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Encephalopathy in acute liver failure resulting from acetaminophen intoxication: New observations with potential therapy

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Abstract

Objective—Hyperammonemia is a major contributing factor to the encephalopathy associated with liver disease. It is now generally accepted that hyperammonemia leads to toxic levels of glutamine in astrocytes. However, the mechanism by which excessive glutamine is toxic to astrocytes is controversial. Nevertheless, there is strong evidence that glutamine-induced osmotic swelling, especially in acute liver failure (ALF), is a contributing factor – the osmotic gliopathy theory. The object of the current communication is to present evidence for the osmotic gliopathy theory in a hyperammonemic patient who overdosed on acetaminophen.

Design—Case report.

Setting—Johns Hopkins Hospital.

Patient—A 22-year old white female who, 36 hours prior to admission, ingested 15 grams of acetaminophen was admitted to the Johns Hopkins Hospital. Physical examination was unremarkable; her mental status was within normal limits and remained so until approximately 72 hours after ingestion when she became confused, irritable and agitated.

Interventions—She was intubated, ventilated and placed on lactulose. Shortly thereafter she was non-communicative, unresponsive to painful stimuli and exhibited decerebrate posturing. A clinical diagnosis of cerebral edema and increased intracranial pressure (ICP) was made. She improved very slowly until 180 hours after ingestion when she moved all extremities. She woke up shortly thereafter.

Measurements and main results—Despite the fact that hyperammonemia is a major contributing factor to the encephalopathy observed in ALF the patient's plasma ammonia peaked when she exhibited no obvious neurological deficit. Thereafter, her plasma ammonia decreased precipitously in parallel with a worsening neurological status. She was deeply encephalopathic during a period when her liver function and plasma ammonia had normalized. Plasma glutamine levels in this patient were high, but began to normalize several hours after plasma ammonia had returned to normal. The patient only commenced to recover as her plasma glutamine began to return to normal.

Conclusions—We suggest that the biochemical data are consistent with the osmotic gliopathy theory – high plasma ammonia leads to high plasma glutamine – an indicator of excess glutamine

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in astrocytes (the site of brain glutamine synthesis). This excess glutamine leads to osmotic stress in these cells. The lag in recovery of brain function presumably reflects time taken for the astrocyte glutamine concentration to return to normal. We hypothesize that an inhibitor of brain glutamine synthesis may be an effective treatment modality for ALF.

Keywords

acetaminophen-poisoning; acute liver failure; astrocytes; encephalopathy; hyperammonemia; intracranial pressure; plasma ammonia; plasma glutamine; osmotic stress

ALF is an uncommon, but nevertheless serious disorder. In 2005, ALF accounted for 6% of liver-related deaths and 6% of orthotopic liver transplants in the U.S. (1). Viral hepatitis was previously reported to be the leading cause of ALF in the U.S., but acetaminophen overdose and idiosyncratic drug reactions are now a more common cause (1). For example, in 2004, data from the U.S. Acute Liver Failure Study Group registry of >700 patients with ALF implicated acetaminophen poisoning in nearly 50% of all cases of ALF in the U.S. (2).

Hyperammonemia and the attendant increase in brain glutamine are major factors in the encephalopathy associated with ALF (3). In addition to ALF, it is apparent that ammonia-induced encephalopathy is a contributing factor to an increasingly recognized spectrum of disorders, including the more common, chronic form of liver disease (4). For example, hyperammonemia-induced encephalopathy is a prominent feature of inborn errors of the urea cycle (5), idiopathic hyperammonemic syndrome after immunosuppression or cytotoxic therapy (6,7), ureolysis in stagnant urine (8,9), valproate therapy (10,11), and essential amino acid total parenteral nutrition (12). Ammonia enters the brain mostly by non-saturable diffusion of the free base (NH_3) (4,13), where it is metabolized almost exclusively to glutamine in a reaction catalyzed by glutamine synthetase (14,15). In the brain, glutamine synthetase is localized largely, if not exclusively, in astrocytes (16,17), and astrocytes are compromised in liver disease/hyperammonemia (18-23). It has been proposed that hyperammonemic encephalopathy in any clinical setting (24) is due, at least in part, to increased ICP, resulting from glutamine-induced osmotic stress in astrocytes – the osmotic gliopathy theory (25). Several magnetic resonance (MR) imaging (MRI) and spectroscopic (MRS) studies are fully in accord with this hypothesis (26). Indeed, based on recent studies, the U.S. Acute Liver Failure Study Group attributes the encephalopathy of hyperammonemia in ALF largely to the synthesis in astrocytes of osmotically active glutamine, resulting in astrocyte swelling, which in turn contributes to the cerebral edema and encephalopathy (27).

