterial ammonia levels, recovery from encephalopathy, and the incidence of cerebral edema. Ultimately, data from 185 patients were included in the per-protocol analysis. Although the drug was well tolerated in ALF, mortality (placebo. 33.3% vs LOLA, 42%), ammonia levels on consecutive days and change in encephalopathy grade were similar between groups, leading the authors to conclude that LOLA was

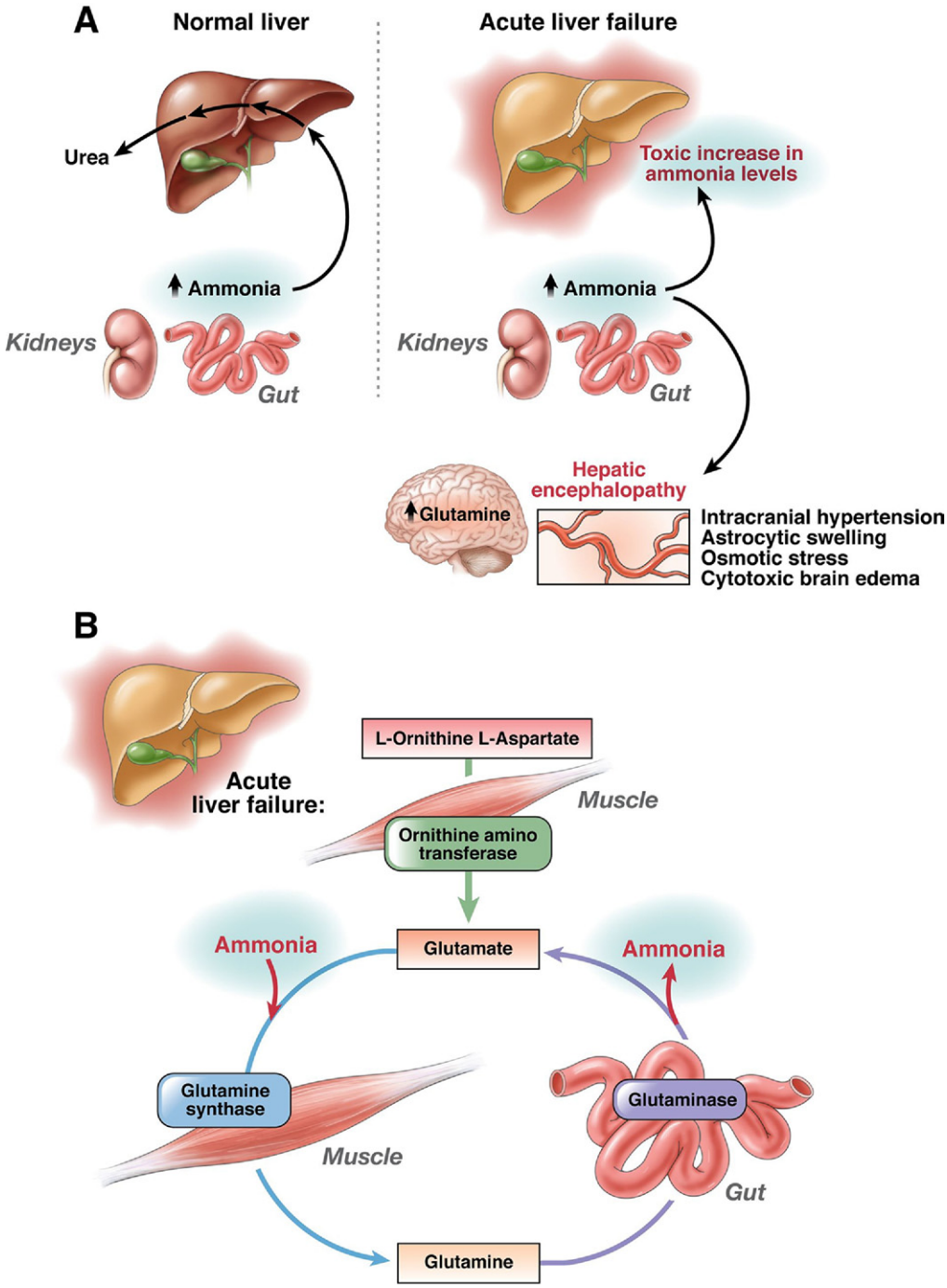

Figure 1. In patients with liver failure, hyperammonemia results from a lack of urea synthetic capacity resulting in increasing quantities of circulating ammonia. The main mechanisms controlling ammonia levels in this situation are the enzymes regulating glutamine, namely, glutamine synthetase and glutaminase, setting up a "futile" cycle. In liver failure, LOLA is thought to act through the conversion of L-ornithine to glutamine in the muscle thereby detoxifying 1 molecule of ammonia. The lack of effectiveness of LOLA in the study by Acharya et al may be explained by the fact that the detoxification of ammonia into glutamine is a transient phenomenon as nitrogen is not actually eliminated from the system. Glutamine essentially acts as a "Trojan horse" for circulation of ammonia and can be readily converted back into glutamate and ammonia by glutaminase present in the gut.

ineffective in ALF. This study again confirmed that the ammonia levels at baseline or the presence of cerebral edema in addition to a prolonged prothrombin time were important predictors of mortality, supporting the rationale for use of an ammonia-lowering agent to improve outcome in ALF.