The increasing awareness of the importance of glutamine in ALF prompted us to describe sequential plasma ammonia and glutamine levels in relation to neurological status in a patient with severe acetaminophen toxicity – data not previously reported. This case allows us to speculate on a possible new treatment for ALF.

CASE REPORT

This was the first Johns Hopkins Hospital admission of a 22-year old white female who, 36 hours prior to admission, ingested 15 grams of acetaminophen. Shortly after admission her plasma acetaminophen level was 179 mg/dL, for which she was treated with N-acetylcysteine. Apart from mild nausea she was asymptomatic. Her plasma AST, ALT and LDH values (IU/L) were, respectively, 7,090, 6,710, 3,110, and her total and direct bilirubin (mg/dL) were 12.9 and 6.3, respectively. Blood pH and pCO_2 were 7.50 and 34 torr, respectively. The serum phosphate level was 1.8 mg/dL (normal value ~2.5 mg/dL). Although it is well known that hyperventilation causes hypophosphatemia (28), that

explanation has not routinely been applied to the hyperventilation regularly seen early in hyperammonemia. Physical examination was unremarkable; her mental status was within normal limits and remained so until approximately 72 hours after ingestion when she became confused, irritable and agitated. She was intubated, ventilated and placed on lactulose. Shortly thereafter she was non-communicative, unresponsive to painful stimuli and exhibited decerebrate posturing. Her ICP was 17-25 mm Hg as measured through an epidural monitor (reference values 7-15 mm Hg). A clinical diagnosis of cerebral edema and increased ICP was made and she advanced to Stage IVA on the liver transplant list; 80 hours after the ingestion of acetaminophen her coma score was 6. She was unchanged for approximately 24 hours when it was noted that she was responsive to painful stimuli. She improved very slowly until 180 hours after ingestion when she moved all extremities; she woke up shortly thereafter.

This patient revealed an informative relationship among neurological status, plasma AST level, plasma ammonia, and plasma glutamine, during and following a single uncomplicated 72 hour episode of acute liver failure (Figure 1). A brief summary of the time course of the patient's symptoms and recovery is given in Table 1.

DISCUSSION

Plasma glutamine and encephalopathy

Inspection of the relationship between plasma glutamine and neurological status reveals that the patient's encephalopathy coincided with increased plasma glutamine concentration and only abated when the plasma glutamine level returned toward normal (Fig. 1). Plasma glutamine was not measured in this patient at admission, but the zero time pre-ingestion value is plotted in this figure as a normal reference value (~0.6 mmol/L). It is clear from figure 1 that she was hyperglutaminemic prior to the first measurement of the plasma glutamine (~1.3 mmol/L) at 102 hours after ingestion, a value three times that measured at 200 hours after ingestion (~0.4 mmol/L). At 102 hours the patient was comatose, whereas at 200 hours she was rapidly regaining normal neurological function.

Brain glutamine: Estimation using plasma levels as a proxy

Although not measured in the patient, her brain glutamine concentration was undoubtedly greatly elevated for a time as a result of the hyperammonemia. In experimental animals, almost all blood-derived ammonia metabolically trapped in the brain is converted to glutamine, even under severely hyperammonemic conditions (15). Moreover, high ammonia levels inhibit the glutaminase reaction, the main route for metabolism of glutamine in the brain (29). These factors result in glutamine accumulation in the brains of hyperammonemic experimental animals (e.g. 4,14,15,30). Increased brain glutamine is also well established for hyperammonemic patients. For example, glutamine is elevated 2.5 fold compared to controls in frontal cortex biopsies of hyperammonemic patients dying with ALF, and this is accompanied by a 3.8 fold increase in blood glutamine (31). Non-invasive proton magnetic resonance spectroscopy (¹H MRS) studies of patients with hepatic encephalopathy have shown the presence of increased brain glutamine (26). Furthermore, glutamine is markedly increased in cerebrospinal fluid in ALF (31,32) and arginase-deficient (33) patients. Finally, a net negative arterio-venous difference (AV) (i.e. net output) has been reported for glutamine across the brain in healthy, adult human volunteers (34) (see also 35) and in experimental animals (36). This output is likely to increase in hyperammonemic ALF patients.