For the most part, the data from this important negative study can be generalized to the Western population. There are striking differences in etiology between the West and India. such as the fact that acetaminophen overdose remains the main cause of ALF in the United States and the United Kingdom as compared with mostly viral hepatitis and a high frequency (~40%) of pregnant patients in India.[1,16] In terms of standard of care, the lack of feeding for the first 3 days is difficult to justify and it is not clear what proportion of patients in both groups had extracorporeal renal support for renal failure. Otherwise, the patients were managed according to a standard protocol with liberal use of mannitol and appropriate exclusion of patients on confounding medications

such as lactulose.[17] The use of intracranial pressure monitoring is controversial,[18] but in a clinical trial such as this, the question of the relationship between potential ammonia lowering and intracranial pressure would have provided invaluable insight. Also, the lack of amino acid data does not help in demonstrating why LOLA was ineffective.

From a pathophysiologic perspective, despite the available data from animal models showing that LOLA may be a useful treatment for ALF, the efficacy of LOLA has been questioned.[19] The only way to reduce ammonia concentration is either to reduce its production or enhance its removal from the body. LOLA, by providing glutamate to muscle, increases the substrate, which should detoxify ammonia into glutamine, as has been suggested; however, the glutamine produced may then act as a "Trojan horse" for circulation of ammonia and can be converted readily back into glutamate and ammonia by the enzyme phosphate-activated glutaminase present in the gut, kidneys, and liver.[12,13,19,20] In a preliminary study, rebound hyperammonemia was observed in patients with liver failure treated with LOLA,[20] leading to the (revised) hypothesis that if the glutamine that is generated from LOLA can be dealt with in some other fashion, then permanent reduction in ammonia might be achieved. The University College London group has hypothesized that the co-administration of L-ornithine (the active component of LOLA) together with phenylacetate could solve this problem. Phenylacetate is effective in treating urea cycle enzyme disorders in children, acting mainly by scavenging glutamine to form phenylacetylglutamine. In the presence of phenylacetate, the glutamine generated from L-ornithine would now be trapped as phenylacetylglutamine and excreted rather than being available for renewed ammoniagenesis via glutaminase.[20] This proposed synergy between L-ornithine and phenylacetate has been confirmed in studies in cirrhotic rats and ALF pigs.[21,22]

Currently, LOLA is not licensed for use in the United States and most countries in Europe. The study by Acharya et al[11] provides no support for the use of LOLA in ALF. Current therapies include mannitol, the cautious use of hypertonic saline, and possibly hemofiltration. Indomethacin and hypothermia are of unproven benefit. *N*-Acetylcysteine has shown benefit in improving overall outcomes in early stage ALF, but it is uncertain whether it has any effect on ammonia metabolism or the development of cerebral edema.[23] Thus, the treatment of hyperammonemia and hepatic encephalopathy remain an unmet clinical need.

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Conflicts of interest

The authors disclose the following: Professor Rajiv Jalan (University College London) has filed for patents surrounding the use of L-ornithine phenylacetate for the treatment of hepatic encephalopathy. The technology has been licensed to Ocera Therapeutics. William M. Lee discloses no conflicts.

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Prominin1 (CD133) as an Intestinal Stem Cell Marker: Promise and Nuance

See "Prominin-1/CD133 marks stem cells and early progenitors in mouse small intestine" by Snippert HJ, Can Es JS, can den Born M, et al, on page 2187.

The presence of long-lived, self-renewing stem cells in intestinal crypts is well established.[1] Although these cells have been studied extensively for their functions and putative hierarchies, molecular markers are as yet unavailable to permit their prospective isolation, their unequivocal identification in tissue sections, or thorough assessment of mechanisms of stem cell replication and differentiation. The self-renewing property of gut epithelium is in many respects reminiscent of cancer, and indeed, cancers are believed to propagate from small subpopulations of stem-like cells that are inherently more tumorigenic than their progeny.[2,3] Identification of intestinal tumor-initiating cells holds special interest because it would allow investigators to address fundamental questions about the cell of tumor origin and its biology in relation to defined normal counterparts. We might learn, for example, to what extent tumor-initiating cells resemble the long-lived stem cell or transit-amplifying cells, knowledge that will shape future approaches toward understanding the disease and defining therapeutic opportunities. These ideas gathered recent momentum when 2 groups reported that human colon cancers could be separated into tumor-initiating and noninitiating cell populations based on expression of the neuronal and hematopoietic cell surface marker CD133 (known as Prominin1 in the mouse). Such advances provoke the need for clarity and consensus on surface markers that reliably distinguish intestinal stem cells from other crypt populations and help to define the relationship between normal and cancerous stem cells. In this issue of GASTROENTEROLOGY, Snippert et al[4] clear the air with respect to CD133/Prominin1.

Stem cells in most tissues are believed to cycle slowly (although this is not imperative a priori); thus, DNA marked during replication retains the label for extended periods, whereas cycling progeny and migrating cells lose the label with turnover. Applying this principle to the small intestine, Potten et al[5] observed that long-term label-retaining cells lie most commonly at crypt position +4, immediately above the Paneth cell zone, with fewer cells present elsewhere in the crypt. By contrast, Bjerknes and Cheng's analysis[6] of migration of labeled cells led to the idea that stem cells may nestle between Paneth cells at the crypt base. Predating identification of candidate stem cell markers, these studies laid important anatomic and conceptual foundations. The RNA-binding protein Musashi-1 is restricted to crypts, but marks a broad population, including transit-amplifying progenitors.[7] Using label-retaining cells as a standard, He et al proposed phosphorylated PTEN as a stem cell marker[8] and phosphorylation of β-catenin on serine 552 as a marker of activated stem cells.[9] DCAMKL1 and telomerase also are proposed as products specific to stem cells in gut epithelium.[10,11] Each of these markers mainly identifies cells in the predicted "+4" position, lying just above the Paneth cell zone (Figure 1), but none has yet been shown directly to mark the operational stem cell.

The Clevers group's characterization of the Wnt target Lgr5 (also called GPR49) provided the first functionally