The blood-brain barrier and choroid plexus contain several glutamine transporters (37). Thus, a possible factor contributing to hyperammonemia-induced increased brain glutamine

is stimulation of glutamine uptake. However, the arrangement and properties of these transporters is such that it is probable the transporters are involved in the net output of glutamine from the brain as a mechanism contributing to nitrogen homeostasis (37). Evidently, however, the efflux rate is not sufficient to keep pace with glutamine synthesis at high levels of ammonia in the astrocyte compartment. Thus, glutamine in the brain accumulates until the astrocyte ammonia levels subside and rate of synthesis is slowed sufficiently to keep pace with glutamine efflux. Taken together, the accumulated findings are consistent with the hypothesis that the plasma glutamine concentration is a likely proxy for glutamine synthesis and output from (rather than input into) the brain, notwithstanding the production of glutamine by other tissues (38). Thus, the correlation between plasma glutamine and neurological status suggests that the encephalopathy in the patient was dependent on increased brain glutamine, particularly in the astrocytic compartment.

If the encephalopathy was glutamine dependent, it may reasonably be asked why the patient did not become fully encephalopathic earlier than 72 hours, especially as it is apparent that she was hyperglutaminemic and her plasma glutamine likely reached its peak prior to the first glutamine measurement at 102 hours. The absence of symptoms in the early stages of cerebral edema may be accounted for by the well known phenomenon of brain compliance in which compression of non-neuronal intracerebral tissue mitigates the early effects of increased volume, thus minimizing the deleterious effects of an increase in ICP (39,40).

In summary, the order of recovery of biological parameters in the patient was: liver function (as deduced from plasma AST levels) < plasma ammonia < plasma glutamine < coma score (Fig. 1). Although ¹HMRS analysis of brain glutamine was not carried out on this patient, one can infer, as noted above, that plasma glutamine is a good surrogate for brain glutamine.

The rapid decline of the patient's mental status occurred at a time (64 – 88 hours after ingestion of acetaminophen) when plasma ammonia was declining, but still above normal. Presumably, during this phase the concentration of brain glutamine continued to increase because, as a result of persistent increased flux of ammonia into brain, glutamine synthesis continued at an accelerated rate in the astrocytic compartment, and glutamine breakdown may have been slowed by the still high ammonia level. As noted above, compliance will protect the brain against astrocyte swelling to some extent. However, this compliance was no longer effective when the increase in astrocyte glutamine reached a critical level and the patient became encephalopathic. Only when brain glutamine levels began to normalize (and relieve osmotic stress) at about ~160 hours did the patient begin to recover, at which time plasma glutamine levels had normalized.

Our studies underscore the need in ALF patients for more correlative studies among blood/plasma parameters (ammonia, glutamine, cerebral A-V difference for glutamine, pH, and liver function indices) and ICP. It is anticipated that positron emission tomography studies and non-invasive MRS and MRI techniques for measuring multiple brain parameters will be a useful adjuncts to such studies.

Brain glutamine synthetase inhibitor as a potential therapeutic target

If excess cerebral glutamine is a major problem in ALF, then interfering with its synthesis in the brain may be of clinical utility. We have suggested that L-methionine-S,R-sulfoximine, a potent glutamine synthetase inhibitor (41), may be useful (24).

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Fig. 1.

Relationship among coma score (yellow triangles), liver function (as assessed by plasma AST; green stars) and plasma biochemistries (NH_4^+ , blue squares; glutamine, red circles). The zero time (pre-ingestion) values of plasma ammonia, glutamine, AST and coma score were assigned normal reference values. Ammonia was measured in the hospital laboratory using the glutamate dehydrogenase method in the Dupont Automatic Clinical Analyzer. The coma scores (Glasgow scale) were measured by the attending nurse. Plasma glutamine was measured by automated amino acid chromatography. AST, aspartate transaminase (aminotransferase) activity. Note that the plasma glutamine was not measured prior to 102 hours. Plasma glutamine probably reached its maximum value coincident with or several hours later than the maximum plasma ammonia level.

Table 1

Chronology of the patient's illness and recovery

Time after ingestion of acetaminophen	Comments
64 h	The patient was neurologically normal, yet her plasma ammonia was 168 $\mu\text{mol/L}$ (more than four times normal), just below its maximum value of 169 $\mu\text{mol/L}$.
72 h	Her neurological status suddenly and rapidly deteriorated in concert with a <i>decrease</i> in the plasma ammonia concentration and an inferred very high plasma glutamine concentration.
110 h	Her deep encephalopathy persisted despite normal plasma ammonia levels, but with plasma glutamine levels two to three times normal. Increased brain glutamine is inferred at this time by exploiting a) plasma glutamine as a surrogate for brain glutamine, b) the known increase in brain glutamine in hyperammonemic animal models, and c) prior studies showing increased brain glutamine in ALF patients by means of MRS techniques and by amino acid analysis of brain biopsies (see the text).
150 h	She showed signs of neurological improvement coincident with a decrease of plasma glutamine concentration toward normal or near normal and an inferred decrease of brain glutamine.
212 h	She was judged to be neurologically normal